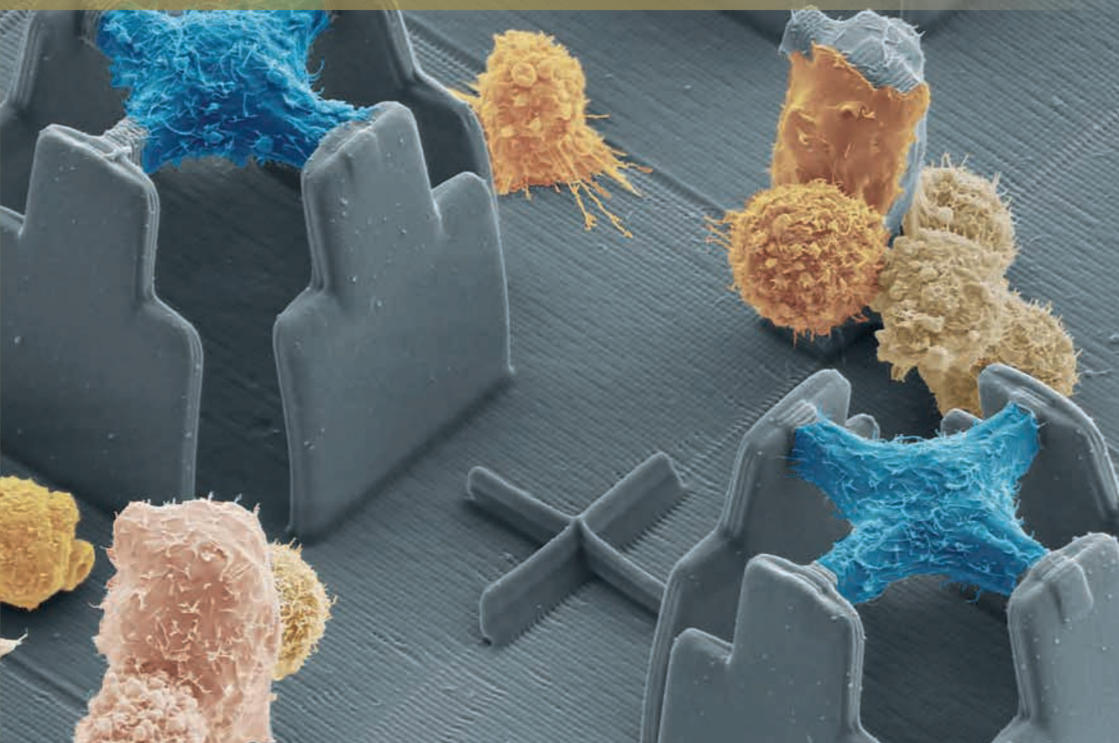
Future 3D Additive Manufacturing | The 3DMM2O Conference

3D Cellular Systems: Synthetic Environments, Mechanobiology and Organoids



Abstract Booklet

April 7 – 11, 2024

Schöntal Monastery, Germany

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Welcome

Dear Colleagues and Friends,

It is our great pleasure to welcome you to

The Future 3D Additive Manufacturing – The 3DMM2O Conference 2024:
3D Cellular Systems: Synthetic Environments, Mechanobiology and Organoids.

The annual conference on topics surrounding 3D Additive Manufacturing is organized by the Cluster of Excellence "3D Matter Made to Order" (3DMM2O), a joint Research Cluster of Karlsruhe Institute of Technology (KIT) and Heidelberg University (Uni HD).

The conference shall be a platform for new approaches in the field of 3D Additive Manufacturing. It aims at bridging the gap between advanced biomaterials design and its application in biological research.

Lectures by international speakers, poster sessions and social networking events will give you an insight in alternating aspects of 3D Additive Manufacturing as well as the opportunity for inspiring and fruitful scientific discussions and exchange.

Martin Bastmeyer
Karlsruhe Institute of Technology

Kerstin Göpfrich
Heidelberg University

Ulrich Schwarz
Heidelberg University

Program

Monday, April 8

Time	Session	Speaker
	Breakfast	
9:00 AM–9:05 AM	Opening & Welcome	
9:05 AM–9:45 AM	Extracellular Matrix Mechanobiology	**Jennifer Young**
9:45 AM–10:25 AM	Fibrous Matrix Mechanics: Orchestrating Cellular Interactions	**Ayelet Lesman**
10:25 AM–10:40 AM	Coffee Break	
10:40 AM–10:55 AM	**Contributed Talk:** Force Transmission is a Master Regulator of Mechanical Cell Competition	**Andreas Schoenit**
10:55 AM–11:10 AM	**Contributed Talk:** Local Matrix Stiffening to Model Lung Fibrotic Remodeling *Ex Vivo*	**Claudia Loebel**
11:10 AM–11:25 AM	**Industry Talk:** NANOSCRIBE: Revolutionizing Biomedical Fabrication: Two-Photon Polymerization for Multimaterial 3D Printing	**Benjamin Richter**
11:25 AM–12:05 PM	Granular Material Approaches for Presenting Tunable Physiological 3D Confinement to Cells	**Andrew Holle**
12:05 PM–12:45 PM	The Mechanobiology of Extracellular Matrix: Thinking Beyond the Youngs Modulus	**Viola Vogel**
12:45 PM–2:00 PM	Lunch	
2:00 PM–2:40 PM	Communication in Synthetic Tissues	**Hagan Bayley**
2:40 PM–3:00 PM	Coffee Break	
3:00 PM–3:40 PM	A High-Throughput and Miniaturized Approach to Creating, Assembling, and Manipulating Cell Spheroids	**Pavel Levkin**
3:40 PM–4:20 PM	Engineering Tissues: Build, Learn, Repeat	**Christopher Chen**
4:20 PM–4:35 PM	Coffee Break	
4:35 PM–5:07 PM	Flashtalks	
5:07 PM–7:00 PM	Poster Session I & Drinks	
7:00 PM	Dinner	

Sunday, April 7

4:00 PM	Check-In
6:00 PM–8:00 PM	Dinner at Individual Time
8:00 PM	Informal Get-Together

Tuesday, April 9

	Breakfast	
9:00 AM	Welcome	
9:00 AM–9:40 AM	Homeostasis of Gut Epithelium Under Mechanical Stress	**Danijela Vignjevic**
9:40 AM–10:20 AM	Mechano-Chemical Bistability of Intestinal Organoids	**Edouard Hannezo**
10:20 AM–10:35 AM	Coffee Break	
10:35 AM–11:15 AM	Cancer Organoid Models for Drug Discovery	**Michael Boutros**
11:15 AM–11:30 AM	**Contributed Talk:** 3D Multiphoton-Lithography Proteinous Scaffolds Moduli for Stem Cell Studies	**Jaroslaw Jacak**
11:30 AM–11:45 AM	**Contributed Talk:** DNA Microbeads for Spatio-Temporally Controlled Morphogen Release Within Organoids	**Tobias Walther**
11:45 AM–12:00 PM	**Industry Talk:** HETEROMERGE: Multi-Material 2-Photon 3D Printing Using an Open-Fluidic *In situ* material exchange	**Robert Kirchner**
12:00 PM–12:10 PM	Group Picture	
12:10 PM–2:00 PM	Lunch	
2:00 PM–2:40 PM	Mesoscale Epithelial Mechanobiology and Cellular Interfaces	**Jacopo di Russo**
2:40 PM–3:20 PM	Bioinspired Strategies and Robotics to Build Tissue with Cell Aggregates	**Daniela Duarte Campos**
3:20 PM–3:35 PM	Coffee Break	
3:35 PM–4:15 PM	3D Biomaterials Processing to Guide Cell Migration and Organization	**Jason Burdick**
4:15 PM–4:55 PM	Injectable Synthetic Molecular and Colloidal Building Blocks to Overcome Challenges in Tissue Engineering	**Laura De Laporte**
4:55 PM–5:05 PM	Coffee Break	
5:05 PM–5:23 PM	Flashtalks	
5:23 PM–7:00 PM	Poster Session II & Drinks	
7:00 PM	Dinner	

Program

Wednesday, April 10

	Breakfast	
9:00 AM	Welcome	
9:00 AM–9:40 AM	Mechanics of Cell Migration *in vivo*	**Roberto Mayor**
9:40 AM–10:20 AM	Anisotropic Flows Can Organize Multi-Cellular Systems	**Matthias Merkel**
10:20 AM–10:35 AM	Coffee Break	
10:35 AM–11:15 AM	Activity Matters in Organoid Systems	**Andreas Bausch**
11:15 AM–11:55 AM	Mechanical Generation of Bicephalous and Toroidal *Hydra* Defines Actin Topological Defects as Force Organizers During Head Regeneration	**Aurélien Roux**
12:00 PM–1:30 PM	Lunch	
1:45 PM–2:00 PM	Meet for Social Program	
2:00 PM–6:00 PM	Social Program	
7:00 PM	Conference Dinner & Poster Award	

Thursday, April 11

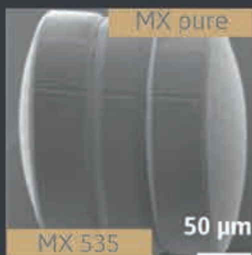
Program

Time	Session	Speaker
	Check-Out	
	Breakfast	
9:00 AM	Welcome	
9:00 AM–9:40 AM	Multiphoton Lithography – Opportunities for Precise Engineering 3D Cell Environment	**Aleksandr Ovsianikov**
9:40 AM–10:20 AM	Material-Based Approaches to Control Cancer Cell Spheroids Dynamics and Invasion	**Ada Cavalcanti-Adam**
10:20 AM–10:35 AM	**Contributed Talk:** Adhesion Mechanics in Breast Cancer Spheroid Spreading	**Grégoire Lemahieu**
10:35 AM–10:50 AM	Coffee Break	
10:50 AM–11:05 AM	**Contributed Talk:** Three-Dimensional Force Inference in Budding Intestinal Organoids	**Oliver Max Drozdowski**
11:05 AM–11:20 AM	**Contributed Talk:** Cell-Instructive Materials: Versatile Biohybrids and their Guided Assembly to Functional Hydrogels	**Jasmina Gačanin**
11:20 AM–11:35 AM	**Contributed Talk:** Combining Two-Photon Polymerization Direct Laser Writing with Soft Lithography for Investigating *Plasmodium* Sporozoite Migration on Triangular Patterns	**Malin Schmidt**
11:35 AM–12:15 PM	Spatiotemporal Order and Dynamics of Tissues and Organoids	**Motomu Tanaka**
12:15 PM–12:55 PM	Printing Cells with Sound	**Peer Fischer**
12:55 PM–1:00 PM	Farewell	
1:00 PM–1:30 PM	Lunch	
	Departure	

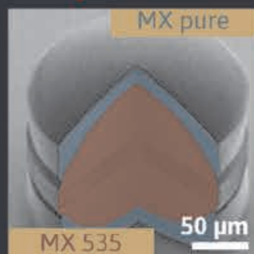
HETEROMERGE

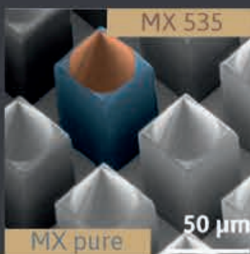
FUNCTIONAL 3D MICRO-PRINTING

PRINTHEAD
FOR MULTI-MATERIAL
TWO-PHOTON PRINTING

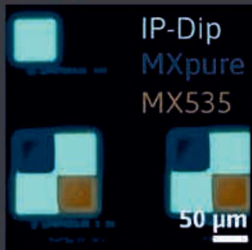


OPTICS





LIFE SCIENCE



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Lighting up high resolution, high throughput 3D bioprinting

Industry talk

“Revolutionizing Biomedical Fabrication: Two-Photon Polymerization for Multimaterial 3D Printing“

Dr. Benjamin Richter, Nanoscribe
8 April 2024 · 11:10 AM

Microoptics | Material engineering | Life sciences | Micromechanics & MEMS | Microfluidics

Wednesday, April 10
10:35 AM–11:15 AM

Andreas Bausch

Technical University of Munich, Germany

ACTIVITY MATTERS IN ORGANOID SYSTEMS

Living matter relies on the self organization of its components into higher order structures, on the molecular as well as on the cellular, organ or even organism scale. Collective motion due to active transport processes has been shown to be a promising route for attributing fascinating order formation processes on these different length scales.

Here I will present recent results on structure formation in organoid systems, demonstrating how mechanical feedback between extracellular matrix, proliferation and cell migration drives structure formation process in these multicellular model systems. I will present results on the developmental phase of mammary gland and pancreatic ducal adenocarcinoma organoids.

Abstracts

Monday, April 8
2:00 PM–2:40 PM

Hagan Bayley

University of Oxford, United Kingdom

COMMUNICATION IN SYNTHETIC TISSUES

By 3D printing, we have assembled synthetic tissues comprising patterned networks of thousands of aqueous droplets joined by lipid bilayers. A related printing technology has been used to pattern a variety of living cells, providing structures that include small tumours and fragments of neural tissue. The mm-scale printed structures can be used as building blocks for cm-scale structures ranging from synthetic axons to hybrid constructs containing both synthetic and living cells.

An important goal is to be able to communicate with these constructs through external stimuli, have them process the incoming signals and accordingly produce outputs that are useful, for example, in medicine. Progress on these aspects of signaling will be described in my talk.

Tuesday, April 9
10:35 AM–11:15 AM

Michael Boutros

Heidelberg University, Germany

CANCER ORGANOID MODELS FOR DRUG DISCOVERY

Patient-derived organoids are stem cell-derived 3D tumor models that can be efficiently established from cancer and normal tissues. The isolation of organoids from human primary tumors and metastases has enabled the establishment of living biobanks. We have performed image-based profiling using a high-throughput microscopy-based methodology to systematically measure phenotypes of *in vitro* models.

When combined with chemical or genetic perturbations, image-based profiling can be a powerful approach to gain systematic insight into biological processes. However, performing large-scale image-based profiling experiments on organoids has been a biological, technical and computational challenge. As a result, the morphological heterogeneity of patient-derived cancer organoids between and within patient donors, their divergent behavior upon pharmacological perturbation, and the underlying mechanisms of cancer organoid morphology are not yet systematically understood.

Here, we established a platform for large-scale image-based phenotyping of patient-derived cancer organoids to understand the underlying factors governing organoid morphology. Colorectal cancer organoids were treated with approximately 500 experimental and clinically used small molecules. We systematically mapped the morphological heterogeneity of organoids and their response to drug perturbations from more than 3,700,000 confocal microscopy images.

We found that the resulting landscape of organoid phenotypes was driven by differences e.g. in organoid size, viability, and cystic vs. solid organoid architecture. We show that induced changes in morphology can be used to cluster small molecules by mechanism of action and infer the biological programs they affect.

Tuesday, April 9
3:35 PM–4:15 PM

Jason Burdick

University of Colorado, USA

3D BIOMATERIALS PROCESSING TO GUIDE CELL MIGRATION AND ORGANIZATION

Cellular aggregates or spheroids are useful to understand basic biological processes or to act as building blocks in the engineering of tissues. Biomaterials are being used to advance spheroids in these applications, such as to guide their fusion or to control their organization. Our laboratory is particularly interested in understanding how engineered hydrogels can be used to support spheroids for use in regenerative medicine or as *in vitro* models of tissues.

In this presentation, I will give several examples of hydrogel technology to guide spheroid behavior. As a first example, we are using shear-yielding suspension baths to support the printing and fusion of spheroids into 3-dimensional tissues, where the bath properties guide print resolution and the stability of the printed structures. Next, hydrogel microparticles are being mixed with spheroids to act as living and non-living granular composites that are shear-thinning for injectability and where inter-particle microgel crosslinking stabilizes the granular structure for development into cartilage tissues. Lastly, by introducing degradation to the microgels, controlled contractility of the composites is possible, including with patterned structures where local contractility guides macroscopic shape evolution.

Thursday, April 11
9:40 AM–10:20 AM

Ada Cavalcanti-Adam

University of Bayreuth, Germany

MATERIAL-BASED APPROACHES TO CONTROL CANCER CELL SPHEROIDS DYNAMICS AND INVASION

Cancer progression is a complex process involving a series of intricate cellular interactions and migrations. Tumor cell spheroids, three-dimensional aggregates of cancer cells, closely mimic the *in vivo* microenvironment and offer invaluable insights into the mechanisms of cancer invasion. In this talk, I will elucidate how different materials can steer the invasion patterns and the solid-to-fluid transitions of cancer cells. By utilizing state-of-the-art bioengineering techniques, we have created innovative *in vitro* models that accurately emulate the tumor microenvironment's physical and biochemical cues.

Our findings demonstrate that specific material properties, such as stiffness, topography, and chemical composition, significantly impact the migratory behavior of cancer cell spheroids. Through advanced live-cell imaging and traction force microscopy, we decipher the intricate interplay between cancer cells and the surrounding material. By employing biofunctionalized materials and targeted drugs, we influence cancer cell behavior, potentially halting their invasive patterns and interfering with their collective responses to the microenvironment.

Christopher Chen

Boston University, USA

ENGINEERING TISSUES: BUILD, LEARN, REPEAT

Mammalian tissues operate as highly integrated systems that link physical structure and biological function. We are attempting to rebuild tissue-like structures *in vitro* as a platform to better understand human physiology and disease, and as a stepping stone toward engineering tissues as therapeutics.

Here, I will describe our recent work in engineering cardiac and vascular structures to understand the complex interplay between forces, signaling, and cellular adhesions in regulating tissue formation, function, and dysfunction.

The presentation will highlight the importance of advancing numerous fabrication approaches to improve our ability to build tissues with ever increasing fidelity and function, and ultimately, how a deeper understanding of the interaction between tissue architectures and biological functionality will provide avenues to build better tools.

Tuesday, April 9
4:15 PM–4:55 PM

Laura De Laporte

RWTH Aachen University, Germany

INJECTABLE SYNTHETIC MOLECULAR AND COLLOIDAL BUILDING BLOCKS TO OVERCOME CHALLENGES IN TISSUE ENGINEERING

We apply polymeric molecular and nano- to micron-scale building blocks to assemble into soft 3D biomaterials with anisotropic and dynamic properties. Microgels and fibers are produced by technologies based on fiber spinning, microfluidics, and in-mold polymerization. To arrange the building blocks in a spatially controlled manner, self-assembly mechanisms and alignment by external magnetic fields are employed. Reactive rod-shaped microgels interlink and form macroporous constructs supporting 3D cell growth or cells are able to use microgels as bricks to build their own house.

On the other hand, the Anisogel technology offers a solution to regenerate sensitive tissues with an oriented architecture, which requires a low invasive therapy. It can be injected as a liquid and structured *in situ* in a controlled manner with defined biochemical, mechanical, and structural parameters. Magnetoceptive, anisometric microgels or short fibers are incorporated to create a unidirectional structure. Cells and nerves grow in a linear manner and the fibronectin produced by fibroblasts is aligned. Regenerated nerves are functional with spontaneous activity and electrical signals propagating along the anisotropy axis of the material. Another developed platform is a thermoresponsive hydrogel system, encapsulated with plasmonic gold-nanorods, which actuates by oscillating light.

This system elucidates how rapid hydrogel beating affects cell migration, focal adhesions, native production of extracellular matrix, and nuclear translocation of mechanosensitive proteins, depending on the amplitude and frequency of actuation. The time spent in the *in vitro* gym seems to affect myoblast differentiation and fibrosis, while actuation seems to induce mesenchymal stem cell differentiation into bone cells.

Jacopo di Russo

RWTH Aachen University, Germany

MESOSCALE EPITHELIAL MECHANOBIOLOGY AND CELLULAR INTERFACES

Mechanical properties regulate tissue functions at a multicellular length scale or mesoscale. These properties depend on the interaction of cells with their interfaces, hence on the balance between intercellular tension and the extracellular matrix (ECM) adhesion forces.

My group aims to dissect the role of cell-ECM and cell-cell communication in epithelial mechanobiology, starting from the medically relevant retinal epithelium. In contrast to the experimental investigation of traditional biological sciences, my laboratory uses cross-disciplinary approaches combining synthetic hydrogels with stem cell-based models. We particularly develop and adapt biohybrid systems where cells interact with hydrogels that are designed to control cell-cell or cell-ECM adhesion. Synthetic material allows the unique reduction of the degree of freedom in the cellular/tissue system, thus helping us to reveal phenotypical tissue plasticity and molecular function.

My talk will first give an overview of published work on understanding how ECM physical (elasticity) and biochemical cues (receptor density) impact epithelial system properties, namely stress heterogeneity and intercellular force coordination. I will show that these properties are not only *in vitro* observations but play pivotal roles in controlling our vision. A density gradient of ECM characterises the contractility of the retinal epithelium *in vivo* and modulates its efficiency in supporting photoreceptor cells' homeostasis.

Furthermore, I will show data from the ongoing work which addresses different aspects of the mechanobiology of tissue ageing. We optimised a phototunable hydrogel as substrates for epithelia to model ECM local remodelling on demand. Moreover, we developed microgels used as phototunable phantom cells to simulate age-related tissue mechanical anisotropy. Altogether, we can dissect the relationship between tissue mechanics and function by controlling the temporal and spatial properties of cellular interfaces.

Tuesday, April 9
2:40 PM–3:20 PM

Daniela Duarte Campos

Heidelberg University, Germany

BIOINSPIRED STRATEGIES AND ROBOTICS TO BUILD TISSUE WITH CELL AGGREGATES

Bioprinting is an exciting technology that holds promise to fabricate tissues and organs both *in vitro* and *in vivo*. Besides engineering lab-grown *in vitro* models, current advances are focussed in bringing bioprinting technologies from the bench to the operating room. Scientists around the world are continuously improving the complexity and precision of bioprinting methods, which still in this decade will not only allow us to engineer new tissues and even organs, but also to change the way transplantation medicine is done.

As with many new fields, this technology comes with challenges, such as controlling cell survival and function for being exposed to shear stress during bioprinting. This is one major hurdle that needs to be addressed by scientists in order to allow this young research field to achieve its first significant impact in medicine.

In this talk, I will show bioinspired strategies and robotics that our lab is developing to minimize or flank the effect of shear stress for the purpose of building tissue using cells and cell aggregates.

Peer Fischer

Heidelberg University, Germany

PRINTING CELLS WITH SOUND

Is it possible to realize an assembler that can assemble biological cells into a defined shape of a 3D object, including cell aggregates and possibly organoids? We have recently developed tools that permit ultrasound fields to be shaped, such that they can be used to form defined pressure distributions in space with which it is possible to assemble cells from a suspension.

In order to realize a 3D assembler, one needs to solve a formidable inverse problem: how to encode an entire 3D object in the form of sound waves that in turn are used to recreate the object in space. This is a complex optimization problem, because of the astronomically large number of computational degrees of freedom. Nevertheless, we have found practical solutions and we have managed to use the 3D pressure distributions to assemble cells in 'one shot'.

An acoustic holographic bioassembly platform will be presented and it is discussed how it can aid biofabrication and tissue engineering. We have also worked on a new type of ultrasound contrast agent – the antibubble – which is a microdoplet of fluid surrounded by air and whose release can be triggered by ultrasound.

Antibubbles are remarkably stable and, importantly, their cargo can be released with low intensity ultrasound fields. They offer significant advantages compared with conventional ultrasound contrast agents and offer new ways to interact with cells and tissues.

Tuesday, April 9
9:40 AM–10:20 AM

Edouard Hannezo

Institute of Science and Technology, Austria

MECHANO-CHEMICAL BISTABILITY OF INTESTINAL ORGANOIDS

How pattern and form are generated in a reproducible manner during embryogenesis remains poorly understood. Intestinal organoid morphogenesis involves a number of mechanochemical regulators, including cell-type specific cytoskeletal forces and osmotically-driven lumen volume changes. However, whether and how these forces are coordinated in time and space, via feedbacks, to ensure robust morphogenesis remains unclear.

Here, we propose a minimal physical model of organoid morphogenesis with local cellular mechano-sensation, where lumen volume changes can impact epithelial shape, via both direct mechanical (passive) and indirect mechano-sensitive (active) mechanisms. We show how mechano-sensitive feedbacks on cytoskeletal tension generically give rise to morphological bistability, where both bulged (open) and budded (closed) crypt states are possible and dependent on the history of volume changes.

We experimentally test key modelling assumptions via biophysical and pharmacological experiments, allowing us to quantitatively demonstrate how bistability can explain several paradoxical experimental observations, such as the importance of the timing of lumen shrinkage and robustness of the final morphogenetic state to mechanical perturbations. This suggests that bistability, arising from feedbacks between cellular tensions and fluid pressure, could be a general mechanism to coordinate multicellular shape changes in developing systems

Andrew Holle

National University of Singapore, Singapore

GRANULAR MATERIAL APPROACHES FOR PRESENTING TUNABLE PHYSIOLOGICAL 3D CONFINEMENT TO CELLS

Self-induced cellular confinement has been shown to play a role in a wide variety of biological processes, including cancer invasion and metastasis, immune cell navigation, and mechanosensitive gene expression and localization. Currently, the standard material-based approaches to presenting physiological confinement to cells, such as sandwich assays and microchannel chips, are frustratingly low-throughput and are not compatible with many emerging molecular biology approaches to elucidating cellular responses to their material microenvironment.

To address this, we have developed a novel approach to presenting confinement to millions of cells in three dimensions that is also suitable for downstream molecular biology experimentation and future cellular engineering-based clinical applications. Inspired by a growing body of annealed granular hydrogel systems, we first constructed a granular system composed of glass microspheres with defined diameters. Due to gravity-induced spherical packing of glass microspheres, we can approximate the void space present in the system, as well as the confinement that cells are exposed to as they migrate within the material.

As our granular system is not annealed, we can harvest cells growing within the material after long-term cell culture via trypsinization and gentle centrifugation. We found that cells grown in this granular material exhibit mechanical memory, as after harvesting their cell and nuclear morphology changes as a function of the dimensionality of the system. qPCR analysis of cells grown in granular materials with different diameter microspheres also reveals a modulation of gene expression as a function of void space and confinement, an experimental approach that was unavailable for conventional 3D confinement assays.

Using this system, we have also found adult stem cells will preferentially differentiate towards an osteogenic lineage when presented with tighter physiological confining spaces. Future work from a materials perspective will focus on controlling the mechanical properties of the individual microspheres, applying systemic strain to the granular system, and building multi-component systems to encourage more complex cellular behaviour.

Monday, April 8
9:45 AM–10:25 AM

Ayelet Lesman

Tel Aviv University, Israel

FIBROUS MATRIX MECHANICS: ORCHESTRATING CELLULAR INTERACTION

The complex fibrous architecture of the extracellular matrix (ECM) facilitates the transmission of mechanical cues over long distances, allowing cells to mechanically interact with one another, even without direct physical contact. This intercellular interaction through the ECM can give rise to intriguing collaborative and collective cellular behaviors. I will present our ongoing efforts aimed at unraveling the role of fibrous networks in mediating long-range cellular interactions.

In our recent biological experiments, we embed cells in predetermined patterns, featuring controlled intercellular distances within 3D fibrous gels composed of collagen or fibrin. This approach enables both visual and quantitative assessments of the mechanical modifications within the ECM, shedding light on the relation between cell-induced mechanical remodeling and establishment of tissue-scale patterns and organizational states. To study the mechanical signals that guide cell behavior, we use optical tweezers microrheology to quantify the microscale mechanical properties of anisotropic and deformed fiber networks, reminiscent of the deformed ECM bands formed between contractile cells.

Drawing inspiration from the dynamic interplay between cells and their microenvironment, we engineer systems to guide and direct cell behaviour for potential applications in tissue engineering.

Pavel Levkin

Karlsruhe Institute of Technology (KIT), Germany

A HIGH-THROUGHPUT AND MINIATURIZED APPROACH TO CREATING, ASSEMBLING, AND MANIPULATING CELL SPHEROIDS

The fabrication of intricate 3D cell architectures is important in various fields including drug screening, tissue engineering, and 3D bioprinting. Despite its significance, achieving high-throughput fabrication of cell spheroids as well as the fabrication and manipulation of complex 3D cell architectures pose considerable challenges.

In this presentation, I will present a method for fabricating complex multi-spheroid architectures in a high-throughput manner, using the droplet microarray platform. Our approach enables the formation of thousands of cell spheroids on a droplet microarray, which are subsequently assembled into multi-spheroid assembloids in an automated, controlled and high-throughput manner. This technique facilitates the formation of double, triple, and multi spheroids, each yielding distinct 3D architectures based on the cell type employed. Furthermore, we introduce a novel method for parallel high-throughput transfer of spheroids from one slide to another.

In addition, a drug screening utilizing glioma cell spheroids formed on the chip and resulted in the identification of several promising drug candidates for the treatment of IDH1 mutant gliomas, will be presented. Finally, the utilization of cell spheroids as bioink additives, enabling their entrapment in 3D hydrogel structures formed using a novel parallel high-throughput 3D printing method will be shown.

The future applications of complex cellular assembloids are vast and include personalized medicine, disease modeling, tissue engineering and regenerative medicine.

1. Assembly of Multi-Spheroid Cellular Architectures by Programmable Droplet Merging. Cui et al. Adv. Mater. 2020. https://doi.org/10.1002/adma.202006434
2. Repurposing FDA-Approved Drugs for Temozolomide-Resistant IDH1 Mutant Glioma Using High-Throughput Miniaturized Screening on Droplet Microarray Chip. Cui et al. Adv. Healthcare Mater. 2023. https://doi.org/10.1002/adhm.202300591
3. High-throughput formation of miniaturized cocultures of 2D cell monolayers and 3D cell spheroids using droplet microarray. Cui et al. Droplet, 2022. https://doi.org/10.1002/dro2.39
4. Simple assessment of viability in 2D and 3D cell microarrays using single step digital imaging. Popova et al. 2022. https://doi.org/10.1016/j.slast.2021.10.017

Wednesday, April 10
9:00 AM–9:40 AM

Roberto Mayor

University College London, United Kingdom

MECHANICS OF CELL MIGRATION *IN VIVO*

Although biomechanics is an active area of research, most of its studies are performed *in vitro*, ignoring the complexity of the 3D environment that cells encounter *in vivo*. We address this issue by analysing cell migration during embryo development, as it represents an excellent model for a complex 3D environment and, at the same time, is amenable to experimentation. We are interested in the mechanisms that control the initiation and directional migration of neural crest cells, a highly migratory and multipotent embryonic cell population whose behaviour has been likened to malignant invasion.

Our observations show that neural crest migration is triggered by an increase in the mechanical stiffness of the surrounding tissue. Once neural crest cells are migrating, they sense a combination of chemical (chemotaxis) and mechanical (durotaxis) cues that control their directionality. We conclude that neural crest cells are able to integrate chemical and mechanical cues to navigate in the complex 3D environment of the embryo.

Matthias Merkel

Université Aix-Marseille, France

ANISOTROPIC FLOWS CAN ORGANIZE MULTI-CELLULAR SYSTEMS

Most animals display one or more body axes (e.g. head-to-tail). In our work, we demonstrate that their formation can be promoted by large-scale tissue flows. We studied aggregates of mouse stem cells, called gastruloids, which are initially spherically symmetric, but later form an axis defined by the polarized expression of specific proteins. We show that advection of cells with tissue flows contribute substantially to the overall polarization, and that these flows are driven by interface and surface tension differences.

We further discuss flows in the context of anisotropic tissue deformation. From a physics perspective, anisotropically deforming tissues can be described as oriented active materials. However, such materials inherently exhibit instabilities, raising the question of how anisotropic tissue deformation during development can be robust.

We show that the presence of a signaling gradient can stabilize the process, but only if it acts to actively extend the tissue along the gradient direction. Conversely, tissues are unstable if they tend to actively contract along the gradient direction. Intriguingly, developing tissues seem to exclusively use the gradient-extensile and not the unstable gradient-contractile coupling.

Our work thus points to a principle of multi-cellular morphogenesis that is directly rooted in active matter physics, and which we expect to also affect multi-cellular engineering.

Thursday, April 11
9:00 AM–9:40 AM

Aleksandr Ovsianikov

Technical University Wien, Austria

MULTIPHOTON LITHOGRAPHY – OPPORTUNITIES FOR PRECISE ENGINEERING 3D CELL ENVIRONMENT

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 micrometres. 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, multiphoton lithography (MPL) is an outstanding one as it can produce features even smaller than a single mammalian cell.

Our recent breakthroughs on the material development side enabled the use of MPL for direct fabrication of cell-containing constructs, placing this technology in the domain of high-definition (HD) bioprinting. In addition, we have recently demonstrated that MPL enabled realization of highly porous biodegradable microscaffolds capable of hosting individual cell spheroids. The resulting tissue units can be used for bottom-up self-assembly of larger tissue constructs with very high initial cell density, paving a way for novel tissue engineering approach.

In this contribution, the recent progress of MPL for biomedical applications, as well as its advantages and limitations will be discussed.

Aurélien Roux

Université de Genève, Switzerland

MECHANICAL GENERATION OF BICEPHALOUS AND TOROIDAL *HYDRA* DEFINES ACTIN TOPOLOGICAL DEFECTS AS FORCE ORGANIZERS DURING HEAD REGENERATION

Liquid crystals are characterized by unique flow properties, dictated by the long-range order of molecules with anisotropic shapes that locally align at high density. Cellular tissues are viscoelastic materials, that have unique flow properties, best exemplified during morphogenesis: oriented flows participate in the formation of shapes and organs. Tissues are composed of cells, and cell shape and forces are dictated by contractile filaments of the cytoskeleton. Thus, both cell assemblies and cytoskeleton assemblies can be described as active nematics, which creates topological defects because of their activity (growth, contractility).

In the small animal *Hydra*, the regeneration of head and foot correlates with the position of integer topological defects in the nematic field of actin, suggesting that topological defects in muscle cells could control or drive morphogenesis.

I will further show that mechanical deformation of the nematic field of the actin in the *Hydra* causes bicephalous regeneration. Moreover, compressing the *Hydra* in different orientations, one can generate toroidal *Hydra*. When toroidal *Hydra* has no defects, as allowed by its unique topology, no head is regenerated, while toroidal *Hydra* with defects regenerate a head and a foot, keeping a hole in their body.

Altogether, our findings support that topological defects in the nematic order of muscles cells or actin control the stress field in developing tissues, driving morphogenesis.

Thursday, April 11
11:35 AM–12:15 PM

Motomu Tanaka

Heidelberg University, Germany

SPATIOTEMPORAL ORDER AND DYNAMICS OF TISSUES AND ORGANOIDS

The formation of tissues and organoids includes many steps, e.g. lineage-specific differentiation of progenitors, cell sorting, and morphological adaptation. In addition to the biochemical cues like signaling, the tissue formation is driven physically, e.g. the mechanical interactions with extracellular matrix and neighboring cells and the intracellular contractile force.

In my talk, I am going to take two cases from our recent studies:
(a) the role of collective cell orders in the maintenance of tissue homeostasis in human eyes, and (b) the interplay of intracellular forces and signaling during the symmetry breaking of Hydra tissues.

For the former, we established numerical indices that represent the spatial order of human corneal endothelium. From several quantitative indices, we found that the principle of colloidal physics helps us to predict the breakdown of the tissue homeostasis much earlier than the widely used diagnostic index. For the latter, we found the interplay of canonical Wnt signaling and dynamic deformation during the cell sorting and symmetry breaking in Hydra organoids.

Our data suggest that the well-defined 3D microenvironments could help us to guide the cell differentiation and pattern formation in organoids and tissues *in vitro*, which can contribute to develop new therapeutic tools.

Danijela Vignjevic

Institut Curie Paris, France

HOMEOSTASIS OF GUT EPITHELIUM UNDER MECHANICAL STRESS

The gut epithelium acts as a barrier between the outside world and the body while at the same time ensuring nutrient absorption. The small intestine epithelium comprises a single layer of columnar cells that line the villi that project into the gut's lumen and the crypts that descend into the connective tissue.

The constant epithelium renewal is achieved by stem cell proliferation in the crypts giving rise to specialized epithelial cell types. Upon exiting the crypt, most cell types migrate towards the villus tip, where they die and are shed into the lumen. The basal surface of the epithelium is underlined by the basement membrane, a thin and dense sheet-like structure on which cells adhere and migrate. While cells migrate collectively, maintaining their apicobasal polarity, they also display a second polarity axis (front-back), characterized by actin-rich basal protrusions oriented in the direction of migration. How this front-back polarity is established and what is the guidance cue for directional migration towards the tip of villi remains unknown.

I will discuss our ongoing research aiming to address if and how the basement membrane provides cues for the directional migration of epithelial cells and what are the roles of adhesive structures in reading those cues. I will also discuss the role of the actomyosin cytoskeleton in maintaining intestinal epithelium integrity and the role of Piezo channels in the maintenance of the stem cell compartment. Finally, I will present our progress on Gut-on-Chip devices.

Monday, April 8
12:05 PM–12:45 PM

Viola Vogel

ETH Zürich, Switzerland

THE MECHANOBIOLOGY OF EXTRACELLULAR MATRIX: THINKING BEYOND THE YOUNG'S MODULUS

While establishing that aggressiveness and malignancy of solid tumors correlates with tissue stiffening, the focus has been on deciphering the underlying mechanisms, which included quantifications of the Young's moduli, the roles of enhanced interstitial pressure, changes in cell contractility, and extracellular matrix (ECM) remodeling and cross-linking.

Yet, rather little is still known today how mechanical factors regulate cell fate in complex organ-specific cell niches. Progress is hampered as tissues are composed of a large variety of cell types which are in close contact to each other and communicate via biochemical and physical factors. Beyond the physical properties of cellular microenvironments, that engineers could easily produce and tune, the reciprocal mechanical signalling between cells and their extracellular matrix environments also involves the stretching of proteins and thus an off-on, or on-off switching of exposed molecular binding sites. Importantly, protein stretching can switch the structure-function relationships of extracellular as well as of intracellular proteins, which defines the underpinning structural principles of mechanobiology.

Translating what has been learned in mechanobiology (mostly on single cells) to real organs, and finally to the clinic, was challenging due to the lack of nanoscale sensors to probe forces or tissue fiber tensions in healthy versus diseased organs. To bring our knowledge to the patient, we developed a nanosensor that probes the stretch-induced destruction of multivalent binding motifs, allowing us for the first time to visualize the tensional states proteins in animal models and in human tissues.

Many unexpected findings were derived that were not anticipated from 2D cell culture studies. Underpinning mechano-regulated structural mechanisms and the significance of these findings in cancer and other pathologies will be discussed.

Jennifer Young

National University of Singapore, Singapore

EXTRACELLULAR MATRIX MECHANOBIOLOGY

It is well appreciated that extracellular cues stemming from the matrix dictate a multitude of cellular functions, from motility to stem cell differentiation. Yet, the extracellular environment is inherently complex and thus hinders our full understanding of specific matrix-based contributions to cellular behavior. Our work focuses on age-related extracellular matrix (ECM) remodeling and engineering materials capable of recapitulating ECM properties *in vitro* at both the micro and nano length scales.

This talk will highlight some of our approaches in ECM tissue characterization and materials design to control cell-matrix interactions in the context of cardiac aging and mechanobiology.

We describe a tunable stiffness gradient polyacrylamide (PA) hydrogel system using a two-step polymerization method capable of spanning the diverse physiological and pathological mechanical landscapes present in the heart (e.g., Young's modulus, E ~10-50 kPa). We apply these stiffness gradient hydrogels to understand the regulation of mechanosensitive processes on cardiac function in an age-dependent manner.

We also describe a new material platform consisting of tunable hybrid hydrogel-decellularized cardiac tissues that maintains age-specific native matrix composition and organization independent of matrix stiffness. Subsequent culture of cardiac fibroblasts (young and aged) show that the matrix 'age' can outweigh matrix mechanics in driving fibroblast activation.

Our strategies ultimately aim to interrogate the cell-matrix interface using highly defined biomaterial systems at different length scales that can inform future matrix-based treatment strategies.

Contributed Talks

Sorted by Date

A **Contributed Talk** consists of a 10-minute talk and a 5-minute Q & A session.

Monday, April 8

Time	Title	Speaker
10:40 AM–10:55 AM	Force Transmission is a Master Regulator of Mechanical Cell Competition	**Andreas Schoenit**
10:55 AM–11:10 AM	Local Matrix Stiffening to Model Lung Fibrotic Remodeling *Ex-Vivo*	**Claudia Loebel**

Tuesday, April 9

Time	Title	Speaker
11:15 AM–11:30 AM	3D Multiphoton-Lithography Proteinous Scaffolds Moduli for Stem Cell Studies	**Jaroslaw Jacak**
11:30 AM–11:45 AM	DNA Microbeads for Spatio-Temporally Controlled Morphogen Release Within Organoids	**Tobias Walther**

Thursday, April 11

Time	Title	Speaker
10:20 AM–10:35 AM	Adhesion Mechanics in Breast Cancer Spheroid Spreading	**Grégoire Lemahieu**
10:50 AM–11:05 AM	Three-Dimensional Force Inference in Budding Intestinal Organoids	**Oliver Max Drozdowski**
11:05 AM–11:20 AM	Cell-Instructive Materials: Versatile Biohybrids and their Guided Assembly to Functional Hydrogels	**Jasmina Gačanin**
11:20 AM–11:35 AM	Combining Two-Photon Polymerization Direct Laser Writing with Soft Lithography for Investigating *Plasmodium* Sporozoite Migration on Triangular Patterns	**Malin Schmidt**

FORCE TRANSMISSION IS A MASTER REGULATOR OF MECHANICAL CELL COMPETITION

Monday, April 8
10:40 AM–10:55 AM

Andreas Schoenit

Lucas Anger, Rene-Marc Mege, Benoit Ladoux

CNRS-Institut Jacques Monod-UMR7592, France

Cell competition is a tissue surveillance mechanism for eliminating unwanted cells and as such is indispensable in development, infection and tumorigenesis. Although different biochemical mechanisms are proposed, due to the dearth of direct force measurements, how mechanical forces determine the competition outcome remains unclear.

In this talk, I will show that across different cell types, mechanical cell competition is regulated by relative force transmission capabilities, exclusively favoring cell types with stronger intercellular adhesion. Direct force measurements reveal increased mechanical activity at the interface of the two competing cell types in the form of large stress fluctuations which can lead to upward forces and cell elimination. A winning cell type endowed with a stronger intercellular adhesion exhibits a higher resistance to elimination while benefiting from efficient force transmission to neighboring cells.

I will demonstrate this competition mechanism in cultured cells and in highly invasive breast tumors, suggesting broad implications of keeping strong force transmission ability for maintaining tissue boundaries and cell invasion pathology.

LOCAL MATRIX STIFFENING TO MODEL LUNG FIBROTIC REMODELING *EX-VIVO*

Claudia Loebel [1,2]

Matthew L. Tan[1], Donia Ahmed[2], Jackson Gabbard[2], Brendon M. Baker[2], Rachel L. Zemans[3]

1 Department of Materials Science and Engineering, University of Michigan, Ann Arbor, MI, USA
2 Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA
3 Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA

Significance: Pulmonary fibrotic remodeling is characterized by spatially heterogeneous aberrant extracellular matrix (ECM) deposition and stiffening within regions of lung alveoli. Ineffectual differentiation from type 2 alveolar epithelial cells (AT2) to type 1 alveolar epithelial cells (AT1) in fibrotic regions has now been associated with the progression of fibrosis. However, it is unclear how ECM crosslinking and mechanics regulate alveolar epithelial stem/progenitor cell fate and function. To address this, we developed a local ECM photo-crosslinking technique within precision cut lung slices (PCLS) as an *ex-vivo* model of early-stage fibrotic remodeling to probe epithelial cell fate in response to ECM stiffening.

Methods: PCLS were prepared from mouse and human donor lungs through agarose infiltration and vibratome sectioning. Local ECM stiffening was induced upon blue light initiated di-tyrosine bond formation of tyrosyl residues and visualized via di-tyrosine bond autofluorescence. AT2 and AT1 cell function were assessed using cell morphology and motility analyses and immunofluorescence of intracellular and extracellular markers.

Results: Blue light exposure resulted in locally enhanced di-tyrosine auto-fluorescence in photo-crosslinked regions indicating ECM crosslinking (Fig. 1). Stiffening of local ECM resulted in increased cell spreading of AT2 progeny with an increase in AT1 differentiation and reduced AT2 marker expression (Fig. 2). In addition, ECM crosslinking resulted in an increase fibrotic ECM deposition (e.g., fibronectin, Fig. 3) and enhanced cell motility (Fig. 4). Perturbing intracellular contractility and cell-ECM interactions, rescued AT2 marker expression with a reduction in AT2 motility and fibronectin deposition. These findings suggest that ECM stiffening regulates AT2-to-AT1 differentiation which depends on cell-ECM mechanosensing.

Conclusion: Local stiffening of native ECM within alveolar regions provides an engineering tool to probe mechanisms of early fibrotic remodeling in the lung including cellular crosstalk and motility of epithelial and mesenchymal cells. Ongoing work is to investigate ineffectual AT2 differentiation and accumulation in response to local ECM stiffening.

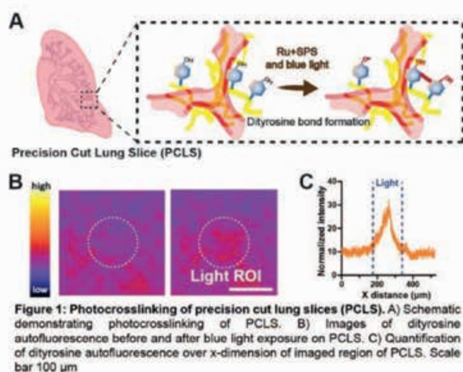

Figure 1: Photocrosslinking of precision cut lung slices (PCLS). A) Schematic demonstrating photocrosslinking of PCLS. B) Images of dityrosine autofluorescence before and after blue light exposure on PCLS. C) Quantification of dityrosine autofluorescence over x-dimension of imaged region of PCLS. Scale bar 100 µm

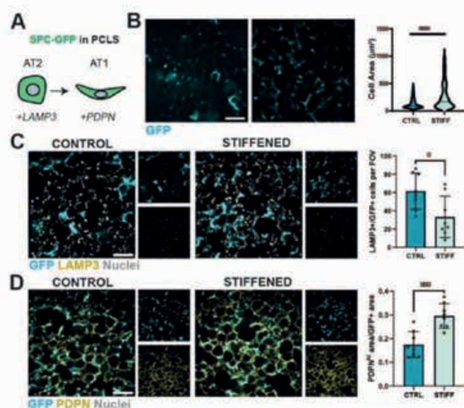

Figure 2: Effect of photocrosslinking on alveolar epithelial differentiation. A) Schematic demonstrating marker expression of lung epithelial differentiation using SPC-GFP lineage traced mice in PCLS. B) Representative images of GFP expressing cells and quantification of cell spreading in control and stiffened regions. C) Representative images and quantification of LAMP3 expression of GFP+ cells. D) Representative images and quantification of podoplanin (PDPN) expression of GFP+ cells. Scale bar 100 µm (*$p<0.05$ ***$p<0.001$ ****$p<0.0001$)

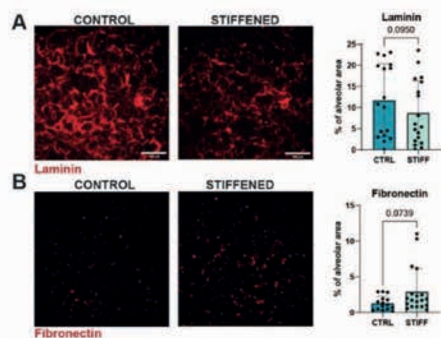

Figure 3: Effect of photocrosslinking on matrix production. A) Representative images and quantification of laminin in control and stiffened regions. B) Representative images and quantification of fibronectin in control and stiffened regions. Scale bar 100 µm.

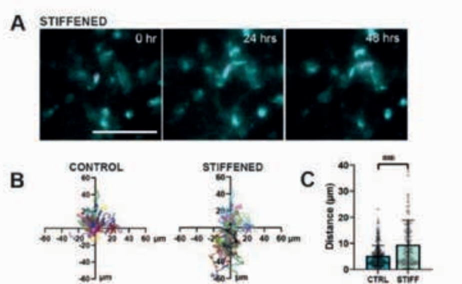

Figure 4: Migration analysis in photostiffened PCLS. A) Representative time-lapse images of epithelial cell migration in stiffened regions B) Migration plots of GFP+ epithelial cells in control and stiffened regions. C) Quantification of distance traveled in control and stiffened regions. Scale bar 100 µm (****$p<0.0001$)

3D MULTIPHOTON-LITHOGRAPHY PROTEINOUS SCAFFOLDS MODULI FOR STEM CELL STUDIES

Jaroslaw Jacak [1]

Christoph Naderer[1], Dmitry Sivun [1], Martina Hoffmann [1], Thomas A. Klar [2],Eleni Priglinger [2]

1 University of Applied Sciences, Linz, Austria
2 Johannes Kepler University, Linz, Austria

In tissue modelling and engineering, three-dimensional (3D) scaffolds play a pivotal role in building biomimetic environments for different cell types. Current research efforts lack a bone-on-a-chip-model, which combines the geometric, functional and mechanical properties required to mimic the complex matrix composed of organic and inorganic components within one scaffold. Multiphoton Lithography (MPL) is especially well suited for rapid prototyping of 3D scaffolds. Feature sizes in the sub-micrometre range are typically achieved with artificial polymer-based materials for 3D tissue engineering however, protein-based biomaterials have garnered increasing interest. So far, modified proteins (e.g. silk methacrylate) have been structured or the direct cross-linking of proteins has been used for 3D printing of hundreds of micrometres large scaffolds.

Herein, we demonstrate the combination of artificial polymer-based and functional protein-based photoresists within one 3D scaffold. 3D grids (up to 315x315x15 μm^3, grid constant 45 µm) with lateral and axial feature sizes below 200 nm and 600 nm were achieved. Protein-based photoresists used for3D nanolithography consisted of methacrylated streptavidin, bovine serum albumin or collagen type I, polyethylene glycol diacrylate or methacrylatedhyaluronic acid as cross-linking agents and a vitamin-based photoinitiator. The functionality of the 3D printed streptavidin and collagen scaffolds have been demonstrated using fluorescently labelled biotin or antibodies, respectively. Different Young's moduli between 80 MPa for the artificial polymer and up to 40kPa for streptavidin-based photoresists were achieved.

We demonstrate the viability of mesenchymal stem cells (MSC) in our 3D scaffolds by imaging the cellular actin cytoskeleton using direct 3D Super-Resolution microscopy. The mechanotransduction of MSCs, a crucial factor in the sensing of mechanicalproperties in cell-matrix interplay, is visualized by imaging of the vinculin protein density, a key player in cell-cell and cell-matrix adhesions. Furthermore,we show the differentiation of MSCs towards the osteoblast lineage within our scaffolds.

DNA MICROBEADS FOR SPATIO-TEMPORALLY CONTROLLED MORPHOGEN RELEASE WITHIN ORGANOIDS

Tobias Walther [1,2]

Cassian Afting[3], Joachim Wittbrodt[3], Kerstin Göpfrich[1,2]

1 Max Planck Institute for Medical Research, Germany
2 Centre for Molecular Biology of Heidelberg University (ZMBH), Germany
3 Centre for Organismal Studies, Heidelberg University (COS), Germany

Organoids have proven to be powerful *in vitro* model systems that mimic features of the corresponding tissue *in vivo*. However, across tissue types and species, organoids still often fail to reach full maturity and function, because biochemical cues cannot be provided from within the organoid to guide their development. The establishment of such tools has been identified as a major goal of the field. Here, we introduce DNA microbeads as a novel tool for implementing spatio-temporally controlled morphogen gradients inside of organoids at any point in their life cycle.

The DNA microbeads are formed in a simple one-pot process, they can be stored for a year and their viscoelastic behavior and surface modification is tunable to mimic the corresponding tissue. Employing medaka retinal organoids and early embryos, we show that DNA microbeads can be integrated into embryos and organoids by microinjection and erased in a non-invasive manner with light. Coupling a recombinant surrogate Wnt to the DNA microbeads we demonstrate the spatio-temporally controlled release of the morphogen from the microinjection site, which leads to the formation of retinal pigmented epithelium while maintaining neuroretinal ganglion cells.

We were thus able to bioengineer retinal organoids to more closely mirror the cell type diversity of *in vivo* retinas. The DNA microbead technology can easily be adapted to other organoid applications for improved tissue mimicry.

ADHESION MECHANICS IN BREAST CANCER SPHEROID SPREADING

Grégoire Lemahieu[1]

Tobias Hub[1], Julian Bauer[1], Leon-Luca Homagk[1], Sam Barnett[1], Andrea Palamidessi[2], Leonardo Barzaghi[2], ChiaraGuidolin[3], Carlo Bevilacqua[4], Federico Colombo[5], Barbara Schamberger[5], Florine Sessler[6], Paulina M. Layseca[7], Manuel G. González[8], FabioGiavazzi[3], Robert Prevedel[4], Christine Selhuber-Unkel[5,6], Johanna Ivaska[7], Xavier Trepat[8], Joachim P. Spatz[1], Giorgio Scita[2], Elisabetta Ada Cavalcanti-Adam[1,9]

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5 Institute for Molecular SystemsEngineering and Advanced Materials, Heidelberg, Germany
6 Biomechanics Core Facility, Institute for Molecular Systems Engineering and AdvancedMaterials, Heidelberg, Germany
7 Cell Adhesion and Cancer Lab, Turku University, Finland
8 Integrative Cell and Tissue Dynamics, Institute forBioengineering of Catalonia, Spain
9 Cellular Biomechanics, Bayreuth University, Germany

Tumor invasion is one of the hallmarks of cancer, upstream of the metastatic cascade. In the first step of epithelial tumor dissemination, a transformed cell escapes from the primary tumor, crosses the basal lamina and enters the peritumoral environment.
In order to model this early stage of invasion, our work involves a breast epithelial ductal carcinoma *in situ* (DCIS) cell line (MCF10.DCIS.com) engineered for RAB5A GTPase overexpression which sparks jamming-to-unjamming transition (JUT) and enhances a flocking-fluid type of motility[1,2,3]. Specifically, we aim at relating the invasive behavior of RAB5A-overexpressing DCIS spheroids to distinct mechanical signatures.
We show that high level of RAB5A expression is sufficient to trigger a fast, flocking-fluid, and isotropic spheroid spreading transition. We observe that the elevated migration speed is mediated by increased focal adhesion assembly and length at the leading cell front. Consequently, we use surface nanopatterning to tune integrins clustering, and show that ligand spacing impacts the type of spreading transition (solid-to-flocking-fluid or solid-to-gas). On top, coupled actin fibers staining and traction force microscopy (TFM) data show the onset of front/rear polarity and increased cell-substrate interaction at the leading edge of RAB5A-overexpressing cell clusters. Moreover, by monitoring the dynamics of mixed cell populations (control and RAB5A-overexpressing cells) in the spreading transition, we model the heterogeneity of interactions between an unjammed, invasive carcinoma, and a jammed, tumor-suppressive tissue.
Finally, using Brillouin microscopy and nano indentation, we show that the transition in dimensionality is accompanied by stiffness plasticity, where single cell nuclear stiffening upon RAB5A overexpression leads to spheroid softening during active wetting. Taken together, these results can help understand the interplay between cell-cell and cell-substrate interactions in epithelial tumor invasion.

[1] C. Malinverno et al., Nat. Mater. 16, 587-596 (2017).
[2] A. Palamidessi et al., Nat. Mater. 18, 1252-1263 (2019).
[3] E. Frittoli et al., Nat. Mater. 22, 644-655 (2023).

THREE-DIMENSIONAL FORCE INFERENCE IN BUDDING INTESTINAL ORGANOIDS

Oliver M. Drozdowski [1,4]

Kim E. Boonekamp[2,4], Ulrike Engel[3], Michael Boutros[2], Ulrich S. Schwarz[1]

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2 Division of Signaling and Functional Genomics,German Cancer Research Center (DKFZ)
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3 NikonImaging Center and Centre for Organismal Studies, Heidelberg University, 69120 Heidelberg, Germany
4 These authors contributed equally to this work

Organoids have become valuable model systems for developmental biology and biomedical research. One of the most successful examples are intestinal organoids, which recapitulate key characteristics of the development of the gut. In particular, with growth and differentiation they undergo a morphological transition from a spherical epithelial shell to a budded shape. Apico-basal forces from the actomyosin system have been proposed to facilitate this structure formation.

Here we introduce a fully three-dimensional (3D) non-invasive force inference technique to indirectly measure such forces. We reconstruct the 3D tissue shape of intestinal organoids and infer individual tensions from microscopy data, assuming a foam-like description of the tissue.

Inference is verified by *in-silico* organoid data simulated with a bubbly vertex model.

From the experimental data for the intestinal organoids, we find an increased global apical tension in budded morphologies, consistent with apical contractile forces effecting the observed budded shapes.

In the future, our force inference technique could also be applied to study the relation between tissue geometry and force generation in other organoid systems.

CELL-INSTRUCTIVE MATERIALS: VERSATILE BIOHYBRIDS AND THEIR GUIDED ASSEMBLY TO FUNCTIONAL HYDROGELS.

Jasmina Gačanin

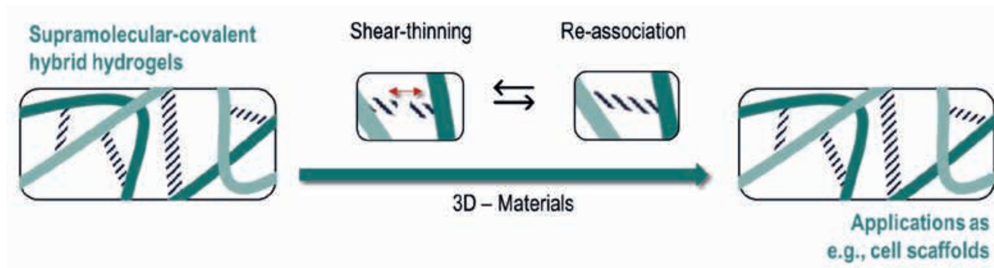
Max Planck Institute for Polymer Research, Mainz, Germany

Hydrogels that instruct cellular functions to e.g., restore damaged tissue while allowing minimally invasive application and therapeutic options are in high demand in regenerative medicine. Current hydrogel systems can rarely cover all highly attractive material properties required, e.g., high definition, biocompatibility, and self-healing. Considering these aspects, we investigate the guided assembly from molecule to macroscopic hydrogels and develop biohybrids as smart cell-instructive materials that can express dynamic soft tissue features.

In this regard, inspired by Nature, we explore the combination of the stability of covalent bonds with dynamic behavior introduced by supramolecular chemistry to achieve a unique fusion of fundamental material characteristics, displaying adaptability, responsiveness, and interactivity: Biopolymer-derived backbones are combined with crosslinking units of either DNA, dynamic-coordinative interactions, or nanofiber-forming peptides yielding highly tunable 3D materials.

In this regard, the programmability of supramolecular interactions achieves multifaceted structure formation, controlled delivery of bioactives to control cell population, or reversible crosslinking to facilitate gel injection minimizing damage to healthy tissue.

The resulting hydrogels have a broad relevance to a variety of tissue engineering strategies as they demonstrate impressive material and biological properties like thixotropic behavior with fast and autonomous recovery as well as biocompatibility, and act as a platform opening various application scenarios.



COMBINING TWO-PHOTON POLYMERIZATION DIRECT LASER WRITING WITH SOFT LITHOGRAPHY FOR INVESTIGATING *PLASMODIUM* SPOROZOITE MIGRATION ON TRIANGULAR PATTERNS

M. Schmidt[1,]

M. Singer[2,3], L. Lettermann[4], S. Geiger[1], U.S. Schwarz[4,5], F. Frischknecht[2,3], C. Selhuber-Unkel[1]

1 Institute for Molecular Systems Engineering and Advanced Materials (IMSEAM), Heidelberg University, Germany
2 Integrative Parasitology, Center for Integrative Infectious Diseases, Heidelberg University Medical School, Germany
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4 Institute for Theoretical Physics, Heidelberg University, Germany
5 BioQuant–Center for Quantitative Biology, Heidelberg University, Germany

Understanding how cells navigate in 3D environments is fundamental to unravelling biological processes. While cancer cells and amoeba movement on triangular and sawtooth patterns are widely studied [1,2], the 3D migration of *Plasmodium* sporozoites, the initial form of malaria parasites entering the human body, remains less explored despite their complex helical migration through the skin and their pivotal role in malaria transmission [3].

Hence, we studied the precise fabrication of differently angled triangular microstructures to investigate the impact of defined 3D environmental boundaries on sporozoite motility and migration dynamics within a hydrogel. Combining two-photon polymerization (2PP) direct laser writing with soft lithography techniques, we replicate 3D printed structures onto non-fluorescent materials like polydimethylsiloxane (PDMS), followed by the attachement of polyacrylamide hydrogels to the PDMS patterns. Pattern accuracy and print parameters were analyzed via AFM and SEM and iteratively improved to meet the requirements of high-quality image analysis. Our investigation focuses on systematically varying the angles (15° to 60°) of the triangular patterns to assess their fabrication accuracy and impact on sporozoite motility and directionality of their helical movement through the hydrogel and on the patterns.

Advanced imaging and tracking methodologies will offer insights into how geometric confinement influences the migration dynamics of sporozoite, shedding light on the underlying mechanisms governing their movement within host tissues. Moreover, this fabrication methodology can also contribute to the broader field of cell migration on triangular and sawtooth patterns, since many different structures can be generated by the large adaptability of the 2PP printed microstructures and replica facilitates their efficient scaling up.

[1] X. Sun et al., Proc Natl Acad Sci U S A, 2015, 112(41): 12557-62.
[2] S. Chen et al., ACS Nano, 2019, 13 (2): 1454-1468.
[3] J. K. Hellmann et al., PLOS Pathogens, 2011, 7(6): e1002080.

Flash Talks

Monday, April 8 — 4:35 – 5:10 PM

Microengineered Perfusable 3D-bioprinted Cancer Model for *in vivo* Mimicry of Tumor Microenvironment	**Dana Venkert**
Brain-Chip: *In vitro* Neuronal Networks with Controlled Polarity	**Stephen Casale**
Percolation in Fibrous Gels by Active Cell Contraction Dominates Tissue Mechanical Behavior	**Ran Glinowiecki**
Tailor-Made, 3D Protein Nanofiber Matrix for Control of Cancer Cell Dynamics	**Zahra Hajian Foroushani**
3D Microfabricated Scaffolds for Investigation of *Plasmodium* Sporozoites Motility	**Zeynab Tavasolyzadeh**
Adhesion-Based Active Gel Model for 1D Cell Migration	**Valentin Wössner**
A Comparative Study of Assays for Quantification of Collagen Deposition in 2D Cell Cultures	**Syuzanna Hambardzumyan**
3D Tumor Model and Cancer Cell Malignancy: Influence of Mechanical Stress and Hypoxia	**Yasmin Antonelli**
Mechano-Regulation of Mesenchymal Stem Cells Using Bio-Metamaterials	**Natalie Munding**
Development of an Optofluidic Platform to Investigate Phototaxis in Cyanobacteria	**Lourdes Albina Nirupa Julius**
From Tissue Engineering to Drug Delivery with 3D Printed Spider Silk	**Kathrin Angelika Neuber**
Dynamics of Nonmuscle Myosin II Isoforms	**Nicolai Schaefer**
Minimal-Invasive 3D Laser Printing of Microimplants in Organisms	**Cassian Maximilian Afting**
Development of Hydrogel-Based Materials to Dissect the Mechanobiology of Retinal Pigment Epithelium (RPE) in Aging	**Felix Reul**
Apico-Basal Polarity in Stem Cells	**Elisa Genthner**
Cellular Mechanobiology in 3D Scaffolds	**Magdalena Fladung**
Mechanobiology of the Outer Retina Deciphered Using a 3D hiPSC-Derived *in vitro* Model	**Vasudha Turuvekere Krishnamurthy**
Late Neuroretinal Organoids Revert to Rudimentary Self-Organization in the Absence of Proper External Signaling	**Christina Schlagheck**
Development of pNIPAM-Actuated Artificial Joints by Direct Laser Writing (DLW)	**Annabelle Sonn**
3D Printed Biosensors for Studying Cardiomyocyte Electrophysiology	**Mahsa Kalantari Saghafi**
Poster Presentation only: The Role of the Extracellular Matrix in Retinal Cell Fate Specification and Retinal Organoid Development	**Anna-Sophia Stanolova**

A **Flashtalk** consists of a 90-secondes talk.

Tuesday, April 9 — 5:05 – 5:23 PM

Title	Presenter
Photoswitching for Controlling Cell Adhesion	**Sophie Geiger**
Heat Shock Treatment Increases Cell Viability After Bioprinting	**Erin Spiller**
Azobenzene-Mediated Photomechanical Stimulation of Cells	**Krishna Ramesh**
Microprinting of Responsive Biomaterials	**Philipp Mainik**
Image-Based Cell Sorting by Photopolymerization	**Tobias Abele**
Real-time Measuring Osmotic Pressure of Micro-tissues with Liposomal FRET Sensors	**Efe Cuma Yavuzsoy**
Mechanical Phenotyping of Colorectal Cancer Organoids	**Kim Boonekamp**
The Role of Mechanosensing in Functionality of Natural Killer Cells and T Cells	**Bin Qu**
Gelatin-Based Hydrogels with Gradient Stiffness for 3D Motility and Durotaxis Studies	**Dimitris Missirlis**
Snake-Skin Inspired Auxetic Design as a Candidate for Mechanically Adaptive Scaffolds for Cells	**Gaurav Dave**
Precision Microactuators: Harnessing Microfiber Skeletal Muscle Cells with Direct Laser Writing	**Mohammadreza Taale**
Post-Bioprinting Delivery of Biomolecules in 3D Tissue-like Constructs Using Synthetic Vesicles	**Ole Thaden**

Poster Presentation

Everyone who gives a **Flashtalk** also gives a **Poster Presentation on the same day**. The corresponding times of the Poster Presentations are as follows:

Monday, April 8:
Poster Session I
5:10 – 7:00 PM

Tuesday, April 9:
Poster Session II
5:23 – 7:15 PM

MICROENGINEERED PERFUSABLE 3D-BIOPRINTED CANCER MODEL FOR *IN VIVO* MIMICRY OF TUMOR MICROENVIRONMENT

Dana Venkert

Tel Aviv University, Israel

Many drugs show promising results in laboratory research, but eventually fail in clinical trials. We hypothesize that one main reason for this translational gap is that current cancer models are inadequate. Most models lack the tumor-stromal cell interactions, which are essential for tumor progression. Such interactions cannot be recapitulated in conventional 2D cultures where cells grow on rigid plastic plates.

Therefore, there is a need to develop a 3D model, which better mimics the tumor microenvironment, and the interactions of the tumor cells with the immune system. Here, we recapitulated the tumor heterogenic microenvironment by creating fibrin glioblastoma-bioink consisting of patient-derived glioblastoma cells, astrocytes and microglia. Additionally, perfusable blood vessels were created using a sacrificial bioink coated with brain pericytes and endothelial cells. We observed similar growth curves, drug response and genetic signature of glioblastoma cells grown in our 3D-bioink platform and in *in vivo* studies as opposed to 2D culture.

Moreover, 3D-bioprinted models have the advantage of a specific cell deposition according to a desired pattern, compared to 3D-spheroids/organoids or 3D-scaffold-based approaches, which mainly rely on cell self-rearrangement.

This 3D-bioprinted model could be the basis for potentially replacing cell cultures andanimal models as a powerful platform for rapid, reproducible and robust personalized therapy screening and drug development.

PROJECT: BRAIN-CHIP: *IN VITRO* NEURONAL NETWORKS WITH CONTROLLED POLARITY

Stephen Casale

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Strategies to direct neuronal growth on 3D micropatterned materials are in high demand due to the need to understand the function of biohybrid neuronal networks. If growth of neurons can be directed by the materials, and signaling activity analyzed, this system could advance brain-chip and related technologies.

PC12 cells, a neuroblastic cell line, are used in conjunction with 3D printing, to provide topological guidance and analysis of network formation. 3D printed materials are made of polymerized IP Visio resin, and structured as hour glasses and disks. Typical structures are approximately 50 microns in height.

Within this project, a protocol for printing 3D structures using direct laser writing, then coating the surface with laminin, was developed. While PC12 cells can adhere to other adhesion proteins, the fastest and most extensive differentiation of these cells occurs on laminin. When seeding cells into hour glass microstructures, our results demonstrate that soma and neurite count, as well as neurite path length, show some increase while neurite path angle shows little to no change over 11 days *in vitro*.

We also show that while the microstructures are printed on glass, more substantial cell differentiation occurs when the cells adhere to laminin coated glass. This suggests that while the 3D printed microstructures can provide topological guidance cues, the same material might not be suitable to accommodate cell adhesion.

PERCOLATION IN FIBROUS GELS BY ACTIVE CELL CONTRACTION DOMINATES TISSUE MECHANICAL BEHAVIOR

Ran Glinowiecki[1,3]

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2 School of Chemistry, Faculty of Exact Science, Tel-Aviv University, Israel
3 Center for Physics and Chemistry of Living Systems, Tel-Aviv University, Israel

Tissues display unique mechanical behaviors arising from interactions among their microscale components, encompassing living cells and extracellular matrix (ECM) fibers. By contracting, cells alter the architecture, orientation and density of the ECM fibers and can form rigid bands of highly aligned and densified fibers that mechanically connect cells over long distances.

We draw inspiration from the ability of cells to interconnect through contraction by forming rigid ECM bands and ask how such multi-cellular connections influence the global mechanical response of the tissue. We use finite element modeling to simulate contraction of particles that mimic active cell contractility. The particles contract in a 3D hyperelastic continuum with material properties of a fibrin gel, which we then probe in shear. We show that active contraction leads to collective inter-particle mechanical interactions, resulting in global stiffening that emerges at relatively low volume fractions of particles and that is not dependent on external loading, rather induced by internal sources.

We show that the stiffening effect depends on the nonlinear material properties and is mediated by clustering of bands that percolate the material. Our work leads to a better understanding of the active cellular interactions that mediate rigidity transitions and mechanical stability in living tissues.

TAILOR-MADE, 3D PROTEIN NANOFIBER MATRIX FOR CONTROL OF CANCER CELL DYNAMICS

Zahra Hajian Foroushani [1]

Boyan Garvalov[2], Stephan Keppler[3], Kentaro Hayashi[4], Christoph Spiegel[5], Stefan Kaufmann[1], Jonathan Sleeman[2],Martin Bastmeyer[3], Eva Blasco[5], Motomu Tanaka[1]

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An increasing number of studies have shown that cell migration and tissue morphogenesis, are guided by physical cues, such as stiffness and the directional order of the fibrous extracellular matrix (ECM). A prominent example is the massive remodeling of ECM in cancer to relieve the invasive migration of cancer cells. However, a quantitative understanding of how stiffness (Young's modulus), density, and directional order of the fibrous ECM influence single-cell behavior is still largely missing. To this end, we designed and fabricated two types of cellular niche models:

(I) stacks of gelatin nanofibers (2.5D) and

(II) electrospun protein nanofibers integrated into 3D printed pillars.

The models designed in this study, based on random and aligned nanofibers with different densities and stiffnesses, will open the possibility of investigating how these three parameters influence the spatiotemporal behavior of single (luminal breast) cancer cells. A variation of crosslinkers was used to mimic the range of healthy (E~ 15 kPa) and cancerous (E~ 50 kPa) ECM stiffness.

As a first set of results, we show that live cell tracking of (+ASAP1/-ASAP1) cells seeded on 2.5D random GNFs, exhibits distinct differences in cell-matrix contact area and spatio-temporal patterns of deformation and motion.

3D MICROFABRICATED SCAFFOLDS FOR INVESTIGATION OF *PLASMODIUM* SPOROZOITES MOTILITY

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Leon Lettermann[3], Mirko Singer[2], Fereydoon Taheri[1], Mohammadreza Taale[1],
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Malaria infections are initiated when an infected Anopheles mosquito injects *Plasmodium* sporozoites from the salivary glands into the host skin. Then they migrate by gliding motility with a speed of 1-2 µm/s through the dermis, searching for a blood capillary to reach the liver.

In vivo imaging studies show that sporozoites commonly circulate along blood capillaries before they enter them. *In vitro* studies investigating the complex sporozoite migration patterns have been mostly performed on 2D substrates.

Here we introduce 3D scaffolds with different geometries inspired by the blood capillary system and printed using two photon polymerization (2PP). This allows us to define the sub-micron spacing within the scaffold to study sporozoite motility. The migration of sporozoites is recorded over time using fluorescence imaging and analysis.

Quantitative analysis of the gained data indicates how the micro-pores of the scaffold are influencing the mean square displacement (MSD) and velocity of the sporozoites.

ADHESION-BASED ACTIVE GEL MODEL FOR 1D CELL MIGRATION

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Active gel theory has demonstrated that actomyosin contractility is sufficient for polarization and self-sustained cell migration in the absence of externalcues.

However, in these models, the dynamic character of substrate adhesion is usually neglected, although it seems to play an important role in more complex migration modes and during motility initiation. Simple models based on bond dynamics have been suggested for the required adhesion dynamics, but these do not include intracellular flows.

Here we show that, in a one-dimensional setting, active gel theory can be extended by such adhesion dynamics and that load sharing is the cooperative effect that is required to obtain symmetry breaking. For intermediate adhesiveness, symmetric actin polymerization then leads to robust motility in a bistable regime. Our model predicts adhesion and flow profiles in qualitative agreement with experimental results.

We also study switching between sessile and motile states by applying nonlinear perturbations as well as cell behavior on adhesive pattern.

A COMPARATIVE STUDY OF ASSAYS FOR QUANTIFICATION OF COLLAGEN DEPOSITION IN 2D CELL CULTURES

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Several methods for matrix collagen quantification in tissue samples are established, e.g., Picro-Sirius red assay, hydroxyproline assay, antibody-based assays, or the 3,4-DHPAA-based fluorometric assay.

The sensitivity of these methods to reliably quantify deposited collagen in 2D cultures, where the collagen is typically available at much lower concentrations than in tissues, has been questioned but not systematically analyzed.

In our study, we compare the performance of the four different methods to quantify deposited collagen in 2D fibroblast cultures at different culture times, under different collagen deposition-stimulating conditions, and as a function of the post-culture processing step (decellularization).

We demonstrate that the available methods can deliver accurate results within different and narrow experimental windows with notable differences in sensitivity, experimental time and cost.

3D TUMOR MODEL AND CANCER CELL MALIGNANCY: INFLUENCE OF MECHANICAL STRESS AND HYPOXIA

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The development of *in vitro* models that accurately mimic the complex tumor microenvironment is crucial for understanding cancer progression and improving therapeutic strategies.

In this work we analyzed the capabilities of a polymer-based 3D tumor model, named 3D tumor-like microcapsules, to effectively stimulate the expression of hallmarks traditionally shown by cancer cells *in vivo*, in particular mirroring the mechanical stimuli sensed by tumor cells in the early stage of pathological progression.

Specifically, we designed alginate-gelatin microcapsules, with dimensions ranging from 300 to 600 µm and an elasticity of around 25 kPa (expressed as Young modulus) to simulate the elasticity of early-stage breast tumors. Using breast cancer cells, we demonstrated that cells embedded in the semicrocapsules exhibit enhanced resistance to the anticancer drug cisplatin compared to cells cultured in traditional 2D flasks or as 3D suspended spheroids.

Our results show that in both 3D models, the resistance becomes apparent within two days post-seeding/immobilization and remains stable at least for four days. Additionally, the growth in the 3D model induces morphological heterogeneity, leading to the presence of polyploid cancer cells and microcells. Genetic assays also showed the upregulation of markers for hypoxia three days following seeding/immobilization, suggesting a potential correlation with the development of chemoresistance.

Furthermore, the study explores alterations in the actomyosin cytoskeleton, a crucial component associated with cell motility, migration, and invasion, all closely linked to the metastatic process. In particular, genetic assays showed the upregulation of actin in the cells preconditioned in the 3D model, suggesting a responsive and active adaptation to mechanical cues.

Overall, the results highlight the importance of 3D models in cancer research, providing a better representation of *in vivo* conditions, and contributing valuable insights into the malignant behaviour of tumor cells during cancer progression.

MECHANO-REGULATION OF MESENCHYMAL STEM CELLS USING BIO-METAMATERIALS

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Although ample evidence has shown that stem cells respond not only to biochemical cues but also to the mechanical properties of their *in vivo* microenvironments, most *in vitro* studies so far have utilized 2D hydrogel substrates. Until now, it is still challenging to fabricate artificial materials with welldefined anisotropic elastic properties for a biological application.

Using mechanical metamaterials fabricated by 3D laser printing based on two-photon absorption we have quantified the mechanical response of human mesenchymal stem cells derived from the human bone marrow to the anisotropic elastic properties (Young's Modulus and Poisson's ratio) of their microenvironment.

In this study, we designed three metamaterials based on unit cells that are sufficiently smaller than the investigated stem cells. A silicone elastomer-like photoresist was used to fabricate the networks, as the use of a "soft photoresist" has been proven to be mandatory for biological cells to experience the elastic properties. In addition to the traditional approach of morphology and immunocytochemical analysis, we also quantified the directional traction forces by converting displacement-vector fields into force-vector fields.

Notably, stem cells showed distinctly different morphological phenotypes and subcellular responses on the three metamaterial nets, indicating that cells feel not only the bulk properties but also the anisotropy of effective Young's Modulus and Poisson's ratio of the metamaterials.

The obtained data provide a positive proof of principle, suggesting the potential of rationally designed "bio-metamaterials" as a new tool to mechanically stimulate and control the shape and functions of various biological cells by the arrangement of unit cells.

DEVELOPMENT OF A OPTOFLUIDIC PLATFORM TO INVESTIGATE PHOTOTAXIS IN CYANOBACTERIA

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Vlad Badilita, Jan G Korvink

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Cyanobacteria, which are among the most ancient microorganisms, perform oxygenic photosynthesis. The high efficiency of photosynthesis incyanobacteria is based, among other things, on their efficient light-harvesting systems. Optimal light absorption is crucial for photosynthetic performance.

Therefore, several cyanobacteria can identify and translocate themselves to spots of optimal illumination. The underlying mechanistic aspects governing bacterial phototaxis have not yet been fully understood. The current hydrogel-based phototaxis assays, which are used to study these aspects, do not enable high precision control over the immediate microenvironment of a bacterium. Complete and precise control over the cell micro-environment would allow us better to elucidate the dependence of motility on various physicochemical parameters. The ability to have precise control over the micro-environment and the applied stimuli helps in recording reliable phototactic responses of the bacteria to optical stimuli.

Thus, there is a need to build novel devices to investigate surface-dependant Type IV pilus (T4P) motility exhibited by cyanobacteria. As a first step, the focus of this work is on understanding the surface properties suitable for twitching motility by rigorously studying different MEMS-compatible surfaces (SU8, PDMS and silane-coated glass) by measuring their contact angle, surface charge, surface chemistry, adhesive and retention force.

Our next goal was to achieve micro-meter precise control of the bacterium to observe its response to external stimuli. We designed and developed ADEPT (Adaptable Dielectrophoresis-based Electronics Platform Tool) to accomplish this, which controls the electrical signal applied to micro-electrodes in a microfluidic chip. The micro-electrodes are arranged circularly, and single cells can be trapped and positioned at different locations by independently controlling the six micro-electrodes phase, frequency, and amplitude. The next step is to integrate the optical stimuli into a microfluidic chip for spatially resolved excitation of a single bacterium.

FROM TISSUE ENGINEERING TO DRUG DELIVERY WITH 3D PRINTED SPIDER SILK

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Spider silk is a fascinating material and spiders are one of nature's best engineers. Human engineers can also utilize the biomaterial recombinant spider silk and use it for one of the most recent engineering skills: additive manufacturing. In this context, the field of bioprinting has an urgent need for a suitable matrix.
Therefore, natural polymers are preferred over synthetic materials, as they often show less toxicity and higher biocompatibility. In contrast to other materials, hydrogels out of recombinant spider silk proteins have the advantage of not needing any additives to be 3D printable. Their shear thinning and viscoelastic behavior, but also non-toxicity, biocompatibility, and biodegradability make them an ideal bioink. [1–3]
In this work, high shape fidelity of spider silk ink is shown by printing a human aortic valve, and high cell viability is shown by encapsulation of the cell line BxPC-3. Moreover, the influence of embedded cells on the rheological properties of the ink has been investigated. Here, we compare hydrogels made of different silk variants concerning their rheological behavior in the absence or presence of cells and their impact on the viability of cells when printed. It is shown that the recombinant spider silk protein eADF4(C16) and the RGD-modified variant thereof are suitable for bioprinting.
Next, spider silk hydrogels can also be used as drug depots. Here, a new processing route using aqueous-organic co-solvents for the incorporation of water-insoluble drugs is presented. The stability, drug release, and customization by 3D printing of these hydrogels are shown. Moreover, the encapsulation of drugs and cells can be combined with the help of producer cells. The fusion protein TNFR2-Fc-GpL can be produced by human embryonal kidney cells. This work shows the encapsulation of these cells in spider silk hydrogels followed by 3D printing. The bioprinted cells showed continuous release of functional TNFR2-Tc-GpL over 14 days. The investigations demonstrate that using spider silk to generate 3D constructs harbors endless opportunities and applications in the fields of bioengineering, regenerative medicine, and drug delivery.

[1] Lechner, A., Trossmann, V. T., Scheibel, T. 2022. Impact of Cell Loading of Recombinant Spider Silk Based Bioinks on Gelation and Printability. Macromolecular bioscience 22, 3, e2100390.
[2] Neubauer, V. J., Trossmann, V. T., Jacobi, S., Döbl, A., Scheibel, T. 2021. Recombinant Spider Silk Gels Derived from Aqueous-Organic Solvents as Depots for Drugs. Angewandte Chemie (International ed. in English) 60, 21, 11847–11851.
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DYNAMICS OF NONMUSCLE MYOSIN II ISOFORMS

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Nonmuscle myosin II (NM II) minifilaments play an essential role in various mammalian cell processes including migration and mechanosensing.

Depending on the cell type up to three isoforms get expressed – NM IIA, NM IIB and NM IIC – that have different characteristics.

To investigate their dynamics and the interplay between the three isoforms in U2OS cells an optogenetic system was used. Through this, minifilament formation could be locally activated and then followed by tracking fluorescence intensity of one of the isoforms. This gave insights into the dynamics of minifilament recruitment during activation and the dynamics of relaxation after stop of activation.

To better understand the interactions between the isoforms, experiments were conducted for each of them using wild type and the three isoform knock out cell lines. This confirmed previous findings that showed that NM IIA is the more dynamic isoform that acts in the short term while NM IIB minifilaments are more stable.

Furthermore, NM IIA plays a role in initiatingNM IIB minifilament formation. Interestingly, while the NM IIC-KO has no phenotype in U2OS cells, it was shown that it does affect the dynamics of the two other isoforms.

MINIMAL-INVASIVE 3D LASER PRINTING OF MICROIMPLANTS *IN ORGANISMO*

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Multi-photon 3D laser printing has gathered much attention in recent years as a means of manufacturing biocompatible scaffolds that can modify and guide cellular behavior *in vitro*. However, *in vivo* tissue engineering efforts have been limited so far to the implantation of beforehand 3D printed biocompatible scaffolds and *in vivo* bioprinting of tissue constructs from bioinks containing cells, biomolecules, and printable hydrogel formulations. Thus, a comprehensive 3D laser printing platform for *in vivo* and *in situ* manufacturing of microimplants raised from synthetic polymer-based inks is currently missing.

Here we present a platform for minimal-invasive manufacturing of microimplants directly in the organism by one-photon photopolymerization and multi-photon 3D laser printing. Employing a commercially available elastomeric ink giving rise to biocompatible synthetic polymer-based microimplants, we demonstrate first applicational examples of biological responses to *in situ* printed microimplants in the teleost fish *Oryzias latipes* and in embryos of the fruit fly *Drosophila melanogaster*.

This provides a framework for future studies addressing the suitability of inks for *in vivo* 3D manufacturing. Our platform bears great potential for the direct engineering of the intricate microarchitectures in a variety of tissues in model organisms and beyond.

DEVOLVEMENT OF HYDROGEL-BASED MATERIALS TO DISSECT THE MECHANOBIOLOGY OF RPE IN AGING

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As an aging population faces a growing challenge with the decline in function of essential senses like sight, the underlying processes in the eye must be understood to access lasting healthcare strategies.

Of special interest for us is the retinal pigment epithelium (RPE) which lies on a specialized extracellular matrix (ECM) and anchors photoreceptor cells in the outer retina. In contrast to most epithelia, RPE is a postmitotic monolayer, and naturally occurring cell death must be compensated either through rearrangement and/or enlargement of cells. Furthermore, in combination with the monolayer remodelling, the ECM dynamically changes, through the localized accumulations of extracellular material between the RPE and the ECM named “Drusen.”

My work aims to develop hydrogel-based tools to dissect the distinct role of mechanics in age-dependent outer retina remodelling both from the ECM and the RPE perspective. To investigate the influence of Drusen formation, we established a phototunable hydrogel, which can produce on-demand topography changes. This dynamic PEG-based hydrogel incorporates a bifunctional photodegradable crosslinker (PD-CL), which undergoes photolysis when illuminated with UV light (~365-385nm) and the subsequent breakdown reduces the mesh density of the hydrogel, leading to a local swelling.

Ultimately, the size of the artificial Drusen can be controlled by the illumination time or intensity and such artificial drusen could be emulated in a cell culture of human induced pluripotent stem cell derived RPE. To be able to control RPE monolayer remodelling as observed in ageing, we optimized hydrogel-based phantom cells containing the PD-CL, thus being able to control their swelling diameter through illumination with UV light.

These phantom cells are coated with recombinant E-Cadherins and then incorporated in RPE monolayers, where we expect that their on-demand softening will lead to an increase in mechanical anisotropy within the monolayer, mimicking age-related RPE remodelling.

APICO-BASAL POLARITY IN STEM CELLS

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The establishment of cell polarity is a key event for cell fate decision during embryogenesis and a prerequisite for differentiation. One factor known to establish apico-basal polarity are intrinsic forces exerted by non muscle myosin II (NMII) through contraction. Human induced pluripotent stem cells (hiPSCs) self-organize apical structures occurring during early embryogenesis *in vitro*. Thus, they could give insights into the interplay of cytoskeletal organization and pluripotency.

The aim of this work is to investigate the apical polarization in hiPSCs and the influence of external cues from the microenvironment on cytoskeletal organization. When cultivated as colonies, assemblies of hiPSCs display a cell-spanning actomyosin ring which encircles an inner population of hiPSCs. This population is defined by an enrichment of Ezrin and PODXL with varying degrees from cell to cell and a network of pronounced actomyosin fibres on the apical side. Co-immuno precipitation of Ezrin with PODXL shows an interaction of the two proteins. Introducing NMII inhibitor results in fracturing of the cell-spanning ring and decreasing in Ezrin signal. In addition, the fracturing shows to affect ratio of transcriptions factor in hiPSCs, leading to early differentiation after 24h of inhibitor treatment. The actin ring is a highly dynamic cytoskeletal structure which is also affected by the microenvironment. This was visible in 2.5D scaffolds, which function as a mechanical barrier, as well as in hemispherical cavities (3D scaffold). The colony integrity is maintained by the stiffness of the microenvironment. Thus, it helps to compensate the contractility tension while sustaining pluripotency.

In hiPSC colonies, the results highlight the importance of contractility tension exerted by NMIIB to maintain integrity, plasma membrane tension and pluripotency. It is interdependent from membrane tension presumably exerted by the Ezrin-PODXL complex. In addition, it was shown that scaffold stiffness plays a crucial role on cytoskeletal organisation.

CELLULAR MECHANOBIOLOGY IN 3D SCAFFOLDS

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It is well known that cells can react to mechanical cues to read out and adapt to the physical properties of their surrounding. By the use of defined microscaffolds, we aim to entangle the effects of the physical environment on the respective cellular response. For this, we employ a set of approaches which target different aspects of cellular mechanobiology.

First, so called metamaterials are used to analyse the influence of substrates with different physical parameters onto cellular force generation. By combining live cell imaging, force field analysis and visualisation of mechanoresponsive biological markers, this novel approach allows the linkage of physical measures to biological observations. Recently, this system was used to demonstrate an effect of metamaterials on the mechanobiology of mesenchymal stem cells

Further, modulators of the cellular force generation are examined regarding their role in sensing and adapting to the the cellular environment. To determine the role of non-muscle myosin II (NMII) isoforms in polarisation, substrates with aligned (aGNF) or random gelatine nanofibres (rGNF), fabricated by electrospinning, are used. Results indicate, that upon knock-out of NMIIA or NMIIB in osteosarcoma cells, polarisation along the aGNF was impaired. Additionally, migration on 2D and within 3D collagen gels was analyzed. Knock-out of NMIIA or NMIIB affected migration speed in 2D and 3D collagen gels, whereas persistance was only impaired after NMIIA KO for migration in 3D collagen gels.

Lastly, one additional factor in 3D migration, confinement, should be analysed. To this end, structured chambers have been fabricated by laser nanoprinting and hot embossing and show sufficient optical properties for live cell imaging.

In conclusion, defined microstructures are fabricated to control aspects of the cellular environment like mechanical properties, geometry and confinement. The individual impact of these parameters on cellular force distribution, polarisation and migration is to be determined, especially in the context of a potential regulatory influence of NMII isoforms.

MECHANOBIOLOGY OF THE OUTER RETINA DECIPHERED USING A 3D HIPSC DERIVED *IN VITRO* MODEL

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The retinal pigment epithelium (RPE) is a monolayer of pigmented cells located between the neurosensory retina and the choroid. The RPE performs several critical functions in the visual process including light absorption, blood retinal barrier formation, processing of vitamin A metabolites, and phagocytosis of photoreceptor outer segments (POS).

On its basal side, the RPE adheres to Bruch's membrane, the extracellular matrix (ECM) layer that separates the epithelial layer from the choroid. On its apical side, RPE closely interacts with the POS, ensuring their homeostasis and vision function. We hypothesize that the composition and age related remodeling of the ECM beneath the RPE can affect the mechanics of the epithelium, in turn affecting the RPE photoreceptor functional interaction.

In a recently published work of our group we have shown that ECM defined RPE contractility modulates the phagocytic efficiency of RPE. The current project aims to deepen our understand ing of ECM's contribution to retinal mechanobiology in a human stem cell derived *in vitro* model. To assemble this model I culture hiPSC derived RPE on a synthetic and tunable polyacrylamide gel, whose stiffness and coating can be controlled.

On top of the RPE, CRX+ photoreceptor progenitors are co cultured after successfully differentiating them from hiPSCs. To sustain the 3 D construct and promote the maturation of POS, I embed the photoreceptor progenitor cells in an injectable hyaluronic acid hydrogel to mimic the interphotoreceptor matrix present *in vivo*. Finally, the hydrogel contains magnetically orientable microgel rods with the aim of guiding the growth of POS towards the RPE.

With the co culture model in place, we will then systematically modify the synthetic RPE substrate and study its effects on the RPE photoreceptor interaction, which is crucial for vision.

LATE NEURORETINAL ORGANOIDS REVERT TO RUDIMENTARY SELF-ORGANIZATION IN THE ABSENCE OF PROPER EXTERNAL SIGNALING

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Retinal organoids (RO) have proven to be powerful *in vitro* models for basic and applied life science research, mimicking a large variety of characteristics of the *in vivo* retina. Yet to this day, RO fail to fully emulate *in vivo* retinas in all aspects like cell type diversity, patterning and ultimately functionality due to a lack of understanding how to guide RO with external cues throughout all developmental steps necessary.

Here we employ RO grown from primary pluripotent embryonic stem cells of medaka fish generating *in vitro* neuroretinal cell types in the developmental time frame of the embryonic retina. We show that the patterning process observed in this neuroretinal tissue leads to a strikingly different organization of the differentiating cells compared to the stereotypic layering of the embryonic retina. In the RO, the differentiating retinal cell types, including photoreceptor cells, organize from broadly distributed neural retinal progenitor cells across the volume of the retinal organoid to forming rosette-like structures comprised of around 6-8 cells. These cellular arrangements along with the reduction of retinal cells, their grouping and prominent spacing indicate self-organizational properties of the retinal cells in the RO likely caused by the absence of sufficient external signaling cues from the surrounding embryo. Over the course of differentiation of the RO, the overall polarity of the tissue gets lost and named rosette-like small substructures, reminiscent of neuroretinal cell arrangements of the evolutionarily lower invertebrates, are formed with a polarized cellular orientation.

By assessing the overall dynamics, organization and potential functional characteristics of the observed process, we hope to unveil the minimal self-organizational arrangement retinal cell types are by themselves capable of and thus, uncover which attributes of retinal patterning are guided by external signaling from a developing embryo. Understanding these features could eventually allow for the design of specialized 3D molecular and physical environments to improve organoid culture at large.

DEVELOPMENT OF PNIPAM-ACTUATED ARTIFICIAL JOINTS BY DIRECT LASER WRITING (DLW)

Annabelle Sonn

Barbara Schamberger, Gaurav Dave, Chantal Barwig, Christine Selhuber-Unkel

Institute for Molecular Systems Engineering and Advanced Materials (IMSEAM), Heidelberg University, Germany

Soft actuators based on responsive materials like polymers, fluids, or hydrogels are flexible components adaptable to dynamic environments.

Thermoresponsive poly-N-isopropylacrylamid (pNIPAM) is an excellent candidate for soft robotic applications. pNIPAM is hydrated and swollen below its lower critical solution temperature (LCST), which is 32 °C, while it shrinks above the LCST resulting in its dehydrated state.

Two-photon-polymerization direct laser writing (DLW) makes use of the absorption of two near-infrared (NIR) photons to initiate the polymerization of the hydrogel ink with high spatial control in a confined volume. This enables the generation of complex 3D structures on the microscale, in our case made from polyacrylamide (PAM) and pNIPAM.

Here, we show first steps towards the design and printing of artificial joints with pNIPAM actuators in the micrometer range. The structures are fabricated in two-steps: First a PAM base is printed, which is then surrounded by pNIPAM, mimicking muscle tissues. After printing, the structures are characterized, and future work comprises the actuation of the joints by temperature change. This proof-of-concept work builds the foundation for multi-material, thermoresponsive microgrippers printed by DLW.

Poster Presentation only

THE ROLE OF THE EXTRACELLULAR MATRIX IN RETINAL CELL FATE SPECIFICATION AND RETINAL ORGANOID DEVELOPMENT

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The extracellular matrix (ECM) is important for many processes occurring in an organism. It gives cells structural integrity, maintains homeostasis and communication between them and plays an important role in stem cell differentiation, which is fascinating, considering the fact that most of the ECM components are highly conserved across species lines.

Here, we look into the specific role the ECM plays in stem cell differentiation especially towards retinal fate and in retinal organoid development. As a model we use embryos of the teleost fish *Oryzias latipes* (Medaka fish) and extract, decellularize and chemically modify the ECM of the embryonic eye (dECM) and focus our attention on two main experimental approaches. The first one is the *de novo* assembly of broken down dECM via multi-photon 3D laser printing after functionalization to investigate the differentiation behavior of embryonic stem cells, presented with this ECM scaffold, creating biomimetic 3D microenvironments that are customizable in their structural arrangements while still retaining their favorable biochemical properties for embryonic retinal tissue development.

In the second experimental approach we focus on retinal organoids derived from *Oryzias latipes* pluripotent embryonic stem cells. Retinal organoids are standardly supplemented with ECM formulations (commonly *Matrigel®*) to assist their development. Interestingly, the embryonal dECM still contains growth factors and differentiation signals that are specific to the embryonal stage and the tissue (retinal tissue in this case), so we aim to use cut-down dECM as a novel cell culture supplement suitable for retinal organoids. In this way we would like to improve the organoids development beyond what is currently achievable with the help of commercially available cell culture supplements.

Implementing these approaches could promisingly expand the retinal organoid bioengineering methods to create a more *in vivo* like model.

3D PRINTED BIOSENSORS FOR STUDYING CARDIOMYOCYTE ELECTROPHYSIOLOGY.

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Printed electronics are an emerging tool to create micro- and nano-hybrid elements for electrical devices, e.g. in flexible and wearable electronics, bioelectronic devices, and biosensors for biological signal detection. In comparison to conventional approaches, the key advantages are low cost and high manufacturing volume. The goal of this research project is to create a fully printed three-dimensional device that can be used to stimulate and measure signals from cardiomyocytes.

Heart disease is a major public health concern with still often unclear causes. Heart failure can result from both chronic and acute cardiac injuries, which are defined by a decrease in cardiac contractility and insufficient blood supply. In North America, heart failure complications are the primary cause of hospitalizations, deaths, and disability. It is critical to study heart cells at many scales, from single cardiomyocytes to 2D cell monolayers and 3D cardiactissues.

Current methods for sensing heart cells, such as Patch Clamp, Voltage Sensitive dyes, Microelectrode arrays (MEAs), and Microfluidic devices, suffer from being (partly) immature techniques, usually rather expensive, and invasive. This project focuses on developing noninvasive biosensors using fully printing techniques to measure the electrophysiological properties of heart cells.

The project is divided into three working packages. First, suitable materials compatible with cardiomyocytes, such as polymers, hydrogels, Au, Pt, ITO, andPEDOT:PSS, will be identified/developed. Second, an electronic device acting as an interfacing probe, specifically Electrolyte-gated transistors (EGTs), will be designed due to their stability, low voltage operation, and signal amplification capabilities. Third, the devices will be fully printed using additive manufacturing methods like inkjet printing, capillary printing, and two-photon polymerization.

Developing such a hybrid bioelectronic structure paves the way toward the construction of advanced devices such as labs on a chip for research and drug-screening, based on fully printed biosensors.

PHOTOSWITCHING FOR CONTROLLING CELL ADHESION

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Cells dynamically adjust their adhesion behaviour to their environment. Mechanical forces can activate a restructuring of adhesion sites. However, most mechanical stimuli directly involve the cytoskeleton.

In our previous work, we apply an oscillatory stimulus on a single adhesion receptor instead [1]. We make use of photoresponsive azobenzenes that oscillate between two isomeres upon irradiation by green (530 nm) light to create a new type of mechanical stimulus on the cell. This stimulation is established by letting the cell adhere to a glass slide functionalised with push-pull azobenzene with an RGDfK headgroup as a binding ligand and a poly ethylene glycol background. While being irradiated, the azobenzenes change rapidly between their two states, creating oscillatory type of stimulus.

Our measurements are based on Atomic Force Microscopy (AFM) to measure how the cell adhesion force changes in response to the mechanical stimulus. We showed that light irradiation lead to a reversible increase in the cell adhesion force.

Our current research focuses on quantifying the effects of mechanical stimulation. We transition to more stable azobenzenes to gain an improved time control of the photoswitching process. Simultaneous AFM and total internal reflection fluorescence (TIRF) microscopy measurements allows an improved resolution of the interface of the cell and our functionalised surface. With the improved setup we will investigate a possible memory effect of the cells after being subject to mechanical stimulation allowing a more long-lasting effect of the azobenzene oscillation.

[1] Kadem, L. F. et al (2017). Angewandte Chemie International Edition, 56 (1), 225-229.

HEAT SHOCK TREATMENT INCREASES CELL VIABILITY AFTER BIOPRINTING

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Bioprinted constructs are becoming more complex as the field progresses, but one limiting factor is the lack of nutrients and O_2 in larger constructs. A potential strategy for increasing the availability of nutrients and O_2 is to incorporate a vasculature.

Using bioprinting to create vascularized biomimetic systems is a promising method, however stress introduced by the printing process can change cell structure and behavior. Heat shock (HS) response is a conserved cellular reaction to environmental stressors resulting in the expression of HS proteins, which prevent or reverse protein misfolding. Here, we use a tube formation assay to determine angiogenic potential of printed endothelial cells with and without HS pre-treatment. Using HS treatment prior to bioprinting, we aim to stabilize the cell cytoskeleton to increase cell survival during the bioprinting process and examine its effects on angiogenic potential.

Endothelial cells were encapsulated in hydrogels, then printed using a drop-on-demand bioprinter, or casted by hand. Morphology and protein levels were analyzed using IF with confocal fluorescent microscopy. Cell painting was used to concurrently stain multiple cellular organelles. Protein expression was examined using qPCR.

Printed cells show less tube formation, whereas HSP samples retain some angiogenic potential post-printing, forming more tubes than printed samples. Levels and location of proteins related to cell adhesion also vary between printed and control samples +/- HS as indicated by IF and quantitative image analysis. Analysis of the number and volume of various organelles across conditions show that printed cells differ from control and HSP cells.

The effects of the shear stress during the printing process cause damage to the cytoskeleton, a reduction of cell adhesion factors, and alters angiogenic potential. HS treatment increases cell viability immediately post-printing and restores other aspects of cellular behavior. Pre-treating cells using HS could be a fast, easy, and inexpensive way to increase cell viability in bioprinted constructs.

AZOBENZENE-MEDIATED PHOTOMECHANICAL STIMULATION OF CELLS

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Stimulus-responsive, biocompatible materials can be used to design platforms to remotely study and manipulate cellular processes for applications in bioengineering and soft robotics. We have developed an azobenzene-based photoswitchable scaffold that can exert nanoscale oscillatory forces on cells by means of rapid cis-trans isomerization of a push-pull azobenzene upon irradiation with visible light.

The biochemical effects of such photomechanical stimulation were analyzed by gene expression studies, which revealed that adhesion-associated genes such as talin, vinculin, paxillin and zyxin get upregulated due to stimulation for as low as 5 minutes.

Besides cell adhesion, we also aim to study the effects of such photomechanical stimulation on the process of stem cell differentiation, which is also crucially regulated by mechanotransductive pathways. We hypothesize that the frequency and duration of the force stimulus, which can be controlled by variable laser pulses, will play an important role in engineering these cellular processes. Such a novel platform opens up possibilities of using light to “write” cellular functions at single-cell or even subcellular resolution without any genetic modifications.

MICROPRINTING OF RESPONSIVE BIOMATERIALS

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Tissue engineering is a rapidly growing field of interest for research and development of biological and biomedical applications. This field focuses on investigating cell interactions in and with biocompatible scaffolds. For this purpose, designing and shaping of these cell scaffolds – especially with modern 3D printing techniques – is getting more and more attention recently. Two-photon 3D laser printing, for example, offers the possibility to fabricate cell scaffolds with micrometer, i.e. subcellular, resolution. New biocompatible inks for the fabrication of these scaffolds based on biomaterials could mimic the surrounding extracellular matrices and increase our understanding of fundamental processes.

Here, we present a new protein-based ink for two-photon 3D laser printing offering stable 3D hydrogels as cell scaffolds. In this work, we have studied the biocompatibility and structure of our biomaterial after printing. Our system facilitates can be processed with existing printing protocols making it suitable for microstructuring in commercial setups and at room temperature conditions. Moreover, we have investigated the intrinsic structure of our printed material which can now be used for designing and tuning future responsive biomaterials.

IMAGE-BASED CELL SORTING BY PHOTOPOLYMERIZATION

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Image-based cell sorting methods are in high demand to isolate subpopulations of cells based on their phenotypical features. However, existing methods often suffer from low throughput and they rely on a single snapshot and/or fluorophore conjugation.

Based on our experience with photopolymerization for synthetic cells[1], we here introduce an automated, high-throughput cell sorting method utilizing spatially controlled photopolymerization within an image-based cell screening and classification framework on a microscope platform. Through targeted illumination, undesired cell populations are encapsulated into hydrogel structures, effectively enlarging them for subsequent passive physical sorting.

We benchmarked our method using fixed cells and demonstrate successful sorting of living cells employing a cytocompatible photoresist. Crucially, hundreds of cells per second can be targeted simultaneously based on microscopy images. This system can potentially be used to sort cells label-free and based on time-dependent processes.

Our synchronized photopolymerization approach offers a high-throughput solution for cell sorting on a microscope, broadening the spectrum of sorting triggers of existing high-throughput techniques to include spatial and temporal dimensions.

[1] Abele et al. (2021); Diedrich, Brosz, Abele et al. (submitted)

REAL-TIME MEASURING OSMOTIC PRESSURE OF MICRO-TISSUES WITH LIPOSOMAL FRET SENSORS

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Internal and external mechanical forces are essential in guiding cell behaviour during tissue formation. It has been demonstrated that cytoskeletal fibres can transmit self-generated forces to the extracellular matrix (ECM) (1). Tissue forces can originate not only from external loads but also internal osmotic pressure. It is known that in collagenous tissues osmotic pressure can generate high tensile stresses (2).

Measuring local osmotic pressure *in vitro* is still an experimental challenge. Our custom-made dye-loaded liposomal osmotic pressure sensors can be used to monitor the role of osmotic effects on tissue formation (3). The working principle of these sensors is to detect shape changes of liposomes in response to changes in osmotic pressure, which can be read out as a change in Förster resonance energy transfer (FRET) efficiency depending on the average distance between the entrapped dyes. We have shown that these sensors are applicable to cell culture in short term experiments (4). With this study, our aim is to apply these sensors to the cell culture during *in vitro* collagen formation.

For this experiment, MCT3T3-E1 pre-osteoblast cell line (seeding density 10^5 cells/cm^2) was used in 96 well plates. Confocal microscope was used to measure the FRET signal and images were taken every 3-4 day over several weeks. After each imaging, new sensors from the same batch were added to the fresh cell medium. The images were evaluated in terms of mean intensities and intensity distributions with a self-written MATLAB code. Knowing the osmotic pressure while new ECM is forming provides information about possible mechanical interactions between the cells and the ECM.

(1) Bidan, C. M., ..., Fratzl, P & Dunlop, J. W. (2016). Journal of the Royal Society Interface
(2) Bertinetti, L., ..., & Fratzl, P. (2015). Journal of the mechanical behavior of biomedical materials
(3) Zhang, W., ..., & Fratzl, P. (2021). Angewandte Chemie
(4) Zhang, W., ..., Yavuzsoy, E. C., ..., & Fratzl, P. (2023). Advanced Healthcare Materials

MECHANICAL PHENOTYPING OF COLORECTAL CANCER ORGANOIDS

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Ina Braun[5], Tim Karrasch[5], Johannes Betge[4,6], Mathias Ebert[4,7], Kerstin Göpfrich[5], Michael Boutros[1-4]

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Colorectal cancer is amongst the most prevalent forms of cancer in the world. Several therapeutic strategies are explored including surgery, radiation and treatment with chemotherapeutic compounds. Lately more tailed treatments towards individual patients are explored. The response to therapy is however not only dependent on the mutational background of the tumor but also based on the physical properties of the tumors.

In the lab we culture patient derived organoids (PDOs) of colorectal cancer and can use this tool to address various points such as the mutational profile, expression profile and drug sensitivity in high throughput screening platforms. We used real-time deformability cytometry (RT-DC) to mechanically characterize colorectal cancer PDOs.

We could identify variable stiffnesses amongst the 8 lines. Furthermore, we performed bioinformatical analysis on available drug sensitivity data, transcriptome data and information on the mutation status to identify correlations between stiffness and drug sensitivity and gene expression.

Future experiments are aimed to sensitize and de-sensitize PDOs to drug treatment by altering the physical properties and correlate stiffness measurements with biological markers.

THE ROLE OF MECHANOSENSING IN FUNCTIONALITY OF NATURAL KILLER CELLS AND T CELLS

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In the context of solid tumor, the tumor cells are often softened relative to their non-malignant counterparts. How softening of tumor cells influences immune surveillance, especially functionality of natural killer (NK) cells and T cells, is not well understood.

Regarding NK cells, we found that NK cell killing efficiency in 3D was impaired against softened tumor cells, while it is enhanced against stiffened tumor cells. Furthermore, we found that the durations required for NK cell killing and detachment are significantly shortened for stiffened tumor cells. Additionally, we identified Piezo1 as the predominantly mechanosensitive ion channel expressed in NK cells.

We observed that perturbation of Piezo1 abolished stiffness-dependent NK cell responsiveness, significantly impaired the killing efficiency of NK cells in 3D, and substantially reduced NK cell infiltration into 3D collagen matrices. Conversely, Piezo1 activation enhances NK killing efficiency as well as infiltration.

Regarding T cells, we found that T cell polarization, characterized by MTOC reorientation towards the T cell/tumor cell contact site, was impaired by softening of tumor cells. This stiffness-regulated T cell functionality was mediated by Piezo1.

Our findings highlight the crucial role of Piezo1-mediated mechanosensing in functionality of both NK and T cells. These findings provide valuable insights into the intricate mechanisms governing immune cell responses and identify potential targets for enhancing immunotherapeutic approaches.

GELATIN-BASED HYDROGELS WITH GRADIENT STIFFNESS FOR 3D MOTILITY AND DUROTAXIS STUDIES

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Cells sense and respond to their mechanical microenvironment through direct physical interactions involving cell surface receptors and the extracellular matrix. Deregulated tissue mechanics are suspected as major drivers of prominent diseases (e.g. fibrosis, cancer). Therefore, the elucidation of mechanotransduction mechanisms, i.e. the processes through which cells interpret mechanical signals and transform them into biochemical signals with functional outcomes, have taken center stage in cell biology research.

On traditional, flat substrates, coated with adhesive ligands, cell motility has been shown to depend on both the ligand density and the viscoelastic properties of the substrate. As a consequence, cells tend to migrate along gradients of these properties. In a more physiological 3D setting, where cells are encapsulated inside a hydrogel material, such processes are confounded by difficulties in independent control over these parameters and the additional consideration of available space for cell movements.

Here, we present a simple methodology to prepare gelatin acryloyl hydrogels of a fixed concentration, but exhibiting a gradient in viscoelastic properties. This is achieved by varying the light exposure of a precursor, cell-laden solution, thereby modulating the conversion of the radical photo-polymerization.

We demonstrate the biocompatibility of our approach and the functional effect on cell motility using fibroblasts as a model cell system. Our results suggest that in absence of any chemotactic gradient, the pore size of the hydrogel material is the dominant parameter that regulates cell motility.

SNAKE-SKIN INSPIRED AUXETIC DESIGN AS A CANDIDATE FOR MECHANICALLY ADAPTIVE SCAFFOLDS FOR CELLS

Gaurav Dave [1]

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It is well established that the interplay between cells and the mechanical properties of the environment plays a important role in tissue engineering. However, traditional approaches have been in the direction of governing/controlling cells via external stimuli (e.g. chemical, mechanical, optical, temperature) delivered by the synthetic scaffolds. In the current project, our aim is taking the alternative route of letting the cells govern the scaffold. To achieve this goal, we design and fabricate scaffolds for cells that can be deformed by cells via traction forces.

The scaffold showcased here is a snake-skin inspired kirigami based auxetic meta-material design that has been adapted into 3D micro-structure. Similar to how, a snake-skin has scales on the surface (stiff motif) which are connected via soft tissue (soft motif); stiff motifs provide structure and protection from the terrain whereas, the soft motifs provide flexibility for movement. Auxetic patterns undergo deformation via bending of soft motifs instead of straining of the material which results in significantly lower stress response to deformation on a structural scale.

The micro-scaffolds are 3D printed via two-photon polymerisation. This technique uses a near-infrared laser to achieve photo-crosslinking of polymer resins. With the technique we can achieve submicron feature sizes for the printed structures. This allows us to fabricate structures that can be perceived as truly 3D environments for cells. Mechanical characterization of the design on the macroscale (mm to cm) for several polymeric materials have shown an 80-90% drop in stiffness as compared to the bulk material. Such low structural stiffness allows us to use a wide range of materials since the mechanical properties can be modulated by design alterations rather than material chemistry. Preliminary results prove that the scaffold is biocompatible and is deformed by cells.

Study of cell-scaffold interaction in such a system will give us an insight into how cells behave in a 3D auxetic environment, and the kind of forces cells exert in an individual as well as collective form on their surroundings.

PRECISION MICROACTUATORS: HARNESSING MICROFIBER SKELETAL MUSCLE CELLS WITH DIRECT LASER WRITING

Mohammadreza Taale

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In the cutting-edge field of biomedical engineering, microactuators have emerged as a cornerstone for developing sophisticated microrobotic systems and precision-targeted drug delivery mechanisms. Among these innovations, the precision movement facilitated by microactuators—through the conversion of various forms of energy into mechanical work—mirrors the essential muscle functions in microrobots for tasks including gripping, movement, and environmental sensing within confined spaces. A forefront of this innovation is the application of bioinspired actuator technologies, especially those utilizing living muscle cells, to emulate natural muscular movements in soft robotic applications. Here, the C2C12 mouse myoblast cell line plays a crucial role, demonstrating significant potential in bio-actuator development by differentiating into skeletal muscle cells, thus providing kinetic energy and enhanced controllability for bio-actuator functionality.

Central to our study is the novel encapsulation strategy of C2C12 cells within a gelatin-based hydrogel, meticulously engineered into microtube structures with a uniform diameter of 50 µm and varying lengths. This microfabrication technique leverages Direct Laser Writing (DLW) to achieve precise structural configurations, ensuring optimal cell viability and the promotion of myotube formation. Our analysis focuses on the impact of the DLW process on cell orientation, nuclear morphology, and the effective actuation of differentiated, multinucleated myotubes through electrical stimulation. By elucidating the technical and biological implications of our microtube encapsulation strategy, we present a significant advancement in microrobotic systems.

Our findings not only underscore the viability and functional potential of C2C12 cells within gelatin-based hydrogel microtubes but also highlight the innovative approaches undertaken to navigate the challenges in bio-actuator development. This abstract offers a glimpse into our pioneering research, emphasizing the methodological innovations, preliminary results, and the broader implications of our work in advancing microrobotic applications through bioengineered actuation mechanisms.

POST-BIOPRINTING DELIVERY OF BIOMOLECULES IN 3D TISSUE-LIKE CONSTRUCTS USING SYNTHETIC VESICLES

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Tissue engineering aims to restore or replace damaged human tissue by fabricating human-like tissue replicas. Bioprinting is a tissue engineering tool used for the automated bioassembly of 3D human-like tissues in a layer-by-layer fashion. The combination of synthetic cell-like constructs, such as micron-sized giant unilamellar vesicles (GUVs), with bioprinting could be of interest in tissue engineering to enhance the post-printed functionality of the fabricated tissue, and potentially replace human cells in the future.

Here, we demonstrated the use of GUVs for the controlled delivery of biomolecules via light in a spatiotemporal manner post-bioprinting. To increase physiological stability, GUVs were PEGylated, and equipped with the photosensitizer chlorin e6 to enable pore formation upon illumination. Photosensitizer and biomolecules were loaded into the lumen of GUVs, then encapsulated in agarose/collagen bioinks, and bioprinted using drop-on-demand (DOD) to fabricate millimeter-sized 3D tissue structures. After bioprinting, the release of biomolecules into the surrounding tissue was initiated using a light source with a 357 nm wavelength.

We demonstrated the feasibility of using GUVs in DOD-bioprinting and delivering of biomolecules post-printing triggered with an external stimulus. This shows that synthetic vesicles can serve as a powerful tool in bioprinting, in conjunction with cells for the delivery of growth factors, drugs, and nutrients, and incorporating additional biological functions. In the future, such synthetic cell-like constructs can even aim to replace human cells in artificial tissues. Overall, synthetic vesicles offer a variety of applications in tissue engineering and regenerative medicine.

We hope to see you again at

Future 3D Additive Manufacturing | The 3DMM2O Conference
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Dear Colleagues and Friends,

We want to take this opportunity to thank everyone involved in this conference: the invited speakers as well as the participants with or without oral and poster presentations, and our sponsoring partners Nanoscribe GmbH & Co.KG, Cellink and HETEROMERGE GmbH. Futhermore, the conference would not have been possible without the funding by the Deutsche Forschungsgemeinschaft (DFG) within the Excellence Strategy as well as the Carl Zeiss Foundation.

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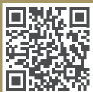
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