2025 International Conference on Biofabrication

14 Eyl 2025 09:00 → 17 Eyl 2025 18:00 Europe/Warsaw

Palace of Culture and Science

Açıklama

Welcome to the 2025 International Conference on Biofabrication

On behalf of the Organizing Committee, it is our great pleasure to welcome you to the 2025 International Conference on Biofabrication in Warsaw, Poland, from 14th to 17th September 2025.

The conference theme ***Revolutionizing Healthcare Through Biofabrication: Challenges and Breakthroughs for a Healthier Future*** reflects the dynamic evolution of our field. Biofabrication holds the potential to revolutionize healthcare by offering innovative solutions to complex medical challenges. Together, we will explore the latest advancements, address pressing issues, and chart new directions that promise to shape the future of healthcare.

Our program will bridge a diverse range of topics, from the latest advances in biomaterials and bioinks to cutting-edge fabrication technologies and their medical applications. We will also address critical challenges and explore paths for future progress, encouraging collaboration and innovation. In addition to the formal sessions, our workshops and discussions will be designed to inspire connections and facilitate the exchange of ideas.

We are excited to host the International Conference on Biofabrication for the first time in the capital of Poland – Warsaw. With its blend of rich history and modern innovation, the city provides the ideal setting for this event. As we engage in discussions on advancing healthcare, we hope you will also take the opportunity to explore the vibrant culture and unique experiences this dynamic city has to offer.

Your active participation is key to the success of this event. We invite you to share your knowledge, engage in meaningful conversations, and build lasting collaborations that will help advance the field of biofabrication.

Sincerely,
Prof. Wojciech Święszkowski
Conference Chair of the Biofabrication 2025

Dr. Marco Costantini
Program Chair of the Biofabrication 2025
Contact
info@biofabrication2025.org

- 14 Eylül Pazar
  - ISBF Board meeting: invited members only — Rudniew
  - Biofabrication in Practice - Hands-on Workshop — Kruczkowski
  - 14:00
    Registration
  - Opening Session — Ratuszowa
  - Awards Session — Ratuszowa

    Toplantıyı düzenleyenler: Carmelo De Maria (University of Pisa)
  - 17:30
    Coffee break & Poster Session I

    https://indico3.conference4me.psnc.pl/event/86/sessions/2096/#20250915
  - Poster Session I: Poster Exhibition — Poster Area

    posters are on display whole day on Sunday and Monday

    Toplantıyı düzenleyenler: Marco Costantini (Institute of Physical Chemistry Polish Academy of Sciences), Carmelo De Maria (University of Pisa)
  - P1 Plenary Session: Prof. Magdalena Żernicka-Goetz — Ratuszowa

    Toplantıyı düzenleyenler: Leonora Bużańska
    1. Modeling Human Development Beyond Implantation Using Integrated Stem Cell-Derived Models

       Our understanding of human development beyond implantation has been historically limited by the lack of suitable in vitro models. I will present our lab's work on developing advanced stem cell-based systems that model post-implantation stages without the use of intact human embryos. Building on knowledge gained from early developmental biology, we have assembled stem cell populations representing embryonic and extra-embryonic lineages into integrated, embryo-like structures. These stem cell-derived models recapitulate key events of early morphogenesis, including the specification of primordial germ cell precursors. By focusing on these highly controlled models, we are uncovering the cellular and molecular mechanisms that shape early human development, and opening doors to studying processes that were previously out of reach.

       Konuşmacılar: Magdalena Żernicka-Goetz (California Institute of Technology)
  - S01 Special Session: 20 Years of Bioprinting: Honoring the Past, Shaping the Future — Ratuszowa

    panelists: Gabor Forgacs, Thomas Boland, Marci Zenobi Wong

    Toplantıyı düzenleyenler: Carmelo De Maria (The University of Pisa), Thomas Boland (The University of Texas at El Paso)
  - 20:00
    Welcome Reception — Marmurowa
- 15 Eylül Pazartesi
  - Rapid Fire Talks 1 — Kisielewski

    Toplantıyı düzenleyenler: Tomasz Jüngst (University Hospital Wuerzburg)
    2. Fabricating microfibrillar fiber bundles as cell-guiding additive for bioprinting

       Introduction

       Two main technologies of Biofabrication are bioprinting and scaffold generation. [1] Bioprinting can be used with cells in the matrix, while scaffold generation is cell-free and cells are attached afterwards. Both have their distinct advantages, e.g. bioprinting enables the generation of complex tissue hierarchies in one step, while scaffolds can guide cell elongation via topographical cues.

       In this regard, we previously established Melt-Electro-Fibrillation, which generates microfibrillar polycaprolactone (PCL) via Melt-Electro-Writing (MEW). These support cell orientation, and it was previously shown that macrophages express anti-inflammatory markers caused by the topology alone. [2]

       To exploit the advantages of bioprinting and scaffold generation, this study aims to converge these methods by utilizing fragmented fibrillar scaffolds as filler material for bioinks. The orientation of fibers after bioprinting with the orientation of the microfibrils led to the generation of a large anisotropic system, while maintaining the ease of application of bioprinting.

       Materials and methods

       The blend of polyvinyl acetate (PVAc) and polycaprolactone (PCL) was processed via melt electrowriting (MEW) and printed on a polyvinyl alcohol (PVA) coated grid. Subsequently the grid was transferred to the laser cutter, where the fibers were cut into bundles of equal length with fused microfibrillar fibers at both ends. Afterwards the

grids with the fibers were immersed in 70% ethanol to dissolve the PVA and PVAc. The pure PCL microfibrillar fibers detached from the grid without damage and were further washed in 70% ethanol. Finally, they were transferred to pure water, lyophilized, and weighed. Then the hydrogel was added to create different fiber-to-hydrogel mixtures.

Results and discussion

Single microfibrillar fibers were laser cut into bundles and after the removal of the PVAc, the microfibrillar PCL structure was uncovered (Figure 1, A). The general form of the bundles consisted of two dome-like caps, which are caused by melting during the laser cut, and straight microfibrillar fibers. The obtained fibers were treated with NaOH to improve the dispersion of single bundles in a variety of hydrogels. Upon incorporation of the fiber bundles into the hydrogel, they were extruded into single lines, and aligned fiber bundles within the hydrogel were obtained (Figure 1, B). As anticipated, seeded cells aligned to the microfibrils of the ribbon (Figure 1, C).

Conclusion

We present a microfibrillar additive for bioinks that is capable to align cells in fiber direction and themselves in printing direction. Therefore, achieving an anisotropy from the cellular level to the macroscopic level, while maintaining the scalability of bioprinting.

References:

[1] J Groll et al A definition of bioinks and their distinction from biomaterial inks Biofabrication 2019 11 013001
[2] Ryma, et.al., Translation of Collagen Ultrastructure to Biomaterial Fabrication for Material-Independent but Highly Efficient Topographic Immunomodulation. Adv. Mater. 2021, 33, 2101228.

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Konuşmacılar: Sven Heilig (University of Wuerzburg)

- 3

Advanced 3D Bioprinted Hydrogels as In Vitro Platforms for Breast Cancer Microenvironment Modeling

Introduction

Breast cancer continues to be one of the leading causes of cancer-related mortality among women worldwide [1,2]. Conventional 2D cultures and animal models fall short in accurately replicating the breast tumor microenvironment, often lacking translational relevance [3]. The development of three-dimensional (3D) in vitro models through hydrogel-based bioprinting offers a promising alternative to better mimic the mechanical, structural, and biological characteristics of tumor tissue [4]. This study aimed to design bioprintable alginate-gelatin (ALG-GEL) hydrogels and evaluate their suitability to serve as advanced 3D platforms for better mimicking the breast tumor microenvironment and enabling testing of cellular behaviors or therapeutic responses in a more physiologically relevant context compared to traditional 2D cultures.

Methods

ALG-GEL hydrogels were prepared mixing different concentrations of alginate and gelatin, to match the stiffness typical of breast tumor tissue (~10 kPa). Swelling behavior, degradation rate, and pH stability were monitored over a 21-day incubation at 37°C. Structural integrity was evaluated via scanning electron microscopy (SEM), and chemical composition was verified through Fourier-transform infrared spectroscopy (FTIR). Rheological analysis assessed the mechanical properties of the hydrogels, while pre-crosslinking printability studies helped determine suitable compositions for bioprinting. MDA-MB-231 breast cancer cells were embedded within bioprinted constructs. Cellular viability and metabolic activity were assessed using CCK8 assays over 21 days. Confocal laser scanning microscopy with live/dead staining was used to confirm cell viability and spatial distribution within the constructs.

Results

All hydrogel formulations exhibited high initial swelling within 2 hours, followed by a controlled degradation profile over 21 days. FTIR confirmed the successful incorporation of gelatin without compromising the alginate backbone. SEM analysis revealed a well-interconnected and homogeneous microstructure. Rheological measurements demonstrated a storage modulus (G′) close to 10 kPa, suitable for mimicking tumor tissue stiffness. Printability studies identified optimal ALG-GEL ratios that ensured structural fidelity and cell compatibility. Importantly, bioprinted constructs showed a marked increase in metabolic activity from day 1 to day 21, suggesting robust cell proliferation. Confocal microscopy confirmed high cell viability and a uniformly distributed cell population throughout the 3D constructs.

Discussion

The combination of structural, mechanical, and biochemical evaluations demonstrated that ALG-GEL hydrogels are highly suitable for biofabrication of tumor-mimetic scaffolds. The progressive increase in metabolic activity suggests that these hydrogels effectively support cell proliferation over prolonged periods. Moreover, the confocal microscopy findings reinforce the scaffold's ability to provide a uniform 3D niche for cell survival and growth. These features are critical for the development of reliable in vitro breast cancer models that may reduce reliance on animal testing and offer new insights into tumor progression and treatment response.

References

[1] M. Arnold, doi.org/10.1016/j.breast.2022.08.010
[2] K. Barzaman, doi.org/10.1016/j.intimp.2020.106535
[3] M. Kapałczyńska, doi.org/10.5114/aoms.2016.63743
[4] A. Guller, doi.org/10.3390/bioengineering10010017

Acknowledgments

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Disclosure Information

The authors declare no conflicts of interest.

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Konuşmacılar: Giovanni Lo Bello (Magna Græcia University of Catanzaro)

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From Ink to Organ Function: Advanced Strategies for the Vascularization of Bioprinted Bionic Organs

Introduction

One of the most significant challenges in organ bioengineering is developing functional vascular networks. Proper vascularization is critical for transporting oxygen, nutrients, and signaling molecules, while also removing waste. In bionic organs, poor vessel formation limits nutrient exchange and cell migration, reducing transplant quality and long-term survival [1], [2]. The traditional use of mature endothelial cells is restricted by their low proliferation and limited angiogenic capacity. Endothelial progenitor cells - particularly endothelial colony-forming cells (ECFCs) - exhibit greater angiogenic potential. This study explores vascularization strategies in a 3D bioprinted pancreas model to advance the functionality of bioengineered organs.

Materials and Methods

A prototype of a bionic pancreas was created using 3D bioprinting, incorporating bioink composed of decellularized extracellular matrix (dECM), pancreatic cells, endothelial cells, and fibroblasts. The selection of biomaterials for the 3D bioprinting of a bionic pancreas integrated with a flow system was guided by comprehensive hemocompatibility assessment tests to ensure optimal interaction with blood components and minimize the risk of thrombogenic or immunological responses. A central pre-designed vascular channel was included to mimic native vasculature. The printed construct was placed in a perfusion bioreactor, simulating physiological flow. After several days of incubation, samples were fixed and analyzed by immunohistochemistry (IHC) using markers such as CD31, insulin, vimentin, and glucagon to evaluate vascular and islet formation. To determine the optimal conditions for vessel formation, microfluidic models with different concentrations of endothelial cells to fibroblasts were designed. Furthermore, isolation and co-culture techniques were improved to enhance the angiogenic potential of the endothelial population. Models are characterized by IHC and qPCR for the following markers: VEGF, Tie2 and Ang1.

Discussion

These early observations highlight the persistent difficulty of achieving sufficient vascularization in bioprinted organs. While the emergence of vessel-like structures is encouraging, further work is needed to improve vascular density and functionality. Adjusting the endothelial-to-fibroblast ratio and improving cell culture protocols could enhance vessel formation. Generating more angiocompetent endothelial populations is expected to support the development of stable and functional microvasculature. Continued optimization will be vital for improving transplant performance and advancing bioengineered organ systems toward clinical application. This study provides initial evidence of successful vascular integration and sets the stage for future investigations focused on enhancing vascular complexity and tissue viability.

Results

Preliminary analysis show microvessel-like structures forming from the central vascular channel in the bioprinted pancreas. These structures tested positive for CD31, indicating early capillary development. Although the number of vessels is limited, their alignment along the direction of perfusion suggests that mechanical flow may promote endothelial organization and sprouting. Microscopic images (to be shown) confirm the physical continuity between the main channel and newly formed vascular outgrowths.

References

[1] Zheng, K., Chai, M., Luo, B., Cheng, K., Wang, Z., Li, N., & Shi, X. (2024). Recent progress of 3D printed vascularized tissues and organs. Smart Materials in Medicine, 5(2), 183–195.
[2] Khan, O. F., & Sefton, M. V. (2011). Endothelialized biomaterials for tissue engineering applications in vivo. Trends in Biotechnology, 29(8), 379–387.

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Konuşmacılar: Palina Milewska (Polbionica Inc.)

- 5

Brain-on-Chip of the 3D Blood–Brain Barrier (BBB) model for Studies of Permeability, Neuroinflammation and Drug Response

Introduction
Depression affects over 350 million individuals globally, with 20–30% developing treatment-resistant depression (TRD), a major contributor to suicide risk. Existing preclinical models inadequately recapitulate the complexity of the human neurovascular unit (NVU) and blood–brain barrier (BBB), thereby limiting the advancement of effective therapeutics. The objective of this study was to develop a three-dimensional (3D) BBB model to enable mechanistic investigations of barrier permeability, neuroinflammation, and pharmacological responses.
Methods
A two-channel polydimethylsiloxane (PDMS) microsystem separated by a porous polycarbonate membrane was fabricated. The lower channel was employed to reconstruct the BBB using the Double-Viscous Finger Patterning (Double-VFP) technique: human brain vascular pericytes (HBVP) and astrocytes (HBVA) were embedded at a 1:3 ratio within a 5 mg/mL collagen I hydrogel to form the lumen, followed by seeding of human brain microvascular endothelial cells (HBMEC) at a 3:1:3 ratio. Culture conditions were optimized for a 10-day period. Cellular viability (AlamarBlue® assay), dextran permeability, CellTracker®-based imaging, and immunostaining analyses were conducted on days 1, 3, 7, and 10; 3D imaging was performed at 24 and 48 hours post-seeding. Cellular morphology within the hydrogel matrix was compared to traditional two-dimensional monolayer cultures via immunocytochemistry to validate model fidelity. To simulate an inflammatory environment, hormonal stimulation using cortisol alone and a combination of cortisol, aldosterone, and angiotensin II—molecules known to be elevated in depression—was optimized in macroscale models employing AlamarBlue® and RealTime-Glo™ MT Cell Viability assays. The upper microchanel allows for the integration of neurons to create together the NVU model (under development).
Results
The microsystem enabled reproducible generation of single- and double-lumen structures, with future compatibility for the incorporation of neurons and microglia. The Double-VFP method supported sustained high cell viability over 10 days, with the formation of a continuous and functional endothelial layer. Relative to the Single-VFP approach, the Double-VFP technique facilitated more rapid organization of cellular components into a functional BBB and exhibited superior barrier integrity, as evidenced by dextran permeability assays. Complete assembly of the BBB architecture was confirmed by CellTracker® imaging and immunostaining within 48 hours. Hormonal stimulation at both low and high concentrations altered cellular metabolic activity, indicating a biological effect that will be corroborated with additional assays, such as ELISA.
Discussion
The developed 3D BBB model—particularly utilizing the Double-VFP method—successfully replicates key physiological features of the BBB and provides a dynamic platform for the study of barrier permeability, neuroinflammatory processes, and pharmacological testing. Its rapid and reproducible assembly, coupled with its structural and functional robustness, positions it as a valuable tool for mechanistic studies in neuropharmacology and for the preclinical evaluation of psychotropic, psychedelic, and anti-inflammatory compounds. Ongoing integration of iPSC-derived neurons and microglia is anticipated to further enhance the model’s relevance for the investigation of depression pathophysiology and therapeutic development.

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Konuşmacılar: Pawel Romanczuk (Warsaw University of Technology)

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Introducing Optical Fiber-Assisted Bioprinting (OFAB) as Novel 3D Bioprinting Method

Introducing Optical Fiber-Assisted Bioprinting (OFAB) as Novel 3D Bioprinting Method
Maximilian Pfeiffle, Alessandro Cianciosi, Tomasz Jüngst
Department for Functional Materials in Medicine and Dentistry, University of Würzburg, Pleicherwall 2, 97070 Würzburg, Germany
Introduction: To accelerate scientific progress in tissue engineering and regenerative medicine, new accessible 3D biofabrication methods must be developed. Optical fiber-assisted bioprinting (OFAB) is such a 3D printing method and was introduced by our team for cell free work.[1] It offers a cost-effective, fast, flexible, and easy-to-use approach to bioprinting with a variety of bioresins, including non-transparent ones. The development and optimization of OFAB as a biofabrication method shown in this contribution presents new opportunities for the free-form creation of intricate 3D structures.
Methods: In OFAB, an optical fiber coupled to a light-emitting diode is used to generate a locally confined area of light intensity high enough to crosslink and solidify photoresins in a defined area around the fiber tip. The size and width of this area are controlled by adding a photoabsorber to the resin and by process parameters. Moving the fiber above or within a vat of resin enables the generation of 2.5D and 3D structures, as the material solidifies along the path of the moving fiber tip, enabling freeform 3D biofabrication. To verify the capabilities of the OFAB platform, resolution is measured using different setups with various optical fibers and resin formulations. Additionally, the cytocompatibility of these resin formulations is evaluated.
Results: This study presents the latest OFAB platform setup and demonstrates its capabilities and versatility. It focuses on identifying the ideal setup and printing parameters. Furthermore, various bioresins, including both biological and synthetic materials are being developed and systematically screened to achieve high resolution and cytocompatibility. While stiffer materials tend to improve print resolution, they are generally less favorable for cell proliferation. To overcome this limitation, the thermoreactive properties of methacrylated gelatin are utilized. Cooled gelatin derivatives provide a stiff and stable support structure during printing, while offering a soft, cell-friendly matrix under in vitro conditions post-print.
Discussion: OFAB offers a highly efficient, reproducible, and versatile method for biofabrication. The establishment of 3D OFAB may represent a significant advancement in tissue engineering and biomimetic scaffold fabrication by making light-based bioprinting more accessible. Additionally, various bioinks are under development to provide a range of cell-friendly formulations, further enhancing the applicability of OFAB across different fields of research.
References: [1] Cianciosi, A.; Pfeiffle, M.; Wohlfahrt, P.; Nurnberger, S.; Jungst, T. Optical fiber-assisted printing: A platform technology for straightforward photopolymer resins patterning and freeform 3d printing. Adv Sci (Weinh) 2024, 11 (32), e2403049.

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Konuşmacılar: Maximilian Pfeiffle (Julius Maximilians Universität Würzburg)

- Clinical Session Ratuszowa

Toplantıyı düzenleyenler: Jos Malda (UMC Utrecht)

- 7

Regenerative transplantation: a paradigm shift

The true need for transplantable organs has been estimated to be in the order of few millions only in the Western world.
As, worldwide, only 172,397 transplants were performed in 2024, we can infer that the current approach to organ transplantation is inadequate.
The idea of replacing a terminally diseased organs with a new, functional one procured from another individual dates to more than a century ago and should therefore be considered obsolete. Thus, transplantation needs a new paradigm to refer to, and a new strategy to move forward.
Historically, the pillars of organ transplantation have been immunology, organ preservation and prevention and management of the complications induced by chronic antirejection therapy. However, the field known as regenerative medicine has shown potential to meet the most urgent needs of modern transplant medicine, namely the identification of an inexhaustible source of organs, immunosuppression-free transplantation, and organ-on-demand.
As we think that we are now transitioning towards the regenerative medicine phase of the transplant history and that transplantation is the major stakeholder in regenerative medicine, we propose a new paradigm consisting in regenerative transplantation, whereby organ bioengineering, repair and regeneration, in association with cell-tissue-organ cryopreservation and biobanking, become the three major pillars of modern transplantation.

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Konuşmacılar: Giuseppe Orlando (Wake Forest University)

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Innovation to Heal: The Critical Path from Research to Real-World Impact

Translating biotherapeutics into clinical practice is essential for enhancing patients' quality of life and reducing therapy costs, ultimately democratizing access to treatments for all. This overview highlights the Mayo Clinic Center for Regenerative Biotherapeutics, dedicated to clinical translation of novel therapies into early-phase clinical trials. With facilities in Minnesota, Florida, and Arizona, our center features 20,000 square feet of clean room space and a team of 140 staff members collaborating with over 50 investigators on approximately 30 investigational INDs across cancer immunotherapy, immune-mediated diseases, organ regeneration, and more.

We emphasize our robust clinical trial infrastructure, with internal teams managing trials supported by project management, grant administration, and business development through collaboration with Mayo Clinic Ventures. Engaging the FDA early through INTERACT opportunities allows us to discuss pilot data and receive critical feedback, while pre-IND interactions enable us to outline our definitive studies, moving toward first-in-human trials. Our approach ensures we align with regulatory expectations, which will be a focus in this presentation as we explore pathways to support late-phase clinical trials. Ultimately, our commitment to translating laboratory discoveries into clinical practice aims to enhance patient care and outcomes.

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Konuşmacılar: Julie Allickson (Mayo Clinic)

- 9

3D Bioprinting: Regulatory and Technological Challenges in Bringing a Disruptive Innovation to the Health Market – European Perspective

3D bioprinting is emerging as one of the most disruptive technologies in modern medicine, offering the possibility to fabricate patient-specific tissues and organs for regenerative therapies, disease modelling, and drug testing. However, its translation from laboratory research to clinical application faces significant scientific, manufacturing, and regulatory challenges—particularly within the European Union's complex legal framework. In addition to overcoming technical barriers such as establishing stable vascular networks, optimizing bioinks, and ensuring long-term construct functionality, developers must navigate a highly specific regulatory pathway. Within the EU, advanced bioprinted products incorporating living cells and structural biomaterials are often classified as combined Advanced Therapy Medicinal Products (cATMPs), subject to Regulation (EC) No 1394/2007 and related directives. This classification requires compliance with stringent Good Manufacturing Practice (GMP) standards, comprehensive preclinical safety and efficacy evaluation, and a centralized marketing authorization procedure through the European Medicines Agency (EMA). Early engagement with regulators via Scientific Advice is crucial to align development plans with EMA expectations, particularly regarding quality attributes, comparators, and clinical trial design. Our 12-year multidisciplinary program has addressed both technological and regulatory milestones in the development of the 3D-bioprinted Bionic Pancreas-ATMP®—from bioink formulation and vascularization strategies, through large-animal studies under GLP-like conditions, to the implementation of ISO 13485 standards. The Bionic Pancreas is now ready for first-in-human trials, supported by a comprehensive risk management plan and a regulatory strategy tailored for parallel EMA and FDA review. The path to clinical adoption of 3D-bioprinted organs in Europe is shaped not only by breakthroughs in tissue engineering but also by the ability to integrate these innovations into the ATMP regulatory framework. Mastering both domains is essential to transform 3D bioprinting from a disruptive concept into a safe, effective, and approved therapy for European patients. This presentation will outline the critical steps and lessons learned, helping other innovators avoid major hurdles along the way.

Konuşmacılar: Michał Wszoła (Polbionica S.A.)

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The Kenzan Method: A Scaffold-Free Biofabrication Approach for Clinical Translation in Japan

We developed the Kenzan Method, a scaffold-free, 3D Biofabrication system that uses multicellular spheroids as building blocks. These spheroids are temporarily pierced on a needle array ("KenZan"), where they fuse, self-organize and secrete their own extracellular matrix, yielding purely cellular, functional tissue constructs without foreign materials.

We have demonstrated its versatility in three areas:
Vascular grafts: Made from patient-derived fibroblast into tube shape, these grafts remodel, endothelialize and self-repair in rat and minipig models. A clinical trial for hemodialysis shunts began in Japan in March 2020.
Peripheral nerve conduits: Bio 3D nerve constructs support significant regeneration and functional recovery in rodent and canine models; Kyoto University's physician-led clinical trial in Japan was successfully completed, demonstrating safety and preliminary efficacy in patients with peripheral nerve injury.
Cartilage repair: iPSC-derived cartilage constructs achieve osteochondral regeneration in animal models; The constructs are expected to regenerate extensive articular cartilage surfaces and Kyoto University is preparing a physician-led clinical trial in accordance with Japan's regenerative medicine legislation.

Key challenges remain reducing culture time (currently 1–2 months) and cost for broader access; navigating Japan's rigorous, precedent-scarce regulatory pathway; and scaling production with long-term outcome data. The Kenzan Method paves the way toward patient-specific, immune-compatible organs and tissues fabricated entirely ex vivo.

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Konuşmacılar: Koichi Nakayama (Saga University School of Medicine)

- 11

3D/4D Bioprinting: Advanced Biofabrication for Clinical Needs

The recent emergence of additive manufacturing/ 3D printing offers novel routes to fabricate implants and tissue scaffolds of complex architecture, which are personalized to meet the patient's needs. In this talk, I will highlight some key ongoing efforts in our group on developing advanced biomaterials and biofabrication strategies to meet clinical needs. These efforts span a variety of biomaterials, from Titanium alloys to bioresorbable polymers, to engineer implants and tissue scaffolds. I will present some recent efforts in fabricating additively manufactured personalized bone plates used in hand surgeries for human patients. We have also developed shape-morphing 4D-printed hydrogels that were used for nerve regeneration in peripheral nerve defects in a rat model. In another study, we have fabricated 3D-bioprinted scaffolds laden with breast tumor organoids from human patients for drug screening toward engineering tumor models for personalized medicine. Thus, this talk will highlight how the application of frontier biofabrication technologies can be further combined with smart materials and design to prepare 4D-printed deployable devices.

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Konuşmacılar: Kaushik Chatterjee (Indian Institute of Science)

- 10:30

Coffee break & Poster Session I

- Poster Session I: Poster Exhibition Poster Area

posters are on display whole day on Sunday and Monday

Toplantıyı düzenleyenler: Marco Costantini (Institute of Physical Chemistry Polish Academy of Sciences), Carmelo De Maria (University of Pisa)

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3D bioprinting of methacrylated xanthan/halloysite nanotubes for wound healing apllications

INTRODUCTION
The skin has restricted regeneration potential in the presence of certain illnesses. In certain circumstances, the using of specific treatments is strongly recommended in order to improve the wound healing process. Polysaccharide-based biomaterials exhibit a high potential for wound healing because this category maintains wound hydration, allows gas exchange and absorbs exudate, all of which are essential components of the healing process [1].
Xanthan, an anionic polysaccharide, is recommended for the production of biomaterials with applications in the treatment of wound healing due to its antibacterial and anti-inflammatory properties. Furthermore, its double helix structure exhibits pseudoplastic activity, making xanthan a promising choice for 3D bioprinting applications. The homogeneous dispersion of halloysite (a natural layered silicate) within a polymeric matrix enhances mechanical characteristics and also promoting cell adhesion and re-epithelialization [2, 3].
METHODS
In this study, multiple bioink formulations based on methacrylated xanthan and various quantities of halloysite nanotubes loaded with vitamin B3 were investigated. The resulting biomaterials were characterized structurally, rheologically, morphologically, and mechanically.
RESULTS AND DISCUSSION
Rheological experiments revealed that all compositions exhibit suitable features for 3D bioprinting, including pseudoplastic behavior and advantageous viscosity recovery. Structural investigations have shown that xanthan was successfully modified and the presence of halloysite in the polymeric matrix was confirmed. The inclusion of halloysite caused an increase of nanomechanical characteristics and hybrid scaffolds are characterized by a modified drug release profile.
REFERENCES

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2. Xu, R. et al. Engineering a halloysite nanotube-enhanced hydrogel 3D skin model for modulated inflammation and accelerated wound healing. Bioact. Mater. 45, 148–161 (2025).
3. Liu, M. et al. The improvement of hemostatic and wound healing property of chitosan by halloysite nanotubes. RSC Adv. 4, 23540–23553 (2014).

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Konuşmacılar: Mădălina Cristina Nicolae (National University of Science and Technology POLITEHNICA Bucharest)

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3D Bioprinted Papillary Dermis Models for Investigating the Impact of Aging on Epidermal Development

The dermal-epidermal junction is critical in maintaining skin homeostasis, providing mechanical support, and facilitating nutrient exchange. One of the key features of this interface is the papillary dermis, which is characterized by a series of undulations that increase surface area for diffusion between dermal and epidermal compartments. During aging, these undulations flatten, resulting in a less effective dermal-epidermal junction. This flattening has been associated with a thinner epidermis, decreased skin elasticity, and impaired wound healing. Despite its importance, the precise contribution of the papillary dermis to epidermal maintenance remains poorly understood.
To address this gap, this study aims to develop a 3D bioprinted model of the papillary dermis using a decellularized extracellular matrix (dECM) derived from porcine skin. Preliminary results have demonstrated the successful production of full-thickness skin equivalents using 5 mg/mL dECM to generate a dermal layer and a keratinocyte suspension seeded to form the epidermal layer. In the next phase, the dECM will be methacrylated to enable light-mediated crosslinking, facilitating the fabrication of structured, villi-like papillary dermis features over a reticular dermis base. Constructs will be fabricated using a digital light processing (DLP) bioprinter (LumenX), aiming for high-resolution features mimicking native papillae in the range of 50–100 µm.
NIH3T3 fibroblasts will be encapsulated within the dECM-based hydrogel to populate the dermal compartment. Following fabrication, dermal constructs will be coated with laminin to promote epidermal attachment. A suspension of immortalized mouse keratinocytes (M4+/+, ABM) will then be seeded on top, and epidermal stratification will be induced by initiating air-liquid interface (ALI) culture with simultaneous supplementation of 1.5 mM calcium to the differentiation medium.
Histological analysis and immunohistochemistry (IHC) will be performed on Day 14 of ALI culture to assess epidermal development. Keratin 14 will be used to visualize basal keratinocytes, while filaggrin will identify cornified layers and terminal differentiation. We hypothesize that constructs with a flat, aged-like papillary dermis will exhibit reduced epidermal thickness and diminished keratinization compared to constructs with a more undulated, youthful papillary dermis, due to decreased surface area for nutrient diffusion.
This study aims to provide novel insights into how age-related changes in the dermal-epidermal interface affect epidermal architecture and homeostasis. A better understanding of these interactions could advance the development of improved skin tissue models for basic research in dermatology and aging biology.

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Konuşmacılar: Gabriel Baldissera (Yale University, Department of Pathology)

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3D electrospun-based hydrogels for skin regeneration

Introduction
In Europe, around 60 million people have diabetes, and approximately 15% will develop diabetic foot ulcers (DFUs), with annual treatment costs of €4–6 billion [1]. Neuropathy hinders early wound detection, complicating DFU management [2]. Electrospun structures mimic the skin's extracellular matrix but their 2D nature limits full regeneration [3]. This study proposes to create a 3D electrospun-based scaffold with nanodetails to fill the entire wound and enhance healing. Polycaprolactone (PCL) short nanofibres (SNFs) will be incorporated into a gelatin bioink combining mechanical strength with biocompatibility. Gelatin also promotes cell adhesion and proliferation through its characteristic RGD peptide sequence [4].

Methods
Electrospun meshes were produced from a 16 wt% PCL solution using a home-made electrospinning apparatus. The fibers were cut with NaOH and dispersed in a 5 wt% gelatin solution to prepare inks with varying SNF contents (100:6.4, 100:10, 100:20). BDDGE crosslinker was added, and the inks were 3D-printed using a home-made BIOMATE system to form scaffolds. Double crosslinking process was carried out in a BDDGE/methanol bath. FTIR-ATR was used to confirm the incorporation of BDDGE and the presence of PCL. Mechanical properties were evaluated via compression tests at 5 mm/s with a 300 N load cell, and 80% of strain on wet samples. Morphological analysis was performed by SEM and µCT.

Results
Different concentrations of SNFs dispersed in the ink were tested to prepare 3D electrospun-based hydrogels by bioprinting, and the processing parameters were optimized. According to the FTIR analysis, a PCL band at 1726 cm-1 and BDDGE at 2943 cm-1 and 1856 cm-1 could be seen in all conditions. SEM images showed the homogeneous dispersion of SNF's, incorporation into the 3D hydrogel filaments and the porous presence in the surface, which was also demonstrated in µCT images. Moreover, with increasing amount of SNFs, the compressive modulus also increased, indicating a higher stiffness, which is relevant to mimic the skin mechanical properties.

Discussion
FTIR-ATR and SEM analysis confirmed the presence of SNF in the hydrogel filaments. Nevertheless, the FTIR-ATR bonds of the primary gelatin amides, associated with the incorporation of the crosslinker BDDGE, are visible. Mechanical analysis indicates the occurrence of viscoelastic behavior with memory, in the present study was demonstrated a direct influence of SNFs in the compressive tests.

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3D Optical Coherence Tomography (OCT) Imaging as a Tool for Quality Assessment of Multi-Material Volumetric Printed Vascular Models

Introduction: The development of biofabrication requires reliable and standardized methods for quantifying a wide range of printing techniques and tissue models to ensure a successful translation into medical applications. With the rise of convergence and the integration of multiple materials, printing processes are becoming increasingly complex, posing challenges for structural analysis. Especially the advancement of volumetric printing (VP) enables the fabrication of complex, three-dimensional vascular models composed of multiple biomaterials.[1] Internal geometries and material transitions are difficult to assess using conventional imaging techniques without destructive sectioning. Reliable and non-invasive quality control methods are essential for the validation of such models, especially given the critical influence of geometry on flow dynamics in vascular applications.[2] Optical coherence tomography (OCT) can offer a non-destructive alternative, combining high-resolution 3D imaging with the ability to differentiate materials based on refractive index differences.[3] This study investigates the applicability of OCT for the quality control of fabricated multi-material VBP vascular models.

Methods: Hybrid vascular constructs are fabricated using advanced multi-material VP strategies that build upon recent developments in the field. The approaches are applied to generate anatomically inspired vascular models with pathological features. Constructs are produced from different hydrogel-based biomaterial resins composed of gelatin methacryloyl, polyethylene glycol diacrylate with distinct refractive properties to enable internal contrast. A commercially available swept source OCT was employed to evaluate structural fidelity and material integration.

Results: The adapted multi-material VP approaches enabled the fabrication of vascular constructs with increased structural complexity and pathological relevance. Internal features, as well as the integration of multiple materials, were reliably visualized and assessed using OCT, where non-destructive standard optical imaging. OCT data revealed high agreement with target geometries and provided volumetric reconstructions of internal architecture. This allowed for qualitative assessment of material distribution, detection of defects such as voids or delamination and for readjusting process parameters to improve printing. The approach was evaluated across different levels of model complexity to assess the detectability of structural features and the potential for quantitative analysis.

Discussion: By combining recent advances in multi-material VP with OCT imaging, a robust platform for the fabrication and non-destructive characterization of vascular disease models was established. OCT proved particularly useful for visualizing complex internal features and heterogeneous material distributions in ways conventional methods could not achieve. The results highlight the potential of this workflow for quality control in advanced tissue model fabrication, especially where functional geometry is essential.

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3D PRINTED BIO-BASED SCAFFOLD FOR CARTILAGE REGENERATION

Cartilage injuries remain a major clinical challenge due to the tissue's limited self-healing capacity. Standard treatments, such as microfracture or autologous chondrocyte implantation, are often insufficient in restoring full function and structure of damaged cartilage. In this study, we present the SmartPiezo scaffold - a novel 3D printed, bio-based, and biodegradable construct designed specifically for cartilage regeneration, with integrated piezoelectric functionality to promote mechanotransduction and tissue remodeling.
The primary goal of this work is to assess the feasibility of using bio-based polymeric materials in a scaffold fabricated via additive manufacturing, capable of delivering not only a suitable structural environment for chondrocyte growth but also a mechanical stimulus mimicking natural cartilage loading. SmartPiezo scaffolds were printed using GelMA with additives to enhance mechanical and piezoelectric properties.
Preliminary results indicate that the SmartPiezo scaffold exhibits promising morphology illustrated by SEM for application in cartilage tissue engineering, combining biocompatibility with mechanical resilience and piezoelectric responsiveness.
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3D-printed Chitosan-Agarose scaffolds for biomedical applications: An in-vitro comparative study of the oxide nanoparticles doped structures

Introduction
Large bone defects caused by trauma or surgical resection often require scaffolds that both promote regeneration and prevent bacterial infection. Chitosan–agarose (CA) hydrogels are biocompatible and printable, but their limited mechanical strength and weak antibacterial capacity remain challenges. Incorporating metal oxide nanoparticles (NPs) such as ZnO, MgO, and CaO offers a strategy to enhance scaffold functionality.
Methods
CA hydrogels (3.5% CS, 1.5% AG) were 3D-printed into porous scaffolds and separately doped with ZnO, MgO, or CaO nanoparticles (0.5% w/v). The inks were assessed for printability, rheological behavior, and wettability. Scaffold degradation, swelling, and cross-linking density were characterized. Antibacterial activity was tested against E. coli and S. aureus by plate count and disc diffusion assays. Bone marrow mesenchymal stem cells (BMSCs) were cultured on scaffolds to evaluate proliferation, ALP activity, osteocalcin gene expression, and mineralization.
Results
All NP-doped scaffolds exhibited improved viscosity, elasticity, and slower degradation compared to pristine CA. ZnO and MgO significantly reduced bacterial colony counts (>10-fold for E. coli) while CaO showed weaker inhibition. BMSCs on ZnO- and CaO-doped scaffolds displayed enhanced proliferation compared to control, with ZnO yielding the highest osteocalcin expression. ALP activity increased most in ZnO and MgO groups by day 21, while CaO scaffolds promoted the most mineral deposition. Together, NP incorporation improved both antibacterial and osteogenic outcomes.
Discussion
Our results show that ZnO provides the strongest antibacterial effect and boosts osteogenic differentiation, MgO mainly enhances mechanical stability and ALP activity, while CaO contributes to mineral deposition and hydrophilicity. This head-to-head comparison within a single 3D-printed CA scaffold system highlights how specific NP types distinctly modulate scaffold performance. ZnO-doped CA scaffolds emerge as the most promising candidate for bone regeneration with infection control.

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3D-Printed Double-nanostructured Scaffolds for Osteochondral Bone Defect Repair

Introduction
Osteochondral defects involve damage to both the articular cartilage and the underlying subchondral bone, often resulting in joint instability and risk of osteoarthritic degeneration [1]. Recent research in treating such complex joint lesions focuses on developing 3D porous biomaterials, engineered with precise size and shape specifications through additive manufacturing techniques. This study reports the fabrication and characterization of double-nanostructured 3D printed scaffolds designed for osteochondral bone regeneration.
Methods
Paste-like inks were prepared using fish gelatin (FG) loaded with a high content of nanostructured biosilica (DE) and a low content of carboxylated nanodiamonds (NDs). The scaffolds were obtained through 3D printing via direct extrusion technology. The double-nanostructured 3D printed scaffolds were investigated regarding stability under simulated physiological conditions, surface mechanical properties, and morphology characteristics. The effect of low concentrations of NDs on MC-3T3E1 behavior was investigated in terms of cytocompatibility and ability to support osteogenic differentiation.
Results
3D printed scaffolds with high fidelity shape accuracy were obtained. All samples showed similar maximum swelling capacity, ranging from 38% to 40%. A slight increase in surface mechanical properties was observed with increasing NDs concentration, with a statistically significant increase for the 1% NDs samples. In addition, the SEM micrographs revealed that the presence of NDs leads to different arrangements of FG macromolecules. All tested compositions were cytocompatible for MC-3T3E1 cells and sustained osteogenic differentiation.
Discussion
In line with previous findings [2], low NDs loadings modulated the surface mechanical properties of the FG matrix and influencing cell viability, proliferation, and adhesion. At 1% NDs the 3D-printed scaffolds showed a significant 16% increase in surface storage modulus. This increase in stiffness may contribute to a more favorable microenvironment for osteogenic differentiation. Furthermore, the scaffolds supported preosteoblast adhesion and promoted osteogenic differentiation over 21 days, with mineralized matrix formation observed across all tested compositions. Remarkably, the pronounced increase in ECM formation at 1% ND loading indicates that NDs significantly enhance cell-matrix interactions even at low concentrations.
Conclusions
The presence of low amounts of NDs in the double-nanostructured 3D printed scaffolds additionally improved the bioactivity of the nanocomposite materials.

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Disclosure statement
The authors declare no conflict of interest.

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3D-Printable Gelatin-Derivatives for the Design of In Vitro Diabetic Skin Models

Diabetes mellitus (DM) is a complex metabolic disease characterized by impaired glucose metabolism (hyperglycemia), leading to severe and long-term complications, such as kidney failure, stroke, peripheral neuropathy, nephropathy and, above all, foot ulcers.
In the last years, 2D in-vitro cell culture systems and 3D in-vivo animal models have been particularly useful in understanding the pathophysiology of diabetic skin. However, these cannot fully represent the disease because of (i) the oversimplification of human physiology and the lack of typical cell-to-cell interactions occurring in an in vivo microenvironment (2D cell culture systems), and (ii) critical differences between species, including metabolic processes, enzymes, and membrane proteins (3D animal models). Trying to overcome these limitations, the use of cell-laden hydrogels, consisting of either extracellular matrix-derived materials or biocompatible natural polymers that provide a microenvironment similar to that of natural tissues, has emerged as a promising alternative for the biofabrication of 3D in vitro models.

In this framework, the research work in the network of D34H project (Digital Driven Diagnostics, pronostics and therapeutics for Sustainable Health care) focuses on the light-assisted bioprinting of a novel 3D in vitro model of diabetic skin useful for drug and therapy screening, using Type-B bovine gelatin methacryloyl (GelMA) derivatives as hydrogel precursors.
In particular, two different strategies are being followed. The first approach involves the biofabrication of cell-laden GelMA-based hydrogels and their subsequent immersion in high glucose culture media in order to evaluate both the cellular behavior and wound-healing mechanisms in hyperglycemic environments resembling those typically associated with DM.
The second strategy aims to further modify the structure of the hydrogel precursor trying to reproduce at the synthesis stage, those structural changes normally affecting the native extracellular-matrix as a result of non-enzymatic glycation of proteins, lipids, and nucleic acids, occurring in diabetic hyperglycemic environments. Accordingly, GelMA is glycosilated with ribose by following the path of Maillard reactions. The so-obtained glycated biopolymer (GLY-GelMA) will be used as innovative biopolymer for the bioprinting of cell-laden hydrogels to be tested in hyperglycemic environments, following the same procedure as described above.

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3D printed scaffolds incorporated autologous growth factor for bone tissue engineering

Successful bone regeneration in clinical research still remains a great challenge due to the complex morphology and the recent advances aim to enhance the rate of bone healing using biofabrication strategies such as 3D bioprinting loaded with growth factors. 3D bioprinting has tremendously boosted the transition from conventional regenerative procedures to customized patient-specific deliverables. Despite the enormous growth of this technology, various factors such as the development and validation of an appropriate biomaterial component of bioinks for a particular tissue, cell sourcing, cell compatibility issues with bioinks, risks associated with unwanted differentiation of loaded cells, etc, have limited the applications in humans. Further, such approaches are expensive, and involves complex regulatory procedures thereby limiting their translation to clinics. Therefore, in the present study, we have incorporated injectable platelet-rich fibrin (iPRF) in the commercially available Cellink bone ink as an alternative to the use of recombinant growth factors and cellular therapy. iPRF is a rich source of autologous growth factors and stem cells and has a significant potential in bone regeneration. We investigated the efficacy of the iPRF incorporated 3D printed scaffolds in the cranial defect models of rabbits. The in-vivo preclinical experiments confirmed the accelerated bone healing with iPRF scaffolds as compared to non-iPRF scaffolds. This disruptive approach may offer the opportunity for tailoring biomaterial inks with a patient's autologous growth factors for 3D printing of bone scaffolds in the operation theatre and subsequent implantation at the defect site as a part of a single stage surgical procedure. This can prove to be a more effective treatment strategy for bone disorders and can help in minimizing the patient recovery time.

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3D-printed two-phase GelMA-dextran hydrogel creates 3D structures with large interconnecting porosity for improved cell-support

Introduction/Objectives
We have previously shown that an aqueous two-phase system (ATPS) composed of gelatin methacryloyl (GelMA) and dextran can form large interconnected pores via phase separation process [1]. As such open porous gels could be beneficial for 3D tissue engineering, we investigated whether the novel material could be processed by microextrusion and drop-on-demand printing without destroying the unique microstructure. In addition, the biocompatibility of the hydrogel was assessed through cell viability and proliferation tests.

Methods
The GelMA-Dextran hydrogel was prepared by inducing phase separation through acidification. Once the phase separation was initiated, photopolymerization was used to arrest the GelMA phase and stabilize the microstructure. The dextran phase was subsequently removed. The hydrogel was tested for its suitability in 3D printing using the microextrusion and inkjet drop-on-demand techniques. After the printing process, cells were seeded onto the printed hydrogel scaffolds. Confocal microscopy was employed to observe the microstructure and stability of the hydrogel, while live/dead staining and proliferation assays were conducted to assess the viability and growth potential of the seeded cells.

Results
The results demonstrated that the GelMA-Dextran hydrogel formed a stable and uniformly interconnected porous structure, which was preserved throughout the printing process. Confocal microscopy confirmed the stability and uniformity of the hydrogel’s microstructure. The printing techniques proved successful in maintaining the integrity of the bicontinuous microstructure, demonstrating the compatibility of the hydrogel with advanced fabrication methods. Cells seeded onto the hydrogel scaffolds exhibited high viability and robust proliferation. Live/dead staining indicated minimal cell death, while proliferation assays confirmed the hydrogel’s ability to provide a suitable environment for cell growth. These findings demonstrated the hydrogel was not only structurally stable but also biocompatible.

Conclusions
We confirmed that the novel hydrogel maintained its structural integrity throughout the printing process and supported high cell viability and proliferation when seeded post-printing. The unique large interconnected porosity may help to provide sufficient support for embedded cells over extended cultivation times without the need for endothelial cell-derived microvascular structures.

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Keywords: bicontinuous microstructure, hydrogel, phase separation, aqueous two-phase system

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A 4D Bioprinting Platform to Engineer Anisotropic Musculoskeletal Tissues by Spatially Patterning Microtissues into Temporally Adapting Support Baths

Tissue engineering (TE) aims to regenerate damaged or diseased tissues by replicating their native structure, composition, and function. This goal is particularly challenging in the context of musculoskeletal tissues—such as articular cartilage, meniscus, ligaments, and tendons—whose unique biomechanical functions are tightly linked to their extracellular matrix (ECM) architecture. Damage to these tissues can accelerate joint degeneration and lead to conditions like osteoarthritis. While traditional TE approaches have attempted to seed stem or progenitor cells into scaffolds or hydrogels to stimulate ECM deposition, these methods often fall short in producing functional tissues with biomimetic collagen architectures, thereby limiting their clinical relevance.

This has led to increasing interest in scaffold-free approaches that rely on the innate ability of cells to self-organize via cell-cell and cell-matrix interactions. These strategies draw inspiration from developmental biology and regenerative processes, where tissues form through the orchestrated fusion and organization of cellular microtissues (μTs) or organoids. Under the right in vitro conditions, stem cells can generate tissue-specific μTs that replicate important structural and functional traits of native tissues. The ultimate goal is to combine these biological building blocks to fabricate large, organized grafts, which requires biofabrication methods that not only preserve cellular phenotype but also guide tissue remodeling and ECM organization.

Developmentally, tissues form within the mechanical and geometrical context of their neighboring structures, which exert compressive, tensile, or shear forces. Such mechanical inputs significantly influence morphogenesis and cell behavior. In vitro, these cues can be mimicked by introducing geometrical constraints and substrate stiffness, which affect cell proliferation, migration, and differentiation. For example, mesenchymal stem cell (MSC) differentiation is strongly regulated by physical confinement and mechanical tension, with stiff substrates encouraging osteogenesis. These mechanical cues offer an additional layer of control over tissue development and can be strategically used to guide the formation of biomimetic tissue constructs.

3D bioprinting has emerged as a powerful tool for spatially organizing cells and biomaterials to recreate the anatomical features of native tissues. Recently, this technology has been adapted for printing cellular aggregates, μTs, and organoids. However, challenges remain, particularly in achieving high-fidelity placement and fusion of microtissues, both of which are crucial for the engineering of large-scale, functional grafts. Moreover, the influence of the bioprinting process on long-term tissue phenotype and structure remains underexplored.

To overcome these limitations, this study employs a 4D bioprinting approach using extrusion-based bioprinting within a methacrylated xanthan gum (XG-MA) support bath with varying stiffness (20-60 kPa). This platform enables high-density microtissue patterning while allowing dynamic control over the bath's physical properties—such as rheology and stiffness—to modulate microenvironmental cues post-printing. By tuning these properties, the platform not only improves print fidelity but also directs microtissue fusion, differentiation, and ECM (re)modeling enabling the biofabrication of highly aligned musculoskeletal tissues, such as articular cartilage, meniscus and ligament. The integration of spatiotemporally controlled mechanical signals into the bioprinting workflow represents a significant advancement toward engineering anatomically scaled, functionally anisotropic musculoskeletal grafts with long-term regenerative potential.

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A Modular Endoscopic Projection System for Spatially Patterned Photocrosslinking in Cartilage Repair

A Modular Endoscopic Projection System for Spatially Patterned Photocrosslinking in Cartilage Repair

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Introduction:

In situ bioprinting enables on-site fabrication of biomaterials directly within defect sites, offering unique advantages for cartilage repair. However, achieving spatial precision and surgical adaptability remains a significant challenge. This study presents a fiber-based light projection system, allowing real-time, patterned photocrosslinking of hydrogels within confined joint spaces and is compatible with standard endoscopes.

Methods:

A 405 nm laser was modulated using a digital micromirror device (DMD) and passed through custom lens assemblies and a beam homogenizer to ensure uniform pattern delivery. The structured light was transmitted through a coherent image guide fiber with minimal resolution loss. This fiber could be used alone or coupled externally to standard 0° or 30° arthroscopes. Light behavior was characterized at working distances of 0.5–2 cm using geometric patterns. To assess biological performance, GelMA hydrogels laden with human chondrocytes were crosslinked into defined architectures and cultured in vitro.

Results:

Structured light crosslinking enabled the fabrication of well-aligned microarchitectures within GelMA-Rhodamine hydrogels. Orientation analysis revealed filamentous features with high directional fidelity, as confirmed by peak alignment at –7.5° in OrientationJ. Cell-laden constructs maintained viability and exhibited robust extracellular matrix production over 54 days. Histological staining (Safranin O, Collagen II) revealed cartilage-like matrix deposition.

Discussion:

This platform offers a compact, spatially precise photocrosslinking strategy compatible with confined anatomical spaces. By enabling the fabrication of structured, cell-laden constructs that support cartilage-like tissue development in vitro, this approach may serve as a foundational tool for minimally invasive bioprinting strategies. The system's modularity allows future adaptation toward intraoperative or image-guided procedures, without compromising biological performance.

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Advanced 3D Bioprinted Hydrogels as In Vitro Platforms for Breast Cancer Microenvironment Modeling

Introduction

Breast cancer continues to be one of the leading causes of cancer-related mortality among women worldwide [1,2]. Conventional 2D cultures and animal models fall short in accurately replicating the breast tumor microenvironment, often lacking translational relevance [3]. The development of three-dimensional (3D) in vitro models through hydrogel-based bioprinting offers a promising alternative to better mimic the mechanical, structural, and biological characteristics of tumor tissue [4]. This study aimed to design bioprintable alginate-gelatin (ALG-GEL) hydrogels and evaluate their suitability to serve as advanced 3D platforms for better mimicking the breast tumor microenvironment and enabling testing of cellular behaviors or therapeutic responses in a more physiologically relevant context compared to traditional 2D cultures.

Methods

ALG-GEL hydrogels were prepared mixing different concentrations of alginate and gelatin, to match the stiffness typical of breast tumor tissue (~10 kPa). Swelling behavior, degradation rate, and pH stability were monitored over a 21-day incubation at 37°C. Structural integrity was evaluated via scanning electron microscopy (SEM), and chemical composition was verified through Fourier-transform infrared spectroscopy (FTIR). Rheological analysis assessed the mechanical properties of the hydrogels, while pre-crosslinking printability studies helped determine suitable compositions for bioprinting. MDA-MB-231 breast cancer cells were embedded within bioprinted constructs. Cellular viability and metabolic activity were assessed using CCK8 assays over 21 days. Confocal laser scanning microscopy with live/dead staining was used to confirm cell viability and spatial distribution within the constructs.

Results

All hydrogel formulations exhibited high initial swelling within 2 hours, followed by a controlled degradation profile over 21 days. FTIR confirmed the successful incorporation of gelatin without compromising the alginate backbone. SEM analysis revealed a well-interconnected and homogeneous microstructure. Rheological measurements demonstrated a storage modulus (G′) close to 10 kPa, suitable for mimicking tumor tissue stiffness. Printability studies identified optimal ALG-GEL ratios that ensured structural fidelity and cell compatibility. Importantly, bioprinted constructs showed a marked increase in metabolic activity from day 1 to day 21, suggesting robust cell proliferation. Confocal microscopy confirmed high cell viability and a uniformly distributed cell population throughout the 3D constructs.

Discussion

The combination of structural, mechanical, and biochemical evaluations demonstrated that ALG-GEL hydrogels are highly suitable for biofabrication of tumor-mimetic scaffolds. The progressive increase in metabolic activity suggests that these hydrogels effectively support cell proliferation over prolonged periods. Moreover, the confocal microscopy findings reinforce the scaffold's ability to provide a uniform 3D niche for cell survival and growth. These features are critical for the development of reliable in vitro breast cancer models that may reduce reliance on animal testing and offer new insights into tumor progression and treatment response.

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Disclosure Information
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A 3D Bioprinted Macroencapsulation System for Efficient Subcutaneous Islet Transplantation

Type 1 diabetes mellitus (T1D) is a chronic autoimmune disease that leads to hyperglycemia due to the loss of pancreatic β cells. One of the most effective methods to treat T1D is pancreatic islet transplantation. However, transplanted islets lose their function because of immune reactions. To prevent immune-mediated damage, semipermeable encapsulation strategies are commonly employed. Nonetheless, macroencapsulation methods face challenges in maintaining long-term islet viability and function due to limited oxygen and nutrient diffusion into the graft. In this study, we developed a macroencapsulation system incorporating surface-layered islets and vascularization-inducing patterns using 3D bioprinting. The islets on the surface-layered were protected by semipermeable alginate and effectively supplied with nutrients and oxygen from host vasculature. As a result, compared to islets embedded in the core, surface-deposited islets were less exposed to hypoxia. In addition, the in vivo function of the construct was evaluated in a diabetic model. After subcutaneous transplantation, the bioprinted construct effectively achieved normoglycemia. Furthermore, active neovascularization was observed around the implanted structure, facilitating improved oxygen and nutrient delivery. This approach offers a promising strategy to enhance the functionality of macroencapsulated grafts for artificial tissue transplantation.

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Advancing Pediatric Bone Tissue Engineering: Dipyriamole-Loaded 3D-Printed Bioceramic Scaffolds Promote Stable Bone Formation in a Growing Pig Model During Facial Maturation

Pediatric bone tissue engineering presents distinct challenges related to the growing patient including: the need for a construct strategy that preserves growth plates; bone generation that remodels in parallel with skeletal development to prevent long-term growth restrictions; and a degradation profile that aligns with the process of bone generation. Despite the recognized limitations of donor-site pain, resorption of autologous bone grafts and complications associated with alternative bone replacement materials, autologous bone grafts continue to be the standard of care for bony defect repair in the pediatric population. Advancements in additive manufacturing have facilitated precise control over the micro- and macro-architecture of biomaterials, enabling the production of tissue engineering devices (e.g., scaffolds) with enhnaced osteogenic properties that can be tailored to accommodate patient-specific defects. The objective of this long term study was to evaluate bone regeneration in a critically-sized calvarial defect using skeletally, immature Gottingen minipigs (5 weeks old), through facial maturity, treated with dipyridamole-augmented 3D-printed bioceramic (DIPY-3DPBC) scaffolds composed of 100% beta-tricalcium phosphate (β -TCP) versus autologous bone graft using a immature porcine model followed. Subjects were set to heal for 24 months post implantation, followed by quantitative and qualitative assessments of bone regeneration bone were conducted using micro-computer tomography (micro-CT) to evaluate volume of regenerated tissue and bridging, alongside two-dimensional histologic analysis to examine the level of regeneration. Volumetric analysis of calvarial defects treated with DIPY-3DPBC scaffolds demonstrated significantly greater bone regeneration, with ~53 ± 8% of total defect volume occupied by new bone, with approximately 3% (±2.0) scaffold remaining, compared to subjects treated with autologous bone graft (44 ± 4%, p = 0.2). Qualitative analysis of the histological micrographs revealed vascularized and organized lamellar bone, along with patent cranial sutures and no evidence of ectopic bone or excess inflammation. Radiographic and histologic analysis revealed patent craniofacial sutures. 3D facial symmetry analysis found that bony growth centers were preserved with no disruption of craniofacial growth. Given the results of longitudinal scaffold osteogenesis, favorable scaffold resorption, with safety of scaffold agents through skeletal maturity in a preclinical model, this DIPY-3DPBC scaffold strategy may serve as promising candidate for future implementation in clinical trials investigating bone tissue engineering alternatives within the pediatric population.

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Anisotropic microfiber reinforcement facilitate tailored mechanical design in large-scale, biofabricated cartilage constructs

Introduction
Cartilage defects pose significant challenges in terms of healing. Current treatments have limitations in size, availability, or durability.[1,2] Biofabrication aims to restore tissue functionality by placing biological active components in a pre-defined 3D organization, typically using soft hydrogels for cell preferences.[3] These hydrogels can be mechanically reinforced with microfiber structures generated with melt electrowriting (MEW).[4] Small scale (diameter 6mm, A=28 mm2) Fabricated osteochondral plugs with cell-laden hydrogel reinforced with MEW meshes are stable in vivo.[5] Translating this towards patient-specific implants, requires consideration of an overall increase in size (>2 cm2) and the local differences in cartilage mechanical properties throughout the articulating joint. This study investigates the local, anisotropic mechanical properties of a large sized MEW-reinforced cell-laden hydrogel scaffold.

Materials and methods
Polycaprolactone (PCL) microfiber box-shaped scaffolds were fabricated using melt electrowriting (MEW) with inter-fiber spacing from 200 x 200 µm to 500 x 500 µm with 100 µm increments. A large-scale (15 cm2) anisotropic scaffold was designed with fiber spacing ranging from 300, 400, and 500 µm, embedded with equine articular cartilage progenitor cells (ACPC) and cell-free gelatine methacryloyl (gelMA), and crosslinked with dichloro-ruthenium (II) hexahydrate and sodium persulfate. Mechanical testing was performed on cell-free constructs, and the compressive E-moduli were measured between 10% and 15% strain. The cell-laden constructs were cultured for 28 days in chondrogenic differentiation medium. Post-culture, the constructs were analyzed for GAG, collagen type I and II, metabolic activity, and cell morphology.
Results
By altering the inter fibre spacing of gelMA embedded MEW-reinforcement, the compressive modulus varied from 0.49 ± 0.18 MPa for 500 µm to 2.52 ± 0.17 MPa for 200 µm. The large sized anisotropic scaffold showed similar mechanical behavior, resulting in an anisotropic mechanical design of the scaffold, which could be distinguished as a high (300 µm), medium (400 µm), and low (500 µm) density fiber zone. The ACPCs in the cell-laden constructs showed homogenous behavior in the different regions and staining showed production of GAGs and collagen type II. Biochemistry showed a lower DNA content in the low-density zone, but higher GAG production compared to the medium density zones.

Conclusion
This study shows the effect of mechanical reinforcement of cell-laden hydrogel scaffolds using scaffolds with anisotropic designs. Manipulating the internal box-spacing enables to regulate the mechanical properties of the scaffold, while showing homogenous behavior of cartilage cells throughout a large-scale mechanically anisotropic scaffold design. These findings allow to produce clinically-relevant sized constructs with personalized features, while providing a viable environment for cartilage cells.

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Bioactive Electrospun Nanofiber Membranes for Dental Tissue Engineering and Regeneration

Bone defects in the oral and maxillofacial region, often resulting from trauma, congenital anomalies, degenerative conditions, or therapeutic procedures, present significant clinical challenges. Guided bone regeneration (GBR) and guided tissue regeneration (GTR) are widely adopted strategies that rely on barrier membranes to spatially direct tissue growth. However, conventional collagen-based membranes frequently lack mechanical integrity, especially in complex or load-bearing defect geometries, while non-resorbable alternatives require secondary surgical removal.

In this work, we present a biofabricated, volume-stable, and fully biodegradable membrane engineered for alveolar ridge regeneration. The membrane is produced via electrospinning of polylactic acid (PLA), functionalised with osteoinductive nanoparticles and bone morphogenetic protein-2 (BMP-2). This approach ensures both mechanical resilience and biological activity, supporting localized, staged regeneration of hard and soft tissues. The nanofibrous architecture enables defect-specific conformability, while the embedded bioactive components facilitate cellular recruitment and differentiation.

This novel membrane design addresses critical limitations of current GBR/GTR technologies by combining structural stability with tailored biodegradation and regenerative potential. The platform holds strong translational promise for advanced dental tissue engineering and clinical bone repair.

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Biofabrication of Tissue-Specific dECM Hydrogels: Unlocking 3D Bioprinting for Regenerative Medicine

Background: The development of functional tracheal tissue requires both mechanical stability and cellular functionality, yet creating biomimetic tracheal structures remains a significant challenge. In this study, we developed tissue-specific hydrogels derived from decellularized extracellular matrices (ECM) of cartilage, submucosa, and muscle using proprietary methods. In tissue engineering, hydrogels made from decellularized extracellular matrix (dECM) are gaining prominence because of their inherent biochemical composition and biocompatibility1. They provide a platform for 3D bioprinting structures tailored to a given tissue. Hydrogels that encapsulate cells in dECM bioinks facilitate the creation of functional structures that have uses in disease modelling, regenerative medicine, and customized therapeutic solutions2.
Objective: To 3D Bioprint Tissue-Specific dECM Hydrogels for Functional Tracheal Engineering
Methods: Muscle, cartilage, and submucosal dECM hydrogels were processed into bioinks and encapsulated with specific cell types. Lattice fidelity and mechanical stability were secured by precisely calibrated 3D bioprinting parameters. While mechanical and biochemical tests evaluated stiffness, bioactivity, and matrix deposition, biological markers were used to evaluate printed structures for cell viability, differentiation, and tissue-specific functionality.
Results: Tissue-specific markers confirmed cell differentiation and functionality, while bioprinted structures demonstrated exceptional cell vitality (>90%). These results demonstrate the adaptability of dECM-based bioinks in producing tissue-specific, functional constructs that may find use in disease models, regenerative medicine, and customized treatments. Our findings pave the way for customizing dECM hydrogels to meet tissue engineering requirements, bringing bioprinting closer to practical implementation.
Conclusion: This work shows how dECM-based bioinks can be used to optimize 3D bioprinting and create tissue-specific, functional structures like . The constructs demonstrated tissue-specific functionality, high cell viability, and structural fidelity, underscoring the versatility of dECM hydrogels for personalized therapies, disease modelling, and regenerative medicine. These results open the door to customizing dECM bioinks to satisfy various tissue engineering requirements.
Acknowledgement
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BIOINK DEVELOPMENT FOR IN SITU OSTEOCHONDRAL REGENERATION THROUGH MICROEXTRUSION AND FILAMENTED LIGHT BIOPRINTING

Introduction
Osteoarthritis (OA) is a degenerative disease affecting osteochondral (OC) tissue, leading to pain and joint dysfunction. Current treatments are often limited by availability, efficacy, and cost, highlighting the need for innovative therapeutic approaches. To address this challenge, we propose a novel tool EndoFLight, an advanced in situ 3D bioprinting platform designed for minimally invasive regeneration of large OC lesions (Figure 1a). This system integrates multi-material, multiscale bioprinting by combining bioink extrusion through standard arthroscopic instruments with filamented light (FLight) printing1 to enable precise deposition and crosslinking of cell-laden photo-resins directly at the injury site.

This study presents the formulation and characterization of photo-resins for EndoFLight technology, which aims to provide a clinically translatable solution for osteochondral tissue regeneration in OA treatment.

Methods
Photo-resins were developed using gelatin methacrylamide (GelMA, X-pure grade, Rousselot), either alone or blended with methacrylated hyaluronic acid (HAMA, LifeCore Biomedical) or hydroxyapatite (HAp, CAM Bioceramics). These formulations were designed to support cartilage and bone regeneration, respectively, with lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) serving as the photo-initiator. The selection of X-pure grade GelMA was motivated by its GMP-compliant production and ultra-low endotoxin content, ensuring its suitability for future clinical applications.

Rheological properties of the photo-resins were analyzed using an Anton Paar MCR 702 rheometer to assess their photo-crosslinking characteristics. The resins were exposed to blue light (400–500 nm, 25 mW/cm²) for 10 minutes at 37°C while simultaneously monitoring the storage and loss moduli. Finally, biocompatibility was evaluated in vitro via LIVE/DEADTM assays. Analyses were performed on crosslinked cell-laden photo-resins and cell monolayers to which uncrosslinked photo-resin droplets were added, with the latter undergoing crosslinking afterwards.

Results
The formulated GelMA/HAMA and GelMA/HAp blends were optically transparent, essential for precise bioprinting via EndoFLight. Photo-rheology tests confirmed their rapid photo-crosslinking behavior, with all resins reaching a stable plateau storage modulus of 4–8 kPa (Figure 1b), which is within an optimal range for cartilage and bone tissue formation, as supported by previous studies.1,2 While HAMA incorporation led to a slight increase in storage modulus, no statistically significant difference were found between the blends and pure GelMA solutions (Figure 1c). Preliminary viability tests indicate that the photo-resins are biocompatible both before and after photo-crosslinking.

Discussion
This project aims to address an unmet clinical need through an innovative, one-step technology to accurately reconstruct the complex architecture of articular cartilage directly in the OC lesion, thereby promoting in situ regeneration. Future research will focus on optimizing the bioinks for the EndoFLight setup, followed by validation of the system through in vitro, ex-vivo and in vivo studies.

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Biofabrication of the osteochondral unit by replicating its tri-layered architecture: hyaline cartilage, calcified cartilage and subchondral bone

Introduction: The osteochondral unit composed of subchondral bone, calcified cartilage and hyaline cartilage fulfills a crucial function in joint homeostasis and load transduction. During osteoarthritis, this unit is severely damaged resulting in impaired joint function. Strategies to regain function range from autologous chondrocyte implantation to tissue engineered cartilage to osteochondral grafts. Osteochondral grafts are advantageous as they can be securely fixed into the defect and have the potential to restore full function. Despite the crucial role of calcified cartilage in anchoring hyaline cartilage onto subchondral bone and regulating crosstalk between the two layers, biofabrication strategies have mainly focused on hyaline cartilage and subchondral bone as calcification is difficult to control. Here we present the biofabrication of the osteochondral unit by replicating its tri-layered architecture from hyaline to calcified cartilage to subchondral bone.
Methods: We first established protocols to mature cartilage grafts into hyaline and calcified cartilage. Chondrocytes were combined with a 0.5% hyaluronan-transglutaminase (HATG) and 0.25% alginate (Alg) hydrogel[2] at a density of 30x106 cells/mL. Hyaline and calcified cartilage grafts were first matured in chondrogenic media (10ng/mL TGF-β3). After 3 weeks of maturation, media for calcified cartilage was switched to hypertrophic media (10ng/mL BMP-2) while hyaline cartilage grafts were maintained in chondrogenic media. We then developed a well-plate insert allowing us to culture osteochondral grafts in two compartments (Fig.1). Sheep articular chondrocytes were combined with the HATG-Alg hydrogel and cast on top of 3D printed trabecular bone ceramics[3] (Sr-HT-Gahnite), which were derived from µCT of healthy sheep joints. The resulting osteochondral grafts were cultured for 3 weeks in chondrogenic media, upon which the lower compartment of the well-plate insert was switched to hypertrophic media, while the upper compartment was maintained in chondrogenic media. All grafts were matured for 9 weeks and their bonding strength, compressive modulus and cross-sectional histological properties (Alizarin Red, Safranin O, collagen I and II) analyzed. SEM and µCT imaging were further performed to confirm calcification.
Results: Under chondrogenic culture conditions grafts matured into hyaline cartilage approaching histological (Fig.1b) and biomechanical properties of native tissue, as evidenced by the presence of glycosaminoglycans and collagen II, and absence of collagen I (compressive modulus: 557±20kPa, articular cartilage: 837±221kPa). Under hypertrophic conditions grafts showed calcification throughout, as confirmed by histology (Fig.1b) and µCT images with a compressive modulus of 647±238kPa. Cartilage further demonstrated good integration into the 3D printed bone ceramics, forming a strong interfacial bond. Ongoing experiments are focused on the generation of a defined calcified cartilage layer within these grafts.
Conclusion: We demonstrate a novel biofabrication technique for creating osteochondral grafts that mimic the micro-architecture of the native osteochondral unit. The ability to replicate hyaline and calcified cartilage approaching native tissue properties, represents a significant advancement in engineering functional osteochondral grafts. Planned in vivo testing in sheep models will provide insight into their structural stability and integration into surrounding tissue, supporting their potential for future translational applications.

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Biohybrid plant extracellular matrix-based humanized bypass grafts for vascular surgery

Peripheral artery disease is the third leading cause for morbidity worldwide, demanding a multimodal therapy for more than 200 million people. Current therapeutic options for more severe cases range from medication therapy to surgical interventions. Synthetic vascular bypass grafts are the current gold standard in the surgical treatment of patients with no suitable autologous graft available. These scaffolds are predisposed to the development and progression of deep wound/graft infection, requiring surgical removal of the implanted bypass. In such cases biological grafts are needed to restore blood flow and successfully confine and treat infection. Here we present non-immunogenic humanized vascular grafts derived from Muscari neglectum (grape hyacinth) stem extracellular matrices employing decellularization. These naturally derived scaffolds retain the mechanobiological characteristics of their native counterparts for successful reimplantation and regeneration of isolated human cells. Morphological integrity and biophysical characteristics were assessed in four biological replicates by visualization and magnetic resonance elastography. In all biological replicates a significant reduction of DNA content and cellular material was observed. In this study, we present biohybrid living vascular grafts by reendothelialization of Muscari neglectum stem extracellular matrix scaffolds with human umbilical vein endothelial cells under dynamic perfusion conditions. By introducing a rice-based hydrogel coating to the fabricated scaffolds, the here presented grafts show adequate hemocompatibility with no hemolytic activity and low thrombogenicity. To further augment fabricated grafts for adequate strength, all coated scaffolds were reseeded with human smooth muscle cells and assessed by their flow dynamics employing ultrasound imaging. In a proof-of-concept experiment, we created a surgical anastomosis using these grafts showing adequate perfusability and sturdiness under pulsatile flow conditions. These biologically derived scaffolds pose as sustainable and cost-effective hybrid living scaffolds, that can be fabricated at large scale for clinical applications, overcoming current limitations in vascular surgery.

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Biphasic Electrospun Structure to Treat Periodontitis

Periodontal disease(PD) is a chronic infection caused by gram-negative bacteria, with a high prevalence worldwide. It is considered the second leading cause of dental problems.The disease begins with an imbalance between the oral microbiota and the host's immune system, triggering vascular changes and inflammation.Common symptoms include bleeding and swollen gums, which compromise tissue integrity and allow aggressive bacteria to penetrate deeper layers, forming periodontal pockets and contributing to bone loss[1].As PD progresses, it results in the destruction of periodontal structures, such as cementum, periodontal ligament, and alveolar bone.Although conventional treatments are available, their effectiveness decreases in advanced stages, prompting the development of innovative therapies like guided tissue regeneration (GTR) membranes.However, current GTR membranes are non-biodegradable and require surgical removal after treatment[2].To overcome this limitation, this project aimed to develop a biodegradable hybrid structure capable of supporting the regeneration of periodontal tissues.
A dual electrospun membrane was created and designed to mimic the native architecture of the bone (zone B) and periodontal ligament(zone L).Zone B incorporated polycaprolactone (PCL) and hydroxyapatite (HAp) to promote osteoconduction and bone integration.Zone L was composed of PCL, gelatin, and chitosan to provide mechanical support, cell adhesion, proliferation, and antimicrobial effects.To fabricate zone L, a solution containing 15 wt% PCL, 5 wt% gelatin, and 1 wt% chitosan was prepared in a 70:30 mixture of acetic and formic acids[3]. For zone B, 16 wt% PCL was mixed with 33–36 wt% hydroxyapatite nanopowder in acetone.Electrospinning was used to produce the fibers, with zone L spun at 18.5 KV and a flow rate of 0.25 mL/h, and zone B at 18 KV and 2.5 mL/h, both maintaining a 10 cm distance between the needle and the collector[4].The resulting membranes were analyzed for their physicochemical and mechanical properties.
The electrospun fibers from zone B showed successful incorporation of HAp, with a homogeneous distribution confirmed by SEM imaging.The fiber diameters 33–36 wt% HAp concentration, was 549.78 ± 164.97 nm, 628.46 ± 254.33 nm, 443.56 ± 239.83 nm,802 ± 497.33 nm, respectively. In zone L, fibers were uniform and bead-free, with an average diameter of 228.71 ± 113.89 nm. Importantly, the dual-layer structure showed strong adhesion between zones without separation, indicating good integration.FTIR analysis confirmed the absence of chemical degradation due to solvent use, and mechanical tests demonstrated structural integrity and tissue-like behavior.
The results indicate that increasing HAp concentration affects both fiber diameter and process stability. Nonetheless, both zones effectively replicate the native extracellular matrix in terms of structure and dimensions.Overall, the developed hybrid membrane shows great potential for periodontal regeneration, offering a biodegradable alternative to traditional GTR membranes and addressing current clinical limitations.Acknowledgements
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  Bioprinted tissues towards standardized industrial applications: Combination of physiological collagen bioink and fabrication techniques in standardizable design

  Introduction
  The standardizability in fabrication of any biological tissues in high numbers is a major promise of the field of biofabrication. The acceptance of biofabrication methods in the industry continues to be hampered by the fact that bioinks are assembled in non-physiological structures (especially for soft tissues) combined with an absent application-orientated model design.
  With Collagen I as the major extracellular matrix (ECM) component and structure-giving element in almost all soft tissues, this material is essential in any physiological bioink. To overcome its drawbacks like uncontrolled gelation during printing or pH, a formulation of collagen was developed, which prevents fibrillation at neutral pH during printing (slightly cooled). By denying any chemical modifications on the collagen molecule, the formulation is based on physical interaction forming polyelectrolyte complexes to prevent fibrillation during printing.
  Printing physiological hydrogels directly into inserts always results in tissue contraction, entailing major issues concerning standardization and incompatibility for current test protocols. To improve these issues, we anchored the tissue by MEW-fibers in the surrounding wall, which stabilizes the tissue and enables the application as transwell insert system.

  Methods
  Collagen I was dissolved in 0.5 % acetic acid and mixed with a solution of an anionic cellulose derivate (CD). The resulting polyelectrolyte complex was lyophilisated. The resulting material was applied as bioink by mixing it directly with cell culture medium and characterized by its gelation and rheological properties, biocompatibility and fibril formation. Thereby, different CDs were tested to find the most promising anionic molecule for collagen I. In the printing process (RGEN 200, RegenHU), gel extrusion of collagen I (and fibroblasts) was combined with MEW (PCL) and FMD (PCL) in a layer-by-layer alternating process. The resulting tissue, surrounded by a plastic ring, was placed in an insert and cultured exemplarily for the application as skin model.

  Results
  By formulating Collagen I with CD, the polyelectrolyte complex was able to shift the fibrillation process at 15 °C from 1h (like pure Collagen) to several hours, depending on the structural properties of the CD (chain length, sulfation, additional substation with other groups) as well as molecular ratio. SEM imaging determined the fibrillar structure of the resulting bioinks. All CD-variations and bioinks showed no negative effects in direct contact with primary human fibroblasts. For printing tissue models, wall printing (FDM), hydrogel printing and anchoring structures (MEW, through the model and wall) were alternated up to five times. The resulting tissue disc was fixed in an empty insert (w/o membrane, Brand), seeded with primary human keratinocytes and matured to generate a highly standardizable full thickness skin model.

  Discussion
  By formulating Collagen I as a polyelectrolyte complex with anionic cellulose sulfate, the applicability of Collagen I was highly improved and generates a basic instrument to be applied on all further Collagen Types as physiological bioinks for complex tissue compositions and any type of soft tissue.

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  Breast cancer cells isolated from the brain form functional cell-cell interactions with brain cells and adapt to the brain extracellular matrix

  Three-dimensional (3D) cellular models to study various kind of diseases mimic a more in vivo-like native environment compared to 2D cellular models. Our study focuses on breast cancer, which represents the second most common cancer to metastasize to the brain. Triple-negative breast cancer, HER2+, and Luminal B breast cancers are common subtypes spreading to distant organs such as the brain, bones, liver, and lungs. The progression of cancer is linked to the interactions between cancer cells and the extracellular matrix (ECM), as well as the interactions between cancer cells and host cells. It has been shown that cancer cells proliferate while remodeling the ECM by secreting excessive fiber-forming matrix proteins, leading to increased stiffness. These mechanical and compositional changes disrupt interactions between the microenvironment and healthy cells, as well as interfere with cell-cell interactions and cell polarity. In this regard, breast cancer cells use the fiber-forming matrix as tracts to invade, e.g. the bloodstream as a prerequisite to reach the host organs. In breast-to-brain metastasis, breast tumor cells extravasate into the brain's perivascular niche by disrupting the blood-brain barrier (BBB). The perivascular niche, which consists of blood vessels, provides a supportive environment for breast cancer cells, allowing them to adapt and proliferate within the host microenvironment. The brain basement membrane is associated with blood vessels, and it is abundant with collagen type IV, laminin, and fibronectin proteins, which assist breast tumor cells to adhere and migrate along the blood vessels. Hyaluronic acid is another important component of the brain's ECM but also exhibits increased expression level during tumor progression at the primary site of breast cancer. Here, we use 3D thiolated hyaluronic acid (HA-SH) as a hydrogel system, which allows us to crosslink ECM components (collagen IV, laminin, fibronectin) to mimic the brain ECM and to investigate how neuronal signaling promotes breast cancer cell growth and proliferation. Breast tumor cells typically form spheroids in an HA-SH environment, maintaining high viability regardless of the stiffness of the hydrogel. When the ECM is supplemented with ECM components such as laminin, the tumor cells change their morphology to a more spread-like form. Additionally, neurons and astrocytes have been shown to interact functionally with breast cancer cells, promoting tumor growth. The cancer cells form pseudo-tripartite synapses with pre- and postsynaptic neurons, utilizing neuronal glutamate release to activate AMPA/NMDA receptors on the tumor cell membrane. The presence of neurons and astrocytes within a 3D network also encourages breast cancer cell spreading and enhances cell-cell interactions. In this regard, we imaged and analyzed colocalization of AMPA subunit GluA2 and vesicular glutamate transporter (vGlut1) on neurons, as well as the gap junction protein connexin 43 on astrocytes, both in the presence and absence of breast cancer cells. In parallel, calcium transients were measured to assess the impact of the breast tumor cells on the functionality of the primary neurons. The degree of structural and functional maturation will serve as a measure of suitability of this distinct 3D disease model for future studies using drug testing strategies.

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  Development of a high-throughput combinatorial microfluidic assisted 3D bioprinter for artificial skeletal muscle tissue screening

  Despite significant advancements in 3D bioprinting, challenges remain in achieving high printing speed, efficiency, and homogeneity in biological samples. These limitations constrain the practical application of bioprinting in tissue engineering, particularly in the fabrication of complex tissues like skeletal muscle. This project introduces a novel high-throughput combina torial microfluidic-assisted 3D bioprinter designed to address these critical gaps. The device is capable of printing 96 distinct material sequences in ap proximately 2 hours, enabling rapid screening for the fabrication of artificial skeletal muscle tissue.
  The bioprinter employs a unique system of open cartridges that store di verse bioinks, each differentiated by cell concentration, medium, hydrogels, and other factors. These cartridges are connected to a pressure controller that applies periodic positive/negative pressure, ensuring gentle mixing to prevent cell sedimentation and achieve homogeneous printing. This method significantly improves upon existing techniques, which often struggle with maintaining bioink homogeneity and cell viability.
  The printer features a printing hat equipped with 12 microfluidic printing heads, each containing a mixing compartment for passive homogenization of 1 aspirated bioinks. These heads are independently controlled by servo mo tors for precise vertical movement, while a robotic arm facilitates horizontal and vertical positioning. The entire process is fully automated via a cus tom python code that synchronizes syringe pumps, servo motors, and the robotic arm, ensuring precise control over bioink aspiration, deposition, and movement. The microfluidic chips within the printing heads are connected to syringe pumps, which regulate bioink flow, while low-density oil minimizes residue and ensures consistent operation.
  The printing process begins with the printing hat moving to the cartridge area, where each head aspirates a set amount of bioink. The hat then moves to a 96-well plate, depositing the bioinks in a spiral pattern into the first 12 wells. After printing, the hat moves to a washing area, where residual bioink is flushed out using oil. This cycle repeats eight times, filling all 96 wells with distinct combinatorial bioink sequences. The printed samples are cultured for 14 days and analyzed using image processing techniques to identify the optimal recipe for skeletal muscle tissue engineering. Key metrics include alignment, a critical feature for muscle contraction and posture, which has been underperforming in prior studies.
  This bioprinter offers significant advantages in speed, simplicity, afford ability, resolution, and homogeneity, making it a transformative tool for tis sue engineering. Its high-throughput capabilities and precision position it as a superior alternative to existing technologies, accelerating the discovery and development of functional artificial tissues.

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  Enhancing keratocyte lineage differentiation of bone marrow-derived mesenchymal stromal cells via 3D culture for in vivo corneal bioprinting applications.

Introduction: Corneal transplantation is the gold standard procedure to cure corneal diseases that can lead to blindness. Fabrication of corneal tissues is a promising solution to overcome the shortage of human donors. Our future goal is to utilize a bioprinter mounted to a robotic arm to print corneal tissue directly onto the eye of the patient. One interesting cell type for in situ and in vivo corneal bioprinting is the use of bone marrow-derived mesenchymal stromal cells (BM-MSC) in their differentiated state toward the keratocyte lineage.
Objective: This study aims to improve the differentiation of BM-MSC into keratocytes using 3D culture techniques, including differentiation post-bioprinting. Specifically, we aim to characterize the expression of primary corneal stromal keratocyte (CSK) specific markers of differentiated cells for their suitability in in vivo corneal bioprinting. To further validate the applicability of the model for in vivo corneal repair, ex vivo experiments were performed using porcine corneal tissue.
Materials & Methods: BM-MSC from 3 donors were cultured in BM-MSC medium. Briefly, BM-MSC were encapsulated in collagen-based hydrogels. After 3 days, differentiation was initiated by subculturing the casted BM-MSC for 14 days in a differentiation medium based on DMEM/F12, 1 % MEM Vitamin Solution, 1 % MEM nonessential amino acids, 1 % Insulin-Transferrin-Selen, 1 mM L-Ascorbate 2-Phosphate, 10 ng/ml FGF-b, and 0.1 ng/ml TGF-b3. The expressions of the CSK-specific markers keratocan, lumican, and ALDH3A1 were investigated by immunofluorescence staining and qPCR. Porcine eyes were cleaned, and the cornea was carefully dissected from the ocular globe. A stromal defect was simulated via central trephination, followed by defect filling using an inkjet-based bioprinter. Cells spreading and differentiation were monitored by IF and qPCR.
Results: 3D differentiation of BM-MSC enhanced their differentiation towards keratocyte lineages compared to traditional 2D culture methods. IF and qPCR analyses revealed an upregulation of CSK-specific markers, indicating successful differentiation, in both 3D cast and 3D printed constructs. Moreover, differentiated cells exhibited typical phenotypes of primary CSK. The ex vivo experiments demonstrated successful filling of the corneal defect with the bioink, along with evidence of cell spreading and keratocyte differentiation as confirmed by protein analysis.
Conclusion: Our work demonstrates the efficiency of 3D culture techniques in promoting the differentiation of BM-MSC into keratocyte lineages for corneal tissue engineering applications. The enhanced expression of CSK-specific markers and functional properties of differentiated cells underscore the potential of this approach for generating bioengineered corneal substitutes. Moreover, the ex vivo tests highlight the potential use of this technique for in vivo corneal bioprinting.

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Engineering of Perfused 3D Hepatocellular Carcinoma Tissue Model

Introduction
For decades, two-dimensional (2D) cell culture systems have been fundamental for studying cellular metabolism, differentiation, and drug efficacy. These simple models enable straightforward experimental design but does not replicate native tissue complexity. Important features such as physiological cell density, structural organization, and vascularization are absent. Recently, three-dimensional (3D) culture techniques have emerged, allowing more physiologically relevant models that better mimic tissue microenvironments [1]. However, most 3D cultures are static, failing to replicate vascular perfusion. To address this limitation, we developed a hepatic carcinoma perfusion model with an integrated canal mimicking physiological blood flow. Transparent biomaterials allow live imaging of cellular development and organization. This system enables real-time studies on tumour progression and therapeutic response. The aim of this study was to develop a perfused 3D hepatic carcinoma model for drug screening applications.
Methods
HepG2, human dermal fibroblasts adult (HDF-a), and human umbilical vein endothelial cells green fluorescent protein (HUVEC-GFP) were cultured under standard conditions. A bioink containing 10% methacrylated gelatin (GelMA), 0.5% methacrylated hyaluronic acid (HAMA), and 1% decellularized liver extracellular matrix (dECM), with HepG2 cells (5–12 × 10^6 cells/mL), was used for bioprinting. Each layer was crosslinked using UV light (405 nm, 28.5 mW/cm^2, 30 s). A 5% dECM paste formed the perfusion canal, which was liquefied and seeded with HDF-a and HUVEC-GFP cells (8–10 × 10^6 cells/mL). Constructs were cultivated with perfusion for 21 days. Viability and proliferation were assessed by enzyme-linked immunosorbent assay (ELISA) for alpha-fetoprotein (AFP) and von Willebrand factor (vWF), lactate dehydrogenase (LDH) release assay, fluorescein diacetate/propidium iodide (FDA/PI) staining, hematoxylin and eosin (H&E) staining, and fluorescent microscopy.
Results
The bioprinted construct exhibited high cell viability, maintaining levels between 90–95%, and sustained secretion of AFP into the culture medium, with concentrations ranging from 2.0 to 2.5 ng/mL after 7 days of cultivation. Owing to the transparent properties of the biomaterials used for the bionic liver fabrication, numerous HepG2 spheroids were microscopically observed within the liver matrix after 14 days of culture, which was confirmed by H&E staining. The secretion of vWF into the culture medium, at concentrations between 2.5 and 5.0 ng/mL, indicated the successful proliferation and expansion of HDF-a and HUVEC-GFP within the perfusion canal. FDA/PI staining performed on day 21 demonstrated a consistently high live-to-dead cell ratio throughout the construct, supporting the sustained viability and structural integrity of the bioprinted model.
Discussion
These findings demonstrate the successful generation of a hepatic carcinoma model, viable for at least 21 days, incorporating a functional perfusion channel. The system's sustained cell viability and three-dimensional tumour spheroid formation suggest its utility for future pharmacological evaluations. Continuous perfusion capability further supports its potential as a platform for preclinical drug screening.
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Konuşmacılar: Michal Uflewski (Polbionica Inc.)

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Expanding the Melt-Electro Fibrillation Polymer Library for Advanced Biofabrication Applications

Expanding the Melt-Electro Fibrillation Polymer Library for Advanced Biofabrication Applications
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Introduction: Melt-Electro Fibrillation (MEF) is an emerging technology derived from Melt Electrowriting (MEW), in which a polymer melt is accelerated by an electric field to achieve a precise micrometer fiber deposition. When two immiscible polymers are co-processed and one selectively removed, highly anisotropic nanofibrillar structures remained1. This collagen-mimetic, topographical guidance can then promote / facilitate the anisotropic distribution and alignment of cells, with the potential to mimic native tissue architectures such as tendon or muscle. However, since each tissue exhibits distinct mechanical properties, such as stiffness or elasticity, as well as different biological requirements, there is a growing need to explore a broader range of polymer combinations to tailor scaffold characteristics for the specific applications. Herein we introduce polymers outside of the original MEF framework and attempt to expand the MEF applicable polymers.
Methods: The polymers were selected based on their melt immiscibility and processing characteristics. In MEF, Poly-ε-Caprolactone (PCL) is blended with Polyvinyl acetate (PVAc) and heated past their melting points. When the PVAc is selectively dissolved via ethanol treatment, the resultant PCL remains. Polyethylene glycol (PEG) was then evaluated as the matrix material, showing capability of the PEG to replace PVAc in select situations. Polypropylene (PP) was also attempted as the fibrillating polymer, as its inert properties has seen its use in the medical industry. PP has low to no miscibility in PVAc or PEG and is possible to MEF. Additionally, polylactic acid (PLA) was also tested for its fibrillation potential. To verify the prints, fibril diameters and morphology were quantified, as well as the initial printing conditions.
Results: This study successfully demonstrates various new polymer combinations outside of the traditional MEF scope and explores potential applications. Furthermore, we measured the parameters that are vital in successful fibrillation of polymers such as their melting points and viscosities at the printing temperatures. While inert materials like PP were immiscible with all tested matrix polymers, other materials like PLA or PCL demonstrated sufficient compatibility to allow an effective fibrillation. The fibrils formed exhibit dimensions on a cellular scale, suggesting strong potential for guiding cellular alignment and the formation of defined, highly anisotropic scaffolds in tissue-specific constructs.
Discussion: Herein we were able to expand the MEF-compatible library and demonstrate the feasibility of generating nanofibrillar scaffolds with both biologically active or inert polymers, The morphological similarities with the traditional PCL/PVAc blend support the versatility of MEF in biomedical applications such as tendon or muscle regeneration.
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- 40

Detergent-free pancreatic decellularization

Introduction
Tissue decellularization is widely used in tissue engineering and regenerative medicine. Currently, most established decellularization methods use detergents, which have long decellularization processes with inconsistent results. Residual chemicals used in decellularization or changes in the biochemistry and structure of extracellular matrix (ECM) proteins may be responsible for lower cell repopulation. Detergent decellularization also necessitates the determination of residual detergent content in the obtained ECM scaffolds. The aim of this work was to develop a simple and cost-effective decellularization protocol without using detergents. The effectiveness of the method was compared with a chemical-based approach.

Methods
Pancreases obtained from a local slaughterhouse were decellularized. After thawing, the organs were homogenized. The homogenate was centrifuged and floating fat was removed. The remaining material was suspended three times in NaCl solution and in water, stirring at 150 rpm at 4°C. At each change of solution, the tissue was ground. Then, the pancreatic material was suspended in phosphate-buffered saline with antibiotic. The drained pancreatic extracellular matrix was frozen at -80°C, lyophilized and cryogenically ground. The powdered final product was subjected to sterilization. DNA from powdered ECM was isolated using the column method and determined spectrophotometrically. Soxhlet extraction was used for yield of lipids. The sulfated glycosaminoglycans (GAGs) and collagen content was quantified using commercial kits. To determine cell viability, hydrogels based on obtained dECM was analyzed by MTT assay.

Results
Experimental results showed that the use of physical and mechanical methods allows for effective decellularization. DNA concentrations of powdered matrix obtained from each were significantly lower than in native tissue by about 98%. The level of fat in the decellularized tissue was significantly reduced by approximately three times. Significant enrichment of the final material in collagen compared to the native tissue was observed. In the obtained material, the content of GAGs was reduced, but at a detectable level. Generally the loss of GAGs is related to the detergent-enzymatic process of decellularization. Cells showed higher viability after contact with the dECM-based hydrogel prepared without detergent than that prepared on the basis of detergent decellularization.

Discussion
The results indicate that detergent-free decellularization has great potential in tissue engineering. Decellularized tissues are expected to retain structural and/or compositional features of the natural ECM, offering more biocompatible and functional matrices for regenerative applications. High and low molar ionic solutions are usually used before the actual decellularization process to induce osmotic shock to disrupt cells. NaCl has been used in combination with detergents such as SDS in skin decellularization or Triton X-100 in lung decellularization. We have shown that aggressive detergents are not necessary for effective decellularization. Further studies are needed to optimize decellularization conditions and fully characterize the biomechanical and biological characteristics of the obtained matrices, as well as integrate them with modern technologies such as 3D printing.

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Development of the mushroom-chitosan-based bioink for bone tissue regeneration

Chitosan, a naturally derived polysaccharide, has gained significant attention in tissue engineering due to its excellent biocompatibility, biodegradability, and bioactivity. However, conventional chitosan sourced from crustacean shells poses limitations such as allergenicity and ethical concerns. In this study, we extracted and characterized mushroom-derived chitosan from Pleurotus ostreatus and developed a bioink for bone tissue regeneration. The mushroom-derived chitosan bioink was evaluated against collagen-based and shrimp shell-derived chitosan bioinks in terms of biocompatibility, antibacterial activity, osteogenic potential, and immunomodulatory properties. Subsequently, a mushroom-derived chitosan/hydroxyapatite-based bioink was formulated based on its printability and cellular activity using MC3T3-E1 preosteoblasts. Since the osteon consists of concentric layers of bone tissue surrounding a central canal and blood vessels, to mimic this unique structure, hydroxyapatite/MC3T3-E1-laden bioink and endothelial cell-laden bioink were printed layer by layer to form a concentric pattern. The bone regenerability of the developed scaffold was then assessed by comparing it to a conventionally printed structure. Cellular activities such as cell proliferation and differentiation were evaluated, and in vitro immune systems were conducted to analyze the expression of inflammatory cytokines. The mushroom-derived chitosan scaffold demonstrated high cell viability (~90%), robust proliferation, and enhanced osteogenic differentiation, as confirmed by immunofluorescence and RT-PCR. Moreover, it induced a lower inflammatory response compared to conventional counterparts. These results highlight that mushroom-derived chitosan is a promising, non-animal-derived biomaterial for bone tissue engineering, offering both functional efficacy and improved biocompatibility with reduced immunogenic risk.

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Development of a Vascularization-Enhancing 3D Printable Fibrin-Gelatin Interpenetrating Network Bioink for Sustained Release Local Drug Delivery

Conventional local drug delivery systems often fail to support sustained drug release, while many biomaterials used in tissue engineering and transplantation lack the necessary stability, biocompatibility, and capacity to support vascularization required for long-term graft survival and function. In order to overcome these restrictions, 3D bioprinting has become a potent technique that makes it possible to create precisely designed structures and gives control over where cells, biomaterials, and medications are positioned. Fibrin, a biocompatible and pro-angiogenic substance, is a promising applicant for vascularization research. However, it has low viscosity and rapid degradation limit its use in 3D bioprinting.
To address these challenges, we developed a 3D-printable fibrin-gelatin interpenetrating network (IPN) biomaterial for developing a drug delivery system. Our strategy makes use of dual crosslinking to improve structural stability while preserving the pro-angiogenic and biocompatible characteristics of fibrin. Our strategy of using dual-crosslinking mechanism improved structural integrity while allowing for sustained control over therapeutic release.
This bioink represents a versatile tool for regenerative medicine, offering a modular approach to tailoring both structural and therapeutic functions in engineered tissue constructs.

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Engineering Human Glomerular Filtration Barrier In Vitro for Disease Modeling and Drug Testing

Introduction: The glomerular filtration barrier (GFB) is a highly specialized structure responsible for blood filtration in the kidney; is composed of podocytes, glomerular basement membrane and fenestrated endothelial cells. Dysfunction of this barrier is a hallmark of many glomerular diseases. However, the current systems in vitro cannot mimic efficiently the 3D organization and the biochemical and mechanical properties of human GFB. This study aimed to develop a biomimetic human GFB in vitro for studying glomerular physiology and pathology.

Methods: To engineer the GFB, human podocyte and glomerular endothelial cell lines were first used to optimize experimental conditions and ensure appropriate cell adhesion and viability. hiPSC-derived podocytes and glomerular endothelial cells were then co-cultured on opposite sides of a porous membrane of the transwell integrated into a 3D millifluidic chip. To more faithfully mimic the physiological condition, the polyester membrane was coated with natural glomerular basement membrane components (i.e. different collagen types, fibronectin etc.) or completely replaced by an extracellular matrix gel. This system was linked to a peristaltic pump to mimic physiological shear stress on cells and to promote cell adhesion, alignment, and maturation.

Results: Our results demonstrated the successful establishment of a 3D biodevice comprising hiPSC-derived podocytes and glomerular endothelial cells separated by a self-assembled basement membrane. Characterization by qPCR and immunofluorescence confirmed the expression of key maturation markers for the GFB. Functional integrity of this barrier was validated through selective permeability assays. To model disease, we induced nephrotoxicity using Adriamycin and incorporated iPSCs derived from patients with Alport Syndrome. Perm-selectivity assays revealed increased permeability in both disease conditions compared to healthy controls. Analysis of samples from Adriamycin-treated and AS-derived models further identified disease-specific alterations in GFB structure and function.

Discussion: This GFB platform represents a significant step forward in the development of human-relevant models for renal research in vitro. It provides a versatile tool for investigating disease mechanisms, enabling personalized medicine approaches, and advancing drug discovery.

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Fabricating microfibrillar fiber bundles as cell-guiding additive for bioprinting

Introduction
Two main technologies of Biofabrication are bioprinting and scaffold generation. [1] Bioprinting can be used with cells in the matrix, while scaffold generation is cell-free and cells are attached afterwards. Both have their distinct advantages, e.g. bioprinting enables the generation of complex tissue hierarchies in one step, while scaffolds can guide cell elongation via topographical cues.
In this regard, we previously established Melt-Electro-Fibrillation, which generates microfibrillar polycaprolactone (PCL) via Melt-Electro-Writing (MEW). These support cell orientation, and it was previously shown that macrophages express anti-inflammatory markers caused by the topology alone. [2]
To exploit the advantages of bioprinting and scaffold generation, this study aims to converge these methods by utilizing fragmented fibrillar scaffolds as filler material for bioinks. The orientation of fibers after bioprinting with the orientation of the microfibrils led to the generation of a large anisotropic system, while maintaining the ease of application of bioprinting.

Materials and methods
The blend of polyvinyl acetate (PVAc) and polycaprolactone (PCL) was processed via melt electrowriting (MEW) and printed on a polyvinyl alcohol (PVA) coated grid. Subsequently the grid was transferred to the laser cutter, where the fibers were cut into bundles of equal length with fused microfibrillar fibers at both ends. Afterwards the grids with the fibers were immersed in 70% ethanol to dissolve the PVA and PVAc. The pure PCL microfibrillar fibers detached from the grid without damage and were further washed in 70% ethanol. Finally, they were transferred to pure water, lyophilized, and weighed. Then the hydrogel was added to create different fiber-to-hydrogel mixtures.

Results and discussion
Single microfibrillar fibers were laser cut into bundles and after the removal of the PVAc, the microfibrillar PCL structure was uncovered (Figure 1, A). The general form of the bundles consisted of two dome-like caps, which are caused by melting during the laser cut, and straight microfibrillar fibers. The obtained fibers were treated with NaOH to improve the dispersion of single bundles in a variety of hydrogels. Upon incorporation of the fiber bundles into the hydrogel, they were extruded into single lines, and aligned fiber bundles within the hydrogel were obtained (Figure 1, B). As anticipated, seeded cells aligned to the microfibrils of the ribbon (Figure 1, C).

Conclusion
We present a microfibrillar additive for bioinks that is capable to align cells in fiber direction and themselves in printing direction. Therefore, achieving an anisotropy from the cellular level to the macroscopic level, while maintaining the scalability of bioprinting.

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Development of Polycaprolactone Fibrous Scaffolds for 3D In Vitro Osteochondral Modelling: Influence of Scaffold Morphology on Cell Distribution and Proliferation

Introduction
Cartilage tissue regeneration has been significantly advanced through the development of artificial scaffolds, including three-dimensional (3D) electrospun structures. A key challenge in designing in vitro osteochondral models is creating scaffolds with a functional barrier that mimics the native tidemark—separating cartilage and bone—while still permitting cellular communication across tissues. In this study, we aimed to develop 3D electrospun polycaprolactone (PCL) scaffolds with fiber architectures that support cell attachment, proliferation, and tissue development, while incorporating a barrier layer to limit cell migration and intermixing between cartilage and bone cells.
Methods
3D fibrous scaffolds featuring interconnected porous networks with gradient designs were fabricated using a 3D fiber printer (3Df-01C, Bious Labs, Lithuania), which integrates melt electrospinning and fused deposition modeling techniques. Surface hydrophilicity was enhanced via non-thermal plasma (NTP) treatment using a dielectric barrier discharge device (DBD-01-V, Bious Labs).
Chondrocyte cell line C28-I2 was cultured on the PCL scaffolds using CellCrown-24NX culture inserts at varying seeding densities over extended time periods. Cell proliferation and viability were assessed using the CCK-8 assay. Scaffold cross-sections were fixed and analyzed microscopically following NucBlue nuclear staining to evaluate cell distribution and scaffold colonization.
Results
The scaffolds exhibited gradient architectures with fiber diameters ranging from 12 µm to 35 µm and pore sizes from 10 µm to 50 µm. A water contact angle of approximately 50° was achieved with an NTP energy dose of 0.39 J/cm².
C28-I2 chondrocytes were successfully cultured on three different scaffold types over an 8-day period. Cells remained viable and demonstrated progressive proliferation, with the highest proliferation observed in scaffolds featuring a gradual porosity and fiber density gradient. These scaffolds supported a more uniform vertical distribution of cells. In contrast, scaffolds with a more porous upper layer exhibited cell aggregation primarily in the denser lower region. Microscopic analysis of cross-sections confirmed these findings. The tightly woven lower layer of the scaffold functioned effectively as a barrier, preventing cell migration, and mimicking the native tidemark. This barrier design shows promise for supporting spatial separation of chondrocytes and osteocytes in osteochondral constructs.
Conclusions
Electrospun PCL fibrous scaffolds provide a suitable environment for chondrocyte adhesion and proliferation. The incorporation of a dense, tightly woven barrier within a gradient porous structure presents a promising approach for engineering in vitro osteochondral models. These scaffolds could facilitate the development of complex tissue constructs for regenerative medicine applications and preclinical testing.

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Development of patient specific composite scaffold using 3D printing for regeneration of craniofacial bone tissue

Development of patient specific composite scaffold using 3D printing for regeneration of craniofacial bone tissue
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Traditional methods for treating bone defects resulting from disease, injury, or congenital conditions often involve autografts, allografts, or xenografts. However, these methods present drawbacks such as the risk of immune system rejection, potential for infection, and limited supply. As a result, tissue engineering approaches, specifically employing scaffolds for material replacement and stimulation of tissue growth, have become increasingly important. Many medical and research facilities are now investigating the use of additive manufacturing techniques to create customized implants for individual patients. Biopolymer-based three-dimensional (3D) printing is proving to be a valuable tool in tissue engineering, the creation of medical devices, and controlled drug release. This method enables the construction of structures that resemble natural cellular environments, which support cell attachment, growth, and ultimately, the restoration of damaged tissues and organs. Alginate and carrageenan are particularly appealing for bone tissue engineering because they are natural, biocompatible, and biodegradable polymers with minimal toxicity. They are easy to process and possess inherent qualities that promote cell adhesion and proliferation. Alginate's ability to readily form hydrogels in mild conditions with multivalent metal ions enhances its bioactivity and makes it a cost-effective and versatile material for scaffold fabrication. Carrageenan, a sulfated polysaccharide, is also biocompatible and water-soluble, forming gels at room temperature. The presence of sulfonic groups in carrageenan can facilitate bone bonding through the integration of calcium ions. Combining alginate and carrageenan with bioceramic materials creates composite scaffolds that capitalize on the beneficial properties of each component. The present research focuses on the creation of

personalized 3D-printed scaffolds using a matrix of alginate/carrageenan (Al/CA) fortified with hydroxyapatite/tricalcium phosphate nanoparticles to promote bone tissue regeneration. The findings demonstrate the effective production of interconnected, porous, and structurally sound 3D-printed Al/CA scaffolds containing various nanoparticle concentrations. The addition of nanoparticles improved the strength and stiffness of the composite scaffolds compared to the pure Al/CA scaffolds. Additionally, the scaffolds promoted apatite formation in laboratory conditions. Studies of cell behavior using MG-63 cells showed that the scaffolds were non-toxic and that the addition of HA/TCP nanoparticles enhanced their ability to promote bone formation. These results indicate that the developed scaffolds are a promising option for creating patient-specific implants for the regeneration of craniofacial hard tissues.

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Creation and optimization of 3D bioprinted perfusable in vitro model to study cancer metastasis to bone

Introduction:
Cancer is one of the leading causes of death worldwide and although developing new therapies can help fight this disease, cancers which origins from breast, lung or prostate can undergo metastasis process leading to development of secondary tumors in other parts of the human body [1]. Bone is the organ to which cancer often metastases and to pioneer cancer therapies, creation of functional bone tissue models is crucial. Several studies aimed at understanding this process have already been conducted using cell cultures and/or animal models. These include basic 3D tissue models representing bone, blood vessels, and cancer. Yet, both 2D and 3D in vitro models have their limitations and currently there are few functional bone in vitro models fully reflecting hierarchical structure of the bone [2]. Here, we propose a new 3D bioprinted perfusable bone model as a promising tool for study cancer metastasis to bone.
Methods:
The developed bioprinted perfusable bone model is consisting of three different compartments, each one built from different materials and reproducing subtissues of the bone: GelMA with β-TCP (calcified region of the bone), GelMA (bone/vessel interface) and fibrin with alginate (vessel). Extrusion bioprinting was used for fabrication of the structures of the model and for verification of obtained structures in perfusion environment the commercially available bioreactor chamber (Polbionica, Poland) was used. The structural, chemical and mechanical properties of obtained bioinks were investigated by FTIR, SEM and microscope imaging as well as degradation and swelling studies. Additionally, for further research the CFD studies were provided in order to optimalize the thickness of printed structures and their distribution inside the bioreactor chamber for future optimal cell seeding and bioprinting with cells. Moreover, as the proof of concept, the simulation studies in what way circulating tumor cells behave in the vessel's lumen of the fabricated perfusable bone model were provided revealing how possibly secondary tumor can develop in the bone.
Results:
All of the prepared bioinks display a proper rheological behavior, making it possible to overcome the negative effects affecting viability of the cells during bioprinting. SEM images allowed to conclude that total porosity and the size of the pores are suitable for cell proliferation. Calculated printing accuracy from microscope images of printed structures revealed that extrusion bioprinting may be successfully applied for creation of such models. The CFD studies allowed to optimize distribution of the printed structures as well as put insight into cancer cells attachment depending on fluid flow inside perfusable blood vessel leading to conclusion what parameters are potentially crucial for secondary tumor development. The presented results add important insight into fabrication parameters as well as optimalization for creation of perfusabale tissue models. Therefore, the proposed 3D bioprinted bone model can be successfully considered for cancer metastasis studies.

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Design and characterization of novel composite hydrogels as bioink base for bone tissue engineering via the endochondral ossification pathway

Among the challenges associated with the regeneration of critical-sized bone defects, the most significant issue is the insufficient delivery of oxygen and nutrients, which impedes the optimal development of osteocytes. A potential solution lies in the use of biomaterials that can induce bone formation from hypertrophic chondrocytes cartilage tissue via endochondral ossification. For the material to adequately perform its function, it must mimic the cartilage tissue and deliver osteo- and angiogenic agents, such as growth factors and ions, to the defect site. In recent years, there has been a shift in focus towards the utilization of advanced biopolymeric materials, including decellularized extracellular matrix (dECM) and multifunctional fillers, such as multielement bioactive glass.
In this study, composite hydrogels consisting of a biopolymer matrix and bioactive glass microparticles were designed and characterized for their potential as bioprintable materials. The matrix was constituted by a solution of dECM isolated from porcine bone (1% w/v) mixed with sodium alginate (1% w/v; Pronova UP VLVG). In this study, the bioactive glass microparticles (BGMPs) of a 13-93-based formula were enriched with osteo- and angiogenetic additives. The BGMPs were synthesized via a water-casting-milling process, and they served as the filler. The filler size was determined by SLS. Composites were examined to ascertain their rheological properties, including dynamic viscosity and viscoelastic modulus. This assessment was conducted to evaluate the feasibility of utilizing the composites for bioprinting applications. The materials were subjected to cross-linking in solutions containing Ca^{2+} and Sr^{2+}. Thereafter, the materials were degraded in culture medium (DMEM F-12). The composition of the materials was examined using Fourier-transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), and dynamic mechanical analysis (DMA).
Bioactive glass microparticles had average size of 4 µm diameter . The composite materials were characterized by the shear thinning behavior with non-zero initial shear stress, viscosity ranges (20-100 Pa·s) at low shear rates and tan(δ) characteristics (from ~0.1 at low frequencies to 0.5 at higher) displaying promising characteristics for further extrusion bioprinting. The FTIR and TGA analyses revealed that the degradation of the composites resulted in slight change in the composition – mostly the filler dissolution/release and dECM degradation .The release was the most prominent on the surface of the composites. The cross-linking environments and degradation process impacted the compressive modulus and ultimate strength of the structures. Higher filler content stabilizes the hydrogels mechanically overtime, possibly by the release of alginate cross-linking agents.
Utilization of novel composites based on dECM, alginate, and BGMPs holds considerable promise as materials for use as bioprinting ink base. This is due to their tissue-mimicking biochemical composition and degradation-tuning properties, which can be tailored by controlling the filler content. The suitability of a material for bioprinting can be determined by conducting cell viability tests following the printing process. In order to evaluate the biological activity of the materials under investigation, it is necessary to utilize appropriate cells (e.g. mesenchymal stem cells) The assessment of cell maturation towards hypertrophic chondrocytes can be conducted through the use of an assay and subsequent microscopic observation.

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HIGH-THROUGHPUT CUSTOM SYSTEMS FOR BIOMATERIALS SCREENING AND BIOLOGICAL ANALYSIS OF CENTIMETRE-SCALE MUSCLES

Skeletal muscle tissue engineering is emerging as a cornerstone in the development of in vitro models, offering unprecedented opportunities to study muscle physiology and pathology. This work aim to develop high-throughput, custom-designed system for fabricating skeletal muscle constructs at a centimetre scale, addressing challenges in scalability, reproducibility, and biological relevance.
Our approach combines innovative biofabrication workflows with advanced imaging. A key feature of the system is its ability to screen and optimize multiple experimental parameters, such as biomaterial selection, cell concentration, mechanical stimuli, and in vitro culturing conditions, thanks to a simple and reproducible methodology. Novel metrics, such as the myo-index, provide quantitative benchmarks for comparing the architecture and maturation of artificial constructs to native tissues.
Furthermore, this versatile system provides a robust platform for analyzing biological processes critical to skeletal muscle development and maturation. Preliminary results highlight the potential of this approach to generate aligned and functional muscle tissue, offering insights into the interplay between C2C12 cellular dynamics and physical stimuli.
Our findings establish a transformative framework, providing scalable tools that bridge the gap between in vitro experimentation and in vivo functionality. This platform offers an easy-to-manage and reproducible in vitro model, opening new avenues for studying biological dynamics, cellular interplay, and developing therapeutic strategies for muscle-related disorders.

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Impact of cellular interactions on microarchitecture, matrix remodeling, and tenogenesis in bioreactor stimulated 3D tendon model

Impact of cellular interactions on microarchitecture, matrix remodeling, and tenogenesis
in bioreactor stimulated 3D tendon model

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ABSTRACT:
Introduction: Tendon injuries are widespread, often leading to tendinopathy due to lack of early recognition, resulting in discomfort and reduced mobility. Despite their mechanically active nature, tendons possess limited self-healing capacity, and current clinical interventions fall short in fully regenerating tendon structure. To address this challenge, an in vitro 3D model is needed to study disease progression and develop an effective tissue regeneration strategy.
Methods: In this study, a 3D electrospun nanofiber tube comprising Poly ε-Caprolactone (PCL) is fabricated and encapsulated with goat tendon decellularized extracellular matrix (tdECM) hydrogel with Adipose-derived mesenchymal stem cells and their spheroids. An amalgamation of these two materials forms an ideal scaffold for tendon tissue engineering. The scaffolds with cells and their spheroids are subjected to mechanical stimulation in a bioreactor to mimic the native tissue conditions, which is essential for effective tendon regeneration. The study compares cellular interactions in the cell and spheroid groups.
Results: The study demonstrates a tissue engineering approach, combining dynamic mechanical cues from a bioreactor and biochemical cues from tdECM to induce tenogenesis in MSCs. The scaffolds exhibit increased cell alignment, strength post 21 days of culture. Gene expression studies reveal an increase in tenogenic markers in response to biomechanical cues in both groups. The encapsulated cell and spheroid groups show varying results in terms of gene expression, protein estimation, and cell viability.
Discussion: While it is a known fact that mechanical stimulation and biochemical cues are important for tendon tissue formation, regeneration, and repair, we emphasize the importance of cellular interactions in an ECM-rich tissue like tendon where the microarchitecture, matrix remodeling and tenogenesis depend on the intercellular signaling and mechanosensation.
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From Ink to Organ Function: Advanced Strategies for the Vascularization of Bioprinted Bionic Organs

Introduction
One of the most significant challenges in organ bioengineering is developing functional vascular networks. Proper vascularization is critical for transporting oxygen, nutrients, and signaling molecules, while also removing waste. In bionic organs, poor vessel formation limits nutrient exchange and cell migration, reducing transplant quality and long-term survival [1], [2]. The traditional use of mature endothelial cells is restricted by their low proliferation and limited angiogenic capacity. Endothelial progenitor cells - particularly endothelial colony-forming cells (ECFCs) - exhibit greater angiogenic potential. This study explores vascularization strategies in a 3D bioprinted pancreas model to advance the functionality of bioengineered organs.
Materials and Methods
A prototype of a bionic pancreas was created using 3D bioprinting, incorporating bioink composed of decellularized extracellular matrix (dECM), pancreatic cells, endothelial cells, and fibroblasts. The selection of biomaterials for the 3D bioprinting of a bionic pancreas integrated with a flow system was guided by comprehensive hemocompatibility assessment tests to ensure optimal interaction with blood components and minimize the risk of thrombogenic or immunological responses. A central pre-designed vascular channel was included to mimic native vasculature. The printed construct was placed in a perfusion bioreactor, simulating physiological flow. After several days of incubation, samples were fixed and analyzed by immunohistochemistry (IHC) using markers such as CD31, insulin, vimentin, and glucagon to evaluate vascular and islet formation. To determine the optimal conditions for vessel formation, microfluidic models with different concentrations of endothelial cells to fibroblasts were designed. Furthermore, isolation and co-culture techniques were improved to enhance the angiogenic potential of the endothelial population. Models are characterized by IHC and qPCR for the following markers: VEGF, Tie2 and Ang1.
Discussion
These early observations highlight the persistent difficulty of achieving sufficient vascularization in bioprinted organs. While the emergence of vessel-like structures is encouraging, further work is needed to improve vascular density and functionality. Adjusting the endothelial-to-fibroblast ratio and improving cell culture protocols could enhance vessel formation. Generating more angiocompetent endothelial populations is expected to support the development of stable and functional microvasculature. Continued optimization will be vital for improving transplant performance and advancing bioengineered organ systems toward clinical application. This study provides initial evidence of successful vascular integration and sets the stage for future investigations focused on enhancing vascular complexity and tissue viability.
Results
Preliminary analysis show microvessel-like structures forming from the central vascular channel in the bioprinted pancreas. These structures tested positive for CD31, indicating early capillary development. Although the number of vessels is limited, their alignment along the direction of perfusion suggests that mechanical flow may promote endothelial organization and sprouting. Microscopic images (to be shown) confirm the physical continuity between the main channel and newly formed vascular outgrowths.
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Fabrication of GelMA/ColMA Hydrogel Microfluidic Model to study vascular endothelial cells

Introduction:
On-chip vascular microfluidic models provide powerful platforms for studying vasculature and its diseases in vitro. These models enable focused investigation of specific vascular layers, such as the endothelium, and the influence of hemodynamics on it. While traditional plastics or glass-based fabrication allows for defined microchannel architecture, its inherent stiffness and low permeability limit biological applications. Thus, hydrogels are gaining interest. Specifically, Gelatin Methacryloyl (GelMA) and Collagen Methacryloyl (ColMA) are attractive due to their porosity, tunable mechanical properties, and inherent bioactivity, closely mimicking the native extracellular matrix (ECM). Here, we present a method combining 3D printing and casting to create hydrogel microfluidic chips with smooth, cylindrical channels.
Methods:

GelMA [1] and ColMA [2] were synthesized according to published protocols. Hydrogels were prepared using 5, 10, and 15% of GelMa and 0.5, 1, and 1.5% ColMa. Compression and swelling tests evaluated physical properties. Porosity was assessed using Scanning Electron Microscopy. Endothelial cell (MS1 and HUVEC) viability on these materials was analyzed. For microfluidic chip preparation, a frame defining the outer geometry was fabricated using Stereolithography printing with a biocompatible resin. Stainless-steel needles (0.8 mm diameter, 3 cm length) were inserted into the frame, and after casting and photocrosslinking the hydrogel, the needles were carefully removed to form smooth, cylindrical lumens. The lumens were then seeded with endothelial cells, which were subjected to pathophysiology-relevant flow.
Results:
Compared to single-component hydrogels, the 15% GelMA / 1.5% ColMA hydrogel exhibited superior mechanical properties, including a higher compressive modulus and lower swelling. The porosity of the hydrogels correlated with the dry content of the gel. All tested hydrogels provided reasonable support for endothelial cells. Formulations with higher solids and 1% ColMA better supported long-term cell culture. The method of chip fabrication produced an optically transparent device, having microchannels with smooth, cylindrical lumens (800 µm diameter; surface roughness ≤ 1 µm). Channels remained stable under shear stresses up to about 70 Pa. Endothelial cells seeded into the channels responded to flow conditions by changes in elongation and orientation.

Discussion:
Our method generated transparent GelMA/ColMA composite hydrogels. Methacrylation enabled tunable mechanical properties; increased ColMa and solids, enhanced the compressive modulus, and reduced swelling. This provided crucial stability, essential for maintaining channel integrity under perfusion in the hydrogel chip. Indeed, the channels with smooth, cylindrical lumens showed excellent stability up to about 70 Pa of shear stress. This platform enables investigation of endothelial mechanobiology under defined pathophysiological conditions.
Acknowledgment:
The study was supported by Ministry of Health of the Czech Republic (grants nr. NW24-08-00064 and NU22-08-00124), and MEDITECH - Centre for multidisciplinary research in cardiovascular medicine (grant nr. CZ.02.01.01/00/23_021/0009171) and Faculty of Medicine of Masaryk University (nr. MUNI/A/1644/2024).

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Fabrication of chitosan/Gallic acid/ Strontium Chloride/ polycaprolactone nanofibers for bone regeneration

Considering the increasing incidence of bone-degenerative diseases and injuries, including osteoarthritis, especially in societies where aging is associated with increased obesity and poor physical activity, the repair of bone defects is one of the major challenges in medical science. Due to the difficulties, high cost, risks of surgery, severe allergic reactions, and ultimately rejection of grafted tissue in surgeries, the demand for tissue engineering methods to replace old and traditional treatment methods has risen significantly. Bone tissue engineering aims to restore bone function through the combination and synergy of biological materials, cells, and therapeutic factors, and the result of using these alternative methods is reduced risks and costs along with better effectiveness in the treatment of the disease. This study aimed to develop a promising bioactive scaffold by combining the structural properties of polycaprolactone nanofibers with the therapeutic features of Gallic acid (GA) and Strontium chloride ($SrCl_2$). The resultant nonporous scaffold was physically characterized, and the outcome indicated that the gallic acid and chitosan enhance the wettability of the scaffold, improving the Sr^{2+} release and, consequently, increasing the adipose-derived mesenchymal stem cells (AD-MSCs) adhesion, proliferation, ALP activity, and biomineralization. The cytotoxicity results also revealed that both Gallic acid and strontium chloride had no adverse effect on the AD-MSCS. Overall, this fabricated scaffold holds promising potential for bone regeneration applications.

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Fabrication of a Biomimetic Scaffold from Decellularized Goat Urinary Bladder for Urinary Bladder Reconstruction

Decellularized scaffolds are becoming important tools within tissue engineering. This work compares the effectiveness of three decellularization protocols—SDS, H_2O_2, and Triton X-100 for goat urinary bladder tissue on the preservation of ECM components and the functionality of scaffolds. The cellular removal and ECM preservation were assessed through histological staining with H&E, Masson's Trichrome, Alcian Blue, and DAPI, and DNA/RNA quantification. Ultrastructure assessment was done by SEM; swelling behavior, tensile properties, and biodegradation rates evaluated the functionality of these scaffolds. Hemolysis assay, MTT assay, in ovo and in vivo assay demonstrated scaffold biocompatibility. Regeneration was confirmed with immunohistochemistry and gene expression analysis. SDS-dGUB has proved to be the most effective protocol for complete cellular removal with minimal damages to the structural component evidenced from the histological analysis, and significantly a lower DNA content of 35 ± 12 ng/mg. In vitro biocompatibility assays revealed better cell proliferation on the SDS-GUB scaffold and low hemolysis of 0.6 ± 0.01%. In vivo implantation studies showed very good scaffold integration, neovascularization, and a minimal inflammatory response. The results of this study clearly show that the SDS decellularization protocol maintains the integrity of the ECM to provide a useful tissue engineering scaffold.

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Histological processing of polycaprolactone scaffolds

Introduction
Cell-seeded scaffolds made from natural or synthetic materials are widely tested in animal models as biodegradable implants for treatment of joint disease (1). One of the most commonly used material for scaffold production is polycaprolactone (PCL), known for its biocompatibility and mechanical properties close to human cartilage. To validate cell distribution within scaffold and its biocompatibility, histological assessment is usually performed. However, processing of PCL polymers is associated with challenges related to temperature sensitivity and solubility in histological reagents (2). Therefore, to overcome them formalin-fixed paraffin-embedded (FFPE) and frozen samples histological techniques were optimised.
Methods
3D fibrous scaffolds featuring interconnected porous networks with gradient design were fabricated using a 3D fiber printer, which integrates melt electrospinning and fused deposition modeling techniques. Surface hydrophilicity was enhanced via non-thermal plasma (NTP) treatment. Modified protocols of standard FFPE and frozen samples histology were used. Cross-sections of PCL scaffolds were acquired with microtome and cryomicrotome, respectively.
Results
Standard FFPE histology caused shrinkage of both NTP-treated and -nontreated PCL scaffolds and disrupted their fiber network. Intact sections were obtained when replacing xylene with D-limonene-based clearing agent ROTI®Histol (Carl Roth) in combination with low-melting paraffin. Paraffin with 51-53°C melting point was applied to nontreated scaffolds, however, for NTP-treated scaffolds 42-44°C melting point paraffin was required, as these scaffolds had lower melting point. The best quality of sections was achieved by processing PCL scaffolds with automated tissue processor LOGOS (Milestone Medical), which employs vacuum to infiltrate paraffin, while avoiding clearing reagents at all.
Furthermore, prolonged incubation in cryoprotective reagents were found to be required to achieve optimal cryosections. To prevent PCL fiber disruption PCL scaffolds were sequentially incubated in 5 and 20 % glucose, mixture of 20% glucose and cryogel, and finally in pure cryogel.
Discussion
In this work we optimised several histological techniques of PCL scaffolds. The best results were achieved by using automated tissue processor with low-melting paraffin for FFPE and prolonged incubation in cryoprotectants for frozen samples. These optimizations extend applicability of histological evaluation to hydrophilic PCL scaffolds.
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Nanofiber-Based Delivery of Crocus Sativus Phenolics Protects Against Chemotherapy-Induced Hepatotoxicity

Introduction: Hepatotoxicity induced by cisplatin (CP), a widely-used chemotherapeutic agent, significantly hampers its therapeutic efficacy. Oxidative stress due to reactive oxygen and nitrogen species plays a critical role in CP-induced liver injury. Crocus sativus (saffron), known for its phenolic compounds and antioxidative properties, has shown potential hepatoprotective effects. To enhance bioavailability and therapeutic efficiency, the present study evaluates the efficacy of nanofiber-based delivery systems encapsulating phenolic compounds extracted from C. sativus petals.   Methods: Phenolic-rich fractions (CPEF) were extracted from dried C. sativus petals using ethyl acetate solvent. These fractions were encapsulated within zein-based electrospun nanofibers (CPN) exhibiting optimal morphology, stability, and encapsulation efficiency (89.6%). Male Balb/c mice were administered CP intraperitoneally to induce hepatotoxicity, followed by dietary supplementation with either CPEF or CPN (100 mg GAE/kg mice body weight). Treatment continued for 30 days post-induction, and mice were evaluated for changes in weight, food intake, biochemical markers, antioxidant enzyme activities, lipid peroxidation, gene expression, and histopathological alterations.   Results: Both CPEF and CPN treatments significantly improved mice weight gain and food intake compared to untreated, CP-exposed controls ($p \leq 0.05$). CPN notably reduced liver enzyme levels (ALT, AST, ALP) and total bilirubin, while elevating antioxidant enzyme activities (GPx, SOD, CAT) more effectively than CPEF alone. Additionally, lipid peroxidation, assessed through MDA levels, decreased significantly following CPN treatment. Gene expression analysis indicated significant downregulation of inflammatory markers (iNOS, INF-γ) and upregulation of SOD expression with CPN administration. Histopathological examination confirmed that CPN markedly improved liver and kidney tissue integrity compared to controls and CPEF treatment.   Discussion: The nanofiber-based encapsulation markedly enhanced the hepatoprotective potential of C. sativus phenolic compounds, likely due to improved bioavailability, controlled release, and heightened antioxidative activity. These findings underscore the therapeutic promise of nanofiber formulations in mitigating cisplatin-induced hepatotoxicity through oxidative stress modulation, inflammation reduction, and enhancement of antioxidant defense systems.   References: Abd Rashid, N., Abd Halim, S. A. S., Teoh, S. L., Budin, S. B., Hussan, F., & Ridzuan, N. R. A. (2021). Alhumaydhi, F. A., Khan, I., Rauf, A., Qureshi, M. N., Aljohani, A. S., & Khan, S. A. (2021). Del Campo, A., Valenzuela, R., Videla, L. A., & Zúniga-Hernandez, ˜ J. (2023). Mykhailenko, O., Ivanauskas, L., Bezruk, I., Sidorenko, L., Lesyk, R., & Georgiyants, V. (2021). Schulte-Werning, L. V., Murugaiah, A., Singh, B., Johannessen, M., Engstad, R. E., Skalko-Basnet, ˇ N., & Holsæter, A. M. (2021). Shamansoori, M. T., Karimi, E., & Oskoueian, E. (2022). Singh, G., Haileselassie, Y., Ji, A. R., Maecker, H. T., Sinha, S. R., & Brim, H. (2022). Tajaddini, A., Roshanravan, N., Mobasseri, M., Aeinehchi, A., Sefid-Mooye Azar, P., Hadi, A., & Ostadrahimi, A. (2021). Videla, L. A., & Valenzuela, R. (2022). Acknowledgments: The authors are grateful to the Islamic Azad University of Mashhad and Arka Industrial Cluster, Mashhad, Iran. Disclosure Information: The authors declare no conflicts of interest.

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Introducing Optical Fiber-Assisted Bioprinting (OFAB) as Novel 3D Bioprinting Method

Introducing Optical Fiber-Assisted Bioprinting (OFAB) as Novel 3D Bioprinting Method

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Introduction: To accelerate scientific progress in tissue engineering and regenerative medicine, new accessible 3D biofabrication methods must be developed. Optical fiber-assisted bioprinting (OFAB) is such a 3D printing method and was introduced by our team for cell free work.[1] It offers a cost-effective, fast, flexible, and easy-to-use approach to bioprinting with a variety of bioresins, including non-transparent ones. The development and optimization of OFAB as a biofabrication method shown in this contribution presents new opportunities for the free-form creation of intricate 3D structures.

Methods: In OFAB, an optical fiber coupled to a light-emitting diode is used to generate a locally confined area of light intensity high enough to crosslink and solidify photoresins in a defined area around the fiber tip. The size and width of this area are controlled by adding a photoabsorber to the resin and by process parameters. Moving the fiber above or within a vat of resin enables the generation of 2.5D and 3D structures, as the material solidifies along the path of the moving fiber tip, enabling freeform 3D biofabrication. To verify the capabilities of the OFAB platform, resolution is measured using different setups with various optical fibers and resin formulations. Additionally, the cytocompatibility of these resin formulations is evaluated.

Results: This study presents the latest OFAB platform setup and demonstrates its capabilities and versatility. It focuses on identifying the ideal setup and printing parameters. Furthermore, various bioresins, including both biological and synthetic materials are being developed and systematically screened to achieve high resolution and cytocompatibility. While stiffer materials tend to improve print resolution, they are generally less favorable for cell proliferation. To overcome this limitation, the thermoreactive properties of methacrylated gelatin are utilized. Cooled gelatin derivatives provide a stiff and stable support structure during printing, while offering a soft, cell-friendly matrix under in vitro conditions post-print.

Discussion: OFAB offers a highly efficient, reproducible, and versatile method for biofabrication. The establishment of 3D OFAB may represent a significant advancement in tissue engineering and biomimetic scaffold fabrication by making light-based bioprinting more accessible. Additionally, various bioinks are under development to provide a range of cell-friendly formulations, further enhancing the applicability of OFAB across different fields of research.

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Integrated MEW-MES Scaffold and Hydrogel for Nasal Septum Regeneration

Introduction: Nasal septum defects, whether resulting from infection, trauma, or prior surgery, remain difficult to repair due to shortcomings in existing implant materials [1]. Conventional implants often suffer from inadequate mechanical strength, rapid degradation, or insufficient bioactive cues for both cartilage and mucosal regeneration [1]. As a result, patients risk poor functional outcomes and recurrent defects. In this study, melt electrowriting (MEW) and melt electrospinning (MES) are harnessed to produce a load-bearing framework seeded with fibroblasts for mucosal regeneration, while an internal 3D-bioprinted hydrogel layer supports chondrocyte proliferation. By simultaneously fulfilling structural and biological requirements, this design aims to overcome the persistent limitations seen in conventional septal implants.

Methods: 1) The nasal septum model was derived from average human measurements [2]. A mold was then designed in Blender and fabricated via stereolithography (SLA) printer (Formlabs 3, Formlabs, Germany) (Fig.1A). 2) Both MEW and MES were conducted employing the GeSiM BioScaffolder 3.1 integrated with a MEW head (GeSiM, Radeberg, Germany). Medical-grade PCL (Purasorb PC 12, Corbion, Amsterdam, Netherlands) was heated to 79°C. For MEW, the printing speed, nozzle-collector distance, pressure and voltage were 70 mm/min, 3.5 mm, 10 kPa, and 7 kV, respectively; MES used 30 mm/min, 15 mm, 5 kPa, and 18 kV. 3) Thebioink was prepared with human chondrocytes. It was printed with a BioScaffolder 3.1 at a speed of 6 mm/s and a pressure of 50 kPa.

Results: The scaffold structure and fabrication process weare shown in Fig.1B-D. With MEW fiber diameters of around 15 µm and MES fiber diameters of approximately 1-2 µm and micropores of about 20 µm were achieved. Live/dead staining of chondrocytes revealed high cell viability within the hydrogel on day 1.

Conclusion: This study presents a multi-layer scaffold combining MEW, MES, and 3D bioprinting to simultaneously support nasal mucosal and cartilage regeneration. MEW and MES layers provide mechanical support and guide fibroblast growth, while a 3D-bioprinted hydrogel promotes chondrocyte activity. The design addresses current limitations in nasal septum repair by integrating mechanical strength with tissue-specific biofunctionality.

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Phycocyanin-Integrated GelMA Hydrogels for Cartilage Tissue Engineering

Osteoarthritis (OA) is one of the most prevalent musculoskeletal disorders globally, with its burden escalating due to the aging population and the absence of disease-modifying therapies 1. Despite its debilitating nature, current treatments remain palliative, focusing primarily on pain management or surgical intervention, with no curative

options. The pathogenesis of OA involves a complex interplay between pro-inflammatory cytokines, dysregulated signaling pathways, and mechanical stress, all contributing to chondrocyte dysfunction and cartilage breakdown2. While anti-inflammatory drugs are routinely used in symptom management, they do not effectively address the structural damage. On the other hand, tissue engineering strategies, particularly hydrogel-based bio-printed constructs, have emerged as promising approaches to promote cartilage regeneration.

In this study, we developed a bioactive hydrogel by integrating phycocyanin3—a natural anti-inflammatory protein derived from Spirulina platensis—into a Gelatin Methacryloyl (GelMA, BIOINX) matrix to mitigate inflammation and promote chondrocyte regeneration. Primary human chondrocytes exposed to phycocyanin (0–200 µg/mL) maintained >90% viability ($p>0.05$), with an IC_{50} of 309.9 µg/mL (10.33 µM). When chondrocytes were cultured under inflammatory conditions (IL-1β/TNF-α stimulation), a marked upregulation of NF-κB expression was observed. Notably, treatment with phycocyanin at concentrations between 100–200 µg/mL significantly suppressed NF-κB expression and reduced inflammatory marker levels, highlighting its anti-inflammatory potential.

Beyond its anti-inflammatory effects, phycocyanin may support chondrogenic differentiation, positioning it as a promising bioactive component for bio-functional printable hydrogel scaffolds aimed at osteochondral repair. Unlike single-target OA therapies, this dual-action hydrogel could have the potential to address both symptom relief and structural repair.

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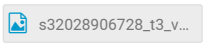
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Microfluidics-Enabled Synthesis of Lipid Nanoparticles for miRNA Cellular Transfection

Introduction

Lipid nanoparticles (LNPs) represent a promising and versatile platform for the non-viral delivery of genetic material, including microRNA (miRNA), which plays a crucial regulatory role in gene expression. While LNPs have already demonstrated clinical success as mRNA carriers in vaccines, most notably in COVID-19 immunization strategies, their application in miRNA delivery opens new opportunities in regenerative medicine, such as wound healing, based on cellular reprogramming. Unlike mRNA, which typically aims to express therapeutic proteins transiently, miRNA can modulate entire gene networks, making LNP-based delivery systems particularly attractive for fine-tuned, endogenous control of cellular processes. The primary aim of this study is to develop effective LNP-based carriers for miRNA delivery and to investigate how various post-processing techniques influence their physicochemical properties. This work focuses on assessing the relationship between processing conditions and key characteristics such as particle size, polydispersity, surface charge, and ultimately, transfection efficiency. Additionally, the performance of the optimized LNPs is compared to a commercial transfection reagent—Lipofectamine™ 3000—to evaluate their potential as a viable alternative in non-viral gene delivery systems.

Methods

miRNA-loaded LNPs (miRNA-LNPs) and blank LNPs (without miRNA) were synthesized using a microfluidic approach. The samples were subjected to four post-processing techniques: sonication, filtration, dialysis, and thermal treatment. Key physicochemical parameters, including average particle size, polydispersity index (PDI), and zeta potential, were evaluated using dynamic light scattering (DLS) and electrophoretic mobility measurements. The optimized LNP formulations were assessed for colloidal stability and homogeneity. Transfection efficiency was tested in L929 fibroblast cells using Cy3-labeled miRNA, with comparative analysis against transfection mediated by Lipofectamine™ 3000.

Results

Post-processing significantly affected LNP properties: sonication and filtration improved size uniformity and colloidal stability, while dialysis effectively reduced PDI without altering surface charge. In contrast, thermal treatment compromised particle stability. The optimized miRNA-LNPs successfully delivered Cy3-miRNA into L929 cells, demonstrating effective intracellular uptake. When compared to Lipofectamine™ 3000, the developed LNPs exhibited comparable—or in some cases superior—transfection efficiency, while showing lower cytotoxicity and better biocompatibility.

Discussion

These findings emphasize the importance of post-processing strategies in tuning the physicochemical properties of LNPs to enhance their performance as gene delivery vehicles. Microfluidic synthesis enabled the fabrication of uniform and scalable LNP formulations suitable for in vitro applications. The comparable performance of the developed LNPs to the commercial Lipofectamine™ 3000 highlights their potential as safer, customizable alternatives for non-viral miRNA delivery in biomedical research. This study provides a systematic framework for designing and optimizing LNPs tailored for nucleic acid delivery, reinforcing the value of microfluidic technology in nanomedicine development.

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Laser-Induced forward transfer (LIFT) for 2.5D deterministic patterning of pre-vascular structures with high spatial complexity in open-top synovial Organ-on-Chip (OoC)

INTRODUCTIO

The synovial lining is responsible to produce synovial fluid into the joint capsule. The synovial fluid acts as a lubricating and protecting layer for the articular cartilage to ensure pain-free frictionless movement of the articular joint. The synovial lining is prone to inflammation from injury, overuse or inflammatory arthritis (1), but modelling these events in vitro, while accurately mimicking the synovial cell composition is challenging. Advances in organ-on-chip (OoC) devices could help to replicate physiological conditions and provide high-throughput in vitro modelling platforms to study novel drugs and their effect. Tissue function correlates intimately with its unique microarchitectural structures. Hence, the faithful incorporation of such microarchitectures in OoC is anticipated to increase their physiological relevance.

In this project, we present a novel strategy to increase complexity and cell disposition in open-top OoC models through LIFT bioprinting directly in synovial lining OoC devices. This approach enables the generation of pre-vascular networks within OoC models that are then closed and perfused at physiological flow rates.

METHODS

Human synovial fibroblasts were isolated from total knee arthroplasty surgeries; human Mesenchymal Stromal Cells (MSC) were isolated from bone marrow. GFP-Human Umbilical Vein Endothelial Cells (HUVEC) were purchased from Lonza. Open-top OoC were fabricated using an SLA 3D printer and Polydimethylsiloxane (PDMS). Synovial fibroblasts were encapsulated in 1mg/mL Collagen type 1 solution containing 2U/mL Thrombin and HUVEC-GFP were LIFT printed suspended in 15mg/mL Fibrinogen. After LIFT printing, OoC were sealed using double-sided tape and cultured in static conditions or under low and high flow rates. Lastly, the production of lubricin and vascular sprouting of the HUVEC was assessed via fluorescent microscopy and ELISA.

RESULTS

Open-top OoC have been successfully fabricated, and synovial fibroblasts were embedded on the cell culture chamber with high viability. Three cell-seeding conditions were studied, synoviocytes only, synoviocytes mixed with HUVEC, and synoviocytes with LIFT printed HUVEC on top of the cell culture chamber. All chips were subjected to both high and low flow rate perfusion. On the LIFT printed condition, the printed vascular pattern was maintained over 7 days of culture and showed interactions between synoviocytes and HUVEC, forming a lumenized structure that attracts cytokines and immature THP1 monocytes upon TGF b and LPS stimulation.

CONCLUSION

LIFT allows rapid and reproducible micropatterning of cells with high viability, as well as multi-cellular multi-material printing. Here, we show the micropatterning of small pre-vascular features in open-top OoC devices to mimic the microarchitecture of native tissue and recreate the onset of inflammation in the synovial lining with THP1 naive monocyte recruitment.

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Melt Electrofibrillation Enables Control over Type II Collagen Alignment in Engineered Cartilage Constructs

Introduction

Cartilage defects present significant challenges in long-term repair, with current treatments, such as cell-based therapies, often failing to restore sustained biomechanical function due to poor integration and/or lack of type II collagen organization[1]. The native depth-dependent fiber orientations in the arched collagen structure provide the mechanical support necessary to withstand cyclic loading in the joints[2]. Melt Electrofibrillation (MEF), a novel biofabrication technique, creates aligned polycaprolactone (PCL) nanofibers through polymer blend phase separation[3]. In contrast to traditional Melt Electrowriting (MEW) microfibers, we hypothesized that MEF nanofibers can guide type II collagen organization during chondrogenesis. This approach offers a pioneering solution for the development of functional articular cartilage tissue.

Methods

PCL microfibers (MEW) and PCL/polyvinyl acetate (PVAc) nanofibers (MEF) were printed using a custom-built MEW platform. PVAc was dissolved by incubating the MEF constructs in 70% ethanol and PBS, thereby exposing the PCL nanofibrils. Fiber diameters were characterized using scanning electron microscopy (Fig.1A). Rhombus-shaped scaffolds were seeded with equine articular cartilage progenitor cells (ACPCs, 5×10^6 cells/ml) to assess cell–material interactions (Fig.1B) using immunofluorescence (DAPI/Actin/Collagen-II). To replicate the native arched collagen architecture of articular cartilage (Fig. 1C), sinusoidal constructs of 288 layers were fabricated with an overall height of up to 1 mm (Fig.1D). ACPCs were seeded and chondrogenically differentiated for 28 days, and collagen alignment analysed by picrosirius red staining and polarized light microscopy, with orientation assessed via Fiji software (Fig.1E).

Results

The MEF generated fibers had significant smaller diameter (560 ± 160 nm) when compared to MEW microfibers (9 ± 1 µm) (Fig.1A). The nanofibers effectively guided ACPC alignment (Fig.1B) and supported organized type II collagen deposition. In contrast, cells on MEW-only scaffolds formed aggregates, with collagen primarily deposited around these clusters. Interestingly, ACPCs seeded on constructs with arched MEF nanofibres were distributed uniformly throughout the constructs and after 28 days of culture type II collagen structures were observed that followed the underlying fiber orientation (Fig.1E), resulting in arched collagen networks.

Conclusion

To the best of our knowledge, this study provides the first evidence of material-guided type II collagen organisation by chondrocytes. Using the MEF technology will thus enable us to control the type II collagen structures within engineered cartilage constructs, which opens new possibilities for engineering implants that can provide long-term mechanical stability.

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Melt electrowriting PCL scaffolds to reinforce 3D bioprinted full thickness cornea

Corneal blindness is one of the leading causes of blindness worldwide. A cornea transplant surgery provides ultimate cure, however, it is restricted by donor tissue shortage. Thus, we aim to develop 3D bioprinted full thickness corneas to address this lack of donor material. To ensure robust transport, handling, and suturing, the bioprinted cornea's mechanical properties needs to be improved. This could be achieved by incorporating a microfiber support structure into the bioink material to reinforce its stability.

Melt electrowriting (MEW) technique allows to produce microfiber scaffolds with high resolution and defined fiber thickness, by electrostatically drawing out a polymer melt of a heated syringe on a moving collector [1]. In this study, medical grade poly(e-caprolactone) (PCL) was selected as a printing material due to its well-established use in MEW and medical applications [2]. PCL is biocompatible, easy to sterilize, and possesses good mechanical properties.

Scaffolds with different parameters were fabricated via MEW. The fiber thickness and density influenced the transparency of the scaffolds. Moreover, the printing parameters, i.e. applied pressure, collector speed, and voltage, had to be balanced to achieve optimal fiber stacking [3]. Loss of fiber alignment resulted in decreased optical clearance, which is of particular relevance in this work.

The PCL microscaffolds significantly increased mechanical stability of the corneal hydrogel. Additionally, the complete structure was successfully sutured in human donor corneas ex vivo. PCL is a biodegradable polymer with various factors that affect its degradation kinetics [4]. Therefore, it will be essential to evaluate degradation of the corneal microscaffolds under the inflamed environment after the cornea surgery.

Overall, this research aims to design and fabricate PCL microscaffolds utilizing MEW, which will reinforce hydrogel-based full thickness corneas. Ultimately, this will allow clinical handling and suturing of bioprinted corneas, which are a promising alternative to donor corneal tissue.

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Multispheroid Bioassembly through Meltelectrowriting of a Precisely Arranged Fibrous Architecture for Tissue Models

Introduction

Over the past few decades, extensive research has been actively conducted for the fabrication of human tissues to, amongst many applications, understand the effects of a wide range of chemicals on human health and the environment (1). Thus, the need for the development of innovative assessment tools that provide reliable results in identifying and regulating the risks of potentially harmful substances is increasingly recognized. This study presents a precisely arranged fibrous architecture, fabricated via MeltElectroWriting (MEW) as spheroid culture support for 3D in vitro models. This platform facilitates multi-spheroid bioassembly, overcoming single spheroid limitations such as hypoxia while promoting cell-cell communication and adaptive interactions with the surrounding Extracellular-Matrix-like fibers (2). The technique has been employed to recapitulate different human tissues, for this study the targeted tissue was the thyroid.

Methods

The scaffolds are printed with an in-house build MeltElectrowriting device by heating a syringe containing melted Polycaprolactone and extruding the material with air pressure while applying voltage to pull a microscale fiber. Fibers are precisely deposited in the shape of squared boxes, in a variety of sizes between 100 µm and 1 mm. The scaffolds perimeter was reinforced with a 3D printed Poly Lactic Acid ring for better handling and coated in polydopamine for improved cell compatibility (3). Human thyroid epithelial cells (huThyrEC) spheroids are formed by cell aggregation in wells to be a similar size as the boxes and after 3 days of culture- supplied with thyroid-stimulating

hormone- are transferred in the scaffold so that each box is occupied by a single spheroid. The assemblies are further cultured for 7 more days followed by evaluating thyroid hormones T3 and T4, thyroid-related protein, metabolomic and transcriptomic changes.
Results
The final scaffolds present a box size of 530 µm and a highly precise architecture, raman analysis confirmed the presence of polydopamine on the surface. Spheroids cultivated in the MEW scaffolds disassembled long the fibers to occupy the box volume and showed significantly reduced hypoxia compared to single spheroid controls that would instead compact. Thyroid hormone secretion was significantly higher in the spheroid groups compared to monolayer with improved results for the MEW ones. From metabolomics it was evident how this tool can bridge the gap between 2D and 3D cultures while transcriptomics resulted in evidence of more upregulated genes in the fibrous environment.
Discussion
Analyzing thyroid hormone levels, protein, metabolomic and transcriptomic changes, provided a comprehensive understanding of cellular responses and metabolic shifts crucial for evaluating thyroid function and response to exposure to harmful or potentially hazardous substances.
Our findings suggest that the microarchitectures platform provides a reliable in vitro model for regulating thyroid function, representing a significant step toward advanced toxicity assessment tools.
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Optimization of dual crosslinked alginate and gelatin bioink for the biofabrication of 3D tumor models

Introduction:
The low success rate of oncological drugs in clinical trials highlights the need for predictive preclinical models because the traditional 2D cultures and animal models fail to replicate human tumor microenvironments fully. The researchers have seen 3D bioprinting as a promising technology for the fabrication of more physiologically relevant tissue models. Bioinks for such applications must possess biocompatibility, biodegradability, non-toxicity, non-immunogenicity, and mechanical integrity. In this context, we investigate the development of alginate-gelatin hydrogels crosslinked with $CaCl_2$ and genipin as potential bioinks for fabricating 3D tissue models.
Methods:
The inks were synthesized using natural polymers such as sodium alginate and gelatin at different concentrations and proportions, crosslinked via ionic ($CaCl_2$) and natural (genipin) methods. The chemical structure was analyzed by attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR). The morphology of the structures was characterized using scanning electron microscopy (SEM). Cell viability studies were assessed through MTT assay using HUVEC and Caco-2 cell lines. Rheological properties, including viscosity and shear-thinning behavior, were preliminarily assessed by oscillatory and flow sweep tests to evaluate the printability and mechanical stability of the hydrogels under bioprinting conditions.
Results:
The ATR-FTIR spectra confirmed successful crosslinking between the alginate, gelatin, $CaCl_2$, and genipin. SEM imaging revealed a porous and interconnected structure favorable for cell proliferation. MTT assays demonstrated promising cell viability rates, with hydrogels crosslinked with $CaCl_2$ reaching 90% viability and those crosslinked with 0.25% w/v genipin reaching 75.07% for Caco-2 cells and for HUVEC cells after 48 hours of culture. Preliminary rheological analyses demonstrated that the g hydrogels with genipin significantly increased storage modulus (G'), indicating greater stiffness and mechanical stability than hydrogels crosslinked with $CaCl_2$ alone. Furthermore, the loss modulus (G'') also exhibited adequate behavior, indicating appropriate viscoelasticity for biofabrication, where fluidity and elasticity must be balanced.
Discussion:
The incorporation of another crosslinker, such as genipin, significantly improved the mechanical stability of the hydrogels. This facilitates better control during the 3D printing process and maintains structural integrity post-printing. The improved rheological properties made the bioinks more suitable for printing complex 3D structures. Compared to traditional 2D cultures, the hydrogels significantly improved the growth and adherence of Caco-2 and HUVEC cells, increasing cell survival and enabling a more accurate modeling of tumor tissue activity.

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Injectable Nanofibrous Microcarriers with Tunable PVA/Tannic Acid Ratios for Controlled miRNA Delivery in Intervertebral Disc Regeneration

Injectable Nanofibrous Microcarriers with Tunable PVA/Tannic Acid Ratios for Controlled miRNA Delivery in Intervertebral Disc Regeneration

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Introduction: Degenerative disc disease (IDD) induces persistent back discomfort resulting from the degeneration of the nucleus pulposus (NP), and existing therapies, such as surgery, fail in restoring disc functionality [1]. Injectable biomaterials that administer therapeutic microRNAs (miRNAs) provide an effective method for nucleus pulposus regeneration[2]. This research formulates injectable nanofibrous microcarriers composed of polyvinyl alcohol (PVA), tannic acid (TA), and miRNA-encapsulated liposomes to enhance extracellular matrix (ECM) production and moderate inflammation, using biofabrication methods for minimally invasive intervertebral disc (IVD) repair.

Methods: Solutions (8% w/v) are formulated in five PVA:TA ratios (95:5, 90:10, 85:15, 80:20, 75:25) beside a pure PVA control (100:0) using distilled water. PVA (6.0–8.0 g) is solubilised at 80–90°C, prevailed by TA (0–2.0 g) at 40–50°C, 1% w/w sulfated alginate (S-ALG, 0.08 g) for extracellular matrix mimicking, and borax (0.1 g) for cross-linking purposes. The synthesis of cationic liposomes (DOTAP/cholesterol/mPEG-DSPE) encasing miR-145/miR-155 involves integrated electrospinning (0.5–1% w/v) and microfluidic mixing (flow rate ratio 3:1). Nanofibers (100–500 nm) are created by electrospinning (15–20 kV, 0.5–1 mL/h) and are cross-linked by the borate-diol linkages in borax. Microcarriers with 60–100 µm and 10–30 µm pores are produced by femtosecond laser micromachining and can then be injected using 30G needles [3].

Results: In all ratios, we expect homogenous nanofibers (100–500 nm), with increased TA content increasing fibre bioactivity. It is expected that microcarriers would have viscoelastic characteristics (Young's modulus ~0.1–1 MPa).With S-ALG facilitating electrostatic binding, liposomes (50–150 nm, zeta potential >10 mV) should attain >85% miRNA encapsulation efficiency and maintain release (80–90%) for 30 days. Pure PVA serves as a reference point for comparison, and the 80:20 ratio is thought to provide a compromise between mechanical stability and TA's anti-inflammatory properties.

Discussion: This polytherapeutic technology goes beyond hydrogel-based systems with transient drug release by combining electrospinning, liposome-mediated miRNA delivery, and laser micromachining. With S-ALG encouraging chondrogenic phenotypes and TA decreasing inflammation, tunable PVA:TA ratios enable bioactivity and mechanical optimization. Water stability is ensured via borax cross-linking, which is essential for NP's aquatic ecosystem. The injectable microcarriers meet the clinical requirements for IDD by enabling minimally invasive delivery.

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Keywords: miRNA delivery, PVA/tannic acid, IVD regeneration, nanofibrous, Biofabrication, microcarriers

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Improved standardization, scalability and convergence of fiber based biofabrication approaches for anisotropic tissues

Introduction: Reproducibility, scalability and adaptability are crucial aspects for any fabrication method that desires to address cell laden materials that shall be used for tissue engineering or drug screening approaches. Biomimetic fiber-based scaffolds are the most prominent among these as they mimic the fibrillar nature of many ECM proteins or other fibrous structures found therein, such as axons or capillaries for instance and can improve cell proliferation and guide cell polarization. In this work, we present a set of complementary fabrication methods that collectively enable the improved production of such scaffolds, thereby expanding the available toolkit for creating biologically relevant fiber-based systems.

Methods: Electrospinning, melt electrowriting (MEW), fused deposition modelling (FDM) and extrusion bioprinting are methods, which can be used separately or combined to generate anisotropic fiber-based scaffolds for tissue engineering. By depositing fibers or fiber infused hydrogels in different arrangements and combinations the generated fibrillar substrates can be used to improve/direct cellular function. A key aspect herein is the standardization and convergence of these methods into a comfortable generally applicable and reproducible system, that is easily adaptable to facilitate the production and screening of various different fibrillar materials.

Results: We present a set of platform technologies that enable flexible and efficient production of fibrous scaffolds using the following methods:

1. Integrating an illustration to G-code approach to generate codes for any core-xyz fabrication machine used to fabricate fibrillar scaffolds such as MEW, FDM or extrusion bioprinting.1
2. To facilitate reproducible fabrication, continuous fiber scaffolds (e.g., MEW or electrospun mats) are collected by depositing them on sacrificial PVA films. This allows for flawless removal, while FDM-printed frames help reinforce the scaffolds for easier handling without deformation.2
3. A generally applicable method has been introduced for producing non-continuous, cut fibrous scaffolds. These can be used as filler materials in bioprinting by depositing them on softer PVA films, which are later cryo-cut into micrometer-sized fibers.3
4. A standardized optical screening approach has been developed to assess the extrusion bioprinting performance of bioinks containing fibrous filler materials. These modified inks exhibit altered printability and are capable of inducing fiber alignment, compared to fiber-free counterparts.4

Discussion: A set of methods has been developed that lays the foundation for standardizing fabrication techniques and analysis for fiber-based materials. These methods have significant implications not only for the production of simple fibrous scaffolds, such as electrospun meshes or MEW scaffolds, but also for more complex scaffolds created through bioprinting or a hybrid approach that combines multiple fabrication techniques. As a result, the range of scaffolds that can be produced could be greatly expanded, offering advantages such as lower development costs, increased production output, and enhanced adaptability. This also enables greater cross-compatibility and comparability across different fabrication methods.

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Production of cell-laden hydrogel microparticle via ruthenium-catalyzed photocrosslinking within coaxial microfluidic system

Introduction
Injectable hydrogels with submicron dimensions that can provide both mechanical support and biological functionality to encapsulated cells are gaining attention for in vivo delivery of therapeutic cells and biomolecules [1,2]. In recent developments, hydrogel crosslinking triggered by visible light using the ruthenium(II) tris-bipyridyl complex (Ru) in conjunction with sodium ammonium persulfate (SPS) has emerged as a superior method compared to UV-based approaches. This is attributed to its benefits, including faster polymerization, lower thermal impact, deeper tissue penetration, and improved cytocompatibility, especially in the fabrication of cell-laden hydrogel microparticles [3].
Method
This study presents a strategy for producing hydrogel microparticles derived from phenol-modified alginate (AlgPh) via visible light-induced crosslinking using the Ru/SPS system in coaxial microfluidic device. The microparticles' structural, physical, and biochemical properties were systematically characterized. Additionally, their compatibility with cells was evaluated through assessment of cell morphology, attachment, and proliferation, aiming to validate the suitability of the fabrication method for potential use in cell delivery and regenerative medicine.
Results
The fabricated AlgPh hydrogel microparticles exhibited a uniform spherical morphology with a nearly monodisperse size profile. Experimental findings indicated that the characteristics of the microparticles could be tuned by adjusting the polymer concentration and flow dynamics in coaxial microfluidic system. Importantly, the produced hydrogel microparticles demonstrated high biocompatibility, largely owing to the mild nature of the Ru/SPS photoinitiation process and the incorporation of a gelatin-based component.
Conclusion
In conclusion, the development of AlgPh-based hydrogel microparticles using a visible light-activated Ru/SPS crosslinking system shows considerable promise as a platform for cell encapsulation and delivery. These microparticles hold significant potential for applications in tissue engineering and cellular therapies.
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Quercetin-derived carbon dots promote proliferation and migration of Schwann cells and enhance neurite outgrowth

Quercetin, a flavonoid known for its antioxidant properties, has recently garnered attention as a potential neuroprotective agent for treatment of the injured nervous system. The repair of peripheral nerve injuries hinges on the proliferation and migration of Schwann cells, which play a crucial role in supporting axonal growth and myelination. In this study we synthesized Quercetin-derived carbon dots (QCDs) and investigated their effects on cultured Schwann cells and the NG108-15 cell line. QCDs was obtained by solvothermal synthesis and characterized via UV–vis absorption spectroscopy, transmission electron microscopy, Fourier transform infrared spectroscopy, and X-ray diffraction analysis. The particles demonstrated significant dose-dependent free radical scavenging activity in DPPH and ABTS radical scavenging assays, supported in vitro proliferation and migration of Schwann cells, expression of neurotrophic and angiogenic growth factors, and stimulated neurite outgrowth from NG108-15 cells. Thus, QCDs could serve as a potential novel treatment strategy to promote regeneration in the injured peripheral nervous system.

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Revolutionizing Tissue Regeneration: An ECM-Integrated Hydrogel System for Next-Generation Biofabrication

Introduction: Advancements in biofabrication are poised to revolutionize healthcare by providing innovative solutions to complex medical challenges. A recent area of excitement is the integration of the extracellular matrix (ECM), which plays a pivotal role in tissue homeostasis, cellular signalling, and regenerative processes, into biofabrication approaches, making it the ECM a critical component in the development of tissue engineering applications. Decellularization techniques enable the removal of cellular components while preserving ECM integrity, allowing for the creation of bioactive scaffolds that support cell attachment, differentiation, and functional integration. However, challenges remain in optimizing ECM processing, bioactivity retention, and mechanical stability for clinical translation. Furthermore, it is known that ECM of various tissue cellular sources present with vastly differing components of ECM, with such differences potentially yielding ECM of varying functionality.

Methods: Herein, we describe the development of ECM-derived biomaterials, with a specific focus on decellularization optimization of tissue and cellular monolayers, hydrogel formulation, and in vitro characterization, to assess their potential for tissue regeneration and cellular applications. We followed a systematic approach to develop ECM-based biomaterials comparatively evaluating chemical, enzymatic, and physical decellularization methods to maximize cellular removal while preserving ECM bioactivity. Following this, we characterised the composition, mechanical properties and ultrastructural integrity of tissue and cellular monolayer-derived ECM. Subsequently, we developed a hydrogel system that incorporated biomaterials with enhanced crosslinking, bioactivity, and ECM integration, assessing gelation behaviour, rheological properties, and swelling dynamics to ensure stability and controlled degradation. Finally, we performed in vitro cytocompatibility and functional assessment, employing live/dead and MTT assays to evaluate cell viability and proliferation within the ECM-integrated hydrogel system. We also performed structural characterization using SEM and fluorescence imaging to confirm ECM integrity and cellular interactions.

Results and Discussion: Our preliminary findings demonstrated a successful decellularization of cell-derived and tissue-based ECM, with histological/cytological and biochemical analyses confirming effective nuclear removal and ECM preservation. Our hydrogel system exhibited strong thermosensitive properties, minimal cytotoxicity, and high transparency, making it suitable for microscopy-based applications and cell culture integration. Subsequent studies will focus on functional validation in complex cellular models, and will further investigate hydrogel-ECM interactions, cellular differentiation potential, and in vivo biocompatibility.

Conclusions: Herein, we present an innovative ECM-integrated hydrogel system designed to support cellular functions and promote tissue regeneration. By refining decellularization methodologies and biomaterial formulations, Our research lays the groundwork for developing biocompatible scaffolds with translational potential in regenerative medicine. Such studies provide significant grounds for further applications in suitability of such ECM-biomaterials for organoid culture, tissue repair, and bioengineered microenvironments.

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Regulatory and Safety Challenges for Natural Biomaterials in Bioprinting and Tissue Engineering for Clinical Applications

Introduction
Natural biomaterials have opened promising avenues in bioprinting and tissue engineering by providing native-like environments for regenerative therapies. These materials offer significant biological advantages but also introduce challenges related to variability, immunogenicity, and contamination risks [1]. The regulatory framework is gradually evolving to address these concerns, but remains insufficient, particularly for decellularized extracellular matrix (dECM) from animal sources. The lack of standardized regulations and acceptance criteria for dECM hinders the clinical translation of dECM-based bioinks and related constructs.
Safety criteria
Effective decellularization is commonly defined as <5 ng DNA/mg dry weight, DNA fragments <200 bp, and absence of visible nuclear material [2]. Ensuring cell removal and biocompatibility is critical for dECM safety, as it prevents immune responses. ISO 10993 provides comprehensive biological evaluation guidelines for medical devices. Safety criteria also include the removal of microbial and viral contaminants, immunogenic epitopes, and residual chemicals. Sterility validation should follow Ph.Eur. 2.6.1 and ISO 11737, requiring a sterility assurance level (SAL) of 10^{-6}. Endotoxin testing must comply with Ph.Eur. 2.6.14 and USP <85>, with a limit of <0.5 EU/mL. ISO 22442 guides sourcing and evaluation of animal-derived materials to mitigate zoonotic risks. Xenogeneic scaffolds must also exclude species-specific antigens (e.g. α-gal), although standardized detection and removal criteria remain undefined. Chemicals, especially detergents, are widely used in decellularization protocols, and their residues must be minimized, yet specific thresholds are lacking. Recent data indicate that Triton X-100 concentrations in influenza vaccines are considered acceptable below 0.05% v/v [3].
Quality, functionality and consistency
The dECM should be evaluated for the preservation of key structural and functional ECM components (e.g. collagen, elastin, GAGs, and growth factors). An often overlooked parameter in dECM characterization is lipid content, which can impair hydrogel formulation. We have defined <4% as the acceptable fat concentration. Functional assessment should also include mechanical testing and in vitro bioactivity assays. Variability in tissue sourcing and processing can impact outcomes, making standardized protocols essential. Batch consistency, reproducibility and regulatory compliance can be supported by GMP practices and ISO 13485, ASTM (F3354-19) standards. From a clinical perspective, implanted dECM-based products should be assigned to Class III medical devices or ATMPs, depending on their composition and function.
Discussion
Despite advances in natural biomaterials preparation and evaluation, regulatory clarity remains limited. Current practices often rely on widely cited but non-binding criteria. DNA thresholds, although frequently referenced, are not officially standardized, and some studies report their exceedance in commercial dECM products with favorable clinical outcomes [4,5]. The use of detergents, particularly Triton X-100 - classified by ECHA as a Substance of Very High Concern due to its environmental impact - highlights the need for safer, more sustainable alternatives. Detergent-free approaches offer potential but often exhibit reduced efficacy. Source materials variability and species-specific risks further hinder standardization. Establishing unified, tissue-specific standards encompassing safety, quality and batch consistency is essential. Moreover, integrating sustainable processing methods and validated quality benchmarks will support regulatory alignment and facilitate clinical translation of dECM-based products.
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Region specific bioprinting of microtissues for meniscus tissue engineering via dual localized growth factor delivery

The meniscus plays a critical role in load transmission, shock absorption, and joint stability within the knee. However, its limited regenerative capacity, particularly in the inner avascular zone, contributes to the progression of degenerative joint diseases following injury. The complex zonal organization of the meniscus—characterized by a fibrous outer zone rich in type I collagen and a cartilaginous inner zone rich in type II collagen—presents a significant challenge for tissue engineering strategies aiming to recapitulate biomechanical function and structure. While previous efforts have leveraged scaffold-based and hydrogel systems to promote fibrocartilaginous differentiation of mesenchymal stromal cells (MSCs), these approaches often fail to mimic the region-specific architecture and biochemistry of the native meniscus.
To address this challenge, we present a refined bioprinting platform that integrates the spatial precision of microtissue printing with localized growth factor delivery. Building upon our previous work with extrusion-based printing of MSC-derived microtissues into methacrylated xanthan gum (XG-MA) support baths, we now introduce a dual-cartridge printing system capable of producing meniscus-shaped grafts with region-specific phenotype. Specifically, we encapsulate connective tissue growth factor (CTGF) or transforming growth factor-β3 (TGF-β3) into PLGA microparticles and embed them within microtissue-laden bioinks for the outer and inner meniscal zones, respectively. These microparticles provide sustained, localized release of either pro-fibrogenic (CTGF-loaded microparticles) or pro-chondrogenic factors (TGF-β3-loaded microparticles), thereby recapitulating the biochemical gradients observed in vivo.
Initial experiments demonstrated the capacity of soluble CTGF and TGF-β3 to guide MSC microtissue fusion and region-specific matrix deposition in fused microtissues grafts . Subsequently, we incorporated the respective growth factor-loaded microparticles into sacrificial gelatin bioinks and assessed their influence on cell phenotype, extracellular

matrix production, and collagen alignment in fused constructs. Finally, dual-cartilage printing was employed to fabricate anatomically relevant, zonally organized meniscal grafts.
This work highlights the potential of combining modular microtissue printing with region-specific growth factor delivery to fabricate biomimetic, scalable meniscal constructs. This bioprinting platform enables the engineering of complex fibrocartilaginous tissues and offers a new foundation for regenerating heterogeneous tissues with localized growth factor delivery.

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The Embryonic Envelope Reimagined: Biofabrication of a Decellularized Chorionic Scaffold for Urinary Bladder Tissue Engineering

Decellularized human chorionic membrane (HCM) offers a promising biomaterial for tissue engineering due to its rich extracellular matrix (ECM), inherent biocompatibility, and accessibility. This study compares the efficacy of different decellularization methods in generating acellular HCM scaffolds while preserving structural and functional ECM integrity. Cellular removal and ECM preservation were evaluated through histological staining (H&E, Masson's Trichrome, Alcian Blue, and DAPI), alongside DNA/RNA quantification. Scanning electron microscopy (SEM) revealed a porous, fibrous ultrastructure conducive to cellular attachment. Mechanical testing showed that the SDS-treated HCM retained tensile strength and elasticity compatible with soft tissue applications. Swelling behavior and degradation rate analysis further demonstrated the scaffold's functional stability. Biocompatibility was validated by low hemolysis percentage (<1%), high cell viability in MTT assays, and positive outcomes from in ovo CAM assays. Preliminary in vivo studies confirmed minimal immune response, good integration, and early neovascularization. Immunohistochemical staining and gene expression analysis further indicated early signs of tissue regeneration. These results suggest that SDS-decellularized HCM is a structurally intact, biologically active scaffold with significant potential in urinary bladder tissue engineering.

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Tailoring Biomaterial Ink Properties with Nanomaterials: POSS and Polydopamine for Next-Generation Bone Tissue Scaffolds

Introduction Significant efforts have concentrated on creating various synthetic and natural biomaterials that mimic the native bone extracellular matrix, promote osteogenic differentiation, and improve effective bone regeneration. 3D printing has become a widely used fabrication method for scaffolds, facilitating the accurate mapping of the 3D structure of bone defects [1]. In this research, we aim to develop a multifunctional ink for 3D printing by combining the biological support of methacrylated gelatin (GelMA), the printability and structural reinforcement of κ-carrageenan, and the bioactive enhancements of polyhedral oligomeric silsesquioxane (POSS) and polydopamine (PDA), creating a composite system that integrates mechanical strength, osteoinductivity, and cellular compatibility [2] [3] [4].
Methods: Hydrogels composed of GelMA or dopamine-grafted GelMA (GelMA-Dopa), κ-carrageenan, POSS, and PDA were initially synthesized to evaluate their physicochemical characteristics, with the most promising formulation selected for subsequent 3D printing. The resulting freeze-dried scaffolds were structurally characterized using FTIR Spectroscopy, their rehydration capacity was assessed, and their morphology was examined via SEM. Additionally, compressive strength tests were performed, and biocompatibility was evaluated to determine their suitability for bone tissue engineering applications.
Results: FTIR Spectroscopy and 1H-NMR analyses confirmed the successful synthesis of GelMA-Dopa, while the formation of PDA was verified by FTIR. Furthermore, AFM and DLS analyses provided insights into the morphology and size distribution of the PDA particles. The incorporation of POSS and PDA significantly influenced the scaffold properties, leading to a reduced swelling degree and enhanced compressive strength. 3D printing experiments demonstrated favorable printability and high shape fidelity, highlighting the potential of these formulations for bone tissue engineering applications.
Conclusion: This research illustrates a multifunctional ink designed for bone tissue engineering, using GelMA, κ-carrageenan, POSS, and PDA to attain an equilibrium among biocompatibility, mechanical strength, and printability. The synthesis and characterization of the hydrogel components validated the structural integrity and functionalization of the materials, whereas the integration of POSS and PDA significantly improved scaffold performance by enhancing compressive resistance and diminishing swelling behavior. Furthermore, 3D printing evaluations validated the materials' capacity to generate constructions with exceptional print quality and structural integrity.
Acknowledgements: A. Dinu acknowledges the funding received from Romanian Ministry of Education and Research and National University of Science and Technology Politehnica Bucharest.
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Rheological characterization of alginate bioinks for application in corneal implant bioprinting

Corneal disorders, including trauma, infections, and degenerative diseases, are among the major causes of blindness. Due to the global shortage of donor corneas and risks associated with transplantation, there is a growing interest in developing bioprinted corneal implants as an alternative therapeutic approach.
The aim of this study was to evaluate an alginate-based bioink. In the first stage of the study, a hydrogel with defined rheological parameters (shear viscosity, storage modulus, yield stress) was used to fabricate a substrate using a conventional extrusion-based method. Despite the optimization of the bioink (shear viscosity, storage modulus, yield stress), the quality of the print with flat geometry was low (Fig. 1a).
In the second stage, the FRESH (Freeform Reversible Embedding of Suspended Hydrogels) method was applied. Pluronic (Fluka) with the addition of 1-3% $CaCl_2$ was used as a support medium, serving as a crosslinking agent for the alginate-based bioink. Three-dimensional structures were fabricated using the FRESH method on a BioCloner Desktop Pro printer. Both simple 2D test substrates (Fig. 1b) and complex structures mimicking corneal geometry were successfully printed. Optimized printing parameters included: extrusion pressure 0,2 MPa number of layers 23, and printing temperature (27 °C).
The selected rheological properties of both the bioink (e.g., viscosity, shear stress) and the support medium (e.g., storage modulus) enabled the bioink to remain in a gel-like state for the required duration (<1 min). The proposed printing conditions allowed the formation of a hydrogel-based substrate with the desired shape and satisfactory print quality (Fig. 1c).
The results indicate the success of the optimization process and provide a solid foundation for further research aimed at developing a fully functional corneal implant.

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Simplified 3D model for invasion testing: complexified interactions between osteosarcoma cells and bone-mimicking PLGA/20TCP scaffolds

Introduction
Osteosarcoma (OS) is a rare and aggressive primary bone tumor affecting children and adolescents. Traditional 2D models fail to replicate the complexity of bone–tumor interactions.
Aim: to develop a hybrid 3D osteosarcoma model replicating interactions between healthy and malignant bone tissues.
Methods
Healthy bone-mimicking scaffolds were fabricated using TCP-enriched (20 wt%) PLGA and PLCL via precise extrusion deposition with a 0/90 fiber orientation and filament shift in the n+2 layer. Scaffolds were characterized for material properties (AFM, SEM, µCT) and degradation (Mw). Scaffolds seeded with hFOB, HUVECs, and hBMSCs in a 1:1:1 tri-culture were evaluated for ALP and CD31 expression via spectrophotometry and immunofluorescence, along with osteocalcin and YAP/TAZ expression. A dual-crosslinked (DC) hydrogel (5% GelMA, G5-DC) was formed via physical (5°C, 1h) and visible light (13s) crosslinking and analyzed for rheological properties, porosity, and biological activity (ALP in hBMSCs). MG63 spheroids (2×10^4 cells) were embedded in G5-DC hydrogels and co-cultured with bone-mimicking scaffolds. The integrated BTM was studied for cell invasion, cytokine production (IL-6, IL-10 (ELISA)), and cytotoxicity (LDH).

Results
PLGA/20TCP scaffolds were chosen for their superior mechanical properties and ability to support bone tissue formation. With a compressive modulus of ~3.70 GPa compared to ~0.64 GPa for PLCL/20TCP, PLGA/20TCP provided the stiffness necessary for osteogenesis. These scaffolds demonstrated enhanced cell adhesion and osteogenic differentiation, shown by increased ALP activity (~120 µM pNP/ng DNA by day 14) and osteocalcin and YAP/TAZ expression alongside robust CD31 expression by day 21. Dual crosslinking strategy produced an osteoid-like stiffness of ~17 kPa. MG63 spheroids were incorporated into G5-DC hydrogels and co-cultured with bone-mimicking scaffolds, forming the integrated BTM. Fluorescence microscopy revealed local cell invasion only into the osteoid-like matrix in the BTM-tumor model (control, spheroids embedded into G5-DC without a seeded scaffold). However, in the full BTM with osteogenically differentiated PLGA/20TCP scaffolds, metastatic spread was inhibited, with IL-6 levels reduced from ~1.6×10³ pg/mL (day 3) to ~0.5×10³ pg/mL (day 32) and IL-10 levels gradually inclining after day 6, a trend in opposite to the BTM-tumor. This suggests that bone-mimicking scaffold plays a crucial role in creating a less permissive environment for tumor progression.
Discussion
Our findings highlight the utility of BTM as a platform for studying osteosarcoma dynamics and testing therapeutic strategies. We are currently evaluating bone-specific markers to gain deeper insights into the mechanisms underlying the lack of tumor invasion when the bone-mimicking scaffold is introduced.
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Reproducible in vitro vascularization via high throughput molding of sacrificial scaffolds

Introduction:
Replicating physiologically relevant tissue environments in vitro requires both sophisticated biological design and reliable scalable fabrication. Current approaches often rely on complex biofabrication methods such as stereolithography (SLA) and melt electrowriting (MEW), which are time-intensive, laborious, and lack reproducibility at scale [1,2]. To overcome these limitations, we developed a vascularized in vitro platform that integrates microchannel networks with industrial manufacturing methods, enabling reproducibility, scalability, and ease of use.

Methods:
The platform incorporates sacrificial template structures embedded within a biocompatible matrix. The dissolution of the template unveils a fully interconnected, perfusable microchannel network within the matrix for seeding with endothelial cells (ECs). This vascular network supports dynamic perfusion from the onset of cell culture, promoting long-term cell viability and tissue development. We transitioned from SLA and MEW to injection molding and vacuum casting, respectively to facilitate large-scale production. Injection molding was used to fabricate precise housing and structural components, while vacuum casting enabled flexible production of complex sacrificial template geometries using biocompatible materials.

Results:
The manufactured platforms demonstrated high reproducibility in microchannel geometry and structural fidelity across batches. Perfusable vascular networks were successfully formed following template dissolution, allowing for continuous media perfusion throughout the culture period. In preliminary cultures, embedded endothelial cells exhibited sustained viability and functionality throughout the entire culture period as confirmed by immunofluorescence staining and permeability assay, respectively. The perfusion system has the potential to enable controlled delivery of compounds, providing a platform for future physiologically relevant drug testing scenarios. Preliminary throughput assessments showed a significant reduction in production time and cost compared to SLA/MEW-based methods, with consistent device quality and usability.

Discussion:
Transitioning to industrial-grade manufacturing significantly improves the scalability of our in vitro system via enhanced reproducibility and reduced costs without compromising biological performance. The integration of injection molding and vacuum casting enables the creation of a ready-to-use, vascularized platform compatible with standard lab workflows. This approach addresses a critical bottleneck in tissue model development by aligning complex biological function with manufacturability. Future work will expand platform adaptability to other tissue types and perfusion modalities, further enhancing its value for pharmaceutical and academic research.

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Towards volumetric scaffold fabrication for regenerative medicine using robotic additive manufacturing

Introduction
Robotic additive manufacturing (RAM) is currently being explored to overcome the limitations of layer-by-layer additive manufacturing technologies in the fabrication of complex constructs for regenerative medicine (RM). A few successful attempts at using RAM for in-situ extrusion-based bioprinting have been reported, where the fabrication is non-planar but still layer-by-layer1,2,3. Alternatively, light based volumetric bioprinting has increasingly been adopted for the generation of complex biological constructs, but it is limited by the narrow selection of biocompatible bioinks and inability to recapitulate anisotropic fiber orientations. Meanwhile, efficient methods to generate volumetric print paths/designs with anisotropic 3D geometries for RAM remains elusive, reducing the effectiveness of RAM for RM. Here, we explore the use of a simple continuous fiber scaffold designs which better utilize the volumetric printing capabilities of RAM as a strategy to fabricate 3D scaffolds for RM.

Methods
Designs
3D space filling curves could be used for efficiently generating volumetric scaffold geometries. However, these designs are impossible to be printed in small scales using extrusion-based 3D printers. Even when using RAM they require the robot to achieve complex orientations and current hardware limitations of industrial robots prevent the printing of these structures. Some simplified versions of space filling curves were chosen for the study.(Hilbert curve and Z-order (Morton) curve). A custom path optimization software was used to calculate the extruder orientation, thus preventing collisions while minimizing changes in orientation of the extruder during the printing. After testing the printability of these designs, larger scaffolds were generated by stacking the repeating units of the curves.

RAM
The RAM system used here couples a 7 degree of freedom robot (Xarm7) with a thermoplastic extruder. The integration of the robot and extruder was handled by an updated version of RAVEN4, which is an open-source RAM package developed in house based on ROS2 (Robot Operating System).

Printing tests
All the printing tests were done using polylactic acid (PLA) on a 0.4mm nozzle at a printing temperature of 180°C. After checking the paths for collisions (in simulation), the effect of print-speed, extruder orientation, cooling fan speed and segment length was studied by printing single unit-cells of each design. The best parameters were used for printing the larger scaffolds using the RAM and a 3-axis printer for comparison.
Results and Discussions
By replacing the fundamental units from layers to complex curves, we are able to achieve extrusion-based volumetric scaffolds while keeping the design process relatively simple. This approach opens new exciting opportunities for RM in terms of biomimicry and advanced devices for stimuli-responsive applications. Results from this study could become the steppingstone for future studies in fabrication of scaffolds with more complex architectures for RM using RAM.

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- S02 3D complex architectures from stem cells Mikołajska

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Tropoelastin as a driver of 3D complex architectures and cell performance

In every species of mammal, bird and reptile, and across almost the entire vertebrate world, the skin, lung, arteries and other tissues require elasticity to function. What bestows this elasticity is the protein elastin, which in turn is assembled from the structural protein building-block tropoelastin. We have found that tropoelastin can promote the repair many types of damaged tissues; and identified collaboratively that tropoelastin can allow stem cells to delay senescence, while retaining phenotype and function. Despite these critical abilities, and even though the tropoelastin gene is essential, paradoxically the production of tropoelastin drops precipitously with age. This presentation will present advances in our mechanistic understanding in the use of tropoelastin to deliver organised elastin in vascular walls during replacement-repair in vivo, promote heart muscle survival and recovery in an in vivo model of ischemic injury, and its ability to compress the inflammatory sequence displayed by macrophages. Collaboratively, we are seeing responses by mesenchymal stromal cells that extend to expression differences accompanied by delayed senescence that fundamentally reflect the role of the elastic extracellular matrix. Also collaboratively, we see that blended methacrylated tropoelastin can be used to rapidly seal interfaces with complex shapes, including damaged lung and heart. In vivo and model studies will be presented that show the value of using tropoelastin to modify cell performance in these cases, in order to help heal surgical wounds, repair arteries and modulate immunity while delivering synthetic tissue-materials for building 3D constructs.

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Engineering Topological and Dynamic Bioinks for Microvascularization

3D bioprinting has emerged as a transformative biotechnology in tissue and organ engineering. While substantial progress has been achieved in fabricating vascular networks with vessel diameters exceeding ~100 μm - a scale primarily constrained by current bioprinting resolution - the engineering of functional microvasculature remains critical for enabling efficient mass transport within local tissue microenvironments. A persistent challenge lies in developing bioinks capable of simultaneously supporting microvascular formation while preserving essential 3D printability and structural fidelity. To address this critical need, we introduce a novel microfiber-templated porogel (μFTP) bioink that enables microvascular engineering down to the building blocks of 3D biopritning. These cell-laden microfibers serve dual functions: acting as sacrificial porogens to create interconnected tubular microchannels while simultaneously delivering endothelial cells for in situ endothelialization. The bioink matrix was further engineered with mechanoresponsive dynamics to facilitate endothelial morphogenesis through the synthesis of a gelatin-based dual-network hydrogel combining a hydrazone-crosslinked dynamic network with a methacrylate-stabilized static framework. Through systematic optimization of bioink formulation and printing parameters, we demonstrate enhanced vascularization outcomes in both in vitro models and in vivo implantation studies. Our work addresses the persistent challenge of tissue microvascularization within bioprinted constructs, eliminating dependence on post-printing cell seeding. The integration of anisotropic hydrogel microstructures (e.g., microfibers) with dynamically tunable matrix properties permits in situ vascular induction - a crucial advancement for engineering clinically relevant tissue volumes.

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Volumetric bioprinting of bi-chamber heart structures using dynamic hydrogels enhances iPSC-derived cardiomyocyte function for in vitro cardiac modeling

Introduction: Cardiovascular disease remains the leading cause of mortality worldwide, with limited success in translating new therapies to the clinic. Existing in vitro models—such as 2D cultures and engineered heart tissues—struggle to recapitulate the complex cell-cell and cell-microenvironment interactions, as well as the structural and functional hallmarks of cardiac pathology within anatomically relevant geometries. To address these limitations, we present a novel in vitro cardiac model (VoluHeart) fabricated using high-speed, contactless volumetric bioprinting (VBP) [1]. This approach enables the rapid generation of scalable, hollow heart-like structures composed of human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs).[2] VoluHeart integrates a newly developed hybrid bioresin capable of forming covalent and supramolecular interactions,[3] resulting in printable hydrogels with enhanced viscoelasticity and structural stability. This dynamic matrix promotes cell-cell interactions and supports network formation. To validate the physiological relevance of VoluHeart, we established a myocardial infarction model via cryoinjury and assessed tissue remodeling using experimental and clinical readouts including histology, beating rate, calcium handling, and RNA sequencing.

Methods: VoluHearts comprising a co-culture of iPSC-derived cardiomyocytes (iPSC-CMs) and cardiac fibroblasts (10:1 ratio, 10x106 cells/mL) were fabricated using VBP within a hybrid gelatin-based resin. This material enables dual crosslinking: covalent bonding via methacryloyl groups under light exposure, and supramolecular assembly through cyclodextrin–adamantane host-guest interactions. iPSC-CM viability and network formation within the hybrid resin was compared to conventional gelatin methacryloyl (GelMA) hydrogels using live/dead assays and immunofluorescence. Functional characterization of VoluHearts included immunofluorescence staining for cardiac (cardiac troponin T, α-actinin) and fibroblast (αSMA) markers, beating rate, and calcium handling analysis. To assess disease modeling capabilities, cryoinjury was applied to the apex of the construct, and post-injury changes were analyzed using the aforementioned readouts along with RNA sequencing.

Results and Discussion: Bi-chambered VoluHearts (~165mm³, printing time=21s) showed high shape fidelity to the original STL file (Figure 1A). VBP's design flexibility enabled asymmetric chambered models and a 4-chambered heart replica (Figure 1B). iPSC-CMs showed >75% viability and superior network formation compared to GelMA over a 2-week culture period in the hybrid bioresin (Figure 1C,D). Within one week, VoluHearts exhibited spontaneous contractions and directional calcium waves. Immunofluorescence imaging confirmed the alignment of iPSC-CM networks along the septum and chamber walls. To model myocardial injury, cryoinjury was applied to the apex of the heart, resulting in localized disruption of calcium signaling. RNA sequencing of injured vs. control samples revealed upregulation of genes associated with fibrotic remodeling and de novo ECM deposition, mirroring clinical patterns observed post-myocardial infarction.
Conclusion: VoluHeart represents a novel and versatile in vitro cardiac model that combines volumetric bioprinting, dynamic biomaterials, and human iPSC-derived cardiomyocytes to recreate anatomically relevant heart structures. Its scalability, compatibility with experimental and clinical readouts, and responsiveness to injury highlight its potential as a platform for disease modeling, high-throughput drug screening, and personalized therapeutic development.

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Preclinical Application of Vascularized 3D Liver Cancer Organoids: Case Study with Kinase Inhibitors and Melatonin

INTRODUCTION: Recent research on cancer therapy has increasingly focused on melatonin as a potential antitumor agent, particularly in liver cancer. Its mechanisms include modulation of apoptotic pathways, reduction of proinflammatory markers, and circadian rhythm regulation - all potentially relevant to inhibiting liver carcinogenesis. Alongside drug development, advancements in tissue models - especially 3D organoids - provide innovative platforms for testing new therapies. Liver-derived organoids can replicate key features of the tumor microenvironment, including cancer cell heterogeneity, stromal components, and vascular characteristics. Integrating melatonin into such models offers a promising path for precision oncology. Therefore, this study aimed to develop vascularized 3D constructs (organoids and tissue model) and evaluate the impact of kinase inhibitors and melatonin on liver cancer cell viability in 2D and 3D culture.

MATERIALS&METHODS: The study was conducted on liver organoids printed using the ink-jet method with bioink composed of GELMA, HAMA, and dECM, as well as HepG2, HDF-a, and HUVEC-GFP cells. To assess the level of secreted metabolites and cell viability, medium samples were collected on days 1, 3, 7, 10, and 14 of the experiment. On day 14, the 3D experimental model was treated with a combination of 4 μM Vemurafenib, 4 μM Cobimetinib, and 5 mM Melatonin to evaluate its effect on the

functional properties of tumor cells in the organoid-based system. At 24, 48, and 72 hours after drug administration take another samples of medium. The collected material was used for LDH assay and AFP level measurement. Additionally, at selected time points, microscopic observations and FDA/PI staining were performed. After the experiment was completed, the material was fixed in formalin for immunohistochemical staining.

RESULTS: During the 14-day incubation period (prior to drug administration), the organoids maintained high viability, exceeding 80%. Around day 10, microtubular structures formed by endothelial cells were observed within the organoids. The organoids exhibited a stable architecture, which enabled their application in more complex tissue constructs incorporating a vascular system (bioprinted tissues). Following the administration of the melatonin and kinase inhibitor mixture, the level of secreted AFP decreased by approximately 80%. This was confirmed by an 82% reduction in the number of viable cells.

DISCUSSION: In summary, the development of advanced tissue models – particularly liver cancer organoids – is an innovative way to evaluate new cancer therapies in vitro. These models provide a comprehensive platform for testing the multifactorial anticancer mechanisms of melatonin, enabling detailed analysis of its effects on cancer cell apoptosis, proliferation, and microenvironment dynamics. The combination of melatonin-based therapy with patient-specific organoid models holds great promise for personalized medicine approaches in liver cancer, offering solid opportunities for preclinical evaluation and clinical translation.
Importantly, the use of melatonin and kinase inhibitors in this study serves as a proof of concept, demonstrating the practical applicability of our biofabricated 3D model. The organoid system itself represents a universal and reliable tool that can be adapted for preclinical testing of various therapeutic strategies beyond the agents tested here. This underscores its potential value in drug development pipelines and precision oncology research.

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Patterned Lumen Enhance Endothelialization of Cell-Laden 3D Bioprinted Vascular Grafts from Human Induced Pluripotent Stem Cells

Introduction
Cardiovascular diseases, responsible for 17.9 million fatalities annually, are the leading cause of death worldwide. Congenital heart disease (CHD) is one of the causes of chronic CVD, which is the most common cause of congenital pathologies and the most common congenital malformation, affecting almost 1% of all live births. The standard treatment of CHD approach often involves graft transplantation, which utilizes autologous or synthetic vascular grafts to bypass and replace diseased vascular segments [1]. However, these options are not ideal for pediatric patients due to their limited ability to grow and adapt alongside the child's developing cardiovascular system. Pediatric patients face additional challenges with these grafts, as they may require multiple surgeries to replace grafts that fail to remodel or expand adequately with the growing body. Synthetic conduits and allogeneic graft replacements have disadvantages such as lack of ability to grow, repair or remodel, and are potentially thrombogenic [2]. This limitation has led to significant interest in tissue-engineered vascular grafts, which offer promising advantages. Unlike synthetic grafts, tissue-engineered options can more accurately replicate the natural structure and properties of blood vessels, including the necessary biological cues for cell attachment, migration, and function. These properties enable the graft to adapt and grow, potentially eliminating the need for multiple surgical interventions [1]. Induced Pluripotent Stem Cells (iPSCs) can solve this challenge as they can be derived from a person's own cells and differentiated into blood vessel cell types.

Materials and Methods
This study presents a new method for vascular grafts made from elastin-like recombinamers (ELR) [3] and gelatin crosslinked by microbial transglutaminase (mTG). Using extrusion 3D bioprinting and casting of developed elastic cell-laden bioink, we constructed three-layered iPSC vascular grafts that included layers of cardiac fibroblasts (CF), smooth muscle cells (SMC), and endothelial cells (EC). We used a combination of techniques—laser-interference lithography, nanoimprint and soft lithography—to achieve patterned lumen of the graft. We seeded iPSC-EC cells inside the graft and exposed them to fluid. Finally, we used confocal microscopy to assess EC alignment and distribution within the graft.

Results and Discussion
We developed ELR-gelatin composite that mimick elasticity of blood vessels. We optimized 3D bioprinting of iPSC-cardiac fibroblasts (CF)-laden tubes and effective casting of SMC-ELR-based hydrogel, crosslinked using mTG. We successfully fabricated the outer and middle layer of the vascular graft with long-term viability of the embedded CF and SMC (Fig 1B). We achieved patterned vessel lumen with dimensions from 300 nm to 8 µm (Fig 1F).
These cues allow for luminal surface patterning resulting in enhanced adhesion, spreading and enable to maintain the monolayer of iPSC-EC under high-flow perfusion (Fig 2E). We confirm the impact of patterns on the increased attachment and spreading of EC along the luminal graft surface (Fig 2F) compared to non-patterned lumen (Fig 2G) under perfusion.

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Bioprinting of Hypoimmune Induced Pluripotent Stem Cell-Derived Pancreatic Islets for Ameliorating Diabetes

Pancreatic islet transplantation holds significant potential for treating insulin-dependent diabetes, but its clinical utility remains limited by donor scarcity, immune rejection, and the need for lifelong immunosuppression. To overcome these challenges, stem cell-derived islets, particularly those differentiated from human induced pluripotent stem cells (iPSCs) engineered for hypoimmunogenicity through CRISPR-Cas9-mediated gene editing, have emerged as a promising alternative source. However, despite their immune-evasive properties, SC-derived islets often exhibit incomplete maturation compared to primary islets, highlighting the need for additional engineering strategies to ensure functional efficacy in vivo. Here, we present a bioengineered islet platform termed hypoimmune iPSC-derived bioprinted islet-like cellular aggregates (HIBICA), which integrates gene-edited hypoimmune iPSC-derived islets with a tissue-specific biochemical niche and a scalable fabrication strategy to generate transplant-ready islet constructs. To promote functional maturation in vitro, we employed pancreatic tissue-derived extracellular matrix (pdECM) as a physiologically relevant microenvironment. Although human-derived pdECM effectively supports insulin-secreting-β cell identity, its limited availability poses challenges for translational scalability. Therefore, we evaluated porcine-derived pdECM as an alternative, and through comparative proteomic profiling and gene ontology analysis, confirmed its compositional similarity to the human-derived pdECM. Notably, porcine-derived pdECM demonstrated significant glucoregulatory support compared to collagen type I, providing a functionally enriched matrix that effectively facilitated the maturation of hypoimmune iPSC-derived islets to levels comparable with those supported by human-derived pdECM. To address limitations in conventional islet aggregate formation methods, such as suspension culture utilizing orbital shakers or spinner flasks, which require at least 24 hours for aggregate formation and often result in heterogeneous aggregate size and unintended clustering that can lead to hypoxic conditions within larger aggregates, we applied embedded 3D bioprinting to rapidly and precisely generate islet-like aggregates within the pdECM bioink. This approach enabled high-throughput fabrication of uniformly sized islet-like aggregates, achieving the production of over one aggregate per second with consistent architecture. The embedded HIBICA maintain their endocrine phenotype, showing stable expression of key hormonal markers (e.g., insulin, glucagon, and somatostatin) along with sustained expression of immune checkpoint markers. Following transplantation into NSG mice, HIBICA outperformed both non-edited SC-islet grafts and hypoimmune islets transplanted without matrix support, exhibiting sustained in vivo insulin secretion and improved graft stability. These findings demonstrate the synergistic effect of immune engineering and biochemical niche optimization coupled with geometrical guidance in enabling long-term graft function without immunosuppression. In conclusion, HIBICA offers a comprehensive platform for advancing SC-derived islet transplantation by combining hypoimmune genetic modifications, tissue-specific matrix support, and scalable fabrication. Furthermore, this platform opens opportunities for allogeneic, off-the-shelf islet therapies, and may serve as a foundation for future combinatorial approaches involving hypoimmune vascularization and integration with microencapsulation systems, expanding therapeutic flexibility in regenerative diabetes care.

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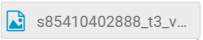
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The role of dynamic, chemical and geometrical mechano-transduction in brain organoid development and advance maturation: A spatio-temporal perspective

Introduction. Mechano-transduction is the process by which cells sense and respond to mechanical stimuli from their environment, converting mechanical signals into biochemical signals that can influence cellular behavior, gene expression, and development [1]. In brain organoids, this mechanical cues are crucial for mimicking developmental processes, from embryonic development to neural stem cell differentiation, spatio-temporal radial glial cell polarization, tissue morphogenesis, homeostasis and the formation of complex brain structures. Thus, understanding of mechano-transduction in unguided and quided brain organoid generation is key for advancing their relevance and accuracy in research [2,3].
Methods. We systematically evaluated the influence of mechano-transduction dictated by geometrical confinement on the development of unguided cerebral organoids. This assessment involved the comparison of the Aggrewell800 microwell plate with the conventional 96-well ultra-low attachment (ULA) plate, aiming to understand the impact of microwell geometry on organoid morphology, growth patterns, and cellular enrichment processes (Fig 1a). Furthermore, we conducted an in-depth investigation into the combined effects of geometrical, dynamic, and chemical cues on the generation of dopaminergic midbrain organoids. This study utilized the Aggrewell800 microwell plate to assess the influence of microwell geometry, while also incorporating orbital shaking to introduce dynamic shear stress. Additionally, we explored the impact of dual-SMAD inhibition as a chemical cue, aiming to elucidate how these factors collectively contribute to the cellular differentiation and development (Fig 1b).
Results. Cerebral organoids derived from the Aggrewell800 platform demonstrated reduced variability in embryoid body formation, with organoid sizes averaging 4–5 mm by day 90. These organoids exhibited more prominent neural rosettes, expanded neuroepithelium, and well-defined cortical plate layers. The basal-apical organization of the cortical plate was evident with enhanced expression of SATB2, CTIP2, and PAX6 markers. Additionally, IBA1+ microglia were significantly improved, reflecting enhanced cellular patterning. On the other hand, both regional OTX2+ progenitors and FOXG1-/TH+ mature midbrain identities were observed, alongside an enrichment of neural and glial cells with a well-defined morphological pattern, indicating enhanced cellular diversity with the combined application of geometrical, chemical, and dynamic cues.
Discussion. Mechano-transduction plays a pivotal role in modulating the development, organization, and functional maturation of brain organoids through key processes such as cellular differentiation and morphogenesis, cortical layer formation, neuronal connectivity, glial and microglial function, and the recapitulation of pathophysiological conditions. By integrating mechanical cues in conjunction with chemical and dynamic signaling factors, it becomes possible to more faithfully replicate the complexity of human brain development and disease in vitro. This integrated approach offers substantial promise for advancing our understanding of brain biology, while also providing a more robust framework for the creation of precise models for drug discovery and disease modeling.

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- S03 4D bioprinting Goethe

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From 3D Printing to Cyborg Heart Tissues: Redefining Tissue Engineering

The field of cardiac tissue engineering is advancing rapidly toward the creation of functional, patient-specific therapies and whole-organ replacement strategies. In this talk, I will present recent progress in developing personalized biomaterials and leveraging state-of-the-art additive manufacturing technologies, including 3D and 4D printing, to fabricate vascularized cardiac tissues and entire hearts. A particular focus will be on a novel 4D printing methodology that enables the generation of cardiac patches with hierarchical vascular networks, spanning from blood vessels as large as 300 µm in diameter down to capillary-scale features. This innovation represents a critical step forward in achieving physiologically relevant perfusion within engineered tissues by overcoming prior limitations in printing resolution. Finally, I will highlight an emerging paradigm in tissue engineering: the integration of micro- and nanoelectronics into living constructs. By merging bioelectronics with engineered tissues, we are creating hybrid “cyborg” tissues and bionic organs with enhanced functionality, paving the way for new therapeutic strategies in regenerative medicine and biohybrid systems.

Konuşmacılar: Tal Dvir (Tel Aviv University Center for Nanoscience & Nanotechnology)

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High-Resolution 3D Printing using fs Lasers – Opportunities for 4D Bioprinting

The most widespread 3D bioprinting technologies are based on computer-controlled deposition of cells or assembly of cellular units, and thus cannot achieve spatial resolution better than few tens of micrometers. Lithography-based methods approach the problem from a different direction, by producing 3D structures within cell-containing materials and can therefore overcome this limitation. Among these methods, high-resolution 3D printing employing nonlinear absorption of femtosecond laser radiation, also referred to as multi-photon lithography, is an outstanding one as it can produce features even smaller than a single mammalian cell.
Numerous publications already demonstrated that MPL can be used to produce stimuli-responsive structures from different materials, including hydrogels. In this talk, the recent progress of MPL for 4D printing applications, as well as its advantages and limitations, along with the perspectives for 4D bioprinting will be discussed. The presentation is supported by numerous examples.

Konuşmacılar: Aleksandr Ovsianikov (TU Wien)

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Xolography for biofabrication: Modulating mechanical properties of hydrogels in 3D

Introduction
Tissues and organs are composed of cells embedded in an instructive 3D extracellular matrix (ECM). Changes in mechanical properties of the ECM act as dynamic cues that guide cells through different stages of development. In synthetic 2D culture systems, matrix stiffness, viscoelasticity, and their variation have been shown to influence cell spreading and differentiation by modulating intercellular traction forces.[1,2] Similar effects have been observed in 3D models [3], but our understanding of how mechanical signals are processed at subcellular level and through specific signaling pathways remains limited. Studying these mechanisms requires synthetic 3D culture systems with precise control over mechanical stimuli - something current technologies struggle to provide.[4] To address this, we introduce the adaption of Xolography volumetric 3D printing into a grayscaled bioprinting process.[5] Xolography employs intersecting light of two different wavelengths and a dual-color photoswitch-photoinitiator to locally crosslink a viscous photoresin. We hypothesize that this method will enable the fabrication of hydrogels with spatially defined mechanical properties like stiffness, offering new opportunities to study 3D mechanotransduction.

Methods
Xolography was used to fabricate hydrogel constructs based on gelatin methacryloyl (GelMA), polyethylene glycol diacrylate (PEGDA) and N-isopropyl acrylamide (NIPAAm). Local reduction of the applied energy dose was implemented by applying intermediate intensity values (i.e. grayscaling) to the visible light projection, one of the two light sources in Xolography. Mechanical properties of printed constructs were assessed by compression testing, nanoindentation, and atomic force microscopy (AFM)-based force spectroscopy. The degree of crosslinking was evaluated with Fourier-transformed infrared spectroscopy (FTIR) and tested as a mechanism to drive reversible, anisotropic shape-changes in simple thermoresponsive hydrogel geometries.

Results
For a hydrogel based on PEGDA and a given set of printing parameters, projections with full exposure of 242 mW cm^{-2} led to constructs with stiffness of 400 ± 100 kPa on macroscale while 50 % decrease of the visible light dose resulted in a lower stiffness of 33 kPa. The same trend was observed on milli-scale: indentation measurements demonstrated a highly significant decrease in Hertzian Young’s modulus from 2.10 ± 0.30 kPa to 0.23 ± 0.03 kPa by decreasing the visible light dose by 30 %. A fivefold decrease of the modulus was observed on microscale within a scan area of 5 µm x 5 µm.
Xolography based on NIPAAm and GelMA allowed printing of shrinkable 4D geometries. Thermo-induced shrinking was found to be reversible to similar extents when compared to cast hydrogels. Anisotropic shrinking based on heterogeneous crosslinking was achieved through grayscaling in hydrogel beams.

Discussion
The capability of grayscaled Xolography to modulate the degree of crosslinking and subsequently stiffness was demonstrated. The effect was confirmed on multiple lengths scales and successfully employed to alter shrinkage spatially. Modulation of local mechanical properties can now be employed to create cell-instructive matrices and eventually unravel 3D mechanotransduction.

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4D-Printed Protein-Driven Smart Hydrogel Actuators for Autonomous Catch-and-Release in Biofabrication and Drug Delivery

4D bioprinting enables the fabrication of dynamic, adaptive materials capable of autonomous shape transformations in response to environmental cues. While most hydrogel-based actuators rely on external control, developing self-regulating soft robotic systems that operate autonomously in physiological conditions remains a significant challenge. Here, we present 4D-printed protein-driven actuators capable of autonomous "Catch and Release" functions in response to gastric fluid conditions (pH ~2, pepsin 0.5–2 mg/ml). Composed of bovine serum albumin (BSA) and polyethylene glycol diacrylate (PEGDA, MW 700), these hydrogel actuators exhibit time-dependent transformations, enabling sequential gripping and payload release without external stimulation.
The hybrid BSA-PEGDA bioink was synthesized via an aza-Michael addition reaction, crosslinked with digital light processing (DLP) 3D printing to achieve tunable microarchitectures and mechanical properties. In simulated gastric conditions, the actuator initially swells as BSA unfolds, allowing it to securely grip its payload. Over time, pepsin-mediated degradation reduces stiffness, enabling a controlled release.
To demonstrate gastric drug delivery potential, doxorubicin (DOX) was incorporated within the hydrogel matrix via non-covalent interactions, ensuring precise, stimuli-driven release. Beyond drug delivery, this system paves the way for biohybrid robotic actuators, autonomous medical grippers, and dynamically responsive biofabricated systems for biomedical applications.
This work advances 4D bioprinting principles by integrating protein-driven autonomous actuation with biofabricated soft robotic functions, offering new directions for autonomous, stimuli-responsive biomaterials in medical devices and biohybrid robotics.

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Development of a photodegradable bioink to recreate multiscale tissue architectures for improved organoid culture

Introduction
Polymer-based hydrogels serve as excellent mimics of the extracellular matrix, enabling the generation of 3D in vitro tissue models.1 To improve the ability of these models to replicate tissue in vivo, there is great interest in enhancing model complexity.2 Towards this goal, the utilization of photoresponsive chemistries (e.g. polymerization/degradation) permits precise user-defined control over hydrogel properties to match tissue structure in vivo. Furthermore, the synergy between photoresponsive chemistries and biofabrication platforms (e.g. 3D extrusion bioprinting, photolithography) permits tissue models to achieve clinically relevant size scales.3 Unlike photopolymerizable bioinks, the generation of photodegradable bioinks has remained cost prohibitive and synthetically challenging, ultimately limiting the achievable complexity of in vitro tissue models. Here, we discuss our use of photodegradable chemistries to probe mechanisms involved in organoid development. We then present an inexpensive and easily accessible photodegradable bioink based on radical induced thiol-maleimide cleavage4 that enables photodegradation of large, cell-laden constructs to generate clinically relevant tissue constructs.

Methods
Intestinal organoids were encapsulated in photoresponsive hydrogels containing either allyl sulfide or nitrobenzyl ether functionalized polymers. A laser scanning confocal microscope was used to apply patterned light (405nm) to organoid-laden hydrogels. Live imaging and immunohistochemistry were used to evaluate organoid behavior following hydrogel photopatterning. Gelatin maleimide (GelMal) was synthesized by reacting a maleimide functionalized small molecule with gelatin using EDC/NHS chemistry. GelMal hydrogels were formed with varying concentrations of a multifunctional thiol. Shear rheology (1 Hz, 1 rad/s) was used to assess hydrogel mechanical properties following thiol gelation, and during photodegradation (400-500 nm, 30 mW cm-2). Centimetre-scale GelMal hydrogel constructs were then formed using an extrusion bioprinter and photodegraded using bulk irradiation.

Results
Bulk and spatially defined irradiation of organoid-laden hydrogels revealed that organoid development is dependent on hydrogel stiffness and epithelial shape, providing variables to direct organoid form and function. With the desire to apply photodegradation reactions to large-scale organoid constructs, we sought to develop a scalable photodegradable bioink. The addition of a multifunctional thiol crosslinker to GelMal induced the formation of thiol-maleimide crosslinks, where hydrogel mechanical properties were tuned using the thiol crosslinker concentration. Following GelMal hydrogel formation, a photoinitiator was added to generate radicals upon exposure to UV light, which cleaved thiol-maleimide crosslinks to facilitate controlled photodegradation. Photoshear rheology revealed rapid GelMal degradation in response to light exposure that was dependent on photoinitiator concentration and light dose. Centimeter-scale constructs were fabricated using extrusion bioprinting and fully degraded within minutes, highlighting the opportunity to utilize photodegradation reactions and biofabrication techniques to generate large-scale tissue architectures.

Discussion
We highlight the importance of photodegradation as a method to explore complex biological mechanisms and introduce the need for scalable photodegradable platforms to enhance the physiological relevance of tissue models.

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Chemical-induced jamming of granular hydrogels as responsive bioinks

Introduction
The development of smart materials for bioprinting unlocks flexibility and control over the final construct characteristics and composition.1 Dynamic crosslinking allows the development of stimuli-responsive inks with intrinsic reversibility triggered by tunable physical and chemical conditions.2 Typically made of densely packed or jammed microgels, granular hydrogels offer unique properties, compared to bulk hydrogels, making them attractive for biofabrication. In the jammed state, the physical interactions between particles result in rheological behaviors (e.g., yield stress, shear thinning behavior) that contribute to their extrudability and shape fidelity post-printing. While microgel jamming is normally done through centrifugation, sedimentation, and vacuum filtration, in this study, we propose a chemical approach through reversible covalent bonds (Fig 1A). We focus on introducing stimuli responsiveness (volume transitions) into individual microgels, by the selective cleavage of disulfide bonds (Fig 1B) and consequently improve the rheological properties and printability of the granular hydrogel-based inks.

Methods
Specifically, we synthesized poly(N-isopropylacrylamide) (pNiPAM) based microgels by surfactant-free radical polymerization and introduced the cleavable dynamic covalent disulfide bonds within the microgels' network, with the crosslinker N,N′-bis(acryloyl)cystamine (BAC). The granular hydrogel inks were prepared by directly dispersing the microgels in 1x-PBS. The reductant agent tris-(2-carboxyethyl)phosphine hydrochloride (TCEP) was used to cleave the disulfide bonds, swell the microgels and consequently jam them (Fig 1C). The inks, before and after swelling/jamming, were characterized by DLS, Wet-AFM, Cryo-TEM, and rheology. A BioScaffolder 3.3 (Gesim) was used to print square-mesh scaffolds up to 32 layers, and shape fidelity was investigated. The obtained 3D printed scaffolds were immersed in a sodium periodate ($NaIO_4$) solution to render back the disulfide bonds and crosslink the 3D printed constructs (Fig 1C). The obtained dimensionally stable scaffolds were used in biocompatibility tests with primary fibroblasts.

Results
pNiPAM microgels were prepared with an average diameter of 818 ± 25 nm at 20°C, and the successful incorporation of the disulfide units in the microgels network was proved by proton NMR and UV-Vis spectroscopy. The addition of TCEP effectively led to microgel swelling by cleavage of the disulfide bonds, which efficiently increases the

yield-stress values of the microgel dispersions (Fig 1D). Moreover, inks that initially exhibited poor shape fidelity after 3D printing, were transformed into stable, multi-layered 3D structures capable of supporting their own weight, up to 32 layers, after reduction (Fig 1E). Post-printing annealing was done by immersion in a NaIO4 solution, to form intra and inter-particle disulfide bonds and obtain a dimensionally stable scaffold in physiological conditions. Its lack of toxicity prospers its use for tissue engineering purposes.

Conclusions
We successfully jammed disulfide crosslinked pNiPAM granular hydrogel systems which printability was tuned, by the weight fraction of particles and by the extent of swelling of the particles. Reversible covalent bonds allowed particle swelling triggered by the addition of reductant, and maintenance of the construct's stability by the immersion in an oxidant solution.

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4D printing of human vascularized cardiac tissues with selective fast-shrinking capillaries

Introduction
3D tissue printing has advanced significantly and can now create controlled vascular networks in engineered tissues for effective oxygen and nutrient transfer. However, a major challenge with cell-containing bioink hydrogels is their limited printing resolution, which affects the creation of small-scale features like capillaries. Here, we present a novel selective-shrinking-living bioink that facilitates the successful 4D printing of functional human cardiac tissue featuring a hierarchical vascular network that includes small-scale capillaries.

Methods
The fabrication of human vascularized cardiac tissue with selectively shrinking capillaries is achieved through a multi-material 4D bioprinting approach that integrates three distinct living bioinks within a supportive matrix. This strategy enables the construction of functional cardiac tissue embedded with an intrinsic, hierarchical microvascular network. The process involves:
1. Endothelial bioink to define the lumens of the microvasculature
2. Selective-shrinking bioink to induce the rapid contraction of capillary-scale structures
3. Cardiac bioink to form the surrounding myocardial tissue

Following printing, the construct is incubated under physiological conditions, triggering a programmed, time-dependent response in each ink. The endothelial bioink first softens and is gently removed, leaving behind adhered endothelial cells. Next, the selective-shrinking bioink contracts in a controlled manner, forming capillary-like vessels. Ultimately, cellular self-organization completes the maturation of a functional, perfusable cardiac tissue with a hierarchically structured vascular network.

Results and Discussion
As a result of the selective-shrinking-living bioink unique 4D behavior, we have demonstrated the first successful printing of a cell-lined capillary-sized blood vessel. To reach this milestone, we have developed a one-step coordinated multi-kinetic 4D printing technique, in which multiple stimuli-responsive bio-inks are printed and activated according to a kinetically controlled sequence. Proper fabrication was achieved by rationally designing the selective-shrinking-living bioink, and the sequential triggering of the living-bioinks. Additionally, by exploiting the ease with which multiple living-bioinks can be deployed in extrusion-based 3D bioprinting, localization of the novel, shrinking-living bioink was demonstrated, resulting in selective shrinkage and capillary formation only in those specific locations, to create a functional and perfusable human cardiac patch with optimal microvasculature that was well anastomized with the host post transplantation. The use of one-step coordinated multi-kinetic 4D printing combined with the novel selective-shrinking-living bioink constitutes a versatile platform that can be used to advance the field of tissue engineering and regenerative medicine, to allow the generation of small-scale tissue units at their correct size.
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Mechanical and biochemical tunability of a photo-editable biomaterial for volumetric bioprinting

Introduction :
Volumetric bioprinting (VBP), enables the fabrication of complex cell laden architectures at high printing speeds in a layer-less fashion. Recently, this technology was refined to precisely pattern (bio)active molecules inside (bio)printed constructs post-fabrication, introducing possibilities for 4D printing. [1] A key challenge in biofabrication lies in replicating the time-dependent biochemical and mechanical changes occurring in the extracellular matrix during tissue maturation or disease progression. Therefore, bioresins for VBP, able to replicate these changes over time, are needed. In this study, a dual-functionalized tyramine and norbornene gelatin (GelTN) is developed for spatiotemporally controlled stiffening and photo-grafting of thiolated compounds within the printed structures. The dual wavelength bioresin is photo-crosslinked via green light-mediated di-tyrosine bond formation, leaving the norbornenes available for post-printing modifications via blue light-mediated thiol-ene click chemistry, allowing precise control over the crosslinked hydrogel's properties.
Materials and Methods :
In VBP, GelTN with photoinitiator system tris(2,2-bipyridyl)ruthenium(II)chloride/sodium persulfate was crosslinked projecting green light (~520nm), tomographic filtered back-projections of the object to be printed. Before photo-grafting, the constructs were infused with thiolated compounds - dithiothreitol (DTT) to induce matrix stiffening, or SH-PEG5000-Cy3, a fluorescent compound, to assess 3D photografting - and a photoinitiator (lithium phenyl-2,4,6-trimethylbenzoyl-phosphinate). An additional volumetric printing step using blue-light (at 405nm) enables a controlled stiffening or patterning of the preformed 3D hydrogel. The stiffening of GelTN gels was assessed through compression testing and the SH-PEG5000-Cy3 photografted gels were imaged via lightsheet microscopy to evaluate grafting accuracy and intensity. Finally, human mesenchymal stromal cells (hMSCs) were encapsulated in the gels (1.5x106 cells/mL) and their viability, metabolic activity, and morphology were compared pre and post photografting.
Results :
VBP-printed 3D constructs were fabricated using green light in less than 30 seconds. A 2-fold increase in stiffness was observed in gels grafted with DTT compared to non-grafted gels and the ability to fine tune the stiffening degree of the gel was further evaluated by varying the thiol-to-norbornene ratios. Furthermore, SH-PEG5000-Cy3 was successfully photografted into complex 3D architectures, enabling the creation of constructs with intricate mechanical and biochemical patterns. Finally, while hMSCs's morphology showed rounder cells in stiffened gels, no significant differences in viability were found between non-grafted (90.5±3.5%) and grafted samples (85.0±5.1%) after a week of culture, confirming the hydrogel's platform biocompatibility.
Discussion :
Utilizing the developed two-wavelength hydrogel, we demonstrated the ability to perform both VBP and volumetric photografting using bio-orthogonal thiol-ene chemistry, distinct photoinitiating systems and light sources. The ability to selectively stiffen gels by grafting different thiol-to-norbornene ratios allows the possibility of controlling the gel's mechanical properties over time. This platform also enables the selective functionalization of the gel with any free thiol-containing compound (e.g. bioactive proteins, growth factors). This control over the gel's properties over time and space allows for studying specific cells' behavior as showed by embedding hMSCs. The combination of the dual wavelengths bioresin and photo-grafting technology shows promise for the development of complex platforms mimicking the dynamic nature of native tissues and organs.
Discussion :
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- S06 Advancing Biofabrication for Regenerative Medicine: From Innovation to Clinical Translation — Kisielewski

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First use of UV-light activated biomaterial in atraumatic hernia repair in patients

Introduction
Surgical mesh implantation represents the current standard of care in inguinal hernia repair; however, conventional fixation using sutures is associated with risks of chronic neuralgia-type pain and local tissue irritation. Alternative fixation methods employing fibrin or cyanoacrylate-based adhesives have been introduced, but each presents inherent limitations in terms of mechanical stability, biocompatibility, and long-term performance. Thus, a novel UV-curable, biodegradable adhesive resin (PhotoBioCure) [1,2] has been developed for soft tissue repair. It was employed for the fixation of a mesh in inguinal hernia repair to reduce tissue trauma and increase the accuracy of a mesh fixation. Following intraoperative placement, the mesh was secured using the adhesive instead of tacks or sutures.

Methods
The first procedure using PhotoBioCure was performed in Poland, at Clinical Hospital in Police (2022-2024). It was employed for the mesh fixation in inguinal hernia repair in patients (n=5) to collect safety and effectiveness data. The mesh was secured using the UV-light activated adhesive instead of tacks or sutures. Patients were monitored over a 24-months follow-up period with comprehensive clinical evaluations, including ultrasonography, thermal imaging, and standardized assessments focusing on whether new biomaterial can reduce postoperative pain and trauma compared to traditional fixation methods: Visual Analog Scale (VAS) for pain, Carolina Comfort Scale (CCS), European Registry for Abdominal Wall Hernias Quality of Life (EuraHS QoL), and the SF-36 Health Survey.

Results
Application of a new biodegradable material does not provoke adverse tissue reactions or impair in wound healing, making it a promising alternative to conventional absorbable sutures for hernia mesh fixation. It significantly reduced operative time relative to conventional suture fixation. Postoperative pain scores (VAS) rapidly declined, with complete resolution of pain by 6 weeks. A progressive reduction in foreign body sensation decreased in 30% at 6 weeks. Throughout the 24-month surveillance, no cases of hernia recurrence, hematoma, infection, or other complications were observed. Patient-reported health-related quality of life (SF-36) improved up to 92.4 at 24 months postoperatively.

Conclusions
PhotoBioCure is an innovative, UV-light activated biomaterial which demonstrated effectiveness for atraumatic surgical mesh fixation in inguinal hernia repair. Its use is associated with reduced operative duration, simplified surgical technique, and favorable postoperative outcomes, without an increased incidence of adverse events.

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Enhancing Osteoarthritis Therapy through RNA Delivery and AI Diagnostics: A Comprehensive Strategy for Early Detection and Targeted Treatment

Introduction
Osteoarthritis (OA) is a debilitating joint disease characterized by the progressive degradation of cartilage, leading to pain, stiffness, and limited mobility. Early detection and effective treatment are critical to improving patient outcomes. In this study, we introduce a combined method that uses a new way to deliver RNA with Silk Fibroin (SF) hydrogels and artificial intelligence (AI) tools to improve the treatment and diagnosis of OA.1 SF was chosen as the ideal biomaterial for RNA delivery due to its superior biocompatibility, biodegradability, and its ability to mimic the extracellular matrix (ECM) of native cartilage. These properties make SF highly effective for controlled RNA release and stabilization, essential for tissue regeneration in OA. 2

Methods
SF-G hydrogel was prepared using the silk solution obtained from Bombyx mori 3 , and crosslinked via mushroom tyrosinase. The interaction between the 28S rRNA from Rattus norvegicus and the N-terminal of SF.3 An AI-driven diagnostic system aims to improve the early detection and grading of OA using knee MRI datasets. By training deep learning models, including ResNet50, DenseNet121, and VGG16, we focused on identifying key features of OA, such as osteophytes, cartilage thinning, bone marrow lesions (BMLs), and eburnation. 4 We introduced a new grading system for OA based on the severity of eburnation, which was validated by expert radiologists from the Apollo Hospital Delhi, India.

Results
The SF-G hydrogel matrix was designed to encapsulate RNA, which provided a stable environment for RNA-based therapies targeting cartilage regeneration. Molecular modeling studies demonstrated that SF interacts strongly with various RNA species—such as ribosomal RNA, transfer RNA, and messenger RNA—outperforming other biomaterials like collagen and chitosan. This finding was corroborated by physical characterization techniques validating SF's potential to support RNA stability and enhance cartilage repair. Concurrently, the AI model demonstrated high diagnostic accuracy, providing a fast, objective, and reliable method for assessing OA progression, minimizing human error, and improving clinical decision-making.

Discussion
We developed SF hydrogel-based RNA delivery with AI diagnostic tools to create a method for managing OA. The RNA delivery system provides a promising therapeutic strategy for cartilage regeneration, while the AI diagnostic tools ensure early and precise diagnosis.
Our AI-based model can classify OA severity from MRI data, enabling early and precise grading. It is particularly useful for advanced OA, such as Grade 3, where RUNX2 is upregulated and SOX9 is dysregulated. These RNA therapeutics will be delivered via an SF-G hydrogel, designed for localized and sustained release. This would normalize gene expression, restore chondrogenic activity, and promote cartilage regeneration in a grade-specific manner.

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Hybrid Bioprinted Dressings for Deep Wound Repair: Harnessing Methacrylated Hyaluronic Acid and Recombinant Proteins for Enhanced Healing in a Porcine Model

Introduction:. An innovative approach combining pre-crosslinked methacrylated hyaluronic acid (HAPrime) with hybrid recombinant proteins was developed to create highly effective dressings for deep wound treatment. Treating deep wounds in animals, particularly pigs, remains a significant challenge due to risk of infection and delayed tissue regeneration. Emerging regenerative strategies, such as bioprinting wound dressings with bioactive components, offer promising solutions. Methacrylated hyaluronic acid (HAMA), a key component of the extracellular matrix, plays a crucial role in wound healing by supporting angiogenesis, tissue regeneration, and reducing inflammation. The bioprinting of HAMA-based dressings enables precise structural tailoring to the wound site, potentially enhancing the healing process. While this study was conducted in a porcine model due to its close resemblance to human skin, the developed strategy holds strong translational potential for future clinical applications in the treatment of complex or chronic wounds in humans.
This study evaluates the therapeutic efficacy of bioprinted HAMA-based dressings with recombinant protein in promoting deep wound healing in pigs, compared to traditional treatment methods.
Methods: The study involved experiments on a group of pigs, to whom bioprinted dressings made of hybrid materials (HAPrime and recombinant protein) were applied on wounds, compared with traditional dressings. Wound healing was evaluated by measuring wound closure and contraction, supported by histological and immunohistochemical analyses of tissue regeneration and inflammation. Gene expression profiling of inflammatory markers was performed, alongside assessment of the dressings antibacterial activity and monitoring of blood biochemical and morphological parameters over the course of the experiment.
Results: Preliminary results suggest that bioprinted dressings may effectively accelerate wound healing and enhance tissue regeneration compared to traditional treatment methods. The obtained outcomes clearly confirmed the utility of the developed approach—treated wounds demonstrated high healing potential, with no observable exudate

and overall maintenance of a clean and stable wound environment. Additionally, the use of bioprinting technology enabled precise dressing conformation to wound geometry, ensuring personalized fit and optimal tissue contact. This likely contributed to improved healing dynamics and supports the clinical relevance of the strategy.
Discussion: The application of bioprinted dressings based on methacrylated hyaluronic acid and recombinant protein demonstrates potential for enhancing the healing of deep wounds in pigs. Improvements in wound closure rates, tissue architecture, and control of infection suggest that this approach could outperform traditional wound care methods. Further investigation will be needed to confirm the long-term benefits and to optimize the material properties for broader clinical application.
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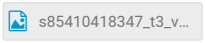
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Biofabricated auricular cartilage grafts for clinical translation

Introduction: Tissue engineering has the potential to revolutionize the treatment of microtia, the congenital malformation of the external ear. Patient-specific auricular grafts could be engineered from a small biopsy of approximately 5 mg, eliminating the need for rib cartilage, and provide better aesthetics through biofabrication techniques, resulting in a real, natural ear. However, despite multiple decades of research (Vacanti mouse: 1997), no tissue engineered auricular graft has made it to the market yet. Key challenges that remain are the uneven maturation of auricular grafts, the development of fibrocartilage as opposed to elastic cartilage and graft stability in vivo. To overcome these challenges, we developed techniques to mature auricular grafts into elastic cartilage matching the histological and biomechanical characteristics of human auricular (elastic) cartilage (Fig.1a) evenly distributed throughout the graft. Finally, we assessed their structural stability by implanting these grafts in a subcutaneous rat model.
Methods: Primary human auricular chondrocytes were obtained from cartilage remnants of otoplasty procedures from patients in the same age range as microtia patients. Auricular grafts were bioprinted using a hyaluronan-transglutaminase (HATG)-alginate (Alg) bioink combined with 30 × 106 auricular chondrocytes/mL utilizing a novel progressive cavity pump-based extrusion system (Puredyne Kit B). After 9 weeks of maturation in vitro, grafts were implanted subcutaneously by creating an incision along the dorsal midline and pocket via blunt dissection in immunocompromised rats. Auricular grafts were implanted and the incision closed with staples. After 6 weeks in vivo, grafts were explanted and analyzed histologically (elastin, collagen II, collagen I, glycosaminoglycans), mechanically and for their ECM composition (elastin, collagen II, collagen I, glycosaminoglycans).
Results: Auricular grafts developed into elastic cartilage showing the deposition of elastin, GAGs and collagen II, while collagen I, a marker for fibrocartilage, was absent. Biomechanically, grafts reached properties of native human auricular cartilage (hAUR) with an instantaneous modulus of 1053 ± 27 kPa (hAUR: 1023 ± 101 kPa). After implantation, the wounds healed well and the animals did not show any signs of pain or discomfort and moved freely, bending the grafts. At explantation, grafts did not show signs of deformation and maintained their structural integrity. Grafts were further able to maintain their biomechanical and histological properties, albeit peripheral changes in the extracellular matrix composition could be observed (Fig.1b).
Discussion: Our results represent the closest approximation to native elastic cartilage reported to date. The ability to tune tissue maturation away from fibrocartilage toward elastic cartilage substantially improved graft stability and in vivo performance. By developing functional auricular cartilage, we anticipate that these grafts will lay the foundation for a clinically viable, long-term treatment for children affected by microtia.

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Towards Clinical Translation: Treatment of Volumetric Muscle Loss with Mechanically Reinforced Engineered Tissues

Introduction
Volumetric muscle loss due to trauma often exceeds the body’s intrinsic regenerative capacity. The gold standard of treatment with autologous transplants carries the significant drawbacks of donor site morbidity and limited availability. Although current tissue-engineered products offer potential, they require improvements in terms of shape fidelity, surgical handling, transport, and functionality to become viable alternatives for clinical use. To address these issues, we designed and 3D-printed a scaffold to enhance translation of biofabricated muscle tissue to the clinic.
Materials and Methods
We designed and printed a polycaprolactone scaffold using fused filament fabrication at 130 °C, with strand diameters ranging from 0.25 to 0.15 mm and a layer height of 0.2 mm. The scaffold is a fine grid featuring rhomboid pores angled at 58°. The grids were then filled with a bioink consisting of the C2C12 myoblast cell line and a collagen-matrigel hydrogel by using the drop-on-demand nozzle of the same printer or by pipetting by hand.
Results and Discussion
FEM simulations optimized the scaffold for elastic deformations up to 15%, which was shown to improve myofiber size alignment and force generation when used during maturation of the tissue. In addition, it is the expected deformation that the tissue will experience when implanted in the body allowing for use after implantation. This elastic elongation was confirmed by cyclic stretching tests.
The scaffold’s anisotropy (horizontal stiffness of 1579 kPa vs. 77 kPa vertical) supports cell alignment and myotube formation. The elastic modulus of the scaffold could be tuned by adjusting strand diameter without affecting pore size. By fine tuning the strand diameter of 3D-printed scaffold the stiffness of the whole structure can be tailored and used as a platform for many different tissues, for example the stiffness of 5 MPa can be used to for tissue engineering of cartilage and tendons, as it is their natural stiffness. The influence of the deformation on the hydrogel’s stiffness was investigated. The scaffold was then filled with a bioink consisting of a hydrogel and myoblasts and its suitability for implantation determined. The integrated scaffold significantly increased shape retention by 400% during cell differentiation into myofibers and improved suture retention. Finally, we showed a scaffold FFF-printing process in combination with a drop on demand bioprinting of the production of patient specific cell laden hydrogel in a single custom-made printer enabling easy handling and the inclusion of printed vasculature. The cells show high post printing viability and good morphology.
Conclusions
This study presents a scaffold that solves a major issue with clinical translation where it’s said TE muscle constructs for VML fail to 33% due to mechanical disruption during surgical implantation. The scaffold in this study is able to be sutured and withstand cyclic loading in vivo. The printing system used is versatile as well as easy to use in a clinical setting as it combines multiple necessary functions. The biofabrication approach enables the fabrication of large patient individualized constructs that demonstrate a good cell viability and shape retention.

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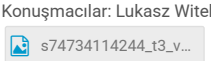
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Advancing Bone Regeneration: 3D Printed β-TCP Implants via LCM in Preclinical Evaluation

Clinical application of beta-tricalcium phosphate (β-TCP) has been limited by lack of bone infiltration within its bulk form. Lithography-based ceramic manufacturing (LCM), a novel additive manufacturing (AM) technique, leverages photopolymerization to create β-TCP structures with higher feature resolution and surface quality than traditional techniques. This modality allows for a more efficient and precise means to control implant micro and macroarchitecture, enabling the production of novel implant configurations. This pilot study explores the bone regenerative capacity of lithography-based ceramic manufactured 100% β-TCP scaffolds for repair of critically-sized mandibular defects in a skeletally mature rabbit model. Quantitative and qualitative analysis of regenerated bone were performed using micro-computer tomography (micro-CT) and two-dimensional histologic analysis, respectively. Three-dimensional volumetric reconstruction revealed bridging bone in sites treated with β-TCP implants, yielding ~10±3.5% of regenerated bone within the construct and ~33±3.2% remaining scaffold volume. Bone regeneration and remaining scaffold quantification were corroborated using traditional two-dimensional histological micrographs and three-dimensional volumetric analysis ($p<0.05$). Qualitative histologic analysis revealed vascularized woven and lamellar bone, with no evidence of ectopic bone, excess inflammation, or fracture. Bone regeneration in this short-term rabbit model following critical-sized mandibular defect repaired with LCM β –TCP scaffolds demonstrated analogous radiographic and histologic properties to native bone.

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  3D BioPrinting: Organ-On-Chips models for drug screening applied to diabetic foot ulcer

  Type 1 Diabetes Mellitus (T1DM) is an autoimmune condition resulting in the destruction of insulin-producing beta cells, leading to chronic hyperglycemia and accumulation of advanced glycation end products (AGEs). These factors contribute to serious complications such as diabetic foot ulcers (DFUs) (1-2), which result from chronic inflammation, vascular damage, and neuropathy. Despite their impact, diabetic skin alterations remain underexplored. Improved understanding of these mechanisms could aid in identifying key pathways underlying DFUs and in developing targeted therapies (3).
  In this study, a 3D in vitro model of diabetic skin was developed to replicate key features of the disease, and to develop a dynamic in vitro model of diabetic skin that recapitulates biological and mechanical features of the pathology. As a preliminary step, human immortalized keratinocytes (HaCaT) and human foreskin fibroblasts (HFF-1) were cultured under normoglycemic (NG, 25 mM glucose) and hyperglycemic (HG, 50 mM glucose) conditions in both 2D and 3D settings. Fibroblasts were embedded in gelatin methacryloyl (GelMA) hydrogels formulated either in with NG or HG medium, with results indicating a loss in metabolic activity under hyperglycemic conditions. Subsequently, full-thickness 3D skin models were fabricated. Fibroblasts were encapsulated in normo- or hyper-glycemic GelMA and seeded onto PET inserts, following by UV photopolymerization of the dermal layer. Subsequently, keratinocytes were seeded on top of the dermal layer. After 3 days of submerged culture, constructs were exposed to air-liquid interface (ALI) conditions for 28 days to promote epidermal differentiation. Morphological and molecular analyses were performed via immunofluorescence staining and droplet digital PCR (ddPCR).
  Immunofluorescence staining revealed reduced epidermal thickness and morphological changes in keratinocytes and fibroblast under hyperglycemia, consistent with features observed in diabetic skin (Figure 1-2, Table 1) . Gene expression analysis (Figure 3) showed decreased COL1A1 expression, indicative of impaired extracellular matrix remodeling, while COL3A1 expression was less affected. Increased IL8 levels in hyperglycemic conditions reflected the diabetic inflammatory profile. Moreover, elevated CDKN1A expression indicated enhanced cellular senescence, hallmark of diabetic skin aging. Finally, VEGF expression was reduced under hyperglycemic conditions, mirroring diabetic impaired angiogenesis and delayed wound healing.
  These results validate the engineered model's ability to replicate critical features of diabetic skin, providing a promising tool for studying diabetic skin pathophysiology and screening treatments.
  Future work will introduce standardized wounds using a custom-designed and 3D printed wounding device, enabling the assessment of healing responses under untreated and treated conditions using therapies such as insulin and metformin. To increase physiological relevance, hypodermal and vascular layers will be integrated, along with macrophages to simulate chronic inflammation. This will enable the creation of a robust and translationally relevant diabetic foot ulcer model for drug testing and disease research.

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  Next-Generation Hemodialysis Membranes: Composites Modified with Nanoparticles for Improved Performance

  Introduction
  The performance of hemodialysis membranes depends on uremic toxin clearance, biocompatibility, mechanical properties, and the ultrafiltration characteristics of the hemodialyzer [1]. The clearance rate can be controlled by membrane properties, such as thickness, morphology, and pore size, as well as the surface properties of the membrane [2].
  Methods
  Novel hemodialysis membranes were fabricated using the phase inversion technique, based on cellulose acetate (CA) and polylactic acid (PLA), and further modified with hydrophilic polymers (PVP, PEG) as well as metallic nanoparticles, including copper, platinum, and selenium. These nano-enhanced materials were comprehensively characterised in terms of their physicochemical and mechanical properties, as well as their biocompatibility. In addition, toxin removal efficiency and membrane flux were also evaluated.
  Results and Discussion
  This work highlights the potential of incorporating metallic nanoparticles into CA/PLA membranes to tailor critical properties for hemodialysis applications. The presence of nanomaterials improves the hydrophilicity and elasticity of membranes while maintaining biocompatibility and high clearance efficiency. Additionally, nano-enhanced membranes exhibit antibacterial activity, which may reduce the risk of potential bloodstream infections—a major cause of death and hospitalisation among hemodialysis (HD) patients [3].
  The findings offer a promising pathway toward next-generation dialysis membranes with enhanced durability, mechanical stability, biocompatibility, and resistance to protein fouling.
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- S08a Biofabrication for cartilage regeneration and joint preservation Ratuszowa

  Toplantıyı düzenleyenler: Jos Malda (UMC Utrecht, NL), Nikita Norkin (EPFL)

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  Biofabrication Strategies for Synovial Joint Regeneration

  Articular cartilage is a specialized hyaline cartilage that covers the epiphyseal surfaces of bones within synovial joints. It functions to reduce friction and distribute mechanical loads over the joint surface. Damage to the tissue leads to increased stress, inflammation, pain and progressive joint degeneration, potentially resulting in the development of osteoarthritis due to the tissue's limited capacity for self-repair. This has led to increased interest in novel biofabrication and bioprinting strategies to develop scaffolds or living tissues capable of regenerating damaged synovial joints. This invited talk will describe two distinct biofabrication strategies for synovial joint regeneration. The first part of the talk will describe how decellularized extracellular matrix (ECM) derived from distinct musculoskeletal tissues can be processed into multi-layered scaffolds capable of directing osteochondral defect regeneration. The second part of the talk will introduce the concept of microtissues as biological building blocks for the engineering of replacement tissue and organs, and then describe how the spatial patterning of growth factors into baths of microtissues can be used to engineer osteochondral grafts.

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  Articular cartilage progenitor cell derived microtissues generate functional articular cartilage when seeded onto decellularized extracellular matrix derived scaffolds

  Osteoarthritis is a progressive inflammatory disease characterized by articular cartilage (AC) degeneration, affecting millions globally. Recapitulating AC's arcade-like collagen structure is key to engineering functional grafts. AC progenitor cells (ACPs) have a unique ability to maintain a stable hyaline phenotype. ACP derived microtissues can potentially be used as biological building blocks for the biofabrication of scaled-up grafts as they support cell-cell and cell-extracellular matrix (ECM) interactions. Here we first explored a temporal BMP-9 stimulation to generate hyaline cartilage microtissues using ACPs and evaluated their fusion and (re)modelling. We then used decellularized AC ECM, known to be chondro-inductive [1], as a scaffold to support ACP microtissues as they self-organise forming a functional osteochondral graft.

  ACP microtissues (1,000 or 2,000 cells/microtissue) were produced using a high throughput microwell mould [2]. After 2 days of maturation, 2,000, 4,000 or 6,000 microtissues were fused in a 6mm cylindrical mould and cultured for 6 weeks (Fig. 1A). We then evaluated the effect of temporal stimulation with BMP-9, with or without TGF-β3 and dexamethasone, for 2 days during microtissue formation, before fusing 2,000 microtissues and culturing for 4 weeks in chondrogenic media without BMP-9 (Fig. 1B). Finally, 4,000 microtissues (1,000 cells/microtissue) were fused for 4 hours in a 5mm cylindrical agarose mould before adding the ECM scaffold. On day 3, the scaffold was flipped and cultured for 4 weeks (Fig. 1C). Chondrogenesis was assessed through histology, immunohistochemistry, and biochemical assays.

ACP microtissues rapidly fused and supported robust chondrogenesis in all conditions as evident by the positive staining for sulphated glycosaminoglycan (sGAG) and collagen (Fig 1A). In the 6000 microtissues group, some necrosis was evident in the centre of the construct. Temporal stimulation of ACP microtissues with BMP-9 induced volumetric expansion during fusion, with an increase in sGAG and collagen synthesis while maintaining a hyaline-like phenotype with an intense collagen type II stain (Fig 1B). Microtissues that were allowed to fuse on the surface of the AC ECM scaffolds formed a white smooth and homogeneous layer of tissue that stained positive for sGAG and collagen. Polarized light microscopy revealed an arcade-like collagen organization with horizontal fibres on the surface and vertical fibres in the middle/deep zone (Fig. 1C).

ACP microtissues exhibited robust fusion and chondrogenesis, with radial confinement in the 6mm cylindrical mould promoting biomimetic collagen alignment, consistent with previous findings [3]. The ACP microtissues formed a smooth, uniform layer of cartilage on the surface of the AC ECM derived scaffold. Temporal BMP-9 stimulation enhanced ECM production, highlighting its potential for improving graft functionality. Future studies will integrate BMP-9 stimulated ACPs with ECM scaffolds, assess mechanical properties, and evaluate graft performance in a goat model of joint injury.

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Acknowledgments
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Konuşmacılar: Giovanni Gonnella (Trinity College Dublin)

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Integrating cartilage microtissues into melt-electrowritten scaffolds for engineering mechanically functional and zonally defined cartilage grafts

Engineering functional, phenotypically stable articular cartilage remains one of the greatest challenges in tissue engineering. Modular tissue engineering strategies that utilize cellular aggregates, microtissues, or organoids as building blocks offer the potential to fabricate complex, hierarchical tissues at scale. However, a key challenge lies in achieving appropriate structural and zonal organization of the graft following fusion of the microtissues, along with the associated development of biomimetic mechanical properties. This challenge has partially been addressed by integrating mesenchymal stem cell (MSC) derived microtissues with 3D-printed polycaprolactone scaffolds (Burdis et al., 2022), however concerns remain regarding the use of MSCs for engineering phenotypically stable and mechanically functional articular cartilage grafts. The aim of this study was to engineer phenotypically stable and zonal defined cartilage grafts by integrating articular cartilage progenitor cell (ACP) derived microtissues into single- and multilayered melt electrowritten (MEW) scaffolds. To this end, ACP and chondrocyte derived microtissues were fabricated using a high-throughput agarose microwell system (Nulty et al., 2021). A total of 2,000 microtissues were seeded onto single-layered MEW scaffolds with pore sizes of 0.3×0.3 mm, 0.4×0.4 mm, and 1.6×0.4 mm, as well as a three-layer MEW scaffold with 0.3×0.3 mm pores in the first layer, 0.4×0.4 mm pores in the second layer, and 1.6×0.4 mm pores in the top third layer in an attempt to mimic the zones of articular cartilage. Scanning electron microscopy analyses confirmed the structural organization and fidelity of the pore ratios after printing (Fig. 1A). Microtissue formation was assessed using cell densities of 1,000, 2,000, and 4,000 cells/µT. Both ACP and chondrocyte microtissues exhibited homogeneity in size and shape. Histological and biochemical analyses revealed a higher deposition of sulphated glycosaminoglycans (sGAG) and collagen in ACP microtissues. The fusion capacity of these microtissues was further evaluated in a bioassembly assay. Chondrocytes displayed poor fusion, confirmed by histological analysis, while ACP microtissues exhibited robust, homogeneous fusion with notable sGAG and collagen deposition. Furthermore, immunohistochemistry revealed stronger collagen II deposition in the fused ACP microtissues. Next, ACP microtissues were seeded into single-layer MEW scaffolds with different pore ratios, where robust tissue formation, along with significant sGAG and collagen deposition, was observed. No calcium deposition was detected in any group (Fig. 1B). The 0.3:0.3 pore ratio MEW scaffold supported the highest levels of sGAG production, while the 0.4:0.4 scaffold supported higher levels of collagen production (Fig. 1D). Next, ACP and chondrocyte derived microtissues were integrated into a multilayer MEW scaffold, which resulted in more zonal tissue organization and superior mechanical properties compared to a control group where microtissues were confined in an agarose mold. Additionally, ACP-seeded multilayer MEW scaffolds exhibited a three-fold increase in ramp and equilibrium modulus. Polarized light microscopy revealed more biomimetic collagen fiber alignment through the depth of the ACP microtissue derived constructs. In conclusion, we successfully engineered functional cartilage grafts by integrating ACP microtissues within MEW scaffolds. When seeded in a multilayered MEW scaffold, the microtissues generated a zonally defined cartilage graft, highlighting the potential of this approach for the treatment of cartilage defects.

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Converging Melt Electrofibrillation and Melt Electrowriting to Engineer Meniscus Implants with Native Collagen Architecture

Introduction: The meniscus is essential for load distribution, shock absorption, and knee joint stability. Its function depends on the organization of collagen fibers in both radial and circumferential directions. Meniscus damage is often a precursor for degenerative conditions to the articular cartilage (AC). Treatment of meniscal tears is limited to the avascular areas[2]. Current artificial meniscus implants (e.g., CMI® or ACTIfit®) [3] offer clinical relief, but lack the mechanical integrity required for long-term function. To address this limitation, we developed a 3D-printed implant that combines the collagen alignment properties of polycaprolactone nanofibers printed with melt electrofibrillation (MEF) [1], with the mechanical properties of melt electrowritten (MEW) PCL microfiber scaffolds.[1]

Methods: PCL microfibers (MEW) and PCL/polyvinyl acetate (PVAc) nanofibers (MEF) were co-printed using dual syringe setup on a RegenHU 3D Discovery printer (Fig.1A). After printing, PVAc was dissolved using 70% ethanol and PBS to reveal the PCL nanofibrils. Scanning Electron Microscopy confirmed nanofiber integration within the reinforcing scaffold (Fig. 1B). The compressive E-modulus of the converged MEW-MEF constructs was measured using a dynamic mechanical analyzer. To assess cell-material interactions, scaffolds were seeded with human meniscus progenitor cells (hMPCs, 5×10^6cells/ml) (Fig.1E). Type I and II collagen deposition were evaluated via immunohistochemistry, cell deposition via H&E, glycosaminoglycan via Safranin-O/Fast-green and collagen alignment via Picrosirius-red and polarized light (Fig.1F).

Results: Nanofibers were successfully integrated into the reinforced MEW scaffold (Fig.1A,1B), and co-printing MEF and MEW fibers yielded meniscus constructs without compromising mechanical stability. Scaled-down (1:2) meniscus implants were fabricated by alternating circumferential and radial nanofiber orientations to mimic native fiber architecture (Fig.1C,1D). MEF nanofibers effectively guided hMPC alignment and promoted organized type I collagen deposition (Fig.1E), without glycosaminoglycan accumulation, consistent with native-healthy meniscus composition. Polarized light confirmed that collagen deposition aligned with the underlying printed nanofibers (Fig.1F).

Conclusion: We present a cell-laden 3D-printed meniscus implant featuring a nanofiber architecture that guides cell-mediated type I collagen alignment. This approach combines the immediate mechanical stability of microfiber reinforcement with the ability of nanofibers to direct organized collagen deposition, offering a promising strategy for achieving long-term structural and functional integrity in meniscus repair.

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Biofabrication of a structurally organised tissue-engineered cartilage-metal implant for osteochondral defect repair

Introduction: Bottom-up tissue engineering strategies, particularly those inspired by developmental biology, hold the potential for creating biomimetic grafts capable of replicating natural tissue structures (1-2). In this work, we leverage the intrinsic ability of cellular aggregates, serving as tissue building blocks, to fuse for engineering biomimetic cartilage. We further develop a new biofabrication framework to integrate this tissue-engineered cartilage with a metallic scaffold to manufacture a hybrid implant for biological joint resurfacing.

Methods: Microtissues were formed using varying ratios of mesenchymal stromal (MSC) cells and articular chondrocytes (CC): (i) MSC-only, (ii) CC-MSC at 1:3, (iii) CC-MSC at 1:1, and (iv) CC-only. Cell suspensions were seeded into custom-made agarose microwell arrays (3). After 2 days, microtissues were harvested and assembled into: (i) scaffold-free constructs and (ii) hybrid cartilage-metal implants. Titanium alloy scaffolds (4 mm × 6 mm) were manufactured via powder bed fusion with a body-centred cubic (BCC) unit infill (pore size: 500 µm; strut diameter: 300 µm). Constructs were cultured for up to 35 days in chondrogenic medium and evaluated using biochemical, histological, and immunofluorescence analyses. The feasibility of the engineered cartilage-metal implants was tested in vivo using a critically sized caprine osteochondral defect model.
Results: In vitro analyses showed that microtissues derived from a co-culture of chondrocytes and MSCs facilitate the engineering of macroscale grafts rich in glycosaminoglycans and collagen type II. The addition of chondrocytes to microtissue building blocks also reduced the macrotissue contraction. The proposed biofabrication framework allowed for growing a self-assembled hyaline cartilage tissue on the surface of a metal scaffold. The metal lattice served as a guiding structure, facilitating the anisotropic self-organisation of the engineered cartilage into a tissue with a biomimetic collagen network. In vivo outcomes from high (medial condyle) and low (lateral trochlea) weight-bearing sites revealed variable integration of the metal implant within the host bone, impacting the overall hybrid graft restoration capacity.
Discussion: The study highlights the fact that promising in vitro outcomes of zonally organised osteochondral implants may not necessarily guarantee their effective performance in vivo. Modular biofabrication approaches successfully combined with emerging manufacturing technologies such as metal 3D printing may enable the manufacture of scalable, biomimetic grafts that replicate osteochondral unit structure.

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Strategies to modulate cell signaling pathways to develop phenotypically stable 3D bioprinted hyaline cartilage tissue mimics using chemically modified silk fibroin-gelatin bioink: an in vitro and in vivo study

Introduction: With a very low success rate of the currently available osteoarthritis (OA) treatment modalities, implanting a phenotypically stable cartilage graft is the only plausible strategy to mitigate cartilage lesions. However, poor understanding of the developmental biology leading to chondrocyte hypertrophy of the implanted construct rendered the attempts futile. The current study aimed at developing a chemically modified silk fibroin-gelatin (SF-G) bioink covalently conjugated with pro-chondrogenic (TGF-β3 and IL-1Ra) and anti-hypertrophic (LDN193189) molecules to evaluate the development of a stable hyaline cartilage phenotype in vitro. Further, in vivo studies were carried out using BMP antagonist (LDN193189)-conjugated SF-G constructs to evaluate the role of BMP inhibition in neo-cartilage regeneration in an osteoarthritic rat model. Methods: The silk fibroin solution (SF) was chemically modified with cyanuric chloride and the diazonium coupling method to conjugate the small molecules and growth factors, and their interaction was validated through computational (docking) and FTIR, and release kinetics studies. Further, the 3D bioprinted chemically decorated SF-G constructs encapsulated with mesenchymal stem cells (MSCs) were cultured in OA-mimicking media and characterized for chondrogenesis using qPCR and Immunostaining. The in vivo implantation of the constructs was carried out in an osteoarthritic rat model, harvested at 4 months and evaluated for neo cartilage regeneration using histology and Immunostaining. Results: The covalent attachment of the small molecules did not significantly alter the rheological and printability profile of the bioink, but rather enhanced their bioavailability and bioactivity by exhibiting sustained release characteristics. The IL1Ra and LDN conjugated groups displayed the maximum chondrogenic traits with simultaneous inhibition of chondrocyte hypertrophy compared to the TGF-β3 and the control. This is marked by the upregulation of the chondrogenic gene (COL2A1, SOX9) and protein expression (COL-II), with decreased expression patterns of hypertrophic markers (COL-X, MMP-13). Post 4 months of implantation, neo-articular regeneration was observed in the osteoarthritic rat models in the LDN-conjugated groups compared to the controls, marked by intense Safranin O staining and COL-II expression. Discussion: Therefore, for the first time, this study underlined the significance of maintaining a stable chondrogenic phenotype under OA-mimicking conditions rather than simply maturing the tissue construct under a conventional chondrogenic medium. Furthermore, the in vivo studies successfully proved the hypothesis that inhibition of the BMP signaling pathway by LDN193189 allows regeneration of articular cartilage tissue in an OA niche. Thus, through this approach, we could successfully fabricate a stable hyaline cartilage tissue as a regenerative therapy for OA.

Keywords: articular cartilage, silk gelatin, hypertrophy, osteoarthritis, signaling pathway
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Konuşmacılar: Nilotpal Majumder (Indian Institute of Technology Delhi)
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Converging laser-induced forward transfer and melt electrowriting for biofabrication of reinforced cartilage constructs

INTRODUCTION
Articular cartilage has limited regenerative potential and therefore does not restore upon damage. Biofabrication technologies offer promising strategies to engineer functional cartilage constructs to address also large defects [1]. Multicellular spheroids, or microtissues, are widely used as building blocks in these approaches to generate large volumes of cartilage-like matrix [1]. Their spatial arrangement within 3D constructs is important for regulating spheroid fusion, guiding tissue formation, and ensuring instant mechanical integrity of the printed structure. Our aim was to enable rapid and automated production of tissue constructs, facilitating future upscaling. Here, we demonstrate for the first time, the successful convergence between laser-induced forward transfer (LIFT) bioprinting and melt electrowriting (MEW) for the deposition of articular cartilage progenitor cell (ACPC) spheroids into reinforcing meshes.

METHODS
ACPC spheroids (125 cells (Æ~80 µm) and 1000 cells (Æ~150 µm)) were produced, pre-matured for 3 days, and mixed with GelMA prior to LIFT bioprinting (NGB-RTM, Poietis). The effects of laser energy (6-15 µJ), spheroid size, and concentration (80,000 - 160,000 spheroids/mL) on printing accuracy and spheroid deposition rate were studied. Post-LIFT viability and morphology were assessed (Day 1, 7, live/dead assay). To evaluate the feasibility of converging LIFT and MEW technologies, ACPC spheroids were deposited into PCL MEW meshes (internal box-size = 400x400 µm). Mesh coverage and printing accuracy were evaluated post-printing, and spheroid-viability was assessed. Furthermore, a LIFT adaptation and an AI-based imaging system, “target-and-shoot”, was used to automate spheroid recognition and control transfer.

RESULTS
Increasing laser energies increased deposition rates, but resulted in decreased printing accuracy and spheroid viability, particularly for the small spheroids. Lower laser energy improved printing accuracy, but resulted in low deposition rates (<10%). Printing at 12 µJ with higher concentrations resulted in adequate printing accuracy and good cell viability (>80%). Converged bioprinting was achieved with deposition rates similar to previous (non-converged) experiments and maintained spheroid viability. The implementation of a “target-and-shoot” system enabled image-guided spheroid selection and transfer to predefined locations within the MEW meshes, significantly improving transfer efficiency and precision.

DISCUSSION
This study presents a novel approach for engineering larger cartilage constructs through integration of LIFT and MEW technologies. Different sizes of ACPC spheroids with

high cell viability and uniformity in size and shape were successfully generated. LIFT parameters, including laser energy, spheroid size and concentration, were optimized to ensure printing fidelity while minimizing cellular damage. Furthermore, we introduced into our printing workflow an AI-based automated detection system that enhances spatial control by selecting the spheroid and directing it to a pre-determined location of a MEW scaffold.

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Konuşmacılar: Núria Ginés Rodriguez (University Medical Center Utrecht)

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Novel bioprintable composite hydrogels for bone tissue engineering via the endochondral ossification pathway

Regeneration of bone defects exceeding the critical size remains a challenge, as bone cells require proper oxygen tension. In contrast, chondrocytes are less susceptible to hypoxic conditions. Consequently, in recent years, many studies have focused on the development of biomaterials for bone regeneration through the endochondral ossification (EO) pathway. This route involves the formation of cartilaginous template, followed by vascular invasion and recruitment of osteoprogenitor cells.
Since cartilage tissue is a highly hydrated structure, hydrogels are widely used for its in vitro engineering. However, during the EO, chondrocytes have to accumulate calcium (Ca) and undergo hypertrophic differentiation. Therefore, we hypothesized that incorporating a Ca-containing inorganic filler into the hydrogel matrix could accelerate the hypertrophic differentiation of chondrogenically primed human bone marrow-derived mesenchymal stem cells (hMSC).
Accordingly, the aim of this study was to investigate the effect of bioactive glass microparticles (BG) on the chondrogenic and hypertrophic maturation of hMSC.
HMSC were encapsulated in a blend of bone-derived decellularized extracellular matrix (dECM, 1% w/v) and alginate (ALG, 1 – 3% w/v), containing 0.0%, 0.5% and 1.0% (w/v) of BG microparticles, and crosslinking using 30 mM CaCl2. The cell-laden constructs were cultured in chondrogenic medium for 3 weeks, followed by hypertrophic medium (chondrogenic medium w/o TGF β3 supplemented with 1 nM L-thyroxine and 2 mM β-glycerophosphate) for up to 3 weeks. Differentiation was evaluated quantitatively by measuring DNA content, glycosaminoglycans (GAG) levels, and alkaline phosphatase (ALP) activity (a marker of hypertrophy), and qualitatively by histological staining (H&E, Alcian Blue, Leukocyte Alkaline Phosphatase Kit, von Kossa).
The addition of the BG microparticles increased ALP activity nearly threefold just one day of culture, regardless of BG concentration. Over the following three weeks, ALP activity increased by two orders of magnitude. However, the effect of BG content was less pronounced (maximum twofold increase for 1.0 % (w/v) BG), while the effect of ALG concentration became more evident during this period. The highest ALP activity was observed in composite hydrogel containing 1% (w/v) of BG and 1 % (w/v) of ALG after three weeks of chondrogenic priming. Alcian blue staining reviled that GAG synthesis continued until week 2 of hypertrophic differentiation, at which point chondrocytes began to enlarge (a sign of hypertrophy). The hypertrophic transition after two additional weeks of differentiation was also clearly visible in the ALP staining, which showed the presence of ALP-positive cells throughout the entire cross-section of the hydrogels. Moreover, the ALP-positive cells were more evenly distributed in the BG-containing hydrogels.
This study demonstrated that addition of BG microparticles into hydrogels enhances the hypertrophic differentiation of hMSC. Therefore, it represents a promising strategy for in vitro bone engineering via EO pathway.

Konuşmacılar: Joanna Idaszek (Warsaw University of Technology)

S14 Biohybrid robotics — Kruczkowski

Toplantıyı düzenleyenler: Minghao Nie (University of Tokyo), Maurizio Gullo (University of Applied Sciences Northwestern Switzerland (FHNW))

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Creating Life-like Robots: From Musculoskeletal Designs to Biohybrid Innovations

Living robots represent a new frontier in engineering materials for robotic systems, incorporating biological living cells and synthetic materials into their design. These bio-hybrid robots are dynamic and intelligent, potentially harnessing living matter's capabilities, such as growth, regeneration, morphing, biodegradation, and environmental adaptation. Such attributes position bio-hybrid devices as a transformative force in robotics development, promising enhanced dexterity, adaptive behaviors, sustainable production, robust performance, and environmental stewardship. Nature's musculoskeletal design can act as an inspiration for both artificial and living robots. We will explore recent advances in artificial electrohydraulic musculoskeletal robots, which employ electrohydraulic actuators to produce lifelike muscle contractions and adaptive motions, as demonstrated in our recent work published in Nature Communications. We will also discuss our breakthroughs in vision-controlled inkjet printing for robotics from our Nature paper, as well as xolographic biofabrication techniques for biohybrid swimmers presented at RoboSoft. Additionally, I'll share insights from our computational optimization of musculoskeletal systems featured at Humanoids. Together, these projects showcase how musculoskeletal, bio-hybrid, and computational techniques are opening new frontiers in robotics interaction and manipulation.

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Konuşmacılar: Aiste Balciunaite (ETH Zurich (Soft Robotics Lab))

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Engineering Biohybrid Robots via Multi-Scale Biofabrication Across Species

Biohybrid robotics integrates living biological components with synthetic systems to create machines that sense, actuate, and adapt in biologically meaningful ways. In this talk, I will present a set of diverse yet complementary projects from our lab that demonstrate a multi-scale and cross-species approach to biohybrid system design. These include robots powered by engineered muscle rings, robot skins with perfusable microchannel networks, microscale soft robots actuated by photosynthetic algae, and plant-driven robotic systems that leverage growth for motion generation. Each system reflects a different scale, material strategy, and biological domain, illustrating the versatility of biofabrication as a unifying framework. Together, these efforts point toward a future where machines are constructed not only with living cells but also through biological growth, remodeling, and self-organization. I will highlight key technical advances, challenges in integration, and emerging applications in soft robotics, translational medicine, cellular agriculture, and green technologies.

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Konuşmacılar: Minghao Nie (The University of Tokyo)

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Hybridization of Melt Electrowriting and Fused Deposition Modeling for the Reinforcement of Textile Scaffolds for Tissue Engineering Applications

Introduction: Melt electrowriting (MEW) has gained considerable popularity in the field of biofabrication due to the unique capabilities it offers to fabricate biologically relevant architectures. Most MEW-generated scaffolds have fiber sizes within the range of 5-50 microns and inter-fiber distances as low as 100 microns. However, that is just close to the maximum pore size where cells uniformly show maximal spreading during their initial attachment. However, no matter how densely woven a MEW layer is, achieving the adequate mechanical properties to withstand suturing is a significant challenge. Additional strength can be provided by using thicker fibers to survive suture stress (e.g., tension, shear, etc.). Eventually, the goal is to have a confluent sheet of cells with the cell's extracellular matrix capable of supporting physiological loads. In the pursuit of this goal, having fiber-bound pores with a predictable geometry that is small enough to allow cells to fully span to two or more polymer filaments becomes crucial. To demonstrate these mechanical and geometric requirements, we tested the hybridization of MEW with Fused Deposition Modeling (FDM) to fabricate reinforced scaffolds that can withstand suturing and at the same time offer an appropriate environment for cell proliferation.
Methods: The hybrid scaffolds used in this study were fabricated in a custom-built printer. It incorporates a dual printhead that uses PID (Proportional–Integral–Derivative)-controlled cartridge heaters to melt polymer contained in a glass reservoir (MEW printhead). For the fabrication of the FDM borders we adapted a pellet extruder mechanism to an Ender Pro hot end (Creality, Shenzen, China) that served as the FDM printhead. Purasorb® PC 12 medical grade polycaprolactone pellets (Corbion, Netherlands) were used for the fabrication of both MEW and FDM fibers. For the fabrication of the MEW textile with a 50-micron pore size, the pellets were melted for 1-hour prior to printing at 80°C and then extruded at a pressure of 0.72 Bar. The high voltage and translating speed of the collector was set to 5 kV and 12.5 mm/s, respectively. For the FDM borders, the extrusion rate and translating speed of the collector was adjusted so that a 0.4-mm thick fiber was printed. The toolpath design and postprocessing of G-Codes for both was done in Fusion 360 (Autodesk, California, US). L929 cells were then seeded on the hybrid scaffolds to study cell viability and confluence during a 7-day period.
Results: Preliminary data on suture retention using the ISO 7198 showed a 166% increase in the peak load (0.655 vs 1.741 N) when comparing a MEW-alone textile vs. the MEW+FDM textile. Moreover, by optimizing the MEW process to print a 50-micron pore size that was reliably and uniformly small, we were able to rapidly achieve cell confluence by day 7 (Figure 1C).
Discussion: FDM fiber incorporation and full confluence of our hybrid textile scaffolds portends future achievement of our mechanical and biological goals. We anticipate that cells stimulated by load carrying FDM borders will thicken and strengthen. The original MEW and FDM fibers will become less relevant over time and eventually resorb.

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Konuşmacılar: David Dean (University of Wisconsin-Madison)

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Harnessing mechanobiology for the engineering of biological machines

Engineered tissues have the potential to serve as sensing, actuation, and mechanical support elements for soft machines that possess biomimetic functionality. Conventional biohybrid constructs involve the use of synthetic structures made from hydrogels or elastomers as support elements because free-standing contractile tissues do not have a stable form. In this talk, I am going to explain how physical principles of connective tissue morphogenesis can be harnessed for the controlled self-assembly of tissues with complex equilibrium shapes. The discovery of these principles involves the use of advanced microscopy, robotic microsurgery, microtechnology, and computational modelling. Combined with efforts in the development of genetically engineered biological actuators, we can finally envision the conception of reconfigurable and self-healing robots that are autonomously assembled from living matter.

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Konuşmacılar: Selman Sakar (EPFL)

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Hydrodynamically Assembled Magnetic Soft Robots for Enhanced Extracellular Vesicle Secretion

Soft robots offer unique advantages in biomedical applications due to their adaptability and biocompatibility. However, scalable, contactless fabrication methods are underdeveloped. We present a novel approach using sound-induced hydrodynamic instabilities to assemble magnetic soft robots within a gelatin matrix. These robots, actuated by a magnetic field, undergo complex shape transformations. Encapsulated human mesenchymal stromal cells (h-MSCs) maintain high viability, and dynamic actuation enhances extracellular vesicle secretion. This scalable, cytocompatible platform has potential in drug delivery and regenerative medicine, with future work focusing on broader biomedical applications.

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Konuşmacılar: Tiziano Serra (AO Research Institute Davos)

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Skin-covered biohybrid robotic finger with embedded perfusable channels

Developing robots covered with living skin tissue can significantly enhance their huamn-like apperance, barrier function, and regenerative capacity. However, maintaining viable skin tissues in air-exposed environments requires a robust internal nutrient supply system. In this study, we propose a method for constructing perfusable skin-covered robotic structures by integrating microchannels into 3D-printed robotic skeletons. These channels serve as internal nutrient delivery pathways for skin tissues, enabling sustained viability and functionality in air.
The robotic finger is designed with microchannels and fabricated using 3D-printers. Human dermal fibroblasts suspended in collagen solution were injected around the skeletal structure, forming the dermal tissue on top of the microchannels. Subsequently, epidermal layers were established by seeding keratinocytes on the dermal surface. The resulting biohybrid structure was cultured in air with nutrient solution perfused through the embedded channels.
Experimental evaluations showed that the formation of a uniformly thick epidermal layer across the tissue. Functional assessments demonstrated significantly improved barrier properties (higher TEER values) and reduced moisture loss compared to conventional static cultures. Also, we demonstrate the functional integration of this biohybrid skin into flexible robotic hands, successfully achieving finger bending through wire-driven actuation, confirming practical applications in advanced robotic systems. This approach highlights a significant step toward durable, functional, skin-covered robotics for diverse biomedical and technological applications.

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Konuşmacılar: Minghao Nie (The University of Tokyo)

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3D Printing Heartbeats: Dual-Scale Conductive Scaffolds for Cardiac Tissue Engineering and Regeneration

Bioengineered cardiac tissue substitutes present immense potential for advancing regenerative therapies for ischemic heart diseases, the leading cause of hospitalization and mortality worldwide. Despite significant research efforts, existing biomaterials and scaffold fabrication approaches continue to face critical challenges, particularly inadequate electrophysiological integration resulting from low cell density within bioprinted constructs, insufficient tissue maturation, and absence of conductive myocardial structures, such as syncytium and Purkinje fibers.
To address these limitations, we developed a dual-scale construct (DSC) that closely replicates the structural and functional attributes of native myocardium by integrating advanced 3D-bioprinting with directional electrospinning. Polycaprolactone (PCL) nanofibers, doped with carbon nanotubes (CNTs) to confer electrical conductivity, were electrospun into highly aligned nanofiber layers that structurally mimic myocardial syncytium. These conductive nanofibers were then sequentially integrated with a novel methylcellulose-gelatin (MCG)-based bioink containing neonatal rat cardiomyocytes (NRCMs), effectively recreating an extracellular matrix (ECM)-like microenvironment.
The custom-formulated bioink exhibited excellent intrinsic cytocompatibility and was highly printable, capable of supporting cell concentrations up to 5.4 million cells per mL. Furthermore, enzymatic crosslinking of the bioink allowed precise modulation of its elastic modulus between 5 and 50 kPa, enabling accurate biomimicry of the mechanical properties of native myocardial ECM. NRCMs encapsulated within the bioprinted constructs maintained exceptionally high viability (>90%) for over three weeks in vitro. Notably, spontaneous contraction of the cardiac constructs initiated between days 5 and 8 post-printing, signifying effective preservation and maturation of cardiomyocyte contractile function. Additionally, the conductive CNT-PCL nanofibers demonstrated high biocompatibility and effectively guided the directional alignment of cardiomyocytes (Figure 1), crucial for optimal physiological tissue function.
This interdisciplinary research successfully established the feasibility of fabricating sophisticated dual-scale biomimetic cardiac scaffolds. Our approach highlights significant opportunities to overcome existing production complexities, including streamlining scaffold assembly processes, optimizing cell health management protocols, and improving overall workflow efficiency. Continued refinement of these strategies promises further enhancements in scaffold functionality, electrophysiological integration, and cellular maturation.
Ultimately, this study represents an essential advancement toward practical and clinically relevant regenerative cardiac applications. The developed dual-scale constructs offer a versatile, cost-effective platform adaptable to diverse tissue-specific needs, thereby significantly advancing cardiac disease modeling, personalized therapeutic tissue substitution, and regenerative transplantation approaches.
Detailed insights into scaffold fabrication methods, bioink optimization strategies, extended cell culture analyses, tissue conditioning via electrical stimulation, and advanced characterization techniques will be comprehensively discussed at the conference.

Figure 1: Micrographs illustrating the dual-scale construct (DSC) approach. Left: CNT-PCL nanofibers transferred onto a previously 3D-bioprinted neonatal rat cardiomyocyte (NRCM) construct (scale bar = 200 µm). Right: Directionally aligned NRCMs guided by the conductive nanofiber architecture (scale bar = 100 µm).

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Konuşmacılar: Maurizio Gullo (FHNW)

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Lunch & Meet the Mentor

- S08b Biofabrication for cartilage regeneration and joint preservation Ratuszowa

Toplantıyı düzenleyenler: Francesca Diletta Spagnuolo (Trinity College Dublin), Jos Malda (Utrecht University, NL)

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IMPACT, a one-stage cell therapy for cartilage defects in the knee joint

Large cartilage defect of the knee joint can cause a major disabilty for young patients. At the UMC Utrecht, a one-stage cell therapy was developed, combining autologous chondrons with allogenieic mesenchymal stromal cells (IMPACT treatment). The IMPACT treatment is a perfect example of how a new brining a novel treatment from bench to bedside, a result of collaboration between (lab)researchers and clinicians. IMPACT has been proven safe in a first-in-man treatment (N=35) and clinical results up to 10 years demonstrate clinical efficacy compared to pre-treatment (figure 1). Furthermore, a phase III randomized treatment demonstrated superior results of IMPACT compared to non-surgical treatment (figure 2). Patients were allowed to cross-over after 9 months of follow-up and also clinically improved after IMPACT, similar to the patients that were immediately randomized in the IMPACT group. IMPACT is classified as an advanced therapeutic medicical product (ATMP). Besides bringing this treatment fo regular care by

obtaining authorisation and reimbursement, the IMPACT treatment can create a platform for other research and treatment, for example combining IMPACT with 3D printed bone structures for the treatment of large osteochondral defects.

Konuşmacılar: Roel Custers (University Medical Center Utrecht)

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TD-198946 Modulates Proliferation, Survival, and Chondrogenesis of iPSC-Derived NC-MSCs via NOTCH3 Pathway Activation

Introduction:
Induced pluripotent stem cell (iPSC)-derived neural crest cells (NC-MSCs), following mesenchymal induction, exhibit strong chondrogenic potential, making them a promising source for cartilage repair. However, like other mesenchymal stem cells (MSCs), their potential decreases with repeated monolayer expansion. TD-198946, a small molecule, has been shown to enhance chondrogenic differentiation in native chondrocytes, synovium-derived stem cells, and iPSC-derived NC-MSCs when combined with BMP and TGF-β3. Additionally, monolayer pre-treatment with TD-198946 has been reported to activate NOTCH3 signaling and promote chondrogenic differentiation. Here, we investigate the effects of TD-198946 pre-treatment on iPSC-derived NC-MSCs.
Methods:
The stemness of TD-198946-treated NC-MSCs was assessed through cell cycle analysis, metabolic activity measurements, and evaluation of chondrogenic differentiation potential. NC-MSCs were pre-treated with varying concentrations of TD-198946 and subsequently used to print scaffold-free, cells-only constructs using a bio-3D printer. These constructs were evaluated for their chondrogenic potential and mechanical properties. To further investigate the role of NOTCH3 signaling, we employed DAPT, a γ-secretase inhibitor, to assess its impact on the cellular phenotype prior to chondrogenic induction.
Results:
TD-198946 played a critical role in the in vitro expansion of NC-MSCs by preventing the gradual onset of G1 arrest observed in passage 4 (P4) and later. This led to significantly enhanced proliferation rates, confirmed by MTT assay, and a reduction in cleaved caspase-3 expression, suggesting decreased apoptosis and improved cell survival. However, when TD-198946 and DAPT, a NOTCH3 inhibitor, were administered together, the improvements in proliferation and cell survival were reversed. Spheroid formation exhibited a clear dose-dependent response to TD-198946, with spheroid size and glycosaminoglycan (GAG) deposition increasing at concentrations of 10, 50, and 100 nM after a single 3-day exposure. Notably, the mechanical strength of the 3D-bioprinted constructs was highest at 50 nM TD-198946, with a slight decrease observed at 100 nM. This trend was also reflected in GAG deposition, emphasizing the importance of an optimal TD-198946 concentration in balancing cellular proliferation and extracellular matrix production, which directly influences construct quality.
Discussion:
Our findings suggest that TD-198946 helps reverse the senescent-like state of MSCs induced by repeated passaging and monolayer culture, thereby restoring their proliferative and chondrogenic potential. However, excessive TD exposure may disrupt the balance between cellular proliferation and extracellular matrix production. Potential mechanisms include overstimulation of signaling pathways, matrix dilution, or early metabolic stress in larger spheroids. Moreover, the inclusion of the NOTCH3 signaling inhibitor, DAPT, abolished the TD-induced enhancement of proliferation and the reversal of G1 arrest, indicating that these effects are mediated through NOTCH3 signaling. By preserving the multipotency of NC-MSCs through TD-198946 pre-treatment, we can enhance the therapeutic potential of NC-MSCs for cartilage repair and regeneration, improving their translational feasibility and clinical applicability.
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Konuşmacılar: Anna Nakamura (Saga University)

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The stiffness of bone extracellular matrix derived scaffolds regulates osteogenesis of mesenchymal stromal cells

Bone extracellular matrix (ECM) shows considerable promise as a material for bone graft substitutes, primarily due to its intrinsic osteoinductive properties, which naturally support the process of bone formation. Bone ECM can be processed into highly porous scaffolds that facilitate effective cell infiltration and nutrient transport. A potential limitation of bone ECM scaffolds is their relatively poor mechanical properties, which may restrict their use to non-load bearing activities. Furthermore, given the well-established role of matrix stiffness in regulating the differentiation of mesenchymal stromal/stem cells (MSCs), the relative softness of current bone ECM derived scaffolds may be suboptimal for supporting osteogenesis. Therefore, the first goal of this study was to develop solubilised bone ECM scaffolds with a range of mechanical properties. We then sought to determine how the stiffness of such bone ECM scaffolds modulates osteogenesis of MSCs.
To this end, we developed a protocol to extract decellularised bone ECM from porcine bone. This method yielded a material suitable for fabricating scaffolds with a unidirectional pore architecture, a design that further supports rapid cell infiltration and alignment within the scaffold structure. By producing scaffolds with varying ECM concentrations, we aimed to control and assess how changes in ECM density influence both their mechanical stiffness and capacity to support osteogenesis of MSCs in vitro. Our findings indicate that scaffold stiffness can be modulated effectively by adjusting ECM concentration: higher ECM concentrations produce stiffer scaffolds while retaining elasticity, as evident by the minimal levels of permanent deformation observed following the application of large compressive strains. All scaffolds were able to retain at least the 95% of their original shape. Additionally, the Young's modulus of the scaffolds increased from approximately 3 kPa for 2% ECM scaffolds to 14 kPa for 6% ECM scaffolds. These stiffer scaffolds supported robust cell proliferation and led to increased mineral and collagen deposition. Aligned collagen deposition parallel to the direction of the scaffold pores was observed, highlighting the role of pore orientation in guiding neo-tissue organisation.
In conclusion, this study demonstrates that by varying ECM concentration, bone ECM-derived scaffolds can be engineered to possess tunable stiffness and tailored porosity, making them highly supportive of the osteogenic differentiation of MSCs. The ability to modulate bone ECM scaffold stiffness provides a valuable tool for optimising scaffold performance for bone tissue engineering applications, where both material stiffness and cellular orientation play essential roles in effective bone regeneration.

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Konuşmacılar: Giovanni Gonnella (Trinity College Dublin)

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Post-Fabrication Development of Extracellular Matrix and Mechanical Properties in Cartilaginous Constructs Made from Hyaluronic Acid-based Bioinks

Introduction
Extrusion-based bioprinting represents a promising alternative to current cell-based approaches in cartilage regeneration. However, a major challenge in the fabrication of cartilage is still to achieve appropriate mechanical properties which are essential for biological functionality. In current biofabrication approaches bioinks are often combined with PCL scaffolds or other synthetic polymers to mimic the mechanical properties of native cartilage [1]. In this study, we utilize a stand-alone bioink consisting of hyaluronic acid (HA) and polyethylene glycol (PEG) derivatives with a dual-stage crosslinking mechanism that has previously been shown, in principle, to enable the deposition of coherent extracellular matrix (ECM) throughout the construct [2]. Here, we aim to analyze the maturation of 3D bioprinted cartilaginous tissues based on chondrogenically differentiated mesenchymal stromal cells (MSC) over a 42-day period. We determine in detail the development of different ECM components as well as the mechanical properties over time.

Methods
Thiolated HA (HA-SH) and PEG derivatives (PEG-DA, PEG-Allyl) were synthesized and characterized using 1H-NMR spectroscopy and aqueous GPC/SEC. MSCs were embedded in the dual-stage crosslinking bioink and differentiated for a 42-day period post-printing. Chondrogenic differentiation was analyzed at multiple time points during the differentiation period and investigated using histology, immunohistochemistry, and wet chemistry-based quantification of ECM components. The mechanical properties were determined over time using multimodal mechanical tests, analyzing the time-matched mechanical response to compression, tension and torsional shear.

Results
Employing our stand-alone HA-SH-based bioink we could prove that the printability and physicochemical properties of constructs immediately after printing were not influenced by incorporation of MSC. During the 42-day period, a marked increase of collagens, glycosaminoglycans, and construct stiffness was observed. Immunohistochemical staining showed that deposition of aggrecan started within the first week of differentiation, with distribution throughout the constructs from day 14 on, while collagen type II showed substantial distribution at day 21. Our data indicated a clear positive correlation between ECM deposition and the mechanical properties of the constructs. This correlation was demonstrated to be valid even when the collagen production was inhibited by ethyl-3,4-dihydroxybenzoate (EDHB). Furthermore, we observed similar mechanical characteristics of our printed cartilaginous tissues compared to human articular cartilage with respect to nonlinearity, hysteresis, conditioning, and stress relaxation behavior.

Discussion
In this study, we could show a strong correlation between ECM development over time and the mechanical response of our bioprinted cartilaginous tissues. While other biofabrication approaches depend on reinforced hydrogels to show an increase in construct stiffness or to achieve printability [1], we observed a marked increase in construct stiffness based on tissue maturation in a stand-alone HA-based bioink. Moreover, we could demonstrate a mechanical behavior with distinct similarities to native articular cartilage.

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Borax-based Self-Healing Gels for Meniscus Tissue Engineering

Borax-based Self-Healing Gels for Meniscus Tissue Engineering
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The meniscus plays a crucial role in force transmission, shock absorption, joint lubrication, and stabilization of the knee joint. One of the major challenges in meniscus biofabrication lies in replicating its highly complex architecture, which consists of regionally distributed and aligned collagen fibers, glycosaminoglycans (GAGs), and a high-water content. GAGs represent the aqueous component of the meniscus and are primarily responsible for its shock-absorbing capacity. Their high swelling ability and fixed negative charges enable them to absorb water during osmotic imbalance, acting as a cushion under mechanical deformation1.
Traditional tissue engineering strategies have often relied on highly crosslinked hydrogels to mimic the hydrated portion of the meniscus2. However, these hydrogels tend to be brittle and restrict polymer chain flexibility, in contrast to the semi-flexible nature of GAGs.
In this study, we introduce a self-healing hydrogel system designed to better replicate the dynamic and hydrated environment provided by GAGs in native meniscus tissue. Self-healing gels form dynamic, reversible bonds that enable rapid structural reorganization (within 10 minutes), leading to a two-fold reduction in loss factor compared to conventional hydrogels. This dynamic behavior enhances mechanical resilience and reduces the risk of long-term deformation (creep). Additionally, the self-healing gels are non-cytotoxic (cell viability >80%) and promote cell migration and spreading. The resulting bioink demonstrates improved mechanical integrity and a closer functional resemblance to the GAG-rich phase of native meniscus tissue.

Figure1. Alginate-Borax based gel stability and self-healing after 1 hour in 37°C (left), frequency sweep of the gel with relaxation spectrum (middle), cell viability in gel after one week (right)

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In vivo evaluation of the long-term stability of printed osteochondral implants based on the reinforcement of soft hydrogels with non-degradable microfiber structures

Introduction
Osteochondral defects present a significant challenge due to the non-regenerative nature of articular cartilage (AC). Current treatments are limited in size, availability and durability.[1,2] Regenerative medicine and bioprinting offer a promising solution to overcome these challenges.[3] Soft hydrogels can be mechanically reinforced with microfiber box structures created with melt electrowriting (MEW).[4] Biofabricated osteochondral implants have demonstrated stability in vivo after six months.[5] In view of the lack of the highly organized arcadic collagen type II structure in the repair tissue and the degradable nature of the polymer fibers, it is likely that the microfiber reinforcement will eventually be compromised. Therefore, this study investigates the application of non-degradable polypropylene (PP) microfiber reinforcement as an alternative to achieve long-term stability in an in vivo equine osteochondral defect model
Methods
Osteochondral implants were fabricated using a 1.2 mm high MEW PP microfiber (Æ 10mm) reinforcement of a cell-free gelMA as the cartilage phase, which was attached to a PCL bone anchor generated using extrusion-based bioprinting. (total dimensions: 6 mm diameter, 7.2 mm height). PP-reinforced constructs were compared to MEW PCL microfiber reinforcement constructs with the same dimensions. The implants were implanted in the trochlea of eight horses (Figure 1C). . The implants were evaluated in vivo in the equine osteochondral defect model. Seven horses received a PP-reinforced implant in one knee and a PCL control implant in the other. After six months, the constructs were explanted and implants, surrounding and opposing tissues were analyzed histologically.
Results
After six months, the fabricated implants demonstrated good integration with the surrounding cartilage tissue in the joint for both PCL and PP reinforced constructs, as was underscored by the abundant ingrowth of bone tissue in the pores of the implant. Histological analysis revealed tissue formation, predominantly fibrotic in nature in the cartilage compartment. Despite being cell-free at the time-point of implantation, cells had invaded the cartilage phase of the implants. Although, glycosaminoglycan (GAG) production was limited compared to the native tissue - with no significant difference between the PCL and PP groups - the PP group provided durable mechanical filling (equal to about 1/3 of the native tissue properties) of the defects to the same extend as the PCL group after 6 months of implantation.
Discussion
The reinforcement of the hydrogel-based cartilage phase with non-degradable PP microfiber structures provides equal mechanical support as PCL reinforced implants, These results highlight the potential of the use for non-degradable materials, such as PP, to provide long term mechanical support for osteochondral defects despite the absence of a highly organized collagen type II network.
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Personalized 3D multi-material printed scaffold for auricle reconstruction

Introduction
Microtia and traumatic injuries are among the primary causes of external ear malformation or absence. Based on epidemiological data, approximately 1.46 out of every 10,000 newborns are affected by microtia, with 22.1% of these cases involving complete anotia [1]. To address these conditions, different ear reconstruction approaches have been developed, including autologous rib cartilage grafts, silicone prostheses, and tissue-engineered polymeric implants [2]. This study presents a novel, patient-specific strategy, involving the design and fabrication of a 3D-printed porous scaffold composed of thermoplastic polyurethane (TPU) and polycaprolactone (PCL).
Materials and Methods

TPU and PCL were selected for their mechanical compatibility with external ear tissues. PCL mimics the auricular cartilage, while TPU replicates the ear lobe's adipose tissue. 3D printing parameters were optimized to achieve the desired porosity and mechanical performance. A laser scanner was used to capture the geometry of the patient's healthy ear. The scanned image was processed to generate a 3D model. The model was split into two parts: the ear lobe and base (TPU printed) and the helix, anti-helix, and concha (PCL printed). MicroCT scans was used to analyze scaffold architecture, including pore size and interconnectivity, and filament dimensions and distribution. DMA was used to evaluate the compressive and torsional properties, with the aim of matching them to the mechanical characteristics of the native auricular cartilage and adipose tissue. Scaffold cytocompatibility was assessed through cell viability and proliferation assays up to 21 days. CondroISO-9 chondrocytes were seeded on PCL scaffold, and 3T3-L1 pre-adipocytes on the TPU one. Cell viability was evaluated by AlamarBlue assay, cell proliferation with DAPI staining. SEM imaging provided qualitative insights into cells morphology and distribution. Adipocytes differentiation and functionality was evaluated by Nile Red staining, evidencing intracellular lipidic droplets.

Results and Discussion

Optimized printing parameters resulted in scaffolds with accurate filament diameter and uniform layer height. The average pore size (390 µm) was suitable for cartilage and adipose tissue regeneration. Compression tests confirmed TPU is the most deformable material with lower elastic modulus, while PCL and the stratified samples exhibited similar mechanical behavior, with the former having a slightly lower elastic modulus. Torsional test highlighted that TPU showed a high flexibility, with the lowest storage and loss moduli. In this case, the material that appears to offer the most resistance to torsion is PCL, and the stratified samples have a behavior that lies in between TPU and PCL, more towards the softer one. Both chondrocytes and preadipocytes seeded respectively on PCL and TPU showed an increasing trend in cell viability up to 21 days. Differentiated adipocytes showed enhanced viability compared to non-differentiated ones, and lipidic droplets production starting from day 14.

Conclusion

This study demonstrates the potential of patient-specific, 3D-printed scaffolds composed of biocompatible polymers as an innovative solution for ear reconstruction in cases of microtia and anotia. This approach offers a promising alternative to conventional surgical methods by combining anatomical precision and tissue-specific mechanics.

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Nano-to-Microarchitectural Design: Intact Collagen Fibril Coatings on MEW Scaffolds to Enhance Chondrogenesis

Introduction

Melt electrowriting (MEW) is an additive manufacturing technique with significant potential in cartilage tissue engineering. While osteoinductive coatings have been employed to functionalize MEW scaffolds, the efficacy of comparable chondro-inductive coatings remains unproven. Type II collagen and sGAGs are key cartilage ECM components that promote chondrogenesis when included in scaffolds [1,2]. However, conventional collagen extractions disrupt native fibril architecture, and without intact fibrils, sGAGs cannot localize in the interfibrillar spaces as in native tissue. Herein, insoluble type II collagen extraction was performed and incorporated into polydopamine coatings on melt electrowritten (MEW) scaffolds. The capacity of these coatings to enhance chondrogenesis of bone marrow derived mesenchymal stem/stromal cells (MSCs) was then assessed.

Materials & Methods

Insoluble and pepsin-soluble type II collagen were isolated from porcine cartilage, and acid-soluble type I collagen from Achilles tendon. Bulk MEW scaffolds (0.6 mm height, 40 layers) with pore sizes of 200–800 µm were printed, and 6 mm discs were punched and treated with 2 M NaOH under vacuum for 30 min to improve hydrophilicity. Scaffolds were coated in a 4 mg/mL dopamine solution (0.02 M Tris, pH 8.5), initiating polymerization, while collagen was pre-activated with EDC/NHS and added to the reaction (final: 2 mg/mL dopamine, 1.5 mg/mL collagen), which proceeded for 24 h at 4 °C. Collagen loading was assessed via hydroxyproline assay and SEM. Scaffolds were seeded with 8×10^5 or 4×10^5 MSCs (1.5 mm and 0.5 mm high, respectively) and cultured in chondrogenic media for 28 days.

Results

SEM revealed that collagen formed composite coatings on MEW scaffolds, predominantly as ~60 µm fibrillar networks and occasional larger D-banded fibrils (~100 µm, 65 nm periodicity). Collagen-functionalised scaffolds also exhibited abundant polydopamine nanospheres, which were sparse in polydopamine-only controls. Biochemical assays showed significantly increased sGAG deposition in collagen-functionalised scaffolds at day 3 (400 µm) and day 28 (200 µm) compared to controls. Histology confirmed widespread sGAG and collagen distribution, with intense staining around MEW fibres. Polarised-light microscopy (PLM) further revealed collagen fibril alignment along the MEW fibres in 200 µm scaffolds.

Discussion

In contrast to the population of fibrils observed in the insoluble collagen-PD coated scaffolds, pepsin-solubilised collagen-PD coatings were more amorphous nature with no clear fibrils observed, suggesting more effective biomimicry following functionalization with insoluble collagen. Polydopamine nanospheres have recently gained interest for their anti-inflammatory properties [3], and the collagen-PD film may have provided an improved pathway for their adhesion to the scaffold through their entanglement in the film compared to using polydopamine alone. The enhanced sGAG deposition in the 200 µm pore size scaffolds may be due to the increased collagen coating loading on these higher surface area scaffolds.

Conclusions

This modified PD coating method was shown to be an effective method for enhancing chondrogenesis in MEW scaffolds.

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- S09 Biofabrication of Anisotropy Goethe

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Harnessing chaotic bioprinting to engineer anisotropic microarchitectures for aligned tissue constructs and prevascularized models

Anisotropy is a defining feature of many biological tissues, from skeletal muscle to vascular networks, and replicating this structural organization is crucial in the design of functional tissue constructs and relevant in vitro models. However, current biofabrication strategies often struggle to produce aligned architectures in a scalable, high-throughput, and versatile manner. Here, we present chaotic bioprinting as a robust and flexible platform for generating anisotropic hydrogel filaments with precisely aligned compartments and microchannels.

This technique leverages Kenics static mixers (KSM)—simple, helicoidal elements placed inside the printhead that split and reorient fluid streams in a deterministic and iterative manner. As materials flow through each KSM element, they are divided and folded repeatedly, creating an exponential increase in internal interfaces and generating a multilayered architecture along the filament axis. This process naturally induces structural anisotropy along the direction of extrusion, enabling the formation of constructs with aligned features at the microscale. The resolution of the internal pattern can be easily tuned by adjusting the number of KSM elements, and the resulting anisotropic microarchitecture supports a wide range of biofabrication applications.

Using this approach, we fabricated hydrogel filaments with bioactive compartments (e.g., GelMA-based inks loaded with myoblasts) flanked by materials lacking cell-adhesive motifs—such as alginate or sacrificial materials like hydroxyethyl cellulose (HEC). This configuration creates cell-instructive corridors that guide cell elongation and fusion along the fiber axis. We demonstrate that this microcompartmentalized architecture supports the formation of aligned multinucleated myotubes and promotes muscle-like tissue maturation.

Furthermore, by co-extruding cell-laden inks with fugitive materials, we generated continuous microchannels aligned along the filaments. These prevascular-like voids—up to 30 per filament and as narrow as 20 µm—significantly enhance mass transport and cell viability over extended culture. Beyond their role in oxygen and nutrient delivery, these channels provide directional guidance for cell migration and serve as a powerful platform to evaluate migratory behavior. For example, highly motile cancer cells such as MDA-MB-231 exhibit directed invasion along the channels, enabling direct visualization and quantification of migration fronts over time. In contrast, less invasive cells like MCF7 or Caco-2 display limited movement, highlighting the utility of this platform for comparative studies.

Beyond biological functionality, chaotic flows can also align functional fillers, such as one-dimensional nanomaterials (e.g., carbon nanotubes), within specific compartments. This capability opens new avenues in the development of anisotropic conductive scaffolds for electrically responsive tissues such as muscle, heart, or nerve, or for the integration of smart materials and biosensors.

Altogether, this work demonstrates that chaotic printing/bioprinting is a simple, scalable, and mathematically predictable tool to fabricate anisotropic constructs with spatially defined architectures. Its plug-and-play nature and versatility in material and cell combinations make it a valuable addition to the biofabrication toolbox for tissue engineering, disease modeling, and beyond.

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Regulating Cellular Behavior in Hierarchical Tissue Engineering

The engineering of hierarchical tissues with controlled cellular behavior is essential for replicating the complexity and functionality of natural organs. In our approach, we employed nanoengineered scaffolds to creat hybrid bioink or controlled macromolecule assembly in pre-crosslinked bioinks (yet not chemically crosslinked) to guide (stem) cell migration and promote anisotropic cellular behavior, resulting in tissues with both structural integrity and key functional properties such as mechanical strength and nutrient flow. Furthermore, we optimized the maturation microenvironment to enhance the regeneration of complex tissue types, including skeletal muscle, nerve, and vascular networks. This work helps bridge the gap between bioengineered tissues and natural organs, advancing biofabrication techniques for more effective organ engineering and regenerative medicine applications.

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Electrofibrillated Nanofibrillar Microbundles as Scaffolds for 3D Muscle Tissue Biofabrication

Recreating the highly aligned and hierarchical structure of native extracellular matrix (ECM) remains a pivotal challenge in musculoskeletal tissue engineering, particularly for skeletal muscle, where anisotropic architecture is critical for function. In this study, we present a novel strategy that integrates melt electrofibrillation and 3D cell spheroid bioassembly to fabricate structurally organized muscle-like tissue constructs. Using a blend of polycaprolactone (PCL) and polyvinyl acetate (PVAc), we generated precisely printed box-shaped scaffolds via melt electrowriting, followed by selective removal of PVAc to yield collagen-mimicking nanofibrillar bundles. These fibrillated scaffolds provided aligned nano-topographical cues, essential for guiding myoblast alignment and subsequent tissue maturation. Murine C2C12 myoblasts were assembled into spheroids using honeycomb-inspired microwell arrays and seeded onto the fibrillated scaffolds. Optimal spheroid size and number per scaffold compartment were established to ensure homogeneous scaffold coverage. Within five days of culture, spheroids disassembled and cells migrated along the aligned fibrils, showing strong infiltration and colonization of the scaffold architecture. Prolonged cultivation (up to 21 days) under both growth and myogenic differentiation conditions resulted in high cellular viability, significant proliferation (as evidenced by DNA content), and enhanced myotube formation. Immunostaining and gene expression analyses confirmed myogenic differentiation, with aligned myotubes expressing myosin heavy chain and elevated levels of myogenic regulatory factors MyoD1 and myogenin, particularly under differentiation conditions. SEM and FIB-SEM imaging further corroborated the formation of tissue-like structures, with cells forming dense, longitudinal bundles throughout the scaffold matrix. Notably, the combination of spheroid-based seeding and aligned nanofibrillar scaffolds led to significantly improved myotube length and width compared to conventional single-cell seeding methods. This work demonstrates the feasibility of using synthetic, collagen-mimicking nanofibrillar scaffolds in conjunction with bioassembled myoblast spheroids to recapitulate key features of skeletal muscle tissue. The melt electrofibrillation approach not only provides precise control over scaffold geometry and fiber alignment but also offers scalability, reproducibility, and mechanical robustness. Altogether, our results underscore the potential of this system as a versatile platform for bottom-up muscle tissue engineering and biofabrication of other anisotropic tissues. Future investigations may expand this model to human muscle cells and further explore its translational relevance for regenerative medicine applications.

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Fibrous bioinks for the bioprinting of anisotropic scaffolds with micro- and nano-scale organization for in vitro skeletal muscle engineering

Introduction
The functionality of skeletal muscle tissue (SMT) hinges on its hierarchical anisotropic microstructure. Conventional 2D cell cultures are poorly biomimetic and unable to properly support in vitro engineering of SMT, triggering the research towards more reliable and predictive in vitro models [1]. Biomimetic fiber-based scaffolds provide topographical cues supporting SMT in vitro engineering, but optimal biomaterials, methods and technologies for a controlled 3D fiber organization are missing. In the biofabrication field, conventional bioinks lack the multiscale anisotropy and the structural fidelity required to guide muscle fiber alignment and maturation. This work addressed such limitations by designing 3D in vitro models of SMT using natural polymers and advanced fabrication techniques.

Methods
Next-generation fibrous bioinks, composed of fragmented, electrospun gelatin fibers (f) uniformly embedded in an alginate/gelatin hydrogel matrix (f-ALG/Gel) were designed. The hydrogel was crosslinked using calcium ions and microbial tranglutaminase. Bioink concentration and composition was optimized through accurate rheological and printability characterization as well as in vitro cell adhesion tests using C2C12 murine myoblasts. The effect of the anisotropic microenvironmental cuesof bioprinted structures on C2C12 cell morphology, phenotype and directional growth was quantified by immunofluorescence analysis. The pipeline for image analysis employed Gaussian filtering and CLAHE to enhance contrast and reduce noise while preserving edge information. Initial segmentation was performed using global thresholding, followed by morphological operations to refine detected structures. A watershed-based algorithm was applied to separate overlapping features, ensuring accurate identification of nuclei and fibers.

Results
Upon extrusion-based 3D bioprinting, shear-induced alignment of f-GFs enabled the fabrication of microfilament-based scaffolds with intrinsic uniaxial anisotropy. The resulting constructs exhibited high shape fidelity, viscoelastic properties, and physiologically relevant stiffness (Young's modulus: 16.1 ± 1.7 kPa). In vitro studies using C2C12 murine myoblasts demonstrated that the embedded f-GFs provided strong topographical guidance, enhancing cell alignment and myogenesis. After 14 days culture, the f-ALG/Gel scaffolds supported a 2.5-fold increase in myotube fusion index and length, alongside reduced angular dispersion, compared to control bioinks. These effects were achieved without the need for biochemical stimulation of cell differentiation through a specific culture medium, underscoring the key role of hierarchical structural cues at the micro- and nanoscale on C2C12 differentiation and maturation.

Discussion
This work proposed a scalable, cell-compatible strategy to recapitulate the hierarchical organization of SMT through 3D bioprinted constructs, offering a new class of structurally instructive bioinks. In the future, the substitution of C2C12 cells with primary human myoblasts or myogenic cells derived from hiPSCs will allow the engineering of human-relevant SMT.
The platform holds broad potential for applications in regenerative medicine, skeletal muscle tissue modelling and the engineering of cultured meat.

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Continuous and Layerless 3D Printing of Aligned Tissues

Introduction:
Biofabrication of highly aligned 3D tissues like nerves, tendons, or muscles remains challenging due to insufficient scaffold cues to guide cell alignment, proliferation, extracellular matrix secretion, and maturation into functional tissues. Previously, we showed the ability to create such tissue constructs using filamented light (FLight) biofabrication approach1. We expanded the method by feeding photoresin continuously into a cuvette while exposing the resin to a speckled laser light projection (Fig. 1a), allowing us to create layerless filamented constructs on a larger scale and overcoming light scattering. This study further demonstrates the ability to encapsulate cells directly into the printed resin with high viability and culture them into aligned tissues.
Materials and Methods:
Gelatin-methacrylate (Gel-MA) with a 99% degree of functionalization was first dissolved in PBS to prepare a 5% w/v solution. Photoinitator (lithium phenyl (2,4,6-trimethylbenzoyl) phosphinate) and photoabsorber (Quinoline Yellow) were added to the Gel-MA solution to reach a final concentration of 0.05 w/v% and 0.0043 w/v%, respectively. C2C12 cells were mixed into the resin at a concentration of 106 cells/ml. The constructs were printed using a 405 nm laser integrated into the custom FLight printer. The resin was extruded constantly at a rate of 11.6 mm3/s at a laser intensity of 34 mW/cm2 into a cuvette with a cross-section of 100 mm2. The light was patterned using a 0.65-inch DLP® chip with a resolution of 1920x1080 pixels. The constructs were cultured in growth media for 7 days and then switched to differentiation media for 5 more days, after which the samples were stained with Hoechst (Nuclei), MF20 (Myosin Heavy Chain), and phalloidin (F-actin) to visualize alignment. Cell viability and cell count were assessed using Propidium Iodide, Calcein AM, and Hoechst staining on days 1,3, and 7.
Results and discussion
4 cm long constructs were printed with C2C12 cells. After 12 days of culture, fusion of cells into contractile myotubes was observed, which were aligned along the filament direction (Fig 1b). Furthermore, this behaviour was found in three different regions of the graft, the top (I), the middle (II), and the bottom (III). The cell viability (Fig 1c) dropped to 70% on the first day after printing, however, it recovered to 90% on day 7. Lastly, we found that the compressive modulus of the printed gel can be modified by varying the energy density delivered by the laser light (Fig 1d) to match material stiffness or even create gradients.
Conclusion
We demonstrate that our biofabrication technique can print cellular constructs with high cell viability. Furthermore, the hydrogel scaffold aids the encapsulated cells in aligning and proliferating along the printed cues, supporting the fusion into myotubes. Lastly, we demonstrate that we can control the stiffness of the printed hydrogels, allowing us to modify cell confinement for the best cell differentiation.

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Advancing Anisotropy in Engineered Skeletal Muscle via Bioactive Porous Hydrgel Interface

Skeletal muscle exhibits a highly organized, anisotropic architecture, where fascicles - bundles of aligned myofibers - are embedded within a connective tissue matrix. Such structural organization is the key for skeletal muscle achieving uniaxial contractions, mechanical stability, and functional performance in vivo.
The presented engineered skeletal muscle (eSM) platform aims the development of biomimetic fascicles through the co-culture of myoblasts and fibroblasts, integrated via a novel aqueous two-phase system (ATPS)-based hydrogel interface formulation. Highly anisotropic in vitro skeletal muscle fascicles were biofabricated using a stable ATPS biomaterial ink for the shell region and a fibrin-based bioink for the core, via rotary wet spinning (RoWS)1,2. The ATPS composition was specifically designed to entrap thermoresponsive hydrogel droplets (THD) within a fast-crosslinkable alginate matrix during fiber formation, allowing THD sacrificial removal under incubation conditions. Upon incubation, the THDs gradually dissolve, creating a porous shell network. This porosity improves nutrient diffusion and metabolic waste removal, and more importantly, it enhances biochemical crosstalk between the external environment and the encapsulated myogenic cells in the fiber core.This porous infrastructure not only facilitates mass transport but also establishes a biologically active interface that supports signal exchange and cellular communication.

The physiologically relevant biocomposition of fascicles was developed by introducing a co-culture system. Myoblasts were embedded within the fibrin-based fiber core, while fibroblasts were seeded onto the porous outer surface of the fibers. This design mimics the native distribution of myofibers encased in a collagen-rich extracellular matrix produced by fibroblasts. The interplay between myoblasts and fibroblasts promotes both myogenic differentiation and matrix remodeling, thereby recapitulating the organization and mechanical support observed in native muscle. Immunofluorescence and proteomic analysis of myogenic markers and extracellular matrix proteins was used to evaluate fascicle maturation and validate the biomimetic co-culture strategy.
Our robust and tunable strategy for the development of highly anisotropic in vitro skeletal muscle models emphasizes biochemical integration, architectural fidelity, and functional maturation. By leveraging ATPS-enabled porosity and co-culture dynamics, our eSM model advances current paradigms in skeletal muscle biofabrication, with implications for biorobotics, cultured meat development, disease modeling, drug screening, and regenerative therapies.

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Hierarchical Structures Biofabricated from Ultrathin Aligned Collagen Membranes Provide Physical Barriers for Molecular Transport

Barrier tissues in the body often form tubular structures that regulate molecular and water transport to maintain physiological function. We present a hierarchical biofabrication strategy to engineer such structures with tailored barrier properties, using ultrathin, wet-spun collagen membranes exhibiting high fibrillar alignment, compaction, and a Darcy permeability of 3.84×10^{-16} m². By stacking or rolling a defined number of membrane layers, followed by drying and genipin cross-linking, structures with tunable transport and mechanical properties are created. A case study demonstrates the design of a collagen-based tube mimicking the dimensions and permeability coefficient of the rat common bile duct. Ex vivo measurements showed a native bile duct permeability coefficient of 4.5×10^{-4} cm s-1 for sodium deoxycholate, a toxic bile acid. To match this while allowing for degradation over two weeks and a safety margin, we fabricated 30-layer collagen tubes (0.7 mm inner diameter, 0.05 mm wall thickness, 12 mm length), achieving up to 240 kPa burst pressure and 70 N mm-2 suture retention strength. The initial permeability coefficient was 0.27×10^{-4} cm s-1, with a Darcy permeability of 0.44×10^{-16} m², effectively limiting bile acid and water leakage. This approach offers a versatile platform for engineering extracellular matrix-based barriers for diverse biomedical applications.

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Konuşmacılar: Axel Guenther (University of Toronto)

- S11a Biofabrication of Soft Tissues Mikołajska

Toplantıyı düzenleyenler: Efsun Şentürk (La Sapienza), Vijayavenkataraman Sanjairaj (New York University Abu Dhabi)

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Biofabrication Strategies for Oral Soft Tissue Regeneration

Gingival recession, a prevalent condition affecting the gum tissues, is characterized by the exposure of tooth root surfaces due to the displacement of the gingival margin. This presentation will emphasize the critical considerations in gingival tissue engineering leveraging on cells, biomaterials, and signaling factors. Successful tissue-engineered gingival constructs hinge on strategic choices such as cell sources, scaffold design, mechanical properties, and growth factor delivery. Recent biofabrication technologies like 3D bioprinting, electrospinning, and microfluidic organ-on-chip systems hold the potential to help elucidate precise control over cell arrangement, biomaterials, and signaling cues. These technologies empower the recapitulation of microphysiological features, enabling the development of gingival constructs that closely emulate the anatomical, physiological, and functional characteristics of native gingival tissues. Further, the parallels between the skin and gingival tissues will be highlighted, exploring the potential transfer of biofabrication approaches from skin tissue regeneration to gingival tissue engineering.

Konuşmacılar: Marco Bottino (University of Michigan School of Dentistry (U-M))

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Challenges and Advances in the Biofabrication of Adipose Tissue

Adipose tissue is a crucial organ involved in energy metabolism, endocrine signaling, and immune regulation, making it a key target for regenerative medicine, disease modeling, and cultured meat applications. While traditional tissue engineering approaches have made significant progress in adipose tissue reconstruction, the biofabrication of functional and physiologically relevant adipose constructs remains challenging. Key obstacles include the need for standardized, animal-free culture conditions, the maintenance of mature adipocytes in vitro, and the development of bioinks that support adipocyte viability and function.

Compared to more structurally robust tissues such as bone or cartilage, soft tissues have been explored in biofabrication at a later stage, likely due to their distinct mechanical properties, which pose specific demands on the biomaterials and processes used. To address these challenges, various groups have developed bioinks based on decellularized extracellular matrix (dECM), leveraging its tissue-specific composition to enhance cell compatibility and functionality.

Recent advancements in biofabrication offer new solutions to these challenges. We have explored various strategies, including the bioprinting of adipose-derived stem cells (ASCs) and mature adipocytes using extrusion-based techniques and methacrylated gelatine or gellan gum bioink. Notably, we have demonstrated the successful printing of mature adipocytes, overcoming one of the major limitations in adipose tissue biofabrication. Additionally, our work focuses on the development of defined, serum-free media formulations to improve long-term cell viability and differentiation. To enhance tissue maturation and function, we are implementing ASC spheroid printing approaches, leveraging their intrinsic ability to self-organize into adipose-like structures.

By integrating controlled biofabrication strategies with optimized culture conditions, these advances contribute to the development of reproducible, scalable, and animal-free adipose tissue models. Such approaches not only support applications in regenerative medicine and disease modeling but also provide valuable insights for alternative meat production.

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Konuşmacılar: Petra Kluger (University of Stuttgart and Reutlingen University)

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Advanced functional 3D bioprinted in vitro model of cortical brain-like tissue

The intricate architecture of the human central nervous system (CNS) presents significant challenges in neuroscience in developing in vitro models that accurately replicate its structure and function under both physiological and pathological conditions. The brain's highly organized and layered regions, each characterized by distinct cellular phenotypes and extracellular matrix (ECM) compositions, are particularly difficult to reproduce in terms of compartmentalization and functional connectivity. In vitro neural models are fundamental for understanding CNS function at the microscale. To develop more physiologically relevant models, innovative technologies are being explored to better mimic in vivo cellular environment and the complexity of brain tissue. In this context, 3D bioprinting has emerged as a leading technique to construct tissue-like architectures that support multicellular organization and enable modeling cell-cell and cell-matrix interactions [1]. At the microscopic level, one of the main challenges lies in identifying materials that can mimic the brain's ECM and providing the mechanical and biochemical cues necessary to support the formation and the maturation of 3D neuronal networks [2].
This study focuses on the development of a chitosan-based bioink suitable for 3D bioprinting of cell-laden constructs embedding different neural cell types to create functional, compartmentalized neuron-glia networks in vitro. Chitosan, a biopolymer known for its similarity to native ECM components, as well as for its low cost and versatility, was selected for its favorable properties in promoting cell adhesion, neuronal growth, and the maturation of neuronal networks derived from human-induced pluripotent stem cells co-cultured with astrocytes [3-4]. The bioink's rheological and mechanical properties were characterized to ensure optimal performance for extrusion-based 3D printing and to evaluate stiffness. The printing protocol and parameters were optimized to enable efficient bioink deposition, resulting in multicompartment personalized constructs with good resolution and shape fidelity. Complex 3D models were fabricated by embedding human-derived cortical glutamatergic and gabaergic neurons and astrocytes, into spatially defined and personalized arrangements. The constructs were functionally and morphologically characterized using high-density micro-electrode arrays and immunocytochemistry technique, respectively, to assess cell distribution and functional neuronal network maturation.
The developed bioink exhibited excellent printability, with shear-thinning behavior and rapid gelation, that ensured post-printing structural stability and preserved good cell viability. Mechanical testing showed that the printed constructs had stiffness values comparable to those of native brain tissue. The bioprinted constructs maintained high shape fidelity with respect to complex geometries. Functional and morphological analyses revealed homogeneous cell distribution and the formation of functional neuronal networks under long-term culture conditions.
In conclusion, this work demonstrates that the developed chitosan-based bioink and the optimized bioprinting protocol enable the fabrication of functionally compartmentalized 3D neural constructs with precise control over the spatial distribution of neural cells. These models closely mimic the structural and functional organization of brain tissue, offering a promising platform for CNS modeling and in-depth studies of cellular behavior, network connectivity, and intercellular communication.

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Ink-jet Bioprinting of Functional Pancreatic Islets for Diabetes Modeling and Transplantation Applications

Introduction
Diabetes is a chronic, globally prevalent disease characterized by impaired pancreatic islet cell function. Existing treatments, such as pancreatic islet transplants or exogenous insulin, do not fully restore physiological pancreatic function. This has prompted the search for alternative therapies. Implementing 3D bioprinting of functional pancreatic organoids that mimic native islet architecture and function can improve disease modeling (e.g., diabetes, pancreatic cancer), toxicity assessment, and drug testing without animal use. These models also hold promise for replacement therapy, reconstructing damaged tissues rather than only treating symptoms. In the future, commercial cells may be replaced by patient-derived ones for personalized models and therapy testing. This represents a major opportunity for bioengineering advancement.
The study aimed to evaluate the functionality of 3D printed pancreatic organoids intended to mimic islets of Langerhans..
Methods
Bioprinted 3D pancreatic organoids were made using electromagnetic inkjet printing technology. The bioink used for printing was based on hydrogels derived from methacrylated gelatin (GELMA) and hyaluronic acid (HAMA) and a cross-linking agent (LAP). A cell suspension from four cell lines was added to the bioink, these were the following cell lines: alpha cells (αTC-1) and beta cells (βTC-tet) of pancreatic islets, endothelial cells (HUVEC) and fibroblast cells (L929) in different concentrations depending on the proposed variant. Six proposed variants of tested organoids:
V1:αTC-1:βTC-tet (ratio 1:2),
V2:αTC-1:βTC-tet (ratio 1:3),
V3:αTC-1:βTC-tet:HUVEC(ratio 1:2:3),
V4:αTC-1 βTC-tet:HUVEC (ratio 1:2:1),
V5:αTC-1:βTC-tet:HUVEC: L929 (ratio 1:2:1:2),
V6:αTC-1:βTC-tet:HUVEC : L929 (ratio 2:4:1:1).
The organoids were cultured in standard conditions for 28 days. Biological analyses determining the functionality of the models consisted of: assessment of viability (FDA/Pi method), microscopic evaluation, H&E staining and immunohistochemistry (glucogon, GSIS (insulin), CD31, vimentin) and immunohistochemical tests.
Discussion
The obtained results demonstrate that 3D bioprinted organoids have significant potential in tissue engineering, especially for supporting diabetes patients by mimicking native islet functions. They create functional islets with insulin-producing β cells, reflecting natural structures. This is a major breakthrough, as previous animal models are limited and classical cell cultures fail to replicate complex tissue architecture. Organoids allow for more realistic testing and will enable personalized medicine by transforming pluripotent stem cells (iPSCs) into α, β, endothelial, and fibroblast cells of a patient. This supports precise disease modeling and therapy testing—a major advance in precision medicine.
Results
In all variants (V1-V6) of bioprinted 3D pancreatic organoids their viability was confirmed for 28 days. In the case of V5 and V6, the earliest spheroid formation was observed (7 days of the experiment). Immunohistochemistry of preparations from individual variants showed the activity of α, β cells, endothelium and fibroblasts. Histological imaging

showed that in the tested models spheroids are spontaneously formed from alpha and beta cells.
Additionally, the GSIS test showed insulin secretion in all tested variants, the highest concentration on day 28 was shown by V6 (170 ng/ml for high glucose). For variants that did not contain endothelium and fibroblasts (V1 and V2), half the secretory value of β cells was observed. The organoids are functional and show the activity of pancreatic islets.

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Impact of laminin variants on neuronal network formation in 3D spinal cord neuron-astrocyte co-cultures

The extracellular matrix (ECM) of the central nervous system is a specialized, ultra-soft structure that provides crucial biochemical and mechanical support for neuronal survival, differentiation, and synaptic connectivity. It consists of a network of biomolecules, including glycosaminoglycans like hyaluronic acid, as well as structural and adhesive proteins such as laminins. Laminins play a pivotal role for spinal cord neurons by promoting cell adhesion, neurite outgrowth, and synapse formation, making it essential for proper neuronal network formation. Laminin-111 is expressed during embryonic development and is involved in axonal guidance and synapse formation. Laminin-211 and 221 are expressed in the neuromuscular system, and laminin-211 enhances neuronal differentiation and survival, while laminin-221 promotes motor axon outgrowth and cell adhesion.[1] Astrocytes, are regulators of the ECM, they modulate its composition by secreting ECM components, including laminins, and influence neuronal adhesion and connectivity through biochemical signaling and direct interactions.
We previously developed a three-dimensional (3D) spinal cord model using MEW-printed scaffolds as reinforcing structures, combined with Matrigel as commercially available ECM.[2] This study demonstrated the importance of the third dimension for neuronal models as not only protein expression differed compared to 2D, but also functional activity in the developed 3D samples started much earlier. To overcome the Matrigel limitations of batch-to-batch variations due to mouse sarcoma identity, and to improve our model we shifted to a thiolate hyaluronic acid (HA-SH)-polyethylene glycol (PEG) hydrogel, which provides a biocompatible ECM-like environment. Previous studies could already show that HA-SH hydrogels, included astrocytes and reinforced with PCL scaffolds successfully supported the network formation of cortical neuron.[3, 4] In addition to a hyaluronic acid based ECM including astrocytes, we added the three mentioned laminin isoforms into our system.
The study investigates how astrocytes and ECM molecules like laminins influence the network formation and function of spinal cord neurons. We compared three conditions: spinal cord neuron-only cultures, spinal cord neuron-astrocyte co-cultures, and laminin-supplemented spinal cord neuron-astrocyte co-cultures. Cell viability was assessed using live/dead assays, while immunocytochemistry was employed to analyze neuronal morphology and synaptic organization. Neuronal network activity was examined via calcium imaging, and the mechanical properties of the constructs were characterized through cyclic compression and tension tests. This model provides a platform to investigate how astrocytes and ECM composition influence spinal cord network formation in a 3D environment. 3D models of the spinal cord will be of interest in future studies to model e.g. neurodegenerative disease mechanisms or in testing therapeutic strategies.

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Towards a magneto-active melt-electrowritten fiber scaffold approach for skeletal muscle engineering

Introduction
Duchenne muscular dystrophy (DMD) results from mutations in the DMD gene, leading to the absence of dystrophin protein and fatal skeletal muscle wasting. Currently, no approved treatments address DMD's root cause. An in vitro skeletal muscle model would provide a platform to study cause and develop therapeutic strategies. However, existent models fail to recapitulate the genetic and pathophysiological characteristics of human skeletal muscle disorders, partially due to the lack of 3D matrices that: 1) offer anisotropic mechanical cues to guide neo-tissue formation by skeletal muscle cells; and 2) enable contactless mechanical stimulation. Here, we present novel magnetoactive 3D fiber matrices that adress these challenges.
Methods
To establish a material that can achieve contactless mechanical stimulation, ferromagnetic powder (MQFP™, Magnequench) was melt-mixed at 80°C with Polycaprolactone (PCL) at concentrations of 0% (MQFP0-PCL), 10% (MQFP10-PCL) and 20% (MQFP20-PCL) w/w, compositions were evaluated for magnetic behavior using by Superconducting Quantum Interference Device (SQUID), and for suitability for melt-electrowriting (MEW) using Small amplitude oscillatory shear (SAOS) test. An in-house MEW system was used, and two sinusoidal microgeometries - "unequally spaced pores" (600x300µm) and "equally spaced pores" (300x300µm), fiber matrices were fabricated using MQFP0-PCL. Mechanical properties of sinusoidal scaffolds and respective control microgeometries were assessed under uniaxial tensile testing. C2C12 myoblasts were statically cultured in 2D wells and 3D scaffolds until differentiation and analyzed via immunostaining and CellProfiler.
Results
SQUID magnetometry results (Figure 1A), show an increasing magnetic moment relationship between MQFP10-PCL (2×10^{-9} A·m²/mm) and MQFP20-PCL (6×10^{-9} A·m²/mm). SAOS measurements indicate compatibility of MQFP-PCL for melt-electrowriting. Increasing MQFP in PCL enhances elastic energy storage. Furthermore, as temperature rises from 100°C to 120°C, complex viscosity (η*) decreases promoting easier processing. Uniaxial tensile test of MQFP0-PCL fiber scaffolds show that leveraging sinusoidal microgeometries unlocks elasticity out of inherently plastic material. Figure 1B tensile tested MQFP0-PCL sinusoidal scaffolds with a total thickness of ≈84µm yielded up to ≈54% strain, and an elastic modulus as low as ≈7,55 MPa, resulting in significantly greater yield strain in addition to a lower tensile moduli than controls (i.e.,square and rectangle). To evaluate anisotropic cell-material interactions immunostaining analysis of myosin heavy chain 4 revealed that 3D sinusoidal fiber matrices influence the alignment and differentiation of C2C12 cells into myotubes along the microgeometry (Figure 1C), while the 2D group displayed disorganized myotubes. The fusion index, used to assess differentiation efficiency, showed no significant difference between 3D groups.
Discussion
MQFP-PCL thermo-rheological compatibility for MEW of organized fiber scaffolds in combination with their magnetic responsiveness display promise for creating adequate stimulation platform to promote skeletal muscle growth and maturation. Melt-electrowritten MQFP0-PCL sinusoidal fiber scaffolds were able to promote the differentiation and organized guidance of C2C12 skeletal muscle myotubes, yet, fusion index results indicate that further differentiation is needed.
Conclusions
Melt-electrowritten sinusoidal fiber matrices were able to modulate the mechanical behavior of MQFP0-PCL scaffolds and guide organization of skeletal muscle microtissue in vitro. Based on these findings, next we intend to magnetically characterize MQFP-PCL to achieve contactless mechanical stimulation, thereby enhancing tissue maturation and differentiation efficiency.

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Dynamic control of the microenvironment enhances tissue maturation

INTRODUCTION
Although a complete understanding of the mechanisms by which ECM stiffness impacts cellular development has not been fully achieved, the biomechanical properties of ECM have been shown to play a significant role in regulating cell proliferation (Mih et al. 2012), migration (Ehrbar et al. 2011), and differentiation (Han et al. 2020) among other phenomena. Furthermore, the ever-changing needs of cells and tissues during development and maturation are reflected in the dynamic nature of their ECM. An ongoing challenge in tissue engineering is that the pared-down complexity present in engineered tissues is generally insufficient to recapitulate these complex, spatiotemporal cell-ECM dynamics. Recently though, we have demonstrated the use of oxidized sucrose (SOx) as a cytocompatible small molecule that can be deployed at different time points during tissue maturation to dynamically modulate the ECM (Silberman et al. 2023).
Here, we show that tissue development and maturation can be controlled by introducing SOx at different time points according to tissues' developmental needs. In particular, we have shown that deploying SOx too early impaired cells' ability to remodel their own microenvironment and to establish essential cell-cell and cell-ECM interactions. By encapsulating cells in a soft hydrogel matrix and dynamically modulating the biomechanical microenvironment to match the cells' evolving needs, on the other hand, we enhanced cell and tissue maturation.

METHODS
Oxidized sucrose (SOx) was synthesized as previously reported (Silberman et al. 2023). Endothelial cells (ECs) and cardiomyocytes (CMs) were encapsulated within an ECM-based hydrogel (Shevach et al. 2015, Edri et al. 2019) and cultured under static conditions. Either on Day 0 or at specified time points during tissue maturation, SOx was added to the culture, incubated overnight, and removed the following day. Tissue morphology, maturation, and functionality were assessed at the end of the culture period.

RESULTS & DISCUSSION
Most significantly, we observed that deploying precisely the same concentration of SOx to manipulate the ECM at different stages of a tissue's maturation led to markedly different outcomes. When exposed to SOx prematurely, ECs failed to organize into discernible blood vessels. ECs cultured in microenvironments dynamically modulated to regulate their ever-changing needs, on the other hand, generated clear capillary networks. Moreover, the timely addition of Sox induced the formation of thicker, more mature blood vessels than was possible to achieve without dynamic tissue modulation. These same observations, that timely SOx deployment is associated with a more mature phenotype, were also evident when working with CMs. Premature deployment led to a lack of synchronization of cellular contractions, while timely deployment led to more powerful cardiac contractions. This work thus demonstrates both why the ability to dynamically control the biomechanical properties of the tissue during maturation is an essential requirement for advancing tissue maturation, and it represents a significant step to achieving more accurate in vitro recapitulation of these complex processes vital for tissue engineering.

Konuşmacılar: Eric Silberman (Tel Aviv University)

- S13 Biofabrication strategies for engineering microarchitectural complexity Kisielewski

Toplantıyı düzenleyenler: Khoon Lim (University of Sydney), Shrike Zhang (Harvard University)

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4D Bioprinting Shape-Morphing Heart Tissues: Sculpting Structure and Guiding Maturation

Bioprinting technology holds tremendous potential for developing artificial tissues and organs that mimic the complexity of their native counterparts. However, despite considerable progress in the field, bioprinted tissues are structurally and functionally immature compared to their native counterparts. This limits their effectiveness as implants for regenerative medicine or predictive platforms for drug screening.

This talk will cover recent advances in my group on creating developmentally-inspired bioprinting strategies that can enhance the structure and function of bioprinted iPSC-derived heart tissues. The presentation will first describe the use of embedded bioprinting in granular support hydrogels to create tissue models that undergo predictable and programmable 4D shape-morphing driven by cell-generated forces. This section will also explore how such 4D shape-morphing behaviours can impact microarchitectural complexity of the developing tissues, including the emergence of global cell and ECM alignment. Next, the talk will describe more recent work on engineering phototunable granular support hydrogels that can control tissue morphogenesis. Finally, the talk will cover work on developing AI-powered bioprinting systems with enhanced precision and reproducibility.

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Conformal In-Situ Bioprinting of Bi-phasic Jammed Bioinks, Independent of Gravity, Orientation, and Curvature

Rapid in situ bioprinting on complex, human-scale anatomical surfaces remain a key challenge for clinical translation. Here, we present a gravity-independent, conformal bioprinting strategy using bi-phasic granular bioink and multinozzle printheads capable of adapting to arbitrary surface curvatures. The bioink comprised of jammed gelatin microgels suspended in a fibrinogen matrix exhibits yield-stress behavior to maintain shape fidelity after extrusion while supporting cell viability and proliferation. Two monolithic multinozzle printhead architectures with identical bioink delivery networks were evaluated: (1) a rigid configuration for handheld bioprinting and (2) a soft robotic variant capable of real-time curvature adaptation via pneumatic actuation. Microgravity experiments aboard a parabolic flight confirmed successful bioink deposition under ~0 g conditions. A ladder-rung microfluidic architecture ensured uniform bioink delivery across printhead nozzles, improving deposition consistency. In situ bioprinting on anatomical facial phantoms confirmed conformal, high-throughput (deposition at 20 mm·s-1) deposition of bioink over physiologically relevant curvatures, both with and without cells. Cell-laden constructs retained >85% cell viability post-printing and supported proliferation. This work introduces a scalable bioprinting platform suitable for clinical, remote, and deep-space environments, enabling autonomous tissue fabrication. The curvature-adaptive printhead advances current in situ bioprinting capabilities, facilitating the generation of personalized grafts with complex anatomical geometries.

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Engineering of cancer microenvironments with embedded interconnected microcapillary networks via in vitro magnetic assembly of endothelial-cell 'seeds'.

Introduction
Despite significant developments in endothelial-cell (EC) manipulation techniques, a proper in vitro model of a functional microvasculature with controlled local interconnectivity under well-defined global architecture is still lacking. Here, we report the generation of such controlled multi-scale vascular networks via manipulation of tens of sprouting EC 'seeds'. We exploit magnetic patterning to assemble EC-coated superparamagnetic microbeads into ordered arrays and establish effective growth rules governing the development of interconnectivity and directionality of the networks depending on the applied seed-seed spacing.

Methods
Our microtissue-assembly method relies on the use magnetic hedgehog-like templates—arrays of permanent neodymium micromagnets arranged underneath a cell culture chamber—which guide the assembly of paramagnetic EC-coated beads on the fibrin-filled chip into a pre-designed quasi-2D pattern. Under long-term culture, the EC-seeds develop radial protrusions or 'sprouts' extending into the surrounding extracellular-matrix(ECM)-mimicking fibrin hydrogel and interconnect to form a percolated network. We use a numerical workflow that we introduced previously [1] to further develop a dedicated custom image processing software allowing multi-parametric morphological and topological characterization of the system of multiple sprouting and interconnecting microvasculatures.

Results
We demonstrated that the spatial organization of the microvasculature can be controlled at both microscopic and mesoscopic level via directed-assembly of EC-microcarriers. We use our system to establish a critical seed-seed spacing below which the neighboring microvasculatures become interconnected, and above which they remain disconnected, even at late times of culture. In the latter case, they can be treated as practically independent which allows systematic monitoring of multiple EC-seeds developing in nearly identical conditions. Developed microvascular networks exhibit characteristics of mature, lumenized vessels. We show that the microvascular arrays co-cultured, e.g., with cancer cells (HeLa in our case), can efficiently serve as a high-throughput platform for functional high-content screening of various anti-angiogenic compounds in full 3D microenvironment. In this respect, our system allows the measurement of a range of morphological and network-topological parameters unavailable with more conventional angiogenesis or vasculogenesis assays.
We showed that the EC-seed-based approach offers a range of advantages over conventional EC-manipulation techniques including: (i) expedited sprouting, (ii) spatial control over interconnections, (iii) reduction in cell consumption by >100x, and (iv) native high-throughput format. We validated our method using both human umbilical vein endothelial cells (HUVECs) as well as human induced pluripotent stem cells (hiPSC)-derived endothelial cells.

Discussion
The magnetic field-driven pre-patterned microvascular arrays offer a uniquely precise and standardized vascular-microtissue engineering tool with broad applications, ranging from angiogenesis research to high-throughput drug testing, and with possible extension to organ-on-chip and tissue-regenerative approaches.

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Integrating Molecular Self-Assembly and Continuous Fibre Extrusion to Fabricate Robust Microenvironments with Tunable Nanofibrous Architecture

Self-assembling peptide amphiphiles (PAs) offer a unique combination of biofunctionality, structural tunability, and nanofibre alignment under shear, making them highly promising materials for advanced in vitro tissue models. Despite this potential, their inherent fragility and the lack of scalable structuring strategies have restricted their wider adoption for in vitro modelling. To address these challenges, we present a novel platform that merges molecular self-assembly with top-down processing - integrating peptide nanofibre gelation with continuous fibre-based extrusion. This enables the scalable creation of robust, hierarchically structured hydrogel coatings with controlled nanostructural alignment, organised concentrically around a mechanically supportive core.

Our system employs a custom-designed, modular extrusion platform featuring a stereolithography (SLA)-printed coaxial nozzle. A syringe pump delivers precise control over peptide solution flow, while a motorised drive module advances a central thermally drawn PLA optical fibre (225 µm diameter). During the process, the peptide amphiphile solution - formulated with thermally annealed E3 (C_{16}–$V_3A_3E_3$) at 2 wt.% - is deposited around the moving fibre and extruded into a calcium chloride bath, where calcium ions rapidly induce gelation. This continuous coaxial setup allows simultaneous alignment of the nanofibre network and solidification of the coating layer.

A key innovation of this approach is the use of power-law rheology in shear-thinning PA solutions to modulate alignment within the gel. By tuning flow rate and fibre velocity, we demonstrate control over shear stress, which directly influences the orientation of nanofibres across the hydrogel thickness. Lower extrusion speeds result in weakly aligned surface layers, while higher speeds generate fully aligned coatings, as confirmed via birefringence imaging (Figure 1). The resulting ~80 µm thick coatings are uniform, reproducible, and stable, with mechanical integrity provided by the inner fibre core.

The suspended fibre geometry supports advanced imaging and characterisation. Using rotationally resolved birefringence microscopy, we map the geometry and internal alignment of the gel layer in 3D. These experimental results are supported by rheological characterisation and will inform computational fluid dynamics (CFD) simulations to establish predictive links between process parameters and structural outcomes.

Preliminary biological validation involved seeding primary mouse cortical neurons onto the coated fibres. Cells exhibited strong attachment and healthy morphology, suggesting excellent compatibility of the aligned peptide hydrogel environment with neuronal culture. This confirms the platform's potential as a next-generation neural model, capable of supporting further development into electrically or chemically stimulated systems.

By bridging bottom-up self-assembly and top-down processing, this fibre extrusion system represents a major step forward in structuring bioactive, aligned hydrogels for tissue engineering. It offers a robust, scalable solution to the key limitations of fragile peptide hydrogel systems while introducing a new approach for using flow dynamics to guide nanostructural assembly. Our future work will explore how alignment and stimuli from the central fibre can modulate neuronal behaviour, enabling the creation of smart, bioactive models for neuroscience and regenerative medicine.

Attached Figure 1: Birefringence images of PA Gel coated optical fibre at 5mm/s (a), 6mm/s (b), and 8mm/s (c), showing increased aligned domains with extrusion speed.

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Biofabrication of the cartilage microtissues: Understanding of collagen type II organization in different shapes

Purpose

The primary role of articular cartilage (AC) is to provide frictionless joint movement while transferring loads to the underlying bone [1]. Thanks to its unique hierarchical arcade-like collagen type II fiber organization, AC withstands extreme mechanical forces. While numerous regenerative medicine approaches strive to replicate the native architecture of AC, none have achieved reproduced this characteristic structure. This study explores collagen type II orientation in engineered microtissues to further understand driving forces for collagen type II alignment.

Methods and Materials

Negative molds of different geometrical shapes (circular, triangular, square) were fabricated in different diameters (diameter =150, 200, and 300 µm) using the stereolithography 3D printing technique (SLA, Formlabs). Subsequently, microwells were prepared using 3D printed molds and agarose. Articular cartilage progenitor cells (ACPCs) were seeded into the microwells and cultured for 28 days. Cells were observed by light (Olympus BX-43) and fluorescence microscopy (Leica Thunder Live Cell Imager). Moreover, deposition of extracellular matrix proteins such as collagen type II and glycosaminoglycans were evaluated by (immuno)histochemistry and biochemistry. Also, samples were stained with picrosirius red and collagen fiber organization was observed with polarized light microscopy (Olympus BX-50, U-POT filter).

Results and Discussion

ACPCs self-assembled into cellular aggregates in geometries reflecting predefined shapes during 28- days cell culture period. Fluorescence images show the formation of the cellular aggregates in triangular, square and circular shapes with cytoskeleton morphology and collagen type II deposition on days 3 and 28 (Figure 1A). Histological evaluations confirmed the production of cartilaginous extracellular matrix, indicating collagen type II deposition (Figure 1B). Moreover, polarized light imaging revealed an initial circular alignment of collagen fibers, regardless of the predefined outer geometries. During maturation of the microtissues, collagen alignment became concentrated specifically at the outer edges of the predefined shapes (Figure 1B). Furthermore, upward tissue growth was observed in the vertical sections of histology slides (Figure 1C).

Consclusion

This study demonstrates a proof-of-principle of guided collagen type II deposition through ACPCs differentiation within predefined shapes, providing insights into how this shape-driven tissue formation can be adopted in biofabrication strategies to engineer functional and long-lasting AC grafts.

Acknowledgements

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Harnessing Geometry to Drive Tissue Formation in Three-Dimensions

INTRODUCTION: The biological world is curved from the subcellular to the continental length scale [1]. Cells sense the complex shapes of their surroundings and respond to these stimuli through the transduction of physical stimuli into biochemical responses. In vitro, designed, cell-scale 2.5D curvatures have been shown to drive cell migration responses [2]. However, synthetic polymer biomaterials with a stochastic microporosity (e.g. those fabricated by emulsion templating or particulate leaching) often show limited cell infiltration into the bulk of the material without surface chemistry modification [3]. Little has been done to translate the physical influence of defined cell-scale curvatures into biomaterial design as a strategy to improve cellularisation rate of 3D scaffolds, partly due to manufacturing technologies previously being too slow to make this a realistic proposition.
Here, we screen a range of cell-scale three-dimensional porous shapes in vitro for their ability to drive mesenchymal stromal cell (MSC) ingrowth. The optimal design is then tessellated into a 3D scaffold for in vitro bone cell culture and implanted subcutaneously to confirm whether these effects are conserved in vivo.
METHODS: Multi-photon polymerisation (MPP) was used to fabricate arrays of 100 µm diameter, wireframe mathematical solids with defined 3D geometries (Fig. 1), then quantitative confocal microscopy was used to identify designs that promote cell ingress across three donors of primary human MSCs. The optimum candidate was tessellated into scaffolds suitable for tissue engineering, assessing cell ingrowth in larger constructs and bone-like matrix deposition under osteogenic conditions. These scaffolds were

then implanted into subcutaneous pouches in Sprague-Dawley rats, histologically assessing ingrowth at days 7 and 28 in comparison to MEDPOR, a clinically available, porous surgical membrane, and a non-porous control.
RESULTS: Across three different synthetic polymers, DesignX demonstrated significantly greater cell infiltration MSCs by day 5, even after normalising for surface area, demonstrating the influence of shape. When tessellated into 3 × 3 × 0.5 mm scaffolds, the influence on cell infiltration was conserved, with DesignX demonstrating greater cellularisation across the full depth of the scaffold by day 5 than control architectures. When maintained in osteogenic medium, scaffolds supported the differentiation of MSCs to osteoblasts, with upregulation of alkaline phosphatase activity and significant deposition of mineralised extracellular matrix. DesignX scaffolds implanted subcutaneously and compared to MEDPOR surgical membranes and solid controls demonstrated superior cell infiltration in vivo by day 7, with evidence of new blood vessel formation around the implant.

DISCUSSION & CONCLUSIONS: This work demonstrates how, with the latest additive manufacturing technologies, we can harness the geometry of a cells 3D physical environment to mediate their response both in vitro and in vivo, paving the way to using bioinstructive shapes to achieve other desirable cell responses in tissue engineering, reducing our reliance on biochemical factors to achieve efficacy.

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Modular Assembly of Multi-Material Constructs via Acoustic Patterning and Filamented Light (FLight) Biofabrication

Light-based biofabrication techniques like filamented light (FLight) offer robust architectural alignment but are often limited in compositional complexity[1]. Conversely, acoustic assembly enables non-contact, label-free patterning of cellular spheroids or bioactive particulates, yet lacks architectural guidance at the microstructural level[2]. To achieve tissue functionality, such as muscle, nerve, and cartilage, the convergence of biofabrication technologies is essential for providing biochemical, mechanical, and architectural cues[3,4].

Here, we demonstrate a modular biofabrication platform that combines bulk acoustic wave (BAW)-based patterning with filamented hydrogels to create hierarchically organised, multi-material constructs. Using 1 mm-thick custom-made cassettes, ceramic particulates are acoustically patterned into defined geometries within various cell-laden hydrogel precursor solutions, including Gelatin, Gelatin methacryloyl (GelMA) and silk mixed with Ruthenium (Ru)/sodium persulfate (SPS). These cassettes were able to serve as an independent unit for spatially resolved biomaterial loading and cellular composition. Critically, distinct cassettes were selectively designed to include either aligned fibrous architectures (via FLight exposure) or bulk, non-aligned hydrogels, introducing deliberate regions of varied topography, biochemical and mechanical properties.
Following vertical assembly, constructs were photo-crosslinked with either laser wavelengths (401 nm or 526 nm) tailored for individual cassette or cross-cassette integration, respectively. This approach enabled precise modulation of filament orientation both within and across several layers, facilitating complex multi-material, multi-cellular environments within a single centimetre-scale construct.

Cell-laden constructs with human dermal fibroblasts and murine myoblasts (C2C12) showed excellent viability (>90%) over 7 days. Moreover, cells demonstrated robust alignment and elongation in FLight-aligned regions, while distinct morphologies and proliferation profiles were observed in non-aligned (bulk) hydrogel and ceramic particle domains, demonstrating effective tailoring of cell-matrix interactions.

This multi-modality strategy enables rapid fabrication of distinct modular engineered tissue units with customizable biochemical, mechanical, and architectural features into a single construct. The versatility to combine diverse biomaterials and structural cues within stackable units offers new opportunities for regenerating muscle-tendon interfaces, creating zonally organised cartilage, and developing physiologically relevant disease models.

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Konuşmacılar: Thomas Robinson (University of Sydney)
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An AI enabled (open-source), eye-in hand technology for robotic additive manufacturing towards the fabrication of complex tissue engineering scaffolds

Introduction
Over the past two decades, robotic additive manufacturing (RAM) has been introduced into fields such as aerospace and construction, and multiple studies have shown the potential of RAM for Regenerative Medicine (RM). However, hardware limitations of current industrial robots reduce the print resolution of small volume RAM hindering the wider adoption into RM. Eye-in Hand (EH) technology, where the robotic arm is equipped with a camera at the end-effector, has been proposed to improve the resolution of robotic arms. Recent studies have used EH robot for in-situ bio-printing1,2, but the possibility to fabricate complex volumetric scaffolds using this technology has not been explored enough. Here, we developed an EH enabled RAM system to demonstrate the potential of the technology to fabricate small scale tissue engineering scaffolds with complex architectures overcoming the robotic hardware errors inherent to current RAM systems.

Methods
RAM
The RAM system used in this study is based on a 7 degree of freedom robot (Xarm7) coupled with a conventional thermoplastic filament extruder. The integration of the robot and extruder was handled by an updated version of RAVEN3, which is an open-source RAM package based on Robot Operating System (ROS2) and Moveit2. A newly developed real-time closed loop control system based on Moveit servo package was integrated into RAVEN for making use of data generated by EH to correct position errors of the robot.

EH
The EH system consists of a black and white camera (B0332, ArduCam) operating at 100 frames per second mounted close to the extruder pointed towards the tip of the nozzle. A computer vision is based on a pre-trained deep Convolutional Neural Network model (YOLOv8, Ultralytics Inc), which was tuned to identify printed filaments in the images captured by the camera. This system identifies the position of the filament in real-time which is then communicated to the RAVEN package, which controls the robot.

Scaffold design
The positional error of the robotic hardware was most pronounced when the orientation of the end-effector was changing. This prohibits printing of non-planar branched structures in 3D where RAM has to join multiple segments printed with different orientation of the extruder. Henceforth, simple designs with non-planar branching paths were used to test the efficacy of EH based position correction for improving printability of branched structures.
Results and Discussions
A novel open-source EH-enabled RAM and a proof of concept, closed-loops error correction system was developed for printing 3D branched structures. The printability improvement of branched structures offered by this approach demonstrates the need for developing complete real-time closed loop control architectures for small scale RAM. Further, the new tools offered by artificial intelligence technologies could make intelligent/adaptive RAM more accessible and efficient for RM applications.

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Konuşmacılar: Vivek Cherian David (MERLN Institute for Technology Inspired Regenerative Medicine)

- S19 Complex architectures and non-planar approaches in Biofabrication Kruczkowski

Toplantıyı düzenleyenler: Pasquale Posabella (Warsaw University of Technology), Pierpaolo Fucile (MERLN Institute for Technology-Inspired Regenerative Medicine)

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Imperceptible augmentation of living systems with organic bioelectronic fibres

The functional and sensory augmentation of living structures, such as human skin and plant epidermis, with electronics can be used to create platforms for health management and environmental monitoring. Ideally, such bioelectronic interfaces should not obstruct the inherent sensations and physiological changes of their hosts. The full life cycle of the interfaces should also be designed to minimize their environmental footprint. Here we report imperceptible augmentation of living systems through in situ tethering of organic bioelectronic fibres. Using an orbital spinning technique, substrate-free and open fibre networks—which are based on poly (3,4-ethylenedioxythiophene):polystyrene sulfonate—can be tethered to biological surfaces, including fingertips, chick embryos and plants. We use customizable fibre networks to create on-skin electrodes that can record electrocardiogram and electromyography signals, skin-gated organic electrochemical transistors and augmented touch and plant interfaces. We also show that the fibres can be used to couple prefabricated microelectronics and electronic textiles, and that the fibres can be repaired, upgraded and recycled.

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Konuşmacılar: Shery Huang (University of Cambridge)

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Truly Omnidirectional 3D Printing

The first publication that presented embedded 3D printing was titled "Omnidirectional Printing". This term elegantly captured the ability to write non-planar and freeform filaments. However, the use of a support bath requires a physical bath to hold the gel, and allows entry only from the top. A truly omnidirectional method of 3D printing would enable printheads to construct parts from all angles, moving from bottom-to-top towards inside-out bioprinting. Here, I will present our collaborative work to create concentric tube robot 3D printheads that can operate through keyholes in the walls of a support bath. In doing so, it can write omnidirectionally with multiple printheads simultaneously.

Konuşmacılar: Mark Skylar-Scott (Stanford University)

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Multi-Axis Out-of-Plane Melt Electrowriting + Fused Deposition Modeling Printing for the Fabrication of Corneal Implants

Introduction: To this date, Melt Electrowriting (MEW) is primarily done on flat collectors for tissue engineering (TE) applications. There are no flat geometries in the body, so it is crucial to find ways to fabricate out-of-plane scaffolds that better conform to the shape of the targeted tissues. 6-axis robots have been used in MEW to move the printhead around a static collector for the fabrication of corneal implants [1]. There were several challenges behind this strategy, such as inaccuracies due to the polymer jet not being parallel to the field of gravity, instabilities of the electrical field and lack of repeatability. These issues prevented accurate fiber deposition. We have explored a 5-axis approach where the movement is provided to the collecting platform to maintain orthogonality between the printhead and the surface of the collecting substrate. As an example of this kinematic strategy, here we explore the fabrication of out-of-plane corneal implants. The standard thickness of MEW scaffolds (25-35 microns) provides insufficient strength to hold sutures, so we also explored the hybridization of MEW with Fused Deposition Modeling (FDM) to produce reinforced implants with thicker FDM-fiber borders.
Methods: The cornea is often perceived to be semi-spherical, however, it is a highly personalized convex surface. In addition to accurately rendering a patient's corneal shape, we wish to render pores with a small enough area that cells can fully spread across the boundary filaments (Fig. 1A). To accomplish these goals, we designed a MEW machine that features 5 axes of combined motion for the collector (XY, rotation around X and rotation around Z) and the printhead (Z) (Fig. 1B). For the fabrication of the corneal implant, Purasorb® PC 12 polycaprolactone pellets (Corbion, Netherlands) were melted for 1-hour prior to printing at 80°C and then extruded at a pressure of 0.33 Bar. The high voltage and translating speed of the collector were set to 5 kV and 22 mm/s, respectively. For the FDM borders, the extrusion rate and translating speed of the collector was adjusted so that a 0.4-mm thick fiber was printed. The toolpath design and postprocessing of G-Codes for both was done in Fusion 360 (Autodesk, California, US).
Results: Fig. 1C shows the out-of-plane corneal textile after printing, which contains the MEW layering of pores that will serve for cell attachment and proliferation. At the border of the textile, the thicker FDM fiber will serve as reinforcement for the retention of sutures.
Discussion: This work shows the fabrication process of personalized corneal implants using a novel multi-axis and hybrid automated approach. It also provides key aspects on MEW parameter optimization for out-of-plane printing and MEW and FDM compatible tooling design. Furthermore, we believe that this work will serve as an inspiration to the growing community of MEW enthusiasts to provide them with key insights that they can incorporate in their future work for the fabrication of scaffolds that are useful for TE applications.
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Konuşmacılar: David Dean (University of Wisconsin-Madison)

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A new Design Interface for Complex Biomimetic Scaffolds and 3D printing Optimization

Tissue Engineering (TE) and Regenerative Medicine (RM) seeks to mimic the complex structure and functionality of natural tissues, where directionally-dependent properties and negative Poisson's ratio behaviors are essential to guide cell migration and activity, ultimately leading to influencing tissue regeneration. These characteristics are fundamentally linked to fiber orientation and dimensions (such as those found in collagen matrices or muscular tissue) in the 3D space, which is non-planar by nature [1, 2]. The biofabrication of 3D porous scaffolds through Additive Manufacturing (AM) is often limited by traditional infill patterns, which prevents the mimicking of complex internal pore architectures needed for biomimetic constructs. Furthermore, commercial and conventional slicing software only generate a set of short and non-continuous printing paths, which negatively affect many properties of the final device, such as shape fidelity, resolution, and mechanical performances.

Our solution to these challenges is an accessible, user-friendly environment for designing more complex, biologically-inspired porous scaffolds (both planar and non-planar) while improving 3D printing outcomes. Our comprehensive design collection, which was developed through Rhino Grasshopper, incorporates many nature-inspired patterns, thus emphasizing biomimetic approaches for tissue engineering applications. All design variables can be individually adjusted and customized to meet specific structural and biological specifications (including filament spacing, layers number and orientation, layer height, and unit cell size), providing versatility across different tissue-specific applications. These designs are refined towards 3D fabrication via our custom-made algorithm GIPPO (Graph-based Iterative Printing Path Optimization), which builds upon Prim's Algorithm principles [3]. This scoring-based randomized approach identifies the optimal printing trajectories by minimizing discontinuous segments. Our platform subsequently generates g-codes compatible with ideally any extrusion-based AM technology. For this particular study, we created g-codes suitable for both commercial Fused Deposition Modeling (FDM) systems and pneumatic extrusion bioprinters.

Differences were identified in terms of printing resolution and quality through stereomicroscopy, while tensile and out-of-plane deformation tests showed improvements, as the minimization of short printed segments resulted in higher mechanical endurance due to less weak points (i.e., caused by the printing process). This was also highlighted by the analysis of the first fracture point.

Our platform can be a powerful tool for advancing tissue engineering and regenerative medicine. It can generate ad hoc bio-inspired designs and optimizing the printing path, thus boosting the final performances of the devices. This will make the process of the generation of functional and nature-inspired scaffolds and cell-laden biological constructs for different tissues applications much easier. Furthermore, it paves the way for complex non-planar applications, such as robotic printing of biomimetic scaffolds "in air", in which the optimization of the segments is crucial.

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Konuşmacılar: Pierpaolo Fucile (MERLN Institute for Technology-0Inspired Regenerative Medicine)

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Biofabrication of a finctional colon using 3D-Bioprinting native-like tissue architecture for drug-response assessment

The advancement of disease modeling and drug testing has been consistently limited by the shortcomings of conventional in vitro and in vivo models, which often fail to accurately replicate the complex microenvironment of human tissues. To address this issue, our study introduces an innovative in vivo-mimicking three-dimensional human colon model that reproduces the structural, mechanical, physiological, chemical, and biological characteristics of native colon tissue more effectively than previously reported models. Our approach has led to the development of a dual-layered construct that replicates the intricate architecture of the colon, including its 3D luminal curvature and spontaneously self-organized crypt-like domains. This design enables the formation of crypt-like structures within a surrounding mucosal architecture, closely mirroring the colon's native tissue composition, layered organization, and 3D topography. As a result, this in vivo-mimicking model provides a highly physiologically relevant platform for investigating colorectal cancer mechanisms and assessing therapeutic responses. By incorporating this model into our research, we have conducted extensive drug efficacy studies, particularly focusing on colorectal cancer treatments. Our findings shows the superior performance of this in vivo-mimicking model in drug response assessments, demonstrating its significant advantages over traditional 2D cultures and other existing 3D constructs.

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Konuşmacılar: Rahim Esfandyarpour (University of California Irvine)

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Biofabrication of a perfusable tumor-on-a-chip model enabled by in situ high-resolution 3D printing

Introduction
Animal models have been the gold standard for testing chemotherapeutic drugs, but less than 5 percent of drugs passed clinical trials between 2000 and 2015 [1]. Furthermore, regulatory agencies are mandating the reduction of animal testing [2]. We propose a tumor-on-a-chip model facilitated by multiphoton lithography (MPL), a high-resolution 3D printing technology that enables the fabrication of structures inside microfluidic chips [3]. By utilizing MPL, we structure a gelatin-based hydrogel within a custom microfluidic chip to create a pattern that enables cells to automatically cluster in a predefined spatial arrangement.

Methods
The microfluidic chip was printed with the Form 3 using the resin Clear V4.1 (Formlabs GmbH). To create the cell distribution structure, a gelatin-based hydrogel (HYDROBIO INX U200, BioINX) was structured using the commercial MPL system NanoOne (UpNano GmbH). L929 fibroblast cells (Sigma-Aldrich) were used to demonstrate the functionalities of the in vitro model. 2000 kDa FITC-dextran (Sigma-Aldrich) was used at a concentration of 1 mg/mL to visualize the microchannels. MDA-MB-231 cells (provided by CeMM, Vienna, Austria) were used to study the effect of cisplatin (Merck KGaA).

Results & Discussion
The printing setup was optimized to achieve a throughput of more than 120 mm3/h, enabling the efficient fabrication of large-scale structures. Using an MPL-compatible gelatin-based hydrogel, we printed a 9 mm-wide structure containing 177 wells (300 µm diameter) with a sloped topography to promote spatial cell clustering.
Clusters were then fully embedded by overlaying a UV-crosslinkable gelatin layer, and by day 3 cells had begun migrating into the surrounding matrix (Figure 1). This modular system affords high-definition control over matrix mechanics and chemistry, enabling highly reproducible studies of cell responses across experimental replicates.

To demonstrate its functional relevance, we examined the response of embedded MDA-MB-231 breast cancer cells to cisplatin. A microfluidic chip was fabricated using stereolithography, featuring an open central chamber for hydrogel loading and in situ structuring via MPL. Microchannels ranging from 50 to 175 µm were integrated at defined proximities to the cell clusters. Channel functionality was verified via FITC-dextran perfusion (Figure 2). Embedded MDA-MB-231 cells were treated with cisplatin, and comparative IC_{50} analyses revealed distinct responses in 2D cultures versus 3D construct, with and without perfusable channels, underscoring the platform's versatility for drug-screening applications

Conclusions
This work demonstrates the fabrication of a gelatin-based hydrogel structure that facilitates the automatic clustering of cells according to predefined patterns. The structure was integrated into a custom-designed microfluidic chip, where microchannels down to 50 µm were successfully perfused. Treatment of MDA-MB-231 breast cancer cell clusters with cisplatin revealed distinct differences when compared to traditional 2D cultures.

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Fast, Precise, and Biocompatible: High Cell Density Bioprinting via Xolography Using Iodixanol and Next-Generation Dual-Color Photoinitiators

Introduction
Xolography is a scalable volumetric 3D printing technology[1] enabling rapid and precise fabrication of complex hydrogel structures[2]. Its recent adaptation for bioprinting has opened exciting possibilities for tissue engineering and regenerative medicine[3]. Yet, standard bioresins like GelMA have shown limited reactivity—a challenge recently addressed through smart formulation strategies using small-molecule additives[4]. Here, we introduce a novel dual-color photoinitiator (DCPI) optimized for use with iodixanol (IDX) in GelMA-based formulations. This combination enables high cell density bioprinting with unmatched precision and speed.

Methods
We synthesized and characterized a new generation DCPI that undergoes photoisomerization to the active photoinitiator molecule with 405 nm, specifically selected to avoid overlap with IDX absorbance (Figure 1a). Its photochemical properties—including thermal half-life, performance at physiological pH, cytotoxicity, and initiation efficiency with visible light—were tailored for bioprinting. Simultaneously, a theoretical model was developed for refractive index tuning of the photopolymer formulation (Figure 1b), incorporating IDX to both match cytosolic optical properties and modulate photoreactivity. Bioprinting was performed on a Xube² platform with hepatocytes, fibroblasts, hMSCs and other cell types with up to 20 million cells/mL.

Results
The integration of IDX enabled precise refractive index matching and unexpectedly enhanced the photopolymerization kinetics, significantly increasing the conversion of GelMA at high cell loads. Constructs of >1 cm³ were printed within 1–3 minutes with channel resolutions as fine as 300 µm. Cell viability exceeded 95% post-print, and long-term culture demonstrated preserved morphology and functionality. After 10 days, primary human fibroblasts showed excellent spreading. Constructs such as bone marrow analogs were successfully perfused and retained spatial fidelity throughout culture. Incorporated hMSCs showed early markers for differentiation.

Discussion
This work demonstrates the feasibility of true high-cell density bioprinting using Xolography, with optimized optical and chemical synergy between a novel DCPI and IDX. The decoupling of light absorption and refractive index modulation enables the design of biologically relevant, photoreactive materials with excellent cytocompatibility. The speed, resolution, and viability achieved suggest a breakthrough in volumetric biofabrication, moving beyond the limitations of layer-based or single-color volumetric methods.

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Konuşmacılar: Niklas Felix König (xolo GmbH)

- 15:00

Coffee break & Poster Session I

- S07 Advancing Biofabrication Methods for Macro-Scale Tissue Engineering Ratuszowa

Toplantıyı düzenleyenler: João Maia (Universidade de Aveiro), Philipp Fisch (ETH Zürich)

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Biofabrication of hybrid cells compartments: from micro to macro

Bioinspired engineered microenvironments provide cells with a holistic “instructive niche” that offers the adequate entourage for cellular control both in space and time. Such approaches are important to design hierarchical constructs with applicability, for example, in tissue engineering or in the development of in vitro models for drug screening. We have been proposing strategies of combining cells and biomaterials in controlled submillimetre compartments, namely: (i) cell encapsulation in liquified capsules with thin biomaterials shells formed by polyelectrolytes complexation for the autonomous development of microtissues; (ii) soft compartments created by the combination of cells and thin micro-membranes, where the biomaterial is crumpled into the shape of miniaturised scaffolds by the traction forces of the cells. Such hybrid elements can be used as building blocks to be assemble into large constructs to produce macroscopic tissues using bottom-up tissue engineering methodologies. We demonstrated the possibility of creating hierarchical constructs based on distinct bottom-up technologies, using hydrogels as the continuous binder of these living hybrid elements. We showed that cells in the original compartments may behave depending on the original microenvironment, and independently of the external milieu.

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Bioprinting with Meniscus dECM, Collagen-Based Nutraceuticals, and Single-Cell Transcriptomics: New Perspectives for Biomaterials and Regenerative Medicine

Collagen-based biomaterials have gained increasing attention in regenerative medicine and nutraceutical applications. This study integrates three complementary approaches: the investigation of collagenous biopolymers from marine spongin, the development of bioinks derived from decellularized collagen-rich extracellular matrix (dECM) of the porcine meniscus for 3D bioprinting, and the creation of a single-cell transcriptome atlas of the meniscus to support tissue engineering strategies.
Our research confirms the presence of collagen types I and III as primary structural components of spongin, with proteomics, solid-state NMR, and Raman spectroscopy demonstrating its compositional similarity to mammalian collagen. Additionally, HPLC-MS analysis identified halogenated di- and tri-tyrosine crosslinking agents, revealing a complex molecular interplay within this ancient biocomposite [1]. In parallel, we present an efficient, scalable method for extracting and processing porcine meniscus dECM for bioink formulation. Given the meniscus's cartilage-like properties and structural robustness, we developed a novel protocol combining homogenization, hydrolysis, supercritical CO_2 extraction, and lyophilization. This method retains native biomolecules while ensuring good printability and cell-supportive properties. Despite DNA content exceeding conventional thresholds, in vitro studies confirmed excellent biocompatibility, challenging current decellularization efficacy standards[2].
Furthermore, we introduce a comprehensive single-cell transcriptome atlas of the porcine meniscus, highlighting four major cell types—chondrocytes, endothelial cells, smooth muscle cells, and immune cells—along with five distinct chondrocyte subclusters (Ch0-Ch4). Notably, chondrocyte subclusters in the red zone exhibit mesenchymal stem cell-like properties, contributing to tissue remodeling, endothelial proliferation, and vascularization, whereas those in the white zone specialize in cartilage matrix deposition and microenvironmental protection. The cellular similarity between porcine and human menisci reinforces the pig model’s relevance for orthopaedic research and regenerative medicine [3].
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Konuşmacılar: Jakub Rybka (Adam Mickiewicz University, NanoBioMedical Centre)

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4D Biofabrication of Scaffold Free Bone Forming Constructs: Towards Stand-Alone Human Size Implants

Introduction
Bioprinting for Tissue Engineered Advance Therapeutic Medicinal Products (TE-ATMPs) recently gaining ground aiming to regenerate defects and restoration of damaged tissues for bone tissue engineering solutions. The efficiency of TE-ATMPs is related to high cell density constructs as obtained via organoid bioassemblies. This has been convincingly demonstrated for engineered cartilage templates while demonstrating positive in vivo outcomes such as bone defect regeneration1. However, large scale self-assembled and complex cartilaginous implants cannot be easily produced via self-assembly processes. Therefore, we explored the use of a high-single-cell-density biofabrication process, using sacrificial alginate bioinks, resulted in the bioprinting of functional 3D scaffold-free cartilaginous tissues, towards Stand-Alone Human Size Implants.
Methods
We developed a chemically modified partially crosslinked alginate (ALG) bioink enabling one-step extrusion 3D bioprinting of high-density human periosteum derived cells (hPDCs). Three conditions were tested regarding chondrogenic differentiation capacity and evaluated by mechanical testing, cartilage specific staining, confocal imaging and qPCR analysis. At the final differentiation timepoint, alginate traces were dissolved with a chelating agent, resulting in scaffold-free stand-alone constructs (SF). Absence of alginate traces validated by FTIR and NMR analysis. Transcriptomics analysis (RNA-sec) was carried out to identify changes in prehypertrophic/hypetrophic phenotype, post and prior to the dissolution process. ALG and SF bioprinted differentiated constructs were implanted ectopically for 4 and 8 weeks. Bone formation and maturation capacity was evaluated by micro-CT and histological analysis. To scale up, hPDCs were expanded in a GMP-grade bioreactor and bioprinted into 2cm cylindrical ring followed by chondrogenic differentiation in a perfusion bioreactor, as well as the demonstrated dissolution process.
Results
Cells exhibited high viability over the time points, while gene markers indicating a prehypertrophic/hypetrophic phenotype were found to be overregulated over time. Volumetric formation of cartilaginous microtissues found exclusively at the highest tested concentration of hPDCs, followed by the secretion of abundant cartilaginous extracellular matrix. Young modulus gradually increased, as indicated by mechanical characterization, linked to ECM secretion. Significant differences were observed between the 2 experimental groups even from the 4th week upon implantation. Bone formation capacity and maturation were significantly improved for SF implants while pure ossicles exhibiting cortical and trabecular bone structures and a fully developed bone marrow cavity. RNA-Seq analysis showed that removal of the alginate traces enhances significantly ECM production and chondrogenic differentiation. We were able to recapitulate those in vitro observations made in smaller scale, towards to a 3D scaffold-free stand-alone human size implant (2.5cm) composed of only the cells and their secreted cartilaginous matrix.
Discussion
We present a novel biofabrication process that enables the functional differentiation of hPDC single cells into 3D competent constructs and post dissolution to a scaffold-free

bone forming implants, towards to human size dimensions. Dissolution process improved structural biomechanical properties and lower immune activation for the SF implants. This scalable biofabrication process as demonstrated by the human size scaffold-free cartilage implant, will support the translation of skeletal TE-ATMPs in clinical applications.

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Konuşmacılar: Andreas Dimopoulos (KU Leuven)

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Interparticle Crosslinked Ion-responsive Microgels for 3D and 4D Bioprinting Applications

Introduction

Materials capable of undergoing shape transformations in response to external stimuli offer significant advantages for a range of applications, including biomedical implants, soft robotics, and medical devices.[1] Microgel-based scaffolds have recently gained attention among these applications due to their interstitial microscale voids, which make them superior in oxygen and nutrient diffusion compared to nanoporous bulk hydrogels.[2] However, conventional microgels often require a secondary hydrogel binder to form stable 3D constructs, which compromises their porosity and limits cell infiltration, vascularization, and nutrition transport.[3] In this work, we report interparticle-crosslinked, shape-transformable microgels that eliminate the need for a binder. These shrinkable microgels, unlike conventional bioinert materials (e.g., pNIPAM), possess intrinsic bioactivity, making them promising candidates for biofabrication.

Methods

A new class of protein/carbohydrate-based ion-responsive microgel was fabricated through Schiff-base chemistry between carbohydrazide-functionalized gelatin-methacrylate and oxidized alginate. Blending and emulsion techniques were used to produce random-shaped (μR) and spherical (μS) microgels, respectively. The microgels retained active methacrylate sites, enabling interparticle photocrosslinking without compromising internal porosity. Rheological characterization was performed to evaluate the microgels' suitability for bioprinting. Chorioallantoic membrane (CAM) assays evaluated the vascularization potential of microgel constructs. Various bioprinting techniques including extrusion-based, embedded, intra-embedded, and aspiration-assisted bioprinting were employed to fabricate the scalable structures. The ion-responsive shrinkage behavior of the microgels was systematically studied in different solutions, and the mechanism was elucidated. Multi-material 4D bioprinting was demonstrated by co-printing ion-responsive and non-responsive microgels.

Results

Both μS and μR were successfully fabricated, with μR exhibiting superior mechanical properties and higher packing density. In an in-ovo model, μR constructs promoted denser vascular networks with thinner blood vessels (10–100 μm), while μS constructs supported the formation of thicker vessels (70–750 μm), due to their lower packing density and larger interstitial voids. The microgel-based bioinks displayed yield-stress behavior, shear-thinning, self-healing capabilities, and excellent printability (Pr~1). Bioprinting techniques demonstrated high process compatibility and biocompatibility (>90%), enabling the fabrication of anatomically relevant structures such as ear, pancreas, and nose. Shrinkage studies revealed ion-induced reversible size reduction up to 62%, governed by the Hofmeister effect. 4D bioprinting enabled defined shape-transformations such as coiling, folding, and gripping in response to ionic stimulation.

Discussion and Conclusion

This work introduces a dual-crosslinked, ion-responsive microgel that overcomes key limitations through a binder-free approach while supporting cell viability and function. μR and μS microgels can be selectively used based on vascularization requirements. Notably, these microgels inherently contain gelatin-derived RGD and MMP-sensitive motifs, eliminating the need for peptide functionalization. Multi-material bioprinting and shape transformations demonstrate the microgels system's versatility for applications in soft-robotics, dynamic implants, and biohybrid devices. Its modular chemistry enables extension to other protein (collagen, silk, dECM, etc) and carbohydrate (cellulose, chitin, starch) systems. Overall, these microgels address key biofabrication challenges, including porosity, structural stability, and bioactivity, while offering programmable shape transformation, positioning them as a next-generation bioink with broad translational potential.

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A 3D Integrated Biosensor Platform for Real-Time Electromechanical Monitoring of Myocardial infarction (MI) model

Cardiovascular diseases (CVDs) remain the leading cause of death globally, with myocardial infarction (MI) as a major contributor. Traditional 2D cultures and animal models fail to recapitulate the native electromechanical properties and pathological responses of the human myocardium. Recent advancements in human-induced pluripotent stem cell (hiPSC) technology and 3D bioprinting have enabled the fabrication of structurally and functionally relevant cardiac tissues that can closely mimic the native myocardium. However, existing platforms still lack robust, real-time monitoring of electrophysiology of cardiac tissue, particularly for simultaneously measuring both contractile force and field potential in 3D cardiac tissues.

In this study, we developed a 3D origami biosensor-integrated platform for simultaneously real time monitoring of 3D bioprinted cardiac tissue contraction force and field potential, enhancing the fidelity and accuracy of the disease model and therapeutic screening. The 3D biosensor system was developed using polydimethylsiloxane (PDMS) as the substrate. Gold strain sensors and a microelectrode array (MEA) were patterned via photolithography and assembled using anisotropic conductive film (ACF) bonding. This multilayer sensor structure allows simultaneous mechanical and electrical signal acquisition. Cardiac tissue was engineered by co-culturing human-induced pluripotent cardiomyocytes (hiPSC-CMs) and Primary cardiac fibroblast (CFs) mixed in a heart-decellularized extracellular matrix (hdECM) hydrogel. The hydrogel was bioprinted and cultured directly on the biosensor. The integrated biosensor system successfully captured real-time contraction profiles and field potential signals from the 3D bioprinted cardiac constructs.

The integrated system can successfully capture high-resolution, real-time data on both mechanical contraction and field potential propagation, with minimal signal interference. Immunofluorescence staining performed on day 14 confirmed tissue maturation, with uniform expression of α-sarcomeric actinin (α-SA) and vimentin. The bioprinted constructs exhibited synchronous beating and stable, functional outputs over time. To mimic native myocardial ischemia, we created a hypoxic environment within the matured cardiac tissue by establishing an oxygen gradient using a gas-permeable barrier, replicating infarct and border zones of the post-MI state. The biosensor detected increased spontaneous beating, reduced contractility, and slowed conduction—hallmarks of electromechanical dysfunction. Treatment with the ARB losartan improved both beating rate and contractile amplitude, demonstrating the platform's capability for real-time drug response assessment.

In conclusion, we develop a next-generation MI-on-a-chip model that integrates 3D bioprinting with a soft, flexible biosensor platform for real-time monitoring of cardiac tissue's contractility and electrophysiological activity. This platform presents a platform bridges 3D bioprinting and sensor technology, enabling continuous, non-invasive tissue maturation and function monitoring. This integrated 3D biosensor is expected to enhance the fidelity of in vitro models and provide more accurate and comprehensive data on tissue responses, thereby advancing cardiac research and therapeutic development.

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Engineering inks and bioinks from human blood components enable enhanced bioactivity in vitro and in vivo

Exploring the natural availability and intrinsic bioactivity of blood-derived proteins opens new avenues for fabricating bioactive and personalized constructs for biomedical applications. However, these biomaterials lack suitable viscoelastic proprieties for 3D printing of complex tissue structures. Ink engineering is progressively being utilized to advance the printability of better therapeutics, with optimized material proprieties. Innovative methodologies to allow 3D printing processability of otherwise low-viscous materials will be presented, with an emphasis on rheological properties and printability behavior. Our hypothesis relies on a dual-step strategy to improve the materials'

viscoelasticity and shear thinning behavior by chemical coupling, and on the maintenance of printed shape and stability by post-printing photocrosslinking.
To achieve this, human-derived Platelet Lysate (PL) inks encompassing both pristine and photo-responsive protein mixtures are developed, using a carbodiimide crosslinking to effectively tune rheological properties of the hydrogels – taking into account the final desired application. Three ink engineering methodologies will be presented: 1) for obtaining soft viscoelastic inks, the crosslinking reaction is tuned to achieve the desired rheological properties; 2) for obtaining granular inks, the resulting pre-gels are mechanically fragmented in small microgels, followed by an effective in-nozzle jamming; 3) for obtaining tough inks, we introduced needle-shaped nano-hydroxyapatite by chemically binding these particles to the protein matrix. Taking advantage of the previously introduced photocurable moieties, post-printing photocrosslinking is used for the stabilization of the printed structures, leading to increased scaffold mechanical stability and robustness.
These strategies could effectively turn an extremely low viscous material into shear-thinning formulations, appropriate for 3D printing technologies. Given its double crosslinking ability, all formulations enabled increasing elastic and viscous modulus in two moments: after primary coupling and after photocrosslinking. The development of microgel/granular inks enhanced printability while the inclusion of nanohydroxyapatite enhanced osteogenic properties and mechanical performance. These inks demonstrated ability of being printed seamlessly, with accurate reproduction of the convoluted models and geometries used, along with injectability and shape recovery behavior. Moreover, multimaterial strategies encompassing different nanohydroxyapatite loaded-inks were successfully 3D printed, attesting once more for the systems' tunability. Bioactivity was evaluated in vitro using primary human adipose-derived stem cells, which demonstrated increased viability and metabolic activity overtime, in all formulations, including in bioink formulations on granular inks. In vivo behavior also demonstrated the superior biocompatibility of these inks in subcutaneous and non-critical femur defect implantation.
The presented strategy denotes an easy-to-handle, tunable, biocompatible and sustainable solution for 3D printing low viscous protein-based materials that can be applied on every extrusion printer, widening the possibilities for the use of these matrices in 3D printing and regenerative medicine. The synergistic combination of photocurable with non-photocurable matrices enabled the development of programmable viscoelasticity and shear-thinning behavior on pre-gels, enabling personalized application of patient and blood-derived materials in precision medicine.

Acknowdedgements: EU's Horizon 2020 research and innovation programme under grant agreement No. 953169, InterLynk, as well as the EU's HORIZON programme under the grant agreement No. 101191729, m2M. Foundation for Science and Technology (FCT), Portugal: DOI 10.54499/2022.04605.CEECIND/CP1720/CT0021, 10.54499/2020.01647.CEECIND/CP1589/CT0034, PRT/BD/154735/2023, SFRH/BD/144520/2019 and 2023.00647.BD, UIDB/50011/2020, UIDP/50011/2020 and LA/P/0006/2020.
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Biofabrication of highly compliant biomimetic tissue-engineered vascular grafts

Introduction: Materials science and regenerative medicine are promising avenues for production of tissue-engineered vascular grafts (TEVGs) with biomimetic extracellular matrices (ECMs) [1–3]. However, many TEVGs exhibit poor mechanical compliance [4,5], with studies often overlooking the complex role of ECMs and mechanics. Here, highly compliant TEVGs were cultured in pulsatile bioreactors using specifically engineered, porous polymer scaffolds as substrates for tissue growth. For 6-weeks in vitro we studied matrix progression and mechanics of TEVGs cultured with mesenchymal stem cells (MSCs) and smooth muscle cells (SMCs) in static or dynamic conditions, followed by post-processing to produce readily-available grafts. Our findings indicate enhanced production of vascular ECM components via dynamic culture via the provision of biomimetic cues induced through mechanostimulation vascular ECM mechanics in line with established studies [6,7], with our findings extending beyond this to advance TEVG biofabrication technologies to improve treatment options.

Methods: Highly compliant small diameter tubular scaffolds produced from medical polycaprolactone (PCL) via melt electrowriting (MEW) were engineered to exhibit biomimetic mechanics with precise sinusoidal fibre architecture and high porosity [8]. Building upon on our preliminary static culture studies [9], high yield, donor-derived placental MSCs [10] were compared against primary SMCs, with TEVGs cultured separately from each cell type for 6 weeks in static or dynamic conditions, prior to decellularization and lyophilization. Subsequent rehydration and seeding of patient endothelial cells was performed to assess endothelialization in vitro. Biochemical analyses, mechanical testing, histology and immunofluorescence imaging enabled assessment of engineered vascular tissue ECM and performance, with comparisons made between cell types and culture conditions.

Results: In 6-weeks, cells and ECM completely filled scaffold pores. Pulsatile stimulation successfully maintained high scaffold compliance (12.4±0.8% per 100mmHg) with negligible loss of mechanics after decellularization. Dynamic TEVGs exhibited burst pressure (1125±212mmHg) and suture strength (3.0±0.4N) significantly greater than static TEVGs (699±171mmHg and 2.3±0.2N, respectively). MSCs produced more dense and collagen-rich ECM reflected by greater GAG and hydroxyproline content. TEVGs were effectively decellularized via TX100 and lyophilized, with negligible influence on ECM performance. Finally, endothelial cell seeding of rehydrated TEVGs achieved intimal endothelialization in 7 days as proof of concept, providing positive indications for potential application in vivo.

Discussion: Here, highly compliant polymer scaffolds cultured with MSCs in pulsatile conditions enabled biofabrication of TEVGs with physiological mechanics and enhanced ECM formation, significantly out-performing SMCs. Additionally, decellularization, lyophilization and endothelialization were assessed with our results providing positive indications toward future preclinical testing. We expect these findings to assist production of the next generation of vascular grafts for improved healthcare outcomes.

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- S11b Biofabrication of Soft Tissues Mikołajska

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Bioprinting Technology for Advanced Tissue Therapeutics

The recent development of bioengineering enables to create human tissues by integrating various native microenvironments, including tissue-specific cells, biochemical and biophysical cues. A significant transition of 3D bioprinting technology into the biomedical field helps to improve the function of engineered tissues by recapitulating physiologically relevant geometry, complexity, and vascular network. Bioinks, used as printable biomaterials, facilitate dispensing of cells through a dispenser as well as support their viability and function by providing engineered extracellular matrix. The successful construction of functional human tissues requires accurate environments that are able to mimic the biochemical and biophysical properties of the target tissue. This talk will cover my research interests in building 3D human tissues and organs to understand, diagnose, and treat various intractable diseases, including degenerative musculoskeletal, cardiovascular, diabetic diseases and cancers. A development of tissue-derived decellularized extracellular matrix bioink will be mainly discussed as a straightforward strategy to provide biological and biophysical cues into engineered tissues. I will also discuss the development of a 3D vascularized tissue construct that is generated by integrating the concept of tissue engineering and the developed platform technologies. Combined with recent advances in human pluripotent stem cell technologies, printed human tissues could serve as an enabling platform for studying complex physiology in tissue and organ contexts of individuals.
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3D-PAN: a novel biofabricated 3D in vitro model of photo-aged skin

Skin is the largest organ of the human body acting as a barrier against physical and chemical stimuli. It is well known that exposure to solar UV rays, UVA and UVB, damages its structure and ages prematurely the skin (a phenomenon called photoaging). In the last decades, three-dimensional (3D) in vitro models were considered to test novel cosmetic and pharmaceutical products against sun-induced photodamage conditions, representing a valid alternative to the in vivo studies [1]. Many types of scaffolds to support cell attachment, proliferation and layers organization were used based prevalently on collagen and chitosan as biomaterials [2]. Here, a novel 3D epidermal skin model based on gelatin micro and nano structured scaffold was realized. Electrospun gelatin scaffolds and non-porous gelatin scaffolds were fabricated to develop a human 3D in vitro model of photoaged skin by UV-exposure. Electrospun scaffolds were fabricated by using gelatin crosslinked with (3-glycidoxypropyl)-trimethoxysilane (GPTMS, Fig. 1A), following a previously published protocol [3]. As the non-porous scaffolds, a 10% w/v solution of gelatin in deionized water with 3.68% of GPTMS was poured into a Petri dish and air-dried to complete the crosslinking (Fig. 1B).
Photo-damage to the scaffolds was assessed by scanning electron microscopy (SEM) and mechanical tensile tests at dry state following a previous protocol [3].
HaCaT cells, human immortalized keratinocytes extensively used for studying epidermal pathophysiology, were seeded onto the two different scaffolds and maintained at an air–liquid interface to promote the development of a stratified epidermis.
Subsequently, cells were exposed to UVA (20 J/cm²) or UVB (40 mJ/cm²) radiation (XX-15L and XX-15M lamps, UVP), to induce the photo-aging process. Cell viability was assessed through fluorescence microscopy using a mixture of calcein-AM and ethidium homodimer-1. The barrier function was evaluated by measuring transepithelial electrical resistance (TEER) with a Millicell ERS-2 voltmeter (Millipore®).
The mechanical properties of samples were analyzed by uniaxial tensile tests in terms of ultimate of Young's modulus, ultimate tensile strength, and maximum elongation. As the tensile elastic modulus and ultimate tensile strength, no statistically significant differences were found between control and UVA/UVB irradiated samples. As expected, the casted scaffolds are stiffer and can withstand higher loads compared to the electrospun ones, but less elastic given the lower maximum elongations. From a morphological point of view, both UVA and UVB irradiation did not significantly impact both scaffolds.
HaCaT cells grown on scaffolds showed sustained basal cell viability and consistently high TEER values after 7 days. After photo-damage, a decrease in cell viability and epithelial resistance was observed. The cell viability and TEER values suggest good quality of the 3D model for the study of epidermal response to UV radiation and possibly other physical damaging agents.
Although preliminary, the overall results of this study confirm the possibility of obtaining a 3D in vitro model of photo-aged skin for studying new skin treatments in cosmetic and pharmaceutical research.

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Fig.1 Electrospun and gelatin scaffolds.

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Multimaterial 3D Bioprinting of Complex Skin-Muscle-Bone Tissue Models with Digital Light Processing

3D multicellular models provide valuable platforms for tissue regeneration, disease modeling, and biomaterial evaluation. However, the recreation of complex stratified systems that combine different tissue types and pathophysiological conditions remains challenging. In this work, we developed a multilayer construct integrating skin, muscle, and bone components using digital light processing (DLP) bioprinting. The bioinks were based on 5% (w/v) gelatin methacrylate (GelMA), with three different cell types: HFF-2 (skin layer), H9c2 (muscle layer), and SaOS-2 (bone/tumor layer) cells. The skin layer was supplemented with extracellular matrix (ECM) derived from dermal tissue for a more realistic environment. The printability, structural integrity, and cell viability of the constructs were evaluated after bioprinting and during culture. Fluorescence microscopy confirmed the successful spatial localization of each cell type within the respective layers, with high viability maintained up to five days. This tri-layer DLP-bioprinted model demonstrates the feasibility of fabricating complex multicellular constructs that not only mimic stratified tissues but also allow the integration of tumor cells, making it a promising platform for cancer research, drug testing, and as an alternative to animal models in preclinical studies.
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Bioprinting of Knee Meniscal Constructs and Corneal Stroma Equivalents

Digital Light Processing (DLP) is a 3D printing method that offers enhanced precision, quicker print times, increased throughput, and better cell viability by minimizing shear stress compared to extrusion-based bioprinting, which depends on mechanical nozzle deposition and tends to be slower with lower resolution. These advantages make it particularly well-suited for fabricating complex tissue structures. We have utilized this technology to fabricate knee meniscal constructs and corneal stroma equivalents (CSE). With Gelatin methacryloyl (GelMA) as the base material for both, we employed two different second crosslinking methods for each of these tissues to achieve the desired properties mimicking the native tissues. For printing meniscal constructs, we employed a post-printing crosslinking with tannic acid. The secondary tannic acid (TA) crosslinking not only resulted in mechanical properties closer to the native meniscal tissue (~200 kPa) but also showed excellent anti-bacterial, anti-oxidant and immunomodulatory properties. TA crosslinking did not affect the chondrogenic potential of GelMA, aided the differentiation of human Mesenchymal Stromal Cells (hMSCs) into chondrocytes. In addition, the anti-inflammatory cytokines such as IL-4, TGF-β1, and IL-10 were upregulated and the pro-inflammatory cytokines such as TNFα, MCP1, and IFNγ were down regulated when macrophages were cultured on TA-crosslinked constructs. We utilized Schiff's base reaction as the secondary crosslinking method for printing of corneal constructs, by adding oxidized carboxy methyl cellulose (OxiCMC) to GelMA. DLP printing not only enabled printing of 6 constructs in 20 minutes with the corneal anisotropy included but also achieved the desirable properties of an ideal CSE. GelMA-OxiCMC bioink had a compressive modulus of ~110 kPa which is in the range of corneal modulus, exhibiting greater than 90% optical transmittance at 500nm. The human corneal keratocytes showed >94% viability post-printing, maintained their stellate morphology, and expressed characteristic markers such as keratocan and lumican.

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3D-Melt-Spin-Bioprinting – A Soft Tissue Engineering Platform Combining Structural Control with hMSC Trilineage Differentiation

Introduction
The field of regenerative medicine increasingly demands scalable, patient-specific soft tissue constructs. While hydrogel-based bioprinting provides a favorable environment for cell viability, it often lacks mechanical integrity - especially critical for replicating the elastic nature of soft tissues. Traditional scaffold-integrated bioprinting has improved structural support but remains largely focused on hard tissue applications due to limited flexibility. To overcome these limitations, we introduce 3D-Melt-Spin-Bioprinting (MSB) - a novel fabrication platform that synergizes textile engineering with drop-on-demand bioprinting to create highly elastic, mechanically tunable scaffolds suitable for soft tissue regeneration.

Materials and Methods
MSB scaffolds were fabricated from various polymers including PLA, TPU, PETG, and PCL. The process enabled precise control over fiber diameter (down to 50 µm), porosity, and mechanical properties, achieving elastic moduli between 0.7 and 212 MPa. TPU scaffolds demonstrated exceptional stretchability (up to 200%) and resilience under cyclic loading. Human mesenchymal stem cells (hMSCs) were printed into these scaffolds using bioinks at a concentration of 1×10^6 cells/ml. Scaffold-bioink compatibility was analyzed via wettability, morphology (actin/DAPI staining), proliferation (CTB assay), and rheological profiling of the bioink. To evaluate the potential for differentiation into multiple cell lines, hMSCs were differentiated into adipogenic, osteogenic and chondrogenic lineages under adapted culture conditions (in Collagen I gels or by spheroid culture). Differentiation was evaluated by histologic staining and qPCR analysis.

Results
MSB technology enabled the fabrication of 1–5 cm³ scaffolds with defined architecture and high elasticity. TPU-based scaffolds outperformed other materials in fatigue resistance and sustained mechanical integrity. The fabricated scaffolds were compared with warp-knitted structures of similar size and density. Bioprinting into PCL scaffolds, followed by culture under varying media conditions, demonstrated effective cellular integration and robust differentiation of hMSC into adipogenic, chondrogenic, and osteogenic lineages. Real-time qPCR and histological staining confirmed expression of lineage-specific markers. For scalability, a custom DLP-printed perfusion bioreactor was developed. Within this system, multiple hMSC islands, cultured in 1 cm³ constructs, maintained viability and proliferation under dynamic conditions.

Discussion

This study presents MSB as a flexible, scalable, and biocompatible platform for soft tissue engineering. The textile-like fabrication process provides unique advantages - precise architectural control and elasticity - unavailable in traditional scaffold or hydrogel systems alone. Trilineage differentiation within these constructs highlights the system's regenerative potential. While the current design addresses many challenges in soft tissue biofabrication, further development is needed to support vascularization for larger tissue volumes. Early experiments incorporating sacrificial bioprinted channels show promise. Future work will focus on integrating endothelial cells (e.g., HUVECs) to facilitate vascular network formation and optimizing long-term culture within dynamic bioreactors.

Conclusion

3D-Melt-Spin-Bioprinting offers a robust and versatile platform for generating personalized, elastic scaffolds capable of supporting complex tissue regeneration. The successful integration and differentiation of hMSCs underscore its potential for clinical applications in soft tissue repair. Ongoing research aims to enhance vascularization and functional tissue maturation to fully realize its therapeutic utility.

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From biomaterials to tubular 3D bioprinted structures as promising solutions for urology

One of the most innovative areas of 3D printing development is bioprinting, the creation of biological structures using living cells and bioinks. This technology enables the production of tissue scaffolds that can serve as templates for regenerative cells. These applications include printing skin and cartilage, as well as preliminary attempts at printing organs such as kidneys, liver, and pancreas. 3D bioprinting of organs and tissues offers a potential solution to the shortage of organs for transplantation and enables the creation of models for pharmacological research, contributing to the goal of minimizing animal testing.
In our work, we focused on increasing the mechanical strength of tubular constructs printed from alginate-gelatin hydrogel for use on the urethra. Considering that this print is to be a fragment of soft tissue with high elasticity and required variable strength (due to the variable pressure of flowing urine), it was decided to undertake research on obtaining increased mechanical strength in a natural way - through cellular hypertrophy.
In order to obtain tubular cross-sections of prints reflecting the shape of the urethra without the need for additional processing (in a single process), while maintaining the best possible viability of cells introduced into the extruded bioink, a special 3D bioprinter with a rotary work table was designed and built.

Conclusions

A specially designed 3D bioprinter, using a rotary table, allows for printing tubular structures from hydrogels in a single process and without supports, while maintaining high accuracy of the obtained path thickness and very high cell survival. The analysis carried out showed that with the increasing culture time of the structures and the degree of their overgrowth by the biological material contained inside, the tensile strength of the prints increases, reaching its maximum after 6 weeks of culture. During long-term culture, the biological material contained inside the bioprints is characterized by excellent survival of fibroblast cells, which create a homogeneous, strongly developed network reflecting the tissue structure. An important aspect of designing analogous hydrogel bioprints is maintaining a balance between the kinetics of degradation of the polymer matrix based on sodium alginate and gelatin in in vitro culture conditions with the corresponding rate of cell growth and proliferation. This correlation eliminates the risk of losing the stability of the structures and creates a prospective possibility of mapping the native tissue of the urethra.

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4D printing of tubular constructs for soft tissue regeneration

There is much interest in biofabrication of tubular constructs for repair and regeneration of several tissues in the human body, such as blood vessels, nerve conduits, gastro-intestinal tract, and bile duct, among others. The rapidly growing field of additive manufacturing and 3D printing technologies is offering new routes for the processing of biomaterials for the fabrication of implants and tissue scaffolds of complex architecture, which are personalized to meet the patient's need. However, the fabrication of thin-walled, hollow tubes of small diameter using soft polymers can be challenging with the currently available strategies of 3D printing. 4D printing is a frontier technology in this field which involves the stimulated shape-morphing ability of the 3D-printed parts. Our group is actively exploring 4D printing approaches to prepare tubular constructs using soft biomedical polymers prepared by extrusion and light-based 3D printing techniques.

We engineered an alginate-based bilayered hydrogel system with defined swelling behaviors, which demonstrated excellent printability in extrusion-based 3D printing and programmed shape deformations post-printing. These 4D-printed hydrogels were used as deployable nerve conduits for the healing of peripheral nerves in rats. In more recent work, we have improved the bioactivity of the nerve conduit by incorporating carbon nanotubes in the hydrogel matrix.

In a different approach, we utilized a single shape memory thermoplastic polymer (SMP), PLMC (polylactide-co-trimethylene carbonate), to achieve programmable shape deformation through anisotropic design and infill angles encoded during 3D printing. Rectangular 2D sheets could self-roll into complete hollow tubes. Furthermore, shape memory properties were demonstrated post-shape change to exhibit dual shape morphing at temperatures close to physiological levels to yield bioresorbable tubes with cellularized lumens for potential use as vascular grafts with improved long-term patency.

We also developed the first cell-laden 4D-bioprinted scaffold prepared by digital light processing (DLP) from a bioink consisting of a blend of gelatin methacryloyl (GelMA) and poly(ethylene glycol) dimethacrylate (PEGDM) operated with visible light (405 nm). DLP-bioprinted gels can change shape-to-complex constructs in response to cell-friendly stimuli, such as hydration, to yield a cell-laden hydrogel tube from a flat sheet.

Taken together, this talk will highlight recent developments in our group that utilize advanced 4D printing for preparing hollow tubular constructs for soft tissue repair and regeneration.

Relevant Publications:

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Bioreactor-Matured, Decellularized Vascular Patches Based on PLCL and Collagen Scaffolds

Cardiovascular surgery continues to face a shortage of suitable biomaterials for full-vessel bypass procedures and vascular patch repair. Current materials exhibit several limitations, including poor patency in small-diameter vessels, limited remodeling capacity, and a high risk of thrombosis [1]. While PLCL (poly(L-lactide-co-ε-caprolactone)) nanofiber sheets are biodegradable over several months, their mechanical properties—particularly suture retention—are insufficient for direct use as patching materials.

However, these sheets serve as an excellent sacrificial substrate for printing collagen-based scaffolds embedded with cells, offering a promising platform for tissue remodeling. In this study, we aimed to develop tissue-engineered vascular patches using PLCL nanofiber carriers combined with printed collagen scaffolds encapsulating adipose-derived stromal cells (ASCs) or smooth muscle cells (SMCs). The constructs were matured in a bioreactor and subsequently decellularized using a custom-built automated system.

PLCL nanofiber sheets were cut into 26 × 76 mm rectangles to fit a custom vacuum fixture in our extrusion-based bioprinter. We employed an optimized collagen bioink at a concentration of 30 mg/mL, incorporating ASCs and SMCs [2]. The bioink was printed onto the PLCL sheets in a rectangular pattern (up to 50 × 20 mm) with a total thickness of 1.5 mm, using 0.25 mm layer increments.

The printed constructs were mounted in a custom-designed bioreactor chamber and connected to a pulsatile flow generator delivering up to 80 mL/min at pressures of 120/60 mmHg. These parameters were selected to generate shear stress up to 10 dyn/cm², promoting cell proliferation, differentiation, and extracellular matrix (ECM) formation [3]. After 7 days of dynamic cultivation, the samples were harvested.

To minimize immunogenicity, the constructs underwent decellularization using our automated system, which performs cycle-based decellularization with integrated rinsing and washing. The process involved four 20-minute cycles with 1% SDS, followed by extensive rinsing—six 10-minute cycles and twenty-three 1-hour cycles [1,3].

Scanning electron microscopy (SEM) confirmed the formation of ECM microstructures resembling native vascular tissue [1]. Mechanical testing revealed a significant improvement in strength compared to unmodified PLCL sheets. However, suture retention remains suboptimal in some samples for in vivo applications. Ongoing optimization efforts focus on crosslinking and mesh reinforcement to enhance mechanical stability.

Nevertheless, the structure developed through this approach demonstrates promising potential as a tissue-engineered vascular patch.

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Konuşmacılar: Roman Matějka (Czech Technical University in Prague)

- S18 Chaotic Printing: A New Bioprinting Paradigm Kruczkowski

Toplantıyı düzenleyenler: Alicia Bonilla Vázquez (Universidade de São Carlos (IQSC-USP)), David Dean (University of Wisconsin-Madison)

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Harnessing Chaotic Flows for Biofabrication: Structuring Complexity with Simplicity

Biofabrication increasingly demands creative strategies to fabricate structured and functional constructs that can meet healthcare challenges. Chaotic printing and bioprinting offer a unique and accessible way to build internal complexity in materials, leveraging the deterministic behavior of laminar flows.
Our journey began with a miniaturized journal bearing system, where controlled chaotic advection allowed fine structuring of hydrogel filaments without the need for nozzle miniaturization. We then expanded these principles into continuous chaotic printing systems based on static mixers, enabling the fabrication of hydrogel filaments with multilayered internal architectures—achieving layer resolutions below 10 µm inside millimetric constructs.
Importantly, in this context, "chaotic" does not imply randomness or turbulence. Originally used in chemical engineering to enhance mixing efficiency under laminar conditions, chaotic flows are here repurposed to organize materials with precision. This reinterpretation opens new opportunities: from fabricating prevascularized skeletal muscle-like tissues and complex tumor microenvironments, to engineering multicellular bacterial ecosystems and functional food scaffolds.
By embracing the inherent dynamics of chaotic flows, we propose a simple, versatile, and reproducible approach to create physiologically relevant architectures. This strategy holds great promise to expand the accessibility of biofabrication and to accelerate healthcare innovation through the fabrication of next-generation tissues, disease models, and therapeutic platforms.

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Konuşmacılar: Grissel Trujillo de Santiago (Tecnologico de Monterrey)

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Chaotic bioprinting of prevascularized tumor niches: A flexible platform for cancer research

In this work, we present our latest advances in the biofabrication of monoculture and polyculture tumor niches using chaotic bioprinting as an enabling strategy for in vitro cancer research.
We first focus on the fabrication of monoculture breast cancer models designed to study tumor cell migration and invasion. Understanding these processes requires sophisticated models that accurately replicate structural and functional aspects of the tumor microenvironment. We employed a chaotic bioprinting approach to create prevascularized tumor niches integrating cancer spheroids and longitudinal vascular-like microchannels within a single construct. Using MDA-MB-231 breast cancer cells, we demonstrate that multichannel scaffolds fabricated via chaotic bioprinting significantly enhance cancer cell migration and directional alignment compared to traditional solid hydrogel constructs. By day 20, migratory fronts in multichannel filaments extended up to four times farther and achieved 88% alignment, indicating robust directional migration.
Gene expression analysis revealed accelerated epithelial-to-mesenchymal transition (EMT) in multichannel constructs, characterized by earlier and stronger upregulation of N-cadherin and vimentin. This enhanced migration was accompanied by increased proliferation, as evidenced by elevated Ki-67 expression. Importantly, the absence of hypoxia in the migratory fronts underscores the role of engineered microchannel architecture in supporting sustained, non-hypoxic migration.
We also report the fabrication of multicellular tumor niches incorporating breast cancer cells, human fibroblasts, and macrophages into chaotically printed hydrogel constructs. These prevascularized polycultures exhibited high viability, dynamic cellular activity, and rich intercellular interactions for over four weeks in culture. Furthermore, treatment with doxorubicin, a commonly used chemotherapeutic agent, revealed distinct responses in terms of viability, cell death, and gene expression profiles, highlighting the model's potential for pharmacological testing.
Overall, this work establishes chaotic bioprinting as a powerful and flexible platform for engineering advanced tumor models with high structural complexity and biological relevance—offering new opportunities to study cancer progression and improve the evaluation of anti-cancer therapies.

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Konuşmacılar: Mario Alvarez (Tecnológico de Monterrey)

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Chaotic Bioprinting of Structured Cocultures: Modeling Bacteria–Host Interactions

Introduction
A substantial body of scientific evidence demonstrates that gut microbiota plays a central role in human health and disease1,2. Understanding bacteria–host interactions in the human gut is essential for advancing microbiome research. Early 3D culture systems relied on physical membranes to separate bacteria from the intestinal epithelium model, which often limited the ability to study bacterial adhesion and invasion3. Most recent in vitro models of host–bacteria interactions rely on a 3D-culture of either the host tissue or the bacterial community, while the counterpart remains in a liquid suspension4,5. These models often fail to replicate the complex spatial organization and dynamic interactions characteristic of native tissues, limiting their physiological relevance for studying microbiota–host crosstalk6. Fabricate constructs in which both components are simultaneously incorporated into a structured 3D environment from the outset of the bioprinting, while ensuring coculture stability over time, is untrivial.

Methods
Structured cocultures fabricated by chaotic bioprinting mimic natural microenvironments by creating defined niches where bacteria and mammalian cells coexist without intermixing (Figure 1a-d). Using a printhead containing Kenics static mixer elements, we bioprinted hydrogel constructs with intercalated layers of Caco-2 cells and bacteria (either Escherichia coli or Lactobacillus rhamnosus) (Figure 1e-f). Characterization, including the analysis of Caco-2 cell dynamics and bacterial community evolution over time, was performed through fluorescence microscopy, colony forming unit counting, and live/dead assays.

Results/discussion
The microarchitecture of printed filaments significantly defines bacterial growth dynamics6. This new approach in which we incorporate Caco-2 cells offers a versatile, cost-effective, and high-throughput platform to analyze how bacterial strains influence mammalian cell behavior within a spatially organized 3D microenvironment. The printed filament preserves spatial compartmentalization over time, effectively preventing significant bacterial migration into the Caco-2 regions. Existing models often struggle to maintain both viable mammalian cells and bacteria in the same system over extended periods4,5. By providing individualized compartments and perfusable channels, our

constructs improve nutrient delivery, waste removal, and overall coculture stability for at least 8 days. To our knowledge, this is the first report of the one-step biofabrication of an architected, membrane-less, gut-like construct containing both bacterial and mammalian cells.

Conclusion
We anticipate that model bacteria-host interactions using chaotic bioprinting will contribute to in vitro studies of microbiome-related therapeutics, probiotic and pathogen dynamics, dysbiosis modeling, and host immune responses in a structured, physiologically relevant environment.

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Konuşmacılar: Ariel Cantoral-Sánchez (Escuela de Ingeniería y Ciencias)

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Using chaotic bioprinting to create a structured multi-strain probiotic.

Introduction
Probiotic therapies offer great potential for addressing gut dysbiosis, but current approaches are limited by low strain diversity, high production costs, and the challenges of culturing strict anaerobes. Some promising approaches include co-culture techniques, but traditional methods have drawbacks, including nutrient competition and instability due to different growth rates among the strains. Alternative strategies that retain the benefits of co-culture while avoiding its limitations are required. One such alternative comes from the field of biofabrication, which allows the creation of complex biological constructs in a high-throughput and cost-effective manner through encapsulation methods. While encapsulation and co-culture can be combined, most approaches create unequal volumes and interface areas, potentially leading to imbalanced growth. Is not an easy task to develop an encapsulation method able to maintain a co-culture that supports the cooperative growth of bacteria while maintaining stability over time.
Methodology
Using a Kenics static mixer–based printhead, we fabricated alginate hydrogel filaments with an internal multilayered microarchitecture containing four probiotic strains: Bifidobacterium bifidum, Bacteroides fragilis, Lactobacillus rhamnosus, and Streptococcus thermophilus (Figure 1). The spatial arrangement of the multilayered architecture was designed to promote cooperative interactions (Figure 1b), particularly by embedding strict anaerobes between facultative anaerobes to create self-sustaining hypoxic niches. The printed constructs were characterized over 72 hours using fluorescence microscopy, colony-forming unit counts, LIVE/DEAD assays, and qPCR.
Results and discussion
Results showed that structured co-cultures exhibited higher viability, enhanced growth, and more balanced population dynamics than the monocultures of each bacterial strain and unstructured (scrambled) co-cultures.This study demonstrates that chaotic bioprinting enables precise spatial control over microbial ecosystems, allowing the rational design of microbial communities with tailored interactions. The approach presents a powerful and scalable platform for next-generation probiotic production and opens new opportunities for engineered microbiomes, synthetic biology, and living material design.
Keywords: biofabrication, probiotics, Next Generation Probiotics, chaotic printing, co-culture.

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Chaotic bioprinting of prevascularized hydrogel scaffolds with bioactive glass: a multimaterial strategy for enhanced cellularization

Introduction:
Internal cellularization of thick tissue scaffolds remains a central challenge in tissue engineering, often requiring complex and costly technologies. In this study, we developed a cost-effective chaotic bioprinting strategy to fabricate compartmentalized hydrogel filaments that simultaneously provide physical cues (hollow microchannels for enhanced mass transport) and chemical cues (sustained ion release from mesoporous bioactive glass [BG] nanoparticles) to support angiogenesis and cellularization.

Methods:
We employed multimaterial chaotic bioprinting, leveraging static mixer-induced flows to co-extrude three distinct hydrogel inks: (1) a soft, cell-friendly ink that naturally contains cell adhesion motifs; (2) a reinforcing ink based on high-viscosity alginate and loaded with BG nanoparticles, providing chemical cues; and (3) a sacrificial ink designed to generate internal void channels. Using these materials, we fabricated four types of hydrogel filaments: (a) solid filaments without internal voids (Sld); (b) filaments with aligned hollow channels (Ch); (c) solid filaments with BG nanoparticles (Sld+BG); and (d) channel-containing filaments with BG nanoparticles (Ch+BG) (Figure 1A). Structural features were characterized via fluorescence microscopy, while physicochemical properties were assessed through swelling assays, tensile testing, and X-ray diffraction. BG incorporation and ion release were evaluated using energy-dispersive spectroscopy (EDS). To assess in vivo-like behavior, printed scaffolds were cultured ex-ovo on the chorioallantoic membrane (CAM) of chick embryos to evaluate vascularization and tissue colonization.

Results:
The hydrogel filaments exhibited structural integrity, enhanced mechanical modulus, and controlled ion diffusion. Filaments containing both void channels and BG nanoparticles showed superior nutrient transport and supported cell migration and adhesion. CAM assays indicated increased cellularization and signs of neovascularization compared to non-structured bulk controls.

Discussion:
Our results demonstrate that chaotic bioprinting enables the integration of functional spatial microarchitecture with bioactive chemical cues within a single filament. The use of void microchannels improves diffusion and supports tissue-like organization, while BG nanoparticles promote cell recruitment (Figure 1B). This combined approach enhanced scaffold performance across mechanical, transport, and biological dimensions.

Conclusion:
This work presents a scalable, multimaterial bioprinting platform that unites prevascularization and biochemical activation for the fabrication of next-generation tissue scaffolds. Applications include wound healing, in vitro tissue models, and small-scale transplantation.

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Konuşmacılar: Sara Pedroza (Tecnológico de Monterrey)

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Mechanized Chaotic Printing of Rapidly Splitting Channels within Microvasculature Sheets

Problem: A fundamental problem for most tissue engineered constructs is the inability to provide a fully perfusing blood supply on implantation. That slows, if not prevents, healing within the treated site. If possible, standard-of-care autologous bone grafts are transplanted with their native vascular pedicle. In these cases, healing is unlikely to be hindered by an insufficient blood supply. Alternatively, if there is no vascular pedicle, a free flap muscle graft, a tissue known to be full of microvasculature, can be used to wrap a bone graft with the muscle flap being connected to nearby blood vessels. That leaves many, if not the majority, of graft-based therapeutic strategies dependent on autologous muscle and tissue grafts, used in tandem, as the primary skeletal reconstruction therapy due to the ready function of the engrafted organ and the presence of

microvasculature in the muscle flap. We have previously shown the ability to use Chaotic Printing Kenics Static Mixer (KSM) technology to create microchannels within hydrogel sheets (Biofabrication 2024 DOI: 10.1088/1758-5090/ad30c8) for use as a microvasculature graft. We have also shown that careful sequencing of KSMs can be used to create a microvasculature graft that begins with the incorporation of nearby, small diameter arteries, as small as 150 µm in diameter, that as printed quickly divide down to capillary branches, at 10 µm diameter, in our CEVIC (Continuously Extruded Variable Internal Channeling), multi-material (i.e., multi-hydrogel), sheet-printing device. More specifically, in that prior study we demonstrated 8 initial channels branching to 512 microchannels with average widths ranging from 621.5 µm to 11.67 µm (Figure 1A). In that study we switched between the KSM elements within the CEVIC manually. In this study, we present the use of a rotary valve, which can be controlled on a panel or remotely. Methods: We paired two rotary valves with our CEVIC chaotic microvascular sheet printer. One handle the cell-bearing GelMA hydrogel, shown with “red” dye in Figure 1(B), and the other rotary valve handles the fugitive hydrogel, hydroxyethyl cellulose, shown with “blue” dye in Figure 1(B). We are fine-tuning the kinematics to ensure that channel splitting occurs with high accuracy. The two inks are carefully designed and prepared so that the viscosity of both hydrogels is similar. Results: Automated switching between KSMs with different diameter microvascular channels is done rapidly over a short printing distance. This is critical for structures such as bone, as microvascular splitting occurs underneath the periosteum and inside the marrow space. Careful design and specialization of the KSM elements of the CEVIC print head is essential to controlling flow during switching. Conclusions: We are completing our in vitro microvascular construct testing in preparation for a mouse hindlimb model which includes a mock femoral bone graft and cell work to see if pre-culturing of cells improves microvascular perfusion of donor bone grafts. Additionally, we will begin seeding different cell types that will more closely match the construct’s surgical applications, such as endothelial cells (inner walls) and pericytes on the outer walls.

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Konuşmacılar: David Dean (University of Wisconsin-Madison)

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Production of micro-compartmentalized constructs for in vitro meat bioprinting using an enzymatic based crosslinking method.

Introduction.
The growing demand for sustainable meat alternatives has accelerated research on culture meat. However, scalable production of fully edible meat-like structured constructs remains a challenge. In this study, we use chaotic bioprinting, extrusive bioprinting enabled by chaotic advection, using an enzymatic crosslinking agent, transglutaminase (TGase), to fabricate compartmentalized gelatin constructs, which induced an accelerated cellular proliferation and differentiation.
Methods.
We developed a chaotic bioprinting strategy, enabled by static mixer-induced chaotic flows, to co-extrude three distinct inks: a hydroxyethyl cellulose (HEC)/TGase sacrificial ink to create hollow channels, a supprotivesupportive gelatin/TGase ink, and a cell-laden gelatin ink. Using this set of inks, with a 2in4e printhead we fabricated (1) a gelatin/TGase solid fiber, (2) a void channeled fiber with a gelatin ink and a HEC/TGase sacrificial ink, and with a 4in4e we fabricated (3) a supported channeled fiber including the support gelatin/TGase ink (Figure 1A). Structural assessment of printed fibers was performed with fluorescence microscopy. Additionally, biological compatibility was assessed in all printed constructs by loading C2C12 murine myoblasts in (1) one gelatin/TGase bioink, (2) a gelatin-based bioink, and (3) in two gelatin-based bioinks (Figure 1B). Cellular viability, proliferation and tissue maturation were evaluated with Live/Dead assays, Actin/DAPI staining, immunostaining, and RT-qPCR.
Results and discussion.
The use of TGase as crosslinking agent proved successful in the formation and maintenance of structural integrity (Figure 1C) in all the three evaluated conditions. Cell-laden bioprinted constructs showed high proliferation and tissue maturation (Figure 1D-E) while maintaining construct integrity and high cellular viability for up to 35 days.
Our results demonstrate that structural construct stability can be achieved when using TGase enzymatic activity as crosslinking agent for gelatin-based hydrogels when using chaotic bioprinting, amplifying the range of materials that can be used with this technique. Additionally, by maintaining the biocompatible properties of gelatin, the bioprinted constructs demonstrated that cellular proliferation can be achieved within the fiber, eliminating the need for extensive expansion of satellite cells prior to bioprinting. Moreover, the inclusion of hollow channels enhanced cell proliferation and tissue maturation, probably induced by improved diffusion within the fiber.
Conclusion.
This work introduces a scalable bioprinting technique that enables the use of highly biocompatible materials (gelatin), to biofabricate prevascularized microarchitected constructs, creating completely edible scaffolds suitable for cultured meat applications and effectively addressing current challenges in the field.

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Konuşmacılar: Héctor Ambriz-González (Tecnológico de Monterrey)

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S20 Emerging Bioprinting Platforms for Musculoskeletal Tissue Engineering — Kisielewski

Toplantıyı düzenleyenler: Eben Alsberg (University of Illinois Chicago), Francesca Diletta Spagnuolo (Trinity College Dublin)

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Bioprinting of phenotypically defined fibrocartilage microtissues into melt electrowritten scaffolds to engineer regionally defined meniscal grafts

Lesions in the menisci are frequently related to sports injuries and dramatically increase the risk of developing osteoarthritis. It is estimated that 1.5 million meniscal repairs are performed annually in the United States and Europe, representing one of the most common clinical procedures performed by orthopaedic surgeons. In the field of tissue engineering there is increased interest in the use of cellular aggregates, microtissues and organoids as biological building-blocks for the biofabrication of scaled-up grafts. Such microtissues can be incorporated within 3D printed polymeric scaffolds to help direct their fusion and (re)modelling and to enhance the biomechanical properties of the resulting construct, making it a promising strategy for meniscus tissue engineering. Therefore, the aim of this study was to deposit fibrocartilaginous microtissues generated using meniscus progenitor cells (MPCs) isolated from the inner and outer region of the meniscus within melt-electrowritten (MEW) scaffolds to biofabricate zonally defined meniscus grafts. We initially explored how the aspect ratio of MEW scaffolds can influence microtissue fusion and remodeling. For this, polycaprolactone (PCL) scaffolds with 0.8×0.8 mm and 0.4×1.6 mm pore sizes were fabricated using a custom-made MEW printer. Next, inner and outer MPC derived microtissues were deposited within the MEW scaffolds. After 24h, we observed that the microtissues successfully fused within the MEW scaffolds, with tissue growth modulated by the pore architecture. Scanning electron microscopic (SEM) analyses revealed the development of a dense extracellular matrix with a preferential fibre orientation when microtissues were seeded in the anisotropic (0.4:1.6 pore aspect ratio) MEW scaffolds. Histological analyses revealed intense staining for sGAG deposition when inner MPC derived microtissues were seeded into the 0.8:0.8 MEW scaffolds. Greater collagen deposition was observed when either inner or outer MPC derived microtissues were seeded in the anisotropic 0.4:1.6 MEW scaffold. All groups were negative for calcium deposition, suggesting the development of a phenotypically stable fibrocartilaginous tissue. Increased type I collagen gene expression was observed in 1.6:0.4 scaffold seeded with outer MPC derived microtissues, while increased aggrecan expression was observed in scaffolds seeded with the inner MPC derived microtissues. Next, outer MPC derived microtissues seeded in the MEW scaffolds were cultured in vitro within a caprine meniscus explant model to evaluate the integration of the constructs with the native meniscus tissue. We observed by histological analyses (hematoxylin and eosin and safranin-O staining) that the MEW-microtissue based constructs robustly integrated with the native tissue. Finally, micro extrusion-based bioprinting was used to deposit outer MPC microtissues into a 1.6:0.4 MEW scaffold, leading to robust tissue formation with substantial sGAG and collagen deposition, as confirmed by histological and biochemical analyses. Additionally, the constructs supported a uniform type I collagen deposition, resembling the outer region of the native meniscus (Fig. 1). In conclusion, this work demonstrates the successful biofabrication of zonally defined meniscus grafts using micro extrusion-based bioprinting of fibrocartilage microtissues into MEW scaffolds.

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Konuşmacılar: Gabriela S. Kronemberger (Trinity College Dublin)

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Biofabrication and Bioprinting Strategies for Musculoskeletal Tissue Engineering

Our musculoskeletal system has a limited capacity for repair. This has led to increased interest in the development of tissue engineering and biofabrication strategies for the regeneration of musculoskeletal tissues such as bone, ligament, tendon, meniscus and articular cartilage. This invited talk will demonstrate how different musculoskeletal tissues, specifically cartilage, bone and osteochondral defects, can be engineered or repaired using emerging biofabrication and 3D bioprinting strategies. Increasingly

complex strategies will be introduced, beginning with relatively simple examples where emerging additive manufacturing platforms are used to produce cell-free biomaterials capable of directing tissue regeneration in vivo, to more complex approaches where microtissues are used as biological building blocks to engineer osteochondral grafts for biological joint resurfacing. Finally, a novel bioprinting platform will be described capable of engineering anisotropic musculoskeletal tissues by spatially patterning microtissues into temporally adapting support baths.

Konuşmacılar: Daniel Kelly (Trinity College Dublin)

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Engineering zonally defined and mechanically robust articular cartilage grafts using melt-electro written scaffolds

Articular cartilage (AC) transmits large mechanical loads in synovial joints. This tissue's properties derive from its unique composition and structure, which consists of glycosaminoglycans and type II collagen arranged into arcade-like structures [1]. AC has a limited capacity for regeneration, hence damage here typically leads to the development of osteoarthritis, a disease impacting the quality of life of millions [2]. Current clinical treatments for AC repair such as mosaicplasty and autologous chondrocyte implantation fail to regenerate AC that mimics the structure and mechanical properties of the native tissue [3]. This has motivated the development of new tissue engineering strategies to recapitulate the zonal architecture of the tissue. We previously explored the integration of melt-electro written (MEW) scaffolds with inkjet bioprinting to generate AC grafts that mimicked some, but not all, features of the native tissue [5]. Challenges remain to scale up such approaches to engineer clinically relevant cartilage and osteochondral grafts. The current study aims to address these challenges by exploring how different cell types and densities, as well as alternative MEW scaffold pore sizes, influence the organization, composition and mechanical properties of the resultant graft, with a view to engineering a tissue with similar properties to native AC.

Bone marrow-derived MSCs were isolated from mature female goats [6]. MEW scaffolds (50 layers) were fabricated from PCL with 0.2×0.2, 0.4×0.4 and 0.8×0.8mm pore sizes using a custom MEW printer [7]. Cells were manually seeded into the scaffolds at a density of 30 million cells per milliliter. Constructs were cultured in chemically defined medium supplemented with 10ng/ml TGF-β3 for 6 weeks and assessed histologically, biochemically and mechanically in tension and compression.
Additionally, articular cartilage progenitors (ACPs) were isolated from mature female goats [1]. MEW scaffolds were fabricated from PCL at a more clinically relevant height of 130 layers with a 0.8x0.8 pore size. Cells were manually seeded into the scaffolds at 15, 30 and 45 million cells per milliliter. Constructs were cultured and assessed in the same manner as described above.

Our results show that MEW scaffold pore size influences collagen organization, mechanical strength and tissue composition, with less arcade-like organization but higher ramp and dynamic moduli for the smallest pore size (Fig. 1A-D). The tensile modulus of the 0.2×0.2 construct approached 6 MPa (average in X & Y directions) after 6 weeks in culture, while its compressive modulus approached 290 kPa. We also observed differences in tissue formation between ACPs and MSCs across different cell densities. Larger, more dense cellular aggregates appear in constructs casted with a higher cell density as compared to lower densities (Fig 1E).

This study indicates that we can engineer scaled-up cartilage grafts with more biomimetic organization and mechanical properties by tailoring cell type, density and scaffold architecture. Results from this study will inform the fabrication of cartilage grafts via electromagnetic droplet printing which will be evaluated using in vitro and in vivo models to assess their potential for osteochondral defect repair. In conclusion, this approach may potentially address current challenges in engineering articular cartilage grafts.

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Konuşmacılar: Max Wirth (Trinity College Dublin)

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Orientation bioprinting using phase-separated microfibrous pore-forming bioink for muscle tissue regeneration

Introduction: Three dimensional (3D) bioprinting provides a wide avenue to design complex and customized constructs for tissue regeneration, disease modelling, and drug testing applications. Bioink formulations in 3D bioprinting usually lack the presence of micrometre-sized and interconnected pores, resulting in reduce cell viability and prevent biological communications with host tissues, which limits the therapeutic efficacy. Previously, we reported porous injectable hydrogels with microcapillary network (μCN) utilizing liquid-liquid phase separation (LLPS).[1] This study aims to design an LLPS bioink and porous 3D scaffold using the extrusion bioprinting technique for muscle tissue regeneration. This strategy enables to form fibrous microporous structures. In this presentation, the orientation of 3D structure, cell differentiation in 3D printed hydrogels, and cell survival in mouse muscle defect model will be presented.
Experiments: Gelatin methacrylate (GelMA) was synthesized by the reaction of gelatin with methacrylic anhydride. Ureidopyrimidinone (UPy) modified gelatin (GUPy) was synthesized by reaction of gelatin with UPy-isocyanate. To prepare bioink (Gel+), GelMA (10 wt%), GUPy (12 wt%) solutions, lithium phenyl (2,4,6-trimethylbenzoyl) phosphinate (LAP), and C2C12 cells were mixed thoroughly using pipette. While for bioink (Gel), GUPy replaced with gelatin. 3D printed μCN structure was engineered through photo crosslinking of GelMA matrix and dissolution of GUPy. 25-gauge (G) needle and 27G nozzle was used to print 3D structures. The stiffness of μCN bioink evaluated using a rheometer. μCN formation, cell adhesion, and differentiation in hydrogels were observed using confocal laser scanning microscopy (CLSM). Additionally, muscle defect models of mice were prepared by cutting the tibialis anterior (TA) muscles and DiI stained MSCs encapsulated in 3D scaffold were transplanted into the defects. Cell engraftment was observed at day 7 after the transplantation.
Results and discussion: Both Gel+ and Gel bioink enabled 3D printing of hydrogels with promising structure fidelity and stability. It was also confirmed that the μCN structures were formed uniformly in printed hydrogels. Moreover, bioink Gel+ exhibits excellent stiffness and less swelling which are necessary for the stability of 3D construct. C2C12 cells showed excellent adhesion, extension, migration, and proliferation in the 3D-printed Gel+ construct due to the presence of μCN, but these traits are not observed in the Gel construct. The CLSM observation revealed that the porous 3D structure not only improved material permeability, but also functioned as a void for cell infiltration which upholds our previous finding.[1] Furthermore, bioink Gel+ represented higher cell survival than that of Gel bioink. Therefore, these 3D printed porous tissues constructs may have versatile applications in the individualized therapy of tissue defects.
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Konuşmacılar: Akihiro Nishiguchi (National Institute for Materials Science)

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Application of Advanced 3D Scaffolds Based on Melt Electrowriting Technology in Bone Tumor Modeling: Geometry Optimization and Mineralization Efficiency Study

Abstract
Introduction
Osteosarcoma (OS), the most common malignant bone tumor, poses significant clinical challenges due to its aggressive progression and limited therapeutic options for metastatic disease[1]. Current preclinical models fail to replicate the mechanical and biochemical complexity of the bone microenvironment[2]. This study explores melt electrowriting (MEW) technology to fabricate polycaprolactone (PCL) scaffolds with optimized geometries and calcium phosphate (CaP) coatings, aiming to create a physiologically relevant model for patient-derived osteosarcoma cell culture.
Methods
PCL scaffolds with rectangular, triangular, and hexagonal geometries were produced using MEW (Fig. 1A,B). Two distinct CaP coating approaches were evaluated: cell-mediated mineralization using SaOS-2 cells and direct calcium phosphate cement (CPC) coating (Fig. 1C). Mechanical properties were characterized via tensile testing, while mineralization efficiency was assessed through calcium quantification (Fig. 1D). Scaffolds were analyzed for their ability to mimic the bone microenvironment by examining calcium deposition and mechanical performance.
Results
Hexagonal scaffolds exhibited superior mechanical performance with tensile strength of 269.9 ± 49.61 kPa, significantly better than triangular (221.4 ± 43.57 kPa) and rectangular (174.7 ± 42.63 kPa) structures. In terms of calcium deposition, hexagonal scaffolds achieved 3.913 ± 0.129 mmol/L in cell culture and 3.933 ± 0.114 mmol/L under CPC coating. For rectangular scaffolds, the cell-coated group showed the highest tensile strength (175.5 ± 35.73 kPa), significantly higher than the uncoated group (160.7 ± 49.92 kPa, $p < 0.05$) and the CPC-coated group (141.2 ± 18.94 kPa, $p < 0.01$). Triangular scaffolds demonstrated the best performance in the CPC-coated group (251.0 ± 44.34 kPa), significantly higher than the uncoated group (214.7 ± 43.62 kPa, $p < 0.05$) and the cell-coated group (231.8 ± 41.92 kPa, $p < 0.05$). Hexagonal scaffolds showed small differences in tensile strength among the three groups, with the cell-coated group (282.8 ± 45.62 kPa) slightly higher than the CPC-coated group (271.6 ± 32.71 kPa) and the uncoated group (269.0 ± 50.41 kPa). In the cell-cultured scaffolds, the mean calcium contents were hexagonal (3.913 ± 0.129 mmol/L), triangular (3.818 ± 0.182 mmol/L), and rectangular (3.738 ± 0.194 mmol/L) structures. For the CPC-coated scaffolds, the mean calcium contents were hexagonal (3.933 ± 0.114 mmol/L), triangular (3.808 ± 0.228 mmol/L), and rectangular (3.831 ± 0.219 mmol/L). Hexagonal scaffolds performed excellently, triangular scaffolds also showed good results, while rectangular scaffolds require optimization.
Discussion

The findings highlight the potential of hexagonal scaffolds to replicate the bone microenvironment, offering a promising platform for advancing osteosarcoma research and preclinical drug testing. Triangular scaffolds, with balanced mechanical and biochemical properties, represent a flexible option requiring both stability and bioactivity. Although rectangular scaffolds exhibited weaker performance, their straightforward design offers room for enhancement. Future research should focus on refining scaffold geometries and coating strategies to meet the diverse requirements of modeling the osteosarcoma microenvironment and improve translational utility.

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3D Biofabrication of human-derived myotendinous junction

The myotendinous junction (MTJ) is a highly specialized interface that connects skeletal muscle to tendon, enabling the transmission of contractile forces and ensuring efficient biomechanical performance of the musculoskeletal system. Functionally, the MTJ plays a pivotal role in maintaining structural continuity between two developmentally distinct tissues—muscle and tendon—while withstanding dynamic mechanical stresses during movement. Due to its complex, interdigitated architecture and constant exposure to high loads, the MTJ is particularly prone to damage under conditions of excessive stretching, repetitive mechanical strain, aging, and neuromuscular pathologies such as muscular dystrophies. In such conditions, microstructural discontinuities at the MTJ can result in compromised force transmission, tissue degeneration, and impaired motor function. Despite its critical physiological relevance, research focused on the MTJ remains limited, primarily due to the scarcity of human tissue samples and the lack of representative in vitro models that can recapitulate its architectural and cellular complexity.

To address this gap, we present the development of a fully human, biomimetic 3D MTJ-like model using rotary wet-spinning (RoWS) technology. This platform allows the precise spatial deposition of different human cell populations into aligned core–shell fiber architectures, enabling the creation of compartmentalized tissue constructs that mimic the anisotropic organization of the native junction. In this study, human primary pericytes (hPeri), representing a muscle-related lineage, and human tendon-derived stem cells (hTDSCs), involved in tendon regeneration, were sequentially loaded and extruded using the RoWS system to form structured, multicellular scaffolds. The resulting constructs demonstrated high structural integrity, anisotropy, and mechanical cohesion between muscle and tendon regions, faithfully replicating key aspects of MTJ organization.

Immunofluorescence analysis confirmed the spatially localized expression of lineage-specific markers. hTDSCs expressed tenogenic markers such as collagen I, collagen III, tenascin-C, and tenomodulin, while hPeri differentiated into myogenic domains positive for myosin heavy chain (MHC), indicative of functional muscle maturation. Notably, cells at the interface region exhibited organized interdigitations between the two compartments, resembling the transition zone of native MTJ tissue. Most significantly, expression of dystrophin—a protein classically associated with muscle fiber stability—was detected not only in the muscle region but also at the MTJ-like interface and within the tendon compartment. This unexpected localization suggests a potential, underexplored role for dystrophin in maintaining MTJ integrity and opens new avenues for studying its involvement in the progression of muscle-tendon degeneration in dystrophic conditions.

The modular and human-based nature of this platform offers several advantages over traditional co-culture or animal-derived models. The use of primary human cells enhances physiological relevance and translational potential, while the compartmentalized RoWS fabrication process ensures reproducibility and spatial control. Furthermore, the construct's compatibility with standard analytical techniques makes it well-suited for downstream applications such as disease modeling, mechanical stimulation studies, and pharmacological screening.

In conclusion, our engineered 3D MTJ-like model provides a robust, reproducible, and scalable platform for investigating human musculoskeletal physiology, injury mechanisms, and therapeutic interventions. This work represents a significant step toward the establishment of clinically relevant in vitro tools to study MTJ pathology and regeneration.

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Konuşmacılar: Marina Volpi (Warsaw University of Technology)

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High-Throughput Fabrication of Vascularized Muscle Micro-Tissues via 3D Bioprinting for Efficient Drug Screening

Abstract

Skeletal muscle plays a critical role in voluntary movement and metabolic regulation, and its dysfunction is implicated in the onset and progression of musculoskeletal and systemic diseases. While interest in the development of therapeutics for skeletal muscle disorders is steadily increasing, conventional in vitro models are limited by low throughput, the complexity of platform fabrication, attributed to the slow prototyping process not ideal for therapeutic drug efficacy evaluation and difficulties in achieving reproducibility. To overcome these limitations, we developed the high-throughput vascularized muscle micro-tissues (VMM) platform utilizing three-dimensional (3D) bioprinting technology. This platform enables the high-throughput fabrication of 3D in vitro skeletal muscle tissues and physiologically relevant vascularized muscle constructs. By employing an automated 3D bioprinting system, rapid and reproducible tissue fabrication is achieved, making the platform highly suitable for scalable drug screening applications. We optimized the fabrication and culture processes to produce highly functional and mature muscle constructs. For platform construction, commercially available 96-well plates were utilized, and mushroom-shaped pin anchors were fabricated by introducing a controlled extrusion delay during the printing process, thereby promoting muscle fiber alignment and functional maturation. The VMM platform facilitates not only quantitative evaluation of muscle functionality but also detailed analysis of muscle-vascular interactions. Furthermore, this platform addresses the persistent challenges of reproducibility in skeletal muscle tissue engineering and holds significant promise for accelerating therapeutic discovery through high-throughput drug screening, ultimately contributing to innovation in healthcare through biofabrication.

Acknowledgement

This work was supported by the Korea government programs as follows: the Ministry of Science and ICT (MSIT) under Grant No. [2020R1A5A8018367], [RS-2022-NR067329], and [RS-2024-00423107], the Ministry of Trade, Industry & Energy (MOTIE) under Grant No. [20012378], and the Ministry of Agriculture, Food and Rural Affairs(MAFRA) under Grant No. [RS-2024-00397026].

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Konuşmacılar: Taeeun Lim (Chonnam National University)

S21 From Bulk to Micro-Architecture: Hydrogel Engineering and Bio-Fabrication for Skeletal Tissue Applications

Goethe

Toplantıyı düzenleyenler: Andreas Dimopoulos (KU Leuven), Ayse Kose (KU Leuven)

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Biofabrication of Dynamic Cell-Instructive Microenvironments for Musculoskeletal Regenerative Medicine

Goethe

Biofabrication technologies, including extrusion bioprinting, bioassembly, digital light processing (DLP) and volumetric bioprinting (VBP), offer the potential to engineer constructs consisting of cell-laden bioinks, tissue modules, and/or bioactive factors that replicate the complex 3D organization of native tissues. Despite rapid advances however, development of individual bioinks for each biofabrication technique and specific tissue niche is required, limiting rapid innovation and scalability for advanced biological applications. Our overall goals are to address major bottlenecks that remain in designing materials that are both cell-instructive and harness 3D biofabrication to deliver multiple modular and dynamic cell microenvironments.

This talk discusses alternative strategies to engineer highly tuneable hydrogel platforms that: 1) promote a specific cell-instructive niche using visible-light photocrosslinking in gelatin-based hydrogels, bioresins and high-throughput microgels, and 2) that can deliver multiple spatio-temporal cell-instructive micro-environments including, cell spheroids, native decellularized extracellular matrix (dECM) materials, oxygen generation, hypoxia.

We describe design of a versatile photoinitiator system (Ru/SPS) and photo-clickable gelatin-based bioinks for biofabrication of 3D in vitro models. Tailoring macromolecular chemistry (eg allylated-gelatin hydrogels; GelAGE), we engineer the cell-instructive tissue niche for multiple cell types via covalent incorporation of thiolated bioactives and/or nanoparticles, dynamic stiffening hydrogels, as well as cross-linking of native decellularized extracellular matrix (dECM) based bioinks in centimetre scale. Example applications discussed include engineering and 3D bioprinting of clinically-relevant human stem-cell microenvironments for osteochondral tissue regeneration, osteoarthritis disease models, tumour microenvironments, and human lipoaspirate grafts.

We further discuss experience in developing hybrid tissue constructs and convergence with 3D spheroid bio-assembly platforms for probing multicellular spheroid fusion, ECM formation and stem-cell niche, offering new paradigms for high-throughput screening and disease modelling in healthy and osteoarthritic spheroid fusion models. Collectively, this work demonstrates the potential of using this cell-instructive platform for development of advanced bioinks and bioresins, advancing biofabrication and regenerative-medicine towards clinical translation

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Konuşmacılar: Tim Woodfield (Univeristy of Otago Christchurch)

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Approaches towards tendon microarchitecture through melt electrowriting and melt-electrofibrillation Goethe

Approaches towards tendon microarchitecture through melt electrowriting and melt-electrofibrillation

Jürgen Groll

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Melt electrowriting (MEW) is a relatively young additive manufacturing technology that exploits the focused deposition of a viscous thermoplast-melt thread onto a grounded target when ejected from a spinneret under voltage [1]. It enables the production of highly defined scaffold geometries built of fibers with diameters in the lower micrometer range [2].
This lecture will first demonstrate how MEW can be used to fabricate regular sinusoidal waved structures that mimic the morphology of tendons and the typical mechanical behavior [3]. The lecture will then shift towards exploiting the use of polymer-blends for MEW with a certain blend-immiscibility and a selective solubility after processing. At the example of PCL/PVAc the lecture will demonstrate that collagen mimetic bundles of nanofibrils with diameters far below the printing resolution of MEW can be achieved this way. A couple of specific topographic effects of the resulting collagen-mimetic PCL fibrillar constructs will be resented and discussed [4], including some most recent and so far unpublished results.

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Konuşmacılar: Jürgen Groll (Univeristy of Würzburg)

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Convergence of Sound-Guided Hydrodynamic Assembly and Supramolecular Self-Assembly to Fabricate Peptide–Fibrin Osteoconductive Membranes with Enhanced Mechanical Properties Goethe

Fibrin-based biomaterials are clinically established for their biocompatibility, resorbability, and hemostatic function, and have found widespread use in surgical sealants and wound repair [1]. However, their application in bone regeneration remains limited due to intrinsic softness, fast degradation, and poor mechanical tunability [2]. To address these shortcomings, we present a novel, multiscale fabrication strategy that integrates sound-guided hydrodynamic assembly with supramolecular peptide self-assembly, enabling the creation of mechanically tunable and osteoconductive peptide–fibrin hybrid membranes.
Hybrid membranes were fabricated using a one-pot process in which bioactive peptide amphiphiles (PAs) co-assembled with fibrinogen during a thrombin-mediated cross-linking. PAs were decorated with bone morphogenetic protein-2 (BMP-2) binding epitopes to promote osteoinductive signaling [3]. Simultaneously, calcined bone particles (CBPs) were patterned within the precursor via Faraday wave-induced acoustic fields (25-143 Hz). The process enabled spatial organization of CBPs during cross-linking while promoting peptide nanofiber integration. A computational model was used to correlate pattern formation with the acoustic parameters. The functional membranes were thoroughly characterized by mechanical properties, cellular infiltration, and particle distribution. Biological response was evaluated through in vitro human mesenchymal stromal cells (hMSC) culture and an immunological profiling was conducted using peripheral blood mononuclear cells (PBMCs) and Olink proteomic analysis.
The fabrication process resulted in radially patterned distributions of CBPs embedded in a peptide–fibrin nanofibrous mesh. The supramolecular assembly of PAs increased the overall stiffness of the membrane, while sound-guided hydrodynamic patterning generated anisotropic mechanical properties tunable via acoustic frequency. A theoretical-experimental relationship was established between wave frequency and resulting membrane stiffness. In vitro, the membranes supported high hMSC viability, promoted cell infiltration, and maintained the integrity of the CBPs pattern. Proteomic analysis of PBMC-conditioned media revealed upregulation of osteogenic and remodeling-associated cytokines, suggesting that the materials can activate pathways associated with osteogenic commitment and bone remodeling.
This study introduces a novel fabrication strategy that converges peptide self-assembly with sound-guided hydrodynamic assembly to yield a mechanically reinforced, biologically active hybrid material. Unlike conventional cross-linking or filler-based reinforcement, this approach provides precise multiscale control over structure and function. The ability to pattern mineral particles and co-assemble peptide nanostructures within fibrin provides new opportunities for designing customizable biomaterials for bone-related applications. While regenerative outcomes remain at a proof-of-concept stage, the method establishes a robust, scalable platform for the biofabrication of mechanically-enhanced, osteoconductive membranes.

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Konuşmacılar: Alessandro Cianciosi (AO Research Institute)

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Microporous injectable hydrogel scaffold for cell transplantation therapy against volumetric muscle loss Goethe

Introduction
Volumetric muscle loss (VML) is a traumatic or surgical injury of the skeletal muscles with irrecoverable functions, which leads to chronic deficits and long-term disability. Regenerative medicine using mesenchymal stem cells (MSCs) is a potent therapeutic approach for VML due to their tissue regenerative ability. However, the therapy for VML remains a challenge in regenerative medicine owing to poor graft survival of cell suspensions injected into the body. Although injectable hydrogels as a cell delivery carrier can enhance cell delivery efficiency, densely cross-linked hydrogels without micro-sized pores often result in poor integration with the host tissues and delayed muscle tissue regeneration.
In this study, we aim to develop micropore-forming injectable hydrogels to deliver MSCs at high efficiency for the treatment of VML (Figure 1). The molecular modification of gelatin with hydrogen-bonding functional group, 2-ureido-4[1H]-pyrimidinone (UPy) units, induced liquid-liquid phase separation (LLPS) when mixed with UPy-unmodified gelatin to form porous injectable hydrogels with microcapillary-like network structures [1]. In this presentation, we will discuss the fabrication of injectable hydrogels, the effect of internal structures of injectable hydrogels on cellular functions, and the therapeutic efficacy against VML.

Materials and Methods
Several hydrogels with different micropore sizes were prepared by varying the concentration of polymer: non-porous, small, medium, and large micropores. MSCs were encapsulated in each hydrogel and cultured for 48 h. The structures of hydrogels and cellular morphology were observed by confocal laser scanning microscopy (CLSM). To verify the cellular function in porous hydrogels, proliferation and secretory function of donor cells and cellular infiltration and differentiation of myoblasts were evaluated. Additionally, VML models of mice were prepared in tibialis anterior muscles and DiI stained MSCs dispersed in PBS or encapsulated in hydrogels were transplanted into the muscle defects. Cell engraftment was observed at day 7 after the transplantation and therapeutic efficiency was evaluated at day 28 after the transplantation.

Results and Discussion

CLSM observation revealed that LLPS structures were formed in hydrogels and microcapillary network-like pores were created by the dissolution of non-crosslinked polymer. Notably, MSCs encapsulated in porous hydrogels with small micropores (approximately 8 μm) showed higher cell adhesion and spreading than that of other hydrogels. Furthermore, this porous hydrogel promoted the proliferation and secretion of paracrine signals of MSCs compared to that in non-porous hydrogels. Porous hydrogels also enhanced cell infiltration and differentiation of myoblasts, indicating that porous hydrogels can promote integration with muscle tissues of host. Additionally, porous hydrogels represent higher cell survival and therapeutic efficacy than that of non-porous hydrogels when MSCs were delivered into VML model of mice. These results suggest that porous hydrogels functioned as not only the scaffold of transplanted cells but also as interconnected voids to enhance cell-material interaction, resulting in improvement of engraftment and therapeutic efficacy against VML.

Reference

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Konuşmacılar: Hana Yasue (Graduate School of Advanced Engineering / National Institute for Materials Science)

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Aqueous Emulsion Bioprinting of Anisotropic Living Architectures with Programmable Porosity and Enhanced Cellular Infiltration Goethe

Bulk hydrogels are comprised of nanoporous polymer networks, and thus restrict cell motility, cell-cell interactions, and nutrient diffusion in bioengineered tissues. To overcome these challenges, interest has shifted toward the fabrication of interconnected microporous hydrogels, which improve nutrient transport, facilitate cell migration, and promote tissue ingrowth. Current fabrication methods, however, often rely on cytotoxic porogenic agents, limiting scalability and biocompatibility. This work presents a tunable and bioprintable microporous hydrogel bioink based on GelMA-HAMA aqueous two-phase systems (ATPS). By leveraging the immiscibility between GelMA and aqueous porogen solutions comprised of polyethylene oxide and xanthan gum, we developed a simple, yet robust method to generate highly interconnected porous networks, achieving porosities up to 70%. Crucially, this approach allows for cell inclusion within the pre-gel solution, minimizing washing steps and providing precise control over the hydrogel microarchitecture. The formulated photocrosslinkable bioinks were 3D printed using a viscoelastic support bath, enabling freeform fabrication of anisotropic structures that supported favorable cell proliferation and alignment. This platform offers a scalable, biocompatible route to fabricate functional tissue scaffolds with controlled porosity and geometry in physiological conditions that uphold cellular activity, advancing the toolbox of bioinks to generate programmable porous constructs.

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Konuşmacılar: Hugo Fernandes (University of Aveiro)

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In-Situ Atmospheric Pressure Plasma-Assisted 3D Printing of Antibacterial PLA Scaffolds for Bone Tissue Engineering

Polylactic acid (PLA) is an eco-friendly and biocompatible polymer commonly utilised in bone tissue engineering. However, its absence of antibacterial properties and susceptibility to wet chemical processes hinder its clinical application. Atmospheric pressure plasma (APP) is widely used for surface activation, but its application is typically confined to shallow surface modifications due to poor penetration depth. In this study, we present an innovative strategy that integrates APP directly into the bioprinting process, enabling in-situ functionalization of PLA scaffolds during fabrication. PLA is printed using a specialized 3D printing system incorporating APP treatment with a hybrid precursor that consists of acrylic acid (AAc) and silver nitrate. During the printing process, the APP facilitates in-situ plasma polymerisation and the reduction of silver ions, resulting in the direct attachment of carboxyl groups and silver ion reduction to the PLA scaffold surface. This quick and dry in-situ method incorporates plasma treatment into the 3D printing process, breaking through the limitation of APP on homogeneously functionalization of 3D substrate, and allowing for real-time functionalization while maintaining the structural integrity of PLA. The final 3D-printed PLA scaffolds demonstrate significantly enhanced hydrophilicity and antibacterial properties while preserving cytocompatibility. This combined printing and functionalization method offers a scalable and effective approach for producing advanced, infection-resistant scaffolds for applications in bone tissue engineering.

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Konuşmacılar: Asnake Ketema (Ming Chi University of Technology)

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In vitro modelling of skeletal muscle tissue and its interfaces using rotary wet-spinning (RoWS) biofabrication Goethe

The regeneration of skeletal muscle (SM) tissue and its interfaces—namely the myotendinous junction (MTJ) and neuromuscular junction (NMJ)—remains a significant challenge in both engineering and clinical domains. Recent advances in biofabrication have begun to address these hurdles, enabling the creation of biomimetic architectures with precise spatial control over cellular organization and mechanical properties.

Here, we present a comprehensive investigation into the use of microfluidics-assisted 3D rotary wet-spinning (RoWS) for the scalable fabrication of functional, anisotropic, and vascularized hydrogel-based constructs tailored for skeletal muscle, MTJ, and microvascular tissue engineering. Our recently developed high-throughput RoWS platform enables the continuous production of core–shell hydrogel microfibers, which serve as modular building blocks for hierarchically structured 3D scaffolds. By optimizing key parameters—including flow rates, rotational speed, and fiber composition—we produced highly aligned fiber bundles that guide uniaxial cell alignment and promote myogenic differentiation.

Using this platform, we demonstrated that constructs encapsulating human pericytes exhibit significantly enhanced myogenic maturation compared to traditional 2D cultures and 3D bulk hydrogels. Proteomic analysis revealed a distinct molecular signature associated with the anisotropic 3D microenvironment, marked by upregulation of contractile and extracellular matrix proteins. In vivo studies in a murine model of volumetric muscle loss further validated the regenerative potential of these constructs, which showed successful engraftment and restoration of muscle architecture.

Building on these findings, we adapted the RoWS system to engineer MTJ-like hydrogel yarns through sequential wet-spinning of C2C12 myoblasts and NIH 3T3 fibroblasts. This strategy recapitulates the graded biological and structural organization of the native muscle–tendon interface. The resulting constructs displayed uniaxial cellular alignment and expression of tissue-specific markers (e.g., MyHC, collagen I/III), and featured hallmark finger-like projections at the muscle-tendon transition zone—mimicking the native MTJ microarchitecture with high fidelity.

To address the crucial need for vascularization in engineered muscle constructs, we also developed a co-culture system comprising human mesenchymal stem cells (hMSCs) and human umbilical vein endothelial cells (HUVECs) within a fibrin-based core. The RoWS facilitated the formation of aligned, capillary-like networks and robust expression of endothelial markers such as CD31, confirming successful microvascularization.

Currently, we are extending our platform to incorporate neuro-mesodermal progenitors (NMPs) for the fabrication of “tubuloids” that may emulate the structural and functional complexity of the NMJ.

Collectively, these findings underscore the versatility and translational promise of the RoWS biofabrication platform for engineering anisotropic, multicellular, and vascularized musculoskeletal tissue constructs. With its high functional performance, biomimetic precision, and scalability, this platform offers a compelling foundation for both next-generation in vitro models and future therapeutic strategies in tissue regeneration.

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Konuşmacılar: Marco Costantini (Institute of Physical Chemistry - PAS)

- 17:00

Coffee break & Poster Session I

- P2 Plenary Session: Prof. Molly Stevens Ratuszowa

Toplantıyı düzenleyenler: Shrike Zhang (Harvard Medical School)

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New bioengineering approaches for advanced therapeutics, regenerative medicine and soft robotics

This talk will provide an overview of recent advances in bioinspired materials for therapeutic and regenerative medicine applications, with a particular focus on establishing translational pipelines to bring our innovations to the clinic [1]. We have developed fabrication methods to engineer complex 3D architectures and biofunctionalized surfaces, incorporating spatially arranged bioinstructive biochemical and topographical cues [2]. Our therapeutic delivery portfolio includes high-molecular-weight polymer carriers for enhanced delivery of saRNA therapeutics, as well as photo-responsive nanoreactors inspired by circadian rhythms [3]. Additionally, we are developing innovative solutions for targeted and controlled delivery using soft robotics with unique bioinspired properties that respond to external stimuli to release therapeutic payloads [4]. Our design approach integrates state-of-the-art fabrication techniques while prioritizing versatility and scalability to maximize translational potential.

Furthermore, we have developed Raman microspectroscopy imaging tools and machine learning algorithms for hyperspectral unmixing of complex biological imaging. These technologies enable us to investigate live-cell and organoid models and visualize the in vivo fate of nanomedicines [5]. I will present recent advances in Raman spectroscopy for high-throughput, label-free characterization of single nanoparticles—an approach we pioneered through the SPARTA™ technique—which allows for the comprehensive analysis of a broad range of nanoparticle-based therapeutics [6].

Finally, I will explore how these versatile technologies can drive transformative biomedical innovations. I will also discuss our efforts in establishing effective translational pipelines to accelerate clinical applications while actively working towards the democratization of healthcare [7].

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Konuşmacılar: Molly Stevens (University of Oxford)

- ISBF General Assembly Ratuszowa
- 20:30

Young Scientist Night

Chłodna Kręgliccy, Chłodna St. 31

- 16 Eylül Salı
- Rapid Fire Talks 2 Kisielewski

Toplantıyı düzenleyenler: Nehar Celikkin (Institute of Physical Chemistry (PAN))

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Anisotropic microfiber reinforcement facilitate tailored mechanical design in large-scale, biofabricated cartilage constructs

Introduction
Cartilage defects pose significant challenges in terms of healing. Current treatments have limitations in size, availability, or durability.[1,2] Biofabrication aims to restore tissue functionality by placing biological active components in a pre-defined 3D organization, typically using soft hydrogels for cell preferences.[3] These hydrogels can be mechanically reinforced with microfiber structures generated with melt electrowriting (MEW).[4] Small scale (diameter 6mm, A=28 mm2) Fabricated osteochondral plugs with cell-laden hydrogel reinforced with MEW meshes are stable in vivo.[5] Translating this towards patient-specific implants, requires consideration of an overall increase in size (>2 cm2) and the local differences in cartilage mechanical properties throughout the articulating joint. This study investigates the local, anisotropic mechanical properties of a large sized MEW-reinforced cell-laden hydrogel scaffold.

Materials and methods
Polycaprolactone (PCL) microfiber box-shaped scaffolds were fabricated using melt electrowriting (MEW) with inter-fiber spacing from 200 x 200 µm to 500 x 500 µm with 100 µm increments. A large-scale (15 cm2) anisotropic scaffold was designed with fiber spacing ranging from 300, 400, and 500 µm, embedded with equine articular cartilage progenitor cells (ACPC) and cell-free gelatine methacryloyl (gelMA), and crosslinked with dichloro-ruthenium (II) hexahydrate and sodium persulfate. Mechanical testing was performed on cell-free constructs, and the compressive E-moduli were measured between 10% and 15% strain. The cell-laden constructs were cultured for 28 days in chondrogenic differentiation medium. Post-culture, the constructs were analyzed for GAG, collagen type I and II, metabolic activity, and cell morphology.
Results
By altering the inter fibre spacing of gelMA embedded MEW-reinforcement, the compressive modulus varied from 0.49 ± 0.18 MPa for 500 µm to 2.52 ± 0.17 MPa for 200 µm. The large sized anisotropic scaffold showed similar mechanical behavior, resulting in an anisotropic mechanical design of the scaffold, which could be distinguished as a high (300 µm), medium (400 µm), and low (500 µm) density fiber zone. The ACPCs in the cell-laden constructs showed homogenous behavior in the different regions and staining showed production of GAGs and collagen type II. Biochemistry showed a lower DNA content in the low-density zone, but higher GAG production compared to the medium density zones.

Conclusion
This study shows the effect of mechanical reinforcement of cell-laden hydrogel scaffolds using scaffolds with anisotropic designs. Manipulating the internal box-spacing enables to regulate the mechanical properties of the scaffold, while showing homogenous behavior of cartilage cells throughout a large-scale mechanically anisotropic scaffold design. These findings allow to produce clinically-relevant sized constructs with personalized features, while providing a viable environment for cartilage cells.

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BIOINK DEVELOPMENT FOR IN SITU OSTEOCHONDRAL REGENERATION THROUGH MICROEXTRUSION AND FILAMENTED LIGHT BIOPRINTING

Introduction
Osteoarthritis (OA) is a degenerative disease affecting osteochondral (OC) tissue, leading to pain and joint dysfunction. Current treatments are often limited by availability, efficacy, and cost, highlighting the need for innovative therapeutic approaches. To address this challenge, we propose a novel tool EndoFLight, an advanced in situ 3D bioprinting platform designed for minimally invasive regeneration of large OC lesions (Figure 1a). This system integrates multi-material, multiscale bioprinting by combining bioink extrusion through standard arthroscopic instruments with filamented light (FLight) printing1 to enable precise deposition and crosslinking of cell-laden photo-resins directly at the injury site.

This study presents the formulation and characterization of photo-resins for EndoFLight technology, which aims to provide a clinically translatable solution for osteochondral tissue regeneration in OA treatment.

Methods
Photo-resins were developed using gelatin methacrylamide (GelMA, X-pure grade, Rousselot), either alone or blended with methacrylated hyaluronic acid (HAMA, LifeCore Biomedical) or hydroxyapatite (HAp, CAM Bioceramics). These formulations were designed to support cartilage and bone regeneration, respectively, with lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) serving as the photo-initiator. The selection of X-pure grade GelMA was motivated by its GMP-compliant production and ultra-low endotoxin content, ensuring its suitability for future clinical applications.

Rheological properties of the photo-resins were analyzed using an Anton Paar MCR 702 rheometer to assess their photo-crosslinking characteristics. The resins were exposed to blue light (400–500 nm, 25 mW/cm²) for 10 minutes at 37°C while simultaneously monitoring the storage and loss moduli. Finally, biocompatibility was evaluated in vitro via LIVE/DEADTM assays. Analyses were performed on crosslinked cell-laden photo-resins and cell monolayers to which uncrosslinked photo-resin droplets were added, with the latter undergoing crosslinking afterwards.

Results

The formulated GelMA/HAMA and GelMA/HAp blends were optically transparent, essential for precise bioprinting via EndoFLight. Photo-rheology tests confirmed their rapid photo-crosslinking behavior, with all resins reaching a stable plateau storage modulus of 4–8 kPa (Figure 1b), which is within an optimal range for cartilage and bone tissue formation, as supported by previous studies.1,2 While HAMA incorporation led to a slight increase in storage modulus, no statistically significant difference were found between the blends and pure GelMA solutions (Figure 1c). Preliminary viability tests indicate that the photo-resins are biocompatible both before and after photo-crosslinking.

Discussion

This project aims to address an unmet clinical need through an innovative, one-step technology to accurately reconstruct the complex architecture of articular cartilage directly in the OC lesion, thereby promoting in situ regeneration. Future research will focus on optimizing the bioinks for the EndoFLight setup, followed by validation of the system through in vitro, ex-vivo and in vivo studies.

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Routine GelMA Characterization: A High-Throughput Approach Integrating Electrophoresis and UV-Vis Spectroscopy

Gelatin Methacryloyl (GelMA) attractes considerable research attention as an important structural component for bioinks.1 The synthesis of GelMA involves the methacrylation of gelatin, wherein methacryl groups are covalently bonded to the amino groups of lysine residues. The degree of methacrylation (DM) is a critical parameter that significantly affects the physicochemical properties of GelMA. For effective bioprinting of organs and tissues, the use of GelMA with a precisely defined DM is essential, as it influences hydrogel stiffness, porosity, swelling behavior, biodegradability, and cellular proliferation. Consequently, there is a need for accessible, cost-effective, precise, and routine methodologies to control and assess the DM of GelMA. This necessity motivated us to develop two novel and complementary methods for determining the DM, both of which can be readily implemented in laboratories equipped with electrophoresis instrument or UV-Vis spectrophotometer.

Currently, the most widely used method for characterizing the degree of methacrylation of GelMA is nuclear magnetic resonance spectroscopy (NMR).2 Due to the high cost of NMR instrument maintenance, the NMR technique is not routinely available in every laboratory. We are proposing alternative approaches to characterizing GelMA that can be used for routine laboratory analysis. The first proposed method relies on measuring the electrophoretic mobility of GelMA samples, which directly correlates with their degree of methacrylation. This approach requires comparison against reference standards with known DM values and can effectively serve as a classification tool for GelMA products based on their DM. The second method we developed focuses on quality assessment after GelMA fabrication, enabling characterization of pure GelMA through analysis of its absorption spectrum. Although this spectroscopic technique does not require the addition of external reagents such as TNBS,3 the construction of a calibration curve remains necessary to achieve precise quantification.
The precision of both newly developed methods is comparable to the NMR technique, exhibiting excellent correlation with NMR data (Table 1). The electrophoretic method enables effective characterization of GelMA even when organic impurities are present. Although this method is somewhat more time-consuming, it can conveniently be substituted with the UV-Vis method for routine applications.

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Multispheroid Bioassembly through Meltelectrowriting of a Precisely Arranged Fibrous Architecture for Tissue Models

Introduction

Over the past few decades, extensive research has been actively conducted for the fabrication of human tissues to, amongst many applications, understand the effects of a wide range of chemicals on human health and the environment (1). Thus, the need for the development of innovative assessment tools that provide reliable results in identifying and regulating the risks of potentially harmful substances is increasingly recognized. This study presents a precisely arranged fibrous architecture, fabricated via MeltElectroWriting (MEW) as spheroid culture support for 3D in vitro models. This platform facilitates multi-spheroid bioassembly, overcoming single spheroid limitations such as hypoxia while promoting cell-cell communication and adaptive interactions with the surrounding Extracellular-Matrix-like fibers (2). The technique has been employed to recapitulate different human tissues, for this study the targeted tissue was the thyroid.

Methods

The scaffolds are printed with an in-house build MeltElectrowriting device by heating a syringe containing melted Polycaprolactone and extruding the material with air pressure while applying voltage to pull a microscale fiber. Fibers are precisely deposited in the shape of squared boxes, in a variety of sizes between 100 µm and 1 mm. The scaffolds perimeter was reinforced with a 3D printed Poly Lactic Acid ring for better handling and coated in polydopamine for improved cell compatibility (3). Human thyroid epithelial cells (huThyrEC) spheroids are formed by cell aggregation in wells to be a similar size as the boxes and after 3 days of culture- supplied with thyroid-stimulating hormone- are transferred in the scaffold so that each box is occupied by a single spheroid. The assemblies are further cultured for 7 more days followed by evaluating thyroid hormones T3 and T4, thyroid-related protein, metabolomic and transcriptomic changes.

Results

The final scaffolds present a box size of 530 µm and a highly precise architecture, raman analysis confirmed the presence of polydopamine on the surface. Spheroids cultivated in the MEW scaffolds disassembled long the fibers to occupy the box volume and showed significantly reduced hypoxia compared to single spheroid controls that would instead compact. Thyroid hormone secretion was significantly higher in the spheroid groups compared to monolayer with improved results for the MEW ones. From metabolomics it was evident how this tool can bridge the gap between 2D and 3D cultures while transcriptomics resulted in evidence of more upregulated genes in the fibrous environment.

Discussion

Analyzing thyroid hormone levels, protein, metabolomic and transcriptomic changes, provided a comprehensive understanding of cellular responses and metabolic shifts crucial for evaluating thyroid function and response to exposure to harmful or potentially hazardous substances.
Our findings suggest that the microarchitectures platform provides a reliable in vitro model for regulating thyroid function, representing a significant step toward advanced toxicity assessment tools.

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HYCON: A Hydrogel-based Conformable Electrode Array for Noninvasive Electrophysiological Recording of Brain Organoids

HYCON: A Hydrogel-based Conformable Electrode Array for Noninvasive Electrophysiological Recording of Brain Organoids

Introduction:

Brain organoids have become an essential tool for modeling human brain development, neurological disorders, and therapeutic interventions. However, designing reliable interfaces for these 3D, delicate structures remains a key challenge. Current electrophysiological approaches either rely on penetrating mesh electrodes that grow into the organoid over time1, or on predefined 3D structures such as basket-shaped micro electrode arrays (MEAs)2 or kirigami-inspired MEAs3 that constrain organoid morphology and eventually penetrate the organoids. While effective in some contexts, these strategies have major limitations: poor adaptability to organoid-to-organoid variability; risk of mechanical stress, and; complex fabrication or integration steps.2

Methods:

To address these limitations, we developed the HYCON array (Hydrogel-based Conformable Electrode Array), a mechanically adaptive platform that allows for conformal contact of electrodes with brain organoids without the need for invasive penetration or rigid 3D structures. The HYCON array consists of a soft, stretchable MEA fabricated using PEDOT-PSS on a styrene-butadiene-styrene (SBS) substrate, which is freely layered on top of a biocompatible hydrogel. The hydrogel with a tunable stiffness of 0.5–2 kPa, acts as a compliant support layer underneath the MEA. A similarly soft hydrogel pocket holds the organoid in place atop the HYCON array. This geometry enables passive conformation of the electrode layer around the curved organoid surface, accommodating organoid shape variation and minimizing compression of the organoid.

Results:

Mechanical characterization of the HYCON array demonstrated that the soft hydrogel layer deforms under mild mechanical loading to conform the PEDOT-based MEA around the organoid without requiring predefined mechanical shaping. Unlike basket-type or rigid supports, the HYCON array adapts to different organoid geometries. The stack is fully flat when fabricated, which simplifies the fabrication process and makes it easier to integrate with standard recording hardware. The conformable interface remains stable even after an organoid has been repositioned on top of it multiple times.

Discussion:

The HYCON array is the first fabricated in-vitro neural interface of its kind, as it relies on hydrogel compliance and passive deformation of the electrodes, rather than predefined or invasive structures, to enable contact with organoids. This design provides mechanical support and matches organoid curvature without risking damage or requiring fixed organoid dimensions. This approach is particularly advantageous for dynamic or large-scale organoid experiments, where shape variability is high and minimal perturbation is critical.

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Reproducible in vitro vascularization via high throughput molding of sacrificial scaffolds

Introduction:

Replicating physiologically relevant tissue environments in vitro requires both sophisticated biological design and reliable scalable fabrication. Current approaches often rely on complex biofabrication methods such as stereolithography (SLA) and melt electrowriting (MEW), which are time-intensive, laborious, and lack reproducibility at scale [1,2]. To overcome these limitations, we developed a vascularized in vitro platform that integrates microchannel networks with industrial manufacturing methods, enabling reproducibility, scalability, and ease of use.

Methods:

The platform incorporates sacrificial template structures embedded within a biocompatible matrix. The dissolution of the template unveils a fully interconnected, perfusable microchannel network within the matrix for seeding with endothelial cells (ECs). This vascular network supports dynamic perfusion from the onset of cell culture, promoting long-term cell viability and tissue development. We transitioned from SLA and MEW to injection molding and vacuum casting, respectively to facilitate large-scale production. Injection molding was used to fabricate precise housing and structural components, while vacuum casting enabled flexible production of complex sacrificial template geometries using biocompatible materials.

Results:

The manufactured platforms demonstrated high reproducibility in microchannel geometry and structural fidelity across batches. Perfusable vascular networks were successfully formed following template dissolution, allowing for continuous media perfusion throughout the culture period. In preliminary cultures, embedded endothelial cells exhibited sustained viability and functionality throughout the entire culture period as confirmed by immunofluorescence staining and permeability assay, respectively. The perfusion system has the potential to enable controlled delivery of compounds, providing a platform for future physiologically relevant drug testing scenarios. Preliminary throughput assessments showed a significant reduction in production time and cost compared to SLA/MEW-based methods, with consistent device quality and usability.

Discussion:

Transitioning to industrial-grade manufacturing significantly improves the scalability of our in vitro system via enhanced reproducibility and reduced costs without compromising biological performance. The integration of injection molding and vacuum casting enables the creation of a ready-to-use, vascularized platform compatible with standard lab workflows. This approach addresses a critical bottleneck in tissue model development by aligning complex biological function with manufacturability. Future work will expand platform adaptability to other tissue types and perfusion modalities, further enhancing its value for pharmaceutical and academic research.

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Konuşmacılar: Patrick Kuntschke (University Hospital Wuerzburg)

- P3 Plenary Session: Prof. Jason Burdick — Ratuszowa

Toplantıyı düzenleyenler: Tim Woodfield (University of Otago Christchurch)

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Additive Manufacturing of Hydrogels for Biomedical Applications

Hydrogels are water-swollen polymer networks that have gained great interest in the field of medicine. While hydrogels are often used as uniform isotropic materials, their processing to include microstructural cues (e.g., porosity, patterning) can further enhance their use. I will provide several recent examples where we have developed methods to introduce microstructure into hydrogels. As one approach, we engineer granular hydrogels through the jamming of hydrogel microparticles, where structure can be altered through the incorporation of anisotropic particles or through the inclusion of cell aggregates. These materials are useful for either endogenous tissue repair (e.g., myocardial infarction) or for tissue engineering (e.g., cartilage). As another approach, hydrogels are processed with lithography-based (i.e., digital light processing, DLP) 3D printing to introduce microstructure. We have been advancing the development of new DLP resins (e.g., responsive nanodomains), as well as DLP-printing techniques (e.g., incorporating chain entanglement) to improve material toughness and fatigue resistance. Such tough hydrogels are being explored as scaffolds for tissue engineering or as tough biomedical adhesives.

Konuşmacılar: Jason A. Burdick (University of Colorado Boulder)

- Debate 1 — Ratuszowa
- 10:30

Coffee break & Poster session II

- Poster Session II: Poster Exhibition — Poster Area

posters are on display whole day on Tuesday and Wednesday

Toplantıyı düzenleyenler: Marco Costantini (Institute of Physical Chemistry Polish Academy of Sciences), Carmelo De Maria (University of Pisa)

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3D Bioprinting Nanofiber-Reinforced Vascular Branches

In this study, we explore a hybrid biofabrication approach combining embedded 3D bioprinting and electrospinning to create bifurcated vasculature. A bifurcated graft is essential during open surgical repair when the aorto-iliac part of the vasculature is diseased. However, fabricating a tissue-engineered vascular graft (TEVG) that closely mimics the native blood vessel network presents significant challenges, namely replicating the complex multi-layered structure of the native vessels, and TEVGs are prone to complications such as thrombosis and intimal hyperplasia. Our approach uses the Freeform Reversible Embedding Hydrogels (FRESH) bioprinting method. Our bifurcated gelatin-based hydrogel structure is printed inside an agarose support bath. Since achieving uniform nanofiber deposition onto bifurcated structures via conventional electrospinning is challenging, our method uses the incorporation of short nanofibers inside the hydrogel matrix prior to bioprinting. Trial runs showed that the composite hydrogel exhibited improved mechanical properties compared to the nanofiber-free hydrogel. Our future work will focus on optimizing the mechanical performance of the printed constructs. Hence, this approach has the potential to serve as a starting point for the biofabrication of complex tissue structures, like vascular branches and soft organs.

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3D Bioprinting of large multilayered blood vessels based on hiMPCs vasculogenesis

Introduction and Aim

The in vitro generation of functional vasculature remains a major challenge. Current models use adult cells to mimic the three layers of native blood vessels. Yet, the coordinated morphogenetic events during vasculogenesis are key for vessel functionality and stability. iPSC-derived mesodermal progenitor cells (hiMPCs) remain viable and undergo vasculogenesis after extrusion; still, their patterning into defined shapes is yet to be established. Our study aims to biofabricate multilayered large blood vessels using hiMPCs. We developed an in-gel bioprinting system to print low-viscosity bioinks into tubular constructs and mimic vasculogenesis.

Materials and Methods

FGX, a blend of cold water fish skin GelMA (fGelMA), porcine skin GelMA (pGelMA) and xanthan gum (XG) was combined with fibrillar collagen type I to produce FGXC, a low viscosity, temperature stable bioink for bioprinting hiMPCs. The bioink was extruded into tubular structures using a XG-based embedding medium. The material rheological behavior and printability was compared to 5% pGelMA, as well as cellular viability and morphogenetic capacity over time.

Results

The rheological evaluation of FGX demonstrated it is a shear thinning, temperature stable biomaterial. The low gelling point of fGelMA contributes to its room temperature handling, while the mechanical strength of pGelMA contributes to its low swelling and stability over time, and XG acts as a viscosity enhancer and porogen. The temperature stability of FGX allows the bioink to be printed under stable conditions at room temperature, contrary to 5% pGelMA, which requires constant adjustment of pressure, temperature and speed. Additionally, cryo-SEM images revealed that the polymeric network of FGX was more homogeneous than in 5% pGelMA.

FGX was combined with fibrillar collagen type I, which can also be handled at room temperature and did not affect the printability of the material. The addition of col I improved cell viability and migration. Differentiation into vascular cell types and formation of vessel-like structures was observed as early as after 3 days of culture. After 7 days of culture, the lumen was fully lined with a layer of endothelial cells (CD31+), which was surrounded by a layer of smooth muscle cells (SMA+), similar to what is observed in native blood vessels. Additionally, the tube wall contained cell populations that expressed markers related to macrophages, immune cells, and hematopoietic stem cells.

Conclusion

FGXC is a novel formulation suitable for extrusion-based bioprinting because of its temperature stability. It was used in an embedding bioprinting setup to produce shape defined and stable tubular structures. hiMPCs have the potential to differentiate into vascular cell types and they were shown as suitable for the biofabrication of large vascular structures. hiMPCs differentiated into endothelial cells and smooth muscle cells in an organized multilayered manner, showing that a single cell type and a single biomaterial can be used to produce multilayered, multicellular structures.

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Konuşmacılar: Nathaly Chicaiza Cabezas (University of Wuerzburg, Department for Functional Materials in Dentistry and Medicine)

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3D Printed Polycaprolactone/Copper-Doped Natural Hydroxyapatite Quantum-Dots Nanocomposite with Superior Osteogenic and Angiogenic Performance in Bone Tissue Engineering

Bone defects arising from trauma, infection, or tumor resection presented a major clinical challenge due to the limited self-repair capacity of large bone defects. In this study, a 3D-printed polycaprolactone (PCL) scaffold incorporating copper-doped natural hydroxyapatite quantum dots (Cu-HA QDs) was designed to accelerate both osteogenesis and angiogenesis. The Cu-HA QDs were produced using biowaste material and a solid-state reaction method. After incorporating Cu-HA QDs into PCL, the scaffold was fabricated via a custom-built 3D printing system. SEM images of the 3D-printed scaffolds showed a highly regular filament architecture and excellent printability of the composite system. Incorporation of Cu-HA QDs markedly increased scaffold stiffness, wettability, and in vitro degradation rate. Cu-HA QD incorporation significantly enhanced cell adhesion and mesenchymal stem cells (MSCs) calcification on the PCL scaffold. Gene expression analysis of MSCs at 14 days demonstrated that the PCL/Cu-HA QD scaffold markedly upregulated osteogenic markers—ALP, RUNX2, and OCN—by 6.8-, 18-, and 21.3-fold, respectively, and angiogenic markers—HIF-1α, VEGF, and Ang1—by 21.4-, 2.1-, and 12.8-fold, respectively. Immunofluorescence staining for OCN confirmed abundant extracellular matrix mineralization, while CD31 staining verified enhanced endothelial marker expression on the PCL/Cu-HA QD scaffold. These dual osteo- and angioinductive effects were attributed to sustained release of Ca^{2+} and Cu^{2+} ions, which synergistically activate BMP, Wnt, and HIF-1α/VEGF signaling pathways. 3D-printed PCL/Cu-HA QD scaffold combined strong printability, controlled degradation, and dual osteogenic–angiogenic bioactivity, providing a promising approach for the regeneration of large bone defects.

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3D Printing of Medical-grade Silicone Elastomer Toward Personalized Soft Tissue Implants

Introduction

Prosthesis and implants are integral parts of modern healthcare, with silicones widely used for their chemical inertness, tissue-like mechanical properties, and adaptability. However, conventional techniques in silicone processing face limitations in structural complexity and patient specificity. 3D printing has emerged as a promising technique for creating personalized medical devices, but the use of medical-grade silicone is limited due to multiple challenges.

Methods

A customized 3D printer was used to print medical-grade silicone. Printing parameters like print bed temperature (e.g., 90°C) and print orientation (e.g., 45°) were varied systematically. The printed samples were characterized for their mechanical performance (tensile properties), defect formation (X-Ray µCT), leachable moieties (NMR), and cytotoxicity.

Results.

Mechanical testing of the samples by tensile mode indicates that the specimens printed at 90°C with 45° orientation showed the lowest tensile strength and modulus. X-ray µCT confirmed increased pore volume fraction for these specimens. These samples also showed lower sphericity values, indicating elongated defects. Both 13C and 29Si NMR detected -CH3 and dimethylsiloxane groups within the leached samples. AlamarBlue and Live/Dead analysis revealed that the leached moieties do not adversely affect the cells. The cells proliferate well from day 1 to day 7 without significant differences between the control and printed samples.

Discussion

The results of this study demonstrated that the print bed temperature and layer orientation influenced the mechanical properties and defect formation of the 3D-printed silicones. Lower tensile strength and modulus for the samples printed at a higher bed temperature and 45° orientation can be attributed to the formation of pores of defects within the 3D-printed samples. This is corroborated by the X-ray µCT analysis, which shows higher pore volume fraction and elongated pores. These defects arise from the printing conditions where entrapped air does not have time to escape before complete curing. The NMR studies show the presence of some precursor moieties that may have

been part of either of the two-part silicone systems. These moieties do not show any cytotoxic effect, as seen from the AlamarBlue and Live/Dead assay.
Conclusion
This study successfully demonstrated the feasibility of 3D printing medical-grade silicones using a customized printer. By optimizing printing parameters, it is possible to achieve desirable mechanical properties, minimize defects, and ensure the biocompatibility of the printed silicone parts. These findings have significant implications for fabricating patient-specific implants, prostheses, and other biomedical devices.
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3D printing of scaffolds on a rotating mandrel for tissue engineering applications

Introduction
One of the main issues limiting the wide-spread application artificial vascular grafts is a high risk of thrombosis due to elasticity mismatch and kinking hazard1. One of possible solutions to these problems is incorporating 3D printed reinforcement to the design of implant2.
The aim of this study is to inspect the possibility of 3D printing with elastic medical grade materials on rotating mandrel. End goal being able to incorporate reinforcements in design of artificial grafts.
Methods
3D printer used allows for deposition of thermoplastic filament onto the rotating mandrel. This approach allows for production of cylindrical geometries without the need of supports3. Printhead with 0.4mm nozzle used during the study was standard commercially available filament printhead (FFF) produced by BioCloner Health.
Filament was extruded from medical grade polyurethane ChronoFlex with variable hardness from 75D to 75A. All materials were extruded using FilaBot NX2 filament extruder.
Printability assessment was carried out while controlling the printhead's nozzle temperature and filament intake flowrate.
Mechanical test where carried out using Instron 2519 with standard 5kN load cell for longitude tensile stress test. For measurements of radial force custom „hook" system was used.
Results
All considered polyurethanes where suitable for filament fabrications. The value of Young modulus of filaments was similar among most of inspected samples. The filament made from the hardest source material (75D) stood out with young modulus value being around x30÷40 times greater than the rest of investigated materials.
It was observed that softness of the filament negatively impacts the maximal federate, due to the risk of bulking.
Discussion
Main observed obstacle for reliable 3D printing of elastic filaments is the high risk of clogging the nozzle or buckling of the material, as reported in the literature4. Those setbacks could be overcome with specially designed printhead or by increasing the diameter of the nozzle, however doing so would negatively impact the resolution of the print.
Frequent peeling off of the model after rapid change in the direction of the movement the mandrel it was concluded that the best practice is to maintain constant spin direction.
This study demonstrates that it is possible to print complex cylindrical structures made of medical grade materials is possible.

Acknowledgements
Rotating mandrel 3D printer and filament extruder were kindly provided by BioCloner Health

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A multiscale and multimaterial bioprinting approach combining photocuring pneumatic extrusion, electrospinning, and hot melt extrusion for the biofabrication of a tracheal construct.

The trachea's complex anatomy presents a challenge in the reconstruction of long-segment tracheal airway defects. Its hierarchical architecture, composed of a fibrous outer layer, several cartilaginous rings providing structural integrity, and an inner mucosal lining, makes it challenging to engineer functional tracheal replacements [1]. The need for viable solutions is ever growing [2] due to long-term endotracheal intubations, trauma and congenital defects, generating an unmet need for tracheal transplants. After their first successful transplantation in 2021 [3], trachea transplants still present challenges due to patient specific complex anatomy, cellular rejection and vascularization. This study demonstrates how bioprinting, combining photocuring pneumatic extrusion (PPE), electrospinning (ESP) and hot melt extrusion (often called fused deposition modelling, FDM, starting from commercial products), can be effectively used to engineer biomimetic tracheal scaffolds by integrating processes that operate across the nano- to microscale with different biomaterials. We used Electrospider (Fig. 1B), a multiscale and multimaterial bioprinting ecosystem, to biofabricate a complex multi-layered construct, in a single printing session, following the design approach showed in Fig. 1C. The first and the fourth layer of the scaffold were produced using ESP to obtain nanoscale fibers, replicating trachea's outer and internal fibrous layers. A 23% w/v solution of polycaprolactone (PCL) (50k molecular weight) in acetic acid and deionized water (9:1) was electrospun at distance from the planar collector of 15cm, voltage 25kV, pressure 300mbar, and environmental temperature of 26°C and 48% relative humidity. A dynamic ESP approach was adopted to obtain membranes of the desired dimensions: the ESP tool was moved along a defined path to recreate a rectangular pattern of 60×30cm with an infill density of 5%, ensuring overlap and continuity of the deposition. Second layer of the scaffold was fabricated using FDM, with 25k molecular weight PCL pellets, replicating the cartilaginous rings. Different infills were tested to enhance the construct's mechanical properties. Third layer of the scaffold was performed using PPE with gelatin methacrylate (GelMa) at 5%w/v and 0.25%w/v of photoinitiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) to support the cartilaginous and mucosal interface and tune the construct's mechanical properties. Moreover, GelMa can also be used to integrate a cellular component. The final construct was then dry-formed using a cylindrical open mould for 24h, allowing also the dehydration of GelMa. Finally, sutures were strategically placed to maintain the anatomical shape of a cylinder with a diameter of 2cm, thickness of 2mm, and a length of 6cm.
Overall results show that by leveraging complementary biofabrication techniques and biomaterials, we established a single step bioprinting method capable of replicating the trachea's layered complexity. Beyond structural fidelity, this approach lays the groundwork for future integration of biological components, offering a novel route toward functional, patient-specific airway grafts, to overcome the issue of large tracheal defects.
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A Silk Fibroin Platform for Enhanced Vascularization in 3D Skin Models

Introduction

One of the major challenges in tissue engineering of three-dimensional (3D) functional tissues is achieving vascularization, which is critical for developing large, viable, and physiologically relevant in vitro models. Constructs exceeding 40 µm in thickness require vascular networks to sustain cell survival1, and blood vessels play a key role in supporting tissue homeostasis through dynamic cell–vessel interactions. In engineered skin models, the lack of an endothelial barrier can significantly impact drug and cosmetic testing outcomes. In native skin, vascularization primarily occurs through angiogenesis — the sprouting of new vessels from pre-existing ones2. To address this, silk-based biomaterials have emerged as promising candidates due to their outstanding biocompatibility, mechanical properties, and ability to support cellular processes critical for tissue integration3.

Methods

We vascularized an already optimized 3D skin model5 using a pure silk fibroin membrane (KLISBio) integrated into a custom holder system. Endothelial cells (ECs) and mesenchymal stromal cells (hMSCs) were co-cultured on the silk membrane, supporting vascular network formation within a biomimetic dermal matrix of methacrylated gelatin (GelMA) containing fibroblasts (HFF-1). Constructs were cultured under pro-angiogenic stimulation with vascular endothelial growth factor (VEGF). Characterization included LIVE/DEAD viability assays, immunofluorescence imaging (whole-mount and cryosectioned samples), and droplet digital PCR (ddPCR) analysis of angiogenesis-related gene expression.

Results

Preliminary results confirmed excellent cell viability and integration across the constructs, as evidenced by LIVE/DEAD staining and immunofluorescence analysis. Endothelial sprouting behavior and early matrix remodeling were observed, indicating active vascular network formation. Comparative ddPCR analysis showed upregulation of key angiogenic markers in the silk-based constructs compared to commercial membrane controls. Immunofluorescence imaging highlighted enhanced endothelial organization and vessel-like structures within the silk platform, supporting its superior ability to promote vascular morphogenesis and functional dermal tissue development.

Discussion

These findings demonstrate the potential of silk fibroin membranes to create robust, vascularized 3D skin models with enhanced physiological relevance. Compared to conventional commercial membranes, the silk platform provides a more supportive environment for endothelial migration, organization, and angiogenesis, likely due to its favorable mechanical and biological properties. This work paves the way for developing next-generation skin models for regenerative medicine, disease modeling, and high-fidelity drug screening applications.

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A study to develop a new therapy for bone defects with scaffold-free cartilage constructs.

Background

Critical-size bone defects (CSDs), resulting from trauma, infection, or tumor resection, pose significant clinical challenges. Autologous bone grafting has limitations like donor site morbidity, necessitating novel strategies. Approaches mimicking endochondral ossification, crucial for bone development and healing, show promise for bone regeneration. Many studies involve seeding mesenchymal stem cells onto scaffolds and subsequently inducing chondrogenesis of these cell-scaffold constructs. However, exogenous scaffolds risk complications like chronic inflammation. This study aimed to develop a novel CSD therapy using scaffold-free cartilage constructs from rat adipose-derived stromal/stem cells (rADSCs).

Material Method

A bio-3D printer fabricated these scaffold-free constructs by stacking cell spheroids. Scaffold-free cartilage constructs from rADSCs underwent chondrogenic induction. A 5-mm CSD was created in rat femurs. Three groups (n=24) were: Defect (no implant), ADSC (undifferentiated constructs), and ADSC-Ch (chondrogenically induced constructs). CT and histological analyses were performed at 6 and 12 weeks post-implantation.

Result

CT at 6 and 12 weeks showed significantly higher Bone Volume/Total Volume in the ADSC-Ch group versus ADSC and Defect groups ($p<0.01$). No significant difference was observed between the ADSC and Defect groups. Histological scoring at both 6 and 12 weeks revealed that the ADSC-Ch group achieved significantly higher scores compared to both the Defect and ADSC groups ($p<0.05$), while no significant difference was noted between the Defect and ADSC groups. Regarding specific tissue findings, the ADSC-Ch group exhibited robust formation of new cortical and cancellous bone, continuous with native bone margins and leading to bony bridging. In contrast, new bone formation in the Defect and ADSC groups was confined to the defect periphery, with central areas predominantly filled by adipose and fibrous tissues.

Conclusion

These findings show rADSC-derived scaffold-free cartilage constructs effectively promote CSD healing in rats. This scaffold-free approach mimicking endochondral ossification holds promise for clinical use, avoiding scaffold-related complications. However, further studies in larger animal models are needed.

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Advancing Tissue-Engineered Neural Interfaces: Impact of Substrate Design on Cell Viability under Electrical Stimulation

The efficacy of neural interfaces relies heavily on the interaction between conductive hydrogels and underlying substrates. However, the impact of substrate selection on hydrogel performance and cell viability under electrical stimulation remains under-explored. This study investigates the electrochemical behaviour of gelatin methacryloyl (GelMA)-based hydrogels interfaced with indium tin oxide (ITO), platinum, and gold mylar substrates to determine how substrate choice influences hydrogel conductivity, cell viability, and proliferation under an optimized electrical stimulation protocol. GelMA hydrogels with varying compositions (GelMA, GelMA-graphene oxide (GO), GelMA-GO-gold nanorods (AuNRs)) were crosslinked onto the substrates. Electrochemical characterization was performed using cyclic voltammetry and electrochemical impedance spectroscopy. PC12 cells were cultured in 2D (on substrates) and 3D (encapsulated within the hydrogels) and subjected to electrical stimulation. Cell viability and proliferation were assessed through metabolic activity assays, DNA quantification, and live/dead staining. Substrate selection significantly influenced hydrogel conductivity and cell behaviour. Notably, ITO substrates consistently supported the highest cell viability and proliferation under electrical stimulation, with at least three times higher metabolic activity compared to platinum and gold mylar over seven days. Electrochemical analysis revealed distinct redox behaviours and capacitive currents for each substrate-hydrogel combination, suggesting varying degrees of charge transfer and storage capabilities. These findings demonstrate that substrate choice critically impacts the performance of GelMA-based hydrogels and the viability of encapsulated cells under electrical stimulation, highlighting ITO as the most promising substrate. This study provides crucial insights for designing effective neural interfaces and advancing neural tissue engineering applications.

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Bone from the Bottom Up: Investigating Dura-Dependent Osteoinduction in Porous 3D-Printed Ceramic Scaffolds

Introduction:
Three-dimensionally (3D)-printed bioceramic scaffolds composed of beta-tricalcium phosphate (β-TCP) have demonstrated the ability to support robust bone regeneration in critically sized calvarial defects. This bone formation is facilitated through two key biological mechanisms: osteoconduction, which guides new bone growth along the scaffold, and potentially dura-mediated osteoinduction, in which the underlying dura mater plays an active role in inducing osteogenesis. However, in clinical settings such as cranioplasty, patients often present with a compromised dura mater that may be scarred, damaged, or completely absent due to prior surgery, trauma, or disease. The absence of an intact dura raises important questions about the efficacy of scaffold-mediated bone regeneration in such scenarios.
To address this clinically relevant concern, the present study investigates whether osteoconduction alone—independent of any contribution from dural osteoinductive signals—is sufficient to support bone regeneration across critically sized calvarial defects. Using a well-established in vivo model, this work aimed to evaluate the regenerative capacity of 3D-printed β-TCP scaffolds isolated from the dura mater, assessing new bone formation, scaffold integration, and vascularization. The findings from this study aim to inform the translational potential of bioceramic scaffolds in complex cranial reconstructions, especially in cases where the native osteoinductive environment is compromised or absent.

Methods:
Unilateral calvarial defects were created in rabbits (n=12) and these defects were filled with 3D-printed bioceramic scaffolds containing one of two structural modifications at the scaffold/dura interface: (a) with a solid nonporous cap or (b) with a fully porous cap. The nonporous cap abutted the dura, effectively isolating the scaffold from direct contact with the osteogenic properties of the dura while the porous cap design permitted dural-mediated osteoinduction. The rabbits were euthanized 8 weeks postoperatively and calvaria were analyzed quantitatively, volumetrically, using 3D reconstructions from microcomputed tomography, as well as qualitatively, using nondecalcified histologic sectioning to assess for differences in bone growth.

Results:
When comparing scaffolds with a porous cap to those with a solid (nonporous) cap, no statistically significant difference was detected in percent bone volume (9.3 ± 4.5 vs. 10.2 ± 4.5; P=0.71), percent volume of soft tissue presence (58.5±7.0 vs. 52.5±2.0; P=0.072), or percent scaffold volume (32.3±3.4 vs. 37.3±4.3; P=0.917). Bridging bone was generated across bone defects treated by both construct designs, independent of design (Figure). Histologic analysis revealed the generation of vascularized bone within the defect with the formation of Haversian canals.

Conclusion:
This study demonstrates that three-dimensionally (3D) printed bioceramic scaffolds composed of beta-tricalcium phosphate can promote bone regeneration across critically sized calvarial defects even in the absence of dura-mediated osteoinduction. These findings suggest that osteoconduction alone may be sufficient for effective bone healing in scenarios where the dura is compromised or absent, as often encountered in clinical cranioplasty. The results provide important insights into scaffold-based tissue engineering strategies and may guide the future design of biomaterial constructs for reliable and durable calvarial reconstruction.

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Brain-on-Chip of the 3D Blood–Brain Barrier (BBB) model for Studies of Permeability, Neuroinflammation and Drug Response

Introduction
Depression affects over 350 million individuals globally, with 20–30% developing treatment-resistant depression (TRD), a major contributor to suicide risk. Existing preclinical models inadequately recapitulate the complexity of the human neurovascular unit (NVU) and blood–brain barrier (BBB), thereby limiting the advancement of effective therapeutics. The objective of this study was to develop a three-dimensional (3D) BBB model to enable mechanistic investigations of barrier permeability, neuroinflammation, and pharmacological responses.
Methods
A two-channel polydimethylsiloxane (PDMS) microsystem separated by a porous polycarbonate membrane was fabricated. The lower channel was employed to reconstruct the BBB using the Double-Viscous Finger Patterning (Double-VFP) technique: human brain vascular pericytes (HBVP) and astrocytes (HBVA) were embedded at a 1:3 ratio within a 5 mg/mL collagen I hydrogel to form the lumen, followed by seeding of human brain microvascular endothelial cells (HBMEC) at a 3:1:3 ratio. Culture conditions were optimized for a 10-day period. Cellular viability (AlamarBlue® assay), dextran permeability, CellTracker®-based imaging, and immunostaining analyses were conducted on days 1, 3, 7, and 10; 3D imaging was performed at 24 and 48 hours post-seeding. Cellular morphology within the hydrogel matrix was compared to traditional two-dimensional monolayer cultures via immunocytochemistry to validate model fidelity. To simulate an inflammatory environment, hormonal stimulation using cortisol alone and a combination of cortisol, aldosterone, and angiotensin II—molecules known to be elevated in depression—was optimized in macroscale models employing AlamarBlue® and RealTime-Glo™ MT Cell Viability assays. The upper microchanel allows for the integration of neurons to create together the NVU model (under development).
Results
The microsystem enabled reproducible generation of single- and double-lumen structures, with future compatibility for the incorporation of neurons and microglia. The Double-VFP method supported sustained high cell viability over 10 days, with the formation of a continuous and functional endothelial layer. Relative to the Single-VFP approach, the Double-VFP technique facilitated more rapid organization of cellular components into a functional BBB and exhibited superior barrier integrity, as evidenced by dextran permeability assays. Complete assembly of the BBB architecture was confirmed by CellTracker® imaging and immunostaining within 48 hours. Hormonal stimulation at both low and high concentrations altered cellular metabolic activity, indicating a biological effect that will be corroborated with additional assays, such as ELISA.
Discussion
The developed 3D BBB model—particularly utilizing the Double-VFP method—successfully replicates key physiological features of the BBB and provides a dynamic platform for the study of barrier permeability, neuroinflammatory processes, and pharmacological testing. Its rapid and reproducible assembly, coupled with its structural and functional robustness, positions it as a valuable tool for mechanistic studies in neuropharmacology and for the preclinical evaluation of psychotropic, psychedelic, and anti-inflammatory compounds. Ongoing integration of iPSC-derived neurons and microglia is anticipated to further enhance the model's relevance for the investigation of depression pathophysiology and therapeutic development.

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Burnishing-Driven Surface Optimization of 3D-Bioprinted PCL Scaffolds for Cell-Responsive Biomedical Applications

Introduction
Biopolymers are widely used in biomedical applications due to their superior biocompatibility and customizable degradability compared to conventional biometals. Among them, polycaprolactone (PCL) is a biocompatible thermoplastic polymer with mechanical properties that make it suitable for a variety of biomedical uses [1]. One of the primary applications of PCL is in bone reconstruction, thanks to its ability to significantly reduce stress shielding. A critical factor for such applications is the osteointegration of the biomaterial, which promotes cell proliferation on the scaffold surface. In this study, PCL samples were bioprinted using an extrusion-based process, followed by a burnishing treatment to improve surface characteristics. The resulting surface roughness was found to be optimal for osteoblast attachment and proliferation [2].
Methods
Polycaprolactone was 3D bioprinted via extrusion following an optimization of the printing parameters. To support this process, a complete thermal and rheological characterization of the biomaterial was carried out using Differential Scanning Calorimetry (DSC) and a strain-controlled rheometer. Specifically, amplitude sweep tests were conducted to define the material's linear viscoelastic region, while frequency sweep tests were performed to evaluate its behavior at the printing temperature. Subsequently, a surface roughness tester was employed to analyze the material's surface profile post-manufacturing. A sequential spherical burnishing process was then applied, assessing the final surface morphology under varying axial forces and directions. The samples were re-evaluated to determine the average surface variation in terms of final roughness.
Results
Data obtained from the surface roughness tester revealed a consistent reduction in roughness for both axial forces tested (25 N and 50 N), with optimal values ranging from 1 to 2 microns. Additionally, the direction perpendicular to the deposition direction of the printing process using 50N as axial force showed the greatest percentage improvement in surface roughness, as illustrated in Figure 1.
Discussion
The surface roughness achieved through the burnishing process is suitable for osteoblast attachment, proliferation, and differentiation. This level of roughness creates a micro-topography that promotes the formation and maturation of focal adhesions, which are critical for cell binding. Furthermore, a direction oriented at 90° influences scaffold

anisotropy, contributing to a more uniform surface and facilitating the consolidation of micropores. These findings highlight the crucial role of finishing processes in applications where biological entities of specific dimensions require high precision.

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Can we make the smallest bone in the body?

Introduction

The auditory system is essential for speech development, spatial orientation, and communication; dysfunction leads to hearing loss, affecting over 466 million individuals globally, making it one of the top five leading causes of the most years lived with disability [1]. Conductive hearing loss frequently arises from tympanic membrane perforation or ossicular chain disruption, commonly managed by ossiculoplasty [2]. Conventional autografts and alloplastic prostheses demonstrate limitations regarding biocompatibility, stability, cost, and long-term efficacy [3, 4]. Advances in three-dimensional printing have enabled patient-specific ossicular prostheses, while emerging bioprinting technologies offer the possibility of fabricating living, functional implants [5, 6]. The present study aims to generate a human stapes via 3D bioprinting, potentially providing a physiological and durable solution for auditory rehabilitation.

Materials and Methods

Human stapes were harvested from cadavers via a transcanal endoscopic approach, fixed in 1% PFA, and imaged with a SkyScan 1176 micro-CT. Stapes-shaped molds (ZA 35 MOULD) were produced for bioprinting. MG63 osteosarcoma cells (1×10^6) were suspended in Cellink Bone bioink, cast into molds, crosslinked with $CaCl_2$, and cultured for 3 weeks in osteogenic MEMα medium; cDMEM served as control. Osteogenic differentiation was assessed by PCR for FN1, BGLAP, BMP2, COL1A1, and SP7. Mineralization was evaluated by Alizarin Red staining with spectrophotometric quantification at 405 nm.

Results

Stapes was shaped in a mould using biogel mixed with M63 (Figure 1). qRT-PCR demonstrated upregulation of osteogenic markers in MG63 cells cultured in osteogenic MEMα compared with cDMEM controls. Expression of FN1, BGLAP, BMP2, COL1A1, and SP7 were elevated after 3 weeks of culture. Among these, BGLAP and COL1A1 showed the highest relative expression, while SP7 only moderately increased. cDMEM control cultures barely expressed the above genes. The results confirm successful osteogenic differentiation within the stapes constructs.

Calcium-containing osteocytes, shown by Alizarin red stain, were only detected in the 3D bioprinted models, incubated in α-MEM, indicating intense mineralization.

Discussion

Bioprinted stapes constructs with MG63 cells in bioink showed osteogenic differentiation, evidenced by upregulated markers and calcium deposition. Future work should explore primary cells, mechanical testing, and, in vivo evaluation for auditory ossicle replacement.

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Carbon Quantum Dots Enhanced GelMA Hydrogel as Novel Bioink for Bone Tissue Engineering Applications

Introduction:

Globally, almost two million bone transplants are performed using traditional methods like metallic implants and bone grafts that have their limitations. In this scenario, BTE has emerged as an advanced field to replace the conventional method by allowing a living tissue to be created within biological framework. Over the past few years, GelMA based hydrogels have been widely used to build scaffold material due to their exceptional biological and physiological properties. However, GelMA faces several limitations like mechanical strength and reduced immunogenicity. In this aspect, CQDs, 0-D carbon nanoparticles have become a promising solution to enhance the bioactivity and physical properties of GelMA by acting as a nanofiller [1]. Thus, this study focuses on dispersing green source derived and flavonoid based CQDs in the GelMA's matrix to synthesize a composite based hydrogel with tunable and enhanced bioactivity and physiological properties.

Methods:

In this study CQDs are synthesized using microwave-based approach. It focuses on using mandarin orange juice as a carbon source, enriched with flavonoids which play a role in promoting bone formation and preventing bone resorption. In addition to this, L-arginine is used as a source of nitrogen to induce nitrogen doping in the CQDs due to its role in promoting osteogenesis, reducing inflammation and oxidative stress. The structural, chemical and optical properties of microwaves synthesized blue luminescent CQDs is confirmed using UV-Visible spectroscopy, FTIR, TEM and PL spectroscopy. Further, CQDs are dispersed in GelMA's matrix in different concentrations to study their effect in the matrix. The morphological feature of the composite is analyzed using SEM and mechanical properties are assessed by DMA, the behavior in buffers is tested by swelling test. Moreover, biocompatibility of CQDs is revealed using MTT assay and staining. Lastly, 3D printing is done to check the effect of composite hydrogel on printing.

Results:

The characterization reveals the presence of various flavonoids on the surface of the CQDs having size around ~4-10 nm with spherical shape and exhibit blue fluorescence [2]. SEM reveals a slight decrease in pore size after addition of CQDs. An increase in compressive modulus is recorded in the composite hydrogel using DMA and the decrease in swelling degree is observed which shows their effect on crosslinking degree of GelMA. Cellular studies reveal the role of composite lack of cytotoxicity of CQDs. Thus, the enhancement in bioactivity, crosslinking and mechanical properties of GelMA after addition of flavonoids based and nitrogen doped CQDs, exhibit their potential to be a promising candidate for bioink to build bioactive scaffold for BTE.

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Cellularly Annealed Tissue (CAT) Structures comprising of magnetic microgel cell carriers.

Introduction

Cell carriers are being utilized in cell culture applications that demand efficiency and large volumes of cells, by utilizing the carriers high surface area in a relatively low volume [1]. Hydrogel microgels, which have been extensively utilized in tissue engineering could offer a platform to build tissue [2], but usually scalability of hydrogel microparticle production and a formulation that can be oil-free and cost efficient has been difficult to combine in one process. In this work we report the fabrication of magnetic hydrogel microparticles using in-air microfluidics [3], their post-processing into magnetic cell carriers and finally their assembly into a composite via magnetic actuation, where cells on the surface of the carriers bond the microgels into tissue composites, which we name Cellularly Annealed Tissue (CAT) Structures.

Methods

1% Alginate particles were produced using In-air microfluidics, briefly we jetted a monodisperse droplet train of of 1% Sodium Alginate in MiliQ polymer solution, supplemented with 5% w/v Fe powder and polymerized it with a 0.2mM CaCl jet, resulting in a monodisperse production 200µm magnetic alginate particles. The produced particles were coated with Gelatin Type A, and crosslinked using 2% v/v Glutaraldehyde in MiliQ, which resulted in a magnetic cell carrier particle suspension. Red

Fluorescent Protein modified Mesenchymal Stem Cells (RFP-MSCs) were seeded on the magnetic carriers, and after 3 days of culture, a neodymium magnet was used below the culture plate to steer the particles into a jammed aggregated construct. After 1 day, the structure was annealed by the cells in the interstitial space, resulting in a CAT that could be steered as one body using magnetic steering.

Results
The coated magnetic particles functioned as cell carriers that a) allowed cell adhesion and b) could be steered using a neodymium magnet to the desired position when the magnet was placed for a short amount of time near the culture plate. Constant magnetic actuation (1 day) of the suspension allowed the cells on the magnetic carriers to be in contact with multiple particles and allowed a CAT structure to emerge.

Discussion
We demonstrate that by functionalizing the core of hydrogel particles with Fe powder and coating with Gelatin in order to enable cell adhesion, the controllable assembly of CAT structures can be achieved. By using in-air microfluidics, no extensive washing steps are necessary and the choice of Fe as the magnetic material, makes the magnetic cell carriers biocompatible. By using different cell types on the magnetic carriers and mixing them together, a novel bottom-up approach of magnetic tissue assembly can be envisioned where the carriers behave as the filler material and the cells as the interstitial phase.

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DEVELOPMENT OF A VERSATILE MUSCULOSKELETAL TISSUE BIOPRINTING PLATFORM TO DIRECT PREFERENTIAL COLLAGEN ALIGNMENT IN HIGHLY CELLULAR BIOINKS

Introduction
Functional regeneration of musculoskeletal tissues requires engineered grafts that mimic the heterogenous and anisotropic structure and mechanics of the native tissue. Existing strategies fail to produce tissues that mimic this structural complexity, often leading to deficits in mechanical properties and repair failure in vivo. 3D bioprinting allows for the freeform patterning of cells and biomaterials at the microscale, potentially enabling the engineering of constructs that mimic the structural complexity of biological tissues. High cell density 3D embedded bioprinting has recently emerged as a versatile biofabrication tool for the generation of spatially organised tissues using high cell density hydrogel composite bioinks [1]. This technique enables the production of complex, free-form constructs using mechanically weak hydrogels. Here we hypothesise that the support bath used in such embedded bioprinting strategies can also be leveraged to direct the growth and spatial organisation of the secreted extracellular matrix (ECM). Our goal is to develop a versatile muscoskeletal bioprinting platform capable of fabricating anisotropic, mechanically functional tissues with preferential collagen alignment, leveraging the spatial cues and physical confinement provided by the embedded support bath.
Materials and methods
Goat bone marrow derived mesenchymal stem/stromal cells (MSCs) were either encapsulated within a partially crosslinked (60mM CaCl2-5min) oxidised alginate (OA) bioink at high cell densities (60x106 / 100x106 cells/mL), or bioprinted as a cell-only bioink. These bioinks were subsequently bioprinted within different concentration oxidised-methacrylated alginate (OMA) support baths using a mechanically driven syringe pump printhead, and ionically and UV crosslinked post-print and maintained in chondrogenic culture for 6 weeks. Various printing parameters such as needle size, print speed, support bath mechanical profile, and construct spatial configuration were systematically varied to in an attempt to produce cartilage and fibrocartilage tissue constructs with defined resolution and preferential alignment within the developing collagen fibres. Assessment was undertaken using brightfield microscopy, biochemical and histological evaluation, polarised light microscopy, immunofluorescence and RT-qPCR.
Results
High cell density bioinks (60/100x106 cells/ml-MSCs-OA), and cell only bioinks were bioprinted within an OMA support bath to a high degree of resolution (200-400 µm), whilst maintaining bioprint fidelity. Print parameters and the supporting bath mechanical profile could be tuned to control bioprint resolution (~200 µm), direct the phenotype of developed cartilaginous tissue, and importantly enhance the preferential alignment of developing collagen fibres (Fig. 1c) whilst maintaining cellular viability (97% viable) (Fib. 1b). MSCs were found to undergo chondrogenic differentiation, as confirmed using an assessment of sulphated-glycosaminoglycans (sGAGs) and collagen production both biochemically and histologically (Fig. 1c). Further assessment using immunofluorescence for specific collagen types highlighted differences in the resulting phenotype of engineered tissue.
Discussion
This body of work has showcased the ability to preferentially control the alignment of developing collagen fibres within cartilage constructs generated using embedded bioprinting, whilst maintaining a high print resolution and cell viability. Future work will focus on scaling up the biofabrication strategies to more native-like geometries.

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Development and use of open-source bioreactor for dynamic cell culture under cyclic tensile strain

Introduction
Bone is a dynamic tissue that experiences a wide range of forces during regular daily locomotion. This environment of dynamic strain strongly influences the architecture of the extracellular matrix, and it can impact the rate that bone adapts or recovers after an injury 1,2. Cell research is commonly performed in mechanically static conditions in the base of well plates, yet this is a far cry from the conditions natural to osteoblasts. To better understand the behaviours of osteoblasts, it is important to ensure that platforms are available to perform cell culture experiments in mechanically dynamic environments 3–5. For this reason, we have designed a bioreactor system that imparts tensile strain onto flat, cell-seeded scaffold constructs in vivo within custom well plates.
Methods
The bioreactor system has 36 separated wells spread across four mechanical actuation units and can apply up to 30% tensile strain to a 15 by 9 mm area of the constructs, operating within an incubator. To be accessible to research groups, the bioreactor has predominantly been constructed from widely available components and materials. Most parts fabricated using 3D printing, and all of the electronics can be found within a typical 3D printer DIY assembly kit. The bioreactor system is modular, allowing for parts to easily be swapped out to account for different sample designs or for maintenance and upgrades. Parts in-contact with cell media are autoclavable to allow for sterilisation and re-use. The device was validated for use in a 28 day in-vitro dynamic culture of osteoblasts on melt-electrowritten polycaprolactone scaffolds. The cell constructs were strained to 4% at 0.5 Hz on days 25-27 and removed on the 28th day.
Results
The entire bioreactor system can be manufactured for under $1000 AUD. Scaffolds could easily be loaded and unloaded from the device as required for assays and experiments. Osteoblasts were found to proliferate within scaffolds stimulated within the bioreactor over the dynamic culture period. The device could be used for multiple studies with sufficient cleaning between studies. Mechanical stimulation was not found to influence metabolic activity. Cells were observed to elongate and align within some regions of high local strain following the 3 days of stimulation.
Discussion
The open-source tensile strain bioreactor is compatible with many types of cell-constructs and can be assembled without the necessity of advanced mechatronics experience. Many bioreactors have been designed for creating biomimetic strain conditions for samples, but as their designs are rarely published comparison between studies is almost impossible. Open-source hardware unlocks experiments for a broad range of research groups and encourages collaboration and reinforcement of results through comparable studies.
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Development of Carboxymethylcellulose Methacrylate and Gelatin Methacrylate for Volumetric Printing of Tubular Scaffolds for Cardiovascular Applications

Introduction
Cardiovascular diseases (CVDs) remain leading cause of mortality worldwide, creating a pressing demand for engineering vascular grafts that can restore or replace damaged blood vessels1. Traditional fabrication techniques often lack the resolution, speed, and geometric complexity required to accurately replicate the intrinsic architecture of native vascular tissues2. In this context, volumetric bioprinting (VP), a next-generation 3D fabrication for ultra-fast, high-resolution creating complex, biomimetic structures3. This study reports the synthesis, optimization, and evaluation of carboxymethylcellulose methacrylate (CMCMA) and gelatin methacrylate (GelMA) hydrogels as dual-component bioresins for volumetric printing of tubular scaffolds, incorporating Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) as the photo-initiator. These scaffolds were assessed for their mechanical properties, print fidelity, and biocompatibility with AC16 human cardiomyocytes.

Materials and Methods
CMCMA was synthesised using methacrylic anhydride under controlled pH conditions, yielding a hydrogel with tuneable mechanical properties and shear-thinning behaviour. GelMA was prepared by functionalising gelatin with methacrylic groups, preserving its cell adhesion motifs and thermosresponsive behaviour. Successful methacrylation was confirmed via FTIR and ^{1}H-NMR spectroscopy (Fig 1A and B). Composite hydrogels were formulated by blending CMCMA and GelMA in varying ratios with LAP as the photoinitiator, chosen for its high photoreactivity and low cytotoxicity under light exposure. The dual bioresin system was optimised to achieve a balance between printability, mechanical integrity and biological compatibility.
Volumetric printing was performed using a digital light processing (DLP)-based tomographic printer. Scaffold with straight tubular geometries, were generated. Critical printing parameters were optimized, including light dose rate, exposure time, and resin refractive index to ensure accurate voxel reconstruction and crosslinking uniformity. The refractive index of the resin was matched to the carrier medium to minimize light scattering and refraction artifacts. Dose rate optimization ensured sufficient polymerization without overheating or overcuring.
Printed scaffolds were assessed for shape fidelity, wall thickness accuracy, and microstructural resolution using optical microscopy. Mechanical properties were evaluated through uniaxial tensile testing. Biocompatibility was assessed using the AC16 human cardiomyocyte cell line. Cell-laden hydrogels were cultured in vitro for 7 day and cell viability was assessed using Live/Dead (Calcein AM, EthD-1) staining at days 1, 3, and 7. In order to investigate cells maturation and morphology, immunofluorescence staining for cardiac-specific markers (e.g., cTnT and α-actinin) was used. Confocal microscopy was used to assess cellular integration and alignment on the scaffold surface.

Results
Both CMCMA and GelMA hydrogels, crosslinked using LAP, exhibited suitable photopolymerization under visible light. The optimized formulation supported high-resolution volumetric printing of tubular scaffolds in under 60 seconds, with accurate geometries and smooth luminal surfaces (Fig 1C and D). Resolution below 100 µm was achieved with minimal printing artifacts.
Mechanically, scaffolds demonstrated elastic moduli within the physiological range of vascular tissue, with CMCMA enhancing structural integrity and elasticity of GelMA. Live/Dead staining confirms the cytocompatibility of the bioresin. Results indicate that CMCMA/GelMA tubular scaffolds a supportive microenvironment for cardiomyocytes and hold promise for cardiovascular tissue engineering.

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Development of Enzymatically Crosslinkable Aphanothece sacrum Polysaccharides for 3D Bioprinting Application

Aphanothece sacrum polysaccharides (ASP) are sulfated polysaccharides derived from a cyanobacterium known as Suizenjinori, which is native to Japan. ASP has potential in food, cosmetics and medical treatments due to its high water retention, anti-inflammatory properties and hydrogel formation ability. Conventional ASP hydrogels are obtained by ionic crosslinking with metal or by physical crosslinking through dry and high temperatures. However, these conditions are not suitable for fabricating cell-laden hydrogels and limit the use of ASP hydrogels for biomedical applications. The objective of this research was to develop ASP hydrogels under mild conditions and investigate their usefulness as a bioink for 3D bioprinting. We synthesized a phenol-grafted ASP (ASP-Ph) that was gelled via horseradish peroxidase (HRP)-catalyzed crosslinking. The hydrogelation time and mechanical properties of the hydrogels could be controlled by the degree of Ph content and the concentration of HRP and hydrogen peroxide (H2O2). ASP-Ph dissolved in medium showed cytocompatibility when using mouse fibroblast cell line (BALB/3T3). In addition, transplantation of hydrogels into mice demonstrated the biocompatibility of the hydrogel. To investigate the potential application of the hydrogel, the angiogenic effect of fibroblast growth factor-2 (FGF-2) released from the hydrogel was evaluated using a chick chorioallantoic membrane (CAM) assay. More number of blood vessels were observed around the hydrogel containing FGF-2 than that around the hydrogel alone. Moreover, we also fabricated the cell-laden constructs using bioinks containing ASP-Ph, HRP and BALB/3T3 with continuous exposure to air containing H2O2. The enclosed cells maintained their viability and mitochondrial activity following 7 days of culture, demonstrating cytocompatibility of the materials and the extrusion-based printing system used in this study. Taken together, ASP-Ph has great potential as an ink component for 3D bioprinting.

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Development of Multifunctional ColMA-Based Scaffolds with MGO and HA for Smart Bone Regeneration

Introduction: Large bone defects present major clinical challenges due to insufficient regenerative capacity. Conventional approaches such as autografts are limited by donor site morbidity and immune complications. Bone tissue engineering (BTE) offers a promising alternative by providing biomimetic scaffolds capable of supporting cellular growth, vascularization, and osteogenesis. Recent studies have demonstrated that magnetically responsive scaffolds can deliver dynamic mechanical cues to enhance stem cell differentiation. This study aims to develop a biocompatible and magnetically responsive scaffold composed of methacrylated collagen (ColMA), polyethylene glycol diacrylate (PEGDA), magnetic graphene oxide (MGO), and hydroxyapatite (HA), further loaded with curcumin, a natural osteoinductive agent with anti-inflammatory properties.

Methods:Type I collagen was methacrylated using methacrylic anhydride, and modification was confirmed by FTIR and 1H-NMR analyses. For the preparation of curcumin-loaded magnetic graphene oxide (MGO), graphene oxide (GO) was initially synthesized by the Hummers method via chemical oxidation. Subsequently, Fe_3O_4 nanoparticles were incorporated onto the GO surface to impart magnetic properties. Curcumin was then loaded onto the MGO surface through physical adsorption, enabling its sustained and controlled release.To enhance the mechanical properties of the scaffold, Poly(ethylene glycol) diacrylate (PEGDA) was used as a co-crosslinker into the formulation. In addition, hydroxyapatite (HA) was dispersed within the ColMA matrix to provide mineral support and promote osteoconductivity. Scaffolds were fabricated via freeze-drying followed by UV crosslinking. Morphological (SEM), physicochemical (XRD, FTIR, TGA), magnetic (VSM), and mechanical (compression modulus) properties were evaluated. In vitro studies included swelling, biodegradation, and drug release kinetics. In vitro cell viability was assessed using the MTT assay.

Results: The resulting scaffolds exhibited a highly porous architecture, and compressive modulus. MGO conferred superparamagnetic properties with negligible remanence. FTIR and XRD confirmed successful integration of MGO and HA. Sustained curcumin release was observed up to 14 days. MGO-ColMA based scaffolds showed high cell viability.

Discussion: The integration of MGO and curcumin into a ColMA/HA scaffold enables a bioactive and magneto-responsive platform that mimics the native bone environment. Curcumin contributes osteoinductive and anti-inflammatory functions, while MGO allows for non-contact mechanical stimulation. Compared to conventional scaffolds, the proposed system presents a tri-modal functionality: osteoconduction (HA), osteoinduction (curcumin), and magnetomechanical stimulation (MGO). The resulting structure supports cell proliferation, osteogenic differentiation, and controlled drug delivery, showing promise for treating critical-sized bone defects.

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Development of a 3D Printing-Based In Vitro Neuromuscular Junction Platform for High-Fidelity Morphological Formation

The neuromuscular junction (NMJ) mediates the transfer of neural signals to skeletal muscle fibers, enabling muscle contraction. The structural organization of the NMJ is critical for efficient signal conduction, and disruptions in its morphology are associated with neuromuscular disorders. However, conventional in vitro models, including 2D culture systems and animal models, offer limited ability to replicate the spatial complexity, structural fidelity, and dynamic interactions characteristic of native NMJs. Moreover, relatively few studies have systematically investigated how defined spatial separation between neural and muscle components affects NMJ formation in vitro, despite its critical role in synaptic development and intercellular communication. These limitations reduce the physiological relevance of mechanistic studies and disease modeling. To address this, a 3D in vitro platform was developed to provide defined spatial arrangement of neural and muscle components and to enable comparative evaluation of NMJ formation under static and interstitial flow conditions. A 3D in vitro platform was fabricated using polyethylene-vinyl acetate (PEVA) via 3D printing, designed to provide defined spatial separation between neural and muscle compartments. Platforms with different fixed neural-muscle distances were fabricated to evaluate the influence of spatial separation on NMJ formation. Neural spheroids were manually placed in the neural chamber, and 3D engineered skeletal muscle tissues were anchored to silicone-based mushroom-type pin structures in the muscle chamber. Culture conditions included static culture and interstitial flow, the latter generated passively by height differences in media reservoirs. Fluorescent tracer experiments were conducted to verify the generation of interstitial flow through the axonal guidance region. NMJ formation was assessed by immunostaining for axonal outgrowth and acetylcholine receptor (AChR) clustering following a defined culture period. This 3D in vitro platform offers a physiologically relevant model for investigating neuromuscular junction (NMJ) formation under defined spatial and mechanical conditions. By enabling a defined spatial relationship between neural and muscle components and applying interstitial flow, the system facilitates the study of morphological development and intercellular cross-talk critical for synaptic function. This approach provides a foundation for advancing fundamental research on NMJ physiology and for developing in vitro disease models with improved structural fidelity and experimental reproducibility.

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Development of a Dual-Bioink Hybrid Scaffold for Tendon Regeneration

Tendon tissue engineering remains a critical challenge due to the need for biomaterials that simultaneously support mechanical load bearing and guide lineage specific cellular differentiation. To address this, we designed a hybrid scaffold system that spatially integrates mechanical reinforcement and tenogenic bioactivity, aiming to mimic native tendon properties more closely than conventional uniform scaffolds.
We developed a dual bioink system engineered to deliver region specific functionality. The first bioink was formulated to enhance mechanical integrity through molecular-level interactions with extracellular proteins, while the second bioink incorporated human adipose derived stem cells (ASCs) and tendon derived extracellular matrix (tECM) components to promote tenogenic differentiation. The two inks were co-delivered using a custom collector system to enable parallel deposition, ensuring microstructural anisotropy. After scaffold fabrication, constructs were cultured in vitro under static conditions.
Cell viability was assessed using live/dead staining and MTT assays at multiple timepoints. Tenogenic differentiation was evaluated by RT-PCR for tendon specific markers including SCX, TNMD, and COL1A1. Mechanical testing was conducted to quantify elastic modulus and ultimate tensile strength, benchmarking against the native tendon range.
The dual bioink scaffold exhibited clear spatial heterogeneity in both mechanical and biological responses. Live/dead staining confirmed high cell viability throughout the biologically active regions, while MTT assays showed sustained metabolic activity. Gene expression analysis revealed a significant upregulation of tenogenic markers in the ASC-tECM regions compared to control hydrogels lacking ECM supplementation. Mechanical analysis demonstrated a significant increase in tensile strength in the reinforced compartment compared to standard gelatin-based constructs, approaching values characteristic of early-stage regenerating tendon.
Our strategy highlights the importance of spatially controlled scaffold design in tendon regeneration. By combining mechanically supportive and biologically inductive regions within a single construct, we achieved both structural resilience and lineage specific differentiation cues. The observed anisotropy is expected to play a crucial role in guiding aligned ECM depositions and long-term tendon remodeling. Importantly, the method avoids reliance on growth factors instead, ECM cues for differentiation, enhancing translational relevance.
This study presents a promising step toward fabricating functional tendon scaffolds with tunable regions tailored to biological and mechanical demands. Further in vivo studies are underway to validate long-term integration and remodeling.

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Development of a Hybrid Gelatin Methacrylate–Carboxymethyl Cellulose Methacrylate Bioink for 3D Bioprinting of Cardiac Patches

Introduction
Cardiovascular diseases (CVDs) are the leading cause of death globally, accounting for approximately 1.6 million deaths annually [1]. Among them, myocardial infarction (MI) is particularly concerning due to its high incidence, mortality, and adverse prognosis. These challenges are amplified in ageing populations, making MI a growing public health issue. Current treatment approaches including pharmacological therapies and surgical interventions can lighten symptoms but do not regenerate damaged myocardium. Heart transplantation remains the only curative option for end-stage cases but is constrained by donor shortages and immune rejection risks. These challenges underscore the pressing need for advance therapeutic strategies to repair and regenerate cardiac tissue.
Cardiac patches (CP) are engineered tissues designed to replace damaged myocardium, thus offering promising alternative to transplantation [2-4]. Therefore, in this study, we developed a hybrid hydrogel composed of gelatin methacrylate (GELMA) and carboxymethyl cellulose methacrylate (CMCMA), tailored to match the mechanical properties of the native ECM. To achieve this, we optimized various parameters including the concentrations of GELMA-CMCMA, crosslinker concentration, and UV exposure time. The resulting bioink was formulated by encapsulating AC16 human cardiomyocyte cells within the CMCMA-GELMA hydrogel. The cytocompatibility of the bioink was subsequently evaluated to ensure a supportive environment for cell viability and function.

Materials and Methods
GELMA and CMCMA were synthesized via methacrylation and characterized using Fourier-transform infrared spectroscopy and nuclear magnetic resonance. Hydrogels were prepared at varying GELMA-CMCMA ratios and crosslinked using a dual enzymatic and photo-crosslinking. Rheological properties, including shear-thinning behavior and viscoelasticity, were analyzed using oscillatory rheometer. Mechanical properties such as stiffness, Young's modulus, and tensile strength were measured through uniaxial compression and tensile tests. Bioprinting parameters such as nozzle diameter, extrusion pressure, print speed, and UV exposure time were optimized to achieve high-resolution, structurally stable constructs. Printability was assessed by evaluating filament formation, shape fidelity, printability index and layer-stacking efficiency (Figure 1). Hydrogel stability post-printing was examined by monitoring swelling, degradation, and gelatin release over time. Further mechanical characterization was conducted using atomic force microscopy to ensure the hydrogels could withstand dynamic cardiac conditions. AC16 human cardiomyocytes were encapsulated within the hydrogels and the developed bioinks were used to assess biocompatibility. Cell viability was analyzed using live/dead staining, and functional performance was evaluated over extended culture periods (Figure 2 A-B).

Results
NMR confirmed successful methacrylation of both GELMA and CMCMA. The hybrid hydrogels exhibited optimal shear-thinning behavior, suitable for extrusion bioprinting. High print fidelity and minimal deformation were observed post-printing. The dual crosslinked hydrogels maintained mechanical stability over time with controlled swelling and degradation. Encapsulated AC16 cells demonstrated high viability, indicating good cytocompatibility. The printed TECPs supported cell attachment, proliferation, and metabolic activity. Future work will evaluate the patches' ability to support CM contractility.

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Development of an anticancer therapeutic tissue adhesive hydrogel post-ovarian cancer resection surgery

Introduction: Globally, ovarian cancer (OC) ranks as the most common cancer among gynecological malignancies. Recent research highlights the critical importance of the perioperative phase in deterring tumor recurrence. During this time, chemotherapy is the predominant treatment method, and numerous drugs have been developed for this purpose [1,2]. However, a major challenge is sustaining the long-term presence of these drugs at the surgical site after the operation to prevent relapse. Implementing such targeted strategies could significantly diminish the residual tumor cells and/or avert micro-metastases during the post-surgical phase. Hence, this study hypothesizes the development of a tissue adhesive hydrogel targeting OC recurring cells, which is encapsulated with drug-loaded carriers that can be used in the resection site to diminish the residual cancer cells post-surgery.
Methods: (1) Development of novel tissue adhesive hydrogel. (2) Preparation of potential drug carriers. (4) Encapsulation of potential carriers with the PARPi (Poly ADP Ribose Polymerase inhibitors) drug. (5) Load the drug-loaded carrier into the tissue adhesive hydrogel and test it in vitro and in vivo.
Results and Discussion. The project is in its mid-phase, and the novel hydrogel, which will serve as the drug delivery platform, is being synthesized first. Different trials and errors were carried out with different natural biomaterials and tuned to crosslink and form a stable hydrogel system at 37 °C without requiring external crosslinkers like UV crosslinking. The following are the different hydrogels stably crosslinked at 37 °C;
Biomaterial Combinations,
The combination IN+PG (1:1) was chosen as the base hydrogel material for this project because it is unmodified, requires no chemical alteration, retains natural bioactivity, supports cell adhesion and proliferation, is cost-effective, and involves simple processing. It also demonstrates proven biocompatibility and low immunogenicity. This base hydrogel was designated as G+OI and was subsequently modified by incorporating additional biomaterials to form G+OI+PGS and G+OI+PGS+BC, aiming to enhance its properties further.
As seen in the results above, A, B, C, D, and E are the Milli-Q water (positive control), phosphate-buffered saline (negative control), hydrogel G+OI, hydrogel G+OI+PGS, and hydrogel G+OI+PGS+BC, respectively. All three hydrogel systems have very few hemolysis percentages, indicating that these hydrogels are highly compatible with body fluid. Also, the hydrogel G+OI+PGS+BC provides a better antioxidant property as seen in Figure H above.
Further, the hydrogels were optimized again to obtain a hydrogel system that will crosslink in a few seconds and have single-layer adhesion.
Fortunately, after many trials of different combinations, the hydrogel was successfully developed to crosslink in seconds and have the property of single-layer adhesion, as seen in figures F and H.
Currently, the newly developed hydrogel has been characterized. Upon completion of this phase, the potential drug carriers will be developed, and PARPi drugs will be loaded into these drug carriers, which will be encapsulated into the hydrogel system to form novel Exos@gel.

Conclusion. A novel tissue adhesive hydrogel encapsulated with PARPi drugs to target the post OC resection to reduce or eliminate the recurrence of OC will be developed upon completion of this project.

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Development of novel poly(2-oxazoline)s-poly(2-oxazine)s based photocrosslinkable hybrid bioink formulations

3D bioprinting is a key methodology in biofabrication, enabling precise spatial placement of cellular, polymeric, organic, and inorganic components to construct three-dimensional biological structures. Extrusion-based bioprinting is widely used, as it supports the printing of hydrogels across a broad viscosity range, accommodates the inclusion of cells and cell spheroids for large-scale constructs, and facilitates relatively high-throughput production [1]. Hydrogels, composed of natural or synthetic polymers, are commonly employed as bioinks to deliver cells into the designed 3D constructs. Natural polymers are favored for their biocompatibility, yet often lack the mechanical strength and rheological properties necessary for effective printing. In contrast, synthetic polymers offer better printability but tend to have limited cellular compatibility.

To overcome these challenges, we utilize synthetic diblock copolymers composed of poly(2-methyl-2-oxazoline) and poly(2-propyl-2-oxazine) (POx-b-POzi) as a smart hydrogel with thermoresponsive and shear-thinning properties. This copolymer serves as a rheology modifier for natural polymers, enabling the creation of hybrid bioinks that combine the favorable characteristics of both material types and facilitate the fabrication of complex 3D biostructures [2].

In this study, we carried out methacrylation on partially hydrolyzed POx-b-POzi, resulting in methacrylated POx-b-POzi. Hydrogel containing both sacrificial and methacrylated POx-b-POzi was supplemented with lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), enabling photocrosslinking of the material. Mouse fibroblasts and pre-osteoblasts were 3D bioprinted with the help of this formulation. Cell-laden constructs showed good cytocompatibility over three weeks in culture, but the cells did not adhere to the crosslinked material and formed spheroid clusters. To address this, methacrylated fish gelatin (fishGelMA) was incorporated into the formulation to provide cell adhesion motifs [3].

Rheological characterizations showed that the adjusted formulation (POx-b-POzi, methacrylated POx-b-POzi, and fishGelMA) was able to retain the synthetic polymer's thermoresponsive gelation features, which enabled 3D bioprinting with high shape fidelity at 37 °C. Fibroblast cells printed with this hydrogel were able to adhere to the crosslinked material. While on the construct surface, cells remained viable over two weeks in culture, the high material stiffness prevented cell growth on the inside. As a next step, the weight percentages of methacrylated POx-b-POzi and fishGelMA, which both contribute to the stiffness of the final structures, will be studied in more detail and optimized.

Overall, we present a hybrid, cytocompatible, and thermoresponsive hydrogel system that enables 3D bioprinting and supports cell adhesion. Further optimization of the material composition will tailor its mechanical properties for applications in tissue engineering and regenerative medicine.

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Engineered Composite Hydrogels for In Situ Tissue Repair: Enhanced Adhesion, Porosity, and Immunomodulation

Introduction/Objectives
Injectable hydrogels can support the body's innate healing capability following defect or lesion removal by providing a temporary matrix for host cell ingrowth and regeneration. However, the clinical adoption of current injectable systems remains low due to issues like product dislodgment during administration, nanoporous structures that limit cell infiltration, and uncontrolled biological responses at the treatment site. This study aims to develop an injectable hydrogel optimized for in situ vocal fold (VF) tissue repair post-defect removal. The ideal hydrogel for this application should (i) cure rapidly to prevent leakage, (ii) adhere strongly to avoid dislodgment, (iii) possess mechanical properties conducive to VF regeneration, (iv) have a microporous structure to support cell infiltration and nutrient/oxygen transport, and (v) contain bioactive properties to promote regenerative healing and minimize fibrosis.
Methods
We synthesized a double-network hydrogel by combining dopamine-grafted hyaluronic acid (DAHA) with silk fibroin (SF), which jellifies rapidly through Fe^{3+}-dopamine coordination and is further strengthened by sonication-induced β-sheet formation in SF. Curcumin-loaded polylactic acid (PLA) particles were incorporated to modulate the inflammatory response. The resultant composite hydrogel, designated as Microporous Double Network Composite (MDNC), comprises an optimized blend of curcumin-loaded PLA particles, 2.5% SF, 2.5% DAHA, and 10 mM iron ions. For comparative analysis, three additional formulations were prepared: Nanoporous Single Network (NSN) containing 5% SF, Microporous Single Network (MSN) composed of 5% DAHA, and Microporous Double Network (MDN) with equal parts of SF and DAHA.

Results

Scanning Electron Microscopy (SEM) and confocal microscopy of FITC-labeled hydrogels illustrated a highly porous structure of MDNC, with an average pore size of 110.21 µm. Rheological analysis revealed rapid gelation within 5 seconds due to iron-dopamine tris coordination, followed by a 50-minute stiffening phase due to β-sheet formation in SF. Oscillatory rheological tests showed that MDNC reached a storage modulus (G') of approximately 1000 Pa, suitable for VF repair, and exhibited a yield strain of about 410%. MDNC hydrogel also exhibited self-healing properties, recovering its original mechanical strength post-deformation. Young's modulus for the MDNC was around 5 kPa, compared to 21 kPa for NSN and 1.7 kPa for MSN. Adhesion tests revealed that MDNC hydrogel had a tensile adhesive strength of 35 kPa and outperformed commercial fibrin glue, which demonstrated a strength of 34 kPa.

Biocompatibility was assessed through Live/Dead assays with over 90% cell viability observed. Phalloidin/DAPI staining confirmed healthy, fibroblast-like cell morphology. Cell migration studies showed a migration index of 19.3%. Anti-inflammatory and anti-fibrotic responses were highlighted by decreased α-SMA and COL1A1 expressions in TGF-β1 stimulated fibroblasts treated with curcumin, and enhanced CD206 expression in THP-1-derived macrophages by 2.9-fold, indicating a shift towards reparative M2 macrophage phenotypes.

Conclusions

Thanks to an unprecedented combination of mechanical, structural, and biological properties, our proposed composite hydrogel is expected to impact broadly the repair and regeneration of various tissues, especially those that are mechanically dynamic.

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Engineering 3D Bioprinted Pancreatic Constructs and Development of Advanced SERS Sensors for Insulin Detection

Abstract:

Introduction:

Islet transplantation is a promising therapeutic avenue for Type 1 Diabetes Mellitus (T1DM), yet its efficacy is limited by inadequate extracellular matrix (ECM) support and microenvironmental cues that affect beta-cell viability and insulin secretion. Furthermore, continuous and accurate monitoring of insulin release is essential for evaluating islet functionality and guiding therapeutic optimization. To overcome these challenges, we developed a 3D-bioprinted islet construct using bioactive, human-derived hydrogels, and an external Surface-Enhanced Raman Scattering (SERS)-based sensing platform for real-time insulin detection from bioreactor-conditioned media.

Materials and Methods:

MIN6 pancreatic beta cells were encapsulated within a composite hydrogel made from human umbilical cord-derived decellularized ECM (UdECM) and hyaluronic acid methacrylate (HAMA). UdECM provided native biochemical cues, including proteins and glycosaminoglycans, promoting cell adhesion, proliferation, intercellular connectivity, and exhibiting low immunogenicity. HAMA enhanced the mechanical stability and printability of the bio ink while maintaining high cytocompatibility. Constructs were fabricated using extrusion-based bioprinting and cultured in vitro. Conditioned media collected at defined time points was analyzed using a customized SERS-based sensor for label-free, high-sensitivity insulin detection.

Results:

The UdECM-HAMA constructs showed high structural integrity and supported the self-assembly of MIN6 cells into spheroid-like clusters. Live/Dead and Alamar Blue assays demonstrated excellent cell viability and proliferation. Immunofluorescence revealed strong expression of N-cadherin and connexin-46, indicating enhanced cell-cell communication. Insulin secretion reached ~60 ng/mL by day 14, significantly exceeding that of control groups. The external SERS sensor enabled sensitive, real-time detection of insulin in the culture supernatant with high specificity and reproducibility.

Conclusion:

The 3D-bio printed islet constructs developed in this study successfully mimicked native pancreatic architecture and supported functional beta-cell behavior with minimal immunogenic response. The incorporation of an external SERS-based insulin sensor enabled continuous, non-invasive monitoring of islet activity. This integrated approach offers a valuable platform for diabetes research, therapeutic screening, and future clinical translation of islet transplantation technologies.

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Engineering Biocompatible Bacterial Cellulose with Salvia Rosmarinus for Antibacterial and Wound Healing Applications

Using natural polymers as biomedical materials for diverse clinical applications has consistently been a focus for scientists. Natural polymers have properties tailored to the specific needs of living organisms and, as a result, have interesting properties of the relevant tissues and are similar to the extracellular matrix. Among the various natural polymers, bacterial cellulose has been considered a promising material for biomedical applications due to its unique properties. BC possesses a high mechanical strength, high purity, hydrogel properties, 3D nanofibrous structures and high-water absorption, biocompatible and non-toxic, which are important factors for biomedical applications, particularly for wound dressing. However, its lack of antibacterial properties limits its usage in this field. In this regard, several research and development studies have been done to create antibacterial properties on BC, including nanoparticles, drugs, and natural materials. In this research, BC was produced using a traditional method and then treated with Salvia Rosmarinus, which has antioxidant, anti-inflammatory, and antimicrobial activity and affects the enhancement of fibroblast proliferation and collagen synthesis. The rate of extract uptake by BC, its antimicrobial effect, cytotoxicity and wound healing were investigated. This study shows excellent antibacterial activities with 100% bacterial reduction percentage against S. aureus and E. coli. Moreover, excellent wound healing properties without cytotoxic effects specified the ability of BC to trap and release Salvia Rosmarinus extract for efficient wound healing.

Keywords: Bacterial Cellulose, Wound Healing, Wound Dressing, Antibacterial, Cell Migration.

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Engineering a 3D model to investigate the role of aging in lung fibrosis

Idiopathic pulmonary fibrosis (IPF) is an age-associated disorder characterized by progressive fibrosis of lung tissue, with TGF-β1 acting as a critical pro-fibrotic factor. Recent studies highlight that epithelial-fibroblast crosstalk, particularly involving senescent lung epithelial (LE) cells, plays a pivotal role in fibrosis progression. Moreover, the extracellular matrix (ECM) and tissue architecture significantly influence cellular behavior and responses to fibrotic stimuli. Despite this, the role of ECM dynamics and 3D culture systems in modeling epithelial-fibroblast interactions and fibrotic progression remains underexplored. This study investigates how senescent LE cells influence lung fibroblasts (LF) in co-culture and examines fibrotic responses in both 2D and 3D culture models, aiming to provide insights into fibrosis mechanisms and the impact of ECM architecture.
Senescent and non-senescent lung epithelial (LE) cells were co-cultured with lung fibroblasts (LF) and stimulated with TGF-β1 to assess fibrosis-associated gene expression over 48 hours. Monocultures of LE and LF cells were similarly stimulated to evaluate their individual contributions to fibrotic responses. Gene expression analyses were conducted to compare the effects of senescent versus non-senescent LE cells in co-culture. Additionally, 2D and 3D (alginate/gelatin hydrogel) culture systems were utilized to examine the influence of extracellular matrix (ECM) and 3D architecture on cellular behavior and fibrosis progression.
Senescent LE cells in co-culture enhanced the fibrotic response of LF cells, particularly under TGF-β1 stimulation, supporting the role of epithelial-fibroblast crosstalk in fibrosis progression. In contrast, non-senescent LE cells exerted a protective effect, significantly reducing fibrosis-associated gene expression in LF co-cultures compared to LF monocultures, even after 48 hours of TGF-β1 stimulation. This protective effect underscores the importance of epithelial integrity in modulating fibroblast activation.
In 3D cultures, fibrotic responses were delayed or attenuated compared to 2D models, highlighting the significant influence of ECM dynamics and 3D architecture on cell behavior. These findings emphasize the limitations of 2D models and the potential of 3D systems to provide physiologically relevant insights into IPF pathogenesis.
This study demonstrates that senescent LE cells amplify pro-fibrotic responses in LF cells, while non-senescent LE cells mitigate these effects, underscoring the critical role of epithelial health in fibrosis progression. Furthermore, the delayed fibrotic response observed in 3D culture models highlights the importance of ECM and 3D architecture in modulating cellular behavior. These findings advocate for the use of 3D models to better study IPF mechanisms and evaluate therapeutic interventions. Future research into matrix remodeling markers and epithelial-fibroblast interactions is essential to develop effective fibrosis management strategies.

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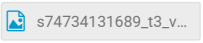
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Engineering a Vascularized Tumor Spheroid Model to Study Angiogenesis in Ovarian Cancer

Introduction: The purpose of this study was to develop an in vitro 3D model of ovarian cancer, which could help investigate potential anticancer drugs and factors related to angiogenesis. Experimental models that capture tumor structure and incorporate angiogenic processes are in high demand. Angiogenesis is a key stage in tumor development, playing a vital role by supporting blood vessel growth to nourish the tumor. Inhibiting this process may aid anticancer therapies. Multicellular 3D in vitro models, with their complex architecture and ability to mimic the tumor microenvironment (TME), allow for more reliable drug testing [1].
Methods: Ovarian cancer spheroids were formed in U-bottom wells. The spheroids consisted of three different cell types: HUVECs (human umbilical vein endothelial cells), HOFs (human ovarian fibroblasts), and A2780 cells (ovarian cancer cells). The appropriate cell ratios, as well as the size and shape of the spheroids (assessed by Feret's diameter and roundness), were evaluated. Cell viability was assessed using Calcein AM and propidium iodide staining. After two days of culture, the spheroids were transferred to 3 mm-diameter wells in PDMS on glass, embedded in a collagen type I hydrogel scaffold. Four model variants (A–D) were analyzed and compared: Model A: HOF and A2780 in a 1:2 ratio, Model B: HOF, A2780, and HUVEC in a 1:2:1 ratio, Model C: HOF and A2780 in a 1:2 ratio, with HUVECs added in the hydrogel, Model D: HOF, A2780, and HUVEC in a 1:2:1 ratio, with HUVECs in the hydrogel. Cell cultures in hydrogel were maintained for four days at 37 °C with 5% CO_2. The culture medium was supplemented with 20 ng/mL of VEGF (vascular endothelial growth factor). Medium was collected daily for VEGF level analysis using an ELISA kit. After three days, the cells were lysed and RNA was isolated to assess the expression of selected angiogenesis-related genes, including ANGPT2, ANG, PDGFβ, PDGFRβ, TGF–β1, and IL–6, using the RT-qPCR method.
Results: In this study, we developed a 3D in vitro vascularized model for future research. The variants containing cells in a 1:2:1 ratio (models B and D) were identified as the most consistent in shape and the most robust to technical variability. VEGF levels in the culture medium increased over time, correlating with the degree of HUVEC self-organization in the hydrogel. Expression levels of selected genes varied between the models.
Discussion: Creating experimental systems that replicate the complex tumor architecture and angiogenic processes is essential for the development of new therapeutic approaches [2]. Our work focuses on engineering a functional model for ovarian cancer research that includes vascularization. This model offers a balance between simplicity and the ability to mimic in vivo conditions, and may have future potential for use in biological and pharmacological studies.
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Enhancing the 3D in vitro models biofabrication with Fibercoll-Flex-N®, a fibrillar native collagen bioink

The role of collagen in regenerative medicine therapies is gaining significant attention. Collagen, particularly in its native, fibrillar form, is essential for creating 3D models that accurately mimic the extracellular matrix (ECM). 3D models in regenerative medicine offer realistic tissue replication, improved cell interactions, personalized treatments, better drug testing, complex tissue engineering, and scalability for clinical use. While soluble collagen is widely available and used, native fibrillar collagen offers a more complex structure that can enhance the rheological properties and biomimicry of scaffolds. This study highlights the application of native, fibrillar collagen bioink in various tissue engineering scenarios, showcasing its potential to improve regenerative medicine outcomes. Fibercoll-Flex-N®, a commercially available 3D fibrillar collagen bioink, offers significant advantages for bioprinting and tissue engineering. This bioink, allows for easy stiffness regulation and supports cell encapsulation at physiological conditions, promoting authentic cell performance, making Fibercoll-Flex-N® a versatile and effective tool for regenerative medicine applications. In this study, human induced pluripotent stem cell-cardiac fibroblasts (hiPSC-CF) and cardiomyocytes (hiPSC-CM), human nasal septum chondrocytes (hCN) and mouse myoblast immortal cells (C2C12) were encapsulated in Fibercoll-Flex-N® to assess the performance of the collagen bioink across different applications. To characterize the mechanical properties of the collagen bioinks, rheological properties such as elastic and viscous modulus (1124 Pa G' and 186 Pa G'') and viscosity (1,5 McP), as well as Fiber length (x50; 400 µm) were determined. Printing speed, pressure and shape were also controlled during 3D scaffold fabrication process to ensure shape fidelity and absence of batch-to-batch variability. Cellular viability and material biocompatibility were confirmed by Alamar Blue® Assay as well as Live/Dead ®Kit. Gene expression completed the analysis of the produced scaffolds. The studies showed high levels of cell viability, harvested DNA and an increase of cell metabolism when compared to the control. Moreover, the gene expression studies also showed an increase of expression in genes of interest. These findings demonstrate that Fibercoll-Flex-N® is well-suited for various in vitro models, showing high cell survival and metabolism rates, along with appropriate gene expression profiles and cellular architecture. The successful application across different in vitro models demonstrates the versatility of Fibercoll-Flex-N®, potentially leading to its use in various regenerative medicine scenarios. These implications highlight the potential of Fibercoll-Flex-N® to advance the field of regenerative medicine by providing a reliable and effective tool for creating high-quality, functional tissue constructs.

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Extracellular Matrix-Based Bio-ink Derived from Porcine Duodenum for Enhanced Wound Healing

The skin, being the body's largest and most exposed organ, is susceptible to various injuries. Overcoming challenges in wound healing necessitates innovative solutions. This study focuses on advancing skin tissue engineering by developing a bio-ink based on an extracellular matrix (ECM) derived from Porcine duodenum, rich in nutrients and growth factors (IGF, EGF, TGF β-1). Porcine duodenum, selected for its abundant active ECM forms, supports crucial wound healing processes such as angiogenesis, cell migration, and proliferation. The project aimed to harness these properties by isolating ECM from Porcine duodenum, incorporating it into a bio-ink, and embedding

therapeutics and growth factors to promote effective tissue regeneration. The Porcine duodenum sample was processed, ensuring sterility, and the resulting slurry underwent polymerization with PVA and Gelatine through mechanical processes and freeze-thaw cycles. Characterization studies included assessments of wetting properties, hemocompatibility, biocompatibility, as well as in ovo and in vitro studies. The bio-ink demonstrated positive outcomes, showcasing biocompatibility, hemocompatibility, and angiogenic potential. In ovo studies confirmed normal angiogenesis, while in vitro studies exhibited a significant increase in cell proliferation compared to control groups. This research establishes that the developed extracellular matrix-based bio-ink, derived from Porcine duodenum, possesses hemocompatible, biocompatible, and angiogenic properties with negligible immunogenicity. These findings contribute to the advancement of bio-ink technology, offering a promising avenue for accelerated wound healing in skin tissue engineering.

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Freeform Printing of Poly(2-cyclopropyl-oxazoline) directly inside perfusion systems for the biofabrication of complex vascular designs

Introduction
The development of functional 3D tissue models has showed significant improvement over traditional 2D cell cultures, as they better mimic the real cell micro-environment. However, in this context, one of the main bottlenecks relates to the difficulty in integrating fully embedded, complex and perfusable vasculature within 3D scaffolds. Even though numerous strategies have been explored, the biofabrication of vascular structures, especially in the micro-scale, still represents an important challenge from a technical point of view. Starting from a previous study [Ryma et al. Adv Mater 2022], in which highly defined micro-scale sacrificial networks were obtained by combining a biocompatible, water-soluble, thermo-responsive polymer with melt electrowriting (MEW) technique, however showing as main limitation the deposition of the sacrificial filaments in a planar substrate, in this work we propose an alternative approach, i.e., Freeform Printing (FFP), to generate 2D/3D freestanding networks of cylindrical filaments directly inside perfusion systems by exploiting the same material.
Materials&Methods
Poly(2-cyclopropyl-oxazoline) (PcycloPrOx) is used as a material for sacrificial scaffolds, being thermoresponsive in aqueous solutions, so that it dissolves on demand by simple temperature reduction. Printing parameters were evaluated by using a piston-driven bioprinter. Different vascular network designs were conceived and analysed through computational fluid-dynamic simulations. Prototypes of perfusion bioreactor and organ-on-chip were designed to allow the direct FFP of sacrificial filaments and then fabricated via DLP printer (Prusa). Three different types of hydrogels (GelMA, ColMA, and fibrinogen) were tested. After casting and crosslinking hydrogels within perfusion systems to embed the printed filaments, sacrificial networks were dissolved and GFP-HUVECs were seeded inside the channels. The vascularized scaffolds were cultured under perfusion over 7 days. Cell viability and attachment inside the channels were studied through confocal to evaluate the channel endothelisation.
Results
Printing parameters was firstly set up: varying the printing velocity (10 mm/min – 100 mm/min) in the G-code was possible to tune filament diameters between around 1 – 0.1 mm. PcycloPrOx filaments were printed directly inside the chamber of perfusion bioreactors and organ-on-chips, demonstrating a practical handling of the whole system. Here, we achieved 2D and 3D free-standing microscale networks defined by different branches, whose channel volumes follow the geometrical ratio between parent and daughter vessel to mimic the vascular tree, for the bioreactor and different channel patterns to create compartments inside the organ-on-chip. Computational simulations allowed to define the oxygen diffusion through 3D vascularized construct in relation to the vascular design and flow rate, and to define velocity field and wall shear rate. Good viability and complete repopulation of channels with all three types of hydrogels was obtained after 7 days.
Conclusion&Future steps
FFP allowed to print highly defined micro-filaments in a 3D hydrogel-based space, overcoming limitation of MEW techniques, directly within perfusion systems in a fast, automatic and straightforward way, opening up the potential of scaling-up the creation of vascularized 3D in vitro models. Moreover, this approach demonstrated to be versatile by using different hydrogels.
Immunostaining and dextran diffusion tests are planned to be performed to assess the formation of a functional endothelium.

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Growth-niche inspired biofabrication by spatio-temporal scaffold modulation using dual sacrificial material casting

Native tissue formation relies on spatio-temporal patterns of cellular proliferation and differentiation occurring over the course of months. In contrast, most biofabrication processes aim to reconstruct full-scale tissue from scratch. This developmental shortcut leads to the well-documented shortcomings in vascularization or innervation. In this work, we present an alternative approach that enables developmentally inspired biofabrication. Two sacrificial materials with complementary gelation behavior and solubility are used. By alternately printing or casting the two materials, granular domains can be generated that can be solubilized with controlled spatio-temporal resolution. Ultimately, this approach allows the controlled growth of voids in 3D scaffolds, e.g. to mimic growing blood vessels.
In this study, three different sacrificial materials, gelatin, Pluronic and polyvinyl alcohol, are investigated and rheologically characterized. Different concentrations of gelatin and Pluronic are studied for their sol-gel and gel-sol transitions to identify complementary gelation behaviors. Interfacial material interactions are studied in a tube inversion assay. To improve material compatibility, different solvent concentrations for the sacrificial materials are tested. To verify the general feasibility of the approach, FFF-printing and digital light processing (DLP) are used to generate molds for the preparation of cast hollow structures. The resulting channels are analyzed for perfusability and shape fidelity.
Gelatine and Pluronic show contradictory sol-gel and gel-sol behavior. The sol-gel and gel-sol transition temperatures of gelatine vary between 28-33 °C and 32-36 °C at concentrations between 10-25 %, i.e. they increase with increasing concentration. For Pluronic at 20-35 % the sol-gel and gel-sol transition temperatures vary between 21.5-5°C and 22-6 °C respectively. The removal of both materials can therefore be carried out successively and at cell compatible temperatures. The results lead to a suitable material selection for joint use. To prevent uncontrolled liquefaction of Pluronic in the presence of other hydrogels, glycerol was added to the surrounding gel. This increased the stability of the material by a factor of 10. The addition of glycerol further improved the shape fidelity of the channels (reducing the deviation from the desired channel width by approximately 25 %).
Finally, to create growing blood vessel-like structures, alternating domains of gelatin-glycerol and Pluronic were cast in a DLP-printed mold. Thermocycling allowed sequential removal of one of the two sacrificial building blocks. Under culture conditions (37 °C), the gelatin building blocks were solubilized, while during cycles of reduced temperature, the Pluronic compartments transitioned to the sol-state. In this way, small elements of both could be removed each cycle, allowing the channel to grow into the newly formed void space.
In conclusion, the study presents the concept of creating growth niches for spatio-temporal control of scaffold structures. To this end, a dual sacrificial material system was developed and tested in a simple blood vessel growth mimicking setup. While the approach has only been tested using 2.5D casting techniques, it holds great promise for application in 3D bioprinting. Here, the ability to generate spatio-temporally modulated growth niches will pave the way for new biofabrication techniques that harness the power of developmental biology for slow tissue growth and conditioning.

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HYCON: A Hydrogel-based Conformable Electrode Array for Noninvasive Electrophysiological Recording of Brain Organoids

HYCON: A Hydrogel-based Conformable Electrode Array for Noninvasive Electrophysiological Recording of Brain Organoids
Introduction:
Brain organoids have become an essential tool for modeling human brain development, neurological disorders, and therapeutic interventions. However, designing reliable interfaces for these 3D, delicate structures remains a key challenge. Current electrophysiological approaches either rely on penetrating mesh electrodes that grow into the organoid over time1, or on predefined 3D structures such as basket-shaped micro electrode arrays (MEAs)2 or kirigami-inspired MEAs3 that constrain organoid morphology and eventually penetrate the organoids. While effective in some contexts, these strategies have major limitations: poor adaptability to organoid-to-organoid variability; risk of mechanical stress, and; complex fabrication or integration steps.2
Methods:
To address these limitations, we developed the HYCON array (Hydrogel-based Conformable Electrode Array), a mechanically adaptive platform that allows for conformal contact of electrodes with brain organoids without the need for invasive penetration or rigid 3D structures. The HYCON array consists of a soft, stretchable MEA fabricated using PEDOT-PSS on a styrene-butadiene-styrene (SBS) substrate, which is freely layered on top of a biocompatible hydrogel. The hydrogel with a tunable stiffness of 0.5–2 kPa, acts as a compliant support layer underneath the MEA. A similarly soft hydrogel pocket holds the organoid in place atop the HYCON array. This geometry enables

passive conformation of the electrode layer around the curved organoid surface, accommodating organoid shape variation and minimizing compression of the organoid.
Results:
Mechanical characterization of the HYCON array demonstrated that the soft hydrogel layer deforms under mild mechanical loading to conform the PEDOT-based MEA around the organoid without requiring predefined mechanical shaping. Unlike basket-type or rigid supports, the HYCON array adapts to different organoid geometries. The stack is fully flat when fabricated, which simplifies the fabrication process and makes it easier to integrate with standard recording hardware. The conformable interface remains stable even after an organoid has been repositioned on top of it multiple times.
Discussion:
The HYCON array is the first fabricated in-vitro neural interface of its kind, as it relies on hydrogel compliance and passive deformation of the electrodes, rather than predefined or invasive structures, to enable contact with organoids. This design provides mechanical support and matches organoid curvature without risking damage or requiring fixed organoid dimensions. This approach is particularly advantageous for dynamic or large-scale organoid experiments, where shape variability is high and minimal perturbation is critical.
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Handheld In situ Bioprinting of Multifunctional Tissue Adhesives for Dermal Regeneration

Tissue adhesives with bioactivity, mechanical integrity, biocompatibility, and rapid sealing are critical for wound healing, especially in severe skin injuries. Traditional suturing/stapling have downsides, such as healing delays, infection risk, and poor aesthetics. Biofabricated tissue adhesives offer minimally invasive, tailored biologically active wound closure. Thus, in-situ bioprinting revolutionizes dermal wound sealing by precisely and adaptably applying biofunctional ingredients. Handheld bioprinting is portable, real-time, and easier to employ in healthcare than conventional bioprinting. In this study, tissue adhesives made from gelatin-methacryloyl (GelMA) and silk-fibroin (SF) were improved with lignin-silver-nanoparticles (L-AgNPs) to have better antibacterial, antioxidant, blood-clotting, and pro-angiogenic properties. Their suitability for in-situ dermal application was assessed by evaluating their structural, mechanical, and biological performances using a handheld bioprinter.

L-AgNPs were synthesized via green method employing lignin as a natural reducing agent and characterized through UV-Vis, FTIR, DLS, zeta potential, DPPH radical scavenging, XRD, and FESEM. SF from Bombyx mori cocoons was SDS-PAGE-analyzed to verify the protein profile. GelMA is tested for methacrylation using TNBS, FTIR, and NMR. UV crosslinking of GelMA/SF mixtures with different amounts of L-AgNP was employed to produce multifunctional tissue adhesives. The structural, mechanical, and biological properties were tested. Lap shear, burst pressure, and porcine skin models were used to analyze tissue adhesion. The antibacterial characteristics of the adhesives were also assessed. Biocompatibility studies were conducted with human dermal fibroblasts. Blood compatibility and hemostatic tests have been performed. The angiogenic potential of the ideal adhesive was evaluated using the in-ovo assay. Furthermore, printability and shape integrity were assessed post-extrusion using a biopen.

L-AgNPs demonstrated a nanometer-scale size (~87 nm), significant colloidal stability (zeta potential ~-31 mV), a crystalline structure (XRD), and spherical morphology (FESEM). SDS-PAGE validated the integrity of SF protein chains, whereas TNBS analysis verified the effective methacrylation of GelMA. The integration of L-AgNPs enhanced the mechanical strength (~1000 kPa), elasticity (~150 kPa), and electrical conductivity (2×10-2 S/m) of the tissue adhesives while maintaining cell viability over 90%. Burst pressure test demonstrated that optimal formulation achieved a compressive strength (~300 mmHg) comparable to that of commercial fibrin sealants. The addition of L-AgNPs considerably enhanced the overlap shear strength (~80 kPa), demonstrating improved tissue adhesion. Antibacterial tests evinced concentration-dependent suppression of Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus. The in-ovo test showed increased neovascularization surrounding L-AgNP-enhanced adhesives. Biopen-mediated printing proved superior extrusion consistency and printability, validating the viability of in-situ wound closure.

L-AgNPs-implemented GelMA/SF tissue adhesives effectively combine mechanical strength, bioactivity, and printability, presenting a viable platform for portable in-situ bioprinting in wound healing therapies. The combination of antioxidant, antibacterial, hemostatic, angiogenic, and adhesive characteristics demonstrates considerable potential for therapeutic application.

Acknowledgments
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Hemispherical 3D culture of lymphoma cells with fibroblasts using Cell Domes fabricated on glass plates

Introduction
Three-dimensional (3D) cell culture techniques have become particularly important for the study of lymphoma cells, which are non-adherent and difficult to culture in traditional two-dimensional (2D) systems, and provide cellular environments that better mimic the in vivo tumour microenvironment. Conventional 2D cultures fail to replicate critical features such as cell-cell and cell-matrix interactions and chemical gradients, leading to discrepancies in drug sensitivity assessments. Co-culturing non-adherent lymphoma cells with adherent fibroblasts presents additional challenges due to intrinsic differences in growth behaviour, adhesion properties and microenvironmental requirements. Nevertheless, the establishment of such a co-culture system is crucial to mimic the complexity of the tumour microenvironment. To address these challenges, we developed a 3D culture system using hemispherical hydrogel structures, termed 'cell domes', immobilised on glass plates.
Materials and Methods
Cell Domes were fabricated by first forming a hemispherical gelatin hydrogel containing diffuse large B-cell lymphoma (DLBCL)-derived KML-1 cells and human dermal fibroblasts (HDFs) on phenol-modified glass plates. A hydrogel membrane composed of alginate and gelatin derivatives was then formed on the hemispherical hydrogel by horseradish peroxidase (HRP)-mediated crosslinking. The internal cavity structure containing the two cells was achieved by incubating the constructs at 37°C, inducing a gel-to-sol transition of the gelatin core. Cell proliferation, hypoxic status, surface marker expression (CD20) and drug sensitivity to doxorubicin were evaluated.
Results and Discussion
KML-1 and HDF cells, initially dispersed within the cell domes, gradually formed aggregates over 10 days, as confirmed by microscopy and section analysis. A hypoxic gradient developed within the domes, with increased HIF-1α expression observed towards the centre of the aggregates, replicating a key feature of in vivo lymphoma tissue. Flow cytometry revealed a decrease in CD20 expression in 3D cultured KML-1 cells compared to 2D cultures. In addition, drug sensitivity assays showed that 3D co-cultured cells were more resistant to doxorubicin, highlighting the influence of the 3D microenvironment on therapeutic response.
Conclusion
The Cell Dome system enables effective 3D co-culture of non-adherent lymphoma cells and adherent fibroblasts, overcoming the traditional challenges associated with their different biological properties. This approach successfully recapitulates critical features of the tumour microenvironment such as hypoxia and drug resistance, providing a promising platform for physiologically relevant preclinical lymphoma research and therapeutic evaluation.

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Konuşmacılar: Shinji Sakai (Osaka University)

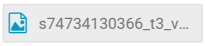
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Hierarchically Structured Multi-Phase Melt-Electrowriting (MEW) Scaffolds for Osteochondral Repair in a Large Animal Model

Introduction
Melt-electrowriting (MEW) is an additive manufacturing technology with the potential to produce regenerative scaffolds that replicate key aspects of the hierarchical structure of musculoskeletal tissues. As small scale polymeric fibres can be accurately deposited using MEW, highly porous 3D scaffolds with well-defined and repeatable pore dimensions can be produced. Herein, we describe the development of biphasic MEW scaffolds with regionally distinct compositions and pore sizes to facilitate the regeneration of osteochondral defects in vivo.

Materials and Methods
The bone phase of the scaffolds consisted of square pores measuring 600 µm with fibres of 18 µm in diameter, while the cartilage phase also consisted of 600 µm pores but with thinner fibres measuring 10 µm in diameter. The MEW scaffold was then placed into a fused deposition modelling (FDM) fabricated tubular structure of 5.85 mm outer diameter, 0.5 mm wall thickness, and 4 mm tall. Slots in the tube wall were designed to catch on the MEW fibres and fix the MEW scaffold in the FDM shell, similar to a previous osteoinductive scaffold [1]. The bone phase of the scaffolds were coated in nano-needle hydroxyapatite (nnHA) which served purpose of double purpose of promoting osteogenesis and consolidating the hybrid structure. The scaffolds were then implanted in a defect made in the trochlear groove of 7 Saanen female goats, and histological analysis was performed after 6 months.
Results
No adverse events were experienced as a result of the surgery or scaffold implantation over the 6 months. The presence of the MEW scaffold enhanced new bone formation and cartilage integration. Histological quantification revealed that the presence of the hybrid scaffold led to significantly more bone formation than the empty control; 40.2% ± 12.9% new bone tissue compared to 22.2% ± 13.1%, respectively. Newly formed cartilage in the defect site stained positively for collagen type II, while newly formed bone tissue stained positively for collagens type I and X.
Discussion
This hybrid MEW-FDM strategy demonstrates the capacity to guide osteochondral repair through biomimetic architectural design. The MEW scaffold promotes targeted tissue integration, while the FDM shell supports surgical handling. However, limited porosity in the FDM component may hinder full tissue infiltration, suggesting future improvements could include increased shell porosity and incorporation of additional scaffold phases to better replicate the osteochondral interface.
Conclusion
These findings highlight the potential of hierarchical MEW-FDM scaffolds as a platform for osteochondral regeneration, and support further optimization of scaffold architecture to enhance integration and long-term functional repair.

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High Temperature Melt Electrowriting for Tissue Engineering

Introduction
Melt electrowriting (MEW) is an additive manufacturing technique in which continuous microfibers are formed from polymer melts using an electric field. The fibers are drawn to a desired thickness by electrostatic forces and deposited on a collector at a different electric potential using a conventional motion system. Due to its origins in tissue engineering, a vast majority of MEW focuses on ε-Polycaprolactone as the sole material due to its low melting point of 60 °C and suitable viscosity for stable long-term processing, which has been extensively studied. A new set of challenges presents itself when processing many polymers of interest with elevated processing temperatures around or above 200 °C.

Methods
We distinguish two behaviors of interest, one being degradation due to the high dwell times of MEW, and the other being the rapid quenching of the jet, causing unexpected deposition behavior or layer bonding to be insufficient to produce structurally sound scaffolds. We investigate these behaviors and adapt our processing conditions for a wide range of different thermoplastic polymers with highly relevant mechanical properties and degradation profiles for tissue engineering via MEW, including PLLA (poly(L-lactic acid)), PLGA (poly(lactic-co-glycolic acid)), PGA (poly(glycolic acid)), PGCL (poly(caprolactone-co-glycolide)) and PBS (poly(butylene succinate)).

Results
We present our findings for the processed polymers, ranging from the preparation and processing conditions for MEW, as well as the resulting scaffold morphologies and mechanical properties, while considering the dwell time in our results, and are investigating common quantitative trends as well as developing guidelines for the printing of demanding polymers based on qualitative data collected herein.

Discussion
We are collecting this data for a growing range of polymers, covering biodegradable polymers, biopolymers, and are pushing towards including as many technical polymers as possible to enhance the processing window for all MEW and to fully understand the printing process as well as its underlying physics and degradation phenomena. We anticipate the materials explored in our work with properties diverging from PCL to have a wide range of adoption in tissue engineering in the coming years, particularly due to increased stiffness, elasticity or faster degradation in vivo.

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High-throughput fabrication of personalized breast cancer organoids by modified microfluidic T-junction tubing apparatus for evaluating patient-specific drug responses

ABSTRACT
In this study, we have developed a modified microfluidic T-junction tubing apparatus to fabricate high-throughput hydrogel-encapsulated personalized breast cancer organoids. We have used mineral oil as a continuous phase and decellularized adipose tissue hydrogel (DAT) encapsulated patient-derived cancer cells as a dispersed phase to generate homogeneous organoids. After assembly, organoids were cultured for 21 days in a 96-well plate format, followed by extensive phenotypic and genotypic characterization, and dose-responsive chemotherapeutic drug cytotoxic evaluation for six different drugs. We demonstrate the successful development of a high-throughput microfluidic platform for generating functional personalized breast cancer organoid models using DAT hydrogel. This innovative approach holds significant potential for future applications in developing personalized cancer organoids and targeted therapeutics.

KEYWORDS: Decellularized adipose tissue (DAT), Microfluidic T-junction, Tumor microenvironment (TME), Personalized cancer organoids.

INTRODUCTION
Recent research has profoundly influenced the development of personalized cancer organoids with complex TME in in-vitro tumor models towards personalized medicine for different cancers [1]. In this prospect, droplet microfluidics has played a significant role in developing high-throughput personalized microtumor models with greater accuracy, homogeneity, and reproducibility [2]. These developed personalized cancer models help us to understand the intricate biological complexity of the patient's tumor growth, differentiation, and metastasis. Thus, developing microfluidic 3D breast cancer organoid models with patient-derived cancer cells with reinforced ECM hydrogel as a component of the TME is essential to improve our comprehensive understanding of cancer biology and screen the most effective and safe chemotherapeutic drugs for individual cancer patients.

EXPERIMENTAL
We used mineral oil as a continuous phase and decellularized adipose tissue hydrogel (DAT) encapsulated cells as a dispersed phase to generate homogeneous cell-encapsulated droplets. After fabrication, we cultured them with essential growth factors in media for 21 days to develop personalized breast cancer organoids. We have thoroughly characterized the fabricated organoids by performing cell viability, metabolic activity, morphological analysis, histological staining, immunostaining for specific marker proteins, and gene expression analysis for specific tumor microenvironment genes by qRT-PCR experiments. We then evaluated the dose-dependent cytotoxicity and calculated the respective IC50 values for six different chemotherapeutic drugs on the developed organoids by Live/dead imaging and MTT assay.

RESULTS AND DISCUSSION
The developed microfluid system is a non-lithography-based device that is easy to assemble and use with a syringe pump. We can use the T-junction multiple times after cleaning and reassembling with the same fittings and PTFE tubes. These advantages make it a cost-effective device that any research laboratory can use for multiple applications to develop personalized organoids. Biological characterization of the developed organoid model shows excellent biocompatibility, metabolic activity, breast cancer-specific marker protein expression, key TME Gene expression, and different dose-response cytotoxicity of anticancer drugs for individual patients. In the future, we aim to validate our DAT hydrogel-based personalized breast cancer organoid models with multiple omics data, followed by biobanking for different breast cancer subtypes. In

the future, hospitals and pharmaceutical laboratories could adopt this microfluidic method as a tool for developing personalized cancer organoids as 3D in vitro cancer models.

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Hybrid Pectin Methacrylate–Gelatin Methacrylate Hydrogels for 3D Bioprinted Cardiac Scaffolds

Introduction

The global burden of cardiovascular diseases, particularly myocardial infarction (MI), continues to rise, driving the need for advanced therapeutic strategies that can restore damaged heart tissue1. Traditional approaches, such as pharmacological interventions and surgical procedures, do not address the fundamental issue of myocardial regeneration. Moreover, the scarcity of donor hearts and immune compatibility issues make heart transplantation an impractical long-term solution for many patients. In light of these limitations, tissue engineering has emerged as a transformative field, offering innovative biomaterial-based platforms for repairing or regenerating cardiac tissue. This study introduces a novel bioink composed of gelatin methacrylate (GELMA) and pectin methacrylate (PECMA), specifically formulated for use in 3D bioprinting to fabricate scaffolds that mimic the native myocardial extracellular matrix (ECM). In this context, we developed a hybrid hydrogel system combining gelatin methacrylate (GELMA) and pectin methacrylate (PECMA), engineered for extrusion-based 3D bioprinting of cardiac scaffolds. The goal was to leverage PECMA's mechanical robustness and GELMA's cell attachment sites to support cardiac cell growth and tissue formation.

Materials and Methods

Both GELMA and PECMA were synthesized through methacrylation reactions, with successful chemical modification confirmed by Fourier-transform infrared spectroscopy and proton nuclear magnetic resonance (NMR). Several hydrogel compositions were prepared by blending the two polymers in varying ratios. A hybrid crosslinking strategy was employed: enzymatic crosslinking using microbial transglutaminase was used to initiate gel formation, followed by photo-crosslinking to stabilize the structure. Rheological analysis was performed to examine viscosity profiles and shear-thinning behavior, which are crucial parameters for extrusion-based 3D printing. Mechanical performance was evaluated through compressive and tensile tests. Human cardiomyocyte-like AC16 cells were encapsulated within the hydrogels to assess cellular responses, including viability, proliferation, and morphology over time.

Results and Discussion

The GELMA–PECMA blends demonstrated excellent printability, characterized by consistent extrusion, filament continuity, and structural retention upon layer-by-layer deposition. Rheological testing confirmed favorable shear-thinning properties, which facilitated smooth extrusion without clogging. Mechanical analysis showed that the elastic modulus of the printed scaffolds could be fine-tuned by modifying the GELMA-to-PECMA ratio, with optimized formulations achieving mechanical properties close to those of native myocardial tissue. Post-printing assessments revealed that the scaffolds maintained their architecture, exhibited controlled swelling behavior, and underwent gradual, predictable degradation under physiological conditions. Cell viability assays indicated high survival rates of encapsulated AC16 cells within the 3D-printed constructs. Fluorescence microscopy showed uniform cell distribution, firm attachment, and evidence of spreading and proliferation (Figure 1 A-B). These results highlight the hydrogel's ability to support cellular functions critical to cardiac tissue regeneration.

Conclusion

This work presents a promising GELMA–PECMA hybrid hydrogel system designed for bioprinting of mechanically stable cardiac scaffolds. By integrating the structural benefits of GELMA with the degradable, biocompatible nature of PECMA, the composite hydrogel offers an ideal environment for cardiomyocyte support and tissue integration. Its favourable mechanical properties, cytocompatibility, and printability make it a strong candidate for future cardiac tissue engineering applications. Future investigations will focus on evaluating the electrophysiological behaviour and contractile function of cells within the scaffolds.

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Hydrogel-Based Microfluidic Lab-on-Chip for Cancer Cell Studies

Introduction

A microfluidic device supported by a hydrogel matrix and carbon nanotubes (CNTs) is a promising tool in developing cancer cell research. These innovative lab-on-chip (LOC) systems enable precise microenvironment control, mimicking in vivo conditions to enhance cancer colony growth and, next, cancer treatment. The integration of hydrogels with microfluidic platforms facilitates the spheroid culture of cancer cells. Moreover, the developed microfluidic environments regulate biochemical and biophysical stimuli, offering a more physiological system compared to traditional 2D cultures [1].

Methods

Hydrogels composed of natural polymers such as sodium alginate and methylcellulose were prepared to provide a matrix that supports cell growth, adhesion, and proliferation. The developed and fabricated hydrogels were mechanically validated by compression test, and degradation evaluation was conducted by in vitro conditions. Furthermore, the hydrogels' cytotoxicity tests were investigated using H69AR lung cancer cells. The designed shape of the hydrogel matrix was obtained by one of the additive manufacturing (AM) technologies - extrusion of the polymer ink. Then, the geometry and structure of the 3D print were stabilized by crosslinking with a CaCl2 solution (1M).

Carbon nanotubes have been incorporated into hydrogels as nanoparticles (powder). Due to the unique properties of CNTs, they have been added to the hydrogel matrix to improve its biological utility. Moreover, CNTs incorporated into hydrogel matrices can also increase their mechanical stability, making them suitable for long-term culture and examination of mutated cells.

Synthetic polymeric light-curing resins (e.g., VisiJet M3 Crystal, GR-10) were used for AM (multi-stream and digital light processing technologies with post-processing requirements) of lab-on-chip substrates and casing. These structures were designed to include, e.g. microchannels and microchambers for spatial and controlled fluid flow, thus enabling cell culture directly on-chip.

Results

The natural hydrogels showed sufficient structural stability in vitro for 7 days (evaluation time). In addition, the low cytotoxicity of the materials used for both hydrogels, substrates, and casing fabrication was indicated. This resulted in a high percentage of survival of H69AR cells in the presence of the aforementioned materials and even enhanced proliferation (an increase in cell viability relative to the control group - cells in a cultivating bottle).

Various AM technologies were used to obtain a ready-to-use microfluidic device. The LOC-type system developed and demonstrated enables precise spatial and temporal control of fluid flow, facilitating 3D perfusion cultures that better simulate physiological conditions.

Discussion

In conclusion, the presented microfluidic LOC system based on hydrogels and CNTs represents a promising and versatile platform for cancer cell research. The ability of the microdevice to mimic the natural cellular environment enables real-time monitoring and future-planned research on photodynamic therapy [2], which underscores its potential to revolutionize cancer diagnosis and therapy.

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INVESTIGATION OF DELAMINATED MXENES AND PCL-MXENES SCAFOLDS IMPACT ON HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS VIABILITY AND ANGIOGENESIS

Tissue engineering methods and regenerative medicine innovations rely on scaffold materials and their fabrication methods [1]. Scaffolds should be biocompatible, biodegradable, and possess mechanical properties suitable for tissue engineering while mimicking the natural tissue structure [2]. Additionally new organic or inorganic incorporated materials could improve scaffold hydrophilicity, conductivity and other mechanical parameters. This study aims to investigate delaminated MXenes, 2D nanomaterials with antibacterial, conductive, and hydrophilic properties, and PCL scaffolds coated with MXenes impact on human endothelial stem cells viability and angiogenesis.

Polycaprolactone (PCL) nanofibers were fabricated using electrospinning with a positively charged needle electrode set at 25 kV, a tip-to-collector distance of 180 mm, and a solution flow rate of 12 mL/h within a controlled atmospheric chamber. Delaminated MXene nanosheets were synthesized from Ti_3AlC_2 MAX-phase precursor. Firstly, material was selectively etched using hydrofluoric acid and then delaminated in a lithium chloride solution and centrifugated to obtain single-layer flakes. Synthesized delaminated MXenes were analysed by X-ray defraction. PCL membranes were pretreated with sodium hydroxide and immersed in an MXene colloidal solution, exposed to sonication, and incubated for three hours to facilitate MXene adhesion. This coating procedure was repeated up to four times to achieve different MXene layer thicknesses. HUVEC cells were exposed to delaminated MXenes for 1 and 3 days or seeded on PCL-MXene and grown up to 3 days. HUVEC viability and angiogenesis was investigated by evaluating cell proliferation, NO production, LDH activity.

Delaminated MXenes were successfully synthesized from Ti_3AlC_2 MAX-phase precursor and characterized by X-ray diffraction (XRD), confirming structural changes distinct from the parent material. The PCL membranes were effectively coated with varying thicknesses of MXene layers through a repeated immersion and sonication process. Human umbilical vein endothelial cells (HUVECs) adhered well to the PCL-MXene scaffolds, while direct exposure to delaminated MXene slurry appeared to reduce HUVEC viability, increased LDH activity and changed NO levels.

These results show that PCL membranes coated with MXenes are more biocompatible compared to direct MXene expose.

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Improved Piezoelectric Response of Poly(L-lactide)-based Nanocomposites Towards Advanced Electrical Cell Stimulation

Introduction

Biodegradable and piezoelectric poly(L-lactide) (PLLA) is gaining attention for biomedical applications, especially in the field of tissue regeneration, based on piezoelectrically-induced electrical cell stimulation. (1,2) However, PLLA possess weaker piezoelectric properties than non-biodegradable poly(vinylidene fluoride) PVDF. (3) Enhancing PLLA's piezoelectricity can be achieved by incorporating biocompatible piezoelectric ceramics barium titanate (BTO) nanoparticles (NPs). (4) However, the high stiffness of ceramic BTO nanoparticle limits stress transfer efficiency and thus piezoelectric effect. This might be overcome by polydopamine (PDA) coating of the BTO NPs (cBTO), what has been shown to increase the piezoelectric response of PVDF nanocomposites. (5) Since for piezoelectric PLLA-based nanocomposites it remains unexplored, this study investigates the piezoelectric improvement of PLLA/cBTO nanocomposite scaffolds due to cBTO NPs.

Materials and Methods

PLLA/cBTO nanocomposite scaffolds with 5 wt.% cBTO NPs of a size of about 50 nm (cat#745952) were electrospun with a drum collector and subsequently post-annealed. PDA-coating on BTO NPs and nanocomposite were examined by XPS and TGA. The fiber morphology was examined by SEM. The degree of crystallinity was determined by DSC and the crystal orientation was assessed by WAXS. Piezoelectric properties of a were examined by PFM and an impact system. Cytocompatibility was assessed via fibroblast metabolic activity.

Results and Discussion

Piezoelectric Enhancement of PLLA/cBTO Nanocomposite Scaffolds: The PFM measurement confirmed piezoelectric behaviour of nanocomposite fibers, depicted in (Figure 1). Moreover, the piezoelectric impact test showed that post-spun annealed PLLA/cBTO nanocomposite scaffolds exhibited the highest voltage output, 2.2 times higher than pristine PLLA and 1.6 times higher than PLLA/BTO scaffolds. PDA coating on BTO NPs improved matrix-particle adhesion, optimizing force transfer and reducing interface defects and thus increasing the piezoelectric response. The improvement in piezoelectricity of PLLA by 120 % due to cBTO NPs is a breakthrough for biomedical applications, particularly tissue regeneration, energy harvesting, ultrasonic transducers, and implantable sensors. The enhanced voltage output (3.2 V) surpasses that of previous PLLA scaffolds used for cartilage regeneration. (1)

Piezoelectric Chain Morphology: High chain orientation, crystal orientation, and a high degree of crystallinity are key factors to induce piezoelectric properties of PLLA matrix. DSC analysis confirmed that all post-spun annealed scaffolds had a similar high degree of crystallinity and 2D WAXS pattern confirmed similar high crystal orientation in all scaffolds.

Conclusions

The present work introduced a new approach to enhancing the piezoelectric properties of PLLA by cBTO NPs. The incorporation of cBTO NPs led to a significant increase in piezoelectric response of PLLA/cBTO nanocomposite scaffold maintaining great cytocompatibility.

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In silico planning of osteoporosis model

Introduction

Osteoporosis (OP) is one of the most common metabolic bone diseases, affecting over 200 million people worldwide. At the cellular level, OP occurs due to an imbalance between resorptive osteoclasts and bone-forming osteoblasts. There are several subtypes of OP, this research focuses on the treatment of post-menopausal osteoporosis.

Methods

The planning of an appropriate in silico analysis model was utilized for both developing a bone model and identifying potential drug targets. The methods involved applying known molecular processes related to bone development and targeting these pathways in osteoporosis. By analysing data from PubMed, KEGG and IPA programs, we aimed to identify targets involved in bone formation and outline different stages of osteoporosis to form the appropriate model systems.

Results

Based on the in silico analysis, bone remodelling and the creation of a viable bone environment suggest using naturally occurring cell types while allowing sufficient time (4D)

for osteoblast progenitor cells to differentiate. After this initial phase, adding osteoclast progenitors along with the necessary factors in a direct co-culture could potentially mimic real-life conditions to bone formation. To develop an OP model from a healthy bone model, the identified pathways indicate that overactivating osteoclasts can be achieved through the addition of Receptor Activator of Nuclear Factor κB Ligand (RANKL), or by utilizing decoy molecules against RANKL inhibiting Osteoprotegerin (OPG). Both molecules are secreted by osteoblast, and are responsible for the regulation of osteoclast differentiation and activation, through RAF1 and MAP3K pathways.
Another common inducer for all OP types is the vitamin D deficiency receptor/retinoid X receptor (VDR/XRX) pathway, which activates cell differentiation and cell proliferation for osteoblasts and regulates cell growth, bone mineralization and calcium homeostasis leading to similar models of necrotic bones.
Discussion
The RANK/RANKL/OPG mechanism is fundamental to our in silico model, as it represents a crucial step leading to osteoclast activation. It's also essential to consider estrogen levels and the signaling pathways of Estrogen Receptor Signaling (ERS) and VDR/XRX. Changes in sex hormone levels—particularly estrogen and testosterone—significantly impact the complex process of bone remodeling through IL-1, IL-6, TNF-α, and OPG. These cytokines and hormones are closely associated with the prevalence of OP, especially among the elderly, with a more pronounced effect on older women. Although several potential drug targets and processes exist within the VDR/XRX, ERS, and RANK/RANKL/OPG systems, our in silico-based design can facilitate the development of appropriate model systems to test both known and novel drugs.

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Intelligent Nanoengineering-Enabled Stem Cell Therapy for Corneal Injury Repair

Introduction
Corneal injuries are a leading cause of vision loss worldwide due to the cornea’s exposed position and precise optical structure.[1] While clinical transplantation is effective, it is limited by donor availability and graft rejection. Mesenchymal stem cell (MSC) therapy offers a promising alternative, promoting corneal wound healing through cell replacement, immunomodulation, and tissue regeneration. However, the long-term survival of MSCs is limited by oxidative stress and inflammatory conditions at injury sites, hindering therapeutic efficacy.
Methods
Here, we present an intelligent nanoengineering strategy to enhance MSC-based corneal repair. We designed ultrasmall, enzyme-free cerium oxide–based nanoparticles functionalized with gold catalytic sites, capable of glucose-driven active motion. This active diffusion improves MSC uptake compared to passive internalization, while the nanoparticles’ intrinsic antioxidant and immunomodulatory properties protect MSCs from oxidative stress, fostering survival and regenerative capacity. [2]
Results
In vitro studies show a two-fold increase in cellular uptake of engineered nanoparticles by MSCs compared to passive controls. In a mouse alkali-burn corneal injury model, the combination of MSCs with these nanoengineered materials significantly accelerates corneal healing and restores transparency, outperforming MSC therapy alone. The synergistic effect arises from enhanced MSC retention, protection against oxidative damage, and promotion of tissue regeneration. Our approach leverages the catalytic activity of nanoengineered materials to mimic enzymatic cascade reactions, avoiding the limitations of natural enzymes such as instability and protease degradation.[3] Cerium oxide provides multiple enzyme-like activities, including catalase, peroxidase, and superoxide dismutase, while the gold catalytic sites enhance glucose conversion to generate active motion.[4] Together, these properties enable precise, glucose-powered transport, improved stem cell delivery, and localized therapeutic effects within complex biological environments.[5]
Discussion
This study demonstrates a biofabrication-inspired platform that integrates intelligent nanoengineered materials with stem cell therapy for ocular repair. The findings highlight the potential of combining nanoengineering and regenerative medicine to develop multifunctional, ultrasmall materials that enhance cellular therapies in oxidative and inflammatory settings. Beyond corneal repair, this strategy provides a blueprint for designing active, protective, and highly efficient delivery platforms for diverse biomedical applications, including tissue regeneration, drug delivery, and precision medicine.
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Interaction of hypoxia-treated human cardiomyocytes and stem cells on nanofibrous scaffolds

Introduction
Cardiovascular disease (CVD) is the most mortality disease globally, and the effective method of its treatment is still unknown. For this reason, there is growing interest in the use of human induced pluripotent stem cells (iPSCs), which can differentiate into all cells in the human body, including induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) which have the potential for future use in regenerative medicine and cardiology [1]. Additionally, it has no appropriate cellular models' mimetic cardiac tissue; therefore, new methods of studying cardiac cell function are still needed. A technique that can potentially produce cellular models that mimic cardiac tissue function is tissue engineering (TE). TE is designed to create a suitable scaffold to mimic the extracellular matrix (ECM). Currently, numerous studies are conducted using nanofibers as a scaffold for culture cardiac cells. However, there is still a lack of research evaluating the impact of polymer nanofibers on the functions of co-culture cardiac cells with stem cells [2].

Methods
We produced polyurethane (PU) nanofibrous mats (Figure 1A) by using the solution blow spinning (SBS) method. Nanofibrous mats were modified with oxygen plasma and protein solutions (0.01% Matrigel solution) to improve cell adhesion. Here, we studied how nanomaterials influence the function of damaged human cardiomyocytes in co-culture with stem cells. First, cell viability (calcein-AM assay) was checked for 3 day-culture (Figure 1B). Next, immunostaining of the levels of OCT4 protein, which regulates the work of induced pluripotent stem cells, and cTnT2 protein involved in the regulation of cardiomyocytes were conducted after 10 days (Figure 2A and B). To confirm the changes occurring in the co-cultures indicative of the differentiation of iPSCs into cardiomyocytes, gene expression analysis of GATA4 (encoding GATA4), TNNI3 (encoding troponin I), SERCA2 (encoding calcium ATPase-type P-ATPase) were performed after 24h and 10 days (Figure 2C).

Results and Discussion
The study checked the viability of the cells, which confirmed that the co-cultures had a high viability (Figure 2). The changes at the protein and gene level (Figure 2) demonstrate that for cultures on polystyrene plates, iPSC cells can differentiate into cardiomyocytes both under iPSC-CMs in normoxia and when iPSC-CMs were subjected to hypoxia. However, at this stage, further studies are required to confirm whether nanofibrous mats can be used for 3D cellular models to study the regeneration of damaged iPSC-CMs. In addition, studies were conducted on immature iPSC-CMs, and further prospects would be to see how mature cardiomyocytes function after hypoxia and in co-culture with iPSCs on nanofibrous mats.

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Linking Rheology with Bioplotting Potential: Chitosan–Sodium Alginate Gels for Biomedical Applications

Introduction

Additive technologies have propelled the popularity of personalized medicine, expanding the adaptation of various biomaterials for additive processing. Sodium alginate (SA) and chitosan (CS) are natural-based, biopolymers that are characterized by simplicity of preparation, ease of modification, and wide availability, and exhibit great potential in additive manufacturing technologies [1–2]. The success of bioplotting dense gel systems relies heavily on understanding and tailoring their rheological and mechanical properties. In this study, we investigate how the incorporation of chitosan into alginate-based gels influences their viscoelastic behaviour, mechanical strength, and overall printability using an extrusion-based bioplotter.

Methods

In this study, a rotational-oscillation rheometer with a parallel plate system was used to analyze the rheological properties of gels. Measurements of viscosity as a function of shear rate were conducted to describe the behavior of non-Newtonian flow in terms of determining the zero viscosity of the solution, the change in viscosity as a function of shear rate, and recovery properties. Oscillation tests were performed to determine the linear range of viscoelasticity, the flow stress, and the relationship between oscillation frequency and viscoelastic properties carried out in variable frequency mode from 0 to 100 Hz at a constant strain rate contained within the LVR range.
Printability was evaluated by printing scaffolds with different fill rates and numbers of layers using a BioX bioplotter (Cellink), analyzing the effect of speed and pressure on print quality. Accuracy was assessed by the width of the printed filament and the size of the pores in the scaffolds measured using a digital microscope. The influence of printing parameters was analyzed using the ANOVA test and η^2 coefficient in Statistica software (TIBCO Software Inc.).

Results

The addition of chitosan changes the nature of the gels from Newtonian to shear-thinning, which was analyzed based on the parameters of the Power-Law rheological model. The results showed a significant effect of chitosan on the rheological properties of gels, which also translated into their processability with a bioplotter.
By adjusting the concentration ratio of sodium alginate and chitosan accordingly, a significant increase in the values of storage modulus, loss modulus, and complex shear modulus G* was obtained, which directly improved printing accuracy. In particular, the higher value of the complex modulus made it possible to print more layers without losing structural stability and collapse of layers.

Discussion

By correlating shear-thinning behavior, flow stress, and recovery properties with printing outcomes, we demonstrate that chitosan serves as a key modifier, enabling the fabrication of stable and precise 3D structures. Our findings highlight the potential of chitosan–alginate composites as bioink candidates for biomedical applications such as tissue scaffolding and wound healing, where both material performance and biocompatibility are critical.

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MAY THE FORCE SHAPE YOU: Harnessing Physical Boundaries In MSC Derived Microtissues To Engineer Scaled Up Cartilage Grafts

Work Title

MAY THE FORCE SHAPE YOU: HARNESSING PHYSICAL BOUNDARIES IN MSC DERIVED MICROTISSUES TO ENGINEER SCALED UP CARTILAGE GRAFTS

Introduction

Articular cartilage (AC) exhibits a unique zonal architecture essential for joint function, characterized by collagen fiber orientation that shifts from parallel at the surface to radial in the deep zone. Tissue-engineered AC grafts using mesenchymal stromal cells (MSCs) often lack this zonal organization when constructed from microtissues (µTs) in unconfined environments, resulting in spherical, structurally disorganized tissues. This study investigates how applying physical boundaries during µT fusion influences tissue morphology and collagen network organization.

Methods

MSC-derived µTs were fabricated using an in-house high-throughput nonadherent agarose hydrogel microwell system as previously described [1,2]. Caprine MSCs were seeded at 2000 cells/µT in chondrogenic media. After 48 hours, µTs were transferred into cylindrical agarose molds (6 mm diameter × 1.5 mm deep) to assemble into unconfined (UC), fully confined cylindrical (CT), or ring-shaped confined (CTM) constructs. Constructs were cultured in normoxia (21% O2 and 5% CO2) for 6 weeks. Histological assessments (H&E, Safranin O, Alcian blue, Sirius red), biochemical assays (DNA, GAG, collagen), and polarized light microscopy (PLM) were used to evaluate tissue morphology, ECM composition, and collagen fiber organization.

Results

Chondrogenesis was evident in all constructs. UC constructs exhibited circumferential collagen alignment at the periphery with a disorganized, cell-depleted core. CT constructs displayed both peripheral parallel and deep radial collagen alignment, along with visible stratification of cell layers. CTM constructs formed rings with radially aligned fibers in the body and circumferential alignment at the outer edge as seen in fig.1. Picrosirius Red staining indicated less intense collagen presence in CT vs. UC groups, while GAG staining (Alcian Blue, Safranin O) was comparable across all groups. Biochemical analysis of preliminary data showed DNA content increasing from CT to CTM (significantly with a p value of 0.0196), significant GAG/DNA content decrease between CT and CTM (p value of 0.0278), and collagen/DNA content showing no significant differences between all groups.

Discussion

Physical confinement significantly influenced the shape and collagen organization of MSC-derived constructs. While UC constructs favoured peripheral alignment and core compaction, additional boundaries in CT and CTM promoted zonal-like collagen distribution reminiscent of native AC. CTM constructs particularly displayed organized fiber orientation mimicking deep and superficial zones, suggesting that macrostructure and extracellular matrix orientation can be tuned via physical guidance of µT assembly. These findings demonstrate the impact of physical boundaries to steer tissue structure in cartilage engineering.

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Disclosure Information

The authors declare no competing financial interests.

Figure 1. Comparison between UC, CT and CTM constructs in terms of collagen staining (histological staining of picrosirius red) and PLM imaging to view the collagen directionality and orientation inside the construct.

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Microfluidic Integration for Real-Time 3D-Bioprinting

Introduction

Three-dimensional (3D) bioprinting offers high precision and flexibility in constructing in-vitro biological environments. Its role in tissue engineering research and applications has grown significantly in recent years1. When combined with microfluidics, bioprinting enables the production of microgels that provide more stable and diverse environments for cells, thereby reducing shear stress-induced damage during printing2. However, many existing systems require additional steps such as oil removal, washing, or post-gelation processing3. These steps increase workflow complexity and limit real-time use.

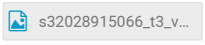
To address these limitations, we developed a simplified microgel-based printing system. This platform enables spontaneous phase separation and direct deposition of oil-phase microgels into an aqueous reservoir, while maintaining the capacity for in-flow cell encapsulation.

Methods
We developed a one-step in-flow printing system4. Microgels are generated inside a microfluidic chip and transferred directly to a 3D printer without any additional processing. The oil-based microgels are then transported through microtubing connected to a modified fused deposition modeling (FDM) printer. Printing takes place in an aqueous reservoir, where the microgels jam and form the printed structure.

Results
Oxidized alginate and gelatin (ADA-GEL) were used as the bioink due to their complementary crosslinking mechanisms. Gelatin undergoes thermally induced gelation, which provides initial structural stability, while the slower crosslinking of oxidized alginate enhances long-term integrity. This combination helps compensate for the delayed gelation of alginate alone.
In addition, ADA-GEL microgels exhibited high stability in aqueous environments. By adjusting the concentrations of the bioink components, we achieved reliable printing performance in water, with the potential to extend this to cell culture media. This represents a notable improvement over conventional systems, which often require microgels to be collected in oil or transferred through additional processing before use.
Using yeast cells as a model, we demonstrated that the system enables reliable encapsulation of cells within the microgels, while effectively minimizing direct contact with the surrounding oil phase. The encapsulated cells were well-distributed and physically separated from 1-undecanol, a solvent known to impair cell viability. These observations suggest that the system can reduce exposure to harmful substances while maintaining the integrity of cell-laden microgels.

Discussion
The in-flow printing system represents a promising advancement in microgel-based bioprinting, offering a simplified and biocompatible workflow by eliminating oil removal and enabling direct aqueous-phase deposition. The use of ADA-GEL ensures structural integrity and compatibility with biologically relevant environments. While further optimization is needed, this platform lays the groundwork for modular, cell-friendly bioprinting systems with broad applicability in tissue engineering.

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Multimaterial 3D-Printed Gradient Scaffolds for Tendon-Bone Interface Regeneration: Novel Infill Design Approaches

Musculoskeletal interface injuries, especially at the tendon-bone junctions, present a significant clinical challenge due to their limited ability to regenerate and the structural complexity of native tissues. Current treatment methods often do not restore biomechanical functionality, highlighting the need for advanced biomimetic scaffolds. Our goal is to engineer tissue-specific constructs using 3D printing technologies, focusing on the functional regeneration of tendon-bone interfaces. We focus on designing and fabricating gradient scaffolds that integrate both macro- and microscale features through additive manufacturing. Our emphasis is on developing innovative infill patterns and concave architectures that provide better cell adhesion and tissue development compared to convex structures [1] and enable tailored mechanical properties.
Our approach combines Fused Deposition Modelling (FDM) with Melt Electrowriting (MEW). MEW is a novel technique that enables highly precise deposition of polymer microfibers under the influence of an electric field. FDM is used to create stiffer scaffold regions that mimic the bone part of the interface, while softer and more flexible structures that imitate the tendon part are produced using MEW.
Concave structures based on small intersection angles and sinusoids were designed. Scaffolds were fabricated from polycaprolactone (PCL) using a multi-head tool (BioScaffolder 3.3, GeSiM). Optimisation of the printing parameters for both techniques was performed. Microstructural, mechanical and biological studies were performed for scaffolds with various fibre thicknesses, geometrical designs, and pore sizes. The tensile strength tests revealed significant differences between varying architectural designs, resulting from fibre density and inter-fibre distance variations. Also, the initial biological test model using primary human dermal fibroblasts revealed distinct cells' preference for concave structures. Enhanced cell bridging was linked to concavities that featured closer fibre connections. In contrast, in more distant fibre connections, cells preferentially aligned along the fibres.
Currently, we are investigating the performance of the tenocites on the gradient scaffolds. Additional data on cell adhesion, morphology, viability, migration, and distribution will provide valuable insights for future studies on the tendon-bone interface structure.

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Multimaterial Core–Shell Bioprinting to Fabricate Cultivated Meat Scaffolds

INTRODUCTION
A sustainable alternative to traditional meat is cultivated meat, which is the growth of animal muscle tissue in laboratories. This technology aims to create a cell-laden product that replicates the texture, composition, and structure of conventional meat.[1] However, the hydrogels commonly used in tissue engineering techniques, whether as cast scaffolds or bioinks, often lack the dual capacity to provide both the mechanical properties of meat and the extracellular matrix (ECM)-like environment needed to support cell viability and muscle fiber differentiation.[2][3] Here, we present a bioprinting method using a custom core-shell nozzle to print a filament with a robust gellan gum (GG) shell and an ECM-mimicking core made of recombinant elastin-like protein (ELP).
MATERIALS AND METHODS
We first tested different extrusion rates and concentrations of GG as a shell material in combination with a core of 3 wt% ELP. Naturally derived, edible thickeners, including methyl cellulose (MC) and cellulose nanofibers (CnF), were blended to achieve different core–shell material distributions. Ink formulations were assessed for printability by calculating a printability index, which was based on quantitative evaluation of printed grid structures, flow behavior, and mechanical properties measured using an oscillatory plate rheometer. The ELP core ink was mixed with a model muscle-like cell type (C2C12 cells) and coextruded with the GG shell to produce a multimaterial scaffold. Cell viability and myotube formation after 7 days in differentiation medium was quantified using confocal microscopy.
RESULTS AND DISCUSSION
The results showed that adding GG as a shell and incorporating thickeners into ELP as the core significantly improved the printability of the material system, enabling the fabrication of a continuous multimaterial filament. This approach also enhanced the mechanical properties of the final construct, better mimicking those of commercially available bovine meat. Adding 3% MC to the ELP improved mechanical properties of the core ink, increasing its stiffness to approximately 100 Pa compared to about 10 Pa for the ELP ink alone. A stable core–shell structure, with the core representing approximately 20% of the total cross-sectional area of the filament and achieving a printability index exceeding 0.85, was consistently obtained under optimized conditions. The printed scaffolds maintained cell viability above 90% and supported myotube formation, as confirmed by immunostaining.
CONCLUSIONS
Multi-material bioprinting allowed for the fabrication of a construct with a bioactive core and a robust shell. This strategy of coaxially extruded filaments was able to overcome key limitations of traditional bioprinting materials with low printability. The mechanical properties of the shell supported the fabrication of mechanically robust 3D constructs, while the core supported tissue-like cell viability and differentiation.
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Mussel-Inspired GelMA/SF-Based Sealants for the Prevention of Air Leakage in the Lung

Introduction
Air leaking is a common problem associated with lung surgery. Surgical sealants implemented to leakage area present a promising solution for postoperative pulmonary air leaks. Particularly, sealant effectiveness depends on mechanical compatibility with the visceral pleura and mismatched mechanical properties can cause leaks, detachment from the tissue, or even tissue damage. A hydrogel sealant inspired by mussels, made from GelMA and silk fibroin (SF), was designed to resemble the structure, strength, and biological properties of lung tissue. Its potential as a surgical lung sealant was assessed in-vitro, ex-vivo, and in-vivo studies.

Methods:
To create a mussel-inspired hydrogel sealant, DOPA-functionalized SF and GelMA polymers were synthesized using a tyrosine modification approach. The tyrosine content was augmented using the Bolton-Hunter reaction. Then, the tyrosine parts on the SF and GelMA structures were changed into DOPA (L-3,4-dihydroxyphenylalanine) using tyrosinase enzyme. The resultant polymers were subsequently crosslinked under visible-light to create tissue adhesives. The final formulation included sodium periodate to facilitate dual-crosslinking. The effectiveness of surgical sealants was evaluated by measuring their pressure resistance in lungs, their capability to close wounds in both wet and dry situations, and their compatibility with MRC-5 cells. Finally, in-vivo performance was evaluated using a rat lung leakage model, where a standard incision was created in lung and the sealant was applied to the incision site. To quantify the in-vivo sealing strength, burst pressure measurements were conducted on DOPA-free and DOPA-containing GelMA/SF sealants applied to lungs on day 0 and day 7. Healthy lungs used as positive controls.

Results
DOPA-containing GelMA/SF sealant exhibited excellent ex-vivo lung tissue adhesion performance in wet/dry environments, with significantly higher performance than the DOPA-free control group. It showed a burst pressure of 140.64±37.72 mmHg in dry and 81.44±11.46 mmHg in wet conditions, and wound closure strength of 226.78±124.26 kPa in dry and 79.40±38.81 kPa in wet conditions. In contrast, the DOPA-free sealant exhibited burst pressure of 100.89 ± 24.83 mmHg in dry and 51.63 ± 13.53 mmHg in wet conditions, and wound closure strength of 99.38 ± 35.61 kPa in dry and 33.40 ± 12.78 kPa in wet conditions. During the 7 days post-seeding period, the MRC-5 cells showed a good attachment and proliferation, indicating a favourable cytocompatibility of the DOPA-containing GelMA/SF sealant even directly contact with the cells. In-vivo results proved its biocompatibility in rat subcutaneous tissue. Two weeks after surgery, the burst pressure of lung tissue treated with DOPA-containing GelMA/SF sealant elevated compared to day 0, reaching the burst pressure values comparable to those of native healthy rat lung tissue (1.90±0.89 kPa vs.1.88±0.49 kPa). In the lung defect group, bleeding in the bronchioles, epithelial cell shedding, and hemorrhagic areas in the pulmonary interstitium were observed. Two weeks after the surgery, DOPA-containing GelMA/SF sealant group resulted in preserved the bronchiole and alveolar structures, and decreased vasocongestion and bleeding.

Discussion
This study illustrates the significant potential of DOPA-containing GelMA/SF sealant for addressing lung air leaks.

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Nippi Inc. – Exhibition Overview

Nippi Inc., a Japanese manufacturer of collagen and gelatin, has spent over a century dedicated to improving people’s quality of life and developing breakthrough products. Guided by our longstanding philosophy of “Quality the First,” we remain committed to supporting the future of human well-being as experts in protein engineering.

At this exhibition, we will showcase our highly concentrated collagen bioink for 3D printing, as well as collagen and gelatin products for medical and research applications. Our products are used across a wide range of fields, from basic research to applied development. We welcome you to learn more about how our solutions can contribute to your work.

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OPTIMIZATION OF SEEDING METHODS ON MELT ELECTROWRITTEN SCAFFOLD FOR THE FIBROCARTILAGINOUS LAYER OF THE TEMPOROMANDIBULAR JOINT CONDYLE

Introduction
Current treatment for temporomandibular joint (TMJ) disorders consisting of pain mitigation or surgical invasion, remain limited. Conventional scaffold fabrication techniques, such as fused deposition modeling, lack microscale resolution necessary to recreate the architecture of TMJ cartilage. In contrast, melt electrowriting (MEW) is a high-resolution additive manufacturing technique that enables the fabrication of microfibrillar scaffolds with precise control over architecture, fiber alignment, and porosity. Providing a highly tunable platform to mimic the mechanical and spatial features of native TMJ tissues.
Conventional cell seeding methods using single cells often result in uneven distribution and poor retention. Therefore, our approach incorporates human nasal chondrocyte (hNC) spheroids, known for their chondrogenic potential and accessibility, as cell source(1). We hypothesize that integrating hNC spheroids into MEW-fabricated scaffolds will enable the development of a hybrid construct that combines mechanical support with biologically driven tissue remodeling. Compared to single-cell seeding, spheroids are expected to provide superior spatial cell distribution, and facilitate zonal cartilage regeneration, critical for TMJ repair.

Methods
A Voron 0.2 printer converted for MEW was used to fabricate polycaprolactone (PCL) scaffolds(2). A trial-and-error approach determined the nozzle-to-collector distance, voltage, pressure, and G Code configurations, with adjustments made based on visual inspection using SEM. PCL’s melting temperature was determined by differential scanning calorimetry.

hNCs were isolated from nasal septum biopsies obtained from three donors. After expansion, cells were seeded at densities of 2400, 3000, or 4800 cells per microwells to form spheroids. Following two days of spheroid formation, they were harvested and seeded onto MEW-scaffolds with a 250um interfiber distance. To support even cell distribution, stepwise cell suspension (300ul +300ul) with centrifugation in between was used. Following spheroid seeding, differentiation was carried out for 21 days in chondrogenic medium (TGF-β1). This was evaluated through immunofluorescence staining for Collagen I and II, as well as glycosaminoglycan (GAG) and DNA.

Results
Optimized printing parameters were 3.1kV, 0.202MPa, 70°C, G27, and a nozzle-to-collector distance of 2.00mm, which gradually increased to 2.02mm over 20 layers to ensure stable and precise fiber deposition. Higher seeding densities achieved larger spheroids of 144.27um (SD39.18; 2400 cells), 187.40um (SD64.46; 3000 cells) to 176.06um (SD78.26; 4800 cells) that fit well into scaffold pores. Single-cell seeding resulted in poor and uneven scaffold coverage, while spheroid-based seeding, using two rounds of stepwise seeding with centrifugation, achieved mean scaffold coverage of 41.0% (SD 5.3%). Immunofluorescence analysis of scaffolds showed higher Collagen I expression relative to Collagen II. Cumulative GAG release in the medium increased steadily over time, with a higher release rate observed in the first half of the culture period.

Discussion
The MEW scaffolds fabricated under optimized parameters provided a stable and consistent platform for spheroid attachment and culture. Scaffold coverage was improved through spheroid-based seeding compared to single-cell seeding. Chondrogenic differentiation was observed, with steady GAG release over 21 days. The higher expression of Collagen I relative to Collagen II may suggest a tendency towards a fibroblastic phenotype. In conclusion, hNC spheroids support more uniform tissue development and present a promising strategy for cartilage regeneration.

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  Optimization of Electrospinning Parameters to Enhance the Solubility of Myricetin

  Introduction:
  Polyphenols are a diverse group of naturally occurring organic compounds with potent antioxidant properties. These compounds play a crucial role in the prevention of various lifestyle-related diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions [1]. Among them, myricetin (MYR) stands out due to its well-documented health-promoting effects such as anti-inflammatory, anticancer, and cardioprotective activities. Despite its high therapeutic potential, MYR's poor water solubility and low bioavailability significantly limit its application in the pharmaceutical and food industries [2]. Electrospinning has emerged as a promising technique for enhancing the solubility and bioavailability of bioactive compounds. This method enables the fabrication of polymer nanofibers capable of encapsulating active substances, thereby improving their physicochemical properties and controlled release [3]. The unique features of electrospun fibers-such as high surface area-to-volume ratio and tunable chemical composition-offer a viable route for enhancing MYR's solubility and therapeutic utility.
  Methods:
  The objective of this study was to evaluate and optimize the electrospinning process to improve the aqueous solubility of MYR by incorporating it into polymeric nanofiber matrices. Various technological parameters, including polymer concentration, applied voltage, needle-to-collector distance, and flow rate, were optimized using the Box-Behnken design. Initially, potential polymers suitable for electrospinning were reviewed and their solubility in methanol, ethanol, and water was assessed. Specific viscosity measurements were conducted to select optimal polymer candidates. Polyvinylpyrrolidone (PVP K30) was chosen for fiber formation with MYR. The identity of the electrospun MYR-PVP30 fibers was confirmed using Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Powder Diffraction (XRPD). Solubility enhancement was evaluated via High-Performance Liquid Chromatography (HPLC), and morphological characterization was performed using Scanning Electron Microscopy (SEM).
  Results:
  The optimized electrospinning process led to the successful fabrication of uniform MYR-loaded nanofibers. The incorporation of MYR into the polymeric matrix resulted in a significant improvement in its apparent water solubility. XRPD analyses confirmed the successful encapsulation and amorphization of MYR within the nanofibers. HPLC analysis demonstrated enhanced solubility, while in vitro antioxidant tests revealed improved bioactivity of the electrospun formulations compared to raw MYR.
  Discussion:
  The study demonstrates that electrospinning is an effective technique for improving the solubility and functional performance of poorly soluble bioactive compounds like MYR. By optimizing key process parameters, it is possible to fabricate nanofibers that not only improve solubility but also preserve or enhance biological activity. These findings suggest that electrospun nanofibers hold considerable promise for use in localized drug delivery systems and potentially broader pharmaceutical applications.

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  PCL electrospun meshes biofunctionalized with chitosan for wound healing

  Introduction
  Wound healing follows a five-stage process when skin tissue is injured, but factors like wound size and patient health may require additional interventions [1]. Tissue-engineered structures, especially electrospun meshes, offer enhanced regeneration by mimicking the skin's extracellular matrix, promoting hemostasis, absorbing exudate, and minimizing scarring [2]. However, electrospinning of natural polymers pose challenges since some polymers are difficult to process [3]. This study focuses on producing polycaprolactone (PCL) electrospun meshes (ePCL) and biofunctionalizing them with chitosan (CS). This approach ensures consistent fiber production while introducing a bioactive polymer on the surface to support in situ wound healing. The biofunctionalized electrospun meshes were analyzed for their physicochemical and mechanical properties.

  Methods
  Electrospun meshes were produced from a 16 wt% PCL solution in acetone using a home-made electrospinning apparatus. After ePCL production, the fibers were modified with sodium hydroxide (NaOH) and then biofunctionalized with CS through carbodiimide coupling (EDC-NHS).

  Results
  The ePCL meshes were produced and subsequently biofunctionalized with different concentrations of CS, namely 0.05, 0.1 and 0.5 wt%, through an optimized procedure. Morphological characterization was performed using SEM at 15kV where different concentrations of CS can be observed encapsulating the ePCL nanofibers without changing typical mesh morphology, although increasing fiber diameter and introducing rugosity. EDX analysis revealed that the condition of ePCL with 0.5 wt% of CS showed a smaller ratio of nitrogen in the sample when compared with the remaining percentages. To confirm the presence of CS on the electrospun fiber's surface ATR-FTIR was used as well.
  The meshes hydrophilicity was evaluated by measuring water contact angles. The biofunctionalized ePCL meshes resulted in more hydrophobic surfaces until a concentration of 0.5 wt% CS, concentration at which a higher hydrophilicity was observed leading to complete water absorption.
  Mechanical properties were evaluated using a texturometer analyzer to assess tensile strength at break (TSB), elongation at break (EB) and Young's modulus (YM). A subsequent increase in CS showed lower YM.

  Discussion
  FTIR-ATR analysis and SEM imaging with EDX confirmed the biopolymer's presence on the fiber surface, without closed pores, for all conditions. Samples with 0.5 wt% CS were more hydrophilic, although 0.1 wt% and 0.05 wt% samples absorbed the water droplet slower due to structure smaller pores. Mechanical analysis demonstrated that adding CS resulted in higher material stiffness compared to ePCL. However, with the increased CS concentration, the material became less stiff, decreasing YM and increasing TSB and EB.

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  Preliminary Evaluation of Soft Material Implant Feasibility for Cartilage In Situ Bioprinting Across Extrusion Orientations

  Introduction
  In situ bioprinting is a field of tissue engineering that employs the additive manufacturing of soft implants directly into the patient. In this biofabrication technique, the implant is printed layer by layer on the site of repair to achieve the desired anatomy. As the body is not a perfectly flat surface, non-planar algorithms for toolpath generation [1] can help

to enhance the success of the interface between the implant and the body, which is vital to feed the implant with cells and nutrients [2][3]. In non-planar bioprinting, it is ideal for the printer's extruder to be perpendicular to the printing surface during the entire implant fabrication. This limitation implies that the nozzle can assume any orientation in the space, from minor deviations of the vertical axis to being completely upside-down. In this work, we investigate the impact of printing soft materials while having the extruder at extreme angles to understand how extruder orientation and gravitational force affect the feasibility and mechanical properties of printed constructs.
Methods
Three ASTM D412 coupons were printed in three different nozzle orientations (regular, horizontal and upside-down) and underwent tensile testing and optical microscopy analysis. The material chosen was Thermoplastic Polyurethane (TPU) hardness 95 Shore A, which approximates the hardness of both healthy cartilage (ICRS grade 0) [4][5] and artificial cartilage hydrogels (e.g. PVA/PEG) [6].
Results
The samples were printed successfully and retained the intended shape and mechanical behaviour in all orientations. Analyzing the stress supported by the samples at 100% strain, the upside-down samples presented a 0.94% decrease in tensile performance, while the horizontally printed coupons performed 2.41% worse than the regular orientation. Upon microscopic visual investigation, all samples showed a similar mesostructure, suggesting that any inverted gravity pull effects on the soft material deposition were not representative.
Conclusion
The results display the feasibility of printing soft materials in all possible nozzle orientations with minimal loss on tensile performance. This conclusion contributes to the field of in situ bioprinting in ensuring that the shape fidelity and mechanical properties of 3D-bioprinted implants are maintained even under extreme extrusion orientations.
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  Printing Complex Structures Using Collagen Bioinks: Optimization of G-code Templates for Enhanced Precision and Cell Viability with FDM Based Slicer

Printing geometrically complex structures requires the preparation of advanced G-code templates to control the printer accurately. Most available slicers are optimized for FDM printing using thermoplastics, which solidify rapidly upon cooling. In contrast, collagen-based bioinks solidify much more slowly and require precise temperature control during printing to prevent degradation while maintaining print accuracy.

The aim of this work was to develop an optimized slicer template for accurate and reproducible printing of models using a collagen bioink with embedded cell cultures. This bioink relies solely on physical gelation mechanisms—specifically, changes in pH and temperature. A collagen bioink at a concentration of 30 mg/ml was used, incorporating porcine adipose-derived stromal cells, and prepared via a two step neutralization method [1].

During slicer optimization, various parameters were tested, including nozzle types (G20, G23), purge lines, print directions and speeds, model geometries, and G-code modifications. The final configuration template included features such as Z-hop activation, perimeter crossing avoidance, randomized seam placement, extended non-extruding travel moves, and a 45-second pause between layers. Geometrically defined solid and hollow shapes were printed and evaluated for shape accuracy, height profile, and defects. To enhance shape visibility and enable automated optical inspection, fluorescein was added to the bioink, and the prints were imaged using fluorescence microscopy.

The results confirmed that print strategy significantly affects print quality and that bioink degradation over time can negatively impact outcomes. A layer-change pause and active heating/cooling of the print bed were implemented to support gelation.

The printed structures were then tested with embedded cell cultures. Fluorescence microscopy and staining of F-actin and nuclei confirmed that cells were evenly distributed throughout the height of the printed constructs. These results demonstrate that a properly configured slicer enables the creation of stable structures.

This optimized template was subsequently used to print microfluidic channels simulating bifurcated vascular networks—structures too complex for manual G-code generation. The printed model was tested in a flow bioreactor, and the remodeling capacity of the printed wall under defined shear stress (5–15 dyne/cm²) was evaluated. The prepared template thus enabled the fabrication of complex structures using a material with a relatively long gelation time. By combining appropriate layering with controlled bed temperature, smooth circular channel shapes were achieved, improving flow characteristics.

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  Protein-Enriched Biomaterials: Strengthening Mechanical Performance and Supporting Cell Proliferation

Introduction:
The development of biomaterials for tissue engineering applications requires a delicate balance between biocompatibility and mechanical performance. While ensuring a material’s compatibility with living tissues is essential to avoid adverse immune responses, mechanical strength and the ability to fine-tune it are equally critical to support tissue regeneration and withstand physiological loads. These parameters directly influence the suitability of biomaterials for various applications, including bone, cartilage, and soft tissue engineering. An emerging and promising approach involves the design of hybrid materials that combine conventional biomaterials with innovative pro-proliferative agents, offering enhanced functionality and new opportunities for regenerative medicine. The aim of the study is to develop a biomaterial composition with high potential for both processing technology - 3D printing, as well as biocompatibility and functionality in the field of biomedical engineering.
Methods:
The material to be analyzed is methacrylated gelatin (GelMA) enriched with recombinant proteins containing elastin, resistin or silk domains. The experiment used the addition of recombinant proteins to GelMA at two concentrations. The reference material was GelMA without additivies. The research methodology included analyses of physicochemical properties using methods such as rheology, printability and static compression test of 3D constructs, as well as basic analyses of biological properties. In the rheological analysis, parameters such as temperature gradient viscosity, phase transition point and complex modulus were determined as a function of shear stress. To evaluate the printability of the material, a three-step procedure was used that included a fiber fusion test, a fiber collapse test and a fiber continuity evaluation test. Mechanical parameters - compressive mechanical strength and Young's modulus were determined using a static compression test of 3D constructs. The biocompatibility properties were evaluated using the MTT and LDH test on the L-929 mouse fibroblast reference line.
Results:
By combining the base material with the selected additives in varying proportions, it is possible to modulate both the mechanical and biological properties of the resulting constructs according to specific application needs. This tunability allows for the adjustment of parameters such as stiffness, elasticity, and degradation rate, as well as the enhancement of cellular responses. The incorporation of tested recombinant proteins, further supports these improvements by enhancing mechanical strength, promoting cell proliferation, and maintaining the biocompatibility and biodegradability of the materials. The materials studied exhibit suitable rheological properties, making them applicable for 3D printing using the extrusion method. Moreover, the viscosity and fiber stability allow for the production of structures with relatively high resolution. This flexible approach broadens the potential for customizing scaffolds for diverse biomedical engineering applications.

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  Routine GelMA Characterization: A High-Throughput Approach Integrating Electrophoresis and UV-Vis Spectroscopy

Gelatin Methacryloyl (GelMA) attractes considerable research attention as an important structural component for bioinks.1 The synthesis of GelMA involves the methacrylation of gelatin, wherein methacryl groups are covalently bonded to the amino groups of lysine residues. The degree of methacrylation (DM) is a critical parameter that significantly affects the physicochemical properties of GelMA. For effective bioprinting of organs and tissues, the use of GelMA with a precisely defined DM is essential, as it influences hydrogel stiffness, porosity, swelling behavior, biodegradability, and cellular proliferation. Consequently, there is a need for accessible, cost-effective, precise, and routine methodologies to control and assess the DM of GelMA. This necessity motivated us to develop two novel and complementary methods for determining the DM, both of which can be readily implemented in laboratories equipped with electrophoresis instrument or UV-Vis spectrophotometer.

Currently, the most widely used method for characterizing the degree of methacrylation of GelMA is nuclear magnetic resonance spectroscopy (NMR).2 Due to the high cost of NMR instrument maintenance, the NMR technique is not routinely available in every laboratory. We are proposing alternative approaches to characterizing GelMA that can be used for routine laboratory analysis. The first proposed method relies on measuring the electrophoretic mobility of GelMA samples, which directly correlates with their degree of methacrylation. This approach requires comparison against reference standards with known DM values and can effectively serve as a classification tool for GelMA products based on their DM. The second method we developed focuses on quality assessment after GelMA fabrication, enabling characterization of pure GelMA through analysis of its absorption spectrum. Although this spectroscopic technique does not require the addition of external reagents such as TNBS,3 the construction of a calibration curve remains necessary to achieve precise quantification.
The precision of both newly developed methods is comparable to the NMR technique, exhibiting excellent correlation with NMR data (Table 1). The electrophoretic method enables effective characterization of GelMA even when organic impurities are present. Although this method is somewhat more time-consuming, it can conveniently be substituted with the UV-Vis method for routine applications.

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Strategies for Antibacterial Properties of Biocompatible PCL and Calcium Phosphate Composite Bone-Regeneration Scaffold

Bone tissue engineering aims to develop biomaterials that can effectively repair and regenerate damaged bone tissue. Polycaprolactone and calcium phosphate, which are used in this field, are among the most widely used biocompatible materials. However, these materials lack antimicrobial properties and are vulnerable to bacterial adhesion and biofilm formation, which can lead to implant failure. To solve this problem, a strategy to improve antimicrobial properties while maintaining existing cell behavior properties is needed. In this study, the antimicrobial properties of scaffolds were improved through two chemical/mechanical antimicrobial strategies. For chemical antimicrobial properties, ZnO was coated on the scaffold surface with thicknesses of 10, 100, and 200 nm, and cell behavior and antimicrobial properties were evaluated. Scaffolds coated with 100 and 200 nm thick ZnO showed high antimicrobial properties against Escherichia coli (E. coli), and considering mechanical properties and cell activity, a 100 nm thick ZnO coating was appropriate. In addition, when calcium phosphate is synthesized in the form of nanostructures on the surface of the scaffold, it sterilizes bacteria (E. coli, Bacillus subtilis (B. subtilis)) mechanically attached to the surface and improves the proliferation and differentiation of bone cells. This antibacterial strategy of the scaffold is a groundbreaking strategy that can impart antibacterial properties while maintaining the cell behavior, shape, and characteristics of the existing scaffold.

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Tunable Collagen Scaffolds Reveal Stiffness-Driven Stromal Activation in 3D Pancreatic Cancer Models

Introduction
Pancreatic Ductal Adenocarcinoma (PDAC) is hallmarked by a dense, collagen-rich stroma, driven by the activation of pancreatic stellate cells (PSCs), which remodel the extracellular matrix (ECM) into a mechanically stiff microenvironment that promotes tumor progression and therapeutic resistance.1,2 To accurately mimic this fibrotic transformation in vitro, we engineered 3D scaffolds using type I collagen — the predominant ECM protein in PDAC — as a tunable biomaterial. Collagen's native bioactivity, cell-binding motifs, and mechanical adaptability make it a powerful tool for biofabricating physiologically relevant tumor microenvironments.3,4
Methods
Immortalized human PSCs behavior was investigated in co-culture with two different immortalized human pancreatic cancer cell lines (AsPC-1 or PANC-1) and compared to the stromal monoculture. Three-dimensional constructs consisting of cells embedded within collagen matrices were prepared varying the collagen concentration from 0.5 to 2.5 mg/mL.
After 48h of culture, activation of the stromal component was studied through the expression of alpha-smooth muscle actin (α-SMA), while N-cadherin and E-cadherin were used as cancer epithelial-to-mesenchymal transition (EMT) markers. Such protein expressions were investigated by immunofluorescence and quantitatively by Western Blot. Moreover, samples stained with DAPI and phalloidin were analyzed to measure variations in cell nuclei morphology and arrangement of the cytoskeleton, respectively, comparing co-cultures of stromal and cancer cells with the single populations.
Results
Hydrogels spanning physiological to pathological stiffnesses induced marked differences in nuclear and cytoskeletal architecture, particularly in stromal cells. PSCs showed stiffness-dependent activation, with α-SMA upregulation in both mono- and co-cultures, more pronounced in AsPC-1 co-cultures. PANC-1 cells acquired EMT markers in stiffer scaffolds, while AsPC-1 displayed inverse N-cadherin trends.
Discussion
This study demonstrates how collagen stiffness modulates stromal and cancer cell behavior in 3D, recapitulating key aspects of PDAC desmoplasia. The observed crosstalk and mechanical feedback highlight the value of engineered ECM scaffolds as predictive platforms for tumor-stroma interactions. Our biofabricated model offers a tunable, physiologically relevant framework to dissect mechano-biological cues in cancer progression.
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Konuşmacılar: Federica Carnevali (University of Salento, Department of Mathematics and Physics “Ennio De Giorgi”)

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Zwitterion-coated PCL scaffold for antifouling implant design

Polycaprolactone (PCL) is a synthetic, biodegradable aliphatic polyester widely used in tissue engineering and has been FDA-approved for various medical devices. However, its long-term performance is compromised by undesirable issues such as surface biofilm formation and a foreign body response (FBR)[1]. These adverse biological processes not only reduce scaffold functionality but also trigger chronic inflammatory responses[2]. To address this challenge, antifouling coatings present a promising strategy to mitigate biological fouling, potentially prolonging the durability of the scaffold and promoting tissue regeneration[3]. This study explores the impact of zwitterionic modification on PCL scaffolds to create a surface water layer that resists biomolecule attachment, resulting in the effectiveness of antifouling coatings in preventing these detrimental effects.
In this study, poly(ethylene glycol) diacrylate (PEGDA) was first coated onto PCL using the solvent casting. Briefly, PCL was soaked in a 10% PEGDA solution in a 1:1 water/acetone mixture for 10 minutes and then dried in a vacuum oven overnight to remove the solvent[4]. Next, sulfobetaine methacrylate (SBMA) was functionalized onto

the PEGDA-modified scaffold using vinyl crosslinking chemistry, combined with lithium phenyl-2,4,6-trimethylbenzoylphosphinate and 405 nm wavelength visible light. The SBMA functionalization will be verified using energy-dispersive X-ray analysis (EDX) or X-ray photoelectron spectroscopy (XPS). The expected binding energy peaks for sulfur and nitrogen are at 167 eV and 400 eV, respectively, which should not be detectable in neither neat PCL nor PEGDA-modified PCL scaffolds in XPS. Preliminary antifouling tests are planned, utilizing BSA, fibronectin, and E. coli adhesion assays.
In future work, a more detailed evaluation of the antifouling effects of these scaffolds will be conducted through in vitro studies of their antifouling capability in a physiological environment. Additionally, the water layer or substrate absorbance dynamics of the SBMA-PCL surface could be investigated using NMR and sum frequency generation spectroscopy (SFG).

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- S04 From single-cell to organoids:building advanced in vitro tissue models Rudniew

Toplantıyı düzenleyenler: Daniel Nieto Garcia (Advanced Biofabrication Laboratory - DNIETO LAB, University of La Coruña, Spain), Joanna Idaszek (Warsaw University of Technology, Poland)

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Photonics-Driven Biofabrication: From Complex Multimaterial Tissue Architectures to Cellular-Scale Manipulation

Photonics-based bioprinting technologies are redefining the frontiers of tissue engineering by enabling the fabrication of complex, multiscale biological constructs with unprecedented precision and speed. This talk will present some recent advancements in light-based bioprinting platforms—including a new technology named Holographic Optical Tweezers Bioprinting (HOTB), Multimaterial Volumetric Bioprinting, AI-driven Digital Light Processing (DLP), and portable hand-held systems—that collectively span a wide range of spatial resolutions and functionalities.

Holographic Optical Tweezers Bioprinting allows for non-contact, three-dimensional manipulation and positioning and printing of individual live cells using holographically shaped laser fields, enabling the assembly of vascular networks and cellular microarchitectures with single-cell precision. Volumetric Bioprinting employs tomographic light projection ans a multimaterial approach to fabricate entire 3D constructs within seconds, offering high-speed production of centimeter-scale tissues. AI-driven DLP-based systems further enhance spatial resolution, material complexity and dinamic correction enabling multimaterial printing with integrated, cell-laden bioinks. Together, these photonics-driven approaches are accelerating the development of architecturally sophisticated, functional tissue models across multiple biological length scales.

Complementing these technologies, a hand-held light-based bioprinter device for in situ skin and soft tissue regeneration will be presented. By enabling direct deposition and photopolymerization of bioinks onto wound sites, these portable systems offer real-time adaptability for point-of-care applications in regenerative medicine.

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From Fields to Form: Wave-Driven 3D Biofabrication of Living Structures

Micro- and nano-scale technologies can have a significant impact on medicine and biology in the areas of cell manipulation, diagnostics, and monitoring. At the convergence of these new technologies and biology, we research for enabling solutions to real-world problems at the clinic. Emerging nanoscale and microfluidic technologies integrated with biofabrication methods in biology offer innovative possibilities for creating intelligent, mobile medical devices that could transform diagnostics and monitoring, soft micro-robotics with broad applications in cancer, tissue engineering, and regenerative medicine fields. We will present interesting applications of microrobotics via 3-D acoustic bioassembly and other fabrication technologies in fluidics such as in engineering 3-D cancer model constructs, cancer tumor imaging, and soft microswimmers.

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Precision biofabrication of prevascular architectures via laser induced forward transfer

Introduction
One of the persistent challenges in the development of reliable in vitro tissue models is the inability to precisely and reproducibly control microarchitectures. This is especially critical for vascularized constructs, where network formation often relies on the stochastic self-assembly of randomly distributed cells, which leads to batch-to-batch variability, limited predictability, and need for high-powered studies to draw statistically meaningful conclusions[1]. To address this, we present a deterministic bioprinting strategy based on laser induced forward transfer (LIFT), enabling single-cell resolution and control over spatial cell patterning[2].

Material and Methods
We leveraged LIFT's versatility to systematically map the printability of a cell medium-like aqueous solution (BSA in PBS) and a polymer solutions (alginate) across a wide viscosity range (0–1000 mPa·s). We analysed printed outcomes (e.g., droplet diameter, circularity, pattern fidelity, and satellite formation) as well as captured jetting dynamics with high-speed imaging. Through a parametric study, we identified optimal transfer conditions by tuning laser energy, donor–receiver distance, ink volume, and composition.
As a model system, we targeted the reproducible biofabrication of vascular-like networks using GFP-labelled human umbilical vein endothelial cells (HUVECs). By tuning droplet spacing and cell density, we printed a series of branching vascular patterns with controlled droplet composition (e.g. cell count).
To further explore the functionalization of pre-vascular designs, we engineered bioprinted micro-architectures composed by co-cultures of HUVECs and mesenchymal stem cells (MSCs) using spatially resolved deposition and distinct ink-substrate combinations (cells printed on fibrin or embedded in fibrinogen and printed onto Matrigel/thrombin with a 3:1 ratio HUVEC/MSC).

Results
We established optimal LIFT biofabrication conditions, enabling the reproducible printing of branching vascular-like networks with precise control over droplet composition and pattern geometry. More precisely, we achieved the transfer of aqueous and polymer droplets with hundreds of picolitre-scale precision. Further, Poisson distribution predictions confirmed controlled cell-per-droplet deposition.
In branching constructs, we observed that patterns with identical total cell number but higher initial spatial density exhibited superior vascular organization and connectivity compared to those with more widely spaced droplets, underscoring the importance of positional control.
Additionally, we demonstrated robust post-printing viability of HUVECs and MSC. The spatial orchestration of HUVEC-MSC co-cultures enhanced construct stability over time and promoted early pre-vascularization, demonstrating that LIFT can serve as a modular platform to engineer microenvironments with tailored cell–cell and cell–matrix interactions.

Discussion and conclusion
Our results establish LIFT as a versatile technology that enables deterministic patterning at single-cell resolution. By addressing the limitations of random cell placement, our approach paves the way for more robust in vitro models for drug screening, disease modelling, and tissue engineering. Future work will focus on enhancing the functionality of the pre-vascularized constructs, such as perfusability to support nutrient supply in large tissue constructs.

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Bioprinting of highly heterogeneous single cell resolution tumor avatars

Understanding how spatial arrangements of diverse cell types within the tumor microenvironment (TME) influence cancer progression remains one of the foremost challenges in oncology. To address this, we have developed a suite of single-cell bioprinting technologies that enable engineering of tumor models with unprecedented spatial resolution in both two and three dimensions. Leveraging a microfluidic dispensing system and two-photon hydrogel printing, we first established a method for bioprinting annotated 2D

maps of breast tumors at single-cell fidelity. By replicating the histology of ductal carcinoma in situ (DCIS) with precise XY deposition of epithelial, stromal, and immune cells —including MCF10A, MDA-MB-231, primary fibroblasts, macrophages, and mesenchymal stem cells—we recreated native tissue architecture with an average print deviation of only 2.4 µm (Figure). These 2D reconstructions allowed real-time tracking of cell dynamics, single cell spatial transcriptomic validation of tumor response, revealing how shifts in stromal phenotype and spatial organization drive early malignant behavior. Building on this framework, we extended our platform into 3D, generating high resolution 3D bioprinted tumor avatars that recapitulate the cellular complexity and organization of human tumors across all spatial axes. These avatars contain up to seven cell types, recapitualting the exact arrangement of tumor neighborhoods of native patient biopsies, and retain high viability, proliferation, and functional heterogeneity. By using patient-derived tumor biopsy maps, we enabled tissue maturation, cell-cell junction formation, and matrix remodeling with or without extracellular matrix supplements (Figure). Perturbation experiments with TGF-β1 revealed fibroblast-driven matrix reorganization and epithelial elongation, validating the avatars' responsiveness and utility for mechanistic studies. We validated the spatially-defined respose of these tumors in comparison to organoids, which inherently lack controlled spatial arragnement, and demonstrate the importance of biopritning spatially defined tumor microenvironemnts to elucidate the complex biology of cancer. Together, these bioprinted models offer a transformative approach to studying the spatial determinants of tumor behavior with unmatched control and resolution. By bridging annotated patient data with controllable experimental systems, our 2D and 3D single-cell bioprinting platforms set the stage for high-content functional interrogation of the TME, and may pave the way for precision oncology, spatial therapeutics, and predictive digital twins in cancer research.

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Biofabrication of Primary Conjunctival Spheroids as 3D In-Vitro Test System

Introduction: To date, no conjunctival spheroids have been reported, despite the conjunctiva's vital role in maintaining ocular surface homeostasis and contributing to various ocular surface diseases. The conjunctiva is a dynamic, multi-cellular mucosal tissue that functions in barrier homeostasis, and tear film stabilization. However, physiologically relevant 3D in vitro models that replicate native structure and function remain lacking. Such models are crucial for advancing mechanistic studies and drug development, while also aligning with the 3Rs principles (Replacement, Reduction, and Refinement). Here, we present a novel approach to generate functional 3D conjunctival spheroids using honeycomb-inspired agarose microwells, enabling scalable and reproducible production for ocular research and therapeutic screening.
Methods: To fabricate honeycomb-inspired microwells, a 2% agarose solution was cast onto 3D-printed micropillars and subsequently sterilized using UV treatment for 4 hr. In this study, we employ primary human conjunctival fibroblast and epithelial cells. Initially, the primary conjunctival fibroblasts were seeded into the agarose molds at densities ranging from 1,000 to 5,000 cells per microwell to generate spheroids of different sizes, followed by centrifugation to promote aggregation. After spheroid formation, conjunctival epithelial cells were seeded on top to facilitate epithelium formation. A series of analyses were conducted, including spheroid size measurement, cell count, viability testing (live/dead staining), and histological staining to evaluate spheroid morphology and epithelial layer formation.
Results and discussion: Honeycomb-inspired agarose microwells facilitated the scalable and robust formation of spheroids. The model demonstrated the ability to mass-produce spheroids of uniform size, which could be easily tuned by adjusting the cell number. The size of the spheroid was tunable with the varying cell number. Live/dead staining indicated excellent cell viability within the spheroids, particularly those formed by 3000 cells. Histological staining confirmed the stromal-like structure and multilayered conjunctival epithelium. Additionally, immunostaining with specific conjunctival markers such as CK13, MUC5AC, and CK19 validated the epithelial identity and functional relevance of the spheroids.
Conclusion: This 3D conjunctival spheroid model enables scalable, reproducible, and cost-effective production of physiologically relevant tissue structures. Using primary human cells, it offers potential for personalized medicine and serves as a valuable in vitro platform for ocular surface research and drug screening.

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Biofabrication approaches for kidney in vitro models

The field of biofabrication has made significant strides in the development of kidney models, offering promising solutions for addressing the growing demand for organ transplants and advancing our understanding of renal physiology and pathology. Biofabrication techniques, including 3D printing, enable the creation of models through sacrificial approaches. 3D printing supports the manufacture of chips with circular cross-sectional regions emulating nephron tubules. Another promising technology for creating in vitro models is bioprinting, which allows for the selective dispensing of cells and biomaterial inks to achieve specific sections of the kidney's functional unit, the nephron. The combination of these approaches with organ-on-a-chip technologies is gradually enabling the creation of complex, functional kidney tissues that mimic the native organ's architecture and function. These models provide valuable platforms for drug testing, disease modeling, and regenerative medicine applications. Recent advancements in induced pluripotent stem cell technology and biomaterials have further enhanced the precision and viability of biofabricated kidney tissues. Despite these advancements, challenges such as vascularization, long-term functionality, and integration with host tissues remain. Continued interdisciplinary research and technological innovation are essential to overcome these hurdles and bring biofabricated kidney models closer to supporting the discovery of new therapies.

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Optimization of osteon-mimic 3D structure and triple co-culture method for bone organoid development

Bone is a dynamic tissue composed of osteoblasts, osteoclasts, and osteocytes, which continuously undergo bone formation and resorption through a process known as bone remodeling. This balance between formation and resorption is tightly regulated by intricate cell–cell interactions. Bone organoid research aims to replicate the characteristics of native bone tissue in vitro; however, the development of bone organoids remains in its early stages due to the bone's complex hierarchical architecture and the multifaceted interactions among its cellular components. In this study, an osteon-mimic three-dimensional (3D) structure was developed to emulate the structural and chemical features of natural osteons. To recreate the cellular microenvironment, a triple co-culture system comprising osteoblasts, osteoclasts, and osteocytes was established, enabling direct cell-to-cell communication. The osteon-mimic 3D structure was fabricated by coating a calcium/phosphate (Ca/P) composite onto a polycaprolactone (PCL) nanofiber membrane, effectively reproducing the lamellar organization of osteons. Morphological and chemical analyses confirmed the resemblance between the fabricated structure and native osteons. Furthermore, by optimizing the triple co-culture conditions, the differentiation and interactions among osteoblasts, osteoclasts, and osteocytes were successfully promoted. As a result, bone organoids replicating the morphological, chemical, and biological characteristics of natural osteons were successfully developed using the osteon-mimic 3D structure combined with triple co-culture.

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- S25 In situ bioprinting Mikołajska

Toplantıyı düzenleyenler: Giovanni Gonnella (Trinity college Dublin), Giovanni Vozzi (UNIPI, Pisa,Italy)

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Sustainable biofabrication: from biomaterials to advanced bioprinting processes

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Konuşmacılar: Giovanni Vozzi (University of Pisa)

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Robotic-based in situ bioprinting: the next frontier in biofabrication

Bioprinting offers several advantages over traditional tissue engineering methods for creating scaffolds used in organ and tissue regeneration, primarily due to its precise and controlled processing of biomaterials. However, this technique—referred to as in vitro bioprinting—faces significant challenges when applied clinically. These include difficulties in scaffold handling, contamination risks, the need for a maturation period in a bioreactor, and discrepancies between the construct's shape and the defect site. To address these limitations, in situ bioprinting has emerged as a promising alternative. This method involves the direct deposition of biological materials into the patient's body, conforming to the complex geometry of the anatomical defect. By leveraging the body as a natural bioreactor, it promotes better maturation and differentiation of the bioprinted constructs. Two main approaches have been developed: a hand-held method using a portable bioprinting device for direct material application, and a robotic method that employs a robotic manipulator with three or more Degrees of Freedom (DoF). The robotic approach minimizes human involvement and offers greater precision, making it suitable for regenerating complex defects.

The technology based on the use of a robotic system has been extensively analysed at the University of Pisa, developing two different robotic platforms, one based on an open-source project (IMAGObot) and one using a commercial robot (Mecademic MECA500). In both cases, the platforms have been re-engineered for bioprinting applications with different technologies (extrusion, inkjet, valvejet), also including different tools for surface mapping (touch probe, laser profilometer). A standardized procedure for in situ bioprinting can be outlined in four key steps: (i) acquiring a digital model of the damaged area, (ii) planning the printing path, (iii) registering the patient within the workspace, and (iv) depositing the biomaterial. The digital model obtained in the first step serves as the basis for generating printing trajectories using a custom-developed algorithm. This algorithm is capable of integrating both planar and non-planar layers, which improves the structural quality and adhesion of the bioprinted construct, while also allowing for photo-curing of the deposited material. Both platform were validated in different simulated clinical scenarios showing promising results and highlighting the potential that a robotic platform can have for in situ bioprinting.

This approach opens the way to a number of possibilities in the field of tissue engineering, especially for the easiest accessible organs such as skin, bone and cartilage. On the other hand, considering the regeneration of internal tissues and organs (e.g. intestine, stomach), the new frontier concerns the development of miniaturised robotic printing systems. These systems can be installed on endoscopes to enable non-invasive regeneration of tissues inside the human body, offering a solution to current surgical treatments.

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Methodologies for arthroscopic in situ extrusion bioprinting for the regeneration of osteochondral defects

Introduction:
Osteochondral (OC) lesions to the knee represent a major burden from a societal and economic point of view, affecting young and active patients and predisposing them to develop post-traumatic osteoarthritis. Current treatments include marrow stimulation, autografts, and cell-based therapies; however, they all suffer from several limitations, including availability, cost and complexity of the procedure [1], highlighting the need for new and effective procedures. In this context, we present the design and fabrication of an extrusion toolhead for in situ bioprinting in the knee, as a regenerative, one-stage solution to treat OC defects.

Experimental section:
A literature survey of the current landscape of commercial arthroscopic devices was conducted to screen the tools currently available on the market. Based on ergonomics considerations, two preliminary designs of the extrusion toolhead were manufactured with Fused Deposition Modelling (FDM). Briefly, the two configurations have different handles depending on the grip type, including precision (pencil-like) and power grips. The extrusion is performed using a leadscrew mechanism and is actuated by a stepper motor with reduced weight to increase the comfort when holding the device. Each configuration includes a CNC milled aluminium block to maintain the ink inside the syringe at 37°C, facilitating the extrusion of gelatin-based inks and enabling their processing. Finally, the extrusion process is controlled using a dedicated board (Ramps v1.4) with the ad hoc configured Marlin firmware.

To test the toolheads in a simulated environment, a human knee phantom was designed and fabricated starting from CT scans of a femur and a tibia. Both bones were manufactured using FDM printing, while the cartilage parts (i.e., femoral and tibia cartilage, menisci) were prepared using ad hoc designed molds. The softer femoral and tibia articular cartilage [2], were prepared with Ecoflex 00-10 silicone (1:1), while the more rigid menisci [3] were obtained using Sylgard-184 silicone (1:10). OC defects of varying size were prepared by coring with a punch directly on the femoral cartilage. Finally, a usability test is currently being conducted with three surgeons at the University of Pisa, experts in arthroscopic procedures. They were tasked with using the two configurations to fill the OC defect prepared on the phantom and asked to provide their feedback through an ad hoc questionnaire.

Conclusions:
Herein, we presented the design and fabrication of custom toolheads for in situ extrusion bioprinting inside the knee, with the aim of regenerating OC defects in a minimally invasive, arthroscopic set-up. The design process was conducted with attention to usability and ergonomics, to facilitate the uptake of the technology in possible future clinical applications. Future developments will include the addition of other bioprinting technologies, like jetting and Filamented Light, to create complex multimaterial and multiscale constructs directly inside the knee.

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Framework for In Situ Bioprinting with Active Patient Tracking, Multi-DoF Deposition and Visible-Light Crosslinking

Introduction
Chronic wounds are skin lesions that fail to heal naturally or through basic care. Pressure ulcers, diabetic foot ulcers and some oncologic and burn wounds present an open gateway for infections to attack the body [1]. Contributing to this scenario, often the patients have only one shot at reparative surgery, due to previous complications and/or the requirement of extended anesthesia. Commercial or surgical skin grafts have mixed results regarding compatibility and post-surgical outcomes [2]. To tackle these issues, tissue engineering was born to manufacture constructs using human cells (e.g. autologous) and/or growth factors, aiming at producing full-sized, functional organs. One of the initiatives inside tissue engineering is 3D Bioprinting, where a bioink with cells/growth factors is used to produce scaffolds (often created in vitro) to fabricate biocompatible anatomies. Among its techniques is in situ bioprinting, where the bioink is directly deposited on the patient's body, using the wound environment as the bioreactor to multiply the cells and fill the wound. Some recent advancements in this field have printed implants in vivo on pigs and mice [3][4]. Others have integrated partial patient tracking to perform intraoperative printing without complete anesthesia [5], or experimented with crosslinking using techniques that don't involve UV light [6]. Still, no published work to date has integrated all these aspects into the same system. To fill this gap, the authors propose a new framework for an in situ bioprinter that integrates active patient tracking and always-perpendicular material deposition on a non-UV crosslinking system, aiming for maximum printability, mechanical properties and cell survival rates for the biofabricated implant.
Methods
The in situ bioprinter will comprise a 6 degrees-of-freedom (DoF) robotic arm and a custom bioink extruder assembled in-house. It will feature: 1) A non-planar slicing algorithm based on previous work from our group [7], capable of generating toolpaths that keep the extruder always perpendicular to the printer surface, while also accounting for tool head collision and compensating for impossible poses for the robot or the end-effector; 2) A tracking system composed of stationary cameras looking at fiducial markers that will recognize all possible 6 DoF of an awake patient, up to natural ranges, velocities and acceleration of motion; 3) a bioink containing liposomes as placeholders for cells, that is formulated to crosslink under visible light.
Results
Expected results are an enhanced implant adhesion and body adaptability due to the multi-DoF material deposition, a live compensation of any possible and feasible patient movement, also reducing the necessity for heavy anesthesia, and the reduction of harm factors due to the use of more natural, visible light during the biofabrication process. Future experimental validation will assess print fidelity, tracking range and cell viability.
Conclusion
The field of in situ bioprinting will be advanced by this work regarding the integration of many factors currently being developed separately, bringing this technology closer to its final, hospital-ready form.
Acknowledgements
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Development of thermosensitive HGC/SdECM bioink for skin tissue regeneration using in situ bioprinting

Introduction

Skin regeneration, especially in large wounds, remains a clinical challenge due to the limited healing capacity of human skin.1 In situ bioprinting enables targeted tissue reconstruction by directly depositing bioinks onto injury sites, supporting the development of personalized regenerative therapies.2 For effective skin regeneration through in situ bioprinting, bioinks must rapidly form stable structures while preserving cell viability.3 However, conventional bioinks for in-situ bioprintng often require post-crosslinking via UV light or chemical agents, inducing cytotoxicity.4

In this study, we developed a thermosensitive bioink by combining porcine skin-derived decellularized extracellular matrix (SdECM) and hexanoyl glycol chitosan (HGC), where the SdECM is expected to provide bioactive signals to enhance cell affinity. The bioink enabled cell encapsulation at low temperatures and rapidly gelation at physiological temperature without additional crosslinker.

Method

HGC was synthesized by N-hexanoylation of glycol chitosan.5 The synthesized HGC was blended with varying amounts of SdECM powder in saline, and the mixtures were stirred overnight. The sol-gel transition temperature, viscosity, modulus of bioink were measured using a rotational rheometer. The printability of the bioink was evaluated through line and circle pattern printing. The biocompatibility of the bioink was assessed by Live/Dead staining and CCK-8 assay. In vivo experiment was conducted by directly printing the bioink on skin wounds constructed on the backs of nude mice.

Result

The sol–gel transition temperature of HGC and HGC/SdECM bioinks decreased from 35 °C to 30 °C with increasing SdECM content, suggesting that enhanced physical crosslinking contributed to improved mechanical properties. The bioinks exhibited shear-thinning and thixotropic behaviors, confirming its suitability for 3D bioprinting applications. Printing tests identified the printable range of each bioink under ambient conditions, with the HGC/SdECM1.5 group exhibiting the broadest range. Live/Dead staining indicated high cell viability across all groups. CCK-8 assay result revealed that higher SdECM content in the bioink was associated with significantly enhanced cell proliferation. In vivo experiments demonstrated that the bioink could be directly applied via 3D printing to wound sites with site-specific deposition, and confirmed its effectiveness in promoting skin tissue regeneration.

Discussion

The thermosensitive HGC/SdECM bioink exhibits biocompatibility and printability, along with structural stability without additional crosslinking agents. The results confirm the bioink's potential for skin regeneration through in situ bioprinting and its applicability to other tissues, providing a foundation for advanced therapeutic solutions.

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Real-time Vertical Motion Compensation in In-situ Laser-Assisted Drop-on-Demand Bioprinting

The field of bioprinting has experienced significant progress in recent years, particularly with the development of methods that enable in-situ bioprinting. Reliable in-situ bioink deposition requires accounting for physiological movements, as even under anesthesia, the body can still move due to breathing or involuntary movements. These motions can interfere with the quality of the printed structure [1]. Advances in sensor technologies and robotic have led to the development of motion compensation techniques that address this challenge. Here, we demonstrate that real-time motion compensation can maintain print quality in Laser-Induced Side Transfer (LIST), a drop-on-demand bioprinting technology [2, 3].

LIST was initially developed using a fixed printing head [2, 3]. Here, we modified LIST for portability by replacing the open-space beam delivery system with an optical fiber (FG105LCA-Multimode Fiber). A water-glycerol mixture was used as model ink. An Optical Coherence Tomography (OCT) fiber-based sensor and the fiber-based LIST printhead were mounted on a Dorna 2 robotic arm for real-time compensation (Fig.1(a)). The OCT sensor measures the printhead-to-substrate distance in real-time, providing feedback to the robot to maintain a constant gap. Microscope slides mounted on a translation stage (Z825B, Thorlabs) simulated breathing motion.

Fig. 1. a) Integration of the robotic arm and OCT sensor, b) The different scenario with their corespond printed pattern.

A square pattern of the model ink was printed at 5 Hz on a microscope slide with each droplet spaced 1.5 mm apart. Fig. 1 (b) shows the printed patterns in three conditions: (1)constant 3 mm printhead-to-substrate distance, (2) substrate movement from 3 to 24 mm, and (3) dynamic compensation in which the substrate moves from 3 to 24 mm while the printhead actively maintains a 3 mm distance. A quantitative analysis of printing quality criteria including position accuracy (mm), circularity, printed droplet area (mm²), and splatter coverage was performed to assess compensation performance. The measured criteria with and without compensation are as follows: position accuracy (0.25 ± 0.09 mm vs 0.38 ± 0.23 mm; p = 0.09), circularity (0.82 ± 0.14 vs 0.69 ± 0.18; p= 0.0029), splatter coverage (2.32 ± 1.83% vs 37.36±13.01 %; p = 0.00038), and printed droplet area (1.27 ± 0.47 mm² vs 1.08 ± 0.31 mm² p= 0.017).

Our statistical analysis showed a significant difference in most metrics for compensated vs non-compensated printing. Metrics from compensated printing closely match those from fixed substrate printing, confirming that real-time compensation preserves print quality under dynamic conditions. This highlights the critical role of compensation in in-situ DoD bioprinting. Our ongoing work focuses on refining the robotic control for fast-moving targets that demand both lateral and vertical compensation.

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FaSt-Light: Fiber-assisted Structured Light Projection for Rapid In Situ Photo-biofabrication

Introduction

Light-based biofabrication techniques have revolutionized the field of tissue engineering and regenerative medicine.[1] Specifically, the projection of structured light, where the spatial distribution of light is controlled at both macro- and micro-scale, has enabled precise fabrication of complex three-dimensional structures with high resolution and speed.[2] However, despite ttremendous progress, biofabrication processes have been mostly limited to benchtop devices which limit the flexibility in terms of where the fabrication can occur.[2] Here, we demonstrate a Fiber-assisted Structured Light (FaSt-Light) projection apparatus (Figure 1A), deploying image guide fiber bundles coupled to multiwavelength projection setup for the rapid in situ crosslinking of photoresins. Through in vitro and ex vivo experiments, we demonstrate potential uses cases of the FaSt-Light approach for in situ biofabrication.

Methods

We developed a bespoke multispectral light engine to generate speckled images at 405, 450, 520 nm (Figure 1B), to which the image guide fiber bundles were coupled. For materials, we used gelatin methacryloyl (GelMA) resins with different photoinitiation systems (Norrish Type I and II) suited for each wavelength, which were investigated with the FaSt-Light system on resulting macro- and micro-scale features and potential biofabrication applications.

Results

FaSt Light enabled control over two aspects of the crosslinked resins: 1. Macroscale structures (> 50 µm) imparted through the light engine which controlled the projection image (Figure 1C), and 2. Microscale control, due to optical modulation instability when the laser speckles interact with the photoresin, allowing introduction of cell-guiding microfilaments (2-8 µm, Figure 1C). Through in vitro experiments with myoblasts, we demonstrated that the microfilamented constructs fabricated in situ guided cellular infiltration (Figure 1D), differentiation and anisotropic matrix production resulting in contractile myotubes. Furthermore, the FaSt-Light approach could be used for in vivo printing (Figure 1E) in selected applications for skin wound and muscle defects. Finally, we demonstrated a new scheme which allowed simultaneous multiwavelength image projection, enabling in situ multi-material biofabrication using a resin blend comprising of Collagen I and Polyethylene Glycol Diacrylate (PEGDA) (Figure 1F).

Discussion

Image guide fiber bundles have traditionally been used for capturing images from hard-to-reach regions such as during endoscopy, and to guide images to camera sensors. [3,4] We demonstrated a reverse approach called FaSt-Light, where the fiber bundles allowed projection of images at multiple wavelengths, enabling flexibility on the location of crosslinking. The proposed FaSt-Light approach could lead to a new range of in situ biofabrication techniques which improve the translational potential of photo-fabricated tissues and grafts.

Acknowledgements

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- S26 Machine Learning and Digital Twin for Predictive Biofabrication — Kruczkowski

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3D printed biomimetic cochleae and machine learning co-modelling provides clinical informatics for cochlear implant patients

Cochlear implants restore hearing in patients with severe to profound deafness by delivering electrical stimuli inside the cochlea. Understanding stimulus current spread, and how it correlates to patient-dependent factors, is hampered by the poor accessibility of the inner ear and by the lack of clinically-relevant in vitro, in vivo or in silico models. Here, we present 3D printing-neural network co-modelling for interpreting electric field imaging profiles of cochlear implant patients. With tuneable electro-anatomy, the 3D printed cochleae can replicate clinical scenarios of electric field imaging profiles at the off-stimuli positions. The co-modelling framework demonstrated autonomous and robust predictions of patient profiles or cochlear geometry, unfolded the electro-anatomical factors causing current spread, assisted on-demand printing for implant testing, and inferred patients' in vivo cochlear tissue resistivity (estimated mean = 6.6 kΩcm). We anticipate our framework will facilitate physical modelling and digital twin innovations for neuromodulation implants.

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AI-driven Biofabrication of advanced in vitro tissue model for immuno-oncology study and psychotropic drug evaluation

Biofabrication is an advanced technology that holds great promise for constructing highly biomimetic in vitro three-dimensional human organs. Such technology would help address the issues of immune rejection and organ donor shortage in organ transplantation, aiding doctors in formulating personalized treatments for clinical patients and replacing animal experiments. Artificial intelligence (AI), with its excellent capabilities in big data processing and analysis, can play a crucial role in handling and processing interdisciplinary data, as well as in better integrating and applying them in biofabrication. We report here our recent progresses in AI-assisted biofabrication of single-cell leveled immuno-oncology model for tumor immunotherapy study, and the neural models for the classification and evaluation of anesthetic and psychotropic drugs.
The immuno-oncology model was constructed through a specialized single-cell printing technique with sequentially delivering dendritic, T and melanoma cells. In particular, we developed a method for analyzing cell movement at the single-cell scale, revealing the consistency between the movement of T cells and their biological functions under different cell types, subtypes, and the effects of drugs. We believe that the established single-cell scaled immuno-oncology model would help to predict cellular immune function for personalized immunotherapy by quantifying contact efficiency with respect to the cell distance and actuation duration.
Referring to the anesthetic and psychotropic drug evaluation, we proposed a high-throughput 3D-printed in vitro neural model. Based on this, opioid drug neurotoxicity dose-response model was established, utilizing multi-dimensional neural toxicity biomarker features. By integrating the fine-grained analysis for quantitative feature contribution screening and combining multivariate analysis method to extract critical multidimensional features, the study achieved accurate classification of opioid drugs. The in vitro neurotoxicity dose-response model and AI-assisted evaluation system proposed in this study are expected to promote scientific advances in the toxicological assessment, risk warning, and judicial decision-making for new psychoactive substances.

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3D-printed mechanically-realistic synthetic kidney models towards digital twin-assisted surgery (DTAS).

Introduction

3D-printed physical organ models have revolutionised the surgical field with applications in training, planning, rehearsal, and patient education[1]. Recent advancements in digital health technologies, such as the introduction of digital twins (DT) in medicine, present further opportunities where these models could play an important role. By developing a mechanically-realistic, anatomically-accurate, and patient-specific physical organ model, a DT framework may be designed to assist surgeons throughout the entire perioperative process[2]. To achieve this, the first step is to develop a 3D-printed model based on human imaging data composed of materials that closely mimic the mechanical behaviour, including elastic and viscoelastic properties. and the tactile properties of human soft tissue. In this work, we developed an interpenetrating polymer network (IPN) hydrogel with tunable mechanical properties produced via light-based 3D-printing for the fabrication of surgical kidney models.

Methods

The resin comprised a synthetic photopolymer (NP; 0.5 M, 1 M), crosslinker (25 mM, 50 mM), photoinitiator (0.2% (w/v)), photoblocker (0.02% (w/v)), and sodium alginate (1% (w/v), 2% (w/v)) in deionised water. Following 3D-printing (LumenX+, CELLINK), the structures were immersed in barium chloride ($BaCl_2$; 66 mM). Mechanical characterisation was carried out via (i) uniaxial compression tests at a rate of 5%/s (Mach-1, BioMomentum, Inc.) and (ii) rheological frequency sweeps from 0.1 Hz to 10 Hz at a fixed shear strain of 0.01% (Kinexus Pro+, NETZCH), to investigate elastic and viscoelastic behaviour, respectively.

Results and Discussion

By varying monomer concentrations, the mechanical properties of the IPN hydrogels could be fine-tuned (Fig.1a-d, f), with some formulations revealing comparable behaviour to both human and porcine kidney tissue reported in the literature [3-5]. Using these hydrogels, scaled-down 3D-printed models of human kidneys were produced as a preliminary step towards fabricating surgical kidney phantoms (Fig.1e).
Ongoing work involves 3D-printing optimisation of the kidney models and surgical tool-tissue interaction deformation studies using an RGB-D camera to feed data to a DT. Overall, this approach shows great promise in producing mechanically-realistic organ phantoms, potentially reducing dependency on cadavers and animals in surgery, while also contributing to the development of cutting-edge digital health technologies, such as the proposed real-time Digital Twin-Assisted Surgery (DTAS)[2].

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Beyond Labels: Transparent Neural Network Decisions for Virtual Immunostaining in Tissue Engineering

Introduction

Immunohistological staining remains the gold standard for visualizing specific cellular and tissue structures, but it involves complex, time-consuming protocols, specialized reagents, and expert personnel. Its application to three-dimensional constructs or organoids is further limited by penetration depth, uniformity, and reproducibility. Virtual staining—digitally generating fluorescence labels in brightfield images via neural networks—has emerged as a promising alternative ([1]-[2]).

Methods

We trained a generative adversarial network (GAN) on paired brightfield and immunofluorescence images (DAPI for nuclei, Phalloidin-488 for F-actin) of osteoblast cultures (2D), optimizing both pixel-wise and perceptual similarity over 150 epochs. To probe the model's decision process, we performed explainability analyses: receptive field mapping to locate critical input regions using guided backpropagation, and feature-map inspection to trace intermediate activations corresponding to nuclei or cytoskeletal structures.

Results

The GAN achieved >80 % structural similarity to ground-truth immunostains within 150 epochs. Targeted data augmentation informed by these insights further improved staining fidelity. Receptive field analysis confirmed network attention concentrated on nucleii and actin regions. Guided backpropagation identified edges and contrast gradients as key predictive features, while feature-map clustering revealed distinct layer sensitivities to nucleii versus cytoskeletal morphologies.

Discussion

Overall, our approach demonstrates the capability of GAN-based virtual staining to reduce reliance on resource-intensive immunohistological protocols and provides valuable methodologies for enhancing the transparency and reliability of neural network-based image interpretation. Compared to recent digital H & E approaches—which transform standard histology into multiplexed channels while similarly leveraging deep learning ([3])—our method directly predicts molecular stains, broadening application towards biofabricated tissues. Ongoing work will validate performance across diverse cell types, scaffold geometries, and explore explainability to guide clinical translation.

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From Pipettes to Prompts: How AI is Reshaping Daily Research in Tissue Engineering and Biomaterials

Artificial intelligence (AI) is rapidly evolving from an experimental curiosity to a robust companion throughout the scientific process. In the context of tissue engineering and biomaterials, AI is not limited to computational modeling or image processing—it now plays a supporting role at nearly every stage of the research lifecycle, from idea generation and literature review to experiment planning, data interpretation, and even preparation for clinical translation.
AI-powered tools offer practical value from day one. During hypothesis development and study design, language models and generative systems can help identify knowledge gaps, summarize complex trends across large corpora, and suggest experimental configurations. They assist in formulating clear, testable research questions, grounded in up-to-date literature—even suggesting relevant citations with contextual summaries. In laboratory work, AI contributes to visualizing scaffold geometries, optimizing bioink compositions, and analyzing imaging or omics data with a speed and scale unattainable by manual methods.
Once data is collected, AI can support statistical analysis, clustering, anomaly detection, and hypothesis refinement. It helps in identifying subtle correlations, guiding follow-up experiments, or even surfacing contradictory results that may have otherwise been overlooked. In parallel, generative image models allow for rapid conceptual prototyping—useful not only in academic contexts but also when communicating complex ideas to cross-disciplinary collaborators or regulatory bodies.
Crucially, the benefits of AI extend beyond research and into the realm of scientific communication and translational planning. AI can support the drafting of reports, abstracts, and graphical summaries; refine the tone and clarity of technical documents; and tailor materials for specific audiences, including clinicians, patients, and funding agencies. As research moves toward preclinical or clinical stages, AI may help in preparing regulatory documents, planning ethical protocols, or exploring design options for implants and devices through simulation.
However, these capabilities come with limitations. AI systems are not infallible—they reflect patterns in data, not understanding. Misleading outputs can arise from vague prompts, biased datasets, or overreliance on automated suggestions. For example, a visual request for "vascularized scaffolds" might yield artistic, anatomically implausible renderings. Citation generators may fabricate convincing but nonexistent references. Thus, human expertise remains essential for validation, interpretation, and direction-setting.
To reflect on these contrasts, this presentation includes a visual panel titled Scientific Intention vs AI Interpretation, showcasing real prompt examples and their often-surprising outcomes. While some misfires can be humorous, they also offer insight into how we prompt, perceive, and shape the AI's contributions.
Ultimately, AI is not a shortcut—it's an amplifier. When used critically and creatively, it becomes a powerful co-pilot across the full arc of scientific work. In a field as

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Mechanical Computational Modeling and Simulation of Biodegradable Implants

Introduction

Medical devices are being revolutionized with the development of new materials and manufacturing processes. Nowadays, technology enables accurate biofabrication of patient-specific parts, which, while holding high potential for providing the best solutions for patients, presents challenges in designing regulatory-approved devices. This becomes even more challenging once the medical device changes its properties over time of use. For example, biodegradability of devices, which can be encouraged by several mechanisms as enzymatic and hydrolysis, will most likely reduce the mechanical properties; 3D lattices that allow tissue ingrowth change their performance according to the rate, characteristics, and maturity of the regenerated tissue. These mechanisms must be monitored in in-vivo studies examining the state of the implant, ingrowth tissue, and the native tissue's reaction.
Recent advancements in computational software and hardware provide quality in-silico tools, leading the main regulatory affairs (RA) to accelerate their efforts in embracing Computational Modeling and Simulation (CM&S) as an alternative to physical tests and to animal studies in the longer term [1, 2]. The impact of incorporating computational tools on reducing the amount of in-vivo testing is significant, translating to reduced development time and cost, and the number of animals to be sacrificed. The potential of CM&S has been covered by RA guidelines for the past two decades, highlighting that a credible in-silico tool can answer a variety of questions of interest already in the early stages of product development.
CollPlant's pioneering development in the field of regenerative medical devices has been rapidly advancing in recent years, enabling the manufacturing of unique lattice-based scaffolds for various applications. The core material of the bioink used to 3D print such products is based on CollPlant's plant-based recombinant type I human collagen (rhCollagen). The collagen-based bioinks offer a range of mechanical properties depending on the selection of additional components of the formulation and their

concentration (e.g., biodegradable polymers, photoinitiator, photoblocker). CM&S tools were incorporated within the design workflow, optimizing both the geometry of the lattice and the material selection, taking into consideration initial and advanced degradation-regeneration states.

Methods
CM&S workflow was developed based on commercial design and Finite Element (FE) software. 'Digital-Twin' models were created, mimicking the implant's geometry, material's hyperelastic response, boundary conditions, and the expected loadings. An iterative design based on the mechanical in-silico performance was performed. Comparison of Finite Element Analysis (FEA) results with physical tests was conducted as part of the verification and validation process.

Results and Discussion
The gross performance of the implants was calculated using FEA and compared against experimental measurements. Stresses, strains, and displacements were tracked by generating field distribution, highlighting potential regions with localized distortion, buckling, and stress concentration. Insights from the simulation results guided the design of the product, leading to improved mechanical properties while maintaining its adaptability for tissue integration and scaffold degradation mechanisms.

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- S30 New Dimensions in Biofabrication: Emerging tools and perspectives Goethe

Toplantıyı düzenleyenler: Jos Malda (Utrecht University), Gosia Wlodarczyk-Biegun (University of Groningen / Silesian University of Technology)

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AI-empowered Bioprinting: From Design to Quality Control

Bioprinting is facing several scientific and technological challenges toward having a clear clinical impact. Together with the complexity of multimaterial and multiscale features required for the fabrication of functional and effective bioprinted constructs, it is necessary to consider the compelling request of a consistent high quality, including inter-batch variability, and safety (e.g., meeting specific standards) of the final products.
In this perspective, Artificial Intelligence (AI) can have a transformative impact on the bioprinting field. A comprehensive view of current advancements and prospects in incorporating AI into bioprinting practices will be provided, from the development of new designs and the optimization of bioprinting protocols, to the enhanced quality control on the fabrication process and on the bioprinted construct.
For example, AI can automatically extract valuable insights from vast literature on bioprinting and Tissue Engineering, fostering innovation in materials and fabrication techniques. Such advancements could mitigate the lengthy trial-and-error approaches and suggest novel material-technology combinations based on scientific literature insights. On the other hand, several AI-based strategies have been described for quality control throughout the bioprinting process, including pre-process quality checks (such as optimizing printing parameters), in-process monitoring (detecting real-time defects), and post-process evaluations (assessing shape fidelity and functionality). Successful applications of ML techniques for each phase of the bioprinting process will be illustrated with a specific focus: the real-time monitoring of extrusion-based bioprinting utilizing a Deep Learning (DL) model to identify printing errors from video streams; and the automatic optimization of printing parameters by leveraging DL evaluations to enhance print quality dynamically. Indeed, AI can make bioprinting more accurate and reliable, facilitating compliance with regulations. In this forward-looking perspective talk, addressing technological obstacles and navigating regulatory frameworks, it is fundamental to consider that AI is in a continuous and accelerated evolution. As such, AI-enhanced solutions for bioprinting come with great potential but also important open questions related to intellectual property, safety, and liability, which need to be taken into account in a holistic view.
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Quantum sensing for assessing the interaction between materials and cells

Quantum sensing for assessing the interaction between materials and cells

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New biomaterials are developed for many applications including drug delivery, tissue engineering implants. All these materials interact with the human body and with cells and it is crucial to understand if they induce a stress response or if the delivered drug actually works. Diamond based quantum sensing is a new tool which allows measuring such stress responses with subcellular resolution. As a result, we can for instance detect where within a cell a drug delivery particle goes and how the cells react to it locally[1]. We have demonstrated this for instance with the anti-cancer drug, diazoxide, that we delivered into cancer cells and monitored the free radical generation in response to the drug. Another example, I will show is how cells react in response to a material with sub-lethal toxicity [2,3]. In both cases, we were able to detect the free radical generation at a single cell level and pinpoint the location of the stress response.

Figure 1: Strategy for detecting free radicals in response to the drug Diazoxide. The drug is delivered with a nanodiamond particle which can also sense the free radical generation in its surrounding

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Fabrication of self-rolling scaffolds for vascular tissue engineering using dual-head melt electrowriting

Self-assembling scaffolds enable enhanced adaptation to the human body environment thanks to a dynamic response to external stimuli. To produce such scaffolds, advanced biofabrication methods are required. Melt electrowriting (MEW) is a high-precision additive manufacturing technique, which enables the creation of fine fibers of molten polymer under the influence of an electrical field. Dual-head MEW approach allows deposition of diverse materials in one printing process, which in turn can increase the complexity of fabricated structures and broaden the applications. In this research, we employ this approach to produce multimaterial self-assembling scaffolds for application in vascular tissue engineering.

Square and triangular structures, with a fiber-to-fiber distance of 300 µm, were designed using FullControl GCode Designer software. The scaffolds consisted of two parts with different swelling properties, where the fiber orientation and layer thickness varied. Polycaprolactone (PCL) or poly(ethylene oxide terephthalate)/poly(butylene terephthalate) with molecular weight of the poly(ethylene oxide) equal to 300 (PEOT-PBT 300) were used as more hydrophobic layer, while PEOT-PBT 1000 was used as more hydrophilic layer. The scaffolds were printed using a dual-head MEW tool (BioScaffolder 3.3, GeSiM). The optimization of fabrication parameters, including material temperature, printing speed, pressure, and voltage, was performed. The printed scaffolds were processed with oven treatment to enhance the connections between fibers. The time and temperature of the process were optimized. The microstructure of scaffolds was characterized by optical and scanning electron microscopy (SEM). The self-assembling properties were investigated by observing the rolling behavior after placing the scaffolds in phosphate-buffered saline (PBS). The tensile strength of dry and wet scaffolds was measured. To assess cell adhesion and distribution inside the tubular scaffolds, preliminary cell study with tenocytes was performed.

The difference in swelling properties between the printed materials enabled self-assembly of the construct. The self-rolling behavior of the obtained scaffold depended on the material combination, the orientation of hydrophobic fibers, as well as on the way of placing scaffolds in PBS. Oven treatment post-processing was crucial to obtain self-rolled scaffolds based on combination of PEOT-PBT 300 and PEOT-PBT 1000. The scaffolds formed tubes with lumen inside. The diameter of the tube, as well as the lumen size, were easily controlled via adjusting number of layers and scaffold dimensions. In the following studies different shapes of tubes were obtained, including gradient (diagonal, spiral) and branched structures. Preliminary test with seeded tenocytes proved scaffolds biocompatibility, however cell studies with endothelial cells will be further investigated.

The conducted research shows successful integration of two different materials into one MEW process. The self-assembling scaffolds is one of the numerous examples how this approach can enhance biofabrication possibilities. The fabricated self-rolling scaffolds are promising solution for vascular tissue engineering applications.

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Spatiotemporal tracking of nascent ECM formation in modular spheroid building blocks to improve 3D bioassembly

Introduction

Three-dimensional (3D) microtissue spheroids replicate native-like microenvironments and thus provide a model platform to study cell-matrix dynamics. Furthermore, modular bioassembly of these cell-dense units allows for precise control over 3D tissue architecture, while maintaining physiological cellular microenvironments. However, successful biofabrication of large-scale tissues from modular microtissue units requires microscale fusion events involving cell migration and cohesive extracellular matrix (ECM) integration [1]. Therefore, understanding interactions between tissue modules is essential for advancing scalable human tissues. As such, metabolic labelling of nascent ECM formation is a promising approach to explore the spatiotemporal dynamics of ECM development. Here, non-canonical amino acids containing biorthogonal functional groups are incorporated into newly secreted proteins and fluorescently labelled to visualize nascent matrix deposition (Figure 1A). In this study, we applied a click chemistry-based approach to metabolically label nascent proteins in 3D cartilage microtissues to investigate the spatial and temporal development of tissue growth and matrix deposition across microtissues and during tissue fusion events.

Methods

High-density human articular chondrocyte microtissues (0.25x106 cells/microtissue) were formed using high-throughput centrifugation in a V-bottom plate and allowed to mature over 14 days. Next, microtissues were bioassembled into 3D-printed, polycaprolactone (PCL) scaffolds and cultured for 14 days to facilitate tissue fusion [2, 3]. During spheroid formation and fusion, microtissues were treated with a non-canonical amino acid, L-azidohomoalanine (AHA; 100 µM), for 1 day at early (Day 1) or late (Day 14) time points. AHA-incorporated microtissues were fluorescently labelled with a dibenzocyclooctyne (DBCO) functionalized fluorophore to visualize nascent ECM formation occurring only during AHA treatment (Figure 1A) [4]. Spatiotemporal nascent matrix deposition was quantified and analysed qualitatively (safranin-O, collagen I/II staining) across microtissues and in fusion regions.

Results

Staining for nascent proteins revealed widespread protein deposition across microtissues, with a 300 % increase in staining intensity at the periphery, suggesting enhanced protein secretion due to greater cellular activity at the microtissue surface. Comparing the protein deposition over time, we observed minimal ECM deposition during the first 24 hours of microtissue formation, followed by a 6.4-fold increase after 14 days of culture, determined by fluorescence intensity quantification (Figure 1B). Application of metabolic labelling during microtissue fusion within the 3D bioassembly model enabled evaluation of nascent matrix formation at the fusion interface, leading a pathway to probe ECM formation across larger constructs (Figure 1C).

Discussion

We present an innovative metabolic labelling approach to study spatiotemporal matrix evolution in complex, multicellular 3D tissues, revealing mechanisms involved in tissue formation, maturation and fusion. Our findings highlight spatial differences and temporal dynamics of ECM deposition across 3D microtissues. Understanding ECM dynamics during tissue fusion is crucial for engineering large-scale 3D constructs. Our fusion model offers a platform to design, evaluate and optimize next-generation tissue spheroid modules and their interaction for scaled-up biofabrication, supporting the development of functional regenerative therapies.

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Bioreactor development and volumetric bioprinting protocol for perfused culture of biofabricated human mammary epithelial ducts and endothelial constructs

Introduction: Tissue function depends on the intricate 3D organization of cells, matrix components, bioactive cues, and dynamic factors like nutrient and oxygen gradients, and mechanical forces. Advances in bioprinting have enhanced cellular organization, yet full tissue maturation, critical for biological function, often requires post fabrication measures. Here, we introduce a novel method to converge volumetrically bioprinted chip constructs with increasing channel complexity and a custom bioreactor to enable microfluidic perfusion. Establishing imaging-based analyses, we provide benchmarks to assess the developed platform and apply this workflow to the creation of a human mammary gland model. Here, the effects of perfusion underline the influence of mechanical stimulation on cell organization. Finally, to increase complexity of the model, we demonstrate the co-culture of two ductal structures—a mammary and an endothelial duct— as a step towards vascularized mammary models.

Methods: To fabricate the chips, volumetric bioprinting (VBP) was performed with gelatin methacryloyl supplemented with photoinitiator lithium phenyl-2,4,6-trimethylbenzoyl-phosphinate. Printing accuracy was assessed through computational nominal actual comparison of the printed object (from lightsheet microscopy images) to the original STL file. Cell seeding optimization used human umbilical vein endothelial cells (HUVECs) and MCF10A cells at varying densities (7.5–60 million cells/mL) with channel coatings of poly-L-lysine, collagen type I, or both. A custom platform enabled automated chip rotation, and cell coverage was analyzed from segmented confocal images. To enable dynamic culture, an autoclavable and transparent CNC-milled perfusion bioreactor was developed. Chips were seeded with MCF10A to assess monolayer formation and cell polarization. As a proof of concept, a dual-channel chip incorporating an MCF10A and an endothelial channel was fabricated.

Results and discussion: Chip constructs with 500-1000µm-diameter channels were printed with minimal deviations (1.94±2.96%) compared to the STL file (Figure 1A). Seeded chips were rotated for 6 hours (90°/10min), and specific coating and density conditions were optimized for both MCF10A and HUVECs (>80% channel coverage). Inclusion of the chips into the bioreactor enabled leak-free perfusion for 21 days, as well as efficient imaging without compromising sterility (Figure 1B). Channels seeded with MCF10A formed a tight epithelial monolayer when cultured dynamically compared to static controls. Furthermore, cell polarization was enhanced in perfused constructs, as observed by CK14 (basal) and CK8/18 (luminal) localization, and cell alignment could be controlled by changing the positioning of the print in the printing reservoir. In a two-channel chip, both endothelial and mammary epithelial cells could be cultured in independent channels, as close as 200 µm from each other, with the mammary channel possessing lobular structures to better mimic mammary duct architecture (Figure 1C,D).

Conclusions: We present a biofabrication pipeline combining VBP and dynamic perfusion in a custom platform. Using a mammary gland model, we highlight the impact of perfusion on cell organization and polarization, offering a valuable tool for studying healthy and diseased tissues. Open source, imaging-based workflows were developed for chip characterization, and the platform's modifiable digital models enable adaptation for diverse tissue engineering applications, advancing disease modeling and drug screening through native like dynamic culture.

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Acoustic droplet ejection enables complex 3D bioprinting and the clog-free transfer of ultra-high cell concentrations due to its nozzle-less principle

Introduction

We have already shown that the Acoustic Droplet Ejection (ADE) technology facilitates the precise, nozzle-less transfer of cell-laden bioinks [1]. This technique offers an alternative to traditional bioprinting methods like microextrusion or inkjet, circumventing nozzle-related issues such as clogging and high wall shear stress. By eliminating the nozzle, ADE significantly reduces physical stress on cells and removes limitations on printable cell densities imposed by nozzle dimensions. Now we further hypothesized that

the technology could enable the fabrication of more intricate structures and the transfer of physiologically relevant cell densities exceeding 108 cells/ml, which is not possible using established nozzle-based bioprinting methods.

Methods
To enhance the complexity of printed 3D structures, two primary improvements were implemented. First, droplet deposition accuracy was increased using an ultrasonic pulse-receiver system to precisely locate the bioink surface and position the transducer accordingly. Second, a slicing workflow, including a Python script to convert pictures and standard G-codes, was integrated to generate complex geometries readable by the custom acoustic bioprinter. Additionally, the limits of the reachable cell concentrations were tested. For this purpose, different cell types pre-stained with Hoechst solution were centrifuged into a pellet. A negligible amount of cell culture medium was added to increase the moisture content of the pellets, which were then pipetted into the acoustic bioprinter. The cells were then printed onto a six-well well plate in a droplet-wise manner, creating a small pool of cell suspension to increase the ejected volume and keep the cells moist. Further experiments were conducted with a lower cell concentration of at least 108 cells per ml. The survival rate of the printed cells was analyzed under a fluorescence microscope.

Results
The Python scripts were used to transform images or common G-Codes into machine readable commands, allowing for the generation of complex two- and three-dimensional structures. The changes in the workflow also significantly increased the printing speed and allowed for the automatic focusing of the transducer to account for reservoir depletion during printing. Reservoir cell concentrations of more than 108 cells per ml were transferred using the acoustic bioprinter (Fig. 1 a. and b.), showing post-printing viabilities above 95 % (Fig. 1 b.).

Conclusions
The changes implemented into the printing workflow allowed for the generation of significantly more complex two and three-dimensional structures. The results of the cell experiments showed that the printer's nozzle-less approach allows for ultra-high cell density transfer with excellent post-printing viability.

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Effects of Aqueous Two-Phase Emulsion (ATPE) as Microporous Matrix for Vascularized Tissue Models

Introduction
3D in-vitro models offer a more accurate simulation of in-vivo conditions than traditional 2D cell cultures. Vascularization is currently a hot topic in tissue modeling, helping to mimic the in-vivo environment, adding physiological relevance, and aiding the supply of nutrient and oxygen as the removal of metabolic waste1. Also, perfused vascular networks allow better biomimicry drug screening, emulating the drug passage through the endothelial barrier2. Different techniques have been employed to introduce a vascular compartment in various tissue models, including self-assembly, microfluidic platforms, sacrificial templating, and 3D bioprinting3.
Microporous hydrogels are promising biomaterials for tissue modeling, offering enhanced biomimicry. Introducing tunable micropores within hydrogels can significantly improve their structural and biological performance. Aqueous two-phase emulsions (ATPE) consist of a matrix material mixed with a porogen, which is later removed after crosslinking. ATPE are appealing systems as they offer a completely aqueous environment, eliminating the need for oil-phase solvents4.
In this study, different combinations of Gelatin Methacryloyl (GelMA) and Polyvinyl Alcohol (PVA) were characterized and tested for different uses, such as Extrusion Based Bioprinting (EBB), demonstrating its versatility in the introduction of a vascularized compartment in tissue models.
Methods
In this study, an ATPE is obtained combining GelMA as the matrix and PVA as porogen. Different PVA molecular weights have been tested based on the application. A preliminary study evaluated porogen removal by means of FTIR analysis, followed by biological metabolic tests on A549 and HUVEC cell. SEM imaging was also employed to visualize differences in the micropore sizes. Rheological and photorheological characterization of different ATPE formulations were conducted to assess their mechanical properties and printability. EBB tests have been performed to evaluate modifications in the micropore morphology induced by the printing process.[CGG2] [CGG3] Sacrificial templating has also been investigated to assess HUVECs response under dynamic conditions. Core-Shell structures obtained via microfluidic EBB are currently under investigation.
Results
PVA is successfully removed after the first washes with PBS, as demonstrated by FTIR analysis. The size of the micropores can be tuned by varying the PVA molecular weight, as demonstrated by SEM imaging, and the rheological properties are in line with a printable biomaterial. Extrusion-based bioprinting has been demonstrated to influence pores morphology, which elongates along the printing direction. Metabolic tests showed enhanced metabolic activities in A549 cell line.
The results demonstrate that the GelMA-PVA ATPE is a versatile and promising alternative to plain GelMA, offering enhanced tunability for specific uses. Potential applications include the use of this ATPE in core-shell bioprinting to create microporous vessel scaffolds for angiogenesis studies.
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- S33 Redefining Cancer Modeling: Patient-Derived Organoids Enabled by Extracellular Matrix (ECM)-Inspired Scaffolds Ratuszowa

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Advancing Bioengineered Solutions for Bile Duct Repair

Biliary complications, such as post-operative biliary strictures, pose serious health risks and place a significant burden on healthcare systems. Conventional treatments, including plastic stents, require frequent replacement, while self-expanding metal stents, despite longer patency, carry risks such as migration and tissue ingrowth. Biodegradable scaffolds offer a promising alternative due to their natural degradation in vivo; however, precisely aligning their degradation rate with tissue regeneration remains a critical challenge. Furthermore, replicating the complex biological and mechanical properties of the native bile duct is essential for functional success.
We recently developed a novel multiphasic tubular scaffold designed to closely replicate the size and function of the human common bile duct. Made of two biocompatible biomaterials and seeded with biliary epithelial cells, the construct demonstrated excellent homogeneity, stability, mechanical strength, suturability, and leak-proof properties. Its ability to transport and modify bile acids highlights its functional maturity and suitability for in vivo applications. As an initial validation, ex vivo human blood assays of the acellular scaffold revealed a favorable immune response characterized by limited inflammation and sustained release of epidermal growth factor—supporting its potential to promote regeneration.

Ongoing work aims to enhance the biliary epithelial component and integrate additional cell types to further improve physiological relevance. In this context, we present preliminary findings on self-organizing biliary organoids composed of epithelial, mesenchymal stromal, and endothelial cells.
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Non-small cell lung cancer tumoroids as tools for translational research

In the last twenty years, the systemic cytotoxic chemotherapy approach left gradually place to a more personalized clinical concept, aiming to identify and target tumor peculiarities which make one tumor different from another. The lack of coherence between experimental results and in vivo effectiveness confirmed the limitation of using clonal 2D cell cultures to predict the efficacy of compounds in clinical applications. To overcome this model inadequacy, mainly in non-small cell lung cancer (NSCLC)

classified as a complex disease with intra- and inter-tumor heterogeneity, the use of patient-derived tumor organoids could be an encouraging tool for precision medicine, due to model ability in retaining most of the original phenotype and genotype.
Our early-stage NSCLC organoids collection (PDO), enriched in both tumor and healthy cultures from each patient, accounts for almost 200 cases. A subset of PDOs has been widely characterized, recalling primary tissue traits and native genomic alterations, even after long-term cultures and freezing/thawing processing.
Drug sensitivity tests revealed extreme variability in PDO responses to a spectrum of compounds used in NSCLC clinical practice, including platinum-derivates and anti-folate agents. Differential responses were also identified when PDO, harboring well defined gene alterations, were treated with corresponding target drugs, confirming that the only presence of the same mutation does not guarantee uniform therapeutic efficacy. Likewise, in vitro testing of PDOs with the same chemotherapeutic compounds administered in vivo, reflected the patients' clinical response.
Tumor organoids represent a promising in vitro model to study NSCLC complexity and to predict patients' clinical outcomes.
Konuşmacılar: Valentina Monica

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3D bioprinted tumoroids: an innovative model to study the role of the Notch pathway in ovarian cancer chemoresistance

Introduction
Ovarian cancer (OC) is the leading cause of death among gynecological malignancies, primarily due to its high mortality rate and frequent recurrence. The standard treatment, platinum-based chemotherapy, often becomes ineffective as patients develop platinum resistance, a process associated with enhanced metastatic potential, epithelial-mesenchymal transition (EMT), and angiogenesis. Notch signaling, particularly through Notch3, plays a critical role in the development of chemoresistance in OC, contributing to cancer stemness, EMT, and tumor-stroma interactions. Additionally, Pin1 has been implicated in stabilizing the Notch3 intracellular domain, modulating its processing and degradation, which further promotes chemoresistance.
Methods
To investigate the role of Notch signaling in OC chemoresistance, we developed a 3D biofabricated tumor model that mimics the ovarian cancer microenvironment. Tumoroids were generated from Kuramochi ovarian cancer cells, where Pin1 expression was silenced via targeted knockdown. The cells were bioprinted into GelMA/alginate (5%/4%) constructs using extrusion-based techniques, optimized for stability and cell viability. Western blot analysis confirmed efficient Pin1 silencing at the protein level. The optimal carboplatin concentration was determined using MTT assays to select sublethal doses that induced cytotoxicity without compromising the integrity of the constructs. Immunofluorescence staining was performed to evaluate tumoroids proliferation (Ki67), cytoskeletal organization (phalloidin), ovarian cancer-specific markers (WT-1, PAX-8), and extracellular matrix deposition (fibronectin).
Results
The biofabricated constructs supported the formation of viable, structured tumoroids with sustained proliferation and high-grade serous OC marker expression. Bioprinted constructs properties allowed for long-term culture post-printing. Pin1 knockdown was confirmed by western blotting, and MTT assays demonstrated a dose-dependent response to carboplatin. Stromal components, including mesothelial and endothelial cells, were successfully integrated into the constructs, recapitulating the spatial tumor-stroma organization of OC.
Discussion
This study demonstrates the potential of biofabricated 3D models for replicating the ovarian tumor microenvironment and studying chemoresistance mechanisms. Pin1 silencing allowed for further investigation into its role in stabilizing Notch3 and enhancing invasive tumor behavior. Future work will expand this platform with patient-derived OC organoids and cancer-associated fibroblasts (CAFs) to evaluate combination therapies targeting the Notch-Pin1 axis and to further dissect the molecular mechanisms driving angiogenesis and metastasis in OC. This model could provide a valuable tool for preclinical personalized therapy screening in platinum-resistant ovarian cancer.
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Disclosure
The authors declare no conflict of interest

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Transforming Pre-Clinical Drug Testing: 3D Bioprinted Patient-Derived Breast Cancer Models on a Microfluidic Device

Transforming Pre-Clinical Drug Testing: 3D Bioprinted Patient-Derived Breast Cancer Models on a Microfluidic Device
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Abstract
Introduction: Cancer shows high genetic and phenotypic diversity between people, generating a need for personalized therapy. Three-dimensional bioprinting represents an efficient and advanced technique for precisely recapitulating the cancer microenvironment. Bioprinting on a microfluidic device addresses the challenge of the availability of a limited number of primary cancer cells derived from biopsy or surgically resected cancer tissue. This study is a step forward in creating a pipeline that uses patient cells to develop models for personalized breast cancer drug testing.
Methods: In this study, primary cancer cells were isolated from biopsy and surgically resected breast cancer tissues. To establish bioprinted constructs for personalized drug testing, we employed an extrusion-based 3D bioprinting method using gelatin methacryloyl-based bioink and primary breast cancer cells. Cell proliferation, protein expression, and anticancer drugs were investigated in these 3D cancer models.
Results: Our results demonstrate the successful long-term expansion of primary cancer cells isolated from cancer tissue. The cells are derived from multiple breast cancer patients, each diagnosed with a different grade of carcinoma. Patient-derived models exhibit dissimilar viability, proliferation, and gene expression. Treatment with well-known breast cancer chemotherapeutic drugs at clinically relevant dosages resulted in heterogeneous drug responses among different patient-derived cancer models.
Discussion: The result of this study supports the establishment of an expandable 3D in vitro cancer model for drug screening. The resulting bioprinted cancer model on a microfluidic chip showed diverse responses elicited by patient-derived cancer cells, reproducing patient-specific cancer conditions and providing robust outcomes. Features exhibited by our bioprinted cancer model correlate with the clinical diagnosis mentioned in the histopathological reports of patients. This study addresses the need for a co-clinical trial platform that can prospectively evaluate the effectiveness of drug profiling on cancer-derived cells to decide patient treatment. By adding other cell types of the cancer microenvironment, this model can be advanced to a heterotypic one.
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Innovative 3D Bioprinted Model to Screen Colorectal Cancer Nanomedical Treatments

Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide. Nanomedicine has shown significant potential in enhancing treatment outcomes and in overcoming the limitation of traditional therapeutic approaches. However, a major challenge lies in translating these advancements into clinical applications. To bridge this gap, we propose a 3D bioprinted model that mimics CRC tissue providing a physiologically relevant platform to test novel therapies. A customized bioink of alginate and

gelatin was coaxially extruded with calcium chloride to bioprint a hollow conduit further crosslinked with microbial transglutaminase (mTG). CCD-18Co fibroblasts from normal colon were coextruded with the bioink, while HT-29 adenocarcinoma cells were seeded inside the lumen of the conduits. After the study of different percentages of bioink's components, viability test over 13 days were conducted to assess bio-stability over long time. To proof the concept, a biodistribution study with lipid-coated zinc oxide nanoparticles with a targeting peptide was carried out and a stimuli-responsive therapeutic treatment using acoustic waves to stimulate the nanoparticles was performed on the co-culture structure aiming at selectively killing the tumor cell and sparing the normal counterpart [1-3]. Different concentrations and combinations of gelatin and alginate were tested, and the optimal bioink was selected based on printability, rheological properties, stability and cellular support. After the tuning of printing parameters, cells were incorporated into the structure, resulting in a model that remained stable for over 13 days; vitality assays showed no significant change in cell viability over the observation period, with cell configuration evolving over time. The final 3D structure replicated CRC with healthy cells in the tube walls and cancer spheroidal structure in the lumen, mimicking the tumor microenvironment. Due to its reproducibility and stability, this model was used to evaluate lipid-coated ZnO nanoparticles, activated by acoustic pressure waves (ultrasound or shock waves), designed to target CRC tumors. By fluorescently labeling these nanoparticles, we monitored and confirmed their preferential uptake by the tumor cells in the model, while avoiding the non-targeted healthy cells. Results further showed a high biocompatibility of both nanoparticles and acoustic waves on healthy cell structures in the tube walls. Then, we observed the efficacy of the therapeutic stimuli-responsive nanomedicine treatment, over long time period (96 hours) against the cancer 3D cell structures. We developed a bioprinted tubular model that represents a promising in vitro system for mimicking CRC and evaluating innovative therapeutic treatments. A 3D biodistribution test confirmed the targeted internalization of nanoparticles by tumor cells, demonstrating the model's potential for nanomedicine screening. This reproducible system offers a cost-effective alternative to animal models, though further improvements, such as vascularization, could enhance physiological relevance and clinical translatability.

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Microfluidics-assisted precision-printing of microencapsulated human tumor microenvironments with gradients in cellular composition for high-throughput evaluation of drug resistance.

The available methods of reproducible formulation of human tumor microenvironments (hTMEs) that is micro-engineered systems incorporating cancer, stroma and extracellular matrix (ECM), that could serve as preclinical models of disease, e.g., in anti-cancer drug testing, currently suffer from excessive complexity (organ-on-chip systems) and/or low-throughput (3D bioprinted constructs). Here, we report microfluidics-assisted microencapsulation of hTMEs inside hydrogel microdroplets which, immediately after generation at a microfluidic printhead, are transferred one-by-one onto the substrate in the form of a linear chain, effectively '1D-printed' with high precision. Due to the elastic neighbor-neighbor interactions, the droplets occasionally rearrange resulting in the generation of unique local patterns [1]. We show that such patterns can serve as highly specific structural 'self-barcodes', allowing for fully non-invasive labeling of the dropelts (i.e., without addition of any chemical labels) with capacity of even N~10^5 droplets.

Our method relies on a new approach to droplet bioprinting [2] wherein double-emulsion W1/O/W2 structure is formed at the tip of the microfluidic printhead such that the inner hydrogel (e.g., gelatin methacryloyl) droplet phase (W1) and the external cell culture medium (W2) are separated by an immiscible (biocompatible) oil-surfactant mixture (O). After transfer to the substrate, the oil forms stabilizing films between the droplets, but can also be removed after the droplets are crosslinked. The double-emulsion approach provides for several advantages over conventional methods of droplet-based bioprinting including: (i) high droplet surface-coverage (>10 droplets/mm^2) and ultra-high throughput, limited only by the available area of the substrate, (ii) high rates of droplet generation and deposition (> 10/s), (iii) possibility of generation of gradients in cellular or biomaterial composition along the printed droplet-lines, and (iv) ease of identification of individual droplets via the self-barcoding mechanism.

As a proof-of concept, following our previous work on microencapsulated hTMEs [3], we show that the viability of chronic myeloid leukemia cancer cells (K562) co-encapsulated with bone-marrow cells (HS-5) under treatment, e.g., with imatinib depends on the cancer-stroma cell ratio which can be fine-tuned in a single high-throughput droplet print. We discuss how the method could be extended to include spatial segregation of cells within each printed microdroplet for more precise mimicry of the hTME and/or high-throughput cell invasion studies.

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Konuşmacılar: Jan Guzowski (Institute of Physical Chemistry Polish Academy of Sciences)

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Mechanically Stimulated Glioblastoma-Derived dECM Bioink for 3D Tumor Microenvironment Model

Glioblastoma (GBM) is a highly invasive and heterogeneous brain tumor, making it particularly difficult to replicate its complex tumor microenvironment (TME) in vitro. One promising approach involves using GBM-derived decellularized extracellular matrices (dECMs), which closely mimic the native TME. These dECMs contain tumor-specific biochemical components such as glioma-associated glycoproteins, growth factors, and ECM-remodeling enzymes that play key roles in regulating cell proliferation, invasion, and stemness. As a result, GBM-derived dECM bioinks are well-suited for modeling tumor heterogeneity, studying cancer cell behavior, and evaluating therapeutic responses. However, acquiring GBM-derived dECM is often challenging due to limited tissue availability. To overcome this, we developed a novel method to generate GBM-derived dECM bioink by culturing glioblastoma cells on collagen-coated substrates and applying mechanical stimulation. This stimulation activated the GBM cells, and RT-qPCR analysis showed significant upregulation of genes associated with invasiveness and proliferation compared to unstimulated controls. The resulting bioconstructs were then decellularized and formulated into bioink for the biofabrication of ex vivo TME models. This study demonstrates that mechanical preconditioning of GBM cells can enhance ECM deposition, offering a scalable and reproducible method for generating tumor-mimetic bioinks. The resulting GBM-derived dECM bioink provides a biologically relevant platform for modeling the GBM microenvironment, investigating tumor progression, and screening therapeutic candidates in a more physiologically accurate context.

Glioblastoma (GBM) is a highly invasive and heterogeneous brain tumor, making it particularly difficult to replicate its complex tumor microenvironment (TME) in vitro. One promising approach involves using GBM-derived decellularized extracellular matrices (dECMs), which closely mimic the native TME. These dECMs contain tumor-specific biochemical components such as glioma-associated glycoproteins, growth factors, and ECM-remodeling enzymes that play key roles in regulating cell proliferation, invasion, and stemness. As a result, GBM-derived dECM bioinks are well-suited for modeling tumor heterogeneity, studying cancer cell behavior, and evaluating therapeutic responses. However, acquiring GBM-derived dECM is often challenging due to limited tissue availability. To overcome this, we developed a novel method to generate GBM-derived dECM bioink by culturing glioblastoma cells on collagen-coated substrates and applying mechanical stimulation. This stimulation activated the GBM cells, and RT-qPCR analysis showed significant upregulation of genes associated with invasiveness and proliferation compared to unstimulated controls. The resulting bioconstructs were then decellularized and formulated into bioink for the biofabrication of ex vivo TME models. This study demonstrates that mechanical preconditioning of GBM cells can enhance ECM deposition, offering a scalable and reproducible method for generating tumor-mimetic bioinks. The resulting GBM-derived dECM bioink provides a biologically relevant platform for modeling the GBM microenvironment, investigating tumor progression, and screening therapeutic candidates in a more physiologically accurate context.

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Konuşmacılar: Seo Hyeon An (Sungkyunkwan University School of Medicine (SKKU-SOM))

S35 Stretching the Frontiers: Advanced Applications of Biofabricated Skeletal Muscle

Kisielewski

Toplantıyı düzenleyenler: Nehar Celikkin (IPC PAS), Sahar Salehi-Müller (University of Bayreuth)

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Perivascular stem cells and 3D printing for human myo-substitutes biofabrication

The skeletal muscle tissue exhibits good regenerative capabilities, which are however limited by injury size. As a matter of fact, large muscle lesions are characterized by poor recovery accompanied by scar formation and functional detriment, condition common to people suffering from volumetric muscle loss and needing reconstructive therapeutic approaches. Even if surgical autologous transplantation is a standardized procedure, the outcomes are often unsatisfactory. Hence, the pressing need to develop engineered artificial tissues to replace wasted muscle. Tissue engineering (TE) is an up-and-coming biotechnology with great potential for muscle repair, but no conclusive strategy has been demonstrated yet. Reconstructing the skeletal muscle architecture and function is still a challenge requiring the parallel alignment of myofibers. Within this

context, we developed a novel approach for the biofabrication of human derived myo-substitutes by exploiting a population of adult myogenic stem cells, namely pericytes, in combination with 3D bio-printing technology to reply the skeletal muscle functional architecture with parallel oriented muscle fibers. The characterization of cell-laden constructs showed a remarkable myogenic activity besides a complete architectural histo-organization, revealing the actual potential of this technology to support human skeletal muscle repair and regeneration.

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Konuşmacılar: Cesare Gargioli (Rome University Tor Vergata)

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Bioprinting Perfusable and Vascularized Skeletal Muscle Flaps for the Treatment of Volumetric Muscle Loss

Volumetric muscle loss (VML) refers to muscle tissue loss exceeding 20% within a functional area due to trauma or surgery, often leading to physical disabilities. VML treatment relies on the transplantation of autologous flaps harvested from a healthy-donor site while minimizing the probability of immune rejection. However, this approach often leads to donor-site morbidity and relies on a restricted supply of muscle tissue. Current solutions in tissue engineering focus on engineered grafts lacking hierarchical vasculature with a feeding vessel, thus limited by diffusion. This study expanded upon a new approach of multimodal bioprinting which enabled the fabrication of thick hierarchical vascular muscle flaps composed of bioprinted and vascularized skeletal muscle tissue, and a 3D-printed engineered macrovessel, which successfully repaired VML injury in-vivo. The flaps are implanted by anastomosing the macrovessel via microsurgery to the femoral artery in proximity to an induced VML injury in Sprague-Dawley rat hindlimbs. Immediate perfusion of the flaps is demonstrated, as is flap endurance to physiological blood pressure, flow, and shear stress. Flap implantation enhanced myocyte differentiation, and vascular ingrowth and facilitated tissue viability and integration. These results obtained by utilizing human-origin cells provide a foundation for fabricating patient-specific flaps for the treatment of extensive soft tissue defects.

Konuşmacılar: Shulamit Levenberg (Technion)

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Non-genetic photostimulation to enhance maturation of biofabricated skeletal muscle.

Introduction. Achieving physiologically relevant maturation of engineered skeletal muscle in vitro remains a major challenge in tissue engineering (1, 2). Traditional stimulation approaches often fail to recapitulate the spatiotemporal precision of native neuromuscular activation (3, 4). In this context, light-responsive molecular transducers represent a promising tool to drive muscle excitation with high spatial and temporal resolution (5, 6). Here, we explore the use of intramembrane azobenzene-based photoswitches for the functional enhancement of 3D skeletal muscle constructs and investigate how photostimulation influences biological changes associated with muscle maturation.
Methods. Engineered skeletal muscle bundles were fabricated using a rotary wet-spinning technique with murine myoblasts embedded in hydrogel-based core-shell fibers. After differentiation, tissues were treated with Ziapin2 (7), a photoresponsive small molecule that integrates into the plasma membrane. Constructs were exposed to photostimulation protocols (variable frequency and duration), and contractile dynamics were evaluated through high-speed video analysis. Structural organization was assessed via confocal microscopy and quantified using maturation indexes. Tissues displaying the most mature phenotype were subjected to molecular characterization.
Results. Preliminary observations suggest that photostimulated muscle constructs may exhibit improved contractile behavior when compared to non-stimulated controls, including more regular contraction patterns and increased responsiveness. Structurally, a trend toward enhanced sarcomere organization and alignment was observed in stimulated samples. Early molecular analyses are being conducted to explore how photostimulation might influence the expression of muscle-specific markers and features linked to fiber-type specification. These investigations aim to identify potential molecular signatures and structural correlates of functional maturation in response to light-based activation.
Discussion. Initial data support the hypothesis that light-driven, non-genetic stimulation could promote both structural and functional maturation of engineered skeletal muscle constructs. This strategy may offer key advantages over conventional stimulation methods, particularly in terms of precision and adaptability. While further validation is ongoing, early results point to biological changes associated with photostimulation, involving molecular pathways related to contractile function and fiber-type differentiation. These findings highlight the potential of photostimulation as a tunable and non-invasive tool for advancing in vitro muscle modeling, with promising implications for applications in regenerative medicine.
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Konuşmacılar: Fabio Maiullari (Polish Academy of Sciences)

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Alginate-dialdehyde–gelatin (ADA-GEL) based composite hydrogels for skeletal muscle biofabrication: development with an ionic medicine strategy

Introduction: Ionic medicine is an approach that proposes the use of therapeutic ions to stimulate cell growth, reduce inflammation, combat oxidative stress, and promote tissue regeneration1. Biofabrication offers a solution for addressing muscle loss in situations where the human body cannot heal itself effectively2. To enhance muscle cell growth by ionic stimulation, this study established a novel bioink system comprising alginate-dialdehyde–gelatin (ADA-GEL) hydrogel, a biocompatible and printable hydrogel widely used in biofabrication3, and ion-releasing bioactive glass (BG) nanoparticles.

Methods: Mesoporous bioactive glass nanoparticles (MBGNs) of calcium silicate composition, selectively doped with boron and cerium, were synthesized, and their physical and chemical properties were characterized. Composite inks were formulated by incorporating 0.1–1% (w/v) MBGNs into 2.5%ADA-5%GEL (w/v). The swelling and degradation behavior, mechanical properties and printability of the hydrogels were investigated. Furthermore, C2C12 myoblast cells were bioprinted and cell viability and morphology were evaluated post-printing.

Results and Discussion: MBGNs were synthesized in three categories: without dopant, with a single doping element, and with dual (Ce, B) dopants. MBGNs were spherical with diameters of 90-180 nm and demonstrated a gradual release of ions into the aqueous environment over time. Dopant ions affected the release rate of silicon ions. Incorporation of MBGNs into ADA-GEL narrowed the available printing window due to accelerated gelation induced by the presence of particles and ions. This effect was particularly noticeable in inks containing 1% (w/v) nanoparticles. Nevertheless, printability assessments showed that all formulations exhibited good processability and printability with well-defined printed structures.
The swelling and degradation of hydrogels showed a similar trend over the incubation period for all compositions. ADA-GEL containing dual-doped MBGNs displayed long-term stability compared to pure ADA-GEL and ADA-GEL containing single-doped MBGNs. The impact of the addition of MBGNs on the compressive modulus was also dependent on ion type and particle concentration. However, the impact diminished after 24 hours of sample incubation.
Finally, bioprinting was conducted with C2C12 cells encapsulated in the hydrogels. Metabolic activity of bioprinted cells increased over a 14-day incubation period, with 0.1 and 1% (w/v) undoped MBGNs to the greatest extent, followed by single-doped nanoparticles. A uniform distribution of cells with elongated morphology was observed. Quantitative analysis of cell alignment demonstrated the highest alignment with 1% (w/v) undoped and single-doped MBGNs.

Conclusions: All ADA-GEL-BG inks, together with optimized printing platforms, enhanced myoblast cell growth and alignment in bioprinted constructs, particularly with 1% (w/v) single-doped MBGNs. The results highlight the potential of the system for muscle tissue engineering strategies and represent an example of application of the emerging field of ionic medicine.

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Acknowledgments: This work is funded by the German Research Foundation (DFG), project number 326998133 – SFB/TRR225 (subproject B03). Hana Kaňková acknowledges funding from the Slovak Recovery Plan under grant agreement No. 09I01-03-V04-00040/2024/VA.

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Konuşmacılar: Hsuan-heng Lu (University Erlangen-Nuremberg)

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Replicating skeletal muscle contraction dynamics in two-photon-3D printed hydrogel fibers

Synthetic muscles that replicate the distinct contraction and force-generating properties of native skeletal muscle are highly sought after. They are used in a variety of applications, including actuators and regenerative therapies such as muscle replacement. Two-photon stereolithography is a promising tool to fabricate bio-scaffolds with micrometer resolution, ideally replicating soft native microtissues in vitro.

(A) We utilize this technique to 3D print synthetic muscle fibers from bovine serum albumin to form a contractile hydrogel structure with tailored mechanical properties. Using a custom microscale tensile strength device, we quantify force-length relations and elastic properties in these 3D printed protein muscle fiber models at a constant temperature of 20 ± 0.1°C.

(B) The observed pH-dependent contraction of the synthetic muscle (non-activated at pH 7, activated at pH 4) resulted in active forces comparable to those in native skeletal muscles. Manipulating the length of the synthetic fiber affects force production, following a parabolic force-length relationship similar to native muscle fibers, characterized by an ascending limb, an optimum, and a descending limb as the length increases. Furthermore, we conducted work loops on the synthetic muscle, which revealed a viscoelastic behavior and a significant impact of velocities on the muscle's mechanical work and power output.

(C) Next, we seek to recapitulate physiological muscle fibers where myocytes align and regenerate to exert contractile forces. Therefore, we studied tissue alignment of C2C12 precursor myocytes in spatially confined 3D printed structures. The results revealed several significant relationships between tissue morphology and the substrates topology.

Ultimately, our two-photon crosslinked synthetic fiber can replicate the contractile dynamics of skeletal muscle tissue. This opens new avenues to further explore contractile synthetic materials, as well as their future refinement by integrating myocytes for a more realistic synthetic muscle fiber.

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Konuşmacılar: Theresa Kuehn (Institute of Biomaterials and Biomolecular Systems)

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G-FLight: Prolonged cell encapsulation and rapid filamented light biofabrication of aligned muscle tissue constructs in microgravity

Introduction:
Biofabrication in space enables the production of patient-specific, structurally complex tissue constructs with customized properties (e.g., cell density, material composition, architecture), essential for future long-duration missions. Although substantial progress has been made in evaluating biofabrication strategies for space applications, the fabrication of anisotropic, highly aligned tissues remains a significant challenge. Additionally, the maintenance and handling of living cells under microgravity conditions require specialized protocols and equipment. Notably, traditional layer-by-layer printing techniques typically rely on gravity, while light-based approaches require specialized resins with active leveling mechanisms to redistribute the material between sequential material depositions during layer shifts. In contrast, the herein developed G-Flight platform employs ready to use resin cuvettes and a single, rapid light projection to fabricate tissue constructs within seconds, entirely independent of gravitational effects.

Methods:
In this study, we developed gelatin methacrylate (GelMA)-based bioresin formulations capable of storage under refrigerated and cryogenic conditions for up to one week. For storage at refrigeration temperatures ("CoolResin"), a commercially available hypothermic preservation medium, Hypothermosol® FRS, was incorporated into the reformulation. For cryopreservation at –80°C ("CryoResin"), additional supplementation with trisaccharide melezitose hydrate and dimethyl sulfoxide was employed.

Figure 1. A. Schematic illustration of the light-engine developed for parabolic flight experiments, during which an approximately 22-second microgravity window was available for biofabrication. B. Light-sheet images of printed constructs, alongside confocal micrographs showing the filamented microstructur. C. On-ground viability assays after seven days of culture, using previously encapsulated Pax7^{+}-nGFP primary myoblasts in various resin formulations. D. Representative immunofluorescence images showing myotube fusion and sarcomere maturation within the printed constructs. E. Constructs fabricated from CoolResin_4C and CryoResin_4C displayed spontaneous contractility which synchronized contractions after electrical stimulation at 1 Hz and 5 H.

Results:
We engineered a custom, small-form-factor FLight biofabrication engine (Figure 1A) equipped with refrigerated resin storage and a 37°C warming block for resin liquefaction. This system was deployed during the 43rd Deutsches Zentrum für Luft- und Raumfahrt parabolic flight campaign (Bordeaux, September 2024) to demonstrate its functionality under repeated microgravity conditions. Across 90 parabolic maneuvers, aligned, filamented hydrogel constructs were successfully fabricated (Figure 1B). Cell viability assays demonstrated excellent survival (>90%) after seven days of culture in constructs fabricated from CoolResin and CryoResin formulations (Figure 1C). Upon induction of differentiation, myoblasts fused into contractile myotubes. Notably, constructs derived from CoolResin4C and CryoResin4C formulations exhibited higher cell densities and significantly increased fusion indices compared to controls. Furthermore, functionality testing via electrical stimulation confirmed synchronized contractility of the tissue constructs (Figure 1D).

Discussion:
This work demonstrates that tailored resin formulations can preserve cell viability of encapsulated primary muscle cells during prolonged refrigerated or cryogenic storage and our custom printer design enables successful fabrication of functional tissue constructs in microgravity. Our optimized resins resulted in high post-printing viability, significantly enhanced myotube formation, and consequently improved contractility compared to controls, highlighting their potential for future biofabrication applications in space environments.

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Konuşmacılar: Michael Winkelbauer (ETH Zürich)

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Biofabrication of a hierarchically organized structure of skeletal muscle tissue

Introduction
Skeletal muscle is a highly organized and heterogeneous tissue composed of multiple cell types and structural components, arranged in a complex hierarchical architecture. This structure is essential for its mechanical strength, contractile function, and ability to regenerate. Skeletal muscles possess a strong capacity for self-repair; however, they are unable to regenerate effectively following substantial tissue loss, which leads to significant functional impairment. While direct injection of cells has been explored as a therapeutic approach, it has shown limited success due to inadequate integration into the host muscle tissue (Salehi et al., 2017). To overcome these challenges, we introduce here various platforms for biofabrication of skeletal muscle tissue with similar heterogeneity, generating a hierarchical functional structure like skeletal muscle (Koeck et al., 2022; Apsite et al., 2020; Sprenger et al., 2024).

Materials and methods
We have shown various fibrous structures for the generation of the anisotropy in combination with 3D bioprinting technology and added living cells to the structure, and analyzed their biological behavior.. Therefore, 1) sub-micron rod-shaped fillers were fabricated using microfabrication and included in the bioink as composite bioinks. 2) electrospun fibrous structures as bilayers were used to engineer self-rolling scaffolds for multicellular tissue generation, and 3) wetspun microfabricated fibers based on collagen and gelatin were used to mimic the muscle tissue interfaces.

Results and discussion
In this work, we demonstrate that injectable, short, and flexible cell carriers can effectively provide contact guidance to cells within the 3D network of hydrogel-based bioinks. Additionally, microfabricated fibers with diameters ranging from 18 to 500 µm successfully align muscle cells, with well-organized internal structures of differentiated myotubes and clearly visible sarcomeric actin. The aligned cells exhibit homogeneous and synchronous contractions, highlighting the potential of these structures to support the formation of large-scale muscle tissue. Enhanced myotube formation and myogenesis, along with improved intracellular organization and functionality, were also observed in response to electrospun scaffolds. These scaffolds are capable of undergoing self-transformation into tubular structures, making them suitable for constructing implants for the treatment of volumetric muscle loss injuries. We anticipated that the studied substrates would promote the formation of organized muscle microtissues.

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Acknowledgement
This work was supported by DFG (SA 3575/1-1, SA 3575/2-1, and project number 326998133—SFB/TRR225 (subproject B03)).

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Konuşmacılar: Sahar Salehi-Mueller (University of Bayreuth)

- Exhibitors' Presentations Mikołajska

TRR225
HealthBiolux
Readily3D

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Konuşmacılar: Taavet Kangur (Readily3D)

- 12:30
  Lunch & Exhibitors' Presentations
- Industrial Session Ratuszowa

Sponsors:
- CollPlant
- Polbionica
- BioCloner Health
- BIOinx
- BIO3DPrinting

Toplantıyı düzenleyenler: Tal Yehiel

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  rhCollagen Bioinks for Biofabrication

rhCollagen Bioinks for Biofabrication

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CollPlant Biotechnologies Ltd., 4 Oppenheimer St, Rehovot, Israel

Introduction

Bioinks are a cornerstone of 3D bioprinting, providing the essential biological and mechanical framework required for the precise deposition of living cells, biomaterials, and signaling molecules. Bioinks enable fabrication of complex, tissue-like structures that mimic native tissues or organs. Effective bioink must support cell viability, promote proliferation and differentiation, and possess the physical properties required for print fidelity.
Extrusion-based bioprinting is widely used due to its simplicity, material flexibility, and cost-efficiency. However, it is characterized by relatively low resolution, shear stress detrimental effects on encapsulated cells, poor interlayer adhesion, and material swelling. Digital Light Processing (DLP) bioprinting, in contrast, offers high-resolution, and light-based fabrication using photo-crosslinkable biomaterials. While promising, DLP is limited by a narrow range of biocompatible photopolymers. Both techniques require advanced bioinks that balance biological compatibility with mechanical and printing requirements.
Collagen based bioinks possess great potential in bioprinting and tissue engineering.
CollPlant has developed a bioink platform for 3D bioprinting of tissues and organs, utilizing a chemically modified plant-derived recombinant type I human collagen (rhCollagen). With its exceptional purity, uniformity and biofunctionality, rhCollagen becomes an ideal building block for biofabrication of tissues and organs. CollPlant’s rhCollagen is fully characterized and does not elicit an immune response.
By utilizing photocurable rhCollagen formulations, it is possible to fabricate scaffolds for production of tissues and organs in a scalable and consistent manner.
Materials and methods
rhCollagen was chemically modified with curable groups to achieve different degrees of functionalization. This enables controlling the physical properties of the printed construct. Various formulations were developed, comprised of functionalized rhCollagen, optionally biocompatible, biodegradable polymers, and printing aids.
These formulations were used as bioinks to support bioprinting technologies such as DLP and extrusion providing extensive rheological properties, low swelling and cell biocompatibility.
Results and discussion
Photocurable collagen based formulations were developed for DLP. Scaffolds printed with such formulations demonstrated optimal physical properties and high resolution. Following printing process, scaffolds were seeded with various cell lines. The scaffolds demonstrated high cell compatibility as evidenced by a high cell proliferation rate.
Collagen-based 'Ready to Use' formulations were developed for extrusion bioprinting, which can be used as bioinks to print with cells. The printed constructs, demonstrated low swelling and convenient handling.
Novel collagen-based bioinks were utilized for printing scaffolds for regenerative medicine, focusing on breast implants. Rheological and mechanical properties of the formulations and the 3D printed models were characterized. The preclinical studies demonstrated progressive formation of natural tissue, neovascularization, and a gradual biodegradation process with no adverse tissue reactions.Top of Form
Conclusions
The unique biological and physical properties of rhCollagen-based formulations provide the flexibility to formulate a broad portfolio of bioinks that are suitable for the production of various tissues and organs. rhCollagen-based formulations can thus offer a superior solution for biofabrication of models and constructs for use in regenerative medicine applications.

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Konuşmacılar: Shirel Cohen (Collplant)

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  Biomaterials and Bioreactor with ISO 13485 Standards – Reliable Tools for Everyday Research

Biomaterials and Bioreactor with ISO 13485 Standards – Reliable Tools for Everyday Research.
One of the biggest challenges in organoid and tissue engineering research is reproducibility. Small changes in bioinks composition or inconsistent bioreactor settings often lead to different results, even when protocols are similar. This slows down projects and makes it hard to compare data across laboratories. Production protocols which undergo strict ISO 13485 procedures can diminish that risk. Our approach is to introduce standardized biomaterials and bioreactor workflows, that make experiments more predictable and easier to repeat, thanks to following strict ISO 13485 procedures. This includes:
Bioinks – based on GelMA, HAMA, CHIMA , dECM and novel recombinant proteins (silk, elastin, resilin - designed to be animal-free), consistent in quality, and supportive for high cell viability.
Perfusion bioreactors – with precise control of CO_2, flow, pressure, nutrient delivery and shear stress, creating conditions that mimic real tissue physiology. Validated calibration protocols ensure that sensor data can be trusted.
With mentioned tools, researchers can create 3D organoids and bioprinted models that are more stable in their mechanical and biological properties. This directly translates

into fewer failed experiments, faster optimization, and easier cross-lab data comparison. In summary, standardized bioinks and bioreactors help scientists save time, reduce costs, and build more reliable models for drug testing, toxicity studies, and disease research.

Konuşmacılar: Maciej Szurek (Polbionica S.A.)

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Multifunctional System for Biofabrication: The BioCloner Health Approach

The rapid evolution of biomedical technologies is transforming both research and clinical practice, creating new opportunities to improve both human and animal health. BioCloner Health develops a multifunctional system for biofabrication that integrates tools, platforms, and processes to bridge the gap between scientific discovery and practical application. The focus is on purposeful innovation and advanced technological solutions that enhance quality of life.

At the core of this approach stands the BioCloner Desktop Pro, a next-generation 3D bioprinter designed to provide researchers and clinicians with a versatile, reliable, and user-friendly platform. The printer enables the fabrication of resorbable implants, tissue models, and other biomedical constructs, supporting applications in regenerative medicine, pharmacology, and translational research. BioCloner Desktop Pro is compatible with a wide range of printheads for processing diverse biomaterials, which allows users to adapt the system to their specific needs. The device is complemented by a dedicated software layer, developed in-house, that provides full control over the process and seamless integration of different material processing strategies. While the hardware itself represents a significant milestone, it functions as part of a broader ecosystem that combines engineering, biology, and clinical expertise to deliver adaptable solutions for diverse healthcare needs.

A distinctive feature of the BioCloner system is its emphasis on interoperability. Technologies are designed to work together in a coordinated way rather than functioning as isolated products. This systemic perspective facilitates the translation of laboratory innovations into practical medical applications and allows for continuous adaptation as biomedical science evolves.

Equally important are the ethical and societal considerations embedded into the development process. Accessibility, sustainability, and inclusivity are treated not as afterthoughts but as fundamental design principles. By integrating these values into the technological framework, the BioCloner approach ensures that progress delivers tangible benefits to individuals, communities, and ecosystems.

This multifunctional perspective demonstrates how strategic vision, technological innovation, and ethical responsibility can be combined into one coherent system. The BioCloner Health approach highlights the potential of biofabrication not only to advance science and medicine but also to shape a more effective, responsible, and human-centered future for healthcare.

Konuşmacılar: Maciej Tabiszewski (BioCloner Health)

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The Role of Standardized Bioinks in Shaping the Future of Biofabrication

Bioprinting has rapidly advanced into a key enabling technology for biomedical research, offering unique capabilities to fabricate complex biological architectures with high precision and adaptability. Its versatility spans a wide range of resolutions, making it possible to construct both large-scale scaffolds and finely detailed microstructures. Central to the success of this approach is the availability of bioinks that are not only functional but also reproducible and standardized, providing reliability and consistency across applications. Such progress has already shown great promise in areas like tissue repair strategies, organ-on-chip technologies, and advanced disease models. Ensuring the development of robust bioinks is therefore critical for translating bioprinting into impactful solutions in regenerative medicine and beyond. This presentation will highlight the significance of standardized bioink systems, with a particular emphasis on gelatin- and poly-ε-caprolactone–based formulations, and discuss their role in advancing the biofabrication field.

Konuşmacılar: Aysu Arslan (BIO INX BV)

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Electrospider series: a significant step towards the practical implementation of multiscale and multimaterial bioprinting.

Bioprinting has made remarkable progress in the fabrication of functional tissues and organ constructs, ushering in a new era of personalized medicine with applications in transplantation, drug testing, research, and disease modeling. Bio3DPrinting has taken a significant step towards clinical translation by introducing Electrospider, a bioprinting ecosystem engineered for versatile multiscale and multimaterial biofabrication. The alignment of this technology with Bio3DPrinting's core values - flexibility, adaptability, and the pursuit of state-of-the-art solutions - is evident in the development of a new product series that adheres to regulations and standards for safety and manufacturing.
Pushing the boundaries of technological advancement, the incorporation of a pioneering multi-tool printhead into the novel Electrospider series marks a transformative leap forward. This multi-tool printhead enables the synergistic use of multiple bioprinting techniques within a single printing session. Combined with advanced management software, it ensures the highest quality and standards in bioprinting complex tissue-like constructs.
The novel Electrospider multitool printhead supports up to five different technologies: a pneumatic extruder, an electrospinning (ESP) tool, a pneumatic melt dispenser, a temperature-controlled pneumatic melt dispenser (range 5 to 40 °C), and a photocuring pneumatic extruder. The synergistic use of these bioprinting technologies enables the processing of a broad spectrum of biomaterials, including hydrogels, photosensitive and/or thermosensitive biomaterials, cell-laden hydrogels, biomolecules, polymeric solutions, and pellets. Furthermore, a suction cup pick-and-place system is integrated into the multitool printhead to automatically seal petri dishes and multiwell plates after bioprinting, preventing scaffold contamination by closing containers with lids.
The innovative multitool printhead operates through a tilting and z-axis translation mechanism that enables the use of one technology at a time and prevents interference from other tools. Its compact, lightweight design preserves the Electrospider’s bioprinting volume and accommodates ESP operations without bulky components. The automated software allows for quick tool changes without user intervention.
By merging these features into a unified ecosystem, Electrospider enables customizable, reproducible, and innovative bioprinting workflows, paving the way for advanced tissue engineering and translational applications.

Konuşmacılar: Ginevra Pegollo (Bio3DPrinting)

- 15:00

Coffee break & Poster session II

- ISBF Early Career Researcher Symposium Kruczkowski

Toplantıyı düzenleyenler: Paulina Nunez Bernal (UMC Utrecht), Philipp Fisch (ETH Zurich)

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New challenges in biofabrication: Starting a research group for cellular agriculture

Standing on the shoulders of a generation of great researchers, our fields of tissue engineering and biofabrication have witnessed an inspiring progress over the past decade. The versatility of innovations and technologies are also creating opportunities for applications and innovations on new research avenues: One of those is cellular agriculture – the animal-free production of dairy and meat products as healthy, ecologically sustainable protein sources and alternative food of the future, by means of tissue engineering and biomanufacturing. Biofabrication principles can play a significant role guiding multi-cellular differentiation in 3D, texture, scalability, and diversity in cultivated meat. Vice versa the biofabrication field can benefit from the ambitions of scale-up, efficiency and cost reduction.
At MERLN, I am currently building an interdisciplinary team for biofabrication and biomaterials in cellular agriculture. During my own shift to food tissue engineering, from lab to desk, and from pipetting to team lead, I have been learning from great mentors and collaborators. I also get to leverage my experience from 3D bioprinting multi-zonal patient-specific tissue substitutes with clinically relevant dimensions and geometries during my PhD (TU Dresden, Germany) and my postdoctoral research of engineered biomaterials, recombinant proteins and diffusion mechanisms to pattern 3D tissue models (Stanford University, USA). Together with my new team, we are working towards the convergence of cellular agriculture and additive bio-manufacturing.
Based on my individual perspective, I am happy to share insights and advice that helped me navigating the academic job market, starting a new research group, and entering a new research field. As scientists, mentors, teachers and people, we all embark on this never-ending learning curve and constant evolution. Aiming to use our skillset towards a real impact for societies, I would like to reflect and discuss crucial points for building resilience, making decisions, seeking advice and leveraging our own individual scientific-personal identities. Let’s all initiate and maintain an open conversation on what new directions and a new generation of interdisciplinary, collaborative biofabrication science can look like – in the lab and beyond.

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Konuşmacılar: David Kilian (MERLN Institute, Maastricht University)

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Building independence and expanding beyond the comfort zone: starting a Research Lab in Europe

In recent years, several new PIs started their groups focused on Tissue Engineering and Biofabrication. Based on our internal discussions, we are all facing the same challenges, such as 1) how to overcome general challenges of the first years while establishing your group; 2) how to create your novel niche as a young PI in such a competitive field; 3) how to balance interactions with previous mentors to have guidance and support without losing independency and identity; 4) how to find space when

applying for funding calls while competing with experienced research leaders. Since January 2024, Dr. Monize C. Decarli has led the Bioprinting and Biofabrication Group at the University Medical Center Groningen/University of Groningen at the Department of Biomaterials and Biomedical Technology (BBT/UMCG, Groningen, Netherlands). Her team works on projects aligned with clinical needs using cutting-edge manufacturing technologies. The group aims to manufacture tissue-like constructs for tissue engineering purposes, which will ultimately act as implants. Likewise, they also manufacture reliable 3D models for drug screening or investigation of tissue formation and pathologies development. Hence, all projects of Dr. Decarli's Lab are closely connected to clinical applications at UMCG and are at the forefront of the tissue engineering field. In her invited talk on Biofabrication 2025, she will discuss the aforementioned challenges, her focus on clinical translation, and many more, sharing her own experience of starting a research group in Europe.

Konuşmacılar: Monize Decarli Decarli

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Leveraging Academic Publishing throughout your career

Academic publishing can be useful in helping to shape your career trajectory, especially in the early stages of research. This presentation offers a practical and strategic overview of how publishing can enhance visibility, credibility, and your professional opportunities. It will explore how to successfully promote your work through social media, academic networks, and institutional platforms as well as how to ensure it reaches the right audiences and has meaningful impact. The session will also highlight the benefits of joining editorial boards, and how to make the most of the opportunities that journal editorial boards provide, such as networking and increasing your personal recognition in the field. Publishing can also impact your career negatively, so we will cover research integrity issues that the field is facing at the moment, what to avoid, what to look for and how you can look out for issues in things you are reading and citing. The publishing landscape is also changing quite rapidly. With large language models and AI on the rise, we will discuss how best to use this to enhance your work, but also what to avoid to ensure your work is adhering to journals ethical policies. Whether you're preparing your first manuscript or planning your longer term publishing strategy for your first big grant, this session will give you an overview of all the things you can keep in mind whilst trying to publish effectively.

Konuşmacılar: Jessica Wheeler

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From BIO INX to BIO Incorporation: The Journey from PhD to Spin-Out

What does it take to transform academic research into a thriving spin-out company? In this talk, Jasper, CEO of BIO INX, shares his journey from PhD research in biomaterials for biofabrication to the creation of BIO INX, a company dedicated to accelerating the translation of biomaterials into practice. He will explore the essential steps along the way—navigating the shift from researcher to entrepreneur, building the right network, identifying funding sources, and aligning with stakeholders who enable innovation beyond the lab. BIO INX's mission is to bring standardization and reliability into the translational pipeline early on, ensuring biomaterials can make real-world impact. This journey highlights both the challenges and opportunities at the intersection of science, entrepreneurship, and innovation.

Konuşmacılar: Jasper Van Hoorick (BIO INX BV)

- S12 Biofabrication of Tissue Interfaces: Bridging the Gap Between Structure and Function Rudniew

Toplantıyı düzenleyenler: Andrew Daly (University of Galway), Felipe Mateus Franco (York University)

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Programming degradation of sacrificial microgels to direct cellular function

Porous scaffolds are fundamental components in the field of tissue engineering and regenerative medicine, serving as essential frameworks that support and guide the growth, attachment, and proliferation of cells. These scaffolds mimic the natural extracellular matrix, providing a three-dimensional environment conducive to tissue development. One of the most critical features of these scaffolds is their porosity, which directly influences nutrient diffusion, waste removal, and cellular migration. The degree and nature of porosity can be finely tuned through various parameters, including the size of sacrificial materials, their volumetric ratios, and the temporal architecture embedded within the scaffold structure. These design elements not only determine the initial physical characteristics of the scaffold but also influence how it evolves over time in response to biological processes.

In this study, we propose that the materials used to create porosity within scaffolds can significantly affect cellular behavior over extended periods. To explore this hypothesis, a flow-focusing microfluidic system was employed to fabricate gelatin-based microgels with precise spatial and temporal control. These microgels were then incorporated into Gelatin Methacryloyl (GelMA) hydrogels to introduce dynamic porosity. This approach allowed for the creation of microporous networks that could evolve over time, facilitating enhanced cell migration and proliferation.
The microfluidic system enabled meticulous control over the size distribution of the gelatin microgels, which is crucial for achieving uniform and predictable porosity within the hydrogel matrix. Furthermore, the degradation rate of these microgels was modulated using dityrosine crosslinks, which were formed through a visible light-induced reaction involving a ruthenium/sodium persulfate co-initiator system. By adjusting variables such as the order of fabrication steps, the concentration of initiators, and the polymer content, we were able to fine-tune the degradation profiles of the microgels, thereby controlling the temporal evolution of porosity within the scaffold.

The biocompatibility of the gelatin microgels was validated through in vitro experiments using human dermal fibroblasts (HDFs) and human mesenchymal stromal cells (HMSCs). Over a seven-day culture period, the microgels supported robust cell adhesion, viability, and proliferation. When these microgels were encapsulated within GelMA hydrogels, they created a delayed microporous structure that significantly enhanced cellular activity compared to bulk GelMA hydrogels without microgels. The composite scaffolds demonstrated superior performance in terms of cell viability, proliferation, and intercellular interactions, highlighting the advantages of incorporating biodegradable microgels into hydrogel matrices.

Overall, this research underscores the potential of using biodegradable gelatin microgels to engineer dynamic, cell-responsive porous scaffolds. Such scaffolds not only provide initial structural support but also adapt over time to meet the evolving needs of regenerating tissues. This approach represents a promising strategy for advancing tissue regeneration by creating more physiologically relevant and responsive biomaterials.

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Konuşmacılar: Khoon Lim (University of Sydney)

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Long-term Cryopreserved Interface-incorporated Microtissues: An Injectable and Printable Solution for Shelf-ready Regenerative Therapies

Introduction
Although cellularized biomaterial constructs have significantly advanced tissue repair and regenerative medicine, their clinical translation remains limited due to labor-intensive preparation processes and inadequate shelf-life. Therefore, creating modular, shelf-ready, cellularized products with long-term preservation capability and immediate clinical utility is critically needed. In this study, we developed an innovative cryopreservable microtissue platform, featuring engineered biomaterial interfaces that support extended cryostorage (over six months). These internal interfaces significantly improve cellular proliferation, and regenerative performance compared with conventional hydrogel constructs. This modular microtissue approach simplifies clinical workflows, enabling rapid, on-demand, injectable or bioprintable applications.

Materials and Methods
We designed modular cryopreserved microtissues utilizing a novel two-step "freeze-then-crosslink" cryo-gelation strategy. Following prolonged cryostorage exceeding seven months, we characterized post-thaw cellular viability, proliferation rates, injectability, and 3D-bioprinting performance. The regenerative potential was validated in vivo through rat models involving subcutaneous implantation and critical-sized skull defect regeneration.

Results
The modular microtissues preserved exceptional cell viability (>85%, human mesenchymal stem cell) after seven months of cryopreservation. Internal biomaterial interfaces significantly improved cellular viability, proliferation, and overall regenerative function, compared to conventional granular mictogels lacking internal interfaces. Specifically, the presence of internal interfaces markedly accelerated biomolecule penetration within the microtissues, achieving over 90% of the peak diffusion density within approximately 10 seconds. In contrast, conventional microgels scaffolds showed substantially slower permeation rates, typically requiring tens of seconds or even minutes to reach approximately 50% of the peak level. Additionally, these modular microtissues were successfully demonstrated as injectable therapeutic formulations and high-resolution bioinks. Their injectability allowed efficient minimally invasive delivery, and precise 3D bioprinting was successfully performed, indicating strong potential for fabricating complex tissues. Animal studies, including subcutaneous implantation and critical-sized skull defect repair in rats, further confirmed that the microtissues effectively promoted tissue regeneration, validating the positive role of the internal biomaterial interfaces.

Discussion
In this study, we address key limitations in current cell-based regenerative therapies by developing an innovative, modular, and cryopreservable microtissue platform. Our design integrates engineered biomaterial interfaces at the microscale, enabling effective long-term preservation with excellent cellular viability and functional outcomes upon thawing. Importantly, this modular approach supports sustained cellular growth across diverse clinical applications, from minimally invasive injections to precision

biofabrication. By significantly simplifying clinical workflows, our platform provides a versatile, accessible, and truly shelf-ready cell therapy solution. This technology holds great promise as a readily available product for both research and clinical applications, particularly in personalized medicine, where patient-derived microtissues can facilitate tailored therapeutic strategies.

Conclusions
In this study, we developed a cryopreserved, interface-incorporated microtissue platform that enables long-term storage, robust cell growth, personalized minimally invasive injection, and rapid on-demand biofabrication for regenerative medicine.

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Konuşmacılar: Chuqian Wang (Tsinghua University)

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An innovative 3D eye in vitro model as an experimental platform for theragnostic: the Tuscany Health Ecosystem approach

Introduction: Retinal pathologies affect more than 350 million people worldwide yearly, impairing visual acuity and quality of life [1-3]. Despite therapy procedures for these pathologies already existing, more effective and patient-friendly ones are the subject of research. Animal models, mainly used in this field, are expensive, time-consuming and present regulatory issues [4]. On the other hand, existing in vitro models lack controlled and reproducible vascularization and micro-macro scale interface [5-6]. For this reason, we propose a biomimetic in vitro model able to mimic the blood-retinal barrier filtration properties and that allows the user to perform drug testing via standard administration routes.

Methods: The model relies on a microfluidic circuit laser-engraved into PDMS, featuring 70-800µm wide and 300µm depth channels functionalized using APTES and gelatin to facilitate cell attachment. It faces electrospun membranes of PLGA or gelatin [7,8], which have already shown good properties for retinal pigmented epithelium cell culture, to mimic choroidal vasculature and Bruch's membrane. These two components are fixed together using an acetic acid-gelatin-based solution. Two 23G cannulas are inserted into the microvascular network input and output, and the assembled component is placed inside a cylindrical PDMS culture chamber. On the top part of the chamber, an alginate-gelatin membrane mimics the corneal stroma, allowing drug injection [9]. Experiments were conducted seeding ARPE-19 cells on the membrane and HUVECs inside the microfluidic channels. Immunostaining techniques were used to address the cell layer proper formation. Antibodies used include ZO-1 to identify RPE tight junctions and CD31 and VE-cadherin to identify HUVECs junctions and specific endothelial markers. Specific drugs as anti-VEGF, were used to perform permeability tests across the membrane to verify the correct functioning of the biological barrier [10].

Results: The device showed the ability of hosting both RPE and HUVECs in both static and dynamic conditions. Moreover, the junctions between cells proved the formation of the biological barrier. The use of specific substances to test permeability of the formed barrier confirmed the active transport performed by the cells.

Discussion: The developed device merges the microstructure of the outer blood-retinal barrier with the macroscopic dimension of the eye. With a reproducible microchannel network mimicking the choroidal vasculature, it overcomes common in vitro model problems. Thanks to its geometry, the device allows the drug testing using standard procedures, like injection, systemic and topical administration, resulting in promising results for the development of in vitro models that can effectively substitute animal models.

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Unlocking natural-based multimaterial 3D printing by engineering the nanocomposite organic/inorganic interface

Unlocking natural-based multimaterial 3D printing by engineering the nanocomposite organic/inorganic interface

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Abstract
Osteochondral defect regeneration remains a clinical challenge with no solution achieving long-term functional restoration. Effective regenerative strategies require hierarchically structured scaffolds that support both bone and cartilage repair. Three-dimensional (3D) printing using natural-based multimaterials and bioactive fillers such as hydroxyapatite or bioactive glasses offer promising approaches for fabricating such biomimetic scaffolds. However, its effectiveness can be limited by inadequate multi-material integration, poor dispersion of bioactive fillers, and suboptimal rheological and mechanical properties of natural-based materials.
In this study, we present an ink engineering approach that enables the 3D printing of nanocomposites (NC), composed of low-viscous natural-derived matrices and bioactive glass nanoparticles. By chemical coupling organic and inorganic phases, we hypothesize to achieve highly reproducible and scalable printability.
We synthesized two photocurable natural matrices: a protein - bovine serum albumin methacrylate (BSAMA), and a polysaccharide - hyaluronic acid methacrylate (HAMA). Bioactive glass nanoparticles (BGNP) were functionalized via aminosilane chemistry. Covalent crosslinking through 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) facilitated homogenous BGNP immobilization, improving rheological properties, dispersion, and printability. EDC/NHS binds carboxylic groups of BSAMA/HAMA and BGNP-grafted amines. Without the phase interaction, the inks were highly heterogeneous and unprintable. Different crosslinker and BGNP amounts were tested. Visible-light photopolymerization (with lithium phenyl-2,4,6-trimethylbenzoylphosphinateensured photoinitiator) was used post-printing to form a mechanically robust construct. The NC's rheological, mechanical, and biological behavior was evaluated. Computational simulation of material properties was performed to validate and predict its applicability.
Our findings demonstrate that the optimized NC inks transform low-viscosity precursors (<1 Pa) into shear-thinning formulations with tunable elastic and viscous moduli (50 - 500 Pa). Post-printing photocured scaffolds exhibited enhanced mechanical stability (1 - 5 kPa) and bioactivity, promoting calcium phosphate deposition in simulated body fluid. In vitro assays with adipose-derived stem cells revealed increased metabolic activity and viability. Notably, BSAMA and HAMA displayed distinct printability, cellular performance, and mechanical properties, which were leveraged for osteochondral regeneration applications. HAMA inks were mechanically more robust, whereas BSAMA inks presented higher cytocompatibility. Further, their seamless integration through photocrosslinkable moieties made them ideal for multi-tissue engineering applications, enabling the obtention of multiple geometries when printing with both materials. Computational simulations validated the performance and feasibility of 3D printed hierarchical scaffolds, confirming clinical relevance.
This work presents a reproducible ink engineering strategy that addressed key limitations of NC inks, derived from low-viscous natural-based biomaterials. This strategy allowed the fabrication of hierarchical, multi-material osteochondral-mimetic scaffolds via extrusion 3D printing.

Keywords: Nanocomposite ink engineering, Extrusion 3D printing, Computational simulation, Osteochondral hierarchical constructs.

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Rapid deep vat printing of tissue interfaces using photoclickable collagen-based bioresins

Introduction:
Deep vat printing (DVP) techniques, such as tomographic (Figure 1A) and filamented light (FLight) (Figure 1B) printing, have advanced biofabrication by enabling high-resolution, layer-free fabrications at unprecedented speeds[1]. Despite, collagen being the most widely used matrix in tissue engineering, its compatibility with DVP remained unexplored. Within this work, we introduce photoclickable collagen-based bioresins for DVP, achieving high printing fidelity (~50 µm), fast throughput (<20 s/cm³), and

excellent biocompatibility as demonstrated by increased integrin β1 (ITGB1) expression in encapsulated C2C12 myoblasts compared to pure gelatin-based resin systems.
Methods:
We synthesized norbornene-functionalized collagen (ColNB), enabling rapid photocrosslinking with thiolated crosslinkers under 405 nm light. As a potential application, we demonstrated multi-material and multicellular DVP using ColNB resins in both tomographic and FLight printing to fabricate facile myotendinous tissue interfaces.

Figure 1. A. Schematic of tomographic printing, enabling the fabrication of entire 3D objects within seconds through volumetric light-exposure. B. Illustration of the FLight biofabrication technique, producing highly aligned filamented constructs. C. Tomographically printed perfusable 3D construct (Scale bar: 1 mm). D. 3D reconstruction of a FLight- construct with microarchitectural analysis (Scale bar: 40 µm). E. Tomographically fabricated muscle–connective tissue constructs after maturation. Immunofluorescence staining for sarcomeric α-actinin (SAA) indicates increased myotube formation in the transition and muscle regions (Scale bar: 500 µm). F. FLight-muscle–connective tissue construct. Myoblasts show highly aligned sarcomere structures with consistent SAA striation and spacing (Scale bars: map: 250 µm; inset: 100 µm; sarcomere detail: 50 µm). G. Quantification of sarcomere spacing in FLight contructs and comparison of myotube diameters.

Results:
Tomographic printing enabled the fabrication of complex, perfusable constructs (Figure 1C) with high spatial control. In contrast, FLight printing yielded high-aspect-ratio constructs with precisely aligned microfilaments throughout the porous gel (Figure 1D). These filament networks acted as structural guidance cues significantly impacting cellular behavior.
While cells aligned in tomographically printed tissues, along stress-induced cues (Figure 1 E), encapsulated myoblasts and fibroblasts within FLight constructs exhibited highly anisotropic cytoskeletal organization (Figure 1F), with ~90% of intracellular f-actin filaments aligned within ±10° of the predominant orientation angle.
Both DVP techniques supported C2C12 myoblast fusion and myotube formation, FLight constructs yielded thicker myotubes (~28 µm) compared to tomographically printed constructs (~16 µm) (Figure 1G). Immunostaining further revealed well-organized sarcomeric α-actinin structures in FLight samples with regular 2.6 µm spacing, a hallmark of contractile function. In contrast, such sarcomere structures were absent in tomographic constructs at identical timepoints.
Additionally, myotendinous tissue interfaces could be fabricated using multi-material printing, showing gradual transitions in MyHC expression and f-actin alignment across zones.

Discussion:
We demonstrate that photoclickable collagen-based resins can be effectively used in DVP for the fabrication of complex, multicellular tissues. Compared to widely used gelatin-based resins (e.g., GelMA, GelNB-GelSH), ColNB offers enhanced bioactivity and ECM-like composition. Our results highlight the potential of collagen-derived materials in advancing DVP-based tissue engineering, especially in applications requiring aligned, functional muscle-like tissues.

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  Hyaluronic Acid-Based Bioink for Cryobioprinting of Anisotropic Neural and Neuromuscular Tissue Constructs

  The structural alignment of cells is a hallmark of functional neural and neuromuscular tissues. However, replicating this anisotropic architecture in engineered three-dimensional (3D) constructs remains a significant challenge in tissue engineering. In this study, we introduce a cryobioprinting strategy capable of fabricating aligned, multi-cell-type scaffolds for modeling neural tissues and neuromuscular junctions (NMJs).
  We developed a hyaluronic acid methacrylate (HAMA)-based cryobioink, incorporating gelatin methacryloyl (GelMA) and the cryoprotectant melezitose, to maintain high cell viability during the extreme freezing and thawing conditions of cryobioprinting and to improve the biomimetic aspect of the neural tissue model. Utilizing a vertical cryofabrication process, we engineered anisotropic scaffolds featuring aligned microchannels that guide the spatial orientation of embedded neural and muscle cells.
  The cryobioink formulation was optimized to maximize post-printing cell survival. Constructs fabricated with the optimized bioink supported up to 95% viability for neural cells, with similarly high viability observed for myoblasts after seven days in culture. Unlike traditional methods where neural cells are seeded onto scaffolds post-cryofabrication, our approach enables direct encapsulation and alignment of cells during the printing process, allowing for precise spatial positioning within the scaffold.
  The microchannel size was tunable by adjusting freezing temperatures and cryoprotectant composition. Following co-culture and differentiation, the constructs exhibited not only sustained high cell viability and alignment, but also functional features of NMJs, such as acetylcholine receptor clustering at the muscle–neuron interface.
  Furthermore, we utilized a vertical cryobioprinting setup with coaxial nozzles to fabricate complex, multi-segmented structures. This configuration enabled the creation of freestanding constructs featuring distinct bioink compositions and consistently aligned microchannels across each segment, providing precise spatial control and enhanced structural design.
  Beyond demonstrating a robust method for fabricating anisotropic neural and neuromuscular tissue constructs, this work highlights hyaluronic acid's role as a functional cryoprotectant. The cryo-printed constructs also exhibited enhanced resistance to swelling and degradation, which is crucial for preserving the fidelity and long-term integrity of the anisotropic scaffold.
  In summary, our cryobioprinting approach and optimized HAMA-based bioink provide a versatile and effective platform for creating 3D neural and neuromuscular tissue models with structural and functional relevance (Figure 1). This strategy paves the way for the development of shelf-stable, anisotropic tissue constructs for applications in disease modeling, drug screening, and regenerative medicine.

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  Broadly accessible 3D in vitro skin model as a comprehensive platform for antibacterial therapies screening

  Background:

  The global rise of skin infections, exacerbated by antibiotic-resistant bacteria, is nowadays a great challenge to healthcare. Traditional treatments are increasingly losing their potential, leading to severe impacts on patient quality of life and survival rates. Consequently, many efforts are made to develop innovative antibacterial therapies. To test this new strategies, conventional methods, such as 2D cell culture and planktonic bacteria culture, often fail to replicate the complexity of infection environments, highlighting the potential of 3D in vitro skin models as a superior alternative.

  Methods:

  The initial focus of the study has been the development of a 3D in vitro skin model. The dermis compartment was biofabricated by embedding human fibroblasts in a gelatin methacryloyl matrix, with human keratinocytes seeded on top the dermis to form the epidermis. The models were cultured for 31 days in an air-liquid interface configuration. The model demonstrated robust barrier formation and extracellular matrix remodelling. A 3 mm biopsy puncher was used to simulate controlled wounding, followed by inoculation of Staphylococcus aureus (Gram-positive) or Escherichia coli (Gram-negative) to mimic infection. Antibiotic treatment was applied after 24 hours. The samples were subsequently analysed for bacterial survival and skin model responses.

  Results:
  Results were remarkable: differences between wounded and unwounded samples underlined the barrier effect: indeed, the bacterial proliferation was lower in unwounded samples, for both bacterial strains, meaning that the developed epidermis was able to partially stop the bacterial proliferation. Moreover, the 3D skin model was able to react to both wound and infection in a complete and complex way in terms of extracellular matrix deposition and remodeling, inflammatory response, antimicrobial peptides production and change in cellular behaviors, from epithelial to mesenchymal and from fibroblasts to myofibroblasts. Another important outcome was the change in skin response during infection, showing the ability of both bacteria, in different ways, to impair the model immune response. Also, the antibiotic interacted with the model, modulating some markers, giving some evidences of the release of endotoxins with Escherichia coli death.

  Conclusions:
  In conclusion the developed 3D in vitro skin model has the potential to become a future landmark as platform for infection investigation and novel therapies testing. Indeed, it

demonstrated to be able to behave in a complex and complete way despite being easily producible and low cost. These properties put it in a prominent position for future standardization of platforms to test antibacterial therapies and strategies.

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Embedded 3D Bioprinting of Perfusable Endothelialized Microchannels for Blood-Brain Barrier Model

The blood–brain barrier (BBB) is a highly specialized neurovascular interface that regulates the selective exchange of molecules between the bloodstream and the central nervous system, playing a critical role in maintaining brain homeostasis (Cruz et al., 2023). Despite advances in in vitro modeling, many existing BBB models lack the structural and functional complexity necessary to fully replicate biomimetic structure. In this study, we propose an embedded 3D bioprinting approach to develop constructs with perfusable endothelialized microchannels to recapitulate the biomimetic structure of the BBB. Our strategy employes high-resolution extrusion-based printing of a sacrificial bioink within a neural cell laden supportive and biocompatible hydrogel (Afghah et al., 2020). The removal of the sacrificial bioink from the crosslinked construct enables the formation of perfusable endotheliazed microchannels, mimicking brain capillary structures. This approach offers a promising platform for applications in drug screening, neurodegenerative disease modeling, and translational neuroscience research.

MATERIALS AND METHODS

A vascularized structure was digitally designed and printed within a biocompatible hydrogel matrix. This supportive matrix, composed of Gelatin Methacryloyl (GelMA) was modified with Xanthan gum to improve the shear-thinning property. A 20% Pluronic F127 (PF127) solution served as sacrificial ink for printing vascularized microchannels. Following the embedded bioprinting process within a GelMA support matrix, the construct was crosslinked under UV light to stabilize its structure. The sacrificial PF127 ink was subsequently removed to create perfusable microchannels. These channels were then perfused with endothelial cells to develop biomimetic endothelialized microchannels. Live/dead assays and structural imaging confirmed both the biocompatibility and fidelity of the endothelial microchannels, demonstrating the potential of the construction for BBB modeling.

RESULTS AND DISCUSSION

Embedded 3D bioprinting technology allowed development of constructs with perfusable microchannels. The hollow channels within the construct remained intact and were effectively seeded with endothelial cells. Live/dead staining confirmed high viability of endothelial cells within the perfused microchannels. Confocal microscopy images revealed well-defined channel architecture and continuous endothelial cell lining. The GelMA hydrogel maintained structural stability while providing a biocompatible matrix for cell growth and neural differentiation.

CONCLUSIONS

The proposed embedded 3D bioprinting approach enabled the development of the constructs with perfusable endothelialized microchannels that effectively recapitulate the biomimetic structure of BBB in a brain-biomimetic structure. This advanced model demonstrates significant potential for enhancing 3D bioprinted blood-brain barrier platforms in neurovascular research and pharmaceutical screening applications.

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- S15 Bioprinting approaches in the EU projects Goethe

Toplantıyı düzenleyenler: Giovanni Vozzi (University of Pisa, Italy), Ayça Bal Öztürk (Istinye University and ADBioink, Turkey)

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Clusters as tools for enhanced collaboration and increased impact of Horizon Europe projects

Konuşmacılar: Giulia Lionetti

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ILLUMINATING in situ bioprinting for joint regeneration

The LUMINATE consortium develops a personalized, one-stage regenerative approach to target large osteochondral lesions, thus preventing post-traumatic osteoarthritis and the associated patient burden. The LUMINATE consortium aims to develop an in situ bioprinting unit, called EndoFLight, which combines three pinting methods (filamented light, micro-extrusion, jetting) to print cartilage and bone photoresins laden with patient-derived cells directly at the site of injury. EndoFLight uses filamented light to bioprint highly architected scaffolds with excellent cell guidance properties. The LUMINATE approach represents a synergy between material development and characterization, custom printer software and hardware and engineering analyses of the mechanical and flow properties of the scaffolds. At the conclusion of the project, the clinical workflow will be validated through GMP processes and a in a large animal pre-clinical model. LUMINATE strives to develop in situ bioprinting therapies which will lessen patient suffering from osteochondral lesions and will pave the way for socioeconomic advantages for our aging society

Konuşmacılar: Marcy Zenobi-Wong (ETH Zurich)

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The m2M project: A bioprinting platform for the rapid, reliable, controlled and quantifiable patterning of cellular aggregates and microtissues into macroscale regenerative grafts with programmable architectures.

A fundamental limitation with current approaches aiming to bioprint tissues and organs is an inability to generate constructs with truly biomimetic composition and structure, resulting in the development of engineered tissues that cannot execute their specific function in vivo. This is perhaps unsurprising, as many tissues and organs continue to mature postnatally, often taking many years to attain the compositional and structural complexity that is integral to their function. A potential solution to this challenge is to engineer tissues that are more representative of an earlier stage of development, using bioprinting to not only generate such constructs, but to also provide them with guiding structures and biochemical cues that supports their maturation into fully functional tissues or organs within damaged or diseased in vivo environments. It has recently been demonstrated that such developmental processes are better recapitulated in ‘microtissues’ or ‘organoids’ formed from self-organizing (multi)cellular aggregates, motivating their use as biological building blocks for the engineering of larger scale tissues and organ. The main goal of micro2MACRO (m2M) is to develop a new bioprinting platform capable of spatially patterning numerous cellular aggregates or microtissues into scaled-up, personalised durable load-bearing grafts and guiding their (re)modelling into fully functional tissues in vivo within damaged or diseased environments. This will be achieved using a converged bioprinting approach capable of rapidly depositing cells and microtissues into guiding scaffold structures with high spatial resolution in a rapid, reliable, reproducible and quantifiable manner. These guiding structures will then function to direction the fusion and remodelling of cellular aggregates and microtissues into structurally organised tissues in vitro and in vivo, as well as providing medium-term (3-5 years) mechanical support to the regenerating tissue. This talk will summarise the main goals of the m2M project and provide an insight into early results obtained by the consortium.

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Innovative in situ 4D bioprinting for regeneration of colorectal mucosa and submucosa

Colorectal diseases are a cohort of pathologies that affect the mucosa and submucosa layers of the anus, rectum, and colon of more than 2 million individuals in the European Union [1]. Among them, familiar adenomatous polyposis (FAP) and ulcerative colitis (UC) seriously compromise the patients’ quality of life. These pathologies could

benefit from the removal of the intestinal mucosa and submucosa, however no strategies exist nowadays for their replacement. Then, when their removal is necessary, the patients undergo a proctocolectomy (i.e., surgical removal of the rectum and all or part of the colon) with a subsequent ileal pouch. Although preserving the patient's continence, the procedure is burdened by significant complications [2].
Thus, in this context of the EU funded projects “TENTACLE”, we envision a radically new strategy for the surgical treatment of UC and FAP, by developing an innovative in situ 4D bioprinting suite to fabricate a shape-morphing layered bioconstructs directly inside the patient rectum/colon, able to replicate the human colorectal mucosa and submucosa (Figure 1). This bioconstructs will promote the regeneration of colorectal mucosa and submucosa thanks to its composition, and the delivery of specific active pharmaceutical ingredients, such as antibiotic and antifibrotic. Moreover, the mucosa layer will possess a shape morphing capability, triggered by humidity, that will induce the creation of crypts on its surface, mimicking the colorectal ones.
TENTACLE in situ 4D bioprinting suite will comprise a first-of-its-kind colonoscopic bioprinter that will be inserted on commercial colonoscopes and will feature an extrusion-based bioprinting unit, a valvejet printhead, and a photocrosslinking apparatus. Multiple artificial intelligent empowered in silico tools will be developed to improve the performance the bioprinting suite. More in detail, an algorithm able to build the bioconstruct’s geometry on the patient defect starting from patient medical image will be developed. Then, a second algorithm for the path planning, namely the ideal distribution of ink, will be developed. Final, an algorithm to real-time monitor the in situ bioprinting process via image analysis will be implemented.
Regarding the inks, two novel bioink formulations containing patients’ cells will be developed, based on photocrosslinkable gelatin and thiolated polysaccharides. Those bioinks will be enriched with engineered micro- and nano-carriers incorporating antibiotic and anti-fibrotic agents.
The entire in situ 4D bioprinting procedure will be validated ex vivo and in vivo, thus paving the way for translating the bioprinting suite toward the clinics.
Collectively, TENTACLE will introduce a minimally invasive alternative to proctocolectomy and is expected to have a high impact on the quality of life of patients affected by FAP and UC.

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3D Bioprinting with Self-Healing, Photocurable Supramolecular Hydrogels without Photo-Initiators

Introduction
Hydrogels are three-dimensional networks of polymers that can absorb and store large amounts of water. Conventional hydrogels are based on static covalent crosslinks, which limits their adaptability to varying mechanical loads and environmental conditions. In supramolecular hydrogels the chains in the network are connected by reversible physical bonds that confer viscoelastic properties to the hydrogels. These hydrogels can be synthesized to include both covalent and dynamic bonds, enhancing their mechanical adaptability. Self-healing, shear-thinning and photocurable hydrogels can be synthesized by forming inclusion complexes between cyclodextrin (CD) host molecules and benzophenone and/or adamantane guest molecules. The inclusion complex formed between CD and benzophenone facilitates the formation of covalent bonds upon UV irradiation, eliminating the need for photoinitiators and resulting in robust hydrogels. This study describes the synthesis of these novel hydrogel materials using dextran as the backbone and provides a detailed evaluation of their physicochemical and biological properties for 3D bioprinting (Figure 1)

Methods
The chemical structures of the modified dextrans were validated by NMR and FT-IR spectra. Hydrogels were formed by mixing the modified dextran solutions and UV irradiation at a wavelength of 365 nm. The rheological properties were systematically evaluated using a rheometer, varying the parameters of chain length, anchor point ratio, solution concentration and hydrogel composition. Printability was tested using an extrusion bioprinter and examined using optical microscopy. Cytotoxicity was determined using a fluorometric assay.

Results
The chemical structures of the modified dextrans were confirmed by characteristic peaks in FT-IR spectra. The degree of substitution, calculated from NMR spectra, was used to determine the extent of modification of the dextran chains, which directly affected the properties of the hydrogels. The viscosity of the hydrogels increased drastically after mixing the different components. The hydrogels exhibited dynamic rheological properties, which could be efficiently adjusted by varying different parameters. The hydrogels were completely cured within 10 seconds by 365 nm UV treatment. The printability of the hydrogels was satisfactory, as shown by tests with a bioprinter. In contrast to GelMA, bioprinting did not require temperature control or a photoinitiator. In vitro tests showed negligible cytotoxicity of the hydrogels, indicating good biocompatibility.

Discussion
The novel supramolecular hydrogels exhibit tunable mechanical properties, making them suitable as a matrix for bioinks to modulate mechanical properties based on different cell types. Since these hydrogels do not require a photoinitiator, they also demonstrate enhanced biocompatibility. In conclusion, these hydrogels have significant potential for bioprinting applications in tissue engineering.

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LUMINATE: lighting the way towards in situ osteochondral regeneration through microextrusion and filamented light bioprinting

Traumatic injuries to the osteochondral tissues of diarthrodial joints like the knee result in pain, functional impairment, and increased risk of developing post-traumatic osteoarthritis (PTOA) and its comorbidities. Strikingly, up to 50% of patients suffering from severe trauma to the knee joint develop PTOA within 10 years from the injury. Current treatments, based on allografts, cell-free grafts or cell-based therapies, are expensive and often with limited availability, ultimately leading to total arthroplasty to relieve the pain and restore function. However, the risk of revision of these implants is unacceptably high in young active patients, predicting a looming epidemic of revision surgeries as these implants start to fail. In this context, there is an urgent need for better therapies to treat the original lesion and prevent its progression to OA. The LUMINATE project proposes a personalized, one-stage regenerative approach to target large osteochondral lesions, thus preventing PTOA development and avoiding costly and invasive arthroplasty surgeries.
LUMINATE will develop a next level in situ bioprinting unit, called EndoFLight, which combines three multimaterial and multiscale toolheads (i.e., micro-extrusion, filamented light, jetting) to print bone and cartilage photoresins laden with allogenic and patient-derived cells directly at the site of injury. Composite photoresins will be based on photo-crosslinkable gelatin and their properties will be extensively fine-tuned to match both the processability, mechanical and biological requirements. EndoFLight will use filamented light to bioprint highly architected scaffolds with excellent cell guidance properties in seconds, resembling the natural organization of the native tissues (i.e., Benninghoff arcades) and enhancing the regeneration process. Together with micro-extrusion and jetting, EndoFLight will enable the deposition at different scales of multiple biomaterials, biomolecules (e.g., BMP2, GDF5) and cell types with light-assisted crosslinking of complex structures directly in vivo in a minimally invasive manner. EndoFLight will feature an optical sensor for segmenting the defect and predicting the correct volume of materials to deposit leveraging Artificial Intelligence and will be compatible with commercial arthroscopic instruments (i.e., rigid trocar for insertion), thus enabling a minimally invasive surgical procedure.
The EndoFLight bioprinted construct will be extensively characterized in vitro and through explant testing (in vitro and in a mouse model), while the bioprinting suite (i.e., cell-laden photoresins, EndoFLight) will be validated for usability in phantoms and for biological performance in a human-relevant preclinical large animal model, paving the way for its clinical translation after the end of the project. The exploitation of the results will be ensured through market analysis, the foundation of a spin-off that will commercialize the project results, the analysis of regulatory aspects of all components of the bioprinting suite and the adaptation of the protocols to Good Manufacturing Practices. Overall, LUMINATE will ensure wide-spread health benefits to the patients suffering from these lesions and will pave the way for enormous socioeconomic advantages for our aging society.

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Konuşmacılar: Giovanni Vozzi (University of Pisa)

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Automated generation of dense, functional, and perfusable bioprinted liver constructs for transplantation

Organ transplantation remains the only curative treatment for patients with end-stage liver disease, yet the chronic shortage of donor organs leaves thousands of patients without timely access to life-saving therapies. Approximately 25% of patients on the liver transplant waiting list either die or deteriorate beyond eligibility. To address this critical gap, the NEOLIVER project aims to develop a scalable, automated, and GMP-compliant platform for the generation of dense, functional, and perfusable bioprinted liver constructs as a potential alternative to organ donation.

Our project focuses on the integration of adult liver stem cell-derived organoids with supporting mesenchymal and endothelial progenitor cells to form multicellular spheroids that recapitulate the complexity of native liver tissue. These spheroids will be produced in large quantities using novel high-throughput microwell platforms and expanded under controlled, xenogeneic-free bioreactor conditions. To meet the clinical threshold of 10–20 billion cells for partial liver function restoration, we are optimizing protocols for mass expansion, differentiation, and cryopreservation that conform to GMP standards.

The spheroids will be encapsulated in synthetic, cell-degradable hydrogels and bioprinted onto a vascular bed using Laser-Induced Forward Transfer (LIFT) technology, enabling high-density deposition with micrometer precision. The printed constructs will include sacrificial microgels to support microvascularization and will undergo maturation in a perfusion bioreactor. In later stages, constructs will be surgically implanted and evaluated in a large immunodeficient pig model to assess perfusion, engraftment, and function.

By combining automation, precision bioprinting, and advanced cell manufacturing, NEOLIVER aims to establish the first modular production line for transplantable liver constructs. This platform has the potential to reduce dependence on donor organs and pave the way for personalized, cell-based therapies in liver disease. The outcomes of this project will also inform broader applications in tissue engineering and regenerative medicine across other organ systems.

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Traceable and verifiable design inputs for AI-powered medical devices: Insights from the LUMINATE projects

Background
Biofabrication technologies are advancing rapidly, offering novel therapeutic strategies for regenerating complex tissue defects. The LUMINATE project, co-funded under the Horizon Europe programme, focuses on the development of an AI-powered bioprinter for intraoperative repair of osteochondral tissue. The device combines high-precision material deposition and AI-powered process control to enable point-of-care clinical interventions. Current EU regulatory frameworks,such as the Medical Device Regulation (EU) 2017/745 and Artificial Intelligence Act, provide both regulatory requirements and a methodological blueprint for ensuring safety, performance, and transparency. Within LUMINATE, these frameworks have guided the implementation of robust traceability and verifiability strategies that underpin both regulatory compliance and clinical effectiveness and guide the design process.
A structured approach to traceable and verifiable design inputs
Within the LUMINATE project, we propose a systematic regulatory methodology for capturing and managing design inputs in a high-risk, AI-enabled, biofabrication medical device. Our approach is based on compliance to ISO 13485 and applies design control principles.
We propose an Integrated design traceability matrix to enhance traceability and verifiability. It systematically lists core design inputs such as clinical requirements, usability requirements, safety requirements, user needs. This explicit mapping then connects the listed design inputs to design outputs, such as biomaterial specifications, device parameters, and AI functionalities, ensuring traceability of decisions.
Structured documentation regarding verification testing enables verifiability, allowing confirmation of risk mitigation strategies, supporting MDR Annex II and AI Act Article 9.
We recommend establishing comprehensive documentation protocols for all AI modules within the LUMINATE suite. This includes detailed records of datasets and records of the design process (model architectures, training-validation processes, and generalization assessments), in alignment with AI Act Articles 10 and 11. Such documentation enhances verifiability by enabling independent assessment of dataset quality, model validity, and reproducibility of performance of the model, consistently meeting clinical and safety expectations.
We advocate systematic integration of international standards to support robust, interoperable system design. These standards enhance traceability through harmonized formats and procedures across hardware, software, and data workflows. They also ensure verifiability by providing benchmarks and tests enabling external reviewers to assess device performance reliably. This approach supports compliance with AI Act Article 15. All planned verification tests are designed to demonstrate conformity with pre-identified standards.
To further improve effectiveness of the design process, we propose embedding iterative co-design processes involving clinicians, biomaterials experts, engineers, and regulators from early development stages. This strategy operationalizes AI Act Article 14 on human oversight, ensuring that human control and interpretability are integrated into the design of AI-assisted bioprinting workflows.Stakeholder engagement improves traceability by linking design to clinical needs and verifiability through documented, validated user input.
Conclusion
The LUMINATE project proposes a replicable methodology to establish traceable, verifiable, and regulatory-compliant design inputs in AI-powered medical devices intended for intraoperative application. By aligning with EU MDR, MDCG guidances, and the AI Act, our approach offers a pathway to de-risk clinical translation and facilitate regulatory approvals. By advancing systematic traceability and verifiability frameworks, we aim to strengthen the reliability and accountability of next-generation biofabrication solutions.

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Advanced 4D biomaterials for mucosa and submucosa treatment in patients affected by colorectal diseases

Colorectal diseases refer to a range of conditions that affect the mucosal and submucosal layers of the anus, rectum, and colon, impacting over 2 million individuals across the European Union [1]. Among these, familial adenomatous polyposis (FAP) and ulcerative colitis (UC) significantly reduce the quality of life of affected patients. While these conditions might benefit from removing the affected intestinal layers, no current solutions exist to replace them. As a result, when removal is necessary, patients typically undergo a proctocolectomy, which involves the surgical removal of the rectum and part or all of the colon, followed by the formation of an ileal pouch.
In this context, the EU-funded project “DAEDALUS” aims at pioneering a novel approach, which combines advanced biomaterials featuring four-dimensional (4D) functionalities (i.e., triggered shape morphing and controlled molecule/drug release) with an innovative delivery system, to in situ reconstruct colorectal mucosa and submucosa. Two injectable biomaterial formulations able to respectively mimic the colorectal mucosa and submucosa will be developed. Both biomaterials will be based on the same photo-crosslinkable biocompatible matrix: star polyethylene glycol - polylactic acid polymers or poly(2-oxazoline)s, mixed with cell-laden photo-crosslinkable gelatine-based solutions. The biomaterials will contain patient-derived cells and will be enriched with magnetic and engineered particles to allow the shape morphing of the structure and a controlled release of drugs/molecules, such as oxygen, growth factors, glucose and anti-fibrotic drugs.
To translate the DAEDALUS concept into a minimally invasive procedure, a multifunctional toolhead compatible with commercial endoscopes will be designed and engineered for in situ biomaterial injection, actuation and crosslinking. The validated advanced materials and methods are intended to be used for clinical applications requiring mucosa and submucosa regeneration; their development will follow the Safe-by-Design (SSbD) approach with the support of in silico models. Additionally, engagement with patients, researchers, professionals, and stakeholders will be promoted to improve the level of understanding of the DAEDALUS solution.
DAEDALUS biomaterials and bioprinting process will be validated both ex vivo and in vivo, setting the stage for translating 4D biomaterials with advanced functionalities into clinical applications.
In summary, DAEDALUS will offer a minimally invasive alternative to proctocolectomy and is anticipated to significantly improve the quality of life for patients suffering from FAP and UC.

Acknowledgment: This project has received funding by the European Union under the call HORIZON-CL4-2024-RESILIENCE-01-36 (acronym: DAEDALUS, number: 101178568).

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- S16 Bioprinting vasculature Ratuszowa

Toplantıyı düzenleyenler: Shulamit Levenberg (Technion), Riccardo Levato (University Medical Center Utrecht)

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Vascular Bioprinting

Vascularization is critical for successful tissue regeneration, as it ensures adequate oxygen, nutrient delivery, and waste removal within engineered constructs. Without a functional vascular network, large or complex tissues are prone to necrosis and poor integration with host tissues. Rapid and stable vascular ingrowth is therefore essential for the survival and functionality of regenerated tissues. To this end, 3D bioprinting offers powerful tools to address vascularization challenges by enabling the spatially controlled deposition of cells, biomaterials, and growth factors. It allows the fabrication of pre-designed vascular networks or microchannel architectures that can guide endothelial cell organization and facilitate perfusion. These strategies not only improve tissue viability but also promote integration with host vasculature, making 3D bioprinting a promising approach for engineering thick, vascularized tissues for regenerative medicine. This talk will specifically discuss our recent efforts in developing a series of advanced vascular bioprinting strategies that tackle some of these problems and improve their capacities towards diverse applications in biomedicine. These platform technologies will likely provide new opportunities in areas from constructing functional tissues and microtissue models for promoting personalizable medicine, all the way to minimally invasive surgical implications.

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Engineering Vasculature: Build, Learn, Repeat

We are attempting to rebuild tissue-like structures from cells as an approach to better understand development, physiology and disease. Here I will describe our recent work in engineering perfusable vasculature to understand the complex interplay between architecture, forces, signaling, and cellular adhesions in regulating vessel formation, function, and dysfunction. The presentation will highlight the importance of novel interdisciplinary approaches to advance the field of vascular tissue engineering, and ultimately, how these technologies will provide avenues to advance regenerative medicine.

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Aqueous Two-Phase Enabled Low Viscosity 3D (LoV3D) Bioprinting of Living Matter with dilating, tunable, and hierarchical channel networks.

INTRODUCTION: Embedded bioprinting is a promising additive manufacturing technique permitting the fabrication of large-scale, freeform, complex 3D tissue constructs. During the development of new (bio)inks, the shape-stability of extruded strands plays a major role. To stabilize strands post-extrusion, while allowing for smooth extrusion with limited pressures, shear thinning inks are commonly employed. For these inks, printing resolution is dictated by nozzle diameter[1], while shear stresses within the nozzle during extrusion determine cell viability[2], which limits bioprinting resolution and speed. Here, we report on a low viscosity 3D (LoV3D) liquid printing approach, which is enabled by the innovative use of an aqueous-two phase system (ATPS). LoV3D allows for highly rapid bioprinting with high cell viability, while allowing for continuous on-the-fly tuning of filament diameter.

METHODS: For 3D printing of several inks, a spatially controlled liquid extruder was combined with a programmable syringe pump to allow flow rate and print speed control. Aqueous two-phase system stabilized 3D bioprinting of low viscous materials in combination with supramolecular complexation of multivalent biotin-avidin bonding was explored to biofabricate intricate, perfusable engineered living materials that are both mechanically and chemically tunable in a single-step manner. To this end, Dextran-Tyramine-Biotin polymer was synthesized and utilized as a highly versatile backbone, and its ability for mechanical and chemical tuning during as well as after printing was investigated. Furthermore, the ability of of LoV3D was to form vascular tree-like hierarchical channel networks via seamless multi-branching as well as integration with monolithic annealed microtissue networks was investigated.

RESULTS: LoV3D allowed for printing of living constructs at high speeds (up to 1.8 m s-1) with high viability due to its exceedingly low viscosity (mPa·s range). Moreover, LoV3D liquid/liquid interfaces offer unique advantages for fusing printed structures, creating intricate vasculature, and modifying surfaces at a higher efficiency than traditional systems. Furthermore, the low interfacial tension of LoV3D bioprinting offers unprecedented nozzle-independent control over filament diameter via large dimension strand-thinning, which allows for printing of an exceptionally wide range of diameters down to single cell widths. Moreover, leveraging biotin-based supramolecular complexation allowed for biofabrication of intricate, perfusable living matter that was mechanically and chemically tunable in a single-step manner. Functionalized channels could be printed directly into syringes containing crosslinkable polymer solution, which remained stable upon ejection thus allowing minimally invasive delivery of (pre)vascularized perfusable living matter. Tuning the bulk's mechanical properties facilitated reversible dilation under physiologically relevant (blood)pressures, which improved nutrient supply to surrounding tissues and mechanically stimulated perivascular tissues via natural hydraulic actuation. Lastly, combining LoV3D printed large channels with annealed engineered microtissues containing perfusable open, interconnected, continuous pore networks yielded hierarchical vascular tree-like channel networks. Advantageously, this produced engineered living matter with a highly dense capillary-like pore space that minimized diffusive distances below 100 micrometer regardless of construct size.

DISCUSSION & CONCLUSIONS: LoV3D bioprinting is introduced as a highly versatile and rapid bioprinting technique, which is inherently compatible with a wide range of biomaterials and crosslinking strategies, which are here explored for the creation of perfusable multiscale vascular-like channel networks.

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Konuşmacılar: Jeroen Leijten (University of Twente)

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Promoting microvasculature development in 3D-bioprinted tissues using saRNA-engineered cells

While three dimensional-bioprinting has already gained global attention as a rapidly advancing field, the creation of properly vascularized tissues remains challenging. Extrusion-based bioprinting is specifically interesting for the fabrication of large tissues, but has a limited resolution. Therefore, with extrusion-based bioprinting, microvasculature formation through self-assembly is currently being explored. In this research project, the potential of self-amplifying mRNA (sa-mRNA) engineered cells in promoting microvascular self-assembly was explored. Sa-mRNA is especially interesting due to its prolonged activity compared to other synthetic mRNAs. A DNA template for VEGF-producing self amplifying mRNA (VEGF-sa-mRNA) was generated by inserting the VEGF165A sequence into a Venezuelan Equine Encephalitis Virus (VEEV)-derived saRNA template plasmid using NEBuilder® HiFi DNA Assembly (NEB). Following linearization, in vitro transcription, and capping of the VEGF-sa-mRNA, cells were transfected with Lipofectamine® MessengerMAX reagent (Invitrogen). First, the VEGF production by the transfected cells was verified together with its functionality. This was done by incubating a co-culture of human adipose stem cells (ASCs) and human umbilical vein endothelial cells (HUVECs) in endothelial medium supplemented with the conditioned medium of baby hamster kidney cells (BHKs), transfected with the VEGF-sa-mRNA. A 7 day incubation in the supplemented medium resulted in significantly higher network length compared to negative control. In a follow-up experiment, a novel scaffold consisting of a recently reported porous bioink was bioprinted1. Within this scaffold, ASCs/HUVECs spheroids were combined with VEGF-sa-mRNA transfected BHKs. After 7 days in culture, vascular spheroids in this construct showed a significant increase in capillary sprouting compared to a negative control lacking the transfected cells. In the context of clinical translatability of the VEGF-sa-mRNA system, transfection of human ASCs was explored as well. More specifically, the ability of an additional cellulose purification step in removing double stranded RNA strands was investigated to reduce the cellular innate immune response and increase sa-mRNA activity. Additionally, the effect of transfection duration on the viability and protein expression of ASCs was analysed. Finally, the VEGF production by these transfected ASCs was monitored across different time periods using ELISA. Cellulose purified sa-mRNA resulted in a significant increase in protein production compared to non-cellulose purified sa-mRNA. A Transfection duration limited to 30 mins was superior in both viability and protein expression. A more detailed analysis using ELISA demonstrated that VEGF production starts as early as 2 hours post-transfection, reaching its peak at 18 hours (59,49 ng/mL, produced by 40,000 ASCs). In summary, this research project took the first steps towards evaluating the potential of sa-mRNA engineered ASCs in stimulating the self-assembly of microvasculature. Future prospects include evaluating the functionality of VEGF produced by transfected ASCs within a bioprinted scaffold. The established VEGF-sa-mRNA system could play a crucial role in applications within regenerative medicine and patient-specific 3D-bioprinted organ models.

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Multiscale and Multimaterial Vascular Bioprinting via Advanced Light-Based Approaches

The fabrication of biomimetic, multiscale, and multimaterial vascular networks remains a central challenge in tissue engineering and organ fabrication. We introduce an advanced light-based bioprinting framework that combines three advanced methodologies - Holographic Optical Tweezers Bioprinting (HOTB), multimaterial Digital Light Processing (DLP), and volumetric DLP bioprinting - to overcome the challenges of multiscale and multimaterial vascular fabrication. The HOTB based methodology enables precise, dynamic manipulation of individual cells and microgels within a photo-crosslinkable matrix, allowing for the active spatial organization of heterogeneous cellular components and microscale features. Complementarily, a custom multimaterial DLP platform facilitates rapid layer-by-layer polymerization of distinct bioinks through wavelength-selective photopolymerization, enabling spatially resolved deposition of materials with diverse mechanical and biological functionalities. Volumetric DLP complements these by enabling ultrafast, continuous fabrication of large-scale, perfusable vascular networks without layering artifacts. Together, these approaches offer a powerful, synergistic platform for generating hierarchically organized, cell-rich, and functionally zoned vascular systems.

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Harnessing Microfluidic Bioprinting for Brain Tissue Vascularization

Introduction

Tissue engineering aims to develop functional tissues for regenerative medicine. To achieve this, vascularization is essential, particularly in thick constructs where diffusion alone is insufficient. Neural tissues are especially challenging due to their high metabolic needs and reliance on dense vascular networks1. Although recent strategies have attempted to recreate vascularized environments in vitro, conventional bioprinting techniques still lack precise spatial control and multi-material integration. Microfluidic bioprinting addresses these limitations by enabling real-time control of material flow and composition, allowing the fabrication of tailored fibers.

To overcome these limitations, we developed a dual-innovation strategy combining (i) core–shell hydrogel fiber bioprinting using separate biomaterial formulations optimized for neural and endothelial compartments, and (ii) a vessel-inspired structural design integrated into a custom perfusable bioreactor. This system enables the fabrication of compact, brain-like tissues incorporating a vessel, paving the way towards the development of a blood-brain barrier (BBB) model.

Methods

Two distinct hydrogel inks were formulated: a soft bioink for neural tissue and a stiffer endothelial bioink composed of gelatin and human placenta-derived decellularized extracellular matrix (dECM). A custom microfluidic printhead enabled continuous extrusion of core–shell fibers where the softer neural compartment was surrounded by a stiffer endothelial ring. By adjusting the inner and outer flow rates, we achieved controlled fiber architecture and stable long-term culture despite the softness of the neural ink, generating microarchitectures that improved cellular organization and compactness2.

The printed fibers were arranged in a computer-aided compact vessel and integrated into a custom bioreactor for dynamic perfusion. Varying perfusion rates were applied to generate different shear stress levels on the inner surface of the printed compact vessel to promote endothelial alignment on the shell of each fiber. Structural integrity, compartmentalization, barrier function, and cell viability and morphology were assessed by fluorescence imaging.

Results

The microfluidic printhead enabled the fabrication of two distinct fiber architectures: core–shell and sandwich. In core–shell fibers, the neural ink was encapsulated by the endothelial shell, while in sandwich fibers the two materials were layered side by side. Adjusting flow rates allowed control over geometry and neural-to-endothelial area ratios.

Both structures maintained integrity during perfusion and supported endothelial alignment and barrier-like formation under flow. Neural cells preserved viability and morphology. These results show that 3D microfluidic bioprinting offers a valuable platform to engineer a neurovascular unit.

Discussion

This platform enables the bioprinting of compact, perfusable vessel surrounded by neural tissue. Microfluidic tuning of fiber architecture allowed optimization of both form and function, while shear stress promoted endothelial organization. The approach offers a versatile foundation for generating dynamic neurovascular models.

Future work will aim to validate long-term tissue maturation under flow and extend the platform for BBB modeling by incorporating microvascular endothelial cells, astrocytes, and pericytes. This strategy addresses the limitations of static culture systems and advances the development of physiologically relevant models for disease research and drug testing, aligning with the conference's vision for transformative biofabrication.

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Biofabrication of Vascular Constructs: Optimization Strategies and Preliminary Validation

The development of physiologically relevant vascular models remains a major challenge in tissue engineering, particularly when using extrusion-based 3D bioprinting with soft, liquid-like biomaterials. To overcome limitations related to structural instability and gravity, the Freeform Reversible Embedding of Suspended Hydrogels (FRESH) method enables mid-air bioprinting within a temporary gelatin-based support bath. However, achieving high shape fidelity while maintaining biological function remains an open issue. This study aims to optimize FRESH bioprinting for vascular model fabrication by systematically analyzing key parameters affecting shape fidelity, mechanical stability, and cell viability.

A composite hydrogel of sodium alginate (SA) and gelatin (GEL) was prepared following a controlled heating and sterilization protocol. The FRESH method was applied using a CELLINK INKREDIBLE+ bioprinter, extruding the hydrogel into a gelatin-based slurry. Variables such as nozzle diameter (0.4 mm vs. 0.6 mm), SA concentration (4% vs. 6%), printing pressure (8–30 kPa), and speed (10 mm/s) were tested to identify optimal printing conditions. After printing, constructs were incubated at 37°C to liquefy the support bath and coated with 1% SA crosslinked in $CaCl_2$ to improve mechanical integrity.

Shape fidelity was evaluated using micro-CT imaging through qualitative and quantitative analysis. Tubular constructs with varying diameters (5, 15, 25 mm) were scanned before and after support removal. Dimensional parameters—including internal and external diameters and wall thickness—were compared to CAD models. Constructs printed with 6% SA showed low standard deviations and high dimensional accuracy. Nozzle size had minimal impact, while coating improved shape fidelity and construct stability, despite introducing slight geometric changes. Larger constructs exhibited shrinkage post-incubation, likely due to calcium-mediated crosslinking, whereas 5 mm structures showed negligible deformation.

Mechanical stability was assessed via uniaxial compression tests on cylindrical samples (9 mm diameter, 10 mm length). Results revealed high intra-batch consistency and moderate inter-batch variability. The mechanical response of bioprinted structures was comparable to native porcine aorta, especially for thinner-walled constructs (~0.8 mm). A minor reduction in mechanical strength was observed after 21 days at 37°C, likely caused by hydrogel swelling and partial degradation. Weekly re-crosslinking is proposed as a strategy to reduce this effect.

Biocompatibility was preliminarily assessed using AG01522 dermal fibroblasts embedded in the bioink. Cell viability was monitored over 21 days via immunofluorescence imaging. Viability remained consistently above 80%, confirming the cytocompatibility of the SA-GEL system and the FRESH process. However, a gradual decrease in cell density was observed, likely due to the limited mechanical strength and retention capacity of the bioink. Future improvements could include enhanced crosslinking and the incorporation of ECM components such as collagen or fibrin to support adhesion and proliferation.

In conclusion, this study validates the use of FRESH bioprinting combined with micro-CT analysis as a viable approach for creating stable and biologically compatible vascular models. Further optimization of bioink formulation and co-culture strategies will be crucial for functional tissue development.

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Konuşmacılar: Franca Scocozza (University of Pavia)

S17 Cellular Agriculture: a new frontier for biofabrication

Kisielewski

Toplantıyı düzenleyenler: Gabor Forgacs (U. Missouri), Daan Luining (Meatable, Inc.)

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Improved product quality of cultivated meat using forward programming for differentation

The use of opti-ox™ as a forward programming method has demonstrated remarkable potential in enhancing the differentiation speed and quality of pluripotent cells, particularly in the context of cultivated meat production. Pluripotent cells represent a promising resource for the development of cultivated meat, offering a sustainable and ethical alternative to traditional meat production. However, a critical challenge in utilizing these cells for cultivated meat is the ability to efficiently and effectively guide their differentiation into muscle and fat tissues, which are essential for replicating the texture, flavor, and sensory attributes of meat. opti-ox™ has proven to address this challenge by pushing pluripotent cells into a desired lineage within 2-4 days, achieving a 100% differentiation efficiency.

opti-ox™ operates as a forward programming technique that optimizes cellular differentiation. This method allows for the accelerated transition of pluripotent cells into the desired specialized tissue types, such as muscle and fat cells, while maintaining high efficiency and consistency. The forward programming nature of opti-ox™ enables

pluripotent cells to bypass many intermediate stages, reducing the time and resources needed for differentiation and enhancing the reproducibility of the process. This rapid differentiation ensures that the resulting tissues closely resemble the complex structure of muscle and fat found in conventional meat, crucial for achieving authentic sensory experiences.
By achieving 100% efficiency in directing cells toward their intended lineages, opti-ox™ minimizes variations in cell populations, ensuring a uniform and reliable output of differentiated muscle and adipocyte tissues. This high efficiency not only accelerates the differentiation process but also improves the consistency of the cultivated meat product. The quality of muscle and fat tissues directly influences the sensory attributes of cultivated meat, including texture, juiciness, mouthfeel, and flavor.
In conclusion, opti-ox™ significantly improves the differentiation speed and quality of pluripotent cells, facilitating the production of cultivated meat with enhanced sensory characteristics. The forward programming approach, which enables a rapid and efficient transition into muscle and fat lineages within 2-4 days, results in a cultivated meat product that closely mimics traditional meat in terms of texture, flavor, and overall eating experience. As the cultivated meat industry continues to evolve, opti-ox™ stands out as a crucial tool in optimizing cellular differentiation and advancing the development of high-quality, sustainable meat alternatives.

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Konuşmacılar: Daan Luining (Meatable)

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Advancing the Biofabrication of Cultivated Meat - Strategies for Scalable and Textured Cell-Based Foods

The development of cell-based foods, including cultivated meat, poultry, and fish, represents a promising solution to address the growing demand for sustainable protein sources. However, scaling up production while maintaining desirable texture and sensory attributes remains a key challenge. Biofabrication techniques offer innovative approaches to automate the processing of large cell masses in a controlled and reproducible manner, making them highly relevant for the industrialization of cultivated meat. Research in this field has gained momentum in recent years, with various bioprinting and assembly-based strategies being explored.

In our work, we focus on primary bovine cells, particularly adipose derived stem cells, while also investigating muscle cell differentiation. Furthermore, we are extending our research to primary chicken and fish cells. A major focus lies in adapting serum-free media for scalable suspension cultures, typically using spheroid-based systems. In parallel, we are developing edible bioinks, predominantly based on food-grade gellan, enriched with essential nutrients such as plant-derived proteins. Using extrusion-based bioprinting, we design structures that enhance the texture and mouthfeel of cultivated meat products. Depending on the approach, cells or cell aggregates can either be pre-differentiated before printing or undergo simultaneous differentiation into muscle and fat within printed constructs. The quality of differentiated fat cells can be assessed through shifts in their fatty acid composition. Another important aspect of our research is the "preparation" of printed samples using conventional cooking methods, followed by analytical characterization to assess their final properties.

Beyond its biomedical applications, biofabrication presents exciting opportunities in the food industry, paving the way for the scalable production of structured, cell-based foods with optimized sensory and nutritional propertie

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Beyond the Blueprint of Nature: Innovera's Path from Biomimicry to Pragmatic Biofabricated Solution

Leather has long been valued for its aesthetic appeal and performance, serving humanity for centuries. However, traditional leather production places significant strain on natural resources, particularly water, and contributes to chemical pollution. While plastic-based alternatives have attempted to replicate leather, they often fall short in craftsmanship and aesthetic appeal.
This presentation traces Modern Meadow's journey in developing Innovera—a next-generation material that not only rivals leather in beauty and strength but also surpasses it in sustainability and performance. Our early biofabrication efforts focused on tissue culture using bovine fibroblasts, followed by the production of recombinant collagen to hierarchically assemble collagen structures through fibrillation. Over time, we shifted toward a more pragmatic approach: deeply understanding the structure and function of leather and recreating its properties using biological proteins and existing fiber and textile technologies.
By leveraging abundant plant protein and recycled polymer sources and integrating expertise from nonwoven manufacturing, textile processing, and coatings, we engineered Innovera—a material that embodies the essence of leather while offering a more sustainable and scalable solution. This work exemplifies the biofabrication, a power of combining biology, materials science, and engineering to create high-performance alternatives that meet and exceed the expectations of traditional materials.

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Thick cultured meat fabricated using hollow fiber bioreactors with uniform fiber distribution

Creating scalable and functional tissue constructs is a critical challenge in tissue engineering and cultured meat production. However, current methodologies are limited by inadequate nutrient delivery, resulting in inconsistent tissue growth and limited scale-up capability.
To address this, we developed a novel hollow fiber bioreactor (HFB) featuring densely packed arrays of semipermeable hollow fibers, serving as artificial circulation systems to uniformly distribute nutrients and oxygen throughout engineered tissues. Microfabricated anchors ensured precise fiber alignment, optimizing nutrient perfusion.
We demonstrated this approach through the biofabrication of centimeter-scale chicken muscle tissue, which exhibited enhanced sarcomere formation, improved texture, and elevated marker protein expression under active perfusion conditions. Robotic-assisted fiber threading significantly increased manufacturing efficiency, demonstrating potential scalability by assembling a bioreactor comprising over 1,000 hollow fibers for the production of large tissue constructs exceeding 10 grams.
This advancement not only enhances the quality and viability of cultured meat but also provides a robust platform for tissue engineering, potentially revolutionizing the mass production of complex, functional tissues and organs.

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A recombinant extracellular matrix-like protein supports myogenesis in 3D bioprinted cultivated meat

Introduction
With a growing world population, humanity faces increasing demands for food and availability of meat. Conventional mass production is inefficient, uses up valuable resources, and leads to detrimental ecological and ethical consequences. Cellular agriculture applies muscle tissue engineering principles to develop future protein sources. While proof-of-concept generation of cultured meat on a smaller scale has been reported, several challenges remain on the path towards biofabrication of whole cuts of beef. Here, we explore the design of sustainable, edible biomaterial inks for scalable bioprinting of nutritious engineered beef. Two major components of the extracellular matrix (ECM) in bovine muscle tissue are elastin and laminin, both of which help guide myogenic differentiation of bovine muscle precursor/satellite cells (bMuSC). To mimic these features in animal-free scaffolds, we designed a recombinant, engineered protein that includes peptide sequences from both elastin and laminin.[1,2] To formulate the engineered protein into an edible scaffold, we developed a vitamin-based strategy to achieve light-based curing of a protein matrix supporting muscle formation.

Materials and methods
An elastin-like protein (ELP) exhibiting a bioactive, cell-instructive, laminin-mimicking sequence was expressed in engineered microorganisms (Escherichia coli).[2] Artificial muscle samples were assembled by seeding or encapsulating C2C12 myoblasts or primary bMuSC into the ELP (2×107 cells per mL). Photo-crosslinking was initiated at 450 nm after riboflavin (vitamin B2) activation.[3] Rheological bioink properties with and without edible viscosity enhancers were investigated using a stress-controlled rheometer (ARG2, TA Instruments). Shear thinning behavior of inks was analyzed by flow curves (0.1-1000 s-1) and frequency sweeps (10-1-102 rad/s). Printability was evaluated after extrusion bioprinting of model test structures and of constructs with multiple, parallel, muscle-like bundles.
Cell survival, myotube formation, and contractility were characterized in serum-containing and serum-free culture conditions. Myogenic differentiation was evaluated by mRNA expression of myogenic markers (e.g. myosin heavy chain; MHC) and by morphology, fusion index, and dimensions of myotubes after immunostaining and confocal microscopy.

Results and discussion
Photo-polymerization of the ELP matrix resulted in a stable gel stiffness of ~100-300 Pa, which was maintained when heating tissue to 80°C to emulate cooking. Vitamin-mediated crosslinking allowed 3D encapsulation of viable cells. Multimaterial printing of ELP fibers with edible gellan gum microgels led to a reinforced stiffness of >104 Pa, in the range of bovine muscle tissue (decellularized: 103 Pa). 3D bioprinting of encapsulated cells in viscosity-adjusted ELP bioinks resulted in high post-printing viability (>90%)

and allowed parallel alignment which is reminiscent to the spatial alignment in muscle fibers. Following 7 days of differentiation, myogenic progenitors produced multinucleated MHC-expressing myotubes. This contributed to the overall tissue stiffness and further compacted the protein-based cultured tissue.

Conclusions
The recombinant protein bioinks we developed by 3D bioprinting supported myogenesis and provided texture to cultured muscle-like tissue with applications to cellular agriculture and beyond.

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Biofabrication of Microarchitected Cell-Based Meat Using Chaotic Bioprinting Strategies

Introduction
The development of sustainable, structured cultivated meat represents a major challenge where tissue engineering and food technology must converge. Despite important achievements in recent years — including the isolation and expansion of satellite cells, and the blending of animal cells with plant-based matrices — replicating the hierarchical microarchitecture of real meat remains a critical bottleneck. Here, we present our advances toward the biofabrication of microarchitected meat using chaotic bioprinting strategies.
Materials and methods
We employed multimaterial chaotic bioprinting strategies to fabricate gelatin-based, meat-like constructs. Custom printheads equipped with 2 to 4 inlets and fitted with 2 to 6 Kenics static mixing (KSM) elements were used to induce chaotic flows and generate highly ordered microarchitectures within gelatin-based hydrogel filaments. Murine myoblasts (C2C12) and fibroblasts (3T3) were used as model cell types.
The progression of the meat-like constructs, from initial printing through to tissue maturation, was evaluated through a series of assays. Cell viability was assessed using live/dead fluorescence staining, while metabolic activity was quantified via PrestoBlue assays and glucose uptake measurements. Muscle-specific gene expression was analyzed to monitor tissue differentiation, and immunostaining was performed to assess protein-level expression of relevant muscle maturation biomarkers.

Results and discussion
We demonstrate that simple chaotic printing, utilizing static mixer-equipped printheads, enables the extrusion of hydrogel filaments loaded with murine myocytes (C2C12 cells) and containing internal void channels1. These channels facilitate myocyte alignment, promote multinucleation, enhance nutrient diffusion to the filament core, and support the formation of highly organized muscle-like fibers.
Through multimaterial chaotic bioprinting2, we also introduce reinforcing scaffolding inks during the extrusion process. These scaffolds enhance the mechanical stability of the printed constructs, enabling long-term culture under dynamic perfusion conditions without compromising structural integrity.
Additionally, the integration of fibroblast-laden inks during chaotic coextrusion adds a biologically active connective tissue-like component to the engineered constructs. Our results show that fibroblast coculture accelerates the consolidation and maturation of muscle-like fibers, supporting tissue organization and contributing to extracellular matrix deposition.
Finally, we describe variations in chaotic bioprinting materials and strategies aimed at improving scalability and manufacturability, setting the groundwork for future translation of microarchitected cultivated meat production.
Conclusion
Our results demonstrate that chaotic bioprinting is a promising platform to engineer complex, organized, and scalable cell-based meat tissues, bridging biological fidelity with manufacturing practicality. We envision the translatability of these techniques to bovine cells to fabricate micro-architected “sashimi” size pieces of eatable meat-like materials in the near future.

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Disclosure:
Grissel Trujillo de Santiago and Mario Moisés Alvarez have submitted patent applications protecting different aspects of a printing/bioprinting technique based on the use of chaotic flows.

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S29 New approaches in tissue engineering — Mikołajska

Toplantıyı düzenleyenler: Aida Fadakarsarkandi (The University of Birmingham), Jan Guzowski (Institute of Physical Chemistry PAN)

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Organ-on-a-Chip systems - a new approach in tissue engineering.

A new approach in tissue engineering is the development of Cell- and Organ-on-a-chip systems. Thanks to the miniaturization, ensuring flow conditions and obtaining specific parameters such as large surface-to-volume (SAV) ratio and effective culture volume (ECV) it is possible to obtain culture conditions closer to in vivo. Microsystems have microstructures, in which different 3D culture models mimicking the function of tissue and organs are developed. Organ-on-a-Chip systems mimic in vivo microenvironment and enable to study response of the cells after the exposure to biophysical and biochemical and pathological factors. We developed 3D models of different tissue/organs such as lung, liver, heart, pancreas, brain, breast, vascular system which mimic organ function in Organ-on-a-Chip systems . These models have been based on the usage of hydrogels, self-organization, membranes, and scaffolds.

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Acetylcholine-loaded Nanoparticles Protect Against Myocardial Injury In Vitro Cardiac Spheroids and In In Vivo Myocardial Infarction Murine Model

Introduction: The production of acetylcholine (ACh), a neurotransmitter regulating cardiac function, is significantly reduced following myocardial damage in vivo. Previous studies demonstrated that ACh administration can be used to reduce infarct size following myocardial infarction (MI) and ischemic-reperfusion (I/R) injury in in vivo animal models. This has been associated with ACh-mediated activation of anti-inflammatory pathways, as well as improved cell survival under hypoxic conditions. Nevertheless, in order to avoid unspecific effects due to ACh’s broad activity on a number of receptors throughout the body, its use in the clinic has been limited, preventing the development of new therapeutic approaches for preventing myocardial damage. Therefore, this project aimed at evaluating the potential protective effects of ACh by combining advanced in vitro models of myocardial damage using vascularised cardiac spheroids. In an effort to translate in vitro findings, we tested the protective effects in an in vivo models of myocardial damage in mice.

Moreover, previously used models have not fully replicated the intricate cardiac microenvironment, limiting the understanding of its clinical potential. In this context, our laboratory has recently developed in vitro cardiac spheroids (CSs) comprised of stem cell-derived cardiomyocytes, fibroblast and endothelial cells to better mimic the molecular, cellular, and extracellular features typical of the human cardiac microenvironment.

Methods: We evaluated the cardioprotective effects of ACh using three different delivery methods: i) freely-dissolved 100μM ACh; ii) ACh-producing cholinergic nerves (CNs); and iii) ACh-loaded nanoparticles (ACh-NPs) (Figure 1). These were tested in vitro by comparing the three delivery methods in cardiac spheroids that modelled I/R conditions.

These were achieved by exposing cardiac spheroids to changes in oxygen levels from normoxia (5% oxygen), hypoxia (0% oxygen) and normoxia (5% oxygen). Additionally, myocardial damage in cardiac spheroids was also achieved by exposing them to doxorubicin (DOX), a well-known cardiotoxic drug. Control and injured (I/R or DOX) cardiac spheroids were tested for changes in viability/toxicity by calcein-AM and ethidium homodimer staining, respectively, as well as for changes in contractile activity by measuring contraction frequency and fractional shortening %. In vivomyocardial damage was achieved by ligating the LAD in a mouse model established in our laboratory. ACh-NPs were directly injected into the muscle wall of infarcted animals right before ligating the LAD. Mice were imaged using ultrasound techniques to measure changes in ejection fraction %. Hearts were also isolated at 28 days to isolate tissue for histological, immunofluorescence and transcriptomics analyses.

Results: Our analyses revealed that increased ACh levels protect against the reduction in cell viability, fractional shortening % (FS%), as well as mitigate changes in genes associated with myocardial damage in cardiac spheroids. Our ultrasound imaging, histology and bulk RNAseq analyses showed that injecting ACh-NPs in the myocardium improved the ejection fraction % (EF%) by 20.24 +/- 2.925 in MI animals, prevented cardiac fibrosis and activated signalling pathways regulating cell survival and proliferation.

Discussion and conclusion: Altogether, our findings support the cardioprotective role of ACh against I/R and DOX-induced myocardial damage, underscoring the potential use of ACh-NPs as a novel therapeutic approach.

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Development of Basement-Mimetic-Substrate via 3D Printing Technology and its applications

The basement membrane (BM) is a crucial extracellular matrix that provides structural support, regulates cell adhesion and migration, and influences cell behavior in various organs. Composed of proteins like collagen and laminin, it plays essential roles in tissue development, repair, and homeostasis. Disruptions in the BM are linked to diseases such as cancer and fibrosis. However, its complex structure makes it challenging to replicate in vitro for organ modeling.
To address this, we developed a thin membrane printing technology to fabricate BM-mimetic substrates. The system composed of precisely controlled temperature environment facilitated not only the crosslinking of the hydrogel but also vitrification to form the thin membrane. This approach allows precise control over the physical and biochemical properties of the membrane, enabling the creation of custom, micro-scale substrates. We used Bruch's membrane (BrM)-derived bioink to fabricate a BrM-mimetic substrate, which was then used to develop a human retinal pigment epithelial (RPE) model.
The RPE model grown on the BrM-mimetic substrate showed enhanced cellular morphology, polarity, and differentiation compared to conventional tissue culture systems, suggesting a more accurate in vitro representation of the retinal environment. This model provides a valuable tool for studying retinal diseases and testing therapies.
In conclusion, our thin membrane printing technology enables the creation of BM-mimetic substrates that better replicate the native tissue environment, improving in vitro organ models for biomedical research and therapeutic development.

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Establishing a human kidney glomerulus co-culture model using melt electrowritten scaffolds under perfusion

Introduction The kidney glomerulus acts as the blood-filtering unit in the kidney and plays a crucial role in maintaining homeostasis. The glomerular filtration barrier is a size-selective filter composed of endothelial cells and podocytes, separated by the glomerular basement membrane. Dysfunction of the barrier can result in proteinuria, often followed by progressive renal damage and kidney failure. Aim of this study was to establish an in vitro human kidney glomerulus co-culture model using endothelial cells (ciGEnCs) and podocytes (PODO/SVTERT152) on collagen coated Melt Electrowriting (MEW) PCL membranes in a perfused bioreactor.
Methods Human urine-derived podocytes (PODO/SVTERT152) were purchased from Evercyte and conditionally immortalized human glomerular endothelial cells (ciGEnCs) provided by the University of Bristol. For the optimisation of the co-culture medium, the effect of pure HPLM (Human Plasma-Like Medium) and medium compositions, consisting of mixtures of HPLM and Endothelial Basal Medium (EBM) were tested. In addition, effects of different FCS (fetal calf serum) concentrations and addition of ATRA (all-trans retinoic acid) were evaluated on both cell types. The microporous PCL membrane was fabricated with the MEW printhead of a BioScaffolder 3.1 bioprinting machine (GeSiM, Germany) and then manually coated with collagen I. Endothelial cells were seeded first on one side of the membrane; after a growth phase of 3 days podocytes were seeded on the opposite side and then both together further cultivated under perfusion conditions in a self-designed and manufactured bioreactor. Three different bioreactors were fabricated having 3 different shear conditions (constant shear and two gradient shear bioreactors) under flow of cell culture media. Gradient shear bioreactors were included in the study to mimic the shear distribution in the native glomerulus. Cell behaviour was investigated utilising fluorescence microscopy and gene expression analyses.
Results & Discussion Both HPLM and an EBM-HPLM 1:1 mixed composition were identified as suitable candidates for the co-culture medium, using 5% FCS for growth and switching to 0.5% FCS for differentiation. The effect of collagen I crosslinking and multiple coatings showed to have a slight impact on scaffold elasticity and permeabilit. In addition, the permeability of the cell-free scaffolds and shear stress simulations of three different bioreactor designs were analysed. After 3 days of perfusion culture, successful attachment of endothelial cells and CD31 expression were observed. In addition, podocytes attached well when seeded on the opposite side of the coated membranes and synaptopodin expression proved successful differentiation. Both cell types could be successfully visualised within the same co-culture and showed typical cell morphologies. Co-culture experiments could be performed with the present set-up for up to 9 days. In conclusion, the research presented in this study establishes a starting point for developing of a novel human kidney glomerulus model. Although the flat morphology of the PCL membrane does not accurately resemble the macrostructure of glomeruli, it may provide an effective 3D microenvironment for cell-cell and cell-matrix interactions.
Acknowledgments The study was funded by the German Research Foundation (DFG, grant No. GE 1133/27-1).

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Biology and Biomechanics for angiogenesis: hand in hand to unveil the possibilities of tissue engineering

The major challenges in new drug development are the low translational efficiency (<10%), soaring costs (>$5 billion/drug) and ethical concerns regarding animal welfare. The emergence of a novel research field with bioengineering promises to offer new strategies to create non-animal models using human cell lines to address these shortcomings. Absence of vascularisation is one of the main bottlenecks to obtaining fully functional bioengineered tissues. The passive diffusion limit for oxygen and nutrients in biological tissues is ~200 µm. Providing a dense and functional microvascular network is key to the formation of larger healthy constructs. The current microfabrication technologies only allow to engineer passive tubular networks in hydrogel matrices, but they lack the biological/cellular components and the dynamic functionality of in vivo capillaries. If angiogenic biochemical cues and their gradients' impact on microvascular tubule self-assembly have been thoroughly investigated, the importance of local mechanical properties in scaffolds has often been overlooked in the development of functional bioengineered tissues. Exploring the impact of local mechanical properties in 3D matrices is one of the next main axes of progress for the field of tissue engineering and being able to intertwine engineering and biological-based knowledge and techniques will be crucial.

Angiogenesis is a very complex and sensitive process, and multiple environmental cues have been extensively studied in 2D systems. In this study, we use a simple, non-inflammatory collagen-based 3D system to study the impact of local mechanical property gradients on HUVECs' self-assembly and lumenisation. This study focused on cross-validating imaging-based observations on properties of the tubules across the system, like morphology or cytoskeleton distribution with biomechanical data obtained through rheological measurements, nano-indentation and optical micro rheology to depict a comprehensive and robust picture of angiogenic behaviours in mechanically heterogeneous matrices, by comparison to homogeneous matrices.

Methods
HUVECs are seeded in different concentrations of 3D Collagen Type I hydrogels and submitted to a constant stimulus of 50ng/mL VEGF via the culture medium. The endothelial cells self-assemble into microvascular networks. The impact of growth factors on the tubule formation has been studied and a reproducible microvascular network formation protocol was established. The microvascular networks were stained and imaged, and automated analysis of morphological (average lumenised tubule length, number of branches, tubule diameter…), cellular (cytoskeleton organisation) and expression profiles were acquired. These biological profiles were put in parallel with bulk and local micromechanical ones.

Results & Future Work
Lumen-presenting microvascular networks have been obtained in a static environment. Exploration of mechanical property gradients have allowed us to highlight the importance of local mechanical property gradients over bulk material properties and to validate the use of different mechanical property measurements' methods for the

exploration of soft 3D hydrogels. Perfusion tests will allow to paint a complete picture of the tubular formation and further optimise the protocol before the functional validation of the microvessels, paving the way to larger, physiologically relevant bioengineered constructs.

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Human model for the ex-vivo perfusion of a free tissue biofabrication substrate using extracorporeal membrane oxygenation

Background
Access to healthy living tissue in the lab has always been an essential substrate for experimentation across the breadth of medical research. Historically limited to live animal studies using small mammals such as rats, and subsequently more anatomically and physiologically analogous animals such as pigs, recent developments in cellular technologies have allowed for the in-vitro production of human tissue, such as the development of spheroid and organoid technologies, which allow for the study of 3D cell structures in a microenvironment that mimics the in-vivo environment. However, they remain unable to completely recreate in-vivo conditions, including issues of scale and maintaining macroscopic tissue architecture.
This project aims to validate a reliable lab-based human model for normothermic perfusion of tissue using human blood products. This would provide a higher fidelity tissue for research applications and incredible scope for use in tissue engineering and biofabrication as a vascularised living substrate for the development of engineered tissue in a simulated in-vivo environment.

Method
We present a case series of 5 consecutive patients undergoing unilateral autologous breast reconstruction using DIEP flap or cosmetic abdominoplasty, with the redundant abdominal flap placed on ex-vivo ECMO perfusion until tissue demise. Tissue viability was assessed using clinical, biochemical and histopathological parameters, alongside demographic and technical factors that influenced flap longevity.

Results
Mean survival time was 3.8 days (max 8 days), with mechanical venous congestion the primary cause of demise in 80% of cases, and progressive venous congestion in 20%.

Conclusion
The preliminary results in this study demonstrate that extracorporeal normothermic perfusion of human free tissue flaps is feasible, with current results in line or exceeding the currently reported survival data in the literature across all forms of ex-vivo tissue perfusion. Ongoing technical improvements of the experimental setup will undoubtedly improve these outcomes further.

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Bioxolography using diphenyliodonium chloride and N-vinylpyrrolidone enables rapid high-resolution volumetric 3D printing of spatially encoded living matter

Objectives: Volumetric bioprinting (VBP) technologies have demonstrated unparalleled potential for printing centimeter-sized engineered tissues, with intricate architectures, in minutes. However, they still face limitations in scalability and resolution. Recently, a next-generation volumetric 3D printing technique named Xolography was introduced. Xolography operates by intersecting a UV-light sheet and a visible-light ultra-HD projection within a moving print-bath containing a dual-color photo-initiator (DCPI). This novel approach was shown to photoprint plastics at unprecedented volume generation rates (55 mm3.s−1) and resolution (<10 µm), while enhancing resin utilization efficiency and demonstrating promising scalability. However, its applications and associated processes have remained limited to the printing of plastics. In this work, we pioneered the bio-Xolographic printing of hydrogels to enable rapid high-resolution bioprinting of living matter.

Methods: Gelatin methacryloyl (GelMA) was blended with different photoreactive compounds to prepare multiple photopolymerizable formulations. Their reactivity was studied using dual-color photorheology and Xolography to identify optimal compositions for Xolographic 3D-printing of hydrogels. Parameters such as component concentration, printing speed, and light irradiance were optimized to achieve high-resolution printing. Cell-free hydrogels were printed in intricate shapes, and both printing fidelity and resolution were evaluated. To enable 3D-bioprinting, the cytotoxicity of DCPI, co-initiator, and different co-monomers was assessed to formulate bioinks that are non-cytotoxic and cell-conducive. Furthermore, cell-laden hydrogels (e.g., with human mesenchymal stromal cells, chondrocytes, and induced pluripotent stem cell-derived cardiomyocytes) were 3D-printed, and cell viability and function were determined. In addition, multimaterial printing, molecular patterning, and grayscale-mediated mechanical patterning are explored to programmably create intricate, biomimetic, and concentration-controlled architectures.

Results: The incorporation of diphenyliodonium chloride and N-vinylpyrrolidone proved essential for enabling Xolographic printing of hydrogels. These additives accelerated crosslinking speed, thereby addressing the reactivity limitations of existing DCPIs. Within 3 minutes, we successfully printed centimeter-scale structures with feature resolutions below 25 µm, as well as biologically relevant architectures containing perfusable interconnected channels. These results demonstrate that the high speed, resolution, and versatility of this emerging technology are translatable to hydrogel printing applications. DCPI maintained high cell viability (>98%) at concentrations suitable for 3D-printing. Moreover, 3D-bioprinted cell-laden hydrogels showed high resolution (<80 µm) and high post-crosslink cell viability (>80%). Repeated print cycles allowed for high resolution encoding of multimaterial designs and molecular patterns in a concentration controlled manner. Moreover, grayscale-mediated movie projection allowed for precise mechanical patterning by controlling crosslink density in a voxelized manner. Utilization of bioresins containing chondrocytes allowed for cartilage formation, while use of induced pluripotent stem cell-derived cardiomyocytes allowed for the biofabrication of electrically-induced contractile tissues. Together, this emphasizes the potential of bioxolography for the rapid biofabrication of large-scale functional tissues.

Conclusions: We here demonstrate that diphenyliodonium bioresin formulations based on chloride and N-vinylpyrrolidone enable Xolographic 3D-bioprinting of complex cm-scale living matter at high speeds and resolutions, while maintaining high cell viability. Bio-xolography is presented as a next generation technology for volumetric bioprinting of engineered tissues, with potential to bioprint and pattern large-sized multi-scale architectures within practical time windows.

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Konuşmacılar: Jeroen Leijten (University of Twente)

- 17:00

Coffee break & Poster session II

- S05 Advancing 3D bioprinting through artificial intelligence Ratuszowa

Toplantıyı düzenleyenler: Nadina Usseglio (Advanced Biofabrication Laboratory - DNIETO LAB, University of La Coruña, Spain), Daniel Nieto Garcia (Advanced Biofabrication Laboratory - DNIETO LAB, University of La Coruña, Spain)

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AI-Enhanced Bioprinting: Intelligent Monitoring, Optimization and Virtual Prototyping

AI-Enhanced Bioprinting: Intelligent Monitoring, Optimization and Virtual Prototyping.
Introduction
The integration of artificial intelligence (AI) and machine learning (ML) into bioprinting is revolutionizing the field of biofabrication. Traditionally based on experimental methods and manual trial-and-error, bioprinting is now evolving into a predictive and adaptive science. AI enables real-time monitoring, autonomous optimization, and virtual prototyping—unlocking new capabilities for precision, efficiency, and reproducibility in tissue engineering. This presentation offers an in-overview of emerging computational approaches structured around three key elements: smart process supervision, data-driven optimization, and digital twin development.
State-of-the-Art Techniques
Smart Process Supervision. Modern bioprinters are increasingly equipped with advanced sensing and vision systems that provide real-time feedback on print quality. Computer Vision Systems: Deep learning algorithms, particularly convolutional neural networks (CNNs), are capable of identifying defects such as nozzle clogging, filament discontinuities, and layer misalignment with high accuracy. These systems allow for automated defect detection and real-time process correction. Adaptive Control Systems, reinforcement learning techniques are being applied to autonomously adjust printing parameters such as temperature, extrusion rate, and print speed. These adaptive systems learn from environmental feedback and compensate for fluctuations in material behavior or ambient conditions, improving consistency across prints.
Data-Driven Optimization. Bioprinting involves the simultaneous balancing of mechanical, biological, and geometric criteria. AI-based optimization enables more efficient exploration of this complex parameter space. Machine Learning Models: Supervised and unsupervised learning models can predict print outcomes based on process variables. These models help optimize parameters to enhance cell viability, print fidelity, and structural performance.

Bioink Optimization. AI models are being trained to predict the rheological, mechanical, and biological properties of bioink formulations based on their molecular characteristics. This approach accelerates the development of custom materials tailored to specific tissue types or printing conditions.
Digital Twin Advancements.
Digital twins offer a virtual replica of the bioprinting process and printed construct, allowing researchers to simulate outcomes before physical fabrication. Physics-Informed Neural Networks: These hybrid models integrate data-driven learning with physical laws governing fluid flow, thermal behavior, and mechanical deformation. They provide accurate predictions of print dynamics and print tissue development. This enables researchers to iterate digitally on scaffold designs, reducing material waste and experimental cycles.
Discussion
Despite rapid progress, several challenges remain. The lack of standardized datasets limits the transferability of AI models across different platforms and bioinks. Furthermore, there is a critical need for experimental validation frameworks to benchmark AI predictions. Regulatory pathways for AI-assisted medical manufacturing are still under development, creating uncertainty for clinical translation. Finally, the scalability of these solutions remains a technical and logistical hurdle.
Future Perspectives
The future of AI-enhanced bioprinting includes the implementation of edge computing for onboard, real-time AI processing, and the fusion of multimodal data—from imaging to biomechanical sensors—to create comprehensive models of bioprinting systems. These advancements aim to transform bioprinting into a truly intelligent, autonomous, and patient-specific manufacturing process.

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Konuşmacılar: Julian Flores (University of Santiago de Compostela)

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AI-Guided Laser-Assisted Bioprinting of Organoids for High-Precision Biofabrication

The manipulation of three-dimensional (3D) cellular structures such as organoids and spheroids plays a central role in modern biomedical applications, including tissue engineering, drug screening, and disease modeling. However, the accurate and reproducible transfer of these fragile structures remains a technical bottleneck. Manual handling is limited by user variability, lacks scalability, and is inherently incompatible with high-throughput workflows. Laser-assisted bioprinting has emerged as a contactless and precise alternative to traditional pipetting or extrusion-based methods. In this context, artificial intelligence (AI) and robotic automation offer the potential to transform cell handling by enabling intelligent decision-making, adaptive control, and real-time feedback. We present PickCell(TM), a fully integrated robotic platform for real-time detection, trajectory optimization, and laser-assisted bioprinting of organoids.

The system integrates a 6-axis robotic arm, a nanosecond pulsed laser (1 ns, 1064 nm, ~30 µJ), high-resolution optics, and a custom software suite. Object detection is performed by a YOLOv11 small model (YOLOv11s)[1], trained on 512x512 grayscale image patches and applied to full 1024x1024 images. Depending on spheroid size and density, the image is divided into 20 to 40 overlapping patches, ensuring robust detection and minimal false negatives. Inference takes approximately 2 seconds per image and can be optimized to under 1 second via batch processing.

Detected coordinates {(x_i, y_i)} are mapped to a user-defined deposition pattern via affine transformation: [x'_i, y'_i]^T = R * [x_i, y_i]^T + t. A Traveling Salesman Problem (TSP) based algorithm computes an optimized sequence: {(x"_i, y"_i)} for i = 1 to N = TSP_Optimize(P_target). Each spheroid is then transferred via a focused laser pulse, with energy adapted to its diameter. Robotic alignment ensures sub-20 µm placement precision.

Validation experiments show over 95% detection accuracy with YOLOv11s and transfer precision below 20 µm. Trajectory optimization reduced operation time by approximately 40% versus unoptimized paths. The platform supports a range of plate formats and accommodates spheroids between 50–500 µm. Post-transfer microscopy confirmed preserved structural integrity.

These results highlight the synergy of AI, photonics, and robotics for advanced biofabrication. Unlike extrusion or aspiration-based techniques[4, 5], PickCell(TM) enables high-speed, contactless, and programmable transfers. Its scalability and modularity make it ideal for automated tissue engineering workflows. Future directions include viability feedback control and multi-material printing capabilities[2, 3].

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Konuşmacılar: Antonio Iazzolino (Poietis BioSystems/Chief R&D Officer)

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In-Situ Defect Detection in Microstructures Fabricated by Two-Photon Polymerization

Biointelligence is an emerging paradigm in manufacturing that integrates bio-inspired principles and biological components with hardware and software to create intelligent, adaptive, and biologically interactive production systems. Two-Photon Polymerization (2PP) is an Additive Manufacturing (AM) technology that supports Biointelligence by enabling the fabrication of high-resolution, three-dimensional (3D) microstructures. Utilizing femtosecond laser pulses to polymerize biocompatible photosensitive materials, 2PP facilitates the creation of sub-micron cell cages, allowing for precise cell manipulation and integration within controlled devices. This work is inspired by the EU-funded BioProS project (Biointelligent Production Sensor to Measure Viral Activity), which focuses on developing a sensor chip for monitoring viral activity during viral vector production. At the heart of the chip is a micro-cage structure produced using 2PP. The cage consists of a circular pillar array, each 30 µm in diameter and 50 µm in height, spaced 30 µm apart to promote efficient cell entrapment. Two parallel guiding lines enclose the pillar, and three V-shaped structures at the inlet and outlet prevent clogging in the initial rows of pillars and ensure uniform fluid flow for subsequent perfusion of cells with viral particles. Despite the high precision of Two-Photon Polymerization, the complex interplay between material properties, process parameters, and print design can lead to defects in the micro-cage, such as edge effects, poor substrate adhesion, and structural distortions, which may compromise the sensor chip’s performance. Process optimization typically relies on time-consuming and resource-intensive trial-and-error methods. In this study, we demonstrate the potential of in-situ monitoring to detect printing defects in real-time, enabling early intervention to prevent faulty prints, reduce waste, save time, and improve efficiency. Layer-wise microscopic images are acquired coaxially through the laser lens while printing the cell micro-cages, with process parameters varied in laser scan speed (µm/s) and laser power (mW). After preprocessing - such as denoising, edge sharpening, and contrast adjustment - the images are

divided into 128×128-pixel patches. Random geometric transformations, including rotation, flipping, and zooming are applied to enhance dataset diversity and generalizability. Then, a deep learning (DL) model for semantic segmentation based on the U-Net architecture is trained using the augmented dataset. The deviation between the layer-wise segmentation and the nominal layer slices, representing ideal printing results, is computed between reconstructed and reference images, enabling evaluation of geometric deviations during fabrication. Finally, a control chart is designed to detect anomalous layers and prints, facilitating the identification of optimal process parameters and early termination of faulty prints.

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Konuşmacılar: Egon Prioglio (Politecnico di Milano)

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Water-Soluble Radical Photoinitiators For Obtaining 3D Object By VPP Printing

The work concerns the development and characterization of novel water-soluble photoinitiators based on benzoin derivatives for the initiation of radical photopolymerization processes in aqueous media. The main objective of the study was to create compounds that combine high performance, good water solubility, low cellular toxicity and spectral compatibility with commonly used UV and VIS light sources, which is crucial for biomedical applications such as tissue engineering and 3D bioprinting.New compounds whose structures have been modified to improve absorption properties and increase water solubility were presented. A series of spectroscopic, photochemical and biological studies were conducted to evaluate their suitability as photoinitiators. Their absorption spectra, photoreactivity, quantum yield of photolysis and photofragmentation mechanism were investigated. In addition, the cytotoxicity of these compounds was evaluated on CHO-K1 cells, which showed their good biocompatibility. The efficiency of initiating the polymerization of acrylate monomers in aqueous media was also tested. It was shown that the developed compounds enable efficient hydrogel formation, and due to their wide range of light absorption, they can be used with various light sources, including Vis-LED at 405 nm. Particular emphasis was placed on the application of these photoinitiators in 3D printing using the VPP method, which makes it possible to create, for example, hydrogel microarrays with complex geometries. Analysis of rheological properties and photocalorimetric studies were also carried out, confirming the suitability of the new initiators in additive technology. Ultimately, the work represents a significant step toward the development of new, safer and more efficient photoinitiators for biomedical applications. The developed compounds combine high initiation efficiency, low toxicity and compatibility with visible light, making them an attractive alternative to current commercial initiators.

Research financed within the framework of the competition no. 2024/ABM/03/KPO/ project no. KPOD.07.07-IW.07-0125/24 entitled: “Title of the Undertaking: Luminescent theranostic compounds with anticancer activity, i.e., combination of photodynamic therapy and diagnostics through imaging in a single molecule and development of 3D printed topical micro-needle systems to provide precise individualized cancer therapy” from the National Plan for Reconstruction and Enhancement of Immunity, part of Investment D3.1.1 Comprehensive Development of Research in Medical and Health Sciences, a project funded by the Medical Research Authority.

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Konuşmacılar: Joanna Ortyl (Cracow University of Technology)

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Automated Pre-Calibration for 3D Extrusion Bioprinting Unlocks Process Consistency and Reproducibility

In 3D extrusion bioprinting, precise and reliable material deposition is essential for fabricating consistent tissue constructs. However, elastic components like disposable syringes and flexible tubing introduce unpredictable deformation and backlash, decoupling piston movement from actual material extrusion. The challenge is further amplified with high-viscosity, non-Newtonian bioinks, whose complex shear-dependent behavior makes predictive calibration difficult. Traditional methods often rely on prior material characterization or time-consuming trial-and-error tuning, limiting flexibility across different print jobs. Additionally, residual pressure decay during pauses can further destabilize printing performance.

To overcome these challenges, we developed a two-stage automated pre-calibration procedure that integrates real-time pressure sensor data with automated imaging analysis to fine-tune print parameters without requiring prior knowledge of material properties.

In stage 1, a series of continuous lines is printed at fixed extrusion and feed rates while monitoring pressure sensor readings (Figure 1). An automated image segmentation pipeline analyzes the resulting line patterns to quantify the diameter of the extruded filaments. By correlating pressure values with the measured filament widths, we identify the pressure needed to achieve a target line width. This pressure value is then used to pre-adjust future prints, ensuring that extrusion begins at the optimal pressure, independent of initial system lag or pressure decay. Without prior pressure adjustment, the system required ~55 seconds to reach a stable pressure, during which extuded filaments were incomplete or fragmented (Figure 1, A; i). Automated analysis of the printed patterns revealed no material deposition during the first ~5 seconds, intermittent line fragments during the following ~25 seconds, and continuous extrusion only after ~45 seconds (Figure 1, A; ii, iii, iv). By contrast, when applying the pre-calibrated pressure, the system maintained stable extrusion throughout the print (Figure 1, B; i, ii). The resulting line patterns showed full and consistent deposition from the first second onward, as confirmed by quantitative image analysis of filament widths across all time intervals (Figure 1, B iii, iv).

In stage 2, lines were printed at constant extrusion rate but alternating feed rates to simulate acceleration, deceleration, and cornering. Without extrusion adjustment, only minimal and delayed pressure increases were observed in response to the changing feed rate. This caused the extruded lines to become non-uniform, wider than target width during slow segments and thinner during fast segments and led to excess material accumulation at corners. By systematically increasing the extrusion correction factor, the system began to react with stronger and more immediate pressure spikes, effectively compensating for changes in motion and yielding uniform filament widths and well-formed corners. However, beyond a certain threshold, the correction factor began to overcompensate, producing extreme pressure spikes that again disrupted line uniformity. Automated detection of line uniformity across test patterns allowed us to identify the optimal correction factor that ensured smooth, consistent lines and corners, which were then applied to print multi-layer scaffold structures.

Overall, this study shows that combining pressure sensing with automated imaging enables quick, material-independent adjustment of bioprinting parameters, improving print uniformity and reducing manual setup.

Konuşmacılar: Maximilian Jergitsch (Biomotion Technologies)

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A machine learning-aided design framework for bone tissue engineering scaffolds

Spherical-based architected scaffolds have gained increased interest in tissue engineering (TE) due to their curved architecture, which inherently reduces stress concentrations and enables controlled mechanical performance. However, designing and optimising such architectures remains challenging because of the intricate relationship between their structural and functional properties.

This work proposes an automated framework for optimising spherical-based porous scaffolds for bone TE. First, neural networks (NNs) were trained using data from finite element simulations to predict their stiffness and porosity based solely on geometrical features. Then, a genetic algorithm (GA) was coupled with the developed NNs to perform an inverse design, exploiting the predictive capability of the NN to tailor the microarchitecture based on the demands of mechanical and porosity. Next, validation through additional FE simulations confirmed the potential and highlighted the limitations of the presented framework. Finally, experimental validation was performed on 3D printed scaffolds.

The proposed tool serves a dual purpose. First, the NNs act as a surrogate model to instantly predict mechanical and porosity characteristics based solely on the scaffolds' microarchitecture. Secondly, it allows the inverse design of spherical-based scaffolds with combined normalised stiffness (between 0.060-0.226) and porosity (between 0.55-0.80). The precise control over porosity and mechanical properties, combined with the intrinsic anisotropic architectures, makes this tool suitable for generating optimised bone scaffolds, replicating the mechanical gradients between cortical and trabecular bone. Moreover, the frameworks can be used as standalone or with additional computational models, such as computational fluid dynamics, to assess nutrient transport in cell-culture experiments.

Further work will expand the tool’s ability to predict scaffold properties under large deformations (e.g., absorbed energy), providing a path for fully optimised bone TE scaffolds.

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Konuşmacılar: Pasquale Posabella (Warsaw University of Technology)

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Generative AI for Fabrication of Large Tissues

The fabrication of large, perfusable tissue constructs remains a major challenge in regenerative medicine due to the complexity of translating vascular networks across multiple scales. Here, we present an agentic generative AI (GenAI) platform capable of autonomously generating STL files for bioprinting macro-to-millimeter-scale architectures that are subsequently colonized by self-organizing cells to form functional microvascular networks. Our approach integrates GenAI-driven design of a hexagonally arrayed macrofluidic scaffold with computationally optimized branching patterns that direct flow from portal-like inlets to central venous-like outlets, mirroring the hepatic lobule's geometry. The scaffolds are bioprinted and seeded with GATA6+ hepatic progenitor organoids, which establish parenchymal domains, followed by ETV2+ endothelial progenitors that line the macro- and millimeter-scale channels. At the microscale, endothelial sprouting and tissue-derived morphogens drive emergent self-

assembly of capillary-level networks that bridge between printed channels, achieving full perfusability without additional external patterning. This multi-scale design strategy closes the gap between macroengineered perfusion conduits and cell-autonomous microvascularization, enabling the generation of large, metabolically active hepatic tissues. The platform highlights how AI-guided macro-to-millimeter structural design coupled with emergent cell-driven microarchitecture formation can be leveraged to accelerate the biomanufacturing of organ-scale tissues.

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Konuşmacılar: Gilad Gome (MIT)

- S10 Biofabrication of Engineered Living Materials Kruczkowski

Toplantıyı düzenleyenler: Michael Gelinsky (TU Dresden), Riccardo Levato (Utrecht University)

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Living Therapeutic Materials for Zero-waste Therapeutics

In many therapeutic treatments, only a small fraction of the administered drug is absorbed, and a large part is wasted and released into the environment. Drug waste means economic loss and environmental risk. We present drug eluting materials with the unique property to self-produce biopharmaceuticals directly at the therapeutic site. The materials are hydrogels that host biofactories of natural therapeutics and maintain and regulate their productivity in-vivo over the long term. The biofactories produce and deliver the drug using energy sources from body fluids. We will present dynamic hydrogel compositions that can contain and control the proliferation and metabolic activity of encapsulated cell biofactories and can be processed into useful living devices with living therapeutic functions. We also present microarrays study multifactorial microbial responses in parallelized experimental formats. Self-replenishable drug eluting devices can deliver drugs continuously at the required concentration and can improve therapeutic outcome at zero-waste.

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Konuşmacılar: Aránzazu Del Campo (INM - Leibniz Institute für New Materials)

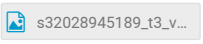
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Photosynthetic living materials for dual-carbon sequestration

Natural ecosystems, such as forests and aquatic systems, offer efficient pathways for carbon (CO_2) sequestration, outperforming industrial carbon capture and storage methods in terms of resilience and environmental impact. These systems operate under ambient conditions, using sunlight and commonly available small molecules as their inputs. Harnessing the capabilities of natural systems offers a compelling alternative to conventional carbon capture approaches. However, confining and controlling natural living organisms or systems outside their native environments remains challenging. Drawing inspirations from nature, we address this challenge by embedding photosynthetic cyanobacteria within 3D-printed hydrogel matrices, to create engineered living materials for efficient carbon sequestration (Figure 1a).
We focused on using Synechococcus sp. PCC 7002, a cyanobacteria strain capable of sequestering atmospheric CO_2 both in the form of biomass accumulation and microbially induced carbonate precipitation. To enable tailored geometries, we encapsulated the cyanobacteria within a hydrogel formulation suitable for multiple additive manufacturing technologies, including direct ink writing and volumetric bioprinting. We used direct ink writing to fabricate structures with improved surface area to volume ratios for surface coatings. This design enhanced light exposure and nutrient exchange for the encapsulated cyanobacteria, and resulted in improved overall viability of the living material (Figure 1b). We also leveraged photo-cross-linking to enable light-based volumetric bioprinting. This approach demonstrated the material's ability to form fine, high-resolution lattice structures. (Figure 1c). During an incubation period of 30 days, the living material sequestrated approximately 2.2 ± 0.9 mg of CO_2 per gram of material, with atmospheric carbon as their main carbon source. Remarkably, the material remained viable for over one year with minimal nutrient input, achieving a cumulative sequestration of 26 ± 7 mg CO_2 per gram of material in the stable carbonate mineral form (Figure 1d). This long-term performance is comparable to that of existing chemical-based carbon capture strategies. Together, the integration of tailored material design and advanced bioprinting methods demonstrates the potential of biofabricated, photosynthetic living materials for carbon-neutral infrastructure and green building materials.

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Konuşmacılar: Yifan Cui (ETH Zurich)



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SporoPrinting - 3D Bioprinting of Functionalized Bacillus subtilis Endospores

Embedding modified microorganisms in polymeric and composite matrices, thereby generating genetically programmable Engineered Living Materials (ELM), is a field of high potential that gained tremendous momentum with the advent of the Synthetic Biology era. Among the numerous methods for designing and manufacturing ELM, 3D bioprinting stands out due to its remarkable ability to precisely control both the structure and integrity of the fabricated constructs and the spatial distribution of cells within them [1]. While bioprinting of engineered microbial cells has been reported for a number of bacterial species, its application for their dormant life stages, so-called spores, is still in its infancy, despite their advantages, being robust, metabolically inactive and long-lived. Endospores of Bacillus subtilis, in particular, hold great potential for ELM, since they provide a second layer to implement engineered functionalities that can be genetically programmed into the DNA of the microbial cells. By translationally fusing a gene-of-interest to a gene encoding a suitable anchor protein, a target protein can be immobilized and hence displayed on the spore envelope during the natural differentiation cycle forming so called SporoBeads [2].

In our study, we combined 3D bioprinting with functionalized SporoBeads to create a dynamic and adaptable ELM. SporoBeads were printed using various alginate-based bioinks [3], and the printing process was optimized with respect to spore density, scaffold stability, and controllability of cell growth. By applying growth medium and sporulation medium to the printed scaffolds, we successfully transitioned the printed spores from their dormant phase into an active vegetative state, and subsequently reverted these vegetative cells back into the spore stage. This process increases the concentration of SporoBeads and regenerates the immobilized proteins on the spore surface. Through the attachment of a fluorescent protein to the surface of the SporoBeads and the labeling of a vegetative gene with a separate fluorescent marker, we demonstrated our capacity to regulate the life cycle stages of B. subtilis within the bioprinted scaffold. This ongoing investigation establishes a foundation for developing a range of ELMs with renewable enzymatic functions, leveraging the robust and versatile nature of SporoBeads.

We acknowledge the German Research Foundation (DFG Priority Programme SPP 2451, grant No. GE 1133/35-1) for founding this project.

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Engineering, characterization, and optimization of fiber meshes with chemically and temporally distinct compartments for ELM fabrication

While the research on Engineered Living Materials (ELMs) is picking up speed, our cooperation project “ContainELMs” in the priority program 2451 of the German Research Foundation focuses on a critical, yet underexplored aspect: biocontainment. While previous studies on hybrid ELMs have demonstrated their general functionality, safe containment of living cells remains a challenge.1,2
This project addresses this gap by designing core-shell fiber structures that provide genetic and physical biocontainment. While the Bioprogrammable Materials group of the INM at Saarbrücken genetically modifies bacteria to only survive in a specific microenvironment by engineering a genetic survival-switch, the task of the Advanced Polymers and Biomaterials group of the IFG at KIT is the design of host structures based on core-shell-fiber scaffolds that maintain this specific microenvironment for extended times, with a possible application for bioremediation. To achieve this goal, 3D-electrohydrodynamic (EHD) Co-jetting is used for the spatially controlled synthesis of multicompartment fiber scaffolds containing bacteria in combination with the Chemical Vapor Deposition (CVD) process, which can provide an additional external shell with custom-tailored surface properties.3,4
The main challenges of this project can be summarized as diffusion and survival control. To ensure the survival of the cells during the production processes, the EHD jetting and CVD parameters need to be optimized and customized for the different bacteria strains. As for the biocontainment, the escape of the bacteria from the fibers is to be prevented, while the diffusion of nutrients and reactants into the fibers must be ensured. For long-term use, the specific microenvironment needs to be maintained

prospectively by controlled diffusion of biofactors out of a core reservoir into the bacteria-containing compartment. These challenges can be addressed by optimizing polymers (-blends), their respective solvents, and hydrogels.

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Konuşmacılar: Meike Koenig (Karlsruhe Institute of Technology)

- 363

Fabrication of Symbiotic Engineered Living Materials for Bone–Fat Interface Modelling

Title

Fabrication of Symbiotic Engineered Living Materials for Bone–Fat Interface Modelling

Introduction

The fabrication of in vitro tissues commonly involves the embedding of mesenchymal stem cells (MSCs) within a hydrogel matrix and the addition of biochemical or physical cues to passively instigate differentiation into a desired lineage. The PRISM-LT project adopts a proactive approach by introducing helper cells—genetically engineered yeast or bacteria—into the 3D construct. These microorganisms are designed to detect metabolic cues characteristic of MSC plasticity and respond by secreting lineage-specific stimulants. This synthetic symbiosis aims to facilitate localised, programmable differentiation, enabling the bioprinting of hybrid tissues composed of spatially compartmentalised voxels of bone, fat, and muscle. Two primary applications are envisioned: (1) the development of a bone/fat interface model for studying age-related bone marrow changes, and (2) the generation of structured cultured meat constructs.

Methods

To initiate the design of the system, helper cells were genetically engineered to constitutively express osteogenic and adipogenic factors using plasmid-based expression systems. In parallel, mesenchymal stem cells (MSCs) were encapsulated in gelatin methacrylate (GelMA) hydrogels of varying mechanical stiffness. Initial characterisation focused on optimising growth factor expression in microbial monocultures, as well as assessing the viability, morphology, and behaviour of MSCs within the hydrogel environment. Co-culture experiments in GelMA are planned as a next step to integrate both components under defined conditions.

Results

Engineered helper cells were successfully transformed with plasmids encoding osteogenic and adipogenic factors. Constitutive expression of growth factors in microbial monocultures was confirmed by SDS-PAGE and ELISA. Growth kinetics and viability assays indicated that both microbial systems remained stable under standard culture conditions. In parallel, MSCs encapsulated in GelMA hydrogels retained high viability over 21 days, with differences in cell morphology observed depending on hydrogel stiffness and density. The project has so far focused on the optimisation of individual components and will next aim to synergise them within a 3D construct.

Discussion

These early findings provide proof-of-concept for the feasibility of symbiotic tissue constructs containing MSCs and programmable microbial partners. The capacity of helper cells to survive in a hydrogel and secrete bioactive proteins presents an opportunity for on-demand, localised MSC stimulation. Challenges remain in regulating microbial growth kinetics. Future efforts will focus on spatially resolved bioprinting, multi-lineage differentiation, and the integration of feedback-controlled genetic circuits. Ultimately, this platform holds potential for constructing complex tissue interfaces for both biomedical and food engineering applications.

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Konuşmacılar: Alexandre Trubert-Paneli (University of Glasgow)

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Investigation of (3D) bioprinting processes for high-scale bioproduction and customized additive manufacturing of engineered living materials

Engineered Living Materials (ELM) harness the extraordinary sensory capabilities and versatile production capacities of living organisms to give materials adaptive functions. The combination of ELM with (3D) bioprinting processes is particularly exciting in this context. Through the targeted application of materials or the construction of complex branched or highly porous multi-material structures, production capacities can be expanded and completely new functionalities implemented. However, the various stresses to which cells are exposed during material transfer in printing processes can pose a challenge to maintaining their functionality.

In this presentation, we will introduce various printing processes and their suitability for processing ELM. These range from industrial printing processes for high-throughput production (e.g., gravure and screen printing) to additive manufacturing of 3D objects (e.g., stereolithography and 3D-bioprinting). Different organisms are used to achieve ELM-specific functionalities. Our work examines the extent to which the organisms are resistant to various printing-related stresses (solvents, UV radiation, temperature, and shear stress). To this end, four different model organisms with defined properties and applications are investigated: (1) Through the use of genetically encoded spores, 3D structures with integrated anti-counterfeiting protection can be produced. (2) The application of 3D bioprinted tobacco plant cell cultures serves as a catalytic production platform. (3) Genetically modified microalgae are used as a growth factor microfactory for the serum-free 3D culture of muscle cells. (4) Finally, mechanosensitive bacteria that can convert mechanical stress into fluorescence signals are investigated.

The results provide important insights into the use of printing processes for the bioproduction of ELM and shed light on which processes are best suited for which type of organism. The work thus provides the basis for the future design of production processes for the functionalization of technical materials or the additive construction of ELM.

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Konuşmacılar: Andreas Blaeser (TU Darmstadt, Institute for BioMedical Printing Technology)

- S23 From Science to Product — Rudniew

Panelists: Shulamit Levenberg, Fabien Guillemot, Michał Wszoła, Utkan Demirci, Aleksandr Ovsianikov

Toplantıyı düzenleyenler: Aleksandr Ovsianikov (TU Wien), Nikita Norkin (EPFL)

- S27 Medical Biotechnology — Kisielewski

Toplantıyı düzenleyenler: Jakub D. Rybka (Adam Mickiewicz Univeristy), Łukasz Matusiak (Warsaw University of Technology)

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Biofabrication of durable cartilage implants

How can we durably regenerate damaged tissues in the human body? Despite major advances in regenerative medicine, this fundamental question remains largely unanswered. Current cell-based tissue engineering strategies allow us to create living implants in the laboratory, and through biofabrication we can design constructs that resemble native tissues in composition and morphology. However, while such approaches provide temporarily stable structures, the collagen-based matrices they rely on typically lack the intricate, anisotropic organization that underpins the biomechanical properties, functionality, and long-term stability of native tissues. This limitation has been a central barrier to developing durable cures for mechanically challenged tissues such as articular cartilage.

Recent progress in the field is beginning to unravel the biological and biophysical triggers that guide the formation, alignment, and integration of structural collagen networks. By harnessing these insights, researchers are now coupling biofabrication with biointerface engineering in an effort to steer tissue development in more physiologically relevant directions. Such strategies hold promise for producing implants with improved resilience, functionality, and integration into host environments.

This keynote will explore how emerging technologies and interdisciplinary approaches are converging to address one of regenerative medicine's most persistent challenges: restoring complex load-bearing tissues with long-lasting function. I will highlight current breakthroughs, ongoing limitations, and the future research directions that may ultimately enable us to move from transient repairs to truly durable regeneration of damaged joints

Konuşmacılar: Jos Malda (UMC Utrecht)

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Advancing Fabrication Techniques for Multi-Layered Tubular Scaffolds in Vascular Tissue Engineering

In the human body, tubular structures are prevalent and exhibit various architectures, such as those found in the vascular and lymphatic systems. For instance, blood vessels can be viewed as tubular constructs with a specific diameter when considering their macroscopic shape. A closer examination reveals that these vessels consist of multiple layers, each composed of different extracellular matrix components and cell types. To replicate the hierarchical structure of blood vessels and enhance the biological function of tissue-engineered vascular models, it is essential to produce multi-layered tubular scaffolds.

This presentation will demonstrate how the combination of diverse materials and fabrication techniques can be utilized to create hierarchical tubular constructs with biomimetic architectures and adjustable mechanical properties. By integrating melt electrowriting and solution electrospinning, bi-layered tubular constructs can be generated

for use as small diameter vascular grafts, featuring tunable mechanical properties and optimized cell orientation in co-culture and perfusion bioreactor setups. Additionally, combining melt electrowriting with the innovative additive manufacturing process of volumetric bioprinting enables the creation of defined fiber-reinforced hydrogel structures with customizable shapes. Traditional casting methods, when used in conjunction with fiber-based scaffolds, offer significant flexibility in material selection, which is particularly advantageous for developing drug-eluting structures. This presentation will showcase the latest advancements in these combined techniques.

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Konuşmacılar: Tomasz Jüngst (Department for Functional Materials in Medicine and Dentistry)

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Contrast-enhanced Micro-CT for improved visualization of ACP-Engineered Osteoblastic Spheroids

Introduction: Bone tissue engineering (TE) aims to develop biomaterials that replicate the specific mechanical strength essential for the functional characteristics of the bone (1). Osteoblastic spheroids provide a three-dimensional (3D) culture model that better mimics in vivo conditions than traditional two-dimensional (2D) cultures. Amorphous calcium phosphate (ACP), derived from eggshell waste, has shown enhanced osteogenic potential, making it a promising biomaterial for TE applications (2). However, due to limited penetration depth and structural distortion, conventional imaging techniques such as confocal microscopy and histology pose challenges in visualizing 3D constructs. This study evaluates contrast-enhanced micro-computed tomography (micro-CT) as a non-invasive alternative for 3D morphological analysis of ACP-engineered osteoblastic spheroids.

Methods: Osteoblastic spheroids were cultivated using flat-bottom, U-bottom, and rotary flask techniques. Spheroids were supplemented with ACP derived from eggshell waste and synthesized ‘control’ ACP to assess its effects on osteogenic differentiation. Contrast-enhanced micro-CT imaging was performed using phosphotungstic acid (PTA) and iodine-based contrast agents. Scans were performed with a laboratory nano-CT (SkyScan 2211 Multiscale x-ray Nano-CT System, Bruker) and at the Synchrotron Radiation for Medical Physics (SYRMEP) beamline in the synchrotron laboratory ELETTRA (Trieste, Italy). Imaging results were compared with confocal laser scanning microscopy, scanning electron microscopy, and classical histology.

Results: The cultivation method significantly influenced spheroid morphology, with rotary flask cultivation producing the most structurally uniform spheroids. ACP incorporation enhanced osteogenic activity and spheroid integrity and altered the spheroids’ morphology in volume and roundness. Contrast-enhanced micro-CT provided superior overall visualization of the spheroid and of internal architecture, allowing detailed analysis of cell and ACP distribution and matrix deposition. Unlike traditional imaging techniques, micro-CT enabled virtual histology without sectioning artifacts or depth limitations (figure in attachment applying pseudo-colors).

Discussion: This study underscores the need for advanced imaging modalities to evaluate engineered tissues effectively. Contrast-enhanced micro-CT offers a non-invasive, high-resolution approach for visualizing cellular interactions within biomaterials, addressing key limitations of conventional imaging techniques. The findings support the integration of ACP into bone TE strategies and demonstrate the potential of micro-CT for enhancing the assessment of biofabricated constructs, particularly for large cell models including a mineralized portion.

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Acknowledgments: We acknowledge financial support from the Baltic Research Programme Project No. EEARESEARCH-85 ‘Waste-to-resource: eggshells as a source for next generation biomaterials for bone regeneration (EGGSHELL)’ under the EEA Grant of Iceland, Liechtenstein and Norway No. EEZ/BPP/VIAA/2021/1 and access to the infrastructure and expertise of the BBCE—Baltic Biomaterials Centre of Excellence (European Union’s Horizon 2020 research and innovation programme under Grant Agreement No. 857287). We also acknowledge ELETTRA Syncrotrone Trieste for the beamtime provided at the SYRMEP beamline under Project Number 20225228.

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Konuşmacılar: Torben Hildebrand (University of Oslo)

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Regeneration of Critically-Sized Full-Thickness Mandibular Defects Using 3D-Printed Bioactive Scaffolds in a Compromised Healing Environment

Despite advances in surgical reconstruction of the head and neck region in recent years, several clinical scenarios continue to pose significant reconstructive challenges such as the reconstruction of complex maxillofacial defects affected by chemotherapy, and/or radiation. The common factor which makes these typically post-traumatic or post oncologic defects difficult to treat is the compromised wound bed. Thus, reliable reconstructive methods to re-establish form and function are needed. The current study hence aimed to elucidate the effect of 3D-printed β–tricalcium phosphate scaffolds coated with a bioactive molecule, dipyridamole (DIPY) - an indirect agonist of the adenosine A2A receptor, via different coating techniques to regenerate critically sized mandibular defects in a previously established (irradiated) rabbit model of compromised wound healing. Eighteen New Zealand white rabbits received a total of 36 Gy radiation followed by surgical intervention to induce unilateral, critically sized, 10 mm full-thickness mandibular defects. Animals were divided into one of three groups and defects were (i) left empty - negative control, or filled with either (ii) DIPY 1 coated scaffolds - scaffolds immersed in a mixture of Bovine Type I Collagen and DIPY (1000 µM) (dip coating), or (iii) DIPY 2 coated scaffolds – individual scaffolds were placed into wells of cell culture plate containing the DIPY solution and allowed to dry/precipitate (drop casting). At the study endpoint (8 weeks), rabbits were euthanized, and their mandibles harvested for micro-computed tomographic (µCT), histological, and histomorphometric processing and analysis.Defects left untreated (negative control) demonstrated fracture of the mandible post-surgery, resulting in partial or complete collapse of the defect site. Scaffolds treated with DIPY 2 yielded significantly higher bone formation at 8 weeks relative to DIPY 1 ($p=0.031$). This was accompanied by increased levels of soft tissue presence in the DIPY 2 group in the intermediary regions of the defect site ($p=0.008$). No statistical differences were observed in scaffold resorption at the 8-week time point. However, irrespective of the DIPY coating technique, qualitative histological analysis at 8 weeks depicted bone at the periphery of defects treated with a scaffold. In addition, bone growth was observed to have proceeded from the periphery towards the center of the defect through the lattice-like, porous structure of the scaffolds. 3D-printed bioceramic scaffolds not only effectively provide adequate mechanical stability at the defect site, but also serve as a viable carrier of DIPY – to elicit the desired osteogenic response, relative to untreated defects, in this compromised model of wound healing.

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Konuşmacılar: Lukasz Witek (NYU College of Dentistry)

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Multicomponent alginate-based microcapsules stabilized by polyelectrolyte complexation with novel biguanide-based polycations for pancreatic islets encapsulation in diabetes treatment

Cell microencapsulation is a widely studied strategy for immunoprotection of transplanted insulin-producing cells used for diabetes treatment. The microcapsule forms a semipermeable membrane around the transplanted cells, serving as a barrier protecting cells from the host immune system while allowing diffusion of nutrients, glucose, and insulin.

The most widely studied microencapsulation strategy uses sodium alginate (SA) as the main microcapsule component, owing to its cytocompatibility and fast ionic crosslinking by divalent cations at physiological conditions. However, simple ionically crosslinked SA microbeads face challenges regarding their long-term stability in vivo [1]. Multiple approaches have been proposed to address this issue, including the introduction of polyelectrolyte complexes stabilizing the microcapsule via interactions with polycations such as poly(methylene-co-cyanoguanidine) (PMCG) [2]. However, the preparation of well-standardized PMCG microcapsules with consistent characteristics and in vivo stability sufficient for clinical translation is still challenging.

Our recent study [2] showed that the microcapsule properties, including the in vivo performance, are significantly affected by the structure of the polycation used for microcapsule stabilization. Herein, we synthesized four different groups of new biguanide-based polycations, similar to PMCG, with side groups showing different levels of hydrophobicity. We determined the polymer molar masses using gel permeation chromatography and studied polymer solubility at conditions required for cell encapsulation, focusing on factors such as polymer concentration, pH, and osmolality.

Selected polycations based on metformin and 1-(o-tolyl) biguanide were successfully used for the preparation of microcapsules based on combination of SA and sodium cellulose sulfate (SCS). Microcapsules were prepared by two different preparation protocols: (i) 1-step protocol, in which microcapsules are prepared by ionic crosslinking of

SA with Ca2+ and polyelectrolyte complexation of SCS with a polycation in one step, and (ii) 2-step protocol, in which SCS-containing SA microbeads are prepared by ionic crosslinking with Ca2+ in the first step, followed by their coating in the polycation solution in the second step.

Empty microcapsules were characterized with respect to their size, morphology, and mechanical stability in compression. The data, obtained for microcapsules analyzed both immediately after their preparation and after 7 day storage in the CMRL medium at 37°C, revealed properties comparable to standard PMCG microcapsules used as a control. Additionally, the high microcapsule resistance to ethylenediamine tetraacetic acid (a chelating agent for Ca2+ cations mediating SA crosslinking) confirmed the stabilizing role of the polyelectrolyte complex. Finally, selected microcapsule types were used for cell encapsulation and subsequently characterized in vitro.

Acknowledgement:
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Native-like biomaterials for organoid culture in a controlled environment

Introduction
Organoids are miniaturized three-dimensional layered constructs offering unprecedent resemblance with the structural, biological and functional characteristics of organs. These models provide a new framework to study the cellular processes, the physiology and the treatment of pathologies at the organ-level. Beyond in vitro modelling, organoids can offer a new alternative for in vivo applications in regenerative medicine.
The standard procedure to grow organoids relies on inducing the self-assembly of stem cells in weak hydrogels, commonly Basal Membrane Extract (BME). However, BME derives from tumorous mouse which hinders the possibility of clinical translation. Beyond that, the micro-environment it provides to organoids is poorly controlled, lacks of hierarchical structure, and offers a composition radically different from the native extracellular matrix (ECM)1. Meanwhile, 2D and prosthetic materials have largely been developed using native proteins, but for organoids, biomimetic materials remain little approached2.
We herein propose new materials for organoid culture, composed solely of ECM proteins, with a porous structure to promote nutrients and oxygen diffusion. We compare the ability to support organoids formation of i) fibrillar native-like collagenous matrices, ii) materials derived from decellularized extra-cellular matrix (dECM), and iii) the standard BME. Our materials represent a new alternative for organoid culture, that is animal-free, compatible with clinical translation, and mimic closely the physiology of tissues.

Methods
Highly concentrated collagen I solutions are ice-templated to allow ice crystals growth and the subsequent collagen segregation in-between the crystals. The ice crystals are subsequently melted at low temperature to reveal pores, while collagen packing is maintained and its self-assembly into fibrils simultaneously induced3. dECM solutions are prepared from corpus spongiosum (dECM-CS), and structured using the same ice-templating process and topotactic gelation. All materials are characterized to assess their composition (IHC, hydroxyproline assay, electrophoresis), native-like features (TEM, PLOM, DMA), and textural aspects (SEM). Urothelial stem cells isolated from patient tissues are seeded and grown on the materials with appropriate culture medium. The organoid formation and properties are characterized and compared between the materials and the control (confocal microscopy, IHC, TEM, qPCR).

Results
We successfully obtained porous yet dense matrices, in the wet state, with both molecular type I collagen and dECM-CS solutions (Fig.1). Preliminary results of urothelial stem cells culture in our models demonstrate their ability to direct the self-assembly of cells into organoids. Additionally, we were able to differentiate bladder and urethral organoids based on cytokeratins makers. Further results are expected to uncover the materials properties that specifically instruct organoids formation.

Discussion
Such biomaterials recapitulate the structural, biological and functional features of biological tissues. By exploring the physics of ice, innovative collagen self-assembly techniques, and more complex materials deriving from native tissues, we tailor the biological composition, the collagen conformation, and the hierarchical and textural aspects of the materials' interfaces to direct the self-assembly of stem cells into organoids.

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Bioprinting of bi-layered cell condensation tubular tissues with defined 3D geometries via a photocurable supporting bath

The development of three-dimensional (3D) cell-only tubular tissue constructs at small lumen sizes is a significant challenge in tissue engineering. This study presents an innovative strategy for fabricating multi-layered living conduits with defined geometries by 3D bioprinting multiple cell-only bioinks along with an oxidized and methacrylated alginate (OMA) microgel ink into a supporting OMA bath using setups of multiaxial nozzles. Single-layered and bi-layered cell-condensation conduits have been fabricated. Single-layered conduits were engineered using a coaxial nozzle with an OMA microgel ink and a cell-only bioink, while bi-layered conduits were produced using a triaxial nozzle with an OMA microgel ink and two different cell-only bioinks. These inks were simultaneously printed via a multiaxial nozzle into an OMA macromer support bath followed by photocrosslinking to stabilize the printed structures. Under in vitro culture, biomaterial-free cell-condensation layers were obtained by removing the OMA microgels and differentiated in a cocktail medium. The differentiated bi-layered tubular tissue constructs were subcutaneously implanted into mice, the bi-layered structures were well-retained over time and integrated with the host tissue. This study demonstrates a promising strategy for fabricating single-layered and bi-layered 3D living hollow cell condensations with controlled architectures through a combination of multiaxial extrusion and embedded bioprinting techniques. The technique has the potential to advance the development of engineered living tubular tissues with improved functionality and integration for future grafting applications.

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Konuşmacılar: Sangjin Lee (University of Hong Kong)

S28 Multicellular 3D models of neuronal diseases in various bioinks: Hypes and falls — Goethe

Toplantıyı düzenleyenler: Efsun Şentürk (La Sapienza), Natascha Schäfer (Institute for Clinical Neurobiology, University Hospital Würzburg, Germany)

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Designing 'fit-for-purpose' neurons for tissue engineering

The human nervous system is one of the most complex to model in vitro. It encompasses the largest diversity of cell types, with the most intricate 'hub-and-spoke' networks. Advancements in stem cell biology have enabled development of more sophisticated 3D models of the 'hub', i.e. central nervous system, with the emergence of organoids and assembloids. However, 3D models of the 'spoke' component, i.e. peripheral nervous system, are less developed. This is partly due the challenges of modelling distinct sensory neuronal responses that are induced by their innervation and functional interactions with an end organ. Here I discuss the convergence of advancing technologies in stem cell biology, genetic engineering and bioprinting to model neuronal-tissue interactions, particularly relating to mechanosensory neurophysiology within the skin and muscle tissues. Specifically, I will focus on the value of having functionally relevant neuronal and cellular tissue cell types to refine model development and enable their application to study human neurophysiology in healthy and diseased states.

Konuşmacılar: Mirella Dottori (University of Wollongong)

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The chemo-mechanical regulation of brain development

During brain morphogenesis, neurons extend axons over large distances along well-defined pathways. Axon pathfinding is regulated by both chemical and mechanical signals. However, we currently know very little about how these signals interact. We here show how local mechanical brain tissue properties contribute to guiding neuronal

axons. In vivo time-lapse atomic force microscopy revealed stiffness gradients in developing brain tissue, which axons followed towards soft. Interfering with brain stiffness and mechanosensitive ion channels in vivo both led to aberrant neuronal growth patterns with reduced fasciculation and pathfinding errors. Tissue stiffness not only directly impacted neuronal growth but also indirectly by regulating neuronal responses to and the availability of chemical guidance cues in the surrounding tissue. The expression of long-range chemical guidance cues in both ex vivo multicellular tissues and in vivo brains was regulated by the stiffness of the environment, strongly suggesting that chemical and mechanical signaling pathways are intimately linked, and that their interaction is crucial for morphogenetic events.

Konuşmacılar: Kristian Franze (Max Planck Zentrum für Physik und Medizin)

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The effect of pure 3D hydrogels on the morphology and fate determination of glioblastoma cell lines

Introduction
A deeper insight into the tumor microenvironment (TME) is crucial for advancing cancer research. In order to study the TME in depth, 3D cell culture models are preferred over traditional 2D cultures, as they offer a more accurate representation of cellular behaviour and functional aspects, when interacting with an extracellular matrix (ECM) like structure. This includes key processes such as proliferation, adhesion, and migration [1]. In 3D cultures, hydrogels are commonly used to mimic the ECM. In order to investigate brain cancers, like glioblastoma, the stiffness of the hydrogel must be low to simulate the human brain tissue in vivo, which typically ranges between 50–200 Pa depending on the region and development [2].
Methods
Two human glioblastoma cell lines (U87-MG and LN18) were cultivated in five commonly used pure hydrogels: collagen, alginate, thiolated hyaluronic acid (HA-SH), oxidized alginate-gelatin (ADA-Gel), and Matrigel. The hydrogels were measured for stiffness by cyclic compression tests up to a 15 % strain. Additionally, the cells were evaluated for their morphology, viability, senescence, as well as gene expression, with a focus on inflammatory markers.
Results
Conducting cyclic compression tests, we demonstrate that these hydrogels displayed shear moduli between 30-110 Pa, similar to slices of native human brains. Both glioblastoma cell lines exhibited a more astrocytic-like morphology in collagen and Matrigel, characterized by spread-out cells with protrusions. In contrast, cells in HA-SH, ADA-Gel, and alginate showed a more rounded spheroid-like morphology without notable outgrowth. This morphological trend was consistent with viability data, with cells showing the highest viability in collagen and Matrigel after six days of cultivation. Establishing a 3D senescence assay (3D-X-GAL), we demonstrated that both cell lines in alginate, ADA-Gel, collagen have a higher cellular β-galactosidase activity than in Matrigel and HA-SH. Additionally, the cells displayed hydrogel-induced inflammatory gene expressions, like IL-6, TNFA and the cytokines CXCL9 and CXCL10 compared to 2D cell cultures.
Discussion
These findings highlight the importance of hydrogel selection for the outcome of 3D culture models. The physical and biochemical properties of the matrix significantly influence cell behaviour. In addition, depending on the hydrogel used differences of a cell cycle arrest or senescence as well as expression of inflammatory genes were found. It should be further evaluated, if the hydrogels cause an inflammation induced senescence or a senescence-associated secretory phenotype (SASP). To better mimic the brain TME, we are currently expanding multiple hydrogel materials and incorporating additional brain-specific components, such as anchoring proteins (e.g. fibronectin, laminin, brevican) or lipids [3,4,]. Co-culturing glioblastoma cells with other brain cell types, such as neurons, astrocytes, or microglia, may also enhance the physiological relevance of the model.

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Konuşmacılar: Vanessa Mößler (University Hospital Erlangen)

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Development of oxidized hyaluronic acid Based Hydrogels for Neuronal Tissue Engineering: Effects of Matrix Stiffness on Primary Neurons

Introduction
External forces, notably in traumatic brain injury, can cause tissue-level damage [1, 2]. Meanwhile, mechanical cues on a smaller scale are pivotal in shaping the development, behavior, and function of individual neural cells [3]. Advancing our understanding of injury and disease mechanisms in the central nervous system depends on investigating how cellular forces and tissue mechanics affect both healthy and pathological states [4]. Due to the presence of hyaluronic acid (HA) in the human body and the extracellular matrix (ECM) mimicking properties of hydrogels, HA based hydrogels are a promising candidate for tissue engineering applications, in particular approaches designed to create scaffolds for repairing or regenerating neurological defects and diseases [5]. In this study, we developed stable oxidized hyaluronic acid (OHA) based hydrogels with tunable mechanical properties with ECM mimicking character for neuronal cells.
Experiment and Methods
HA was oxidized using sodium metaperiodate ($NaIO_4$) and lyophilized. Hydrogels were formed by combining various OHA and gelatin (GEL) ratios, with microbial transglutaminase (mTG) as an enzymatic crosslinker. Crosslinking occurred at room temperature for 30 minutes. Mechanical properties were evaluated via parallel plate compression testing. Swelling, degradation, and mechanical stability were monitored over 7 or 28 days.
Three hydrogel compositions were chosen for biological testing. Cell encapsulation and viability were first assessed using WST-8 assays and live/dead imaging with NIH-3T3 cells. Primary E18 rat cortical neurons were then encapsulated to evaluate suitability for neuronal tissue engineering. Neuronal development was assessed via immunostaining, confocal microscopy, and Scholl analysis.
Results and Discussion
Mechanical testing showed that higher OHA and GEL concentrations increased stiffness and long-term stability, while lower concentrations produced softer gels. mTG levels significantly influenced stiffness; higher concentrations improved mechanical integrity, particularly with low OHA/GEL ratios. NIH-3T3 cells showed high viability in all formulations.
Neuronal experiments revealed that hydrogels with intermediate stiffness (~0.5 kPa) supported optimal neuron survival and outgrowth. These conditions, achieved with lower OHA/GEL ratios and high crosslinking, promoted network formation and neurite extension.
Conclusion
OHA/GEL hydrogels with diverse material properties were synthesized. The addition of mTG enhanced long-term stability, stiffness, and temperature stability. These hydrogels could be customized to mimic neuronal extracellular matrix (ECM) properties, making them suitable for 3D neuronal cell cultivation.
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Konuşmacılar: Markus Lorke (Friedrich-Alexander-Universität Erlangen-Nuremberg)

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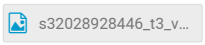
Glioma Hetero-spheroids embedded in ultra-soft Brain-like ECM composites: a platform for investigating glioma-microglia interactions

Glioblastoma multiforme (GBM), the most aggressive brain tumor, interacts with its tumor microenvironment (TME) through complex mechanisms. GBM-TME interactions are primary drivers of tumor malignancy and progression. The TME consists of the extracellular matrix (ECM) and various cells of the brain. Novel research strategies to expand on the understanding of these interactions as a prerequisite to develop new therapeutic avenues are imperative for this tumor type.
We have previously established a triculture system that includes glioma cells, neurons and astrocytes using an ultra-soft hyaluronic acid-based (HA-SH) hydrogel. We found that native molecular mechanisms described for glioma cells in vivo were recapitulated in the 3D composite; including direct cell-cell interactions between glioma cells and neurons or astrocytes. Moreover, the system provided a brain-like ECM that allowed the functional maturation of a neuronal network. In the presence of glioma cells, the

neuronal network had a hyperexcitable phenotype with increased firing rates and synchronicity. The developed platform proved to be a viable option for the study of complex systems and interactions for GBM-TME models.
Here, a modified version of the previous hydrogel supplemented with collagen type IV, laminin and fibronectin is utilized to improve mimicry of the brain ECM. In this modified hydrogel, ANG (astrocytes-neurons-glioma) tri- and MANG (microglia-astrocytes-neurons-glioma) tetraspheroids are embedded to investigate the role of microglia-glioma interactions in a high-cell density configuration. Microglia cells are part of the innate immune response representing the primary line of defense against external agents of the central nervous system . Beyond their immune system function it has been extensively shown that microglia cells are involved in processes to maintain proper neuronal function. Microglia cells have been demonstrated to directly interact with glioma cells thus we want to analyze in MANG spheroids. Furthermore, we aim to investigate the functional impact of microglia on the primary neurons. As shown in the figure, in the presence of microglia cells in the MANG spheroid configuration, neurons generate spontaneous and robust calcium transients. With an assessment of neuronal calcium transients in ANG and MANG spheroids together with the expression profile, we seek to recapitulate and understand the role of the immune milieu of GBM in a high-throughput 3D composite system. Increasing the complexity of the basic building blocks of our previously developed system, for both ECM and cell types, we take a step towards reproducing the complex TME of GBM in an in vitro model.

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Konuşmacılar: Mateo Andrade Mier (University Hospital Würzburg)



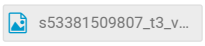
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A mechanically stable and soft electroactive hydrogel interface for neural cellular stimulation and neural growth factor delivery

Introduction: The translation of signals between the human body and biomedical implants is a critical challenge in advancing tissue engineering, regenerative medicine, and diagnostics. Minimizing the inherent differences between neural biological components and synthetic interfaces, particularly in stiffness, biocompatibility, and cellular cues, is crucial for effective neural interfaces1. In this regard, Electroactive hydrogels (EAHs) are promising due to their biomimetic properties: elastic networks, high water content, and electroactive behavior1. These materials can better replicate the mechanical, electrical, and biological properties of the native extracellular matrix1-3. Given the electroactive nature of neural tissue, interfaces interacting with this activity are of greatest importance. This work addresses the need for a mechanically stable, printable, and electroactive soft interface for neural applications1. Its objectives include developing such a material and evaluating its efficiency in interacting with growth factors and modulating neural cell behavior through electrical stimulation to enhance proliferation3 and differentiation2. Successful interfaces could significantly advance our understanding of cellular responses to electrical stimulation, improve tissue repair, and lead to innovative therapeutic and drug delivery systems.
Methods: An electroactive hydrogel composite was fabricated using Gelatin Methacryloyl (GelMA) as the hydrogel matrix and graphene oxide (GO) as the conductive nanofiller. In vitro studies were conducted using neural cells, cultured on the electroactive hydrogel interface. These cultures were subjected to various electrical stimulation protocols to assess cell proliferation and differentiation. The interaction of specific growth factors with the electroactive hydrogel was also investigated, including encapsulation and release characteristics, and the influence of electrical stimulation on growth factor delivery and cellular response. Electrochemical impedance spectroscopy was used to evaluate the electroactivity of the composite hydrogel. Rheological properties were assessed to confirm printability and shape fidelity.
Results: Incorporation of graphene oxide into GelMA significantly enhanced electroactivity, evidenced by reduced impedance and increased charge injection capacity. GO also improved GelMA's rheological properties, enabling the development of an electrically active bioink with high-fidelity 3D printing. The electroactive hydrogel, combined with electrical stimulation, modulated neural cell behavior, enhancing proliferation and potentially differentiation of neuronal cells. GO in GelMA positively influenced cell viability and metabolic activity. The study also demonstrated successful growth factor encapsulation and controlled release, with the hydrogel and electrical stimulation affecting growth factor efficacy on neural cells.
Discussion: These findings highlight the potential of mechanically stable, printable, and electroactive GelMA/GO hydrogels for neural interfaces. The ability to modulate neural cell behavior through the combined effects of electroactive material, electrical stimulation, and controlled growth factor delivery offers advantages over traditional methods. Also, the printable electroactive hydrogels enable biofabrication of complex 3D neural interfaces with customized functionalities, potentially improving in vivo integration in future. This advancement is promising for a variety of neural tissue engineering applications, such as spinal cord injury repair and drug delivery.
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Konuşmacılar: Alexandre Mendes (Swinburne University of Technology)



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Modeling Neuroinflammation-Driven Extracellular Matrix Remodeling Using a Cerebral Organoid-on-Chip Platform

Introduction
The infections, exogenous chemicals, such as drugs1, environmental pollutants and industrial chemicals, may affect the biological processes of the central nervous system as well as its structural, cellular, and molecular function2 and eventually lead to neuroinflammation3 as well as neurotoxicity4. Neuroinflammation is the common cause of numerous neurological disorders, including Alzheimer's disease, and multiple sclerosis. Despite its clinical significance, the intricate cellular and molecular events underpinning neuroinflammation remain incompletely understood, partly due to the lack of physiologically relevant human-based models. Cerebral organoids5 have emerged as powerful three-dimensional in vitro models that recapitulate key aspects of human brain development and architecture. However, conventional organoid systems lack the dynamic microenvironment and mechanical cues present in vivo. Here, we present an advanced cerebral organoid-on-chip platform that enables the controlled study of neuroinflammatory processes, with a particular focus on the often-overlooked but critical remodeling of the extracellular matrix (ECM) within the brain parenchyma.
Methods
Herein, we describe an organoid-on-chip system, which integrates microfluidic control to mimic vascular perfusion and interstitial flow, thereby better simulating the mechanical forces that influence ECM dynamics in the inflamed brain.
Results
Using this platform, we demonstrate that exposure to pro-inflammatory stimuli induces substantial remodeling of the ECM, including collagen, elastin and glycosaminoglycans (GAGs), alongside changes in expression levels of cytokines. Importantly, we show that ECM remodeling precedes and amplifies canonical cellular responses associated with neuroinflammation, such as microglial activation.
Discussion
By emphasizing the role of ECM alterations in the progression of neuroinflammation, our cerebral organoid-on-chip model provides a transformative platform for dissecting the complex interplay between the cellular and extracellular compartments of the human brain. This system not only advances our fundamental understanding of neuroinflammatory mechanisms but also offers a promising avenue for preclinical testing of novel therapeutics aimed at preserving ECM homeostasis and promoting brain tissue resilience. For future work, biosensor integration might be considered for real-time monitoring of soluble factors6.

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- S36 Support baths for embedded bioprinting of biomimetic tissues and in vitro models Mikołajska

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  3D bioprinting of tissue specific high cell-density bioinks for biofabrication of cell condensation-based engineering tissues

  Bioprinting with high cell-density bioinks holds great promise for cellular condensation-based tissue engineering and regenerative medicine. However, achieving precise control over complex tissue structures and organization using high cell-density bioinks remains a significant challenge. Here, we introduce a novel approach for fabricating tissue-specific constructs by directly assembling high cell-density bioinks via three-dimensional printing into a photocrosslinkable and biodegradable hydrogel microparticle supporting bath. Three types of tissue-specific high cell-density bioinks were developed using either individual stem cells or stem cell aggregates, incorporating growth factor-loaded gelatin microparticles to guide differentiation. Once bioprinted into the photocrosslinked microgel bath, the bioinks undergo cellular condensation and differentiate along tissue-specific lineages, forming multiphasic structures such as osteochondral tissues. By varying the incorporated growth factors and cell types, this platform enables the engineering of diverse functional tissues with precisely controlled cellular architecture and organization. Furthermore, by printing patterned, cell-only prevascular bioinks, we demonstrate the formation of complex prevascular networks. These networks, combined with osteogenic microgels in the supporting bath, enhance the development of bone-like tissues.

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  Rebuilding Tendon Architecture and Function with Magnetic 3D Bioprinting

  Current clinical approaches for tendon injuries and disorders remain limited, often leading to suboptimal outcomes such as poor healing and high reinjury rates. Tissue engineering holds promise as an alternative, yet the unique characteristics of tendon tissue—its complex hierarchical architecture, distinct biomechanical properties, sensitivity to mechanical stimuli, and inherently low regenerative capacity—pose major challenges for the design of effective regenerative therapies. Critical requirements include reproducing the fibrillar, hierarchical extracellular matrix, enabling remote activation of mechanotransduction pathways, and providing the biochemical signals necessary to initiate regenerative processes.

  Our group has been developing cell-laden, three-dimensional magnetically responsive platforms that emulate essential features of native tendon tissue. These constructs can be remotely stimulated during in vitro culture or after in vivo implantation through external magnetic fields. Using both conventional and advanced fabrication approaches, such as multimaterial 3D bioprinting, we design magneto-responsive systems that replicate aspects of tendon architecture, composition, and mechanical performance. When combined with appropriate stem cell populations, these systems are capable of guiding cellular behavior toward tendon regeneration.

  We have shown that magnetic stimulation at different intensities and frequencies can promote tenogenic differentiation of human adipose-derived stem cells (hASCs) and modulate inflammatory responses across multiple cell types. At the same time, these 3D cell-laden magnetic platforms function as advanced tissue models, offering insights into the mechanisms of tendon homeostasis and repair. Such knowledge provides the foundation for rational design principles in the biofabrication of living tendon substitutes, with the ultimate goal of enabling effective tendon regeneration rather than mere tissue repair.

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  Construction of vascularized liver tissue model with embedded Bioprinting

  Constructing an in vitro vascularized liver tissue model that mimics the human liver plays a key role in promoting cell growth and biomimetic physiological heterogeneous structures and cellular microenvironments. However, the layer-by-layer printing method is greatly limited by the rheological properties of the bioink, making it difficult to form complex three-dimensional vascular structures in low-viscosity soft materials. To overcome this problem, in this study, we mixed low-viscosity biomaterials with gelatin microgels to form a cross-linkable biphasic embedding medium. This medium has the yield stress and self-healing properties, which is conducive to the efficient and continuous three-dimensional forming capability of the sacrificial ink. We controlled the filaments diameter by controlling the printing speed to adjust it from 250μm to 1000μm, and can accurately control the ink deposition position and filaments shape. We used in situ endothelialization to construct complex vascular structures and achieve close adhesion between hepatocytes and endothelial cells. In vitro study results showed that vascularized liver tissue model showed higher MRP2, albumin synthesis function, and higher enzyme activity than the mixed liver tissue. In summary, this method can quickly construct three-dimensional vascular structures in low-viscosity materials, and the resulting vascularized liver tissue model has good biological functions, opening up new opportunities for clinical applications.

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  A novel support bath for the 3D (bio)printing of highly aligned collagen fibers

  The development of innovative bioinks and bioprinting strategies is critical for advancing tissue engineering and regenerative medicine. Collagen, a major structural protein in the extracellular matrix, is widely used in bioink formulations due to its biocompatibility and ability to support cell growth and differentiation. However, a primary challenge with collagen-based inks is their low viscosity, which can lead to undesirable spreading during the printing process and insufficient structural integrity of the printed constructs. To address these challenges, we developed a novel support bath for the (3D) bioprinting of collagen-based inks. This support bath not only prevents the spreading of low-viscosity inks, but also promotes collagen fiber formation and alignment, which is crucial to many tissue engineering applications.
  The support bath was developed by modifying a previously designed buffer containing phosphate buffer and polyethylene glycol in distilled water. To increase the viscosity of this buffer, we added either nanofibrillated cellulose (NFC) or microfibrillated cellulose (MFC) at different concentrations. Cellulose was selected primarily due to its minimal impact on collagen fiber retention within the solution and its role as a versatile thixotropic agent. To evaluate the efficacy of these viscosity enhancers, we prepared three groups—2% MFC, 10% NFC, and 15% NFC—and compared their effects on final solution opacity and viscosity. As a test ink, we used solubilised articular cartilage extracellular matrix (ECM), which is rich in type II collagen.
  Initial rheological data indicated that the 15% NFC solution exhibited the highest viscosity and stability at the application temperature of 37°C, making it the most promising candidate for the support bath. Furthermore, at higher concentrations, NFC produced a clearer bath than MFC, which could be beneficial for specific printing applications. We evaluated the baths' suitability with 10 mg/ml and 50 mg/ml ECM-based inks to confirm its effectiveness with both low-viscosity and more viscous inks. Among the three groups, only the 15% NFC supportive bath demonstrated adequate support for both high- and low-viscosity inks during and after the printing process.
  To create stable constructs using this 3D printing process, we subjected them to freeze-drying and cross-linking using dehydrothermal (DHT) treatment post-printing. The printed constructs were successfully retrieved by solubilizing the NFC bath in distilled water, demonstrating compatibility with collagen-rich inks. To assess fiber alignment, we used histological analysis with Picrosirius Red staining and scanning electron microscopy (SEM). Both methods confirmed enhanced collagen fiber alignment in the constructs printed within the 15% NFC support bath. Furthermore, SEM demonstrated the successful formation of collagen fibers with D-band periodicity.
  In conclusion, our novel supportive bath containing 15% NFC significantly improves the stability and printability of collagen-based inks, while promoting fiber alignment in a programmable manner. It also supported the formation of collagen fibers with D-band periodicity. This support bath has potential for various tissue engineering applications, offering a robust solution for 3D printing complex collagen constructs.

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  Supported 3D-bioprinting of spheroid-ladden cartilage constructs

  Introduction
  3D-bioprinting for cartilage tissue engineering can provide controlled cell localization and complex tissue shapes fabrication to meet clinical needs. Extrusion-based 3D-bioprinting has demonstrated resolution limitations owing to surface tension effects. Therefore, confined printing techniques such as Freeform Reversible Embedding of Suspended Hydrogels (FRESH) have been explored to improve the manufacturing resolution. Common FRESH techniques rely on a gelatin bath and require separate steps of crosslinking or post-processing. To further explore the use of supported bioprinting setups and include in situ crosslinking, here we explore the use of pluronic and CaCl2-based support baths to 3D-bioprint alginate-based constructs for cartilage tissue engineering applications.

Methods
Hydrogel mixes were explored as bioinks; composed of alginate, gelatin and methylcellulose. Firstly, acellular bioink bioprinting optimisation was performed in unsupported and supported setups by assessing the filament resolution.
Secondly, HMSC-based spheroids (1000-1200 cells/spheroid), were embedded in the bioink at various concentrations (1000, 5000, 10000 spheroids/mL) following previous literature1 and 3D-bioprinted using a support bath setup. These constructs were cultured in chondrogenic differentiation medium for 21 days. Post-bioprinting spheroid viability was assessed through LIVE/DEAD staining and chondrogenic differentiation was evaluated through immunofluorescence staining of specific markers (collagen type-I, collagen type-II, SOX-9, RUNX2, collagen type-X) as well as histological assessment (alcian blue and alizarin red).
Results
The explored FRESH-like bioprinting set up, composed of a pluronic and CaCl2 bath, demonstrated an increase in filament resolution of up to 35% (1600 µm vs 600 µm fiber diameter using 22G needle). Furthermore, the in situ crosslinking of the bioprinted constructs was demonstrated by obtaining stable constructs post-bath dissolution without the need of an additional crosslinking step.
Spheroid-based bioprinting of constructs showed bioink stability across the 21 days of chondrogenic differentiation. Furthermore, spheroid viability post-printing was observed to be high, showing a majority of alive cells and minimal cell death on the spheroid surface. Lower spheroid concentrations in the bioink demonstrated a lower spheroid fusion. The highest concentration of printed spheroids was observed to have high levels of spheroid fusion and matrix production.
Immunofluorescence staining of the differentiated spheroids showed the production of collagen type-II around the spheroids and minimal collagen type-I. SOX-9 expression was still observed after 21 days of differentiation with no expression of RUNX2 or collagen type-X. Alcian blue staining showed the high level of glycosaminoglycan production around the spheroids with no calcium deposits visible through alizarin red staining.
Discussion
Acellular bioprinting optimisation demonstrated an improvement of the bioprinting resolution and a quicker manufacturing process. This opens the door to further exploring pluronic based support baths for 3D-bioprinting applications. Moreover, the inclusion of spheroids in the bioprinting process showed the possibility of using these bioprinting support set ups and the subsequent potential to differentiate them into chondrogenic lineage. Future experiments should focus on increasing the number of spheroids and assess the effect this has on cartilage tissue production.
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Cell-Only 3D Bioprinting of Articular Cartilage Progenitor Cells within a Physically Constraining Support Bath to Engineer a Structurally Organized Tissue

Engineering functional articular cartilage (AC) remains a challenging goal in tissue engineering. Since the function of AC is derived from its depth-dependent organization, the field has typically focused on developing multilayered scaffolds that mimic specific zonal aspects of the native tissue. Scaffolds have succeeded in recapitulating some aspects of native AC, however, they have generally failed to regenerate its intricate architecture, including the arcade-like collagen network [1]. Computational modelling has shown that achieving this collagen organisation is the most important factor in determining the functional success of engineered AC [2]. This motivates the need for innovative strategies to direct collagen alignment. To address this challenge, this study leverages embedded 3D bioprinting to provide spatially controlled physical cues to AC progenitor cells (ACPs) facilitating their self-organization into a structurally organized tissue with an arcade-like collagen architecture.

ACPs were isolated through differential adhesion to fibronectin. Passage 4 ACPs were centrifuged and loaded into a syringe with a 25G needle without a supporting ink (cell-only bioprinting). A methacrylated xanthan gum (XGMA) support bath was prepared by dissolving xanthan gum (0.5% w/v) in deionized water, adding glycidyl methacrylate (7.41% v/v), and stirring overnight at 60°C. The solution was dialyzed (MWCO 6–8kDa), freeze-dried, and stored at -20°C. For bioprinting, 1% w/v XGMA was used, and filaments of 270, 400, and 700µm in diameter were bioprinted. Post-bioprinting, the bath was crosslinked with UV light (Fig. 1A). ACPs were bioprinted into a single-layered sheet in the XY plane with horizontal and vertical filaments to achieve a biomimetic AC collagen organization. Afterwards a two-layered graft was bioprinted with horizontal filaments in the XY plane overlaying vertical filaments in the Z-axis. Following 4 weeks of culture chondrogenesis was assessed through histology, immunohistochemistry, and biochemical assays.

All bioprinted filaments demonstrated robust chondrogenesis as evident by the positive staining for sulphated glycosaminoglycan (sGAG) and collagen. With polarized light microscopy (PLM) it was evident that the thinner bioprinted filament (270µm) supported superior collagen alignment throughout the depth of the tissue (Fig. 1B). ACPs in the bioprinted sheet also stained positive for sGAG and collagen deposition (Fig. 1C). PLM imaging revealed a horizontal collagen alignment in the superficial zone and a vertical collagen alignment in the middle/deep zones (Fig. 1C). Similarly, in the two layered graft a vertical collagen fibre alignment was observed in the vertically bioprinted filaments, while a horizontal alignment was observed in the horizontal filaments (Fig. 1D).

The findings demonstrate that external XGMA boundaries effectively guide neotissue alignment deposited by bioprinted ACPs. Thinner filaments promoted greater collagen alignment throughout the tissue. Spatial confinement of bioprinted vertical and horizontal filaments enabled the engineering of grafts recapitulating aspects of the arcade-like collagen organization of native AC. A limitation of this study is the presence of the bath in the final graft. Hence future work will investigate the use of degradable support baths. In conclusion, this approach emphasizes the potential of 3D bioprinting for replicating the collagen architecture of AC, paving the way to engineering truly functional grafts.

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Enabling Materials and Technologies in a lung-on-chip-device investigating pulmonary fibrosis onset.

Introduction
Pulmonary fibrosis (PF) is a debilitating disease with a poor prognosis, often linked to long-term exposure to harmful substances. Understanding the role of pollutants in PF onset requires sophisticated in vitro models capable of replicating lung physiology and pathology. In this contribution we present the realisation of an innovative approach based on new materials and 3D bioprinting to create a bioengineered lung-on-chip (LOC) platform aiming at mimicking the microstructure and extracellular matrix function.
Methods
The 3D lung model was manufactured by using advanced bioprinting techniques. Embedded bioprinting was employed, exploiting microtissues precursors (µTPs), obtained by dynamic seeding of human lung fibroblasts inside biodegradable porous gelatin microcarriers, as previously reported (1,2).As sacrificial inks, a plethora of materials based on a poly(ethylene glycol)-based poly(ether urethane) (PEG-PEU) and α-cyclodextrins (a-CDs) was developed.(3) Different a-CD concentrations were tested, while keeping PEG-PEU concentration constant at 4% w/v and the hydrogels were characterized through rheology and stability tests. To further modulate their performances, the PEG-PEU-based support baths were enriched with a Pluronic®– based PEU (P407-PEU).
Results
Among the sacrificial ink formulations, the hydrogel with the highest proportion of P407-PEU exhibited superior mechanical and self-healing performances and was thus selected for further investigation as a support bath. Stability tests under culture-mimicking conditions demonstrated structural integrity for up to 7–10 days. To evaluate bioprinting potential, qualitative printing tests were performed using a 3D bioprinter (Dr. Invivo 4D6 - Rokit). The selected hydrogel demonstrated shear-thinning behavior and rapid structural recovery. Furthermore, it showed easy extrusion through an 18G needle while maintaining the filament shape (Fig. 1). These characteristics make it suitable not only as a support matrix but also as injectable biomaterial ink.
The ink demonstrated to be able to support µTPs dispersion to deliver lung tissue models, to be hosted in a microfluidic platform to test the toxicity of pollutants after aerosol exposure.
Discussion
This contribution aims at illustrating the collective use of discipline and expertise such as bioprinting, biomaterial science, customized polymer synthesis, biomaterial processing, in vitro model design, microfluidics, bioengineering, epidemiology and biostatistics (the latter to identify relevant pollutants to be tested) to develop a novel in vitro platform to evaluate the effect of inhaled harmful substances in respiratory disease onset, contributing to the progress of healthcare through advanced techniques and

overcome existing challenges in health management.

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Acknowledgment

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Multimaterial Core–Shell Bioprinting to Fabricate Cultivated Meat Scaffolds

INTRODUCTION

A sustainable alternative to traditional meat is cultivated meat, which is the growth of animal muscle tissue in laboratories. This technology aims to create a cell-laden product that replicates the texture, composition, and structure of conventional meat.[1] However, the hydrogels commonly used in tissue engineering techniques, whether as cast scaffolds or bioinks, often lack the dual capacity to provide both the mechanical properties of meat and the extracellular matrix (ECM)-like environment needed to support cell viability and muscle fiber differentiation.[2][3] Here, we present a bioprinting method using a custom core-shell nozzle to print a filament with a robust gellan gum (GG) shell and an ECM-mimicking core made of recombinant elastin-like protein (ELP).

MATERIALS AND METHODS

We first tested different extrusion rates and concentrations of GG as a shell material in combination with a core of 3 wt% ELP. Naturally derived, edible thickeners, including methyl cellulose (MC) and cellulose nanofibers (CnF), were blended to achieve different core–shell material distributions. Ink formulations were assessed for printability by calculating a printability index, which was based on quantitative evaluation of printed grid structures, flow behavior, and mechanical properties measured using an oscillatory plate rheometer. The ELP core ink was mixed with a model muscle-like cell type (C2C12 cells) and coextruded with the GG shell to produce a multimaterial scaffold. Cell viability and myotube formation after 7 days in differentiation medium was quantified using confocal microscopy.

RESULTS AND DISCUSSION

The results showed that adding GG as a shell and incorporating thickeners into ELP as the core significantly improved the printability of the material system, enabling the fabrication of a continuous multimaterial filament. This approach also enhanced the mechanical properties of the final construct, better mimicking those of commercially available bovine meat. Adding 3% MC to the ELP improved mechanical properties of the core ink, increasing its stiffness to approximately 100 Pa compared to about 10 Pa for the ELP ink alone. A stable core–shell structure, with the core representing approximately 20% of the total cross-sectional area of the filament and achieving a printability index exceeding 0.85, was consistently obtained under optimized conditions. The printed scaffolds maintained cell viability above 90% and supported myotube formation, as confirmed by immunostaining.

CONCLUSIONS

Multi-material bioprinting allowed for the fabrication of a construct with a bioactive core and a robust shell. This strategy of coaxially extruded filaments was able to overcome key limitations of traditional bioprinting materials with low printability. The mechanical properties of the shell supported the fabrication of mechanically robust 3D constructs, while the core supported tissue-like cell viability and differentiation.

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Development of novel poly(2-oxazoline)s-poly(2-oxazine)s based photocrosslinkable hybrid bioink formulations

3D bioprinting is a key methodology in biofabrication, enabling precise spatial placement of cellular, polymeric, organic, and inorganic components to construct three-dimensional biological structures. Extrusion-based bioprinting is widely used, as it supports the printing of hydrogels across a broad viscosity range, accommodates the inclusion of cells and cell spheroids for large-scale constructs, and facilitates relatively high-throughput production [1]. Hydrogels, composed of natural or synthetic polymers, are commonly employed as bioinks to deliver cells into the designed 3D constructs. Natural polymers are favored for their biocompatibility, yet often lack the mechanical strength and rheological properties necessary for effective printing. In contrast, synthetic polymers offer better printability but tend to have limited cellular compatibility.

To overcome these challenges, we utilize synthetic diblock copolymers composed of poly(2-methyl-2-oxazoline) and poly(2-propyl-2-oxazine) (POx-b-POzi) as a smart hydrogel with thermoresponsive and shear-thinning properties. This copolymer serves as a rheology modifier for natural polymers, enabling the creation of hybrid bioinks that combine the favorable characteristics of both material types and facilitate the fabrication of complex 3D biostructures [2].

In this study, we carried out methacrylation on partially hydrolyzed POx-b-POzi, resulting in methacrylated POx-b-POzi. Hydrogel containing both sacrificial and methacrylated POx-b-POzi was supplemented with lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), enabling photocrosslinking of the material. Mouse fibroblasts and pre-osteoblasts were 3D bioprinted with the help of this formulation. Cell-laden constructs showed good cytocompatibility over three weeks in culture, but the cells did not adhere to the crosslinked material and formed spheroid clusters. To address this, methacrylated fish gelatin (fishGelMA) was incorporated into the formulation to provide cell adhesion motifs [3].

Rheological characterizations showed that the adjusted formulation (POx-b-POzi, methacrylated POx-b-POzi, and fishGelMA) was able to retain the synthetic polymer's thermoresponsive gelation features, which enabled 3D bioprinting with high shape fidelity at 37 °C. Fibroblast cells printed with this hydrogel were able to adhere to the crosslinked material. While on the construct surface, cells remained viable over two weeks in culture, the high material stiffness prevented cell growth on the inside. As a next step, the weight percentages of methacrylated POx-b-POzi and fishGelMA, which both contribute to the stiffness of the final structures, will be studied in more detail and optimized.

Overall, we present a hybrid, cytocompatible, and thermoresponsive hydrogel system that enables 3D bioprinting and supports cell adhesion. Further optimization of the material composition will tailor its mechanical properties for applications in tissue engineering and regenerative medicine.

Acknowledgement: This work was supported by Business Finland R2B funding.

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Konuşmacılar: Ekaterina Takmakova (University of Helsinki, Soft Matter Chemistry)

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A Modular Endoscopic Projection System for Spatially Patterned Photocrosslinking in Cartilage Repair

A Modular Endoscopic Projection System for Spatially Patterned Photocrosslinking in Cartilage Repair

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Introduction:
In situ bioprinting enables on-site fabrication of biomaterials directly within defect sites, offering unique advantages for cartilage repair. However, achieving spatial precision and surgical adaptability remains a significant challenge. This study presents a fiber-based light projection system, allowing real-time, patterned photocrosslinking of hydrogels within confined joint spaces and is compatible with standard endoscopes.
Methods:
A 405 nm laser was modulated using a digital micromirror device (DMD) and passed through custom lens assemblies and a beam homogenizer to ensure uniform pattern delivery. The structured light was transmitted through a coherent image guide fiber with minimal resolution loss. This fiber could be used alone or coupled externally to standard 0° or 30° arthroscopes. Light behavior was characterized at working distances of 0.5–2 cm using geometric patterns. To assess biological performance, GelMA hydrogels laden with human chondrocytes were crosslinked into defined architectures and cultured in vitro.
Results:
Structured light crosslinking enabled the fabrication of well-aligned microarchitectures within GelMA-Rhodamine hydrogels. Orientation analysis revealed filamentous features with high directional fidelity, as confirmed by peak alignment at –7.5° in OrientationJ. Cell-laden constructs maintained viability and exhibited robust extracellular matrix production over 54 days. Histological staining (Safranin O, Collagen II) revealed cartilage-like matrix deposition.
Discussion:
This platform offers a compact, spatially precise photocrosslinking strategy compatible with confined anatomical spaces. By enabling the fabrication of structured, cell-laden constructs that support cartilage-like tissue development in vitro, this approach may serve as a foundational tool for minimally invasive bioprinting strategies. The system's modularity allows future adaptation toward intraoperative or image-guided procedures, without compromising biological performance.
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3D Optical Coherence Tomography (OCT) Imaging as a Tool for Quality Assessment of Multi-Material Volumetric Printed Vascular Models

Introduction: The development of biofabrication requires reliable and standardized methods for quantifying a wide range of printing techniques and tissue models to ensure a successful translation into medical applications. With the rise of convergence and the integration of multiple materials, printing processes are becoming increasingly complex, posing challenges for structural analysis. Especially the advancement of volumetric printing (VP) enables the fabrication of complex, three-dimensional vascular models composed of multiple biomaterials.[1] Internal geometries and material transitions are difficult to assess using conventional imaging techniques without destructive sectioning. Reliable and non-invasive quality control methods are essential for the validation of such models, especially given the critical influence of geometry on flow dynamics in vascular applications.[2] Optical coherence tomography (OCT) can offer a non-destructive alternative, combining high-resolution 3D imaging with the ability to differentiate materials based on refractive index differences.[3] This study investigates the applicability of OCT for the quality control of fabricated multi-material VBP vascular models.

Methods: Hybrid vascular constructs are fabricated using advanced multi-material VP strategies that build upon recent developments in the field. The approaches are applied to generate anatomically inspired vascular models with pathological features. Constructs are produced from different hydrogel-based biomaterial resins composed of gelatin methacryloyl, polyethylene glycol diacrylate with distinct refractive properties to enable internal contrast. A commercially available swept source OCT was employed to evaluate structural fidelity and material integration.

Results: The adapted multi-material VP approaches enabled the fabrication of vascular constructs with increased structural complexity and pathological relevance. Internal features, as well as the integration of multiple materials, were reliably visualized and assessed using OCT, where non-destructive standard optical imaging. OCT data revealed high agreement with target geometries and provided volumetric reconstructions of internal architecture. This allowed for qualitative assessment of material distribution, detection of defects such as voids or delamination and for readjusting process parameters to improve printing. The approach was evaluated across different levels of model complexity to assess the detectability of structural features and the potential for quantitative analysis.

Discussion: By combining recent advances in multi-material VP with OCT imaging, a robust platform for the fabrication and non-destructive characterization of vascular disease models was established. OCT proved particularly useful for visualizing complex internal features and heterogeneous material distributions in ways conventional methods could not achieve. The results highlight the potential of this workflow for quality control in advanced tissue model fabrication, especially where functional geometry is essential.

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Fabrication of GelMA/ColMA Hydrogel Microfluidic Model to study vascular endothelial cells

Introduction:
On-chip vascular microfluidic models provide powerful platforms for studying vasculature and its diseases in vitro. These models enable focused investigation of specific vascular layers, such as the endothelium, and the influence of hemodynamics on it. While traditional plastics or glass-based fabrication allows for defined microchannel architecture, its inherent stiffness and low permeability limit biological applications. Thus, hydrogels are gaining interest. Specifically, Gelatin Methacryloyl (GelMA) and Collagen Methacryloyl (ColMA) are attractive due to their porosity, tunable mechanical properties, and inherent bioactivity, closely mimicking the native extracellular matrix (ECM). Here, we present a method combining 3D printing and casting to create hydrogel microfluidic chips with smooth, cylindrical channels.
Methods:
GelMA [1] and ColMA [2] were synthesized according to published protocols. Hydrogels were prepared using 5, 10, and 15% of GelMa and 0.5, 1, and 1.5% ColMa. Compression and swelling tests evaluated physical properties. Porosity was assessed using Scanning Electron Microscopy. Endothelial cell (MS1 and HUVEC) viability on these materials was analyzed. For microfluidic chip preparation, a frame defining the outer geometry was fabricated using Stereolithography printing with a biocompatible resin. Stainless-steel needles (0.8 mm diameter, 3 cm length) were inserted into the frame, and after casting and photocrosslinking the hydrogel, the needles were carefully removed to form smooth, cylindrical lumens. The lumens were then seeded with endothelial cells, which were subjected to pathophysiology-relevant flow.
Results:
Compared to single-component hydrogels, the 15% GelMA / 1.5% ColMA hydrogel exhibited superior mechanical properties, including a higher compressive modulus and lower swelling. The porosity of the hydrogels correlated with the dry content of the gel. All tested hydrogels provided reasonable support for endothelial cells. Formulations with higher solids and 1% ColMA better supported long-term cell culture. The method of chip fabrication produced an optically transparent device, having microchannels with

smooth, cylindrical lumens (800 µm diameter; surface roughness ≤ 1 µm). Channels remained stable under shear stresses up to about 70 Pa. Endothelial cells seeded into the channels responded to flow conditions by changes in elongation and orientation.

Discussion:
Our method generated transparent GelMA/ColMA composite hydrogels. Methacrylation enabled tunable mechanical properties; increased ColMa and solids, enhanced the compressive modulus, and reduced swelling. This provided crucial stability, essential for maintaining channel integrity under perfusion in the hydrogel chip. Indeed, the channels with smooth, cylindrical lumens showed excellent stability up to about 70 Pa of shear stress. This platform enables investigation of endothelial mechanobiology under defined pathophysiological conditions.
Acknowledgment:
The study was supported by Ministry of Health of the Czech Republic (grants nr. NW24-08-00064 and NU22-08-00124), and MEDITECH - Centre for multidisciplinary research in cardiovascular medicine (grant nr. CZ.02.01.01/00/23_021/0009171) and Faculty of Medicine of Masaryk University (nr. MUNI/A/1644/2024).

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Development of a Dual-Bioink Hybrid Scaffold for Tendon Regeneration

Tendon tissue engineering remains a critical challenge due to the need for biomaterials that simultaneously support mechanical load bearing and guide lineage specific cellular differentiation. To address this, we designed a hybrid scaffold system that spatially integrates mechanical reinforcement and tenogenic bioactivity, aiming to mimic native tendon properties more closely than conventional uniform scaffolds.
We developed a dual bioink system engineered to deliver region specific functionality. The first bioink was formulated to enhance mechanical integrity through molecular-level interactions with extracellular proteins, while the second bioink incorporated human adipose derived stem cells (ASCs) and tendon derived extracellular matrix (tECM) components to promote tenogenic differentiation. The two inks were co-delivered using a custom collector system to enable parallel deposition, ensuring microstructural anisotropy. After scaffold fabrication, constructs were cultured in vitro under static conditions.
Cell viability was assessed using live/dead staining and MTT assays at multiple timepoints. Tenogenic differentiation was evaluated by RT-PCR for tendon specific markers including SCX, TNMD, and COL1A1. Mechanical testing was conducted to quantify elastic modulus and ultimate tensile strength, benchmarking against the native tendon range.
The dual bioink scaffold exhibited clear spatial heterogeneity in both mechanical and biological responses. Live/dead staining confirmed high cell viability throughout the biologically active regions, while MTT assays showed sustained metabolic activity. Gene expression analysis revealed a significant upregulation of tenogenic markers in the ASC-tECM regions compared to control hydrogels lacking ECM supplementation. Mechanical analysis demonstrated a significant increase in tensile strength in the reinforced compartment compared to standard gelatin-based constructs, approaching values characteristic of early-stage regenerating tendon.
Our strategy highlights the importance of spatially controlled scaffold design in tendon regeneration. By combining mechanically supportive and biologically inductive regions within a single construct, we achieved both structural resilience and lineage specific differentiation cues. The observed anisotropy is expected to play a crucial role in guiding aligned ECM depositions and long-term tendon remodeling. Importantly, the method avoids reliance on growth factors instead, ECM cues for differentiation, enhancing translational relevance.
This study presents a promising step toward fabricating functional tendon scaffolds with tunable regions tailored to biological and mechanical demands. Further in vivo studies are underway to validate long-term integration and remodeling.

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Konuşmacılar: Byungjoon Choi (Sungkyunkwan University School of Medicine (SKKU-SOM))

- P4 Plenary Session: Prof. Wei Sun Ratuszowa

Toplantıyı düzenleyenler: Jinah Jang (POSTECH)

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Biofabrication for in vitro modeling

Biofabrication uses biomaterials and other biological compounds (cells, aggregates, organoids, etc) as building blocks to create functional in vitro models. This presentation is intended to report some saliant advances in this field, particularly on fabrication of cell-free-3D tissue scaffolds, printing cells, aggregates and organoids, along with the development of bioinks. Examples include printing biomaterials for constructing tissue scaffolds, printing ESC, hiPSC and neuron-cells for regenerative medicine, disease study and drug testing, and printing patient-derived cancer cells and tumor organoids for anti-cancer drug evolution and personalized cancer treatment. Some personal thoughts on challenges and opportunities of the field will also be shared at the end of the presentation.
Konuşmacılar: Wei Sun (Tsinghua University)

- Debate 2 Ratuszowa
- 10:30

Coffee break & Poster session II

- Poster Session II: Poster Exhibition Poster Area

posters are on display whole day on Tuesday and Wednesday
Toplantıyı düzenleyenler: Marco Costantini (Institute of Physical Chemistry Polish Academy of Sciences), Carmelo De Maria (University of Pisa)

- S22a Microfluidics and Biofabrication for Next-Generation bioengineering Rudniew

Toplantıyı düzenleyenler: Ozlem Yesil-Celiktas (Ege University), Fabio Maiullar (Institute of Physical Chemistry, Polish Academy of Sciences)

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Advanced micromaterials and modular bio-inks for biofabrication of engineered living materials

The modular design of tissues is of indispensable importance for proper organ function. Yet, most tissue engineering strategies are based on creating homogeneous tissues, which has limited are capacity to create viable and functional tissues. I will discuss several novel micromaterials and biofabrication strategies we developed to create engineered living matter with modular designs that allow for unprecedented control over cell fate and drive the engineering of functional multiscale tissues. Specifically, we used innovative ultra-high throughput microfluidic droplet/plume generation to fabricate microgels with on-demand tunable biophysical and biochemical properties to controllably program stem cell differentiation along chosen lineages in a temporally controlled manner. These mass produced microgels range from single celled microgels that act as pericellular matrices to multi-celled hollow microgels that acts as organoid forming picoreactors. Microgels were then used to create a variety of advanced bioinks for the biofabrication of engineered living materials with innovative properties, which includes microporous tissues containing high density capillary networks. Moreover, to enable the survival and function of large engineered living matter, we developed a variety of oxygen and nutrient releasing microgels to endow the biofabricated constructs with self-oxygenating and self-feeding properties that allow for the bridging of the prevascular phase. Finally, we have developed several 3D printing techniques including low viscous 3D bioprinting and Xolography to endow engineered constructs with vascular channels. Together this material and technology toolbox allows for unprecedented control over the design and behavior of engineered living matter. In short, I here present several (micro)material and (micro)technology-based concepts that are designed to advance the engineering of multiscale hierarchically organized living matter.

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Clean-Cure Technology: Light-Driven [2+2] Cycloaddition for Initiator-Free and Highly Biocompatible Biomaterial Engineering.

Introduction
The research concept is based on the [2+2] cycloaddition reaction of double bonds embedded in the modified polymer structure, enabling efficient and initiator-free hydrogel crosslinking upon light exposure. It is widely recognized that degradation by-products of photoinitiators exhibit cytotoxicity, which is a major limitation in the field of biomaterials engineering. To overcome this challenge, we initiated the development of materials capable of undergoing crosslinking independently of photoinitiators. The key strategy involved identifying compounds containing functional groups prone to UV-Vis light sensitization, incorporating them into polymer structures via coupling agents, and

optimizing operational parameters for bioprinting applications. A library of hybrid materials was developed to enable the fabrication of highly biocompatible 3D scaffolds by tuning the combinations of different building blocks

Methods
To create a base of new materials, a series of optimization reactions were carried out to functionalize natural polymers. Biomaterials were obtained by functionalizing gelatin with acids such as coumarin-3-carboxylic acid, cinnamic acid or 4-vinylbenzoic acid using coupling reagents. In addition, a series of experiments were conducted to study the properties of the biomaterials. The functionality of the obtained biomaterials was demonstrated by determining the crosslinking profile of the materials, their rheological properties and printability. MTT tests were performed to determine the cytotoxicity profile of the materials, as well as comet assays to determine the level of cellular DNA damage in the obtained material compared to commercially available biomaterials. In addition, microscopic observations were performed and cell viability in the constructs was assessed using the FDA/Pi assay.

Results
As a result of conducted experiments, a class of biomaterials capable of crosslinking under UV-Vis light was created by taking advantage of their ability to perform cycloaddition reactions [2+2]. The materials showed excellent printability, as well as good biophysical properties, high biocompatibility, no cytotoxicity and low genotoxicity. In addition, the biomaterials exhibited photoprotective properties against cells inside the cross-linked structure. The concept used made it possible to eliminate the use of initiators in the crosslinking process.

Discussion
Focusing on the fundamental knowledge related to the application of cycloaddition reactions in material engineering, the research was mainly focused on crosslinking polymers by reacting identical groups with each other in a homodimerization reaction. The study was further extended to the cross-dimerization reaction of various derivatives. The selection of appropriate substituents introduced into the polymer structure makes it possible not only to eliminate the biohazardous photoinitiator, but also to modify the material's crosslinking parameters or its physicochemical, rheological, mechanical and biological properties for a specific application. As a result, the Clean-Cure product series was created, which has a wide potential in materials engineering and regenerative medicine applications.

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Microfluidic Biomimetic Platform to elucidate Stromal-Driven Chemoresistance in 3D Breast Cancer Co-Culture Spheroid Models

Abstract:
Background:
The development of physiologically relevant tumor models is critical for understanding the tumor microenvironment (TME) and its influence on therapeutic response. In breast cancer, cancer-associated fibroblasts (CAFs) are key stromal players that remodel the extracellular matrix (ECM), promote tumor progression, and confer drug resistance. However, conventional static cultures lack the dynamic, spatial, and biochemical complexity of in vivo tumors. To address this, we developed a microfluidic biomimetic platform integrated with confocal imaging to investigate CAF-mediated/ stromal-driven chemoresistance in 3D breast cancer co-culture spheroids.
Methods:
MCF7 breast cancer cells were cultured as monospheroids and co-cultured with either primary human normal fibroblasts (NFs) or TGF-β1–induced CAFs in a 1:3 ratio to generate heterotypic spheroids. Spheroids were generated within Pluronic-coated microchambers of a custom PDMS microfluidic device designed for continuous medium perfusion and real-time optical access. This setup maintains physiological shear and simulates interstitial flow. Before microfluidic loading, IC50 values for Cisplatin (10 μM), Paclitaxel (10 nM), and 5-Fluorouracil (8.5 μM) were derived using MCF7 monospheroids in 96-well plates. Post-compaction, spheroids were treated with single agents and with dual- and triple-drug combinations at 0.25x and 0.5x IC50 under flow for 72 hours. Drug response was measured using MTT and Alamar Blue assays, while live/dead viability was visualized using Calcein-AM/PI staining. Confocal microscopy enabled spatial analysis of proliferation (Ki67), apoptosis (Caspase-3/7), and necrosis (PI). Endpoint qPCR was performed to assess gene expression for ECM remodeling (MMP2, MMP9), anti-apoptosis (Bcl-2), apoptosis (Bax, Caspase-3), and drug efflux (ABCG2).
Results:
CAF-MCF7 spheroids exhibited 1.8-fold higher compaction and denser ECM matrix compared to NF-MCF7 and MCF7-only controls, visibly reducing dye and drug diffusion into the spheroid core. Following monotherapy treatment, CAF-MCF7 spheroids maintained significantly higher viability (Alamar Blue: 72% ± 3.6) compared to NF-MCF7 (48% ± 4.9) and MCF7-only spheroids (42% ± 3.3). Dual-drug combinations improved efficacy (viability reduced to ~55% in CAF-MCF7), while triple combinations were most potent, reducing viability to 38%, though still less effective than in stromal-free controls (22%). Confocal imaging revealed peripheral apoptosis and viable, proliferative cores in CAF-spheroids, indicating spatial drug resistance. qPCR data showed elevated Bcl-2 (2.6-fold) and ABCG2 (3.1-fold) expression in CAF-MCF7 spheroids, suggesting strong anti-apoptotic and drug-efflux activity. MMP2 and MMP9 expression was significantly higher (4.2 and 5.4 fold respectively), correlating with ECM remodeling and increased physical drug barriers. Caspase-3 and Bax were upregulated in treated groups but dampened in CAF-containing conditions, supporting CAF-mediated attenuation of therapeutic apoptosis.
Conclusion:
This study demonstrates a dynamic and modular microfluidic platform for modeling stromal-tumor interactions and drug resistance in breast cancer. The integration of live imaging, shear-based perfusion, and molecular analysis offers a physiologically relevant system for dissecting TME dynamics and evaluating mono- and combination therapies. CAFs markedly influence drug penetration and treatment efficacy through both biochemical and biophysical mechanisms, underscoring their value as therapeutic targets in precision oncology.
Keywords:
Cancer-associated fibroblasts, breast cancer, microfluidics, 3D spheroids, chemoresistance, tumor microenvironment, confocal imaging, combination therapy.

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Microfluidic-Assisted 3D Printing of Functionally Graded Ceramic Scaffolds for Bone Tissue Engineering

Human bone exhibits exceptional mechanical properties due to its hierarchical architecture, which span from the nano/microscopic to the macroscopic scale. The increasing incidence of orthopaedic disorders, as fractures, osteoporosis-related bone loss, and joint degeneration is a growing concern, especially among the aging population. This trend has increased the demand for effective bone graft substitute that promote regeneration, mechanical stability, and biological integration. Bone scaffolds need to provide a porous matrix with interconnected porosity to enhance tissue growth as well as sufficient strength to support physiological loads. Additionally, they must be compatible with the physiological remodelling processes mediated by osteoclasts and osteoblasts. [1] In response to these clinical and biological challenges, researchers are focusing on scaffold-based techniques. A key objective is the development of scaffolds with precise shape, mechanical strength, porosity, and biofunctionality, that replicate the hierarchical and functional complexity of natural bone. Among the techniques used for bone tissue engineering, microfluidic-assisted 3D printing offers unique advantages by enabling the fabrication of customized structures with controlled architecture. [2]

In this study, we present a microfluidic-assisted 3D printing method to fabricate functionally graded ceramic scaffold for bone regeneration. The system employs a custom flow-focusing microfluidic printhead to generate size-controlled oil-in-water emulsion. By adjusting the relative flow rates of the continuous and dispersed phases, the emulsion droplet size, and consequently the local porosity, can be modulated on-demand during printing, enabling the fabrication of scaffolds with spatially controlled gradients. A composite ink based on gelatine methacrylate (GelMA) and hydroxyapatite (HA) was selected for its biocompatibility, osteoinductivity and suitable rheological properties. Notably, the bioactivity of HA allows direct interaction with bone tissue, stimulating growth and accelerating bone healing. [3] Due to its low-viscosity, the ink was extruded into an agarose fluid gel serving as supporting bath. Through strategic emulsion generation and fiber deposition, this method enables precise control over scaffold morphology and architecture, leading to the fabrication of functionally graded porous materials (FGPMs). Morphological characterization via scanning electron microscopy (SEM) revealed that the ceramic grains fused during sintering, while micro-computed tomography (μ-CT) analysis demonstrated well-defined hierarchical internal porosity

architecture of the scaffolds. Mechanical testing confirmed the stiffness of the resulting ceramic scaffold. The sintered scaffold was then seeded with human mesenchymal stem cells (hMSCs) to demonstrate how adhesion and spreading were influenced by scaffold architecture, in particular pore size, interconnectivity, and surface topography, and to assess osteogenic differentiation. These results confirmed the platform's ability to create supportive microenvironments.

In conclusion, microfluidics-based systems combined with rapid prototyping, allow the replication of bioinspired scaffolds that closely mimic the structural and mechanical features of native bone. Mechanical and morphological investigations validated the novel platform's ability to deposit high-resolution 3D structure with density gradients. This platform has great potential to progress the field of bone tissue engineering through the development of biomimetic, high-performance constructs.

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Functional maturation of hepatic organoids on a novel microfluidic chip platform: Optimization of experimental design

Introduction. The liver is a key organ that plays a crucial role in metabolism and is responsible for various functions in the body, including homeostasis, synthesis of essential components, nutrient storage, and detoxification [1]. With the growing need for reliable and effective in vitro liver models for toxicological studies, recent advances have been made in tissue engineering, biomaterials, microfabrication, and cell biology. Among the models developed using bioengineering techniques, induced pluripotent stem cells (iPSCs) derived three-dimensional (3D) hepatic organoids and liver-on-a-chip platforms have shown promising results by mimicking in vivo liver tissue behavior, providing a controlled physiological microenvironment, and eliciting cellular metabolic responses [2,3]. However, a significant limitation of these models is their retention of immature, fetal hepatoblast characteristics. Additionally, they fail to fully recapitulate the complete spectrum of mature hepatic cell types and exhibit substantial inter-culture variability, hindering their capacity to accurately model mature liver physiology [4]. So, in this study, we aimed to evaluate the molecular and functional maturation of hepatic organoids on a newly designed microfluidic chip platform that simulates low shear stress conditions with in vivo-like physiological dynamic laminar flow. The goal was to develop a model that closely approximates human liver functionality, as assessed through various functional analyses, including albumin secretion.
Methods. To this end, the Box-Behnken module in the Design-Expert v7 software was employed to identify the independent variables; organoid passage number (P/3-15), perfusion flow rate (5-15 µl/min), and maturation duration (7-13 days) that would optimize albumin level, a key functional marker of hepatic organoid maturation. These variables were selected as inputs, and a predicted experimental design was generated.
Results. Based on this design, iPSCs-derived hepatic organoids were cultured on-chip platforms during both expansion and maturation period after embedding in hydrogel (Figure 1), with albumin concentration data serving as output for statistical analysis. Using response surface methodology, the program determined the optimal parameters as independent variables, and further characterization of the optimal on-chip maturation experiments were carried out based on fat accumulation, ammonia and urea levels, CYP3A4 cytochrome activity and confocal imaging for specific markers after FunGI tissue clearing.
Figure 1. a. Hepatic organoids in culture and on b,c. layer-by-layer designed perfusable microfluidic platform
Discussion. Our newly designed perfusable liver organoid-on-a-chip model holds significant potential for preclinical pharmacokinetic high-throughput drug development and screening studies, due to its ability to serve as a functional perfused interface compatible with other organ-specific organoid-on-a-chip systems and to be integrated into humanoid-on-chip platforms.
Acknowledgments
This study was financially supported by the Scientific and Technological Research Council of Turkey (TUBITAK) – Science Fellowships and Grant Programmes (BIDEB) 2218 through number of 123C325.
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Bioengineered Model for investigating the role of the microenvironment in human testicular cell function

Spermatogenesis is a highly coordinated process occurring within the seminiferous tubules, where germ cells develop in close association with somatic Sertoli cells (SCs). These cells provide essential structural, metabolic, and regulatory support to developing germ cells, and their functionality is tightly regulated by the surrounding extracellular matrix (ECM). However, replicating the complex human testicular niche in vitro remains a major obstacle for advancing in vitro spermatogenesis (IVS), particularly with respect to long-term SC function and proper spatial organization. This study presents the development and characterization of a three-dimensional (3D) connective tissue equivalent (CTE), enriched with key ECM components, as a biomimetic platform to support human SC (hSC) function and maturation.
We employed a two-step tissue engineering strategy to fabricate an ECM-rich CTE using human stromal fibroblast-derived microtissues (µTPs). Porous gelatin microbeads, produced through double emulsion and crosslinked with glyceraldehyde, served as scaffolds for stromal cell attachment. These µTPs were dynamically cultured for 9–11 days in spinner flask bioreactors to promote matrix deposition, and subsequently fused in silicone molds under dynamic conditions to generate dense, collagen-rich CTEs containing testis-relevant ECM proteins such as laminin and fibronectin. hSCs were introduced either as single cells or pre-formed 3D spheroids onto matured CTEs and maintained under air-liquid interface (ALI) culture with hormone supplementation (FSH and testosterone) for up to 28 days.
The CTE-supported cultures demonstrated progressive cellular integration and ECM remodeling. Histological analyses revealed more efficient retention and 3D organization of hSC spheroids compared to single-cell seeded constructs. Masson's Trichrome staining confirmed increased collagen deposition over time, particularly around spheroids. Immunofluorescence revealed progressive upregulation and spatial organization of tight junction proteins ZO-1 and OCLN, with more pronounced barrier integrity observed in the spheroid-based group by day 28. Gene expression analyses via qRT-PCR supported these findings, showing significantly higher expression of Sertoli-specific (SOX9, ABP, GATA4) and blood-testis barrier-related markers (CLDN11, ZO-1, FSHR) in the spheroid-seeded constructs at 28 days. These results underscore the synergistic role of ECM composition and spatial cell pre-organization in promoting SC maturation and niche remodeling.
In conclusion, the engineered CTE provides a functionally supportive testicular microenvironment that sustains hSC viability, phenotype, and barrier formation in vitro. The use of pre-formed hSC spheroids proved superior to single-cell approaches, enabling more effective recapitulation of the in vivo-like 3D architecture and function. This platform holds strong potential for future applications in IVS research, disease modeling, and fertility preservation strategies by offering a reliable and physiologically relevant model of the human testicular niche.

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Programming motion and shape-morphing in bioprinted tissues via dynamic magnetic fields

Introduction
Shape changes during heart development, known as looping, are crucial for its morphogenesis [1]. Conventional bioprinting techniques produce static structures that bypass the shape-morphing cascades, essential for tissue maturation during development. 4D materials that can undergo shape changes due to external stimuli such as magnetic fields, light, etc., can play a key role in mimicking these dynamic shape changes, which is critical in understanding heart development [2]. They can also regulate the spatial arrangement of cells in 3D-engineered cardiac structures. Magnetic bioinks for 4D shape-morphing have been studied before and proven to improve hydrogel cell-seeding efficiency [3]. However, the complexity of motion that can be achieved with this technology has been restricted.
Herein, we have developed controlled geometries using collagen-based magnetic inks and actuated them by an external magnetic field to mimic the cardiac shape changes occurring at the embryonic stage. Further, we have studied the patterning and assembly of human iPSC-cardiomyocytes encapsulated in the ink. We believe that the 4D shape-morphing due to the programmed magnetic actuation would improve the functionality of the human iPSC-cardiomyocytes.

Methods
Fe3O4 magnetic nanoparticles (MNPs) of varying concentrations (0-5 mg/ml) were mixed with 4.8 mg/ml of neutralized collagen to form the acellular bioink. We conducted biocompatibility and rheology analysis with these concentrations to determine the optimal MNP concentration for further studies. 5 mg/ml of the MNP was chosen to print multimeric geometries with collagen and magnetic ink in a 0.5% agarose support bath. The constructs were then actuated with the help of an external magnetic field built within an in-house 3D bioprinter with an integrated magnetic probe with XYZ control. Further, the viability of the human iPSC-cardiomyocytes encapsulated within the bioinks in a support bath was determined by a live-dead assay. Conjugation of the Fe3O4 to collagen was prepared for docking using UCSF Chimera, followed by docking with Autodock Vina.

Results
The developed collagen-based magnetic ink exhibited superior printability and rheological properties. As shown in Figure 1C, upon programmed magnetic actuation, the multimeric U-shaped geometry bends or forms a closed loop mimicking the shape changes like bending, buckling, and twisting that happen during embryonic cardiac looping. The straight tube with magnetic ends buckles together to form a U-like geometry, with the tail ends attracted to the magnetic probe.

Discussion
The programmed magnetic actuation to the multimeric geometries replicated the embryonic shape change during cardiac looping. Ongoing work involves the actuation of the human iPSC-cardiomyocytes encapsulated within the magnetic bioink, which is believed to enhance its functionality.

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Development of a Patient-Specific Drug Testing Platform Using Vascularized 3D Bioprinted Spheroids and Liver dECM Bioink

Introduction:
Accurate preclinical drug evaluation relies on physiologically relevant models that replicate the native tissue microenvironment, including structural architecture and vasculature. Liver cancer, a major global health burden, often requires complex and individualized treatment strategies. However, current therapeutic approaches are typically generalized, failing to address patient-specific responses, which contributes to suboptimal outcomes. Developing reliable liver cancer models that mimic the biological environment is therefore crucial. In particular, the liver's highly vascularized and metabolically active nature necessitates specialized modeling to capture its complex microenvironment. Traditional 2D cultures and Matrigel-based models fall short in replicating these complexities. Recent advances in 3D bioprinting enable uniform fabrication of multicellular constructs with spatial control, allowing for the integration of functional tissue and vasculature. This study demonstrates the feasibility of using liver decellularized extracellular matrix (dECM) to recreate the tumor microenvironment in hepatocellular carcinoma models. Building on this, we present a 3D bioprinted spheroid platform incorporating tumor cells and endothelial cells, designed for patient-specific drug screening in liver cancer.

Methods:
Liver dECM was used as a bioink to fabricate adherent cells or spheroids containing iPSC-derived hepatocytes, HepG2 cells, or patient-derived liver cancer cells. To evaluate the feasibility of dECM, iPSC-derived hepatocytes were cultured on dECM and Matrigel in both 2D and 3D environments. Albumin and CYP gene expression levels were quantified. For drug screening, HepG2 cells were cultured in 2D with dECM and treated with a panel of 10 anticancer agents. Subsequently, HepG2 and patient-derived tumor cells were encapsulated in liver dECM and bioprinted into 3D spheroids for evaluation with three selected drugs. To assess the effect of vascular proximity, endothelial cells were printed at varying distances from the spheroids, and angiogenic behavior was analyzed.

Results:
In both 2D and 3D conditions, iPSC-derived hepatocytes cultured with liver dECM showed elevated albumin expression compared to those in Matrigel. Notably, CYP gene expression was significantly increased under 3D culture conditions with dECM, indicating enhanced hepatic metabolic function. In case of 2D cultured HepG2, several anticancer drugs showed heightened sensitivity in the presence of dECM. Drug testing in 3D spheroids using HepG2 and patient-derived tumor cells revealed different responses, suggesting improved predictive relevance. Endothelial cells exhibited distance-dependent angiogenic behavior when printed near spheroids, highlighting the spatial influence on vascular growth.

Discussion:
Liver dECM supports hepatic functionality more effectively than Matrigel, especially under 3D conditions that promote metabolic gene expression. The use of 3D bioprinting enabled precise spatial control in fabricating uniform spheroids and integrating vascular components. The observed distance-dependent angiogenesis emphasizes the importance of vascular organization in shaping the tumor microenvironment. Furthermore, modulating spheroid size could induce hypoxic conditions, potentially enhancing angiogenic signaling and promoting the formation of more physiologically relevant vasculature. Because we observed distinct inter-patient variability in drug responses, this integrated platform offers a promising strategy for personalized drug evaluation in liver disease and oncology.

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- S22b Bioprinting From Earth to Space Ratuszowa

Toplantıyı düzenleyenler: João F. Mano (Universidade de Aveiro), Marina Volpi (Warsaw University of Technology, Poland)

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Bioprinting in space

In the last decades crewed space research was limited to the International Space Station (ISS) from where a fast return to Earth in case of severe health issues of the astronauts always is possible. This will not be the case anymore in the upcoming space missions to Moon and finally maybe to Mars. As a consequence, health care support mechanisms must be developed that are completely independent from Earth. Biofabrication technologies and specifically 3D bioprinting methods are seen as such solutions as they enable the opportunity for fabricating human tissues that can be utilised to treat injured or otherwise ill astronauts on-board the spacecraft or in extraterrestrial human settlements. Several international space agencies and research teams have started therefore to look into the possibilities to establish biofabrication technologies in space (1,2).
However, current research focus is the investigation of opportunities - but also limitations - of operating such systems like extrusion bioprinters under microgravity (µg) conditions. Respective studies also can be performed in parabolic flight experiments in which short µg phases can be realised. Another important aspect is the logistics of bioprinting experiments in space as crew time is very limited and astronauts not specifically trained for the multiple procedures. Especially preparation of bioinks might be complicated and impeded by the lack of equipment like sterile benches and centrifuges. We therefore have looked into opportunities to store pre-mixed bioinks or process them using frozen cell suspensions (3).
The lecture will provide an overview about first experiments that already have been performed during parabolic flights, at the ISS or using fully automated satellite-based systems. In addition, possible applications both in research and support of astronauts' health will be discussed.

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Development of a Microgravity Simulating Platform for 3D-Bioprinting Applications

Introduction: Experiments on the International Space Station (ISS) involve extremely high costs, often amounting to several million euros. These expenses primarily arise from transportation costs and the valuable time required from astronauts. Additionally, long-term missions to the Moon or Mars face significant challenges due to limited resource availability.1 Due to the high costs of conducting experiments in space and the complexity of addressing potential physico-chemical challenges in such an environment, it is crucial, to conduct realistic pre-tests on Earth before transferring experiments to space. This strategy maximizes scientific output while minimizing both time and monetary expenditure in space. In this context, our project aims to develop a simulation platform that enables the analysis and optimization of 3D bioprinting processes under simulated microgravity (µg) conditions on Earth. Successfully implemented, this platform holds great potential to improve the cost-effectiveness and success rate of bioprinting experiments in space.
Methods: Our simulation platform utilizes a droplet-based 3D bioprinting technology in a submerged setup, also known as submerged bioprinting.2 In this approach, bioinks and hydrogels are printed into an unpolar liquid, balancing gravity with buoyancy to create a levitation effect. By adjusting density ratios, different gravitational levels, such as those found on the Moon or Mars, can be simulated. To optimize the printing process, we investigate interactions between bioinks and immersion liquids, focusing on wettability, droplet dynamics, and coalescence behavior. Highspeed cameras capture the droplet movement and coalescence, which are then compared to analytical models. Additionally, experiments conducted during parabolic flights provide real µg effects to validate and enhance the simulation platform. A test stand was developed and successfully tested during the 85th ESA Parabolic Flight Campaign in November 2024.
Results: The findings indicate that the immersion liquid effectively modulates buoyancy similar to a µg environment but also leads to significant changes in wettability (e.g., doubling of the contact angle) and droplet dynamics. Highspeed imaging indicates that droplet velocity is substantially reduced in the immersion liquid, leading to a 10–35 % reduction for standard settings. A particular challenge arises from the formation of complex droplet shapes at higher kinetic energies, which do not correspond to droplet behavior in air. Results from the parabolic flight campaign suggest that the influence of µg decreases as substrate wettability increases. In µg conditions, droplet height varied approximately between 0–20 %, depending on substrate wettability.
Discussion: These results provide valuable insights into validating the simulation platform and expanding the understanding of fluid dynamics in 3D bioprinting. The detailed analysis of material-fluid interactions enhances process comprehension and enables targeted optimizations. The simulation platform is expected to facilitate the planning, validation, and optimization of future bioprinting experiments in space. This could increase the scientific relevance and technological maturity of such experiments while offering economic advantages through cost reduction and improved resource efficiency. Long term, this technology has the potential to simplify bioprinting experiments in space, contributing to biotechnological research, medical applications, and food production on long-duration missions.3

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PULSE - Magneto-acoustic 3D bioprinting onboard the International Space Station

Introduction
Microgravity provides a unique environment for advancing tissue engineering and biofabrication by eliminating gravitational constraints such as sedimentation, buoyancy, and hydrostatic pressure gradients. These factors enable 3D bioprinting of tissue and organ constructs of more complex geometries in three dimensions, offering structural and functional fidelity that is difficult to achieve under terrestrial conditions. The PULSE (3D Printing of Ultra-fideLity tissues using Space for anti-ageing solutions on Earth) project aims to leverage these advantages for bioprinting high-fidelity cardiac tissue models in space for ageing research and drug development.
Methods
The PULSE project includes the development of a novel levitation-based bioprinting system that combines acoustic and magnetic fields to position and fuse cellular spheroids into complex tissue constructs. This system will be validated under real microgravity conditions during a mission to the International Space Station in 2027. For the implementation of the PULSE mission, science requirements were first identified and translated into system requirements and specifications. Then, an accommodation study was conducted with the aim of identifying the most feasible solution for the accommodation of the PULSE device. Interface requirements with ISS systems include electrical power, data communication, crew interaction procedures, and launch safety compliance. Biological protocols are being developed to ensure tissue viability throughout all steps of the mission: from ground preparation and launch to in-orbit experiment execution and sample return.
Results
The requirements of the PULSE project led to the selection of a concept able to operate within a self-contained payload unit with integrated environmental controls and autonomous medium exchange and sample fixation capabilities. To minimize the need for crew time, crew interaction will be limited to the introduction of the bioink compartment containing the biological samples into the main unit of the PULSE device prior to installation in the ICE Cubes Facility inside the Columbus module of the International Space Station. All other functionalities will be performed autonomously by the PULSE device or controlled from ground with near-real time interaction between the science team on Earth and the PULSE device in space through the ICE Cubes Mission Control Center. Preliminary ground-based bioprinting tests using the levitation system have demonstrated the ability to successfully perform sample levitation. Biological tests showed compatibility of the science protocols with the proposed mission scenario. The experiment is planned to have a duration of 4-6 weeks in orbit. At the end of the experiment, samples will be returned to Earth for post-flight analysis.
Discussion
This work demonstrates the technical feasibility and scientific potential of using the microgravity environment towards the development of new biofabrication technologies with benefits for Earth and space exploration. This project is also a case study in the end-to-end process of translating terrestrial biofabrication platforms into operational spaceflight payloads, highlighting the challenges and opportunities of interdisciplinary convergence between biomedical science and aerospace engineering.

Acknowledgments
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Disclosure
The authors declare no competing financial interests.

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Bioprinting of dense, biomimetic type I collagen coacervates for long-term 3D cell culture

Type I collagen, is the central component of the extracellular matrix (ECM) and its use in the context of regenerative medicine is expected to mark an important turning point towards the use of biomimetic materials in regenerative medicine. Collagen provides mechanical support and biological cues for cellular adhesion and proliferation making it an ideal choice for fabricating biomimetic scaffolds1. In vivo it presents a hierarchical organization in which collagen triple helices self-assemble into highly ordered fibrils which are necessary for cell adhesion and to in a process called fibrillogenesis. However, these structures are seldom found in vitro since their formation requires extremely high collagen concentrations 2.
Integrating collagen's intrinsic ability to self-assemble with a bottom-up fabrication technique such as 3D printing could bridge the gap in recreating a new generation of biological tissue analogues, able to reproduce their hierarchical nature. Despite the potential to overcome traditional tissue engineering limitations, this combined approach is extremely difficult to implement 3 due to the dramatic viscosity increase with concentration.
Here, we report a new strategy for formulating highly concentrated collagen bioinks with drastically reduced viscosity able to bypass current limitations in 3D bioprinting type I collagen and ensure an ideal environment to support cellular processes.4
The bioink relies on the interaction between collagen and ATP, a small electrolyte, causing the partition and concentration of collagen molecules inside droplets. Rheological measurements allowed to assess the impact of the coacervation process on printability. The formulation and the rheological properties of the bioink were correlated to the viability, proliferative status, morphology and migratory ability of Normal Human Dermal Fibroblasts (NHDFs) in a collagen matrix during 21 days of culture.
Rheological analysis revealed a ten-fold decrease in dynamic viscosity with respect to collagen in solution at equivalent concentration. Importantly, collagen coacervation does not impair the fibril formation process and allows the formation of ordered collagen motifs.
The biocompatibility was assessed by NHDFs' metabolic activity in dense collagen matrix at 60 mg/mL. Under the confocal microscope (Figure 1) fibroblasts display physiological characteristics (spindle shape and cytoplasmic projections) and colonized and remodelled the matrix the entire printout volume. Because of the biomimetic nature of the resulting materials the final geometry is constant even up to one month of cell culture, a rare feature since fibroblasts tend to contract sub-physiological collagen matrices.
Our formulation offers an innovative collagen bioink, with drastically reduced viscosity, compatible with extrusion-based bioprinting at physiologically relevant concentrations. Moreover, our bioink differentiates itself from existing literature as the only available alternative to process highly concentrated collagen solutions (60-80 mg/mL) by 3D bioprinting, while encapsulating cells. This work provides a compelling formulation to significantly improve the design of 3D cell culture scaffolds and tissue engineering constructs, enabling a better reiteration of tissue macro and microanatomy and enhancing biological function with extended lifespan.

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Multifunctional Neural Tissue-Specific Bioadhesives for Regeneration and Long-Term Stability of Peripheral Nerve Interfaces

Implantable peripheral neural interfaces (PNIs) have demonstrated considerable versatility by facilitating direct access to targeted nerves with high signal specificity, thereby enabling both the acquisition of physiological information through electrical recording and the modulation of organ function via controlled electrical stimulation. However, the long-term performance of most implantable devices remains suboptimal due to persistent mechanical and procedural challenges. A primary cause of tissue damage arises from the modulus mismatch between the compliant neural tissue and the rigid conventional electrode materials. Moreover, in practical settings, the epineurium is often excised to improve the signal-to-noise ratio, a procedure that further exacerbates mechanical trauma and incites inflammatory responses, resulting in tissue encapsulation. Collectively, these adverse biological reactions—ranging from physical damage to fibrotic tissue formation—diminish electrical signal transmission and ultimately compromise the chronic stability and functional integrity of PNIs. To overcome these limitations, we propose a neural tissue-specific adhesive hydrogel engineered to support peripheral nerve regeneration and preserve sustained neural signal fidelity at the interface. We developed neural protein-enriched extracellular matrix adhesives (NeuPEA) by incorporating recombinant Annexin A2, which is a pivotal protein in peripheral nerve repair, into ECM hydrogels enriched with proteins associated with peripheral neural tissue. Schwann cells cultured within NeuPEA exhibited markedly upregulated neural-specific gene expression and enhanced functional activity compared to those within non-nerve-specific ECMs, validating its superior bioactivity and regenerative potential. To impart tissue adhesiveness, tyrosine residues were chemically converted into L-3,4-dihydroxyphenylalanine (L-DOPA), a natural catechol-based adhesive moiety. This transformation was achieved via visible light-induced oxidation using a ruthenium/sodium persulfate photocrosslinking system, facilitating rapid and robust hydrogel adhesion to both neural tissues and electrode substrates. Notably, the L-DOPA content increased quantitatively in direct proportion to the NeuPEA concentration. Additionally, NeuPEA exhibited a lower modulus compared to native nerve tissue, offering improved compliance and thereby alleviating modulus mismatch-induced tissue injury. This mechanical softness further contributed to reduced viscosity and lower injection force, enabling easy administration within confined peripheral nerve regions. Importantly, the hydrogel maintained high electrical conductivity and low impedance, both of which are essential for the reliable acquisition of neural signals without interference. Finally, we successfully stimulated the rat sciatic nerve, as evidenced by ankle movement, and acquired sensory signals in response to various stimuli such as brushing and pressing. Overall, NeuPEA represents a promising neural adhesive hydrogel that combines regenerative capability with multifunctional properties, offering integrated material strategies for enhanced long-term stability and performance of PNIs, with potential applications in neural interface engineering and regenerative bioelectronic systems.

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Bioprinted Breast Cancer-Stroma Model for Real-Time Analysis of Tumor Cell Migration Dynamics

Invasion of cancer cells into surrounding tissue represents the first step of metastasis and is strongly controlled by the tumor microenvironment. In breast cancer, adipose-derived stromal cells (ASCs) from adjacent adipose tissue are increasingly recognized as important regulators of this process, but the underlying mechanisms remain unclear. While bioprinting offers unique advantages for investigating tumor-stroma interactions, its potential has been limited by a lack of bioinks that support cell migration. To address this, we developed a migration-permissive bioink and generated a fully 3D bioprinted breast cancer-stroma model to analyze the migration and invasion dynamics of tumor cells in response to neighboring ASCs.

Various methacrylated collagen type I (ColMA)-based bioinks were characterized regarding printability, rheology, mechanics, and ultrastructure. Cell viability and migration capacity of MDA-MB-231 breast cancer cells in ColMA bioinks were analyzed by live/dead staining and spheroid invasion assays. Utilizing extrusion-based bioprinting, we generated compartmentalized co-culture constructs. Printed constructs were imaged over 4 days, capturing brightfield and fluorescence z-stacks. After image deconvolution and segmentation, cancer cell migration in x/y/z-direction was tracked for computing key migration parameters including track length, speed, sphericity, displacement towards and invasion into the stromal compartment.

After bioprinting, constructs displayed a fibrous collagen network morphology and high cell viability (>90 %). Migration of MDA-MB-231 cells was shown to be highly dependent on matrix remodeling via matrix metalloproteases and influenced significantly by ASCs. 3D real-time tracking of migrating tumor cells within the bioprinted co-culture model revealed that ASCs within the stromal compartment markedly enhanced migration and invasion parameters, as evidenced by an increase in mean track speeds by 29 %, a distinct displacement towards stroma, and a 2.5-fold increase in the number of invading cells. Further, a linear correlation between cell speed and cell sphericity suggests that ASC-driven morphological changes boost migration speed. This 3D bioprinted model reveals new insights how stromal cells from adipose tissue influence the invasion dynamics of breast cancer, and thus holds strong potential for anti-metastatic drug screening.

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Unexpected Pathways: Non-Medical Explorations in Biofabrication on the Journey Toward Therapeutic Impact

Biofabrication technologies have been largely developed to address medical needs, with the ultimate goal of enabling regenerative therapies. However, in our effort to advance toward that goal, we often generate tools, insights, and applications that reveal their own value in complementary, non-medical domains.
In this keynote, I will share how our work in chaotic bioprinting—originally designed for engineering aligned, vascularizable, and functional tissue constructs—has unexpectedly opened doors to alternative applications outside of traditional therapeutic frameworks.
For instance, our pursuit of structured musculoskeletal tissues and prevascularized constructs led us to develop methods for fabricating hydrogel-based architectures with internal microchannels. These channels enhance mass transport and offer a highly accessible surface-area-to-volume ratio. While initially conceived as a strategy to improve the survival and function of living tissues, this approach also revealed opportunities for scalable cell expansion—relevant for both regenerative medicine and future applications in cultured meat.
Inspired by this crossover potential, we adapted our chaotic printing platform to create structured, plant-based meat analogues through FORMA Foods. By translating lessons from tissue engineering into food-grade materials, we are now exploring the fabrication of fibrous, texturally complex constructs that reach consumers sooner and offer insights into scale-up, material behavior, and structure-function relationships—insights that may eventually inform biomedical strategies.
We also explored the biofabrication of microbial consortia using spatially controlled hydrogel constructs. By positioning bacterial species according to their known physiological preferences—such as oxygen sensitivity, acid tolerance, or metabolic compatibility—we aim to promote coexistence and collaborative behavior within defined 3D arrangements. This work opens new directions for advanced probiotics and the engineering of microbial ecosystems.
Finally, I will highlight two interdisciplinary collaborations sparked by our biofabrication platform: one in aquaculture, where layered chaotic fibers are being explored as functional implants to help regulate fish metabolism and behavior for production purposes; and one in materials science, where co-printing nanocellulose-based hydrogels embedded with magnetic and conductive elements results in cryogels with electromagnetic shielding properties.
These explorations, while seemingly peripheral to traditional tissue engineering, have taught us valuable lessons in material design, patterning, biological interaction, and manufacturability. Far from distractions, these paths enrich our perspective and may ultimately feed back into solving key challenges in regenerative medicine. In this way, even the most unexpected trajectories can bring us closer to meaningful therapeutic impact.

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Advanced 3D-Bioprinted Grafts for Bypass Surgeries

Small-diameter vascular conduits for the treatment of cardiovascular diseases are in high demand. Bioprinting a patient-specific blood vessel is an attractive alternative, however, the inferior mechanical properties of cell-laden hydrogels is a major drawback. Here, we present a combined electrospinning and bioprinting technique in which electrospun nanofibers and cell-laden hydrogels are used to fabricate a layered vascular graft similar to those found in natural blood vessels. Polycaprolactone (PCL) nanofibres loaded with heparin offered an active surface for the endothelial cells (ECs) to grow in a rotating bioreactor. The PCL nanofibrous layer also acted as a barrier to

restrict the migration of smooth muscle cells (SMCs) toward the lumen of the engineered vascular graft. The cell survival rates of ECs and SMCs at week 2 and week 4 were found to be greater than 80%, along with the substantial amount of collagen deposition after 4 weeks of cell culture. Hence, the presented method could be used to biofabricate a vascular graft for the treatment of cardiovascular disease.

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Konuşmacılar: Faraz Fazal (The University of Edinburgh)

- S24 ISBF-ESB: How biomaterials design can help bioprinting? Mikołajska

Toplantıyı düzenleyenler: Gianluca Ciardelli (Politecnico di Torino), Debby Gawlitta (UMC Utrecht)

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Innovative blends for Chronic Lymphatic Leukemia in vitro models

Introduction

Chronic Lymphocytic Leukemia (CLL) is the most common hematological malignancy in the Western World [1], caused by the expansion and accumulation of B lymphocytes in peripheral blood, bone marrow, lymph nodes and spleen. To date, the comprehension of the interactions between CLL cells and the tumor microenvironment (TME) is challenging to implement novel therapiesIn this study, we designed and prepared 3D bioprinted in vitro models to investigate CLL cells behavior in TME with different stiffness.

Materials and Methods

Low, medium, and high viscosity alginate was dissolved in DPBS (2, 4% w/v). Calcium carbonate (CaCO3) and D-glucono-lactone (GDL) were mixed with deionized water and added dropwise (alginate:CaCO3:GDL=2:1:1) [2]. Rheological analyses of pre-crosslinked alginate inks were performed to investigate the optimal pre-crosslinking condition to ensure adequate printability. Thixotropy tests were performed to evaluate recovery ability of the inks after the printing. Methacrylate gelatin (GelMA) was synthetized as reported elsewhere [3]. Biomaterial inks were prepared using pre-crosslinked alginate at high viscosity, obtaining ALG_1 and ALG_2. Additionally, a blended bioink (ALG_2_GELMA_0.4RF) was obtained by mixing ALG_2 with GelMA, using a visible light specific photoinitiator (RF/SPS). Cylindrical samples were 3D printed by optimizing the printing parameters. ALG_1 and ALG_2 hydrogels were ionically crosslinked, whereas ALG_2_GELMA_0.4RF samples were photocrosslinked during the printing process. Stability in vitro tests and compressive mechanical tests were performed to evaluate stability in RPMI medium and elastic modulus. CLL cell line MEC1 were collected and suspended in ALG_1, ALG_2, ALG_2_GELMA_0.4RF. 3D bioprinted samples were cultured up to 14 days. AlamarBlue assay and Live/Dead staining were performed to investigate cells' behavior in the hydrogels.

Results and Discussion

Alginate gelation occurred in 48 h at 4°C and at room temperature. Herschel-Bulkley model evidenced that printable inks can be obtained from high viscosity alginate and CaCO3/GDL gelation at 4°C. Thixotropy tests evidenced the highest recovery rate at 4°C (Figure 1a). The biomaterial inks composition affected stability and mechanical properties. Alginate low concentration (ALG_1) resulted in weaker hydrogels, whereas ALG_2 and ALG_2_GELMA_0.4RF were stable up to 10 days. Mechanical tests on ALG_1, ALG_2 and ALG_2_GELMA_0.4RF exhibited an elastic modulus of 7.51, 13.67 and 30.68 kPa, respectively (Figure 1b). MEC1 cells proliferated in cell-laden bioprinted scaffolds. Cell metabolism progressively increased in time for ALG_2 and ALG_2_GELMA_0.4RF, whereas decreased for ALG_1, due to a faster degradation. Live/Dead staining evidenced how cells differently behaved in microenvironments with different stiffness. By increasing hydrogel stiffness, cells formed tumor-like clusters, especially after 7 days of culture. Moreover, in ALG_1 and ALG_2, MEC1 cells assumed a rounded shape, whereas the presence of RGD motifs in the blended bioink allowed cells to adhere onto the surrounding microenvironment.

Conclusion

Varying the composition of the bioink it was possible to obtain in vitro models that allow a different CLL cell behavior. In particular, ALG-GelMA bioprinted models resulted promising 3D in vitro models for CLL study.

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Light-based volumetric bioprinting and imaging-driven manufacturing: shining a new light on engineered tissues and organoids

In the quest to capture the complex environment of living organs within lab-made tissues, light emerged as a uniquely powerful stimulus for enabling dynamic and spatio-temporal control over cell and biomaterial properties, opening new avenues in regenerative medicine and tissue engineering. Light-responsive moieties permit to non-invasively trigger mechanical actuation and shape-changes in cell-laden constructs, to modulate stiffening or softening of the extracellular milieu, and to enable spatio-temporal control over cell behavior. Previously, we introduced volumetric bioprinting (VBP), an ultra-fast, layer-less visible light-based biofabrication approach, to resolve virtually any 3D geometrical patterns in less than 20 seconds by projecting tomographic patterns onto photosensitive hydrogels making it possible to sculpt cell-laden materials with unprecedented geometrical freedom into high resolution architectures. Using visible light volumetric bioprinting technologies and protein-derived photoresponsive hydrogels, complex mini-organ models, also termed organoids, can be safely assembled into centimeter scale living tissues in a matter of few seconds. Herein, the most recent advances in light-driven biofabrication will be presented, together with our efforts to engineer functional blood vessels, breast gland tissue, and pancreatic tissues as advanced biological models, using organoids as living building blocks. Challenges in recreating vascularized environments can be addressed converging light-based volumetric printing with microgel-based printable materials, as well as via combining VBP other fabrication techniques, such as extrusion-based bioprinting and melt electrowriting. Moreover, spatial-specific functionalization with growth factors and biochemical signals can be achieved via a secondary VBP process to graft bioactive proteins in specific locations. Further advancing this technology, precise imaging strategies are leveraged to for enhanced metrology, quality control, and for introducing the concept of context-aware printing. In context-aware manufacturing, making printers that are able to detect objects, cells and features of interest within the printing vat, enables the creation of constructs that match the metabolic demands of the embedded cells, facilitates multi-material printing and overprinting, and permits to print across opaque, light-occluding elements, allowing for the creation of complex composite materials and living tissues. Overall, introducing anisotropic, multicellular/multimaterial patterns within volumetrically bioprinted constructs enables the biofabrication of freeform tissue models that more closely mimic the complex biochemical and structural composition of native tissues, to more precisely guide cell fate and maturation.

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Development of bioinks containing functionalized silk nanofibers that simultaneously improve printability and cell proliferation

Introduction

For 3D bioprinted structures to function effectively as tissues, it is essential to promote the proliferation of encapsulated cells. At the same time, it is important to be able to print with high structural fidelity to the blueprints designed to perform biological functions. Different techniques are currently used to meet each of these requirements. Cell proliferation is supported by a porous scaffold that facilitates the diffusion of nutrients and oxygen and provides sufficient space for growth. Conversely, structural fidelity to blueprints can be improved by incorporating nanofibers into bioinks to enhance shear-thinning properties. However, it is still difficult to achieve both improvements simultaneously. In this study, we propose a novel strategy to achieve enhancements in both cell proliferation and structural fidelity to blueprints using a single bioink component.

Our strategy involves immobilizing gelation-inhibiting molecules on nanofibers and incorporating them into the bioink. For gelation, we used a phenol-modified polymer that can be crosslinked by a horseradish peroxidase (HRP)-catalyzed reaction in the presence of hydrogen peroxide (H2O2). To inhibit gelation around the nanofibers, we immobilized catalase on silk fibroin nanofibers (SFNFs), which are known as cytocompatible material, to decompose H2O2 around the nanofibers. This is referred to as SFNF-catalase.

Method

Catalase was immobilized on SFNF and silk fibroin fiber (SFF) using carbodiimide chemistry. To observe crosslinking inhibition around catalase-immobilized SFF (SFF-catalase), phenol-modified hyaluronic acid (HA-Ph) (0.5% w/v) and HRP (5 U/mL) were mixed with SFF or SFF-catalase, and hydrogel films were prepared with H2O2 supplied from the air. The formation of dityrosine bonds through the crosslinking of phenol moieties was observed using a fluorescence microscope (ex: 320 nm, em: 404 nm).

Cell-laden square structures were printed from a mixture of SFNF or SFNF-catalase (0.1 mg/mL), HA-Ph (0.5% w/v), HRP (5 U/mL), and HepG2 human liver cancer cells (1.4×106 cells/mL) with H2O2 supplied from the air and cultured for 7 days. The proliferation rate of the enclosed cells was evaluated by Calcein AM/PI staining.

Result and discussion
Fluorescence mapping of dityrosine formed via HRP-catalyzed crosslinking showed that the fluorescence intensity was lower around SFF-catalase than farther from the fibers. In contrast, no difference was observed when catalase-free SFF was used. This result demonstrates that the catalase immobilized on SFFs inhibited crosslinking of phenol groups around the fibers. As shown in the attached figure, independent of the immobilization of catalase, SFNFs contributed to achieving a structure close to the blueprint. Interestingly, the cells in the printed constructs containing SFNF-catalase showed greater proliferation compared to those in the constructs containing catalase-free SFNF. This result also supports the above-mentioned result, indicating the inhibition of gelation around SFNF-catalases.
In summary, our SFNF-catalase bioink formulation enables both enhanced cell proliferation and high structural fidelity through a biocompatible strategy, addressing a key limitation of current bioprinting technologies.

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Volumetric Additive Manufacturing of Gelatin Hydrogels to Guide Stem Cell Differentiation

Introduction
Volumetric additive manufacturing (VAM) is emerging as a powerful biofabrication approach, allowing the rapid generation of complex, cell-laden hydrogel scaffolds. Unlike traditional layer-by-layer 3D printing, VAM enables high-speed fabrication, offering significant advantages for tissue engineering (TE).[1] Hydrogels derived from gelatin-based polymers, particularly those crosslinked via thiol-norbornene photo-click chemistry[2], provide tunable mechanical properties that can be tailored to influence cellular behavior. This study investigates photo-crosslinkable gelatin hydrogels (gelatin-norbornene (GelNB) and thiolated gelatin (GelSH)) for VAM, focusing on their potential to guide mesenchymal stromal cell (MSC) differentiation.

Methods
Hydrogels were developed using GelNB and GelSH precursors with varying degrees of substitution (DS).[3] GelSH with DS values of 39%, 54%, and 63%, and GelNB with a DS of 60% were developed. GelNB-GelSH formulations were characterized by rheological analysis to determine the storage and loss moduli (G' and G"). The GelNB-GelSH63 (10%(w/v)) formulation was selected for further study due to its favorable mechanical properties and printability. VAM was optimized to ensure uniform crosslinking and enhanced structural integrity. Mechanical characterization was conducted using compressive testing and frequency sweep rheology, while computer aided design/manufacturing (CAD/CAM) mimicry was assessed via optical and scanning electron microscopy. MSC viability and proliferation were evaluated over 21 days using metabolic assays, and differentiation potential was assessed through ALP activity and calcium deposition (osteogenic), Alcian Blue staining for glycosaminoglycans (chondrogenic), and Oil Red O staining for lipid accumulation (adipogenic).

Results
Hydrogel characterization demonstrated a clear correlation between DS and mechanical properties, with GelSH DS and hydrogel concentration influencing mass swelling ratio and G' (range: 206 Pa to 12.5 kPa). This tunable behavior highlights the versatility of these hydrogels in replicating diverse tissue environments. VAM was exploited to fabricate 3D scaffolds, incorporating casted films as reference. VAM-printed GelNB-GelSH exhibited superior mechanical characteristics (compressive strength: 507.63 ± 53.15 kPa; E-modulus: 21.04 ± 2.14 kPa) compared to film-casted samples (E-modulus: 6.47 ± 0.15 kPa). VAM scaffolds demonstrated excellent structural fidelity and mechanical integrity, supporting sustained MSC proliferation and enhanced osteogenic differentiation, evidenced by increased ALP activity and calcium deposition. Conversely, film-casted hydrogels promoted chondrogenic and adipogenic differentiation.

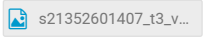
Discussion
VAM enabled the fabrication of complex, high-fidelity 3D structures from the optimized GelNB-GelSH formulations, demonstrating their capacity to encapsulate MSCs within a biomimetic matrix (Figure 1). The significantly higher ALP activity and calcium deposition in VAM-printed scaffolds underscored enhanced osteogenic differentiation. In contrast, softer, less crosslinked film-casted hydrogels favored chondrogenic and adipogenic differentiation, highlighting the influence of scaffold mechanical properties on MSC fate.

Conclusions
The study emphasizes the significant role of hydrogel physico-chemical properties in directing MSC differentiation. VAM shows substantial promise for developing customizable, complex 3D scaffolds tailored for specific TE applications.

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Engineering spatially patterned and controllable hydrogel matrices in organ-on-chip platforms

Introduction: Biological tissues exhibit intricate spatial variations, stiffness gradients, and complex niche environments critical to their biological function and implicated in various pathologies. Organ-on-chip technologies offer advanced biomimetic culture conditions compared to traditional culture methods[1-2]. However, replicating native tissue complexity remains challenging, requiring techniques to spatial patterning biomaterials within chips. This study introduces translatable micromanufacturing and biofabrication techniques to create controllable, tissue-like constructs for organ-on-chip platforms using interpenetrating polymer networks (IPNs). Techniques include digital light processing (DLP) bioprinting, extrusion-based bioprinting, and grayscale photomask gradients. Muscle-tendon and bone-cartilage interface models are used as exemplars of this spatial patterning

Methods: A gelatin-based IPN biomaterial library was developed, integrating methacryloyl and thiol-ene chemistries to modulate mechanical properties, macromer densities, and binding sites for tethered growth factors and ligands. Gelatin biomaterials were functionalised using established methods to achieve gelatin methacryloyl (GelMA; 40 and 80% degrees of functionalisation - DoF)[3-4], gelatin norbornene (GelNB; 27% DoF)[5-6], and thiolated gelatin (GelSH, 0.368 mmol/g thiol content). Precise control over synthesis parameters, including macromer concentration, molar ratios of thiol and alkene groups, norbornene/thiolated gelatin or multi-arm polyethylene glycol-norbornene (PEG-NB) or thiol (PEG-SH), and crosslinkers (dithiothreitol – DTT, photoinitiators – LAP and Igracure), produced IPNs with defined stiffness gradients in response to UV light (λ365 and 405 nm). Comprehensive characterisation involved rheological analysis, bioprinting parameters, swelling studies, mechanical assessment for localised stiffness, and bulk compression testing in confined and free-swelling conditions.

Results: Preliminary findings demonstrated GelMA's inability to crosslink within PDMS chips due to oxygen inhibition, resulting in incomplete photopolymerisation. To address this, thiol-ene chemistry was employed using GelSH or GelMA with DTT or multi-arm PEG crosslinkers. Incorporating PEG-4SH into GelMA enabled rapid crosslinking (<1 minute) and increased the storage modulus (2.7kPa) compared to 5% (w/v) GelMA control (1.4kPa). Similarly, 5% GelSH combined with PEG-8NB exhibited rapid crosslinking (~30 seconds) and an increased modulus (14kPa), illustrating tuneability of IPNs. Initial extrusion-based studies demonstrated multi-printhead extrusion with GelMA concentrations of 7.5%, 10%, and 15% to produce constructs featuring ~two-fold stiffness gradients. Coaxial printing provided an alternative approach to fabricate gradients by modulating GelMA concentration or DoF through precise control of extrusion speed and pressure.

Discussion: Our findings demonstrate the versatility of the gelatin-based IPN library in controlling physiochemical properties. Ongoing studies are characterising IPN combinations and evaluating the influence of multi-arm PEG-NB or PEG-SH on cell behaviour, mechanical properties, and bioprinting assessment. Preliminary pilot studies using 10% (w/v) PEGDA with grayscale DLP photomasks have shown effective stiffness modulation through gradient tuning, with gelatin-based materials planned for further

assessment. These approaches will be optimised for integration across diverse organ-on-chip platforms, including closed-channel systems, open-chamber designs, and custom-fabricated chips. By tailoring micromanufacturing techniques to overcome chip-specific limitations like oxygen inhibition, this work enables reproducible fabrication of tissue gradients and interfaces using a thiol-ene chemistry, improving the predictive capabilities of organ-on-chip models.

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Bioinks based on human-derived proteins

We have been proposing the use of human-derived proteins that, upon chemical modification, could be used to generate adequate microenvironments to interact adequately with cells. We have selected two sources of such materials: (i) platelet lysates, containing mostly globular proteins including relevant growth factors with highly regenerative potential; and (ii) proteins from amniotic membrane or from the entire placenta, composed of fibrical proteins such as collagens and other compoents of the extra-cellular matrix. Due to their hydrophilic nature and richness in chemically active groups, these proteins can be chemical modified to generate materials with new or improved properties, while maintaining the biochemical features of human tissues. Hydrogels from these materials can be obtained from different crosslinking procedures, including photocrosslinking or supra-molecular assembly, to be used in a variety of forms, including injectable/bioprintable systems. The globular nature of some of the proteins, in particular those from platelet lysates lead to low-viscosity solutions that are not adequate to be directly used in extrusion-based 3D-bioprinting. We suggested solutions to bioprint such systems namely by: (1) using gel-like supporting baths allowing the free-form processing in the thridimensional space; (2) using two sequential crosslinking, where the first step permits the obtention of bioinks with adequate rheological characteristics, followed by photocrosslinking during extrusion; (3) by pre-processing covalently crosslinking hydrogels in the form of an extrudable granular system, followed by a final photocrosslinking fixation during bioprinting.

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Development of a nutrient-rich glycogen-gelatin composite polymer for embedded 3D printing of vascularized tissue engineered constructs via aqueous three phase separation

Introduction:
Despite major advances in tissue engineering one key challenge remains: to ensure cell survival inside large, engineered constructs. Diffusion alone cannot sustain cell viability to the construct core, making it critical to develop strategies that support cells until a functional vascular network forms, regardless of construct size. Recent approaches to address this issue involve in situ metabolic support that provide oxygen or nutrients throughout the engineered tissues [1]. Although promising, their clinical use is limited by reliance on xenogenic enzymes that often trigger an unwanted immune response [2].
Within biofabrication, insufficient nutrient delivery in large constructs is often addressed by pre-vascularization of constructs using 3D printing methods such as freeform 3D printing (FRESH) [3]. Nevertheless, despite the ability to generate complex vascular architectures, a large limitation is the scalability required for clinically relevant tissue volumes. Here, we report on an alternative: the innovative use of glycogen methacrylate (GlyMA) bound to the backbone of gelatin methacryloyl (GelMA). Glycogen is a naturally occurring nutritional nanoparticle found within mammalian cells that reversibly stores glucose [4]. Surprisingly, we show that glyMA offers metabolic support for human mesenchymal stem cells (hMSCs) for clinically relevant time periods in anoxic conditions.

Materials and Methods:
Cell and hydrogel culture: hMSCs were cultured within gelMA and gelMA+glyMA hydrogels (6x2mm) crosslinked using ruthenium/sodium persulfate. The encapsulated cells were either cultured upon serum starvation or normal serum supplementation and cultured for clinically relevant time periods in anoxic or normoxic conditions. Viability and metabolic activity were assessed using a live/dead stain and Alamar blue. Toxicity was assessed using a lactate dehydrogenase assay.
Microresin water bath and FRESH printing: GelMA hydrogels were supplemented with varying concentrations of glyMA. The polymers self-organized through aqueous two-phase separation to form glyMA microresins dispersed throughout the gelMA matrix. A subsequent dextran-based separation step enabled spatial compartmentalization of both polymers. This led to a more complex separation system that is aqueous three phase separation (A3PS). The glyMA-gelMA microresin bath was used to FRESH 3D print pre-vascularized, large tissue engineered constructs.

Results:
H-NMR analysis confirmed that glyMA was synthesized with a consistent degree of functionalization of approximately 25%. Live/dead, Alamar blue, and LDH assays confirmed that glyMA was non-cytotoxic. Interestingly, during a long-term culture in anoxia, hMSCs supplemented with glyMA remained viable. Moreover, the addition of glyMA favored pro-angiogenic potential as endothelial cells formed a tight monolayer on top of the hydrogel. In contrast, no confluent endothelium formed on gelMA hydrogels. Using the glyMA-gelMA polymer, a new way to generate nutrient-rich gelMA microgels was developed using A3PS. This approach enables the extraction of glyMA-gelMA microresins that can be used to formulate a FRESH-compatible 3D printing bath. This platform was designed to support the printing of macro-vessels with complex geometries.

Conclusion:
In this work, we established a non-toxic, multi-functional material applicable as a self-feeding metabolic support hydrogel for engineering of large size tissues. Moreover, we demonstrated the first use of A3PS to generate a nutrient-rich microresin bath compatible with FRESH 3D printing of vasculature.

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S31 Open-Source Hardware for Biofabrication Goethe

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Light-Pipe FRESH - Bringing the Power of Photochemistry to Open-Source Embedded 3D Bioprinting

Recreating the spatial and functional heterogeneity of native tissues remains a central challenge in tissue engineering. Native tissues exhibit complex gradients in mechanical properties, extracellular matrix (ECM) composition, and biochemical cues, which are difficult to replicate using conventional biofabrication methods. Extrusion-based embedded 3D bioprinting techniques, such as Freeform Reversible Embedding of Suspended Hydrogels (FRESH), enable high-fidelity deposition of multiple bioinks but are limited by the number of extruders and resolution id dependent on nozzle diameter. Conversely, light-based bioprinting provides precise spatiotemporal control of photochemistry for tuning mechanics and biochemistry but struggles to integrate multiple biomaterials. To address these limitations, we developed Light-Pipe FRESH 3D bioprinting, a hybrid approach integrating optical fiber-delivered photochemistry with embedded extrusion printing. By rastering light from a fiber-optic cannula within a photocrosslinkable support bath, Light-Pipe FRESH achieves programmable modulation of crosslink density, biochemical patterning, and multi-material integration within a single construct.

Light-Pipe FRESH integrates a custom-designed optical pathway into an open-source FRESH 3D bioprinter. A high-intensity UV light source (365–405 nm) was filtered, coupled into a 100 µm fiber-optic cannula (NA = 0.1), and rastered within a photocrosslinkable gelatin methacryloyl (GelMA)-based granular support bath containing photoinitiator (LAP) and photoabsorber (tartrazine). GelMA microspheres (~10 µm) were synthesized via complex coacervation and combined with a GelMA-based photofiller.

Rheology confirmed yield-stress, shear-thinning, and self-healing properties essential for embedded printing, while photorheology quantified controllable light-induced gelation. Scaffolds with programmable mechanical domains were fabricated by varying print speed (20–40 mm/min) or light power (6–10 μW). C2C12 myoblast-laden collagen hydrogels were cast onto printed scaffolds and cultured for up to 21 days. Remodeling, compaction, and contractility were assessed via confocal microscopy, custom microindentation, calcium imaging, and electrical stimulation. Multi-material scaffolds combining collagen and GelMA mimicked myotendinous junctions, and spatial photoconjugation demonstrated programmable biochemical patterning.

Light-Pipe FRESH enables precise spatial control over scaffold architecture and mechanical properties. Modulating print speed tuned the effective elastic modulus from approximately 5 to 30 kPa, allowing creation of scaffolds with stiffness gradients. Myoblast-seeded scaffolds exhibited stiffness-dependent remodeling: softer scaffolds showed greater compaction and matrix remodeling, whereas stiffer scaffolds preserved architecture and promoted aligned myotube fusion. Calcium imaging and field stimulation revealed domain-dependent contractility, with softer regions exhibiting higher contractile displacement. Dual-head bioprinting combining light-pipe photopatterning of extrused collagen filaments were used to engineer myotendinous junction-like scaffolds, integrating collagen-based tendon regions with GelMA-based muscle domains. Multi-wavelength photochemistry and spatial photoconjugation enabled programmable biochemical heterogeneity within a single construct. In Summary, Light-Pipe FRESH combines high-fidelity multi-material deposition with on-demand spatiotemporal control photochemistry, offering a versatile platform for engineering tissue constructs functional gradients in mechanical, chemical, and biological properties.

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Printess 2.0: A 10-axis low-cost multimaterial bioprinter

Open source and low cost bioprinters improve access to biofabrication and allow for a common hardware language for easier sharing of materials, code, and approaches. We recently developed the Printess, a 6-axis low-cost 3D bioprinter that could perform multimaterial, gradient mixing, multimaterial multinozzle, and embedded multinozzle 3D bioprinting. Here, we expand upon the design of our Printess with the Printess 2.0, a 10-axis machine with 4-material extruders with independent z-actuation. Using this new platform we demonstrate multimaterial mixing and gradient multinozzle capabilities. With new firmware updates, we enable spindle control of extrusion to simplify GCode writing for students and scientists, such that extruder motion does not need to be calculated for every line of GCode. The Printess 2.0 will be an accessible, open source, and powerful new tool for educational and research environments.

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Adoption of the MEWron as An Open-Source Platform for Education, Training and Increased Productivity for Studies of Melt Electrowriting Scaffolds

Introduction: As new biofabrication technologies emerge, the possibilities for research into new applications expand rapidly. However, for young researchers with limited experience in the relevant areas (i.e., regenerative medicine, robotics, 3D printing or G-code postprocessing) and constrained financial resources, the application of new technologies may be challenging. From the very beginning, the prohibitive price of commercially available Melt Electrowriting (MEW) machines prevented access to this versatile fabrication technique. Additionally, the information needed to build and operate a non-commercial MEW printer was limited and, by definition, non-standard for more than a decade, which prevented many interested researchers from commencing scientific exploration in MEW. These barriers to the utilization of MEW fell with the publishing of a low-cost and open-access platform, the MEWron [1]. Since its unveiling, the number of MEW-based research has grown considerably. Additionally, a new community is forming around the National Science Foundation-sponsored HAMMER (Hybrid Autonomous Manufacturing) Engineering Research Center's (ERC) which is tasked with developing inexpensive desktop fabrication units (PET-Fabs), and short learning curve suites of hardware and software that can be used for education and research in emerging manufacturing technologies [2].
Methods: To build our MEWron, we followed the guidelines that describe the conversion of the Voron 3D printer [1]. While the printer itself can be assembled for less than $1,000 USD, one of the most expensive components to procure is the high voltage power supply (HVPS), which may cost a few thousand dollars. We integrated an inexpensiv
250 USD using a 12V to 10kV voltage converter. It also integrates a YT Meter (Shenzen, China) 10 kV indicator, and an easy-to-build electronic board priced at about $20 USD for voltage control. We added to the MEWron suite a 100 USD plug-and-play, pellet-fed Fused Deposition Modeling (FDM) print head. For this, we adapted a pellet extrusion mechanism to a Creality Ender Pro hot end (Shenzhen, China) that is compatible with the MEWron.
Results: The adoption of the MEWron as a HAMMER ERC PET-Fab has allowed it to serve as a training platform for undergraduate students in electronics, 3D printing, G-code programming, and the analysis of mechanical systems. For many students it is also a gateway to the fields of biofabrication, tissue engineering, and regenerative medicine.
Discussion: The MEWron has allowed MEW research on regenerative medicine to quickly scale. Previously, our lone MEW machine was committed to a single study at a time due to its small throughput. The adoption of the MEWron has allowed us to expand our research capabilities, while simultaneously disseminating knowledge of these techniques to a broader scientific community. This dynamic environment has also fostered the development of new tools, such as the inexpensive HVPS and the integration of a FDM pellet-fed printhead. Finally, extended access has also resulted in a maker community coming together around MEW to lower costs, set performance standards, provide training opportunities, and collaborate on research into new applications.
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The MEWron: Open-source Melt Electrowriting

Introduction
There are several emerging direct-writing 3D printing technologies whose development can benefit from established, low-cost open-source ecosystems. Such 3D printing technologies include Freeform Reversible Embedding of Suspended Hydrogels (FRESH) and melt electrowriting (MEW). The latter technology is a high-resolution technique capable of depositing micron-scale fibers into ordered macroscopic scaffolds. Originating in tissue engineering research, many groups depend on expensive and inflexible commercial printers or custom devices built by individual scientists, slowing down the sharing, proliferation and advancement of MEW technology even though the underlying physical principles are simple to harness and suitable open-source hardware based on fused filament fabrication (FFF) exists.

Methods & Results
Our work adapts existing open-source hardware from the Voron ecosystem into capable, low-cost MEW printers while retaining the open access ideas they were built on. We demonstrate the power of this approach by building a fully functioning "MEWron" printer for below $2,000 capable of processing thermoplastic filaments at up to 300°C melt temperature. We produced fiber diameters of 7 μm from medical-grade ε-Polycaprolactone (PCL) as part of this proof-of-concept study. We also introduced MEWron variants to solve material-specific challenges and used them to process Nylon-12 and silk fibroin successfully in these configurations while maintaining modularity to combine and assemble a machine for a given research task.
Building on this established platform, our work now improves key areas of the MEWron by offering alternatives to improve supply chain constraints, ease of assembly and use, or enhanced functionality of the electronic hard-and software, printhead and high voltage contacting components such as the collector.

Discussion
The Voron open-source hardware ecosystem is a readily accessible, modifiable, and powerful system to facilitate the development of emerging manufacturing approaches. Since the initial release, the low cost and flexibility of this platform has attracted numerous research groups to become involved in the development or refinement of the MEWron to accelerate and proliferate the technology in the coming years.

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ACTISCULPT: ACTIVE SCULPTING OF MULTILAYERED FLOW FOR NEXT GENERATION 3D PRINTING NOZZLES

Introduction: Additive manufacturing has transformed material science by enabling 3D printing of tunable structures for applications like micro-robotics, sensors, and tissue engineering [1]. However, conventional extrusion-based printing uses fixed nozzle shapes, which limit control over the extruded material compositions and printed geometries.

Recent methods offer some composition and flow control but rely on bulky, complex systems [2]. Passive techniques like co-axial nozzles, Dean vortices, and hydrodynamic focusing lack dynamic modulation [3–4]. Here, we present Active Flow Sculpting (ActiSculpt), a method for spatiotemporal modulation of multi-layered laminar streams using on-demand acoustic streaming, enabling dynamic flow structuring. We developed software to predict the cross-sectional flow patterns using a comprehensively enhanced acoustic streaming model that accurately depicts the experimental conditions. We achieved a five-fold increase in the moment of inertia (MoI) of the sculpted cross-sectional flow profile, translating into a wide range of bending and torsional strengths of a cured fiber.

Methods: Surface acoustic waves from interdigital transducers (IDTs) generate strong body forces to shape laminar flow in the ActiSculpt platform (Fig. 1a). Four straight IDTs, offset by 250 µm, are placed beneath a 1 mm × 1 mm PDMS microchannel. Each IDT (37–48 MHz) is actuated independently via time-division multiplexing (TDM) (Fig. 1b). A low-cost numerical model is developed to simulate the fluid transformation based on Eckart's streaming (Fig. 1c). Red and blue photo-curable precursors are polymerized via stop-flow lithography[5,6] to reveal cross-sectional flow profiles (Fig. 1d). The TDM allows combinatorial actuation of the multiple IDTs.

Results and discussion: Distinct sculpted flow shapes are achieved in a precisely controllable manner by selectively varying the input power to IDTs 1–4. The sculpted shapes are characterized using a diffusion parameter based on standard deviation, with values remaining above the mixing threshold even at higher power levels (Fig. 2a). The sculpted shape uniformity is assessed by comparing results across different cycles. The uniformity exceeds 75% (up to ~95%) for different actuations by IDTs 1-4, confirming the robustness of our method (Fig. 2b). Transformation of the center of mass for each labelled channels quantified by cumulative dislocation parameter as swiping the power values (Fig. 2c). We measured the MoI of the sculpted shapes to vary by 5-fold using a single ActiSculpt platform (not shown), which highlights the versatility of our method. A combinatorial actuation of actuators reveals more unique shapes and opportunities for cross-sectional sculpting (Fig. 3).

Conclusion: The ActiSculpt platform offers highly flexible and unique capabilities to actively manipulate multi-layered flows on-demand, which will unveil new possibilities in the field of microfluidic flow control, 3D printing, and additive manufacturing.

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- S34 Smart Materials in Biofabrication Kruczkowski

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3D Printed Smart Biomaterials for Pediatric Reconstruction

Smart Materials, broadly defined here as any biomaterial that alters its shape and material properties over a time period ranging from minutes to years in response to externally applied (light, heat, etc) or host (fluid, cells, etc.) stimuli hold tremendous promise for fabricating resorbable, implanted devices for pediatric reconstruction applications. Regulatory approval requires understanding how these effects depend on material composition, 3D printing modality, host clinical site application, ability to meet design requirements and how material alterations occur in a pre-clinically and clinically. This paper reports the affect of printing modality on polycaprolactone (PCL), a widely used clinical material, and Poly Glycerol Dodecanedioate (PGD), a shape-memory biomaterial developed by our group. It also reports in vivo responses in devices 3D printed from both of these smart biomaterials.
We first demonstrate that elastic and post-elastic mechanical properties (i.e. plastic and damage behavior) depend on both the raw material source and the 3D printing modality (e.g. extrusion, DLP, or laser sintering) used to make a device. PCL shows elastic-plastic behavior that depends both on medical grade material supplier and the 3D printing modality (extrusion vs laser sintering) used to print the device. PGD exhibits shape memory nonlinear elastic post-elastic damage behavior whose whose shape memory, elastic and failure stress properties also depend on 3D printing modality (extrusion vs DLP). PGD This is critical information for iterating device design.
Second, we show that that shape memory and mechanical properties change significantly in vivo due to fluid penetrating the polymer matrix that itself depends on tissue coverage of the device. Suprisingly, both PCL and PGD become stiffer (increased elastic properties) and demonstrated increased yield and failure stress during degradation before complete resorption. PCL demonstrated a transition from a highly ductile material after 3D printing to a completely brittle material with no plasticity after degrading 2 years in vivo. This process has been termed "hydrolic embrittlement" and is demonstrated here in a large pre-clinical animal model.
Third, in pre-clinical and clinical implantation, we demonstrate that clinical outcomes in pediatric applications depend on a complex interaction between smart biomaterial property changes during degradation (especially elastic stiffening and yield/failure stress changes), host tissue remodeling, and host anatomic growth. Tissue growth over the device limits mass transport, thereby inhibiting polymer chain exudation from the device. Polymer chains reorganize into lamellar structures stiffening the device. In turn, a stiffnened device places greater stress on growing tissue, potentially affecting pediatric growth and development after device placement. The tissue growth in turn stresses the implanted device and can lead to the fracture. The key is designing for eventual fracture after the device has fulfilled its function. Rapid fractures is more likely in rapidly growing pre-clinical models than in patients, as shown for a 3D printed tracheal repair device. Such complex interacting phenomena (smart biomaterial property changes couple with in vivo tissue remodeling and growth) must be better understood and incorporated in long term (months to years) pediatric device design and simulation to improve 3D printed biofabricated pediatric device development and translation.

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Chromogenic plant extracellular matrix-derived scaffolds as smart wound dressings

Burn injuries and related wound infections are the leading cause for >150.000 deaths worldwide each year. Skin defects caused by burns or infection may necessitate grafting to augment wound healing. As early identification of relevant contaminants is essential to adequately treat wound/graft infection as a potential life-threatening complication, real-time monitoring of bacterial overgrowth will have extraordinary value in regenerative and septic surgery. Here we present Zantedeschia aethiopica spathe-derived extracellular matrix scaffolds as biological and non-immunogenic skin grafts for reconstructive surgery. By employing decellularization to retain the natural three-dimensional microstructure of the spathes matrisome, we have successfully preserved the physiological bioarchitecture of Zantedeschia which has long been acknowledged for its inherent anti-inflammatory and anti-oxidative properties for wound healing and tissue reconstruction. As a result of decellularization, five biofabricated translucent scaffolds are free of cellular material and DNA content has significantly been reduced producing non-immunogenic grafts. By augmenting these scaffolds with anthocyanin infused rice-based hydrogel coatings, in all five biological replicates these scaffolds visualize spatiotemporal chromogenic changes to pH shifts caused by cellular/microbial overgrowth. In recellularization experiments employing human epidermal keratinocytes, we have shown biocompatibility of the engineered graft by adequate proliferation of reseeded cells visualized by histological imaging. These scaffolds of natural origin can be seamlessly stacked together by their hexagonal shape to address existing defects meeting individual needs while being readily available. By further providing the opportunity to supplement these coated scaffolds with bioagents and therapeutics, we intend to enable personalization of these stackable epidermal wound patches for surgical applications. We hereby present a naturally sourced, cost-effective, and sustainable alternatives to currently available therapeutic options with bioactive and smart material properties for enhanced wound healing.

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4D printing of multilayer bio-based devices for the non-invasive treatment of intestinal ulcers

Introduction: Ulcers are a breach in the membrane of the stomach or intestine caused by inflamed necrotic tissue. When they develop in the ileum and jejunum, ulcers represent a burden clinical challenge, since they are not accessible through colon- or gastroscopy[1]. To solve this clinical need, in the context of the PRIN2022 project Prometheus, we studied the fabrication of a multi-layered structure, designed for oral administration, physically programmed to reach a bleeding ulcer, self-deploy, wirelessly communicate, and promote tissue regeneration, thanks to bio-enabled materials processed via 4D printing.
Structure Design: To achieved all the envisioned functionalities, the structure comprises (from outer to inner, FigureA): i)a gastrointestinal protective layer, allowing the device to overcome the stomach without degrading; ii)a self-deploying scaffold able to attach to a bleeding ulcers, self-unfolds and promote tissue regeneration, iii)a cylindrical mandril for printing support and on which a biocompatible antenna for external communication could be inserted.
Gastro-intestinal protective layer: Alginate (5% w/v in dH20) and Acyl-EZE (20% w/v in dH2O) were identified as potential gastro-resistant materials.100 µm thin films of the sole solutions and their mixture in a ratio 1:1 were prepared by solvent casting and tested according to the European Pharmacopeia (3 hours in 0.1M HCl, 1 hours in KH2PO4 Buffer Solution). The wettability and the permeability of the films were also tested in 0.1M HCl. Tests revealed that the mixture solution is compliant with the standards and easily printable via extrusion-based bioprinting.
Self-deploying scaffold: The core part of the device is a bilayer C-shaped scaffold based on gelatin and silk[2], programmed to self-deploying when hydrated(FigureB), thus allowing to cover the entire ulcerated area. The shape-morphing over time was achieved through the differential swelling properties of the two layer of the structure that

creates a deformation gradient. To allow the structure to anchor to a bleeding ulcer the silk solution was functionalized with a peptide able to bond the P-selectin, marker of activated platelets in the bleeding ulcers. The C-shaped structure was 4D printed on a rotating spindle made of a polyvinyl alcohol, able to degrade at different pHs upon 150 minutes.
Biocompatible antenna design and fabrication: PEDOT:PSS was chosen ad biocompatible conductive materials for the fabrication of the antenna. To enhance its conductibility, the addition of glycerol (1, 2.5, 5 and 10% w/v in dH20) and the implementation of thermal treatment to the structure were investigated (2h at 120°C). Tests revealed the PEDOT/Glycerol2.5% solution after the thermal treatment has the highest conductivity. To optimize the design of the antenna, computational models were performed to study the response of structures with different shapes(FigureC) hit by a plane electromagnetic wave.

Discussion and Conclusion
We are currently focusing on the fabrication of the entire structure in a single fabrication process, exploiting a customize multi-technology bioprinter[3] and an ad hoc slicing software[4], and its subsequential tests on an intestine phantom.

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3D Printing of Biomaterial Inks based on Hyaluronic Acid-Chitosan Complex Coacervates

Introduction
3D printing of hydrogels usually relies on a combination of fine-tuned material chemistry and polymer chain architecture to produce inks with adequate viscoelastic properties such as yield-stress flow, shear-thinning and self-healing behavior. [1] Complex coacervates are versatile materials obtained through an associative liquid-liquid phase separation phenomenon driven by electrostatic attraction between oppositely charged macro-ions (e.g. polysaccharides, proteins etc.) that show a great potential for the use as biomaterial inks. Indeed, for a given polyelectrolyte couple, depending on the salt concentration of the medium, a complex coacervate either behaves as a free-flowing viscoelastic fluid or a rigid polyelectrolyte complex or anything in between [2]. In our work, we leveraged complex coacervation between biological polyelectrolytes either as linear chains or as jammed hybrid microgels, so-called granular hydrogels [3], to develop 3D printable scaffolds with responsive biocompatible and contractible properties without need of post-printing crosslinking (Figure 1).

Methods
In this presentation, we first focus on the use of complex coacervates made of hyaluronic acid – chitosan linear chains (HA-CHI) as a biomaterial ink for 3D printing. By carefully optimizing, the physico-chemical parameters of the system, meaning pH, salinity and molecular weight of the polymers, we were able to produce a set of biomaterial inks that can be used in different environmental conditions. In parallel, we also synthesized hybrid microgels composed of methacrylated HA (HAMA) and chitosan (CHIMA) that were co-crosslinked by UV-irradiation in a batch emulsion process. Once jammed by centrifugation, the salt-reponsiveness of the resulting complex coacervate granular hydrogels was investigated in a range of physiologically relevant salt concentrations.
Results
The developed inks based on HA-CHI can not only be dried and re-hydrated without loss of shape fidelity, but also be printed into a liquid medium (fresh-printing) without the need of any chemical modification or post-printing curing process. We also show that an additional responsive dimension can be obtained by using the HAMA-CHIMA granular hydrogels. By decreasing the salt concentration of the medium below the critical concentration for electrostatic association between the polyelectrolytes, microgels with decreasing size leading to hydrogels with tunable stiffness, packing density and size were obtained. The 3D printed scaffolds exhibit long-term stability without need of chemical crosslinking and post-printing modulation of the resolution is also rendered possible.

Discussion
By combining oppositely charged biopolymers in aqueous conditions, either as linear chains or as hybrid microgels, we could highlight the potential of complex coacervates to be used as 2D and 3D scaffolds in cell culture studies.This approach also opens up the way to the design of more functional 3D hydrogel constructs with biocompatible and dynamic mechanical properties.
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Pectin-Enhanced Albumin Foam for Stable and Biocompatible Support Baths in Embedded Bioprinting

Introduction
Embedded bioprinting enables the deposition of bioinks within a supportive matrix, traditionally composed of viscoplastic gels. While these materials offer mechanical stability, they often compromise nutrient and oxygen transport to embedded cells [1]. To address these limitations, our group introduced in-foam bioprinting, a novel approach that utilizes a nutrient-rich, albumin-based foam as the support medium [2]. Despite its advantages, this albumin-based foam suffers from rapid degradation, limiting its application for longer prints. In this study, the incorporation of pectin as a stabilizing agent to enhance foam stability is investigated. The effects of pectin on foam properties, print fidelity, and cell viability are evaluated to assess its suitability as an improved support material for embedded bioprinting.

Materials and Methods
The foam was prepared by dissolving albumin and pectin powders in DMEM and mechanically mixing at 2000 rpm for 2 minutes (Figure 1A). Three compositions were studied: 8% w/v albumin (Alb8), 8% w/v albumin with 1% w/v pectin (Alb8Pec1) and 8% w/v albumin with 2% w/v pectin (Alb8Pec2). A rheometer with concentric cylinder geometry was used to perform rheological characterizations of the foam. Chitosan 2% w/v with gelling agent (0.1M β-glycerol phosphate, 0.075M sodium hydrogen carbonate) hydrogel was used for printability studies using an extrusion bioprinter and was embedded with L929 fibroblasts for cell studies. The cell-laden bioink was bioprinted into each foam composition as well as a standard gelatin microparticle support bath as used in Freeform Reversible Embedding of Suspended Hydrogels (FRESH) bioprinting [1] to ensure and compare cell viability. All cell viability tests were measured through live/dead assays using calcein and ethidium homodimer-1 to stain the live and dead cells respectively. The dissolved oxygen levels of the support baths were measured over time using a non-invasive optical oxygen sensor.

Results and Discussion
The foams containing pectin maintained the important rheological properties required by support baths such as shear thinning behavior and recovery properties (Figure 1B). The addition of pectin increases foam stability in terms of delaying bubble coalescence and liquid drainage when compared to the Alb8 foam presented in our previous work [2] (Figure 1C-F). Low-viscosity and slow-crosslinking chitosan hydrogels were successfully printed in the foams (Figure 1G). Cell-laden constructs printed in the foams containing 1% w/v and 2% w/v pectin exhibited higher cell viability compared to those printed in albumin-only foam (Figure 1H). When compared to FRESH support baths, in-foam bioprinting had higher cell viability when left in the supports for 3 and 5 hours with Alb8Pec1 exhibiting the highest viability overall (Figure 1I). Dissolved oxygen level measurements over time demonstrated that foam supports have slightly higher oxygen levels potentially contributing to their increased viability (Figure 1J).

Conclusions
Pectin enhances foam stability and preserves key support bath properties, while also enabling higher cell viability than conventional methods similar to FRESH. Combined with high dissolved oxygen content, these advantages position in-foam bioprinting as a strong candidate for complex tissue engineering.

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Extrusion-based bioprinting with phenylboronic acid-grafted hyaluronic acid inks via HRP-mediated crosslinking in vaporized hydrogen peroxide

Introduction
To advance 3D bioprinting, it is essential to develop bioinks with appropriate rheological (e.g., flow behavior, yield stress) and gelation (e.g., kinetics, storage modulus) properties to enhance printability. Previous studies have incorporated additional components into bioinks, such as rheology modifiers (e.g., nanofibers, nanoparticles) and secondary crosslinking agents (e.g., radical generators, other polymers). However, these additional components can complicate printing systems and potentially compromise biocompatibility. Therefore, improving printability by developing bioinks without additional components is necessary.
Here, we developed a bioink consisting of horseradish peroxidase (HRP) and phenylboronic acid-functionalized hyaluronic acid (HA-nAPBA), and applied it to a printing method that utilizes exposure to air containing ppm-level H2O2 (Figure 1). This printing system utilizes two key features: (1) shear-responsive rheological behavior via dynamic boronic acid–diol bonds, and (2) gelation via H2O2-responsive boron–carbon (B–C) bond cleavage followed by HRP-mediated crosslinking. In this study, we first investigated how the type of n-aminophenylboronic acid (nAPBA; n = 2, 3, 4) grafted onto HA, as well as the concentrations of HRP and H2O2, affected the rheological and gelation properties. Then, the relationship between these parameters and printability was evaluated. Finally, mammalian cells were cultured within the fabricated constructs.

Methods
Each nAPBA was grafted onto the HA by amide coupling. The binding affinity of each nAPBA for diols was spectroscopically evaluated using fluorescent Alizarin Red S. Gelation was tested by mixing PBS (500 µL) containing HA-nAPBA (0.80 w/v%), HRP (1.25–125 U/mL), and H_2O_2 (1.25–10 mM). Printability was assessed by printing a 32 × 32 mm, 4-layer lattice under ppm-level H_2O_2 using a 3D printer (BioX, CELLINK, Gothenburg, Sweden) with a 27G conical nozzle. Cell-laden constructs were printed with a bioink with HepG2 cells (1.0 × 10^6 cells/mL), HA-3APBA (1.5 w/v%), and HRP (50 U/mL), and cultured for 14 days.

Results and Discussion
The binding affinity between nAPBA and diols followed the ratio HA-2APBA: HA-3APBA: HA-4APBA = 1: 18.8: 7.2, and the viscosity of 1.0 w/v% solutions followed the same order. HA-2APBA solution did not form a hydrogel, while HA-3APBA and HA-4APBA solutions formed a hydrogel. Increasing the HRP concentration from 2.5 to 125 U/mL shortened the gelation time of HA-4APBA from 15 ± 3.3 s to 2.7 ± 0.7 s, and that of HA-3APBA from 119 ± 5.3 s to 12 ± 2.4 s. These gelation kinetics are probably explained by the combined effects of B-C bond cleavage and HRP-mediated reaction. Subsequently, HRP and H2O2 concentration-dependent printability of HA-3APBA ink was evaluated. Increased HRP and H2O2 concentrations improved printability, but also resulted in non-uniform line width and ink clogging. HepG2 cells in the printed constructs proliferated and formed aggregates until day 10, and cell-laden constructs were stable until day 14 (Figure 2).

Conclusion
This study demonstrated that bioinks composed of HA-nAPBA and HRP can improve printability without the need for additional components. In the future, printability may be further improved by combining polymers with phenylboronic acid derivatives that have higher binding affinity.

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Porous Conductive Hydrogels Toward High-Sensitivity Bioelectronic Interfaces

Introduction: Hydrogel electronics have emerged as promising alternatives to traditional rigid metallic electronics for bioelectronic and human-machine interfaces, owing to their intrinsic biocompatibility and physicochemical similarities to biological tissues1. Despite their promise, most conductive hydrogel systems rely on metallic fillers or nanomaterials to achieve sufficient conductivity, which can compromise flexibility and biocompatibility. Additionally, conductive hydrogels are limited to conventional fabrication techniques that are planar and require multiple processing steps. Embedded three-dimensional printing (E3DP) has emerged as a promising method for freeform patterning of soft materials, enabling the fabrication of complex and intricate hydrogel circuitry within insulating substrates2. However, most conductive hydrogels are insulated within elastomer-based matrices, which suffer from mechanical mismatch with tissues, poor biocompatibility, and low permeability. Here, we propose a single-step E3DP method for fabricating isolated, conductive hydrogel constructs within a highly porous, insulating support matrix by leveraging selective phase transitions of albumin.

Materials and Methods: The conductive hydrogel (albumin (8 wt%) treated with NaOH (10 mM)) is printed into a foam precursor support bath (mechanically foamed at 2500rpm, 2mins) (Fig.1a). Following printing, the printed construct (conductive hydrogel and foam support matrix) is thermally treated (60°C, 10 mins) to initiate selective hydrolysis of the printed albumin hydrogel, yielding conductive albumin channels3, while thermally crosslinking of the surrounding matrix, forming a porous dielectric, through the denaturation of proteins4. The air bubbles and self-healing properties of the foam support bath are characterized by brightfield imaging and cyclic recovery tests, respectively. The effects of thermal treatment on the mechanical properties of albumin (foamed, NaOH-treated, and heat-treated forms) are studied. Furthermore, the printability, sensitivity (ΔR/R), and permeability of the hydrogels are studied.

Results: Treating albumin with NaOH slightly increases its shear moduli; however, subsequent thermal treatment induces hydrolysis, thus decreasing its shear moduli (Fig.1b). In contrast, albumin foam exhibits a significant increase in shear moduli upon heating, confirming hydrogel foam crosslinking. Although NaOH-treated albumin liquefies upon heating due to peptide bond hydrolysis, our results show that when printed into the foam support matrix, the hydrogel remains spatially confined and does not diffuse (Fig.1c). This is due to the concurrent thermal denaturation and crosslinking of the surrounding foam matrix. Hydrogels fabricated using EF3DP exhibit high printability (Pr=0.9) and sensitivity under cyclic strain (~500% ΔR/R, ε = 100%; Figs.1c–d)), which is comparable to values (100-1200%) in literature2. The foam precursor is highly porous (~200–500 µm air bubbles; Figs.1e,f), which is hypothesized to enhance its insulating properties. Moreover, the foam precursor exhibits self-healing properties (Fig.1g), making it ideal for EF3DP. Lastly, thermally treated foams show significantly higher moisture permeability than non-porous hydrogels (Fig.1h), potentially improving skin compatibility and reducing irritation associated with elastomer-based systems.

Conclusion: The proposed EF3DP strategy enables the fabrication of all-hydrogel hybrid ionic–dielectric system by spatially controlling protein phase transitions within a biocompatible, insulating matrix, with enhanced sensitivity and permeability for next-generation bioelectronic interfaces.

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Four-dimensional (4D) Printing of Porous Shape Memory Polymers for Tissue Engineering

Shape memory polymers (SMPs) are a class of smart materials capable of undergoing programmed shape changes in response to external stimuli. Polyglycerol dodecanoate acrylate (PGDA), a thermally responsive SMP, has demonstrated promise in biomedical applications due to its biocompatibility.[1] However, its high viscosity and the requirement for harsh thermal curing hinder its use in vat-based 4D printing. Additionally, the dense, nonporous nature of PGDA limits cell infiltration, posing a challenge for tissue engineering. In this study, we formulated novel ink for 4D printing using a mixture of PGDA, polyglycerol sebacate acrylate (PGSA), and polyethylene glycol (PEG). PEG functions as a porogen to induce porosity and as a rheological modifier. PGSA, structurally differing from PGDA by a two-carbon-shorter dicarboxylic acid, offers a lower melting point, facilitating processing.
PGDA and PGSA were synthesized following established protocols.[2,3] Equimolar amounts of glycerol and dodecanedioic or sebacic acid were reacted under nitrogen for 24

hours. Acrylation was performed by dissolving 20 g of prepolymer in a basic solution (methoxyphenol, 4-dimethylaminopyridine, triethylamine, and methylene dichloride), followed by the dropwise addition of acryloyl chloride under inert conditions for 24 hours. The reaction mixture was purified via rotary evaporation and ethyl acetate washing. PGDA and PGSA were then blended at ratios of 3:1, 1:1, and 1:3 with 0–30 wt.% PEG at 60°C. The rheological measurements were carried out using a TA Instruments HR20 rheometer with a 100 µm gap, in triplicate for each study group. Rectangular shape (3×5×3) 3D-printed using Cellink Lumen X Gen3 with 20mW.cm-2 UV intensity and 10 seconds exposure for 100 µm layers. The PEG leached out from the samples, programmed at 37ºC and fixed at 0ºC before the shape memory behavior test.
The rheological measurements showed a decrease in the viscosity of PGDA with the addition of 15 and 30 wt% PEG at 40ºC (Figure 1A). Additionally, the incorporation of PGSA by 3-fold resulted in a lower viscosity at 40ºC (Figure 1B). Adding PEG to the 3:1 group further decreased the viscosity to reach the printable region. A similar trend was also observed using 1:1 and 1:3 ratios of PGDA:PGSA to achieve more printable resins (Figure 1C and 1D). The 3:1 formulation with 30 wt.% PEG was used to 3D print SMP strips, which showed shape memory behavior upon exposure to 37ºC water (Figure 2). Future studies will focus on characterizing the porosity percentage, the pore size, and the degradability behavior of the printed structure.

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Lunch & Exhibitors' Presentations

- S22d General Symposia Mikołajska

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Biofabrication based on combined 3D bioprinting and fiber spinning

Biofabrication is an emerging field of engineering aimed at creating tissues and tissue-like structures. A key technology in biofabrication is 3D bioprinting, which utilizes methods of precise layer-by-layer deposition of cell-containing bioingredients to form active 3D structures. While 3D bioprinting allows for the creation of some biologically relevant shapes and structures, its reliance on the use of isotropic hydrogels limits the mechanical properties of the formed structures, making them unsuitable for tissues such as cartilage, bone, and skin. The hydrogels themselves also lack the complex hierarchical ECM structure of native tissue. Combining 3D bioprinting with fiber fabrication can produce 3D hybrid structures with improved mechanical and biological properties that more closely mimic the architecture of native tissue at different scales. There are attempts to combine 3D bioprinting and electrospinning/electrospinning which, however, are limited in terms of how fibers are produced, how they are deposited, and what types of structures can be obtained.
We have developed a fundamentally new approach that combines mechanical fiber spinning and 3D bioprinting, offering significant advantages over methods such as electrospinning and electrospinning. Key advantages include (i) ultra-fast deposition of continuous fibers, (ii) deposition of freestanding fibers, and (iii) precise control of fiber direction. These fibers provide strong support for the structures created, eliminating the need for crosslinking hydrogels. This ensures cell motility, facilitating efficient cell migration and tissue formation.

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Development and Characterization of Alginate-Based Bioinks incorporating Nicotiana tabacum Cells for Bioprinting

Development and Characterization of Alginate-Based Bioinks incorporating Nicotiana tabacum Cells for Bioprinting
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Plant cells are valuable for producing various molecules due to their secondary metabolism [1]. Immobilization allows their use in continuous systems, improving product yield and simplifying downstream processing [2, 3]. Further enhancement can be achieved by printing a defined three-dimensional geometric shape from a hydrogel filled with plant cells, known as green 3D bioprinting [4]. This can improve the distribution of oxygen and nutrients and the transport of the final product. For successful printing, the hydrogel must be biocompatible and have appropriate rheological properties. A potentially suitable gelling agent is alginate, which is derived from plants and cross-links with CaCl2.
To assess the biocompatibility of alginate-based hydrogels, the components alginate and CaCl2, as well as the cross-linked gel, were tested with Nicotiana tabacum cells from a suspension culture. CaCl2 concentrations ranged from 10 to 450 mM and alginate concentrations from 0.5 to 1.5%. In the immobilization procedure, plant cells were mixed with the alginate media solution and added dropwise to a CaCl2 solution. The beads were analyzed by measuring fresh/dry weight and by staining with FDA/PI.
To estimate the suitability of the bioink for different printing processes, such as drop-on-demand bioprinting, the rheological properties were investigated. Therefore, the pure 1.5% alginate and the bioink with N. tabacum cells were investigated by measuring the viscosity at different shear rates. To determine the maximum tolerated stress for plant cells, shear rates up to 800 s-1 were applied and the cell viability assessed. After gelation, the mechanical stability of the hydrogel cell constructs was investigated using compression tests.
The addition of uncross-linked alginate to the medium has a strong effect on the viscosity and a strong growth inhibition was observed. CaCl2 has no or little effect up to a concentration of 20 mM. Based on these findings, the final gel used for immobilization consisted of 1.5% alginate cross-linked with 20 mM CaCl2. Here the tobacco cells showed growth with a sixfold increase in dry weight after 10 days and high viability with more than 85% viable cells after 17 days of culture, while the hydrogel remained stable. The bioink showed the desired shear-thinning behavior for bioprinting even stronger than pure alginate. No loss of cell viability was observed after shearing compared with the control.
In conclusion, 1.5% alginate hydrogel with 20 mM CaCl2 is a promising bioink-base for following 3D bioprinting approaches with N. tabacum, due to its biocompatibility, shear-thinning behavior and mechanical stability.
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From microtissues to organized constructs via extrusion-guided bioassembly

Physiologically relevant in vitro models of connective tissues are critical for advancing tissue engineering and disease modeling. However, replicating the hierarchical organization and extracellular matrix (ECM) richness of native stromal environments remains a significant challenge. Traditional scaffold-based approaches often lack the resolution and biological complexity to support proper tissue organization and maturation. In this study, we introduce a versatile extrusion-guided bioprinting strategy for the spatial organization and fusion of biofabricated connective microtissue precursors (µTPs) into structured, ECM-rich constructs. This bottom-up approach is compatible with stromal microtissues derived from different human sources, including pulmonary and breast fibroblasts, and enables the fabrication of scaffold-free tissues through controlled fusion post-printing.
Connective µTPs were generated by culturing primary human fibroblasts onto porous gelatin-based microcarriers under dynamic conditions in spinner flasks. After 18–20 days, the resulting microtissues reached sizes up to ~500 µm for lung-derived fibroblasts (L-µTPs) and ~800 µm for breast-derived fibroblasts (B-µTPs), with early ECM deposition evident in both cases. The µTPs were suspended in a 30% w/v thermoresponsive Pluronic® F127 hydrogel (P30) to create a printable bioink. This formulation demonstrated shear-thinning behavior, homogenous µTP distribution, and temperature-responsive gelation, ensuring filament stability and high print fidelity.
Bioprinting was performed using an extrusion-based system into a 40% w/v Pluronic® F127 support bath (P40), which provided mechanical support and promoted fusion of adjacent µTPs. The P40 bath exhibited self-healing and thixotropic properties, allowing the fabrication of stable, high-resolution constructs with minimal deformation. Multiple

geometries were printed to assess fusion kinetics and structural remodeling. Post-printing, constructs were cultured dynamically for up to 28 days to support tissue maturation and ECM remodeling. Tissue fusion, compaction, and matrix organization were monitored via confocal microscopy, live/dead staining, second harmonic generation imaging, and immunofluorescence analysis for fibronectin and collagen.
Both L-µTP and B-µTP constructs exhibited rapid fusion behavior within the first week post-printing. Quantitative imaging confirmed progressive compaction and continuous tissue formation by day 8. Matrix deposition intensified over time, with increasing alignment along the print direction. Importantly, live/dead analysis revealed high cell viability (>90%) throughout the culture period. Structural remodeling was confirmed by second harmonic generation and mechanical stiffening of the constructs, consistent with functional ECM development. Fusion efficiency was closely linked to initial µTP proximity and pattern resolution, demonstrating the importance of extrusion control in directing construct architecture.
This work establishes a modular, scaffold-free strategy to fabricate engineered stromal tissues from biologically active microtissues. The platform is adaptable across tissue types and enables precise control over microtissue organization and fusion, key features for building complex connective tissue models. The method's flexibility, printability, and biological performance support its potential use in both fundamental studies and translational applications, such as organ-on-chip systems and fibrotic disease modeling. By demonstrating this approach with lung and breast fibroblast-derived microtissues, we highlight its robustness and extendability for connective tissue engineering beyond a single anatomical context.
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Integration of Human Primary Hair Follicle Dermal Papilla Cell Spheroids in Melt Electrowritten-Reinforced Hydrogel Scaffolds for Engineering Skin Substitutes

INTRODUCTION

Skin possesses a complex structure with diverse components. The epidermis consists of tightly packed epithelial cells, while dermis contain fibroblasts, blood vessels, sensory neurons, immune cells, and hair follicles. Both in vitro and in vivo models serve as useful tools for studying skin biology and uncovering the cellular and molecular processes involved in degenerative skin conditions. [1] However, these models have significant limitations because they often lack key biological and structural components of native skin. The most notable drawback of current lab-grown skin models is the absence of critical skin appendages, such as hair follicles (HFs) which limit our understanding of proper micro-environmental factors that contribute to tissue organization. In this study, we aimed to integrate human primary hair follicle dermal papilla cell (HFDPC) spheroids (hair follicle germs) within a fully functionalized vascularized bi-layered construct to biofabricate skin substitute with natural mechanical strength and flexibility and an ability to develop hair follicles.

MATERIALS AND METHODS

A biofabrication platform was developed to incorporate human primary hair follicle dermal papilla cell (HFDPC) spheroids within three-dimensional melt-electro written (3D-MEW) scaffold embedding human umbilical vein endothelial cells (HUVECs) and human dermal fibroblasts (HDFs) within a gelatin-based hydrogel to construct a full skin substitute with hair follicles. Briefly, a functionalized Polycaprolactone (PCL) skin graft with a 0-90° architecture was fabricated using an advanced 3D-MEW technique. Following this, the dermal layer was engineered by embedding HUVECs and HDFs within a gelatin-based hydrogel to create a vascularized dermal construct. HFDPC spheroids were generated and then precisely incorporated into the scaffold using a novel bioprinting method for further evaluation. Subsequently, keratinocyte cells were seeded to form the epidermis layer for a full-thickness skin construct with hair follicles.

RESULTS AND DISCUSSION

The developed platform enabled the construction of a 3D biomimetic structure of a full skin substitute with hair follicle germs. Confocal microscopic images revealed that the HFDPC spheroids were located within the center of the PCL filaments in the dermis, with high cell viability throughout the 3D hybrid structure (Figure 1-I). The distinct spindle-shaped morphology clearly showed that HDFs extended along the PCL filaments and were directed toward the surroundings of the spheroids. This suggested that the cell-spheroid interaction could be effective in the hybrid structure and that the PCL scaffold geometry can have an important role in hair follicle development. After 21 days of in vitro incubation, hair follicle induction within the hybrid structure was observed (Figure 1-II).

CONCLUSIONS

The results demonstrated that the developed 3D platform effectively supported the construction of highly viable hybrid structure recapitulating structure of a full-thickness skin substitutes with hair follicles.

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Achieving Meat-like Texture in Plant-Based Analogues through Chaotic 3D Food Printing

Bovine meat is one of the preferred sources of protein around the world due to its remarkable nature. Meat exhibits a high density of high-quality protein, is well-balanced in essential amino acids, and has a very distinctive set of textural and flavor attributes. However, traditional methods of meat production have been heavily criticized for their poor sustainability, particularly in terms of land and water use, and for being a major contributor to greenhouse gas emissions (i.e., carbon dioxide and methane). The fabrication of plant-based meat analogues has been proposed as a more sustainable alternative, and many commercial embodiments have emerged in the past five years. Despite this, consumer acceptance of these products has been lower than expected, primarily due to dissatisfaction with their organoleptic attributes.
In this study, we demonstrate the use of printheads equipped with Kenics static mixers (KSMs) capable of inducing chaotic f lows (i.e., chaotic food printing) to fabricate plant-based meat analogues with a highly organized and aligned microstructure that resembles the layers of protein, fat, and connective tissue in real bovine meat. We employed 3D-printed chaotic printheads—tubes containing four KSM elements, two top inlets, and one side inlet—to coextrude three highly viscous pastes emulating the main components of meat: protein fibers, fat, and connective tissue. The protein ink was based on pea protein and methylcellulose, the fatty ink on coconut oil and methylcellulose, and the third on konjac and methylcellulose. To coextrude these high-viscosity pastes, we developed a food printer (Figure 1A) using high-torque stepper motors coordinated by an Arduino-based controller, synchronized with an x-y-z positioning system derived from a commercial plastic filament 3D printer. Using this lab-made printer and chaotic printheads, we produced a plant-based analogue with an internal architecture closely resembling the fibrillar structure and textural properties of real bovine meat cuts (Figure 1B).
This chaotic food printer can print 2 kg of plant-based meat per hour, exceeding the throughput of traditional cattle raising by two orders of magnitude. The printed plant-based meat was characterized in terms of bromatology aspects, mechanical properties, cooking performance, and organoleptic attributes (texture, flavor, color, among others) (Figure 1C). A comparative analysis between real meat and this 3D printed meat analogue (prepared in two different presentations) is presented and discussed. Overall, this 3D printing process enables the development of a highly organized, layered, and micro-architected product that closely resembles the texture of soft meat and effectively mimics the perception of meat fibers to a trained panel of tasters. Chaotic printing allows for the creation of plant-based meat analogues that are perceived as highly acceptable by consumers and closely resembling real bovine meat in terms of palatability.

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Merging on-demand single cell dispensing technology with camera controlled DoD-bioprinting enables time and resource efficient cross-scale 3D-biofabrication

Single cell printing techniques are extremely valuable for the precise and controlled construction of tissue precursors. However, existing methods for printing single cells are subject to rheological restrictions. In addition, the high resolution required for printing individual cells conflicts with the desire for time-efficient construction of 3D structures. This work addresses the question of how both compromises can be overcome by integrating a high-speed single cell printing method with high-resolution drop-on-demand 3D bioprinting.
To this end, a newly developed method of on-demand single cell printing is used. The process combines precise inkjet printing (down to pL range) with a microfluidic cell trap. The cell trap allows single cells to be captured above the nozzle and dispensed on demand. In this way, single cells can be printed at a frequency of > 2 Hz and with an accuracy of less than 5 µm. Due to the inkjet technology, the method is limited to the processing of relatively low-viscosity fluids. In addition, despite the high frequency, the picoliter droplets produced result in low material output, which limits the efficient construction of 3D structures. To solve this problem, the single cell printer head is combined with a drop-based printing process. A piezo-electrically controlled open nozzle printer head is used for this purpose. The drop volume and drop quality can be monitored and controlled with an integrated camera system.
As a result, macroscopic gel structures with a resolution of a few hundred micrometers (DoD) can be produced with intermediate cell layers with single cell resolution (single cell dispensing). The process offers great potential for use in regenerative medicine or the production of miniaturized organ-on-a-chip models.

Konuşmacılar: Andreas Blaeser (TU Darmstadt, Institute for BioMedical Printing Technology)

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3D bioprinted semi-synthetic liver-like tissues

INTRODUCTION: The integration of biomaterials with living cells presents a critical bottleneck in the field of tissue engineering and regenerative medicine. Despite the rapid progress in biofabrication technologies, designing functional and biologically relevant tissue constructs that mimic native organ complexity remains challenging. Here, we assembled semi-synthetic liver-like tissues using alginate microgels and hepatocytes. We employed 3D bioprinting to construct centimeter-scale semi-synthetic tissues made of either HepG2 cells or HepaRG cells along with functionalized microgels. These microgels were loaded with a small molecule that mimicked a cytochrome P450 (CYP450) enzyme activity (Figure 1a).1 Notably, HepaRG cells, which exhibit more pronounced hepatic characteristics compared to HepG2 cells, were used to develop a more representative liver model (Figure 1b).2 Upon differentiation, HepaRG cells gave rise to both mature hepatocytes and biliary cells, offering a minimal yet biologically relevant semi-synthetic liver tissue analog.
METHODS: Alginate microgels were fabricated using an Encapsulator, optionally incorporating artificial enzymes. Cell aggregates of HepG2 cells or HepaRG cells were cultured for 48 hours before being suspended in a bio-ink mixture containing alginate, gelatin methacryloyl (GelMA), and microgels. This composite ink was then 3D-bioprinted and maintained in culture for up to 35 days. Cellular proliferation was assessed via dsDNA quantification and LIVE/DEAD staining. Expression of relevant hepatic markers was evaluated using RT-qPCR. To model liver steatosis, differentiated HepaRG cell-based tissues were treated with free fatty acids for two weeks and analyzed for lipid accumulation through fat staining techniques.
RESULTS: Cell aggregates were successfully formed and integrated into 3D-bioprinted constructs using a composite ink made of alginate/GelMA and microgels. HepG2 cells and HepaRG cells maintained viability and proliferated over a 35-day culture period. Differentiation of HepaRG cells initiated at day 14 resulted in upregulated markers of mature hepatocytes and biliary cells by day 28, confirmed by immunostaining. The steatotic tissue model, created via fatty acid incubation, exhibited pronounced lipid accumulation and elevated reactive oxygen species (ROS), as demonstrated through confocal imaging and gene expression analysis. Functional assays using tert-butyl hydroperoxide (tBuOOH) and resorufin ethyl ether revealed enhanced CYP1A2 activity in the presence of catalytic microgels.
DISCUSSION & CONCLUSIONS: Incorporating metalloporphyrin-functionalized microgels into 3D bioprinted semi-synthetic tissues successfully simulated CYP450 enzymatic activity, enhancing the metabolic capacity of co-cultured HepG2 cells over extended periods. Furthermore, employing HepaRG cells enabled the generation of differentiated liver-like tissues. The addition of free fatty acids produced a high-fidelity steatotic model, replicating key pathological features such as lipid accumulation and oxidative stress.
Altogether, these findings demonstrate that engineered semi-synthetic tissues combining functional microgels and liver cells support prolonged viability and hepatic function. This platform represents a significant step forward in developing modular, biologically integrated liver models for research and therapeutic applications.

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Smart pH-sensing scaffolds for Single-Cell Metabolic Flux Analyses in tumor models

Abstract
The tumor microenvironment is characterized by an elevated hydrogen ion concentration, which is the result of increased cellular metabolic demand and altered perfusion, e.g., oxygen availability or acidic metabolic waste products. The acidity of the tumour microenvironment, which is spatially and temporally heterogeneous, affects cancer initiation and progression, but also the efficacy of anti-cancer drug treatments. [1] Therefore, monitoring the local pH metabolic fluctuations is critical for understanding the basic biology of the tumour, and can also be used as a valid metabolic readout for cancer diagnosis and treatment.
Ratiometric fluorescence-based pH sensors represent reliable tools for spatio-temporal pH detection, thanks to their minimal invasive features and high reliability in terms of measurements, which are independent from probes concentration changes, instrument sensitivity and environmental conditions.[2]
To realise a 3D tumor-like platform enabled with ratiometric pH sensing capabilities, we developed a spherical in vitro 3D hydrogel seeded with tumor and stomal cells integrating a microparticle based pH-sensor system compatible with live cell confocal laser scanning microscopy (CLSM), allowing non- invasive visualization and detection of acid-base metabolic variation at single cell level over time and space to predict and to quantify anticancer drug efficacy. In particular, the extracellular acidification is more pronounced after drugs treatment, resulting in increased antitumor effect correlated with apoptotic cell death [3,4] In addition, we devised a new method to precisely quantify single-cell fermentation fluxes over time by combining high-resolution pH sensing electrospun nanofibers with constraint-based inverse modelling. We applied our method to cell cultures with mixed populations of cancer cells and fibroblasts and found that the proton trafficking underlying bulk acidification was strongly heterogeneous, with maximal single-cell fluxes exceeding typical values by up to 3 orders of magnitude.[5] Our method addressed issues ranging from the homeostatic function of proton exchange to the metabolic coupling of cells with different energetic demands, allowing for real-time non-invasive single-cell metabolic flux analysis.

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Konuşmacılar: Valentina Onesto (CNR Nanotec)

S32 Post Bioprinting Process

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Toplantıyı düzenleyenler: Koichi Nakayama (Saga University), Denisa Kaňoková (Czech Technical University in Prague, Faculty of Biomedical Engineering)

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Opening Remarks: Post-Bioprinting Process

Bioprinting technologies, including those developed by our group, have already entered the stage of clinical application. However, most of these achievements remain in the Proof of Concept and/or phaseI/II, and many challenges must be overcome before bioprinting can become a widely adopted medical practice. One critical aspect is the series of steps that follow the industrial printing process—commonly referred to in the printing industry as “post-printing.” In traditional printing, commercial success does not depend on printing alone but also on subsequent processes such as finishing, binding, marketing, and distribution. Similarly, in our field, while research has largely focused on pre-printing preparation and the printing itself, the path to clinical adoption requires greater attention to the post-bioprinting phase. By highlighting this analogy, the aim of this session is to bring awareness to the diverse post-bioprinting processes that are indispensable for translating printed constructs into functional and clinically relevant therapies. Through this session, we hope to encourage the research community to expand its perspective beyond the printing step and accelerate progress toward the realization of bioprinting as a standard medical practice.

Konuşmacılar: Koichi Nakayama (Saga University)

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  Advancing 4D Bioprinting with Tailored Bioreactors: Successes, Challenges, and Future Directions

  Introduction: Customized bioreactors can replicate diverse physiological conditions, such as shear stress, pulsatile pressure, and strain, while enhancing diffusion and nutrient exchange. These conditions stimulate cellular processes including proliferation, differentiation, gene expression, and substrate remodeling. However, in two-dimensional cultures, these stimuli promote adipose-derived stromal cells (AdSC) and endothelial co-culture into smooth muscle-like cells, improving cell-substrate and cell-cell interactions, and promoting ECM production. Despite these advancements, the result is still only a few layers of cells rather than fully developed tissue. Introducing a third dimension with collagen bioprinted scaffolding enables time- and stimuli-dependent remodeling, leading to structures that more closely resemble real tissue [1].

  Methods: In our studies, we used porcine-based collagen bioink at concentrations of 20–50 mg/ml, incorporating porcine AdSCs at densities of 10–20 million cells/ml. The collagen was mixed using a custom-built mixing system. Substrates were printed with a custom bioprinter utilizing syringe-based extrusion. Gelling was achieved through pH neutralization and temperature change [2]. Samples were printed onto glass or PLCL nanofibers with defined rectangular dimensions (up to 25 x 75 mm) and varying thicknesses ranging from 0.5 to 2 mm. Printed samples were mounted either as fixed or freely floating in custom cultivation bioreactor chambers connected to a customized programmable pulsatile pressure flow generator. We focused on varying multiple conditions, including generic perfusion for nutrient replacement, increased partial pressures to promote diffusion, pulsatile pressures to enhance diffusion and provide mechanical stimulation, and defined shear stress. These conditions were applied continuously or with intermittent resting pauses. Culture media were programmatically altered to promote cellular proliferation, differentiation, and ECM remodeling.

  Discussion: Perfusion with altered partial pressures of CO2 and O2 improved cell viability. Depending on cell densities and cultivation time, the initial collagen was remodeled into compressed, more stable, and stiffer structure, resulting in an increased compression modulus [2]. Differentiation media with pulsatile stress promoted smooth muscle cell (SMC) differentiation and new extracellular matrix (ECM) formation. Controlled shear stress oriented the cells, and with the addition of arterial endothelial cells (ECs), vessel-like formations were created. The initial 3D bioprinting provided a shape that was remodeled over time. By controlling these parameters during cultivation, it is possible to create functionalized tissue constructs for various applications, such as vessel patching and grafting, with defined microstructure and biomechanical properties, unlike static culture [3].

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  Konuşmacılar: Roman Matějka (Czech Technical University in Prague)

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  Enhancing 3D Bioprinting of High-Concentrated Collagen Bioinks through Active Media Perfusion and Microvascular Flow Simulation

  3D bioprinting has emerged as a promising technology in tissue engineering and regenerative medicine. Collagen, given its natural abundance in the extracellular matrix and excellent biocompatibility, serves as an ideal biomaterial for the preparation of printable bioinks. This study explores the optimization of collagen bioink properties as well as cultivation strategies and microvascular simulation to enhance the functionality of bioprinted constructs. We focused on improving cell viability, morphology, and metabolic activity within bioprinted collagen structures by optimizing the neutralization protocol of highly concentrated collagen hydrogels (up to 50 mg/mL), which included two successive neutralization steps using a 2× concentrated culture medium and NaOH for precise pH adjustment [1]. This process was supported by a semi-automated, custom-built mixing system featuring colorimetric pH estimation, ensuring high consistency and reproducibility in bioink preparation, and printed using a custom bioprinter utilizing syringe-based extrusion. Alongside, we investigated the impact of active media perfusion during cultivation. Compared to static culture, active perfusion significantly improved cell viability and metabolic activity within highly concentrated collagen constructs [2]. The dynamic perfusion environment enhanced nutrient and gas delivery and waste removal, which supported the development of more viable, structurally robust, and functional bioprinted tissues. To further support cell proliferation and tissue maturation in thick (>1.5 mm) samples, we developed a novel microchannel fabrication strategy, since achieving a high proliferation rate in thick constructs remains a challenge. By printing highly concentrated collagen bioink directly onto a horizontal array of thin needles, we generated continuous, organized microchannels throughout the constructs. These structures significantly expanded the diffusion surface area, which improved cell viability in thicker samples. Overall, the integration of optimized bioink preparation, active media perfusion, and precise microchannel engineering offers a strategy for enhancing the viability, structural integrity, and functionality of thick, bioprinted collagen-based constructs. These advancements hold significant promise for future cardiovascular applications, potentially revolutionizing treatments and therapies in this field.

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  Controlled perfusion of a vascularized microenvironment within a 3D printed bioreactor to study leukemia cells trafficking ex-vivo

  INTRODUCTION
  Advanced in-vitro systems are fundamental to investigate cell dynamics and crosstalk occurring within the same or different districts of the human body.
  In the case of chronic lymphocytic leukemia (CLL), given the importance of the microenvironment, there is a strong need for new tools to unveil key pathogenetic mechanisms and perform more reliable drug testing ex-vivo. Little is known about the mechanisms regulating CLL cells intra- and extravasation from lymphoid tissues, including the role played by endothelial cells and local vasculature1.

  METHODS
  We designed and 3D-printed a modular bioreactor (VesselBox) to selectively perfuse a 3D-bioprinted and casted vascularized scaffold, made by the biomaterial GelXA Laminink 411 and embedded with human lymphatic fibroblasts (HLF) mimicking the lymph node microenvironment. The full-thickness of the lumen is populated by endothelial (HUVEC) and bone marrow mesenchymal (BM-MSC) cells, mixed in GelMA-Fibrin or Self-Assembling Peptides (SAPs). A CLL cell-line (MEC1-GFP) and CLL primary cells were perfused at 100uL/min for 1, 3 and 7 days (Fig.1).

  RESULTS
  We first generated 3D vascularized scaffolds by 3D-bioprinting the external microenvironment constituted by HLF and casting the vascular compartment made of HUVEC and BM-MSC. We demonstrated the functional organization of the vascular compartment through CD31/VE-cadherin/VWF detection by IF after 14 days of culture, together with the homogeneous distribution and proper morphology of HLF in the microenvironment2.
  Computational analysis supported the bioreactor validation, predicting oxygen diffusion (from 0.2 to 0.0265 mol/m3), pressure distribution (0.1Pa) and shear stress (0.017 dyn/cm2) within the perfused scaffold, resembling in-vivo situation.
  Scaffolds were then perfused with MEC1-GFP and patient-derived CLL cells (n=3) for 1, 3 and 7 days, monitoring their extravasation and immunophenotype by confocal microscopy and flow cytometry, respectively.
  By flow cytometry, we detected differences in circulating cells in the presence or the absence of the microenvironment in the VesselBox as compared to CLL cells isolated

from the peripheral blood (baseline CLL), specifically in markers involved in cell homing (CXCR4 and CD62L) and activation (CD23)3. In particular, we observed the presence of a CXCR4high/CD5high population in the complete VesselBox, which is supposed to be more prone to tissue homing, that isn't present in baseline CLL and in empty VesselBox. Also, CD62L+ cells followed the same trend as CXCR4high/CD5high population, thus increasing in the presence of the vascularized scaffold. Lastly, when circulating in empty VesselBox, CD23high cells decreased if compared to baseline CLL, while they almost completely recover in complete system. CXCR4 and CD62L expression levels were also validated by RT-PCR, showing coherent results. We are now testing with promising results the compatibility of BM-MSC with SAPs to improve the vascular compartment of our model.

DISCUSSION

The VesselBox device enables the investigation of CLL cells dissemination ex-vivo, following their extravasation and mobilization processes with tissue-specific microenvironment components and an endothelial barrier. This platform allows to study patient-specific response to targeted and combinatorial therapies, and can be adapted for other applications customizing microenvironment and endothelial barrier.

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Perfusion Platforms with Engineered Surfaces for Vascular Modeling and Hemodynamic Investigations

Perfusion platforms are increasingly used to replicate in vivo vascular environments and to investigate how the interplay between vascular geometry, surface properties, and flow dynamics influences the physiology of endothelial cells lining the lumen of vasculature. Here, we present an approach that combines 3D printing, soft lithography, and advanced surface modification to create perfused vascular platforms designed for both long-term endothelial cell culture and hemodynamic analysis, with particular relevance to cerebrovascular pathologies such as intracranial aneurysm rupture.

In the first part of our work, we developed cylindrical-channel PDMS microfluidic chips using 3D-printed molds and soft lithography. To address the hydrophobic nature of PDMS and its poor cell-adhesive properties, we applied high-frequency low-pressure air plasma treatment. This enabled uniform and reproducible oxidation of the luminal surfaces in enclosed channels. When followed by collagen IV coating, the modified surfaces supported robust endothelialization, enabling the formation of stable, biomimetic endothelial monolayers under continuous perfusion.

The optimized methodology was then adapted for hemodynamic studies platforms. We employed computational fluid dynamics (CFD) and experimental validation using particle image velocimetry (PIV) to analyze flow patterns and wall shear stress distributions in vascular geometries derived from patient-specific intracranial aneurysms. Surgical and imaging data allowed identification of rupture points in six clinical cases, which were then correlated with local hemodynamic parameters. Our findings suggest that rupture sites often coincide with regions of abnormal wall shear stress and high oscillatory shear index.

Future work will focus on adapting the platform for stereolithography (SLA) printing of hydrogels to replace PDMS with more physiologically relevant materials. This transition is expected to enhance fabrication precision and throughput, while also introducing new challenges in post-fabrication processing, particularly with respect to surface modification. Additionally, we aim to establish cell-based models of intracranial aneurysms within these systems to facilitate mechanistic studies and screening of candidate therapeutic compounds.

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From Structure to Function: Enabling Intelligent Biofabrication with Sensor Integration and AI workflows

Biofabrication has revolutionized the way we design in vitro models of human physiology. Advances in 3D bioprinting, scaffold engineering, and stem cell biology have enabled the creation of increasingly sophisticated tissues. Biofabrication permits spatiotemporal control over cell-cell and cell-extracellular matrix communication and thus the recreation of tissue-like structures [1,2]. Yet, construction of tissues often remains blind to their function, as it lacks real time feedback as the process itself is not monitored. This can be enabled by measurement of various process parameters which allow real time adaptation, self-monitoring and interfacing with analytical or therapeutic workflows. Sensors provide a continuous, non-destructive readout of key qualitative and quantitative parameters of tissue models [3]. Microphysiological systems (MPS) are facing similar challenges as the data generated by these models still mainly relies on end point measurements. In this talk, I will focus on examples from imec's MPS program in how sensor embedding and real-time feedback offers multi-modal output such as electrical impedance sensing, electrical activity monitoring, metabolite sensing and protein detection in combination with novel imaging techniques. The co-integration of these sensors allows not only capturing structure of the tissues but also dynamics and heterogeneity, yielding more powerful data of the model under study. Additionally, I will outline how the sensor platform can interface with AI algorithms to automatically classify tissue states and predict responses — offering a feedback loop that is very useful for biofabrication.

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Predictive Rheological Insights into Shear and Elongational Stress Effects on Cell Viability in Extrusion-Based Bioprinting

Extrusion-based bioprinting enables fabrication of living constructs with tissue-like features but poses significant challenges for maintaining post-printing cell viability due to complex mechanical stresses. In this study, we leverage the integrated in-line rheological modules of the RevoBITs Byte 1 bioprinter to directly quantify shear and elongational stresses during printing and correlate these measurements with cellular outcomes.

Two xanthan gum–PEGDA bioinks (3 % and 5 % w/v xanthan) were characterized via rotational and capillary rheometry (CaRheo) with Bagley entrance correction, and with the novel BERIT elongational rheology setup, all seamlessly integrated into the Byte 1 system. Both formulations exhibited pronounced visco-elastic solid like behaviour in shear and elongation; the 5 % ink showed higher oscillatory viscosity throughout the tested regime and exhibited elongational stress plateaus of 400–550 Pa, compared to 300–450 Pa for the 3 % ink. Capillary measurements revealed wall shear stresses of 30–150 Pa for the 3 % ink versus 20–100 Pa for the 5 % ink, despite the latter's higher rotational viscosity, indicating bioink-specific flow regimes such as plug flow or wall slip in the 5 % formulation.

BJ fibroblast-laden droplets were printed using eight print configurations per bioink (27 G/30 G, 0.5 in/1.5 in) and feedrates (77 µl/min and 154 µl/min). Metabolic activity was assessed immediately (day 0) and at days 1 and 4 post-printing. While bioink handling alone induced notable stress (control vs. printed), cells in the 5 % ink consistently showed up to 30 % higher viability compared to the 3 % ink, suggesting protective effects from reduced local shear gradients. Larger nozzle diameters and shorter lengths further enhanced viability; feedrate remained a minor factor.

Linear regression modeling identified time, xanthan concentration, nozzle diameter, and their interactions as the most significant predictors of metabolic activity. In contrast, nozzle length and feedrate remained minor factors. Gradient analysis within the parameter space highlighted optimal settings for maximal cell health.

By integrating direct in-line rheometry within the Byte 1 bioprinter and correlating stress profiles to biological metrics, this work demonstrates a pathway toward predictive bioprinting workflows, reducing trial-and-error and showcasing the capabilities of RevoBITs' platform for high-quality tissue fabrication

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Konuşmacılar: Ruben Gerrit Scheuring (Institute of Polymer Materials, FAU Erlangen-Nuremberg)

S37 Celebrating 25 years of gel-MA: The importance of standardization in gelatin materials towards clinical translation

Kruczkowski

Toplantıyı düzenleyenler: Jasper Van Hoorick (BIO INX BV), Jos Olijve (Rousselot Biomedical)

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The origin of gel-MA, a short history from PhD to industry

Konuşmacılar: Jasper Van Hoorick

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Gelatin-based bioinks and bioresins for advanced light-driven biofabrication

Photoresponsive gelatin derivatives are among the most used biomaterials to produce bioink and bioresins for biofabrication. The unique thermoreversible gelation properties of gelatin, combined with covalent crosslinking strategies via the incorporation of i.e. acrylates groups offers a broad range of rheological performances suitable for both extrusion and light-based printing applications. This lecture will focus on highlighting the relevance of accurately controlling gelatin-based materials composition and physico-chemical properties for various technique in light based bioprinting. Modulating viscosity and gelation points offered a powerful tool for digital light projection (DLP) fabrication of complex 3D geometries embedding convoluted channels reflecting anatomical features of blood vessels. Moreover, with the advent of ultra-fast volumetric bioprinting (VBP) strategies, which rely on hihglz viscous polymers to prevent cell sedimentation during printing, GelMA and thiol-ene gelatin have greatly facilitated the biofabrication of structures embedding both single cells and organoids, using both single-components gelatins or microgel-based bioresins. At the same time, methods for multi-material printing are needed for broad VBP adoption and applicability. Although converging VBP with extrusion bioprinting in support baths offers a novel, promising solution, further knowledge on the engineering of hydrogels as light-responsive, volumetrically printable baths is needed. Recently, we investigated the tuning of gelatin macromers, in particular leveraging the effect of molecular weight and degree of modification, to overcome these challenges, creating a library of materials for VBP and Embedded extrusion Volumetric Printing (EmVP). Bioresins with tunable printability and mechanical properties are produced, and a novel subset of gelatins and GelMA exhibiting stable shear-yielding behavior offers a new, single-component, ready-to-use suspension medium for in-bath printing, which is stable over multiple hours without needing temperature control. As a proof-of-concept biological application, bioprinted gels are tested with insulin-producing pancreatic cell lines for 21 days of culture. Leveraging a multi-color printer, complex multi-material and multi-cellular geometries are produced, enhancing the accessibility of volumetric printing for advanced tissue models. In future developments, heterocellular and anisotropic tissue models that can be volumetrically produced in a high-throughput fashion could have far-reaching implications for biomedical and pharmaceutical research, paving the way for the next generation of in vitro tissue models.

Konuşmacılar: Riccardo Levato (Utrecht University)

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Finetuning the properties of GelMA-dextran aqueous two-phase systems to create unique interconnected porous hydrogels via inkjet 3D printing

Hydrogels with tailored porosity and microstructure are essential for biomedical applications such as drug delivery and tissue engineering. However, controlling their internal architecture remains a challenge. A promising approach leverages polymer phase separation in water to create hydrogels with large interconnected pores, enhancing cell growth or migration, nutrient transport, and cellular waste removal.

This work presents a straightforward strategy to regulate the phase separation of gelatin methacryloyl (GelMA) and dextran in water to produce hydrogels with tunable interconnected pores. By introducing glucono delta-lactone (GDL) into the aqueous two-phase system (ATPS), a gradual pH decrease is triggered, inducing segregative phase separation between the two biopolymers. The in-situ acidification delays and moderates phase separation kinetics. UV photo-crosslinking of the GelMA-rich phase at different time points arrests the microstructure at different stages of phase separation, allowing fine control over the length scales of the interconnected GelMA-dextran channels. After washing out dextran, porous GelMA-based hydrogels are obtained.

The approach is effective for both casting and inkjet 3D printing. Initially, the GelMA-dextran mixture is in solution state at 37°C, with GDL delaying phase separation to ensure that the system is fully mixed and that there is sufficient time to set up and calibrate the printer before inkjet printing. The delay duration is tunable by adjusting GDL concentration, and the higher the GDL concentration, the faster the pH drops. Additional parameters such temperature, ionic strength and viscosity have also been investigated to optimize ATPS ink.

In figure (A), confocal laser scanning microscopy (CLSM) is used to monitor the in-situ evolution of the GelMA-dextran solution at 37 °C. Labeled GelMA appears red, while dextran-rich regions are dark. With 5 mg/mL GDL, bicontinuous GelMA-dextran interconnected channels begin forming after a 26-minute delay, and spinodal decomposition is complete within 4 minutes. Photo-crosslinking during this time window allows the formation of permanent hydrogels with varied microporosity. In figure (B), the temporal evolution of the width of the elongated interwoven channels of GelMA and dextran is shown. The quantitative analysis was performed with the software “Aquami” and depicts the distinct parallel growth of GelMA (orange symbols) and dextran (black symbols) domains.

The proposed method is simple and the production of these unique porous hydrogels is easily scalable. It provides enhanced reproducibility and control over spinodal decomposition, which is typically sensitive to experimental procedures. Importantly, the printing process does not interfere with phase separation or disrupt the targeted microstructure. The tailored design of the length scales of the interconnected GelMA-dextran channels, hence the resulting porosity of the hydrogels, is promising for biomedical applications with different requirements regarding cell types or sizes.

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GelMA-Based Hydrogels for in vitro Cholangiocarcinoma Modeling and Drug Testing

Introduction

Cholangiocarcinoma (CCA) is a rare but deadly disease that arises from epithelial cells in the liver (intrahepatic) or the biliary tract (extrahepatic). The absence of specific symptoms leads to late diagnosis, which is associated with a poor prognosis due to disease progression, metastasis, and the emergence of drug resistance. The heterogeneity of CCA makes the drug discovery process longer and more complex, partly due to the lack of reliable in vitro models for preclinical studies 1. CCAs are characterized by a dense, desmoplastic microenvironment with complex crosstalk between various stromal components and the extracellular matrix 2,3. Designing an in vitro model that mimics physiological conditions could improve the evaluation of drug efficacy.

Methods

To model the CCA tumor niche, gelatin methacryloyl (GelMA) hydrogels served as a 3D supporting matrix for co-culture of an immortalized CCA cell line (TFK-1) and human Mesenchymal Stem Cells (hMSCs). Matrix effects on cell behavior were investigated by assessing cell proliferation (viability assays), 3D structure formation (immunofluorescence), and gene expression (digital PCR). Furthermore, the influence of matrix composition on drug sensitivity was evaluated by assessing the effects of Gemcitabine and Curcumin 4 on cell viability.

Results

Consistent with the design of supportive matrices, high cell viability was observed, along with the formation of tumor spheroids within seven days. Furthermore, the matrix stiffness appeared to play a significant role, as softer hydrogels supported higher proliferation. Overall, RNA expression analyses revealed the maintenance of the epithelial phenotype in TFK-1 cells, demonstrating that methacryloyl residues and the photopolymerization process did not induce phenotypic modifications. On the other hand, the presence of hMSCs promoted the expression of genes associated with ECM remodeling (e.g., MMP9) and signaling pathways (e.g., VEGF). Furthermore, when the established 3D cultures were challenged with chemotherapeutic agents, we observed reduced sensitivity compared to 2D cultures, with the specific matrix influencing the response.

Discussion

Taken together, this study demonstrates the suitability of GelMA hydrogels for modeling the CCA niche, revealing significant influences of matrix stiffness on cell proliferation and drug sensitivity that are not observed in 2D cultures. These initial findings warrant further investigation into the specific matrix cues driving these differential responses and the potential for exploiting these interactions for improved therapeutic interventions in CCA.

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Towards scaling up two-photon 3D bioprinting of gelatin-based polymers

Introduction
Bioprinting offers the opportunity to reproduce tissue structures in-vitro to support the reconstruction of functional tissue constructs. Among the different bioprinting techniques, two photon polymerization (2PP) allows the generations of the smallest features up to the submicron range. However, the low volume throughput is the main drawback to obtaining constructs of a relevant size.[1] Moreover, the materials for cell encapsulation using 2PP can be printed in low concentrations, with consequently low viscosities that over long printing times can cause cell sedimentation.[2] When scaling up, ensuring adequate cell spreading within the structure is pivotal to allow high cell viability and functionality.[3] In this work, these challenges of applying 2PP to bioprinting with cells are addressed, using fibroblasts and photorosslinkable gelatin as reference substrates.
Methods
Gelatin was functionalized with norbornene (NB) moieties according to Van Hoorick et al. [4] and used with a thiolated PEG as a crosslinker. A gellifier was added to enable printing in a solid state, after measuring its sol/gel transition temperature via rheological measurements. The polymer blend solution was mixed with L929 fibroblasts to a final concentration of 2x106cells/mL, and the bioink was printed using an UpNano NanoOne printer.
The processability window of the ink was identified by printing a reference “multigrid” structure and varying the main process parameters in a full factorial DoE, followed by the estimation of shape fidelity and swelling via image analysis on confocal images after 1 day of incubation at 37 °C in PBS. To estimate the printing time, a full factorial DoE was prepared by printing cubes of different sizes at varying printing parameters.
Within this window, the effect of different lattice geometries and pore size on cell viability was tested using Live/Dead staining with fluorescence imaging 1 day post-printing. Cytoskeleton remodelling in larger structures (side 500 µm) with different porosities was assessed at day 7 via confocal imaging after actin-F/DAPI staining.
Results and discussion
The addition of the gellifier avoided cell sedimentation of the cells in bulk polymer, which enables the production of constructs with higher thickness (500 µm) with a homogeneous cell distribution of the cells along the z-axis.
The processability window of the material at varying process parameters was identified in a wider printing domain than previously reported, and a simple model for the printing time was used to exclude the printing parameters corresponding to a volume throughput higher than 1 mm3/hr. This reduced printing time while maintaining resolution.
Finally, a wider region of the processability window was identified as compatible with the bioprinting process. Moreover, the introduction of micro (pore size<cell size) and macro (pore size>cell diameter) porosity showed to improve both cell viability, cytoskeleton remodelling, and cell spreading, compared to bulk, non-porous structures obtained with the same printing parameters.
These strategies are promising for the scale-up and production of relevant tissue structures with viable cells present throughout the hydrogel.
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Decellularized brain tissue combined with GelMa as a novel hydrogel emulating extracellular matrix in cerebral organoid-on-chips

Decellularized brain tissue combined with GelMa as a novel hydrogel emulating extracellular matrix in cerebral organoid-on-chips

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Introduction

In recent years, advances in biotechnology have significantly contributed to the development of more physiologically relevant in vitro models. Among these innovations, the differentiation of functional cells from induced pluripotent stem cells (iPSCs), the emergence of three-dimensional organoid models, and the design of dynamic organ-on-chip platforms that replicate physiological fluid flow and cellular behavior have garnered remarkable attention. Furthermore, the engineering of tissue-specific biomimetic extracellular matrix (ECM) formulations has further enhanced the fidelity of these models through various strategies. Decellularization is one such approach, involving the removal of cellular and nuclear components from tissues or organs while preserving the native protein and biochemical complexity of the ECM, with the aim of preventing immune responses and replicating natural tissue architecture1. Brain-derived decellularized ECM (dECM) has gained considerable interest in neuroscience due to its ability to more accurately mimic the native brain microenvironment2, thereby providing a favorable environment for organoid survival and maturation. The successful development and long-term maintenance of cerebral organoids critically rely on the ECM, which not only offers mechanical support but also delivers essential biochemical cues that regulate cell adhesion, migration, and differentiation. In the brain, the neuronal ECM creates a highly specialized and dynamic microenvironment that modulates neural-glial interactions, synaptic stability, and plasticity3. In light of these insights, the present study aims to develop a novel brain derived dECM-GelMa biomaterial and integrating it with cerebral organoids for further applications (Fig 1).

Figure 1. Schematic representation.

Methods
Brain tissues were decellularized using novel method. Protein retention and DNA removal efficiency were evaluated through biochemical assays to assess the preservation of ECM integrity. Formulated ECM pre-gel is mixed with GelMa and optimized for further organoid applications.

Results
Biochemical analyses demonstrated that the decellularization process preserved almost 50% of the total brain ECM proteins while removing approximately 80% of the native DNA content.

Discussion
These findings highlight that preserving ECM integrity is crucial for supporting cerebral organoid viability, enhancing neural-glial interactions within the cerebral microenvironment.

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Advancing Cartilage Regeneration with Reproducible GelMA Bioinks

Gelatin Methacryloyl (GelMA) is widely used in biofabrication, yet its lack of reproducibility remains a major barrier for translation. This issue originates from a variety of reasons including inconsistent selection of raw materials, variations in modification strategies, varying degrees of substitution, and differences in solvent and photoinitiator concentrations employed in various studies. To overcome these issues, a new bioink is presented based on porcine gelatin where these issues are tackled through a combination of batch control, significant QMS protocols and a purification step to remove endotoxins from the gelatin, and significant Quality control in the following bio ink production, resulting in a true medical grade bioink. From the production of gelatin to the final formulation of ink, quality assurance protocols are implemented at each stage of material development. The printability of the ink was optimized to provide high reproducibility in 3D bioprinting. For biological validation, primary human chondrocytes were mixed with the bioink and UV-crosslinked. Biological assays for cartilage regeneration were conducted on the resulting constructs, which included metabolic activity assays, live/dead cell viability tests, and histological analysis. The bioink's compatibility with human chondrocytes was suggested by high cell viability and metabolic activity of the constructs, as indicated by preliminary results. This study introduces a GelMA-based bioink that is reproducible and ready-to-use by implementing rigorous quality assurance protocols and tackling the reproducibility issues at the earliest phases of bioink development. In the future, the primary objective will be to enhance biofabrication techniques for patient-specific constructs, with the ultimate objective of clinical translation for cartilage repair.

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- S38 Advanced Organ-on-Chip Technologies for Pulmonary and microenvironment Studies — Rudniew

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The transformative role of organ-on-chips for evaluating the exposure of airborne particles

Introduction

With the escalating challenges of environmental pollution and climate change, research on chronic non-communicable diseases arising from exposure to various pollutants such as inorganic particles and micro-/nanoplastics has gained significant momentum. Among these, respiratory exposure to particulate matter, a major component of air pollution, has been strongly implicated in the pathogenesis of chronic respiratory diseases. Microplastics, another ubiquitous pollutant, have been detected in both indoor and outdoor environments[1] and even within human lung tissues[2], raising concerns about their potential health impacts. This increasing plastic burden heightens human exposure through inhalation, ingestion, and dermal contact. Moreover, microplastics can further degrade into nanoscale fragments, which have an even greater capacity to penetrate biological barriers, exacerbating their potential health risks. Among these biological barriers, the airway epithelial barrier serves as the first line of defense against airborne pollutants. However, exposure to microplastics compromises the integrity and functionality of this barrier (Fig. 1), thereby increasing susceptibility to respiratory diseases. Disruption of the airway epithelial barrier is not only linked to chronic respiratory conditions but may also contribute to the development of neurodegenerative diseases through systemic inflammation and neuroimmune interactions.

Figure 1: Transition of inhaled nanoplastics through the alveolar epithelial barrier into the vascular system. (The image has been created using smart.servier.com)

Current in vitro and ex vivo models range from conventional Transwell systems to advanced organ-on-chip platforms3,4, which enable the co-culture of human lung epithelial cells and endothelial cells. Additionally, more sophisticated organotypic lung tissue cultures incorporating both tissue-specific and resident immune cells5 provide valuable insights into the complex interactions between pollutants and the pulmonary microenvironment.

Methods

We designed and fabricated an epithelial barrier-on-a-chip platform5 consisting of easily moldable polydimethylsiloxane layers along with a thin, flexible, and transparent membrane to evaluate exposure to various airborne particles.

Results

The administration of silica and polypropylene nanoparticles to the epithelial barrier-on-chip platform under static and dynamic conditions demonstrated the detrimental effects. Cell viabilities were altered, resulting in increased permeabilities, decreased ZO-1 expressions and increased proinflammatory cytokines.

Discussion

Understanding how exposure to these pollutants disrupts airway epithelial integrity is crucial for elucidating the etiopathogenesis of respiratory and neurodegenerative diseases. Advancing robust preclinical models will not only enhance our mechanistic understanding but also facilitate the development of novel therapeutic strategies to mitigate the health impacts of environmental pollution. The organ-on-chip models are envisaged to serve as robust and reliable substitutes in this context.

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Physiologically Relevant Lung-on-Chip Models for Studying Airborne Toxicants and Drug Responses

Over the past 15 years, our group has focused on the development of advanced lung-on-chip (LOC) models that closely mimic the human lung parenchyma with high physiological relevance. Our early systems[1],[2], which replicated the three-dimensional deflection associated with physiological breathing motions, have since been commercialized by our spin-off company, AlveoliX. These foundational models paved the way for a second-generation LOC platform featuring a membrane composed of collagen and elastin—two key extracellular matrix proteins—structured into an array of alveoli-sized compartments, thereby replacing the flexible and porous PDMS membrane[3],[4].

More recently, we have employed these platforms for the toxicological assessment of airborne substances such as disinfectants[5] and cigarette smoke (Sengupta et al., in revision). Our studies revealed a critical synergy between mechanical stress and the air-liquid interface in disrupting the integrity of the alveolar air-blood barrier, as evidenced by significant reductions in trans-epithelial electrical resistance (TEER), among other indicators.

Additionally, we have developed an idiopathic pulmonary fibrosis (IPF) model on-chip, which not only recapitulates fibrotic-like tissue remodeling but also demonstrates how cyclic mechanical strain significantly influences the therapeutic response to nintedanib (Weber et al., submitted). Together, these findings highlight the essential role of biomechanical cues in shaping both toxicological outcomes and drug efficacy, underscoring the need for physiologically dynamic in vitro pulmonary models in respiratory research.

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Modeling and Biofabrication of Lung Epithelial Barrier-on-a-Chip

Introduction

The main defense mechanism against inhaled airborne particles is the epithelial barrier, consisting of lung epithelial cells connected by adherent junctions. While some airborne particles are eliminated by the innate defense system, those that are not detected, continue to progress in the body. In the long-term and high-concentration exposures the particles may escape the radar of the immune system1 which in turn triggers oxidative stress, inflammation, and apoptosis mechanisms that lead to the disruption of the barrier integrity2. Impairment of homeostatic interaction of cell-level to system-level defense mechanisms increases susceptibility to respiratory diseases and can contribute to disease initiation in other tissues of the human body. Physiological and anatomical mimicry models of the respiratory system have been proven to be essential for drug development and elucidating the pathophysiological-mechanisms of diseases triggered by epigenetics and hereditary genetic factors. Lung epithelial models can be organized as monoculture/multicultural cells with supporting scaffolds such as trans-wells and bio-gels. Notably as a novel study niche, organ-on-a-chips provide controllable physiological conditions that recapitulate tissue specific features. Epithelial cells, endothelial cells, their basement membranes, and interstitial space between these two monolayered cells provide an extracellular matrix of the lung epithelial barrier. The hierarchical structure of extracellular matrix (ECM) can be emulated by two chambers containing a biological or synthetic membrane, which is functionalized with epithelial-endothelial cells. Hydrogel-based systems such as type-I-collagen scaffolds, commercial matrices (Matrigel, gelMA) and decellularized-tissue-derived-scaffolds are also employed for housing cells3. Interestingly, decellularized leaves have been utilized as vasculatures4 or scaffolds5. Thus the choice for fabrication would heavily rely on the specific requirements of the model.

Methods

Polydimethylsiloxane based soft molded organ-on-a-chip platform was designed and fabricated to emulate epithelial barrier micro-physiology for evaluating the effects of aerosol exposure.

Results

Optimized model in terms of flow mechanics and the transport of microplastic particles were numerically modeled using the finite element calculation method. TEER measurements validated the barrier integrity has been successfully achieved in organ-on-a-chip platform while the exposure causes the disturbance of barrier integrity and cell viability demonstrated with IF-stainings.

Discussion

Obtaining a proven and repeatable model for epithelial barriers allows us to investigate the toxicology of pollutants and study new therapeutic breakthroughs for a healthier future. Innovative approaches such as the organ-on-a-chip systems stand out as promising candidates for these models

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Novel Approach Methodologies in Modeling Complex Bioaerosol Exposure in Asthma and Allergic Rhinitis Under Climate Change

The undeniable impact of climate change and air pollution on respiratory health has led to increasing cases of asthma, allergic rhinitis and other chronic non-communicable immunemediated upper and lower airway diseases. Natural bioaerosols, such as pollen and fungi, are essential atmospheric components undergoing significant structural and functional changes due to industrial pollution and atmospheric warming. Pollutants like particulate matter(PMx), polycyclic aromatic hydrocarbons(PAHs), nitrogen dioxide(NO_2), sulfur dioxide(SO_2) and carbon monoxide(CO) modify the surface and biological properties of atmospheric bioaerosols such as pollen and fungi, enhancing their allergenic potentials. As a result, sensitized individuals face heightened risks of asthma exacerbation, and these alterations likely contribute to the rise in frequency and severity of allergic diseases. NAMs, such as precision-cut lung slices(PCLS), air–liquid interface(ALI) cultures and lung-on-a-chip models, along with the integration of data from these innovative models with computational models, provide better insights into how environmental factors influence asthma and allergic diseases compared to traditional models. These systems simulate the interaction between pollutants and the respiratory system with higher precision, helping to better understand the health implications of bioaerosol exposure. Additionally, NAMs improve preclinical study outcomes by offering higher throughput, reduced costs and greater reproducibility, enhancing the translation of data into clinical applications. This review critically evaluates the potential of NAMs in researching airway diseases, with a focus on allergy and asthma. It highlights their advantages in studying the increasingly complex structures of bioaerosols under conditions of environmental pollution and climate change, while also addressing the existing gaps, challenges and limitations of these models.

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Surface Modification of PDMS and PET Membranes for Improved Integration and Biocompatibility in Organ-on-a-Chip Systems

Introduction

Polymeric membranes, such as polyethylene terephthalate (PET), are widely used in Organ-on-a-Chip (OoC) systems due to their mechanical strength, porosity, and compatibility with microscopic analysis.[1] However, effective integration of these membranes into PDMS-based devices remains a technical challenge, as native PET does not readily bond with PDMS.[2] Surface modification is therefore necessary to ensure mechanical stability and functional integrity of the system during long-term culture and under flow conditions.

Methods

A two-layer poly(dimethylsiloxane) (PDMS) OoC system was developed, consisting of a top channel the culture of ovarian cancer cells and a bottom channel for the culture of non-malignant fibroblasts, separated by a thin porous PET membrane. To enhance bonding, PET membranes were surface-modified using two organosilanes: (3-Aminopropyl)triethoxysilane (APTES) and (3-Glycidyloxypropyl)trimethoxysilane (GPTMS) at concentrations of 1% and 5% v/v. As an alternative approach, due to the potential cytotoxicity of silanes, a polydopamine coating was tested.. Surface modification was confirmed by water contact angle measurements and Fourier Transform Infrared Spectroscopy (FTIR). Bonding strength was evaluated by manual peeling and under continuous flow. Cytotoxicity was assessed using cell viability and morphology on modified membranes.

Results

Bonding efficiency varied with the type and concentration of silane. Microsystems modified with 5% silane exhibited stronger bonding, often tearing the PDMS during peeling, while 1% silane-bonded devices failed more predictably along PDMS–PET interfaces. Under flow, silane-modified devices failed at rates >5 µL/min, whereas polydopamine-modified systems remained sealed at up to 30 µL/min. Contact angle measurements confirmed successful surface modification: unmodified PDMS and PET showed high hydrophobicity (110° and 65°, respectively), while oxygen plasma treatment greatly increased hydrophilicity (30° and 25°). Subsequent silanization partially restored hydrophobicity (APTES ~45°, GPTMS ~35°), whereas polydopamine maintained low contact angles (~27°), indicating sustained hydrophilicity. In cytocompatibility assays, MTT tests performed on extracts from the modified membranes revealed no significant cytotoxicity. However, direct cell culture on silane-modified membranes showed limited cell adhesion and abnormal morphology, suggesting surface-related effects despite the absence of toxic leachables. In contrast, polydopamine-coated membranes supported

abundant cells with normal morphology, indicating superior biocompatibility and more favorable conditions for cell growth.

Discussion

Although the literature supports the use of APTES and GPTMS for polymer surface modification and PDMS bonding [3], our results suggest that the outcomes is not always consistent and may lead to cytotoxic effects, possibly due to residual unreacted silane or non-uniform coating. Polydopamine offered a more reproducible and biocompatible alternative, ensuring strong bonding and healthy cell growth under flow conditions.

Acknowledgemets

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Microfluidic-Assisted Biofabrication of Tunable Hydrogel Vascular Fibers with Dual Crosslinking for Tissue Engineering Applications

Efficient vascularization is critical for ensuring adequate nutrient and oxygen transport as well as metabolic waste removal in engineered tissues. Conventional strategies often depend on angiogenesis from existing vasculature, inherently limiting construct size and functional complexity. Nonetheless, the generation of structurally stable and perfusable constructs remains a significant hurdle in tissue engineering. Among fabrication strategies, microfluidic-based techniques provide precise modulation over fiber geometry, dimensions, and surface chemistry.In this study, we employed a coaxial microfluidic-assisted wet spinning approach to fabricate compliant, vessel-like hydrogel fibers composed of alginate and tyramine-functionalized gelatin (Gela-Ph). This system harnessed the biocompatibility and bioactivity of gelatin alongside the rheological advantages of both core and shell alginate/Gela-Ph solutions to produce continuous fibers in a calcium chloride coagulation bath. Post-spinning, the fibers were further stabilized via a visible-light-triggered crosslinking process, wherein phenolic groups on Gela-Ph were covalently linked using riboflavin as the photoinitiator and persulfate as the electron donor.The resulting hydrogel vessels exhibited uniform morphology and remained free of polymer aggregation or channel occlusion under optimized flow and reagent conditions. By modulating inner and outer flow rates, vessel diameter and membrane thickness were finely tunable. Spectroscopic analyses (FTIR and NMR) verified the successful conjugation of tyramine to gelatin and its subsequent covalent crosslinking. The photo-crosslinking strategy conferred enhanced mechanical resilience, elasticity, and sustained cell viability within the fibers for up to 24 days. Perfusion assays demonstrated flow rates from 0.1 to 20 mL/min, replicating physiological conditions observed in native venous microvasculature.The integration of Gela-Ph with visible-light-mediated crosslinking within alginate-based hydrogel fibers presents a promising platform for vascular tissue engineering. The system offers mechanical flexibility, biocompatibility, and functional flow capacity, though further refinement is necessary to advance toward clinical applicability and therapeutic use.

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Exploring Two-Photon Lithography for Microfabrication in Biological and Microfluidic Applications

Two-photon lithography (TPL) is a high-resolution technique capable of fabricating complex three-dimensional microstructures with sub-micrometer precision. Unlike conventional lithography methods, TPL allows freeform fabrication under ambient conditions, making it especially useful in biological applications where microenvironmental control is essential[1]. Its ability to produce structures with variable porosity, multilayered architecture, and internal heterogeneity makes it ideal for use in tissue engineering and microfluidic systems[2].

In our work, we used custom-developed photoresists optimized for two-photon polymerization to fabricate microstructured components for biological applications. Scaffold architectures were designed with controlled porosity and spatial dimensions suitable for integration into microfluidic devices. Structures were written using a two-photon lithography system under ambient conditions, and fabrication parameters were adjusted to match the mechanical and geometrical requirements of each application. To expand functionality, TPL was combined with complementary techniques—such as soft lithography for chip fabrication and replica molding for layer integration—allowing us to align scaffolds and functional microstructures directly within microfluidic layouts. This modular approach enabled the production of complete microsystems incorporating filters, barriers, and culture supports.

We present our approach to fabricating well-defined porous scaffolds designed for integration into microfluidic devices, intended to serve as a framework for future biological studies within organ-on-chip platforms. Furthermore, we demonstrate the fabrication of functional microelements, including membranes with defined architecture and precise alignment within microfluidic chips, enabling compartmentalization or filtration. We also show the feasibility of fabricating microneedles with sharp tips and controlled dimensions, which hold potential for pharmaceutical research.

Our findings confirm that TPL is well-suited for building multifunctional microsystems for biological research. Beyond scaffolds, the method offers a pathway to fabricate a wide range of internal chip components, such as biosensors and microneedles, that expand the utility of microfluidic systems. The ability to integrate multiple features with sub-micrometer accuracy supports more faithful modeling of tissue microenvironments and paves the way for next-generation organ-on-chip devices. Future work will focus on biological validation and further exploration of material biocompatibility and mechanical properties.

References

[1] S. O’Halloran, A. Pandit, A. Heise, A. Kellett, Advanced Science 2023, 10, 2204072.
[2] C. Maibohm, O. F. Silvestre, J. Borme, M. Sinou, K. Heggarty, J. B. Nieder, Sci Rep 2020, 10, 8740.

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Konuşmacılar: Mario Kandra (IQSnano)

- 15:00

Coffee break & Poster session II

- Closing Session and Awards Ratuszowa