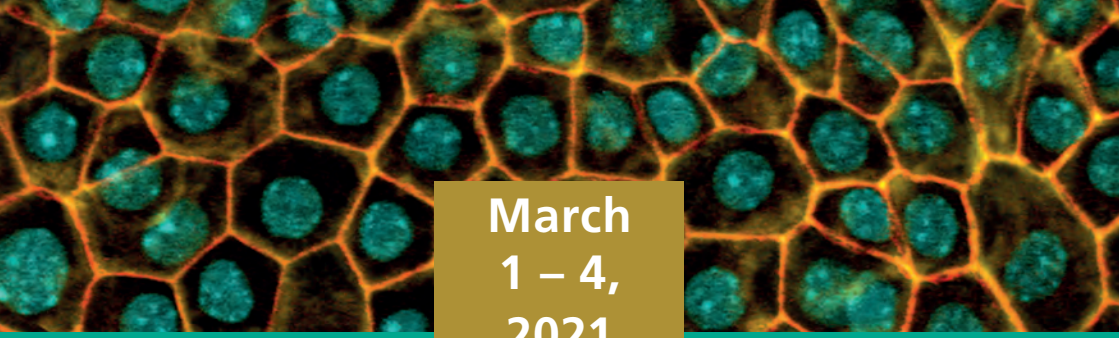
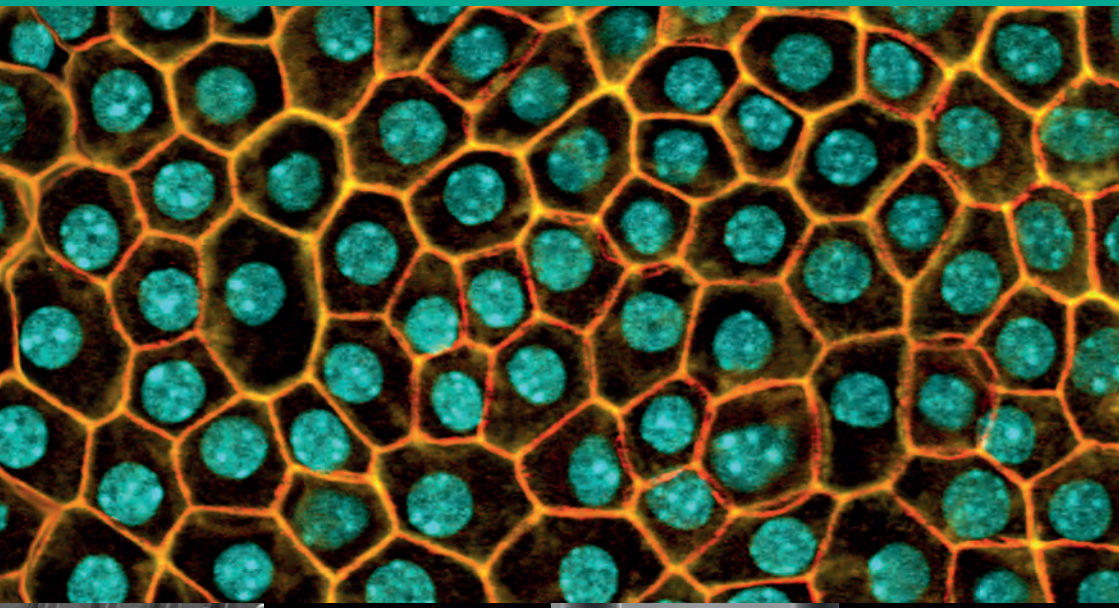
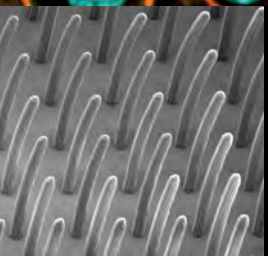


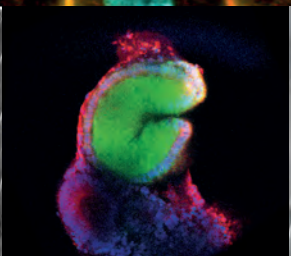
**March 1 – 4, 2021**

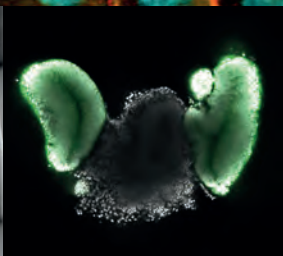
**Future 3D Additive Manufacturing |** The 3DMM2O Conference 2021

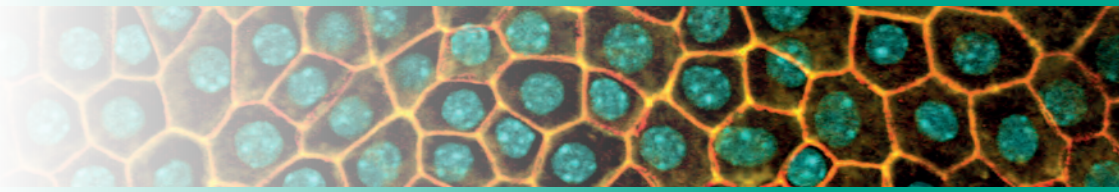
# 3D Hybrid Organotypic Systems
## Abstract Booklet











# 3D Hybrid Organotypic Systems

**2021 ONLINE!**

**March 1 – 4, 2021**

## Welcome

## Dear Colleagues and Friends,

it is our great pleasure to welcome you to the virtual
**Future 3D Additive Manufacturing – The 3DMM2O Conference 2021: 3D Hybrid Organotypic Systems.**

This is the first iteration of the annual conference on topics surrounding 3D Additive Manufacturing 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 biological research field of the Cluster of Excellence focuses on next-generation 3D scaffolds, which allow to direct the assembly of 3D organotypic systems starting from pluripotent stem cells. The mammalian retina has been selected as a model system, since it is the best understood vertebrate neuronal sensory organ with most cell types and their connectome being described.

The conference aims to foster discussions on innovative and interdisciplinary approaches in the fields of bioprinting, neuronal organoids, development and physiology of the retina. Lectures, poster sessions and informal gatherings will give you the opportunity for inspiring and fruitful scientific discussions and networking.

**Martin Bastmeyer** and **Joachim Wittbrodt,**
Organizers

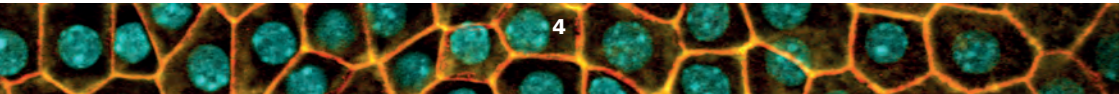
# Conference Structure

The program offers oral presentations of international experts as well as sessions for poster presentations, selected talks and flash talks of participants. They will cover a broad range of different topics of the conference research field, future trends and emerging technologies.

The conference also provides the opportunity for discussion and networking during the Q & A sessions after each talk, the lunch breaks or the coffee breaks. The chat functions offer the best conditions for virtual meeting and exchange.

## March 1

| | | |
|---|---|---|
| **8:00 AM – 8:30 AM** | Registration | |
| **8:30 AM – 8:45 AM** | Introduction to Technical and Organizational Details | Jorinne Sturm |
| **8:45 AM – 9:00 AM** | Opening & Welcome | Prof. Dr. Martin Bastmeyer & Prof. Dr. Joachim Wittbrodt |
| **9:00 AM – 9:35 AM** | The Cluster 3DMM2O: 3D Additive Manufacturing Driven Towards the Molecular Scale | Prof. Dr. Martin Wegener |
| **9:35 AM – 10:10 AM** | Formation of the Vertebrate Retina – an Engineering Challenge | Prof. Dr. Joachim Wittbrodt |
| **10:10 AM – 10:45 AM** | 3D Laser Nanoprinting for Cell Biological Applications | Prof. Dr. Martin Bastmeyer |
| **10:45 AM – 11:00 AM** | Coffee Break / Active Break | |
| **11:00 AM – 11:35 AM** | Piconewton-Sensitive Biosensors to Investigate Adhesion Mechanics in Cells | Prof. Dr. Carsten Grashoff |
| **11:35 AM – 12:10 PM** | Standardized Hydrogel Beads and Their Applications in Mechanobiology | Prof. Dr. Jochen Guck |
| **12:10 PM – 1:00 PM** | Lunch Break | |
| **1:00 PM – 2:00 PM** | Flashtalks | |
| **2:00 PM – 3:00 PM** | Poster Session I | |



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## March 2

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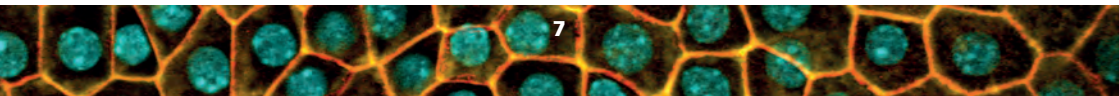
|---|---|---|
| **1:30 PM – 1:35 PM** | Welcome & Introduction Day 2 | Prof. Dr. Martin Bastmeyer & Prof. Dr. Joachim Wittbrodt |
| **1:35 PM – 2:10 PM** | Modelling Cell Shape and Dynamics in Three-Dimensional Scaffolds | Prof. Dr. Ulrich Schwarz |
| **2:10 PM – 2:45 PM** | Collective Cell Order Maintains Homeostasis of Human Cornea: A New Physical Biomarker for the Quality Control of Cell Culture and the Prediction of Long-Term Prognosis | Prof. Dr. Motomu Tanaka |
| **2:45 PM – 3:20 PM** | Integrated Organ-On-A-Chip Systems: Microphysiological Platforms Recapitulating Complex Human Tissue | Jun.-Prof. Dr. Peter Loskill |
| **3:20 PM – 3:55 PM** | Building the Retina One Cell at a Time | Seth Blackshaw, Ph.D., Professor |
| **3:55 PM – 4:10 PM** | Coffee Break / Active Break | |
| **4:10 PM – 5:10 PM** | Poster Session II | |
| **5:10 PM – 5:45 PM** | 3D Models of Retinal Development: Organoids and Retinospheres | Thomas A. Reh, Ph.D, Professor |
| **5:45 PM – 6:20 PM** | Retinal Organoids – Non-Invasive Testing of Metabolic Function and Sheet Transplantation | Magdalene J. Seiler, Ph.D., Associate Professor |
| **6:20 PM – 6:55 PM** | User Programmable Hydrogel Biomaterials to Probe and Direct 4D Stem Cell Fate | Cole A. DeForest, Ph.D., Assistant Professor |
| **6:55 PM – 7:15 PM** | From Visions Towards Scenarios for Future 3D Additive Manufacturing in Society | Prof. Dr. Andreas Lösch, Max Roßmann, Dr. Christoph Schneider |

# March 3

| | | |
|---|---|---|
| **1:30 PM – 1:35 PM** | Welcome & Introduction Day 3 | Prof. Dr. Martin Bastmeyer & Prof. Dr. Joachim Wittbrodt |
| **1:35 PM – 2:10 PM** | Macro-Scale Tissue Self-Organization Through Organoid Bioprinting | Prof. Dr. Matthias Lutolf |
| **2:10 PM – 2:45 PM** | 3D Organ-On-A-Chip Models: Reconstruction of Retinal Blood Barriers | Prof. Dr. Ute Schepers |
| **2:45 PM – 3:00 PM** | Selected Talk: 3D Bioprinting of Functionally Graded Biomaterials | Aurelien Forget, Merve Kuzucu, Grace Vera, V. Prasad Shastri, Marco Beaumont |
| **3:00 PM – 3:15 PM** | Selected Talk: Programmable DNA Materials for Life Science Applications | Carmen M. Domínguez, Yong Hu, Christof M. Niemeyer |
| **3:15 PM – 3:20 PM** | Break | |
| **3:20 PM – 3:35 PM** | Selected Talk: Membrane-Coated Lipid Bilayer Architectures for In-Vitro Synthetic Biology | Hiromune Eto, H. Franquelim, M. Heymann, P. Schwille |
| **3:35 PM – 3:50 PM** | Selected Talk: Crosslinking Materials for Responsive Hydrogels Designed for Use in Regenerative Medicine | Jasmina Gačanin, Christopher V. Synatschke, Tanja Weil |
| **3:50 PM – 4:10 PM** | Coffee Break / Active Break | |
| **4:10 PM – 4:25 PM** | Selected Talk: Niche-Guided Tissue Patterning by Chemomechanical Flow Lithography | Peter L. H. Newman, Pierre Osteil, Tim Anderson, Jane Q. J. Sun, Daryan Kempe, Maté Biro, Patrick P.L. Tam, Jae-Won Shin, Hala Zreiqat |
| **4:25 PM – 4:40 PM** | Selected Talk: Microscopic and RNAseq Analysis of Retinal Organoids With Optic Nerve Outgrowth | Philip Wagstaff, Anneloor ten Asbroek, Jaco ten Brink, Aldo Jongejan, Nomdo Jansonius, Arthur Bergen |
| **4:40 PM – 4:45 PM** | Break | |
| **4:45 PM – 5:00 PM** | Selected Talk: Bottom-Up Assembly, Actuation and Division of Synthetic Cells | Kevin Jahnke, Yannik Dreher, Kerstin Göpfrich |
| **5:00 PM – 5:15 PM** | Selected Talk: 3D Printing of Inherently Nanoporous Polymers Via Polymerization-Induced Phase Separation | Zheqin Dong, Haijun Cui, Haodong Zhang, Fei Wang, Xiang Zhan, Frederik Mayer, Britta Nestler, Martin Wegener, Pavel A. Levkin |
| **5:15 PM – 5:20 PM** | Break | |
| **5:20 PM – 6:20 PM** | Poster Session III | |
| **6:20 PM – 7:00 PM** | Dinner Break | |
| **7:00 PM – 9:00 PM** | Social Program | |

# March 4

| | | |
|---|---|---|
| **8:30 AM – 8:35 AM** | Welcome & Introduction Day 4 | Prof. Dr. Martin Bastmeyer & Prof. Dr. Joachim Wittbrodt |
| **8:35 AM – 9:10 AM** | Towards Reconstituting Human Somitogenesis In-Vitro | Cantas Alev, M.D., Ph.D., Associate Professor |
| **9:10 AM – 9:45 AM** | Making Retinal Organoids, a Voyage of Discovery | Majlinda Lako, Ph.D., Professor |
| **9:45 AM – 10:00 AM** | Coffee Break / Active Break | |
| **10:00 AM – 10:35 AM** | Micro-3D Printing to Understand Biological Dynamics | Jun.-Prof. Dr. Michael Heymann |
| **10:35 AM – 11:10 AM** | Gastruloids: An ESC Based Model for Mammalian Gastrulation and Axial Organization | Alfonso Martinez-Arias, Professor |
| **11:10 AM – 11:25 AM** | Poster Award Ceremony & Farewell | Prof. Dr. Martin Bastmeyer & Prof. Dr. Joachim Wittbrodt |



# Speakers

## Towards Reconstituting Human Somitogenesis *In-Vitro*

**Cantas Alev, M.D., Ph.D., Associate Professor**

Institute for the Advanced Study of Human Biology (ASHBi), Kyoto University, Japan

## 3D Laser Nanoprinting for Cell Biological Applications

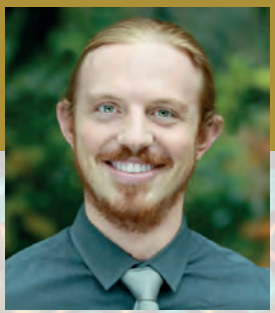
**Prof. Dr. Martin Bastmeyer**

Zoological Institute, Cell and Neurobiology, Karlsruhe Institute for Technology (KIT), Germany

## Building the Retina One Cell at a Time

**Seth Blackshaw, Ph.D., Professor**

Department of Neuroscience, Johns Hopkins University School of Medicine, USA



## User Programmable Hydrogel Biomaterials to Probe and Direct 4D Stem Cell Fate

**Cole A. DeForest, Ph.D., Assistant Professor**

Department of Chemical Engineering & Department of Bioengineering, Institute for Stem Cell & Regenerative Medicine, University of Washington, USA

## Piconewton-Sensitive Biosensors to Investigate Adhesion Mechanics in Cells

**Prof. Dr. Carsten Grashoff**

Institute of Molecular Cell Biology, University of Münster, Germany

## Standardized Hydrogel Beads and Their Applications in Mechanobiology

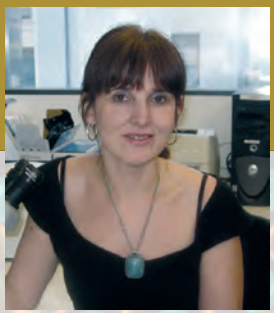
**Prof. Dr. Jochen Guck**

Max Planck Institute for the Science of Light, Germany

## Micro-3D Printing to Understand Biological Dynamics

**Jun.-Prof. Dr. Michael Heymann**

Institute of Biomaterials and Biomolecular Systems, University of Stuttgart, Germany



## Making Retinal Organoids, a Voyage of Discovery

**Majlinda Lako, Ph.D., Professor**

Institute of Genetic Medicine, Newcastle University, United Kingdom

## Integrated Organ-On-A-Chip Systems: Microphysiological Platforms Recapitulating Complex Human Tissue

**Jun.-Prof. Dr. Peter Loskill**

Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Germany
Faculty of Medicine, Eberhard Karls University Tübingen

## Macro-Scale Tissue Self-Organization Through Organoid Bioprinting

**Prof. Dr. Matthias Lutolf**

Laboratory of Stem Cell Bioengineering, Ecole polytechnique fédérale de Lausanne (EPFL), Switzerland

## Gastruloids: An ESC Based Model for Mammalian Gastrulation and Axial Organization

**Alfonso Martinez-Arias, Professor**

Department of Genetics, University of Cambridge, United Kingdom

## 3D Models of Retinal Development: Organoids and Retinospheres

**Thomas A. Reh, Ph.D, Professor**

Health Sciences Center, University of Washington, USA

## 3D Organ-On-A-Chip Models: Reconstruction of Retinal Blood Barriers

**Prof. Dr. Ute Schepers**

Institute of Toxicology and Genetics (ITG), Karlsruhe Institute of Technology (KIT), Germany

## Modelling Cell Shape and Dynamics in Three-Dimensional Scaffolds

**Prof. Dr. Ulrich Schwarz**

BioQuant Center for Quantitative Biology / Institute for Theoretical Physics (ITP), Heidelberg University, Germany

## Retinal Organoids – Non-Invasive Testing of Metabolic Function and Sheet Transplantation

**Magdalene J. Seiler, Ph.D., Associate Professor**

Department of Physical Medicine & Rehabilitation, Department of Ophthalmology, Sue and Bill Gross Stem Cell Research Center, University of California, Irvine, USA

## Collective Cell Order Maintains Homeostasis of Human Cornea: A New Physical Biomarker for the Quality Control of Cell Culture and the Prediction of Long-Term Prognosis

**Prof. Dr. Motomu Tanaka**

Institute of Physical Chemistry (PCI),
Heidelberg University, Germany

## The Cluster 3DMM2O: 3D Additive Manufacturing Driven Towards the Molecular Scale

**Prof. Dr. Martin Wegener**

Institute of Applied Physics (APH) / Institute of Nanotechnology (INT), Karlsruhe Institute of Technology (KIT), Germany, Spokesperson of the Cluster of Excellence "3D Matter Made of Order" (3DMM2O)

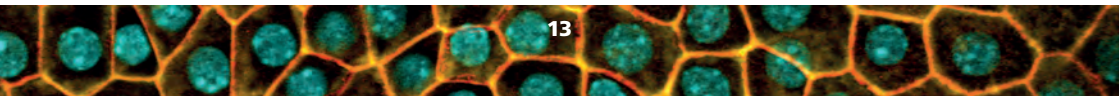
## Formation of the Vertebrate Retina – an Engineering Challenge

**Prof. Dr. Joachim Wittbrodt**

Centre for Organismal Studies (COS),
Heidelberg University, Germany

# Speakers' Abstracts

## In Alphabetical Order



# Towards Reconstituting Human Somitogenesis *In-Vitro*

**Cantas Alev, M.D., Ph.D., Associate Professor**

Institute for the Advanced Study of Human Biology (ASHBi),
Kyoto University, Japan
alev.cantas.8m@kyoto-u.ac.jp

Pluripotent stem cells (PSCs) have increasingly been used to model different aspects of human embryogenesis and organ formation. Despite recent advances in the *in vitro* induction of major mesodermal lineages and mesoderm-derived cell types, experimental model systems that can recapitulate more complex biological features of human mesoderm development and patterning are largely missing.

Here, we utilized induced pluripotent stem cells (iPSCs) for the stepwise *in vitro* induction of human presomitic mesoderm (PSM) and its derivatives to recapitulate distinct aspects of human somitogenesis. We focused initially on the *in vitro* recapitulation of the human segmentation clock, a major biological concept believed to underlie the rhythmic and controlled emergence of somites, which give rise to the segmental pattern of the vertebrate axial skeleton.

We succeeded to observe oscillatory expression of core segmentation clock genes, including *HES7* and *DKK1*, determined the period of the *in vitro* human segmentation clock to be around five hours and showed the presence of dynamic traveling wave-like gene expression within *in vitro* induced human PSM. We furthermore identified and compared oscillatory genes in human and murine PSC-derived PSM, which revealed species-specific as well as common molecular components and pathways associated with the mouse and human segmentation clocks. Subsequent analysis of patient-derived and patient-like iPSCs targeting genes associated with segmentation defects of the vertebrae (*HES7, LFNG, DLL3, MESP2*) revealed gene-specific alterations of different properties of the *in vitro* human segmentation clock.

Taken together, these findings indicate that our *in vitro* system recapitulates key features of the human segmentation clock and may be used to provide novel insights into normal and abnormal development of the human axial skeleton.

# 3D Laser Nanoprinting for Cell Biological Applications

**Prof. Dr. Martin Bastmeyer**

Zoological Institute, Cell and Neurobiology,
Karlsruhe Institute for Technology (KIT), Germany
bastmeyer@kit.edu

Cell behavior and differentiation are not only influenced by biochemical cues but also by physical properties like adhesive geometry, topography, and stiffness of the 3D extracellular environment. In this talk I will discuss how 3D laser nanoprinting can be applied to design 3D cellular microenvironments in the µm range with defined geometries and adjustable flexibility. To achieve a precise and patterned functionalization with biomolecules in 3D three approaches are chosen:

(i) By sequential printing of two different photoresists, composite-polymer scaffolds with distinct protein-binding properties can be fabricated and selectively bio-functionalised thereafter. Cells cultured in these scaffolds selectively form cell-adhesion sites with the functionalised parts, allowing for controlling cell adhesion and cell shape in 3D. Since the elastic modulus of the scaffold material varies between $E$=140-350 MPa, measurements of cell adhesion forces in relation to adhesion geometry are also feasible. In addition, these scaffolds can be used to mechanically stimulate cells at single defined adhesion sites.

(ii) By combining nanoprinting with an efficient surface photochemistry, also amenable to two-photon activation, it is possible to generate structurally complex 3D microstructures with 3D resolved chemical patterns. Microscaffolds with lattice constants of 10–20 microns can be patterned with protein ligands with a resolution close to one micron using a phototriggered cycloaddition. These techniques have been applied to guide cell attachment in 3D-microscaffolds selectively functionalized with two distinct adhesion proteins.

(iii) By using stimuli-responsive hydrogels, 3D scaffolds can be transferred from passive to dynamic systems. We have fabricated composite 3D scaffolds that allow for the micromanipulation of single cells. These scaffolds allow to directly correlate displacements to cellular forces and to quantify the effects with high throughput.

In summary, the above described 3D scaffolds enable to study the influence of spatial ligand-distribution on cellular differentiation, allow visualizing and measuring cell adhesion forces, and can be used to mechanically stimulate single cells at defined adhesion sites.

# Building the Retina One Cell at a Time

**Seth Blackshaw, Ph.D., Professor**

Department of Neuroscience, Johns Hopkins University School of Medicine, USA
sblack@jhmi.edu

The retina is widely used as a model system for functional studies of neural fate specification in model organisms such as mouse and zebrafish.

The development of retinal organoids, moreover, potentially allow such studies to be extended to humans. However, we still lack a comprehensive picture of the gene regulatory networks that control both evolutionarily conserved and species-specific aspects of retinal development, and it is still unclear how well retinal organoids actually mirror the process of retinal development as it occurs *in vivo*.

I will discuss our groups recent application of single cell RNA- and ATAC-Seq analysis to identify gene regulatory networks that control retinal development in zebrafish, mouse and human. We will discuss functional studies that have arisen from this work, which have identified new genes that control temporal patterning, neurogenesis and specification of both photoreceptor and inner retinal cells.

In addition, we will present new work in which we have extended this analysis to human retinal organoids of various ages, and identified key similarities and differences between the gene regulatory networks that control retinogenesis *in vitro* and *in vivo*.

# User Programmable Hydrogel Biomaterials to Probe and Direct 4D Stem Cell Fate

**Cole A. DeForest, Ph.D., Assistant Professor**

Department of Chemical Engineering & Department of Bioengineering,
Institute for Stem Cell & Regenerative Medicine, University of Washington, USA
profcole@uw.edu

The extracellular matrix directs stem cell function through a complex choreography of biomacromolecular interactions in a tissue-dependent manner. Far from static, this hierarchical milieu of biochemical and biophysical cues presented within the native cellular niche is both spatially complex and ever changing.

As these pericellular reconfigurations are vital for tissue morphogenesis, disease regulation, and healing, *in vitro* culture platforms that recapitulate such dynamic environmental phenomena would be invaluable for fundamental studies in stem cell biology, as well as in the eventual engineering of functional human tissue. In this talk, I will discuss some of our group's recent success exploiting bioorthogonal photochemistry and chemoenzymatic reactions to reversibly modify both the chemical and physical aspects of polymeric cell culture platforms with user-defined spatiotemporal control.

Results will highlight our ability to modulate intricate cellular behavior including stem cell differentiation, protein secretion, and cell-cell interactions in 4D.

# Piconewton-Sensitive Biosensors to Investigate Adhesion Mechanics in Cells

**Prof. Dr. Carsten Grashoff**

Institute of Molecular Cell Biology,
University of Münster, Germany
grashoff@uni-muenster.de

To investigate the molecular mechanisms underlying cell adhesion mechanics, we developed a set of single-molecule-calibrated biosensors that are sensitive to physiologically relevant forces in the low piconewton range and characterized by fast folding/unfolding transitions and reversibility.

All biosensors are genetically encoded and can be utilized to determine molecular forces acting across individual molecules in cells. Their application to the focal adhesion protein talin and the desmosomal molecule desmoplakin reveals intriguing differences in how distinct adhesion molecules modulate intracellular force transduction.

# Standardized Hydrogel Beads and Their Applications in Mechanobiology

**Prof. Dr. Jochen Guck**

Max Planck Institute for the Science of Light, Germany
jochen.guck@mpl.mpg.de

Tissues are defined not only by their biochemical composition, but also by their distinct mechanical properties, which cells can sense and respond to. Studying this mechanosensitivity *in vivo* is often descriptive and correlative. *In vitro* assays are either only 2D, or in 3D convolve mechanics with porosity and biochemical heterogeneity. This convolution renders testing the relative importance of mechanosensitivity in realistic environments challenging.

Here, we present novel colloidal crystals as modular 3D scaffolds where these parameters are principally decoupled. By using monodisperse, protein-coated PAAm microgel beads with well-defined elastic properties as building blocks, variable stiffness regions can be realized by an additive process within one 3D colloidal crystal. Using these mechanically patterned colloidal crystals, we have demonstrated durotactic fibroblast migration and mechanosensitive neurite outgrowth of dorsal root ganglion neurons in 3D.

Further, the PAAm hydrogel beads also find many other applications in mechanobiology, for example as standardized mechanical cell mimics for calibration of cell mechanics measurements and for assaying the importance of deformability in vascular circulation, or as cell-scale stress sensors in developmental processes.

# Micro-3D Printing to Understand Biological Dynamics

**Jun.-Prof. Dr. Michael Heymann**

Institute of Biomaterials and Biomolecular Systems, University of Stuttgart, Germany
michael.heymann@bio.uni-stuttgart.de

Living systems have a stunning ability to self-organize in space and time. Many remaining grand challenges in biology and medicine come from our inability to comprehend the underlying molecular-scale phenomena in a complex context such as a multi-component mixture or a cell. We advance two photon stereolithography based microfabrication to create and comprehend biomolecular structure and function across scales.

This entails novel ultracompact microfluidic approaches to time-resolved structural biology to record 'molecular movies' of macromolecular conformational changes at the atomic scale. This allows to determine the structures of transient states and thereby kinetic mechanisms of substrate turn-over during enzyme catalysis. We could follow the catalytic reaction of the *M. tuberculosis* β-lactamase with the 3$^{rd}$ generation antibiotic ceftriaxone with millisecond to second time resolution at 2 Å spatial resolution.

In extending this technology to synthetic biology, we can reconstitute functional biological and biomimetic systems from the bottom up with unprecedented precision and throughput. For instances to compartmentalize the *E.coli* MinDE protein oscillator, that positions the cell division machinery at mid-cell, into physiologically relevant three-dimensional model compartments, such as lipid vesicles exhibiting active shape changes. In current efforts, we are developing novel protein photoresists to nano-3D-print sub-cellular compartments with the highest achievable functional conformity to cellular structures *in vivo*. In first proof-of-principle experiments we structured a contractile eukaryotic cell division model.

Such model systems will allow to design and program dynamic biological states far from equilibrium to investigate spatiotemporal self-organization principles in biology that by lack of suitable tools have previously been inaccessible to experimental quantification. Our ultimate objective is to decipher the principles of synchronization, morphogenesis, and differentiation in confined geometries, as well as biochemical information processing and chemo-mechanical coupling at scales ranging from the nanoscale to the full organ. This will enable previously inconceivable avenues to investigate and to program fundamental aspects of biological self-organization and disease, to uncover new biophysical principles, as well as healthcare and biotechnology applications.

# Making Retinal Organoids, a Voyage of Discovery

**Majlinda Lako, Ph.D., Professor**

Institute of Genetic Medicine, Newcastle University, United Kingdom
majlinda.lako@ncl.ac.uk

Prof. Majlinda Lako completed her PhD studies at the Human Genetics Department of Newcastle University in 1998. Following her postdoctoral training at Durham University, she returned to Newcastle to create her own independent research group in 2003 working in human pluripotent stem cells.

The research aims of Lako's group are to understand and define the early events occurring in human embryogenesis with special focus on eye formation and developing new treatments for eye disease. They are engaged in several large research programmes that aim to define good manufacturing protocols for deriving functional corneal and retinal cells that can be used for drug testing, disease modelling and cell based replacement therapies.

This talk focuses on their efforts to optimise the generation of light responsive retinal organoids and their application to disease modelling, photoreceptor transplantation and drug discovery/repurposing.

# Integrated Organ-On-A-Chip Systems: Microphysiological Platforms Recapitulating Complex Human Tissue

**Jun.-Prof. Dr. Peter Loskill**

Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Germany
Faculty of Medicine, Eberhard Karls University Tübingen
peter.loskill@uni-tuebingen.de

Drug discovery and development to date has relied on animal models, which are useful, but fail to resemble human physiology. The discovery of human induced pluripotent stem cells (hiPSC) has led to the emergence of a new paradigm of drug screening using human patient- and disease-specific organ/tissue-models. One promising approach to generate these models is by combining the hiPSC technology with microfluidic devices tailored to create microphysiological environments and recapitulate 3D tissue structure and function. Such organ-on-a-chip platforms (OoCs) or microphysiological systems combine human genetic background, *in vivo*-like tissue structure, physiological functionality, and "vasculature-like" perfusion.

Using microfabrication techniques, we have developed a variety of OoCs that incorporate complex human 3D tissues and keep them viable and functional over multiple weeks, including "Retina-on-a-chip", "Choroid-on-a-chip", "Heart-on-a-chip", "Pancreas-on-a-chip and a "White adipose tissue(WAT)-on-a-chip".

The OoCs generally consist of three functional components: organ-specific tissue chambers mimicking *in vivo* structure and microenvironment of the respective tissues; "vasculature-like" media channels enabling a precise and computationally predictable delivery of soluble compounds (nutrients, drugs, hormones); "endothelial-like" barriers protecting the tissues from shear forces while allowing diffusive transport. The small scale and accessibility for *in situ* analysis makes our OoCs amenable for both massive parallelization and integration into a high-content-screening approach.

The adoption of OoCs in industrial and non-specialized laboratories requires *enabling technologies* that are user-friendly and compatible with automated workflows. We have developed technologies for automated 3D tissue generation as well as for the flexible plug&play connection of individual OoCs into multi-organ-chips. These technologies paired with the versatility of our OoCs pave the way for applications in drug development, personalized medicine, toxicity screening, and mechanistic research.

# Macro-Scale Tissue Self-Organization Through Organoid Bioprinting

**Prof. Dr. Matthias Lutolf**

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Bioprinting promises enormous control over the spatial deposition of cells in three dimensions, but current approaches have had limited success at reproducing the intricate micro-architecture, cell-type diversity and function of native tissues formed through cellular self-organization.

We introduce a three-dimensional bioprinting concept that uses organoid-forming stem cells as building blocks that can be deposited directly into extracellular matrices conducive to spontaneous self-organization. By controlling the geometry and cellular density, we generated centimetre-scale tissues that comprise self-organized features such as lumens, branched vasculature and tubular intestinal epithelia with *in vivo*-like crypts and villus domains.

Supporting cells were deposited to modulate morphogenesis in space and time, and different epithelial cells were printed sequentially to mimic the organ boundaries present in the gastrointestinal tract. We thus show how biofabrication and organoid technology can be merged to control tissue self-organization from millimetre to centimetre scales, opening new avenues for drug discovery, diagnostics and regenerative medicine.

# Gastruloids: An ESC Based Model for Mammalian Gastrulation and Axial Organization

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When small, specified numbers of mouse Embryonic Stem Cells are placed in defined culture conditions they aggregate and initiate a sequence of pattern forming events that mimic the events that take place in the embryo: they undergo symmetry breaking, gastrulation like movements, axial specification and germ layer organization.

We can culture them for up to seven days to reach a stage comparable to E9.0 in the mouse embryo and exhibit a similar organization including three orthogonal axes with associated asymmetries. This experimental system can be used to gain insights into the process of gastrulation and axial organization as well as the emergence of the primordia for tissues and organs.

I shall be discussing specific examples and the implications these have for the theoretical and practical understanding of developmental events in mammals and our efforts to extend the system to human Pluripotent Stem Cells.

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# 3D Models of Retinal Development: Organoids and Retinospheres

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The neural retina is subject to a wide range of neurodegenerative diseases, including inherited degeneration and dysfunction, as well as age related diseases.

Although great progress has been made in understanding the genetics and risk factors for many of these diseases, translating these approaches to clinical trials and approved therapies remains challenging. This is in part due to the lack of a suitable *in vitro* screening system or cell line for identifying compounds that can effectively engage targets and for lead optimization and off-target effects.

A key breakthrough in the field was the development of retinal organoids from iPSCs. Retinal organoids are a particularly attractive model, since these develop many features of normal retina; organoids mimic the lamination of the retina, and have been successful in generating the major retinal cell types. Retinal organoids have been shown to be quite similar to human fetal retina using histological methods and genomic approaches (eg. RNAseq at the bulk and single cell level, DNAseq, ATACseq, etc).

Retinal organoids, however, are still not a perfect substitute for the human retina. Organoids lack non-neural cell types, a continuous layer of RPE, microglia and endothelial cells, cells that are well known to participate in major retinal pathologies.

To better understand the limits of *in vitro* retinal development, we developed an organoid-like model using primary retinal tissue, which we call retinospheres. Retinospheres can be generated from any age of retina we have tested to date, and a detailed comparison using histology and scRNAseq between retinospheres and fetal retina have shown they are remarkably similar. Retinospheres also retain their lamination much better than stem cell derived organoids and they retain characteristic features of the region of the retina from which they originated, eg. fovea. Importantly, retinospheres contain the non-neural cells not typically present in organoids. Moreover, fusions between organoids and retinospheres allows us to better understand their differences and develop optimal models from stem cells.

# 3D Organ-On-A-Chip Models: Reconstruction of Retinal Blood Barriers

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During the last decade many approaches have been made to generate novel materials for the 3D recnstruction of neuronal tissues such as the blood brain barrier, the neurovascular junction or even the retina by tissue engineering through 3D bioprinting.

The materials have to be tailored to resemble all the properties to allow for stem cells such as iPS cells to initiate their differentiation program to the neuronal tissue. In addition they have to be biocompatible and even degradable for long periods of time during tissue generation. 3D-printed tissues often resemble organoids from different cells without the appropriate vascularization enabling the tissue to be connected to nutrition. Endothilium of blood vessels also prime the tissue for the expression of maturation factors.

Many groups have established a model of 3D-retina organoids.However, they often miss the correct vascularizationand the reconstruction of the inner and outer bood-retina barrier. 3-D retina organoids are initiated by induced pluripotent stem cells outside of traditional 2D culture systems to result in multiple cell types that organize along three dimensions into microphysiological systems. One goal is the development of materials that allow for the stimulated and directed growth and differentiation of iPSCs into a 3D retinal tissue that resemble a nature like 3D neuronal environment.

The blood-retinal barrier (BRB) is essential to maintaining the eye as a privileged site and is essential for normal visual function. The BRB, separated in inner and outer barrier, is a particularly tight and restrictive physiologic barrier that regulates ion, protein, and water flux into and out of the retina. The inner BRB being formed of tight junctions between retinal capillary endothelial cells and is similar to the blood brain barrier. Based on our experience on the reconstruction of the BBB we developed a blood vessel system that allowes together with our novel ultrafast curing material the reconstruction of the retina barriers by combined approached from direct laser writing and 3D bioprinting.

# Modelling Cell Shape and Dynamics in Three-Dimensional Scaffolds

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Cells look and behave differently in three-dimensional scaffolds than on two-dimensional surfaces, but the most important underlying processes determining shape, mechanics and movement are the same: membrane protrusions due to actin polymerization and myosin-based contractility of the actomyosin cortex and stress fibers.

I first will discuss the interplay between tension and elasticity that characterizes the cell envelope in two dimensions, and then extend this viewpoint to three dimensions. Next I will address models that allow us to formulate dynamical versions of tension-dominated systems, namely cellular Potts and phase field models.

Given that we can model the forward problem, we finally can ask how to control cell shape by solving the inverse problem and designing scaffolds that result in a desired functionality.

For 3D hybrid organotypic systems that mimic the retina, such a desired functionality might be light scattering determined by the contrast between cell nuclei and cytoplasm.

# Retinal Organoids – Non-Invasive Testing of Metabolic Function and Sheet Transplantation

**Magdalene J. Seiler, Ph.D., Associate Professor**

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**Purpose.** Human embryonic stem cell (hESC)-derived retinal organoids (ROs) improve visual function after transplantation into retinal degeneration (RD) models. Advanced imaging techniques (fluorescence lifetime microscopy [FLIM] and hyperspectral imaging [HSpec]) provides non-invasive data for quality control and long-term *in vitro* follow-up. In this work, hESC-derived retinal organoids, produced by a cGMP compatible process, were followed by 2-photon microscopy *in vitro* prior to surgical transplantation; and *in vivo* by OCT imaging after transplantation to the subretinal space of nude RD *rho S334ter-3* (RN) rats.

**Methods.** A scalable cGMP compatible process was established for the generation and characterization of a Working Cell Bank (WCB) of CSC-14 hESCs (NIH 0284). hESC-derived retinal organoids were characterized by immunohistochemistry (IHC), flow cytometry and qPCR. 2-photon excitation microscopy (2PE) was used to collect metabolic information from intrinsic fluorophores: NADH (FLIM), and retinol (HSpec) inside organoids with subcellular resolution (Browne et al, 2017, IOVS) up to 6 months *in vitro*. FLIM images were taken using 740nm pulsed excitation (Zeiss LSM 710). HSpec fluorescence emissions were taken in the range of 420 nm to 690 nm. Data were analyzed by SimFCS (Global Software) via the phasor approach. Retinal organoid sheets (differentiation day 30-90) were transplanted to the subretinal space of RN rats (P31-51). Transplants were monitored *in vivo* by Optical Coherence Tomography (OCT). Visual function was accessed by optokinetic tests (OKT) and superior colliculus (SC) electrophysiology. *Ex vivo* sections through transplants were stained with hematoxylin & eosin (H&E), or processed for IHC to label human donor cells, retinal cell types and synaptic markers.

**Results.** The WCB of CSC-14 hESCs was characterized using the following metrics: viability, identity (Oct4); karyotype stability; sterility and neural differentiation potential. This WCB was used to generate all ROs. Long-term imaging data of retinal organoids (>180 days) demonstrated metabolic activities confirming overall cellular viability. Initially, a shift from glycolytic to oxidative metabolic activities was observed. As time progressed, glycolysis became predominant on the surface of the organoids. HSpec images showed retinol distribution on the surface. IHC of retinal organoids shows early lamination and development of retinal cell progenitors. Organoids selected for transplantation showed early lamination. Post-transplantation OCT imaging revealed transplant development and photoreceptor rosettes.

Transplanted eyes showed vision improvement by OKT and SC recording. Transplants developed different retinal cells including photoreceptors; and integrated with the host retina.

**Conclusions.** A WCB of CSC-14 hESCs was successfully established and meets FDA requirements. Retinal organoids showed a metabolic shift in long term culture, from glycolytic (proliferative) to oxidative (differentiated) state, and back to the glycolytic surface (indicating a photoreceptor layer). Retinal organoids mature further after transplantation, develop photoreceptors, integrate into the host retina, and improve visual function.

**Support:** California Institute for Regenerative Medicine (CIRM) TR1-10995;
RPB unrestricted grant to UCI Department of Ophthalmology; ICTS KL2 TR001416

# Collective Cell Order Maintains Homeostasis of Human Cornea: A New Physical Biomarker for the Quality Control of Cell Culture and the Prediction of Long-Term Prognosis

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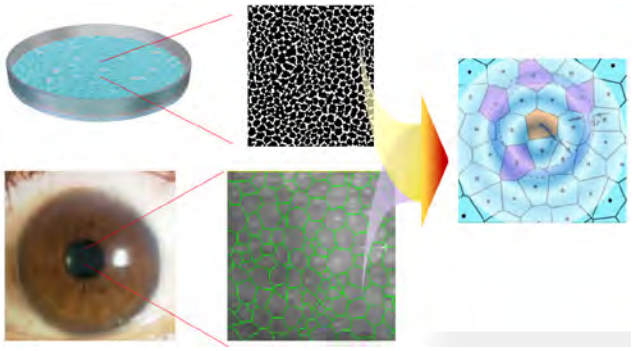
Dysfunction of the corneal endothelium reduces the transparency of the cornea and can cause blindness. Currently, the clinical treatment inevitably involves the transplantation of donor corneas, as human corneal endothelial cells have an extremely low proliferative capcity *in vivo*.

The successful *in vitro* expansion of endothelial cells enables the restoration of a functional cornea via intraocular injection of endothelial cells in suspension, yet a substantial amount of the cultured cells is lost by destructive quality assessment. Recently, we established a quantitative measure (physical biomarker) by shedding light on the collective order of the cells by treating them as 2D colloidal assemblies. The second derivative of potential of mean force (spring constant) calculated from phase contrast imaging and from specular microscopy can be used as a noninvasive index for the quality assessment of corneal endothelial cells *in vitro* and for the long-term prognosis of corneal restoration in patients *in vivo*, respectively.

Our data suggest that this new biomarker may enable a shift from passive monitoring to pre-emptive intervention in patients with severe corneal disorders, which is a major health issue in the aging society.



Quantification of collective order of human endothothelial cells in *in vitro* culture (upper panels) and in restoring *in vivo* corneas in patients (lower panels) using one physical biomaker.

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# The Cluster 3DMM2O: 3D Additive Manufacturing Driven Towards the Molecular Scale

**Prof. Dr. Martin Wegener**
Institute of Applied Physics (APH) / Institute of Nanotechnology (INT),
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In this talk, I shall gave an introduction into and an overview of the activities of the Excellence Cluster 3DMM2O.

On the technology side, the scientific challenges pursued by the Cluster can be nicknamed as "finer, faster, and more", i.e., advance molecular materials and technologies in terms of resolution, speed, and multi-material printing by orders of magnitude.

On the application side, the Cluster aims at functional 3D hybrid optical and electronic systems, 3D artificial materials called metamaterials, and at reconstructing functioning organotypic systems by using 3D scaffolds for cell culture.

In the talk, I will emphasize manufacturing technologies relevant for 3D organotypic systems, especially 3D laser nanoprinting.

# Formation of the Vertebrate Retina – an Engineering Challenge

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The vertebrate eye is formed in a very stereotypic manner and the neuronal and non-neuronal cell types are arranged in an almost crystalline array. I will touch the molecular on morphogenetic aspects of vertebrate eye formation and will in particular address the life-long growth in non-mammalian species.

In fish and amphibia the eye is formed in two phases, initially from multipotent retinal progenitor cells that evaginate from the lateral diencephalon. Those undergo a major morphogenetic rearrangement and this retinal flow eventually transforms the vesicle into the characteristic optic cup. Concomitant with the flow, differentiation of retinal cell types is initiated in the central retina by signals emanating form the optic stalk. The second phase of retina formation starts subsequently and is driven by a stem cell niche in the periphery of the just formed eye cup, the ciliary marginal zone.

I will illustrate the properties of stem and progenitor cells and will eventually touch upon how the action of stem cells is particularly canalised by the immune system. It will be challenging to disentangle the evolutionarily tightly coupled processes into individual building blocks that, relying on the properties of self-organisation, can be recombined in a new context.

Results on self-organising retinal organoids from diverse species give first encouraging insights and open a perspective for systematically tackling the impact of the physical environment.

# From Visions Towards Scenarios for Future 3D Additive Manufacturing in Society

ORAL PRESENTATION

**Andreas Lösch, Max Roßmann, Christoph Schneider**

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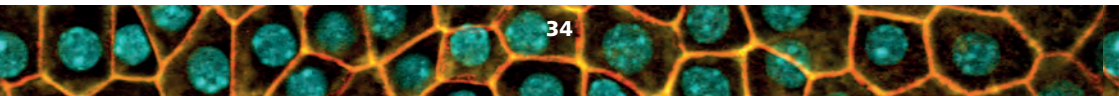
Visions of the future influence decisions today with impacts on the future of technologies. This presentation from the Carl Zeiss Vision Assessment Study in the Cluster of Excellence 3DMM2O offers an argument on the role of on different visions of 3D printing and their influence in technology development as a basis for a process of co-creating scenarios that combine technological and social change.

Future 3D Additive Manufacturing is considered an enabler of fundamental changes to production and consumption, i.e., a disruptive technology with increasing potential in the future. Given the variety of different technologies subsumed under the term 3D printing, the success of this generalized vision is astonishing. To provide an overview of prominent pathways from technology features in development to imagined social benefit, we distinguished the most popular key-narratives based on interviews and mass media analysis. By distinguishing, i.e., the role of "hybrid-organotypic systems" in the narrative "prospects for individualized medicine" from "rapid manufacturing at the push of a button," or "saving resources for sustainability," it becomes more clear which technology properties and actors might play a role.

Based on this understanding of visions of the future in the present we use scenario-methods to shape different imagined futures based on the insights of social scientific and technological experts. These scenarios will be used to reflect on current ideas, beliefs, and desires with stakeholders and citizens to make visions of the future usable to shape 3D additive manufacturing in society. We will show how the methodological construction of scenarios can enable more robust and diverse usages of images of the future in science and society. Through combining these perspectives and bringing different actors into dialogue on the scenarios the future of 3D additive manufacturing can be turned from an unknown into a resource for the present: What technological possibilities are expected in the future and how could patterns of consumption, structures of regulation, expectations of health or intellectual property change in tandem with them?

In short, how can we envision pictures of a future society that uses 3D additive manufacturing in responsible ways? And what can we learn from these pictures for our present?

# Selected Talks



# In Scheduled Order – Overview:

| | |
|---|---|
| **3D Bioprinting of Functionally Graded Biomaterials** | Aurelien Forget, Merve Kuzucu, Grace Vera, V. Prasad Shastri, Marco Beaumont |
| **Programmable DNA Materials for Life Science Applications** | Carmen M. Domínguez, Yong Hu, Christof M. Niemeyer |
| **Membrane-Coated Lipid Bilayer Architectures for *In Vitro* Synthetic Biology** | Hiromune Eto, H. Franquelim, M. Heymann, P. Schwille |
| **Crosslinking Materials for Responsive Hydrogels Designed for Use in Regenerative Medicine** | Jasmina Gačanin, Christopher V. Synatschke, Tanja Weil |
| **Bottom-Up Assembly, Actuation and Division of Synthetic Cells** | Kevin Jahnke, Yannik Dreher, Kerstin Göpfrich |
| **Niche-Guided Tissue Patterning by Chemomechanical Flow Lithography** | Peter L. H. Newman, Pierre Osteil, Tim Anderson, Jane Q. J. Sun, Daryan Kempe, Maté Biro, Patrick P.L. Tam, Jae-Won Shin, Hala Zreiqat |
| **Microscopic and RNAseq Analysis of Retinal Organoids With Optic Nerve Outgrowth** | Philip Wagstaff, Anneloor ten Asbroek, Jaco ten Brink, Aldo Jongejan, Nomdo Jansonius, Arthur Bergen |
| **3D Printing of Inherently Nanoporous Polymers Via Polymerization-Induced Phase Separation** | Zheqin Dong, Haijun Cui, Haodong Zhang, Fei Wang, Xiang Zhan , Frederik Mayer, Britta Nestler, Martin Wegener, Pavel A. Levkin |

# 3D Bioprinting of Functionally Graded Biomaterials

**ORAL PRESENTATION**

**Aurelien Forget,**[1] **Merve Kuzucu,**[1] **Grace Vera,**[1] **V. Prasad Shastri,**[1] **Marco Beaumont**[2]

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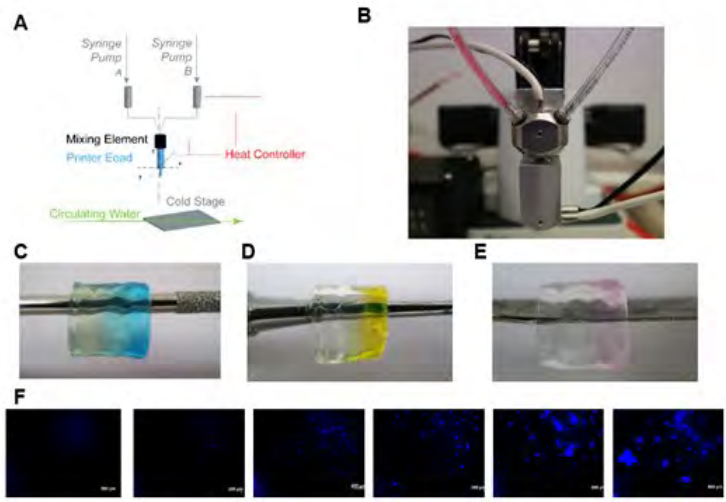
Many biological events during development and later in mature tissues are governed by gradients which provide information on the direction of cellular movements, so called taxis. If we want to study biological processes in vitro, engineering solutions to reproduce the graded architecture of mature and diseased tissue are needed.

With this objective, we present herein a platform for the 3D bioprinting of functionally graded biomaterials based on carboxylated agarose, a bioink amendable by fuse deposition modelling and drop-on-demand. Using a custom-made robot-arm bioprinter equipped with a mixing printing head, we created objects made of carboxylated agarose exhibiting gradient of mechanical properties and gradient of cell concentration. Functionalization of carboxylated agarose with maleimide moieties that react in minutes with cysteine-terminated cell adhesion peptide allowed us to print objects with gradient of immobilised biological signal.

The presented approach for the manufacturing of 3D graded objects pave the way towards the development of complex graded tissue mimics such as the osteochondral interface or tumour environment.



# Programmable DNA Materials for Life Science Applications

ORAL PRESENTATION

**Carmen M. Domínguez,**[1] **Yong Hu,**[1] **Christof M. Niemeyer**[1]

1 Institute for Biological Interfaces (IBG-I), Karlsruhe Institute of Technology (KIT), Eggenstein-Leopoldshafen, Germany

Biomedical applications require substrata that allow for the grafting, colonization and control of eukaryotic cells. Currently available materials are often limited by insufficient possibilities for the integration of biological functions and means for tuning the mechanical properties.

We report on tailorable nanocomposite materials in which silica nanoparticles are interwoven with carbon nanotubes by DNA polymerization. The modular, well controllable and scalable synthesis yields materials whose composition can be adjusted to give rise to different mechanical, electrical, optical or biological properties. The materials were exploited as substrata that outperform conventional culture surfaces in the ability to control cellular adhesion, proliferation and transmigration through the hydrogel matrix.

The composite materials also enable the construction of layered cell architectures, the expansion of embryonic stem cells by simplified cultivation methods and the on-demand release of uniformly sized stem cell spheroids.

Furthermore, they can also be functionalized with proteins, such as agonists or antagonists of cell membrane receptors, to influence the cellular activation, thereby proving their appropriateness for the establishment of hierarchically structured interfaces to living cells.

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# Membrane-Coated Lipid Bilayer Architectures for *In Vitro* Synthetic Biology

ORAL PRESENTATION

**Hiromune Eto,**[1] **H. Franquelim,**[1] **M. Heymann,**[1,2] **P. Schwille**[1]

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In bottom-up synthetic biology, one of the major methodological challenges is to provide reaction spaces that mimic biological systems with regard to topography and surface functionality. Of particular interest are lipid membrane interfaces, as many protein functions take place in association with the lipid bilayer.

With the advent of 3D printing by two-photon direct laser writing, structurally defined 3D microenvironments with length scales relevant to cellular and biological processes can be fabricated. Using such micron-scale features, we are able to shape lipid membranes according to the imposed 3D architecture, from which we create environments and 3D geometries relevant to *in vitro* synthetic biology.

By employing appropriate surface modifications, we can manipulate charges on the polymer surface, which enables the formation of both negative and positive charged lipid membranes supported by the printed polymer. On these 3D lipid architectures, we in vitro reconstitute dynamic protein systems that are geometry sensitive, and we observe their self-organisation in response to the 3D geometries of the printed environment. To this end, we developed a novel tool to explore the geometrical and topographical effects of complex 3D shapes on protein function.

# Crosslinking Materials for Responsive Hydrogels Designed for Use in Regenerative Medicine

ORAL PRESENTATION

**Jasmina Gačanin,**[1] **Christopher V. Synatschke,**[1] **Tanja Weil**[1]

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New biomaterials that can support cellular adhesion, growth, and differentiation to replace damaged tissue, while allowing for minimally invasive administration, are strongly required in regenerative medicine. To meet these demands, we developed biohybrid materials consisting of a biopolymer backbone that is grafted with two types of supramolecular gelators, namely DNA or nanofiber-forming peptides, thus combining the stability of covalent bonds with supramolecular chemistry. Both types of supramolecular crosslinkers yield hydrogels with remarkable material and biological properties, such as thixotropic behavior and high biocompatibility. While the programmability of DNA is exploited to enable the controlled delivery of bioactive protein for cell population control, the second set of hydrogels benefits from grafted peptides, which can undergo a triggered molecular rearrangement that induces self-assembly into nanofibers and subsequent gelation with exceptional self-recovery.

We further expand our research into customized crosslinking materials and improve an existing hydrogel platform based on human serum albumin. Here, the main objective of our recent joint work with cooperation partners is to create the basis for novel treatment options for inflammatory joint diseases. We present our results for investigating suitable crosslinkers for biocompatible and injectable hydrogels with adjustable mechanical properties which are envisioned to support a local, controllable release of therapeutic molecules. These include covalent crosslinkers such as bifunctional polymers as well as supramolecular crosslinkers like self-assembling peptides. By providing new crosslinking materials, we expand the available toolbox of albumin-based hydrogels and create tailored hydrogels with a unique fusion of advantageous properties enabling excellent performance in regenerative medicine.

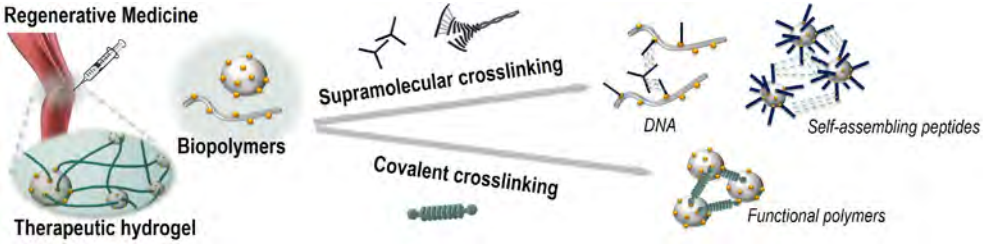


Figure 1: Crosslinking strategies and materials for responsive hydrogels from biopolymers

# Bottom-Up Assembly, Actuation and Division of Synthetic Cells

ORAL PRESENTATION

**Kevin Jahnke,**[1,2] **Yannik Dreher,**[1,2] **Kerstin Göpfrich**[1,2]

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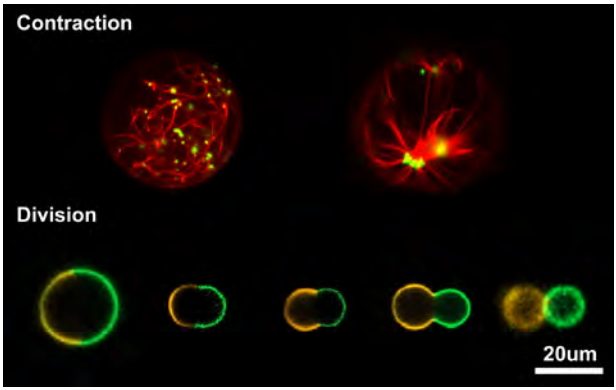
The future of 3D additive manufacturing will, no doubt, entail the construction of biological systems and synthetic cells from the bottom up. Instead of relying exclusively on biological building blocks, the integration of new tools and new materials may be a shortcut towards the assembly of active and eventually fully functional synthetic cells [Göpfrich et al., *Trends in Biotechnology*, 2018]. This is especially apparent when considering recent advances in DNA nanotechnology, microfluidics and 3D printing. Exemplifying this approach, we demonstrate the assembly of synthetic cellular compartments that we equip with natural or synthetic cytoskeletons. We use light as a non-invasive stimulus to trigger their symmetry-breaking contraction [Jahnke et al., *Advanced Biosystems,* 2020] (Figure 1, top).

We further demonstrate the division of giant unilamellar lipid vesicles (GUVs) as synthetic cell models based on phase separation and osmosis rather than the biological building blocks of a cell's division machinery (Figure 1, bottom). We derive a parameter-free analytical model which makes quantitative predictions that we verify experimentally [Dreher et al., *Angewandte Chemie*, 2020]. Remarkably, we show that caged compounds provide full spatio-temporal control to increase the osmolarity locally in an illuminated area, such that a target-GUV undergoes division whereas the surrounding GUVs remain unaffected. All in all, we believe that additive manufacturing for synthetic biology, and light-triggered actuation in particular, provides not only creative freedom but can also help to accelerate synthetic biology research.



# Niche-Guided Tissue Patterning by Chemomechanical Flow Lithography

ORAL PRESENTATION

**Peter L. H. Newman,**[1] **Pierre Osteil,**[2,3] **Tim Anderson,**[1] **Jane Q. J. Sun,**[2,3] **Daryan Kempe,**[4] **Maté Biro,**[4] **Patrick P.L. Tam,**[2,3] **Jae-Won Shin,**[5] **Hala Zreiqat**[1]

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2 Embryology Unit, Children's Medical Research Institute, Sydney, Australia
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4 EMBL Australia, Single Molecule Science Node, School of Medical Sciences, UNSW, Sydney, Australia.
5 Department of Pharmacology, University of Illinois at Chicago, Illinois, USA

Pluripotent-stem-cell-derived tissue-models have been established with increasingly physiological shape, size and function, however, the histo-and-morphogenetic processes present in these models proceeds stochastically. This reflects an absence of technologies able to produce complex supportive cell niches that can reproducibly guide tissue patterning and generate well-defined tissue structures.

To address this, have developed chemomechanical flow lithography (CMFL), a printing technology that delivers orthogonally programmable chemical and mechanical properties to microstructured niches that drive the differentiation of selective cell types and spatial emplacement of these cells in a micropattern.

We print microstructured niches with conjugated of peptides, proteins and macromolecular morphogens across a range of Young's Modulus. Using such niches, we generated tissue patterned constructs from a single cell source with regionalised cell differentiation, including a bone-fat osteoid from stromal mesenchyme, and a patterned assembly of germ-layer tissues derived from pluripotent stem cells. Thus, CMFL is a valuable tool for generating orthogonally and chemomechanically defined niches able to guide spatially defined tissue patterns.

This approach enables studies to better understand how extrinsic niche factors regulate histogenic and morphogenetic processes,towards engineering complex structured tissue and organ systems with higher-level emergent function.

# Microscopic and RNAseq Analysis of Retinal Organoids With Optic Nerve Outgrowth

ORAL PRESENTATION

**Philip Wagstaff,**[1] **Anneloor ten Asbroek,**[1] **Jaco ten Brink,**[1] **Aldo Jongejan,**[2] **Nomdo Jansonius,**[3] **Arthur Bergen**[1,4]

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Retinal degenerative diseases such as glaucoma form a major global health problem. There is currently no cure for these diseases, largely due to the lack of suitable models. A lot of recent research is focused on the development of a new type of *in vitro* model representing all retinal cell types: organoids. In order for these models to represent the *in vivo* eye they must mimic the proper environment, whilst also being comparable to the *in vivo* situation in gene expression and the developmental timeline.

We constructed retinal organoids using an in-house developed protocol. First, we encased hESCs in 3D matrigel drops. After 4 days, we plated embryoid bodies for a further 7 days. We scraped off neurospheres at day 11 and allowed them to form retinal organoids in a 3D floating environment. We harvested samples at different timepoints and followed development microscopically, by RNAseq analysis and selected immunohistochemistry.

We found that the encasement of embryoid bodies in matrigel boosted early differentiation of organoids. We could generate retinal ganglion cells within 28 days without the addition of fate determining factors, whilst generating all other cell types found within the retina. We observed massive neural outgrowth when whole organoids were plated, managing to grow long optic nerve-like growth when using unidirectional scaffolds.

Our results present a method of generating retinal ganglion cells in a short timeframe. We observed interesting outgrowth when plating whole organoids, and managed to dictate this growth using scaffolds. This will allow us to study the development and morphology of the emerging eye with different disease models, as well as neurodegenerative diseases affecting the eye and optic nerve.

# 3D Printing of Inherently Nanoporous Polymers Via Polymerization-Induced Phase Separation

ORAL PRESENTATION

**Zheqin Dong,**[1] **Haijun Cui,**[1] **Haodong Zhang,**[2] **Fei Wang,**[2] **Xiang Zhan,**[3] **Frederik Mayer,**[4] **Britta Nestler,**[2] **Martin Wegener,**[4] **Pavel A. Levkin**[1]

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2 Institute of Applied Materials - Computational Materials Science (IAM-CMS), Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany
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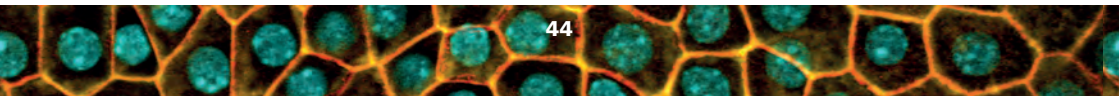
3D printing offers enormous flexibility in fabrication of polymer objects with complex geometries. However, it is not suitable for fabricating large polymer structures with geometrical features at the sub-micrometer scale. Porous structure at the sub-micrometer scale can render macroscopic objects with unique properties, including similarities with biological interfaces, permeability and large surface area, imperative *inter alia* for adsorption, separation, sensing or biomedical applications.

Here we introduce a method combining advantages of 3D printing via digital light processing and polymerization-induced phase separation, which enables formation of 3D polymer structures of digitally defined macroscopic geometry with controllable inherent porosity at the sub-micrometer scale.

We demonstrate the possibility to create 3D polymer structures of highly complex geometries and spatially controlled pore sizes from 10 nm to 1000 µm. Produced hierarchical polymers combining nanoporosity with micrometer-sized pores demonstrate improved adsorption performance due to better pore accessibility and favored cell adhesion and growth for 3D cell culture due to surface porosity.

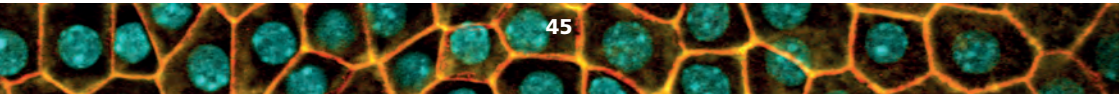
This method extends the scope of applications of 3D printing to hierarchical inherently porous 3D objects combining structural features ranging from 10 nm up to cm, making them valuable for a wide variety of applications.

# Flash Talks



# In Scheduled Order – Overview:

| | |
|---|---|
| **Additive Design of a Bioreactor for Electrical *In Vitro* Cell Stimulation** | Johann Schorzmann, Claudia Müller, Christian Bay, Aldo R. Boccaccini, Frank Döpper, Sahar Salehi |
| **Fish Primary Embryonic Stem Cells Self-Assemble Into Retinal Tissue Mirroring *In Vivo* Early Eye Development** | Lucie Zilova, Venera Weinhardt, Tinatini Tavhelidse, Thomas Thumberger, Joachim Wittbrodt |
| **Magnetoelectric Nanoelectrodes for Wireless Deep Brain Stimulation in Freely Moving Mice** | Kristen L. Kozielski, Hunter Gilbert, Ali Jahanshahianvar, Yan Yu, Önder Erin, David Francisco, Faisal Alosaimi, Yasin Temel, Metin Sitti |
| **Mechanical Stimulation of Single Cells by Reversible Host-Guest Interactions in 3D Microscaffolds** | Marc Hippler, Martin Bastmeyer |
| **Stable and Functional Integration of Synthetic Cells into Living 3D Cultures** | Oskar Staufer, Julia Csatari, Ilia Platzman, Joachim P. Spatz |



# Additive Design of a Bioreactor for Electrical *In Vitro* Cell Stimulation

**ORAL PRESENTATION**

**Johann Schorzmann,**[1] **Claudia Müller,**[2] **Christian Bay,**[3] **Aldo R. Boccaccini,**[4] **Frank Döpper,**[1] **Sahar Salehi**[2]

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For *in vitro* differentiation toward functional electroactive cells external electrical stimuli is required. This study reports on the use of Additive Manufacturing (AM) fabricating a bioreactor with selective electrical conductivity by material extrusion technique. The challenge was to integrate the electrodes in the bioreactor housing out of additively manufactured, biocompatible and electrically conductive materials.

The two components of the bioreactor were manufactured simultaneously in one part composed of two different materials. Due to the contact of the cells to the bioreactor housing and electrodes, the biocompatibility (ISO 10993-5) of several polymeric filaments was tested. Of the tested polymers, Polypropylen was chosen as the housing polymer for the bioreactor. Testing the electrical conductivity (ISO 3915), of 12 polymeric filaments, Polylactic acid with 20 % carbon black was selected ($R = 1.51 \times 10^1$ Ωcm with 140 €/kg). The construction of the electrodes is based on the functionality of a parallel-plate capacitor to create an electric field inside the growth chamber of the bioreactor.

In order to test the functionality of the bioreactor, differentiated myotubes (aligned C2C12) were developed on micro-patterned gelatine methacryloyl substrates printed on the glass bottom of the bioreactor after 3 days of culture and 7 days of differentiation. The perfusing bioreactor was incubated at 37 C and 5 % $CO_2$ and bipolar rectangular pulses were applied (f=1 Hz, $t_{on}$=1 ms, $U_{app}$=6 V) to the myotubes formed on the micro-grooved substrates. The synchronized beating of the aligned myotubes were detected and recorded using time laps imaging under the microscope.

# Fish Primary Embryonic Stem Cells Self-Assemble into Retinal Tissue Mirroring *In Vivo* Early Eye Development

ORAL PRESENTATION

**Lucie Zilova,**[1] **Venera Weinhardt,**[1] **Tinatini Tavhelidse,**[1] **Thomas Thumberger,**[1] **Joachim Wittbrodt**[1]

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Organization of pluripotent stem cells into organ-like structures (organoids) represents rapidly growing field providing a unique platform to study normal development as well as mechanistic and genetic roots of pathologies in a controlled environment. While human organoids are the most desired, they also take the longest time to develop, lack direct comparison to the *in vivo* biological processes and their systematic analysis is limited by technical challenges in genetic manipulations. To overcome these limitations, we turned to rapidly developing teleost species medaka and zebrafish. Although fish embryonic stem cells have been established, their ability to self-organize into high complexity tissues has not been elucidated.

In our manuscript, we used blastula stage medaka and zebrafish embryos as a source of pluripotent stem cells and established the conditions to generate anterior neural structures, particularly the retina. Within four days, blastula-stage cell aggregates reproducibly execute key steps of eye development: retinal specification, optic vesicle morphogenesis and the onset of differentiation. The number of aggregated cells as well as genetic factors crucially impacted on concomitant morphological changes that were intriguingly similar to *in vivo* situation.

We anticipate that fast development in combination with advanced techniques of genome editing will establish fish-derived organoids as a versatile tool enabling quicker ways to address multiple aspects of development and disease, and systematically probe interactions of organoids with variable physical environments.

Furthermore, derivation of organoids from a wide range of evolutionarily diverse species provides the opportunity to systematically address and distinguish intrinsic and extrinsic constraints contributing to organ morphogenesis and self-organization to ultimately tackle the question of the "conservation" of cellular self-organization.

# Magnetoelectric Nanoelectrodes for Wireless Deep Brain Stimulation in Freely Moving Mice

**ORAL PRESENTATION**

**Kristen L. Kozielski,[1,3] Hunter Gilbert,[1] Ali Jahanshahianvar,[2] Yan Yu,[1] Önder Erin,[1] David Francisco,[1] Faisal Alosaimi,[2] Yasin Temel,[2] and Metin Sitti[1]**

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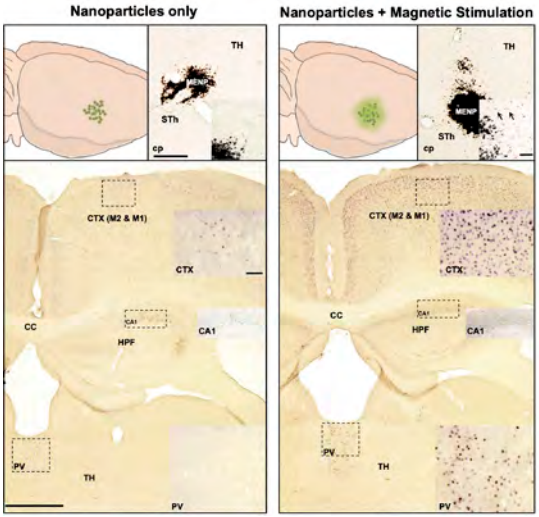
Deep brain stimulation (DBS), a clinical procedure that uses electrodes wired into the deep brain, has provided patients with symptom relief from Parkinson's disease and other disorders for more than 30 years. Recently, efforts to make neural intervention less invasive and safer have generated interest in smaller and wireless devices.

Our goal was to wirelessly modulate the deep brain of mice using injectable materials. We used magnetoelectric nanoelectrodes, which couple magnetic and electric fields, in order to generate electric signals in the brain. Magnetoelectric nanoparticles (MENPs) were synthesized using magnetostrictive $CoFe_2O_4$ nanoparticles strain-coupled to piezoelectric material $BaTiO_3$. MENPs were injected into the subthalamic region of mice, and magnetic stimulation was carried out while mice were awake and freely moving. We found significantly more activated (cFos positive) cells when MENPs were stimulated with a magnetic field versus MENPs only. cFos expression was also significantly higher in the motor cortex and limbic thalamus versus MENPs alone. Importantly, there was not a global change in cFos expression, such as in the CA1 region of the hippocampus.

This suggests that the cFos increases were due to local subthalamic stimulation of basal ganglia circuitry, and not a nonspecific, global modulation of neuronal activity via the magnetic field. Subthalamic modulation in mice led to modulation of the motor cortex and limbic thalamus, and subsequently, behavioral change. This work demonstrates MENPs as a versatile platform technology for less invasive, deep brain modulation.



# Mechanical Stimulation of Single Cells by Reversible Host-Guest Interactions in 3D Microscaffolds

ORAL PRESENTATION

**Marc Hippler,**[1,2] **Martin Bastmeyer**[2,3]

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Cell culture in three-dimensional (3D) environments is a thriving topic, which is driven by the desire to mimic physiological conditions as close as possible. In the recent past, 3D laser lithography was successfully employed to manufacture cell scaffolds with tailored geometry and spatially functionalized matrix. The next major goal is the transition from passive to dynamic systems that can be tuned on demand.

Herein, we present stimuli-responsive hydrogel microstructures fabricated by 3D laser lithography. The material is based on supramolecular host-guest chemistry that can be altered by addition of competitive guest molecules in solution. This system allows us to reversibly swell and shrink the hydrogel under physiological conditions. We characterize the stimuli-response as a function of concentration and time via atomic force microscopy and laser scanning microscopy.

We combine this material with conventional photoresists to create dynamic composite scaffolds consisting of protein-passivating base structures, protein-adhesive parts and the stimuli-responsive hydrogel. Using this design, we demonstrate spatially and temporally well-defined displacements of individual cell adhesions in a 3D environment. In a single experiment, it is possible to simultaneously record live cell data from a multitude of cells under different experimental conditions. The response of the cells is precisely tracked via digital image correlation to analyze the cell behavior as a function of time. With this versatile technique, we will study the mechanoresponse of cells and the role of different proteins in the mechanotransduction.

M. Hippler, K. Weißenbruch, K. Richler, E. D. Lemma, M. Nakahata, B. Richter, C. Barner-Kowollik, Y. Takashima, A. Harada, E. Blasco, M. Wegener, M. Tanaka, M. Bastmeyer, Science Advances 6, eabc2648

# Stable and Functional Integration of Synthetic Cells into Living 3D Cultures

**ORAL PRESENTATION**

**Oskar Staufer,**[1,2,3,4] **Julia Csatari,**[1] **Ilia Platzman,**[1,4] **Joachim P. Spatz**[1,2,3,4]

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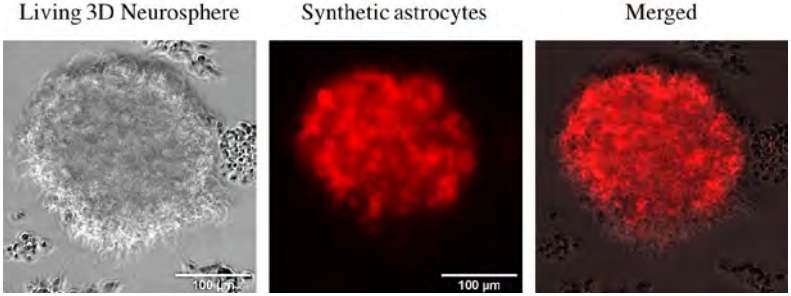
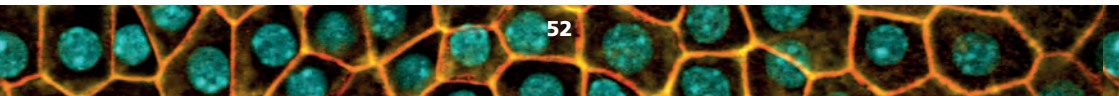
Native-state tissue architectures can be closely mimicked *in vitro* by applying 3D cell cultures. The constricted environment inside such collectives, fosters cellular interactions and promotes signalling. However, tuning individual biochemical and biomechanical properties of individual components of such complex 3D architectures (*e.g.* single cells) is so far only achieved by genetic engineering approaches or invasive probes. Therefore, concepts that allow to integrate addressable components like synthetic cells into 3D cultures, provide new means to direct and order 3D models.

I present complementary microfluidic-based approaches to create 3D cultures that self-assemble together with fully synthetic cells into hybrid-spheroids. I present how these stably integrated, GUV-based synthetic cells communicate with their natural neighbours by presenting cell surface proteins. Synthetic cells provide precisely defined properties to the 3D cultures, by which morphological plasticity or emerging signalling dynamics inside 3D cultures can be probed systematically. I show how introducing synthetic cells that present astrocyte-like adhesion proteins, mimic the natural, astrocyte-rich microenvironment and boost differentiation of immature neuronal spheroids.

Furthermore, I will take a more global view on how integration of artificial cell-like agents into living systems allows to perform cellular Turing-tests. The integration of artificial cells into living collectives could test their ability to exhibit life-like behaviour equivalent to, or indistinguishable from, living cells. This considers the question, 'Can matter be alive?'. When bidirectional information exchange is established and natural cells consider artificial cells as real parts of their collective, life-like properties could be assigned to the synthetic construct.



# Flash Talks & Poster Presentations



# In Scheduled Order – Overview:

| | |
|---|---|
| **Near-Field Electrospinning as Future Organotypic System** | Ahsana Sadaf, Monsur Islam, Dario Mager, Jan Gerrit Korvink |
| **High-Resolution, 3D-Printed Cell Niches Mimicking Native Tissue in Composition and Mechanics** | Amelie Erben, Stefanie Sudhop, Hauke Clausen-Schaumann, Michael Heymann |
| **A New Technology for the Generation of Perfect Cell Spheroids for Cell-Based Assays** | Annamarija Raic, Anett Ullrich, Nadine Kaiser, Véronique Schwartz |
| **YAP Nuclear/Cytoplasmic Relocation in 2D, 2.5D and 3D Constrained Fibroblasts** | Enrico Domenico Lemma, Marc Hippler, Sarah Bertels, Kai Weißenbruch, Stephan Keppler, Martin Bastmeyer |
| **Adhesion Miniproteins for Tissue Engineering** | Florian Häge, Franziska Thomas |
| **Assembly of Multi-Spheroid Cellular Architectures by Programmable Droplet Merging** | Haijun Cui, Xianxian Wang, Janine Wesslowski, Tina Tronser, Jakob Rosenbauer,Alexander Schug, Gary Davidson, Anna A. Popova, Pavel A. Levkin |
| **NMR Metabolomic Profiling for Efficient Derivation of Retinal Organoids** | Hannah Soergel, Lucie Zilova, Felix Loosli, Claudia Muhle-Goll, Joachim Wittbrodt |
| **Peptide-Based Hybrid Hydrogel as Novel Scaffold** | J. Luisa J. L. L. Wiechmann, Jasmina Gačanin, Christopher V. Synatschke, Tanja Weil |
| **Co-Cultivation of mESC-Derived Retinal Pigment Epithelium with Retinal Progenitor Cells** | Kai Patrick Richler |
| **Engineering and Actuation of Synthetic Cells With Light** | Kevin Jahnke, Noah Ritzmann, Julius Fichtler, Tobias Abele, Rasmus Schröder, Joachim P. Spatz, Daniel Müller, Kerstin Göpfrich |
| **Bioprinting by Light Sheet Lithography: Engineering Complex Tissues With High Resolution at High Speed** | Louise Breideband, Francesco Pampaloni, Levin Hafa, Gustaf Mårtensson, Robert Eklund, Helmut Wurst, Brigitte Angres, Núria Torras, Elena Martinez, Ruby Shalom-Feuerstein |
| **Sensitive Multi-Photon Photoresists for Rapid Parallelized 3D Laser Nanoprinting** | Pascal Kiefer, Vincent Hahn, Martina Nardi, Liang Yang, Eva Blasco, Christopher Barner-Kowollik, Martin Wegener |
| **Niche-Guided Tissue Patterning by Chemomechanical Flow Lithography** | Peter L. H. Newman, Pierre Osteil, Tim Anderson, Jane Q. J. Sun, Daryan Kempe, Maté Biro, Patrick P.L. Tam, Jae-Won Shin, Hala Zreiqat |
| **Cultivation and Differentiation of Retinal Cells in 3D Scaffolds** | Stephan Keppler |

# Near-Field Electrospinning as Future Organotypic System

ORAL & POSTER PRESENTATION

**Ahsana Sadaf,**[1] **Monsur Islam,**[1] **Dario Mager,**[1] **Jan Gerrit Korvink**[1]

1 Institute of Microstructure Technology (IMT), Karlsruhe Institute of Technology (KIT), Eggenstein-Leopoldshafen, Germany

Here we present preliminary results towards fabrication of 3D scaffolds for organotypic systems using near field electrospinning. Electrospinning is a technique of drawing nanofibers from a charged polymer droplet under a high electric field. Electrospinning has been demonstrated as a promising technique for scaffold fabrication for tissue engineering applications [1-2]. Here we aim to enable electrospinning to fabricate 3D architectures of nanofibers with controlled geometries, which can be suitable for scaffolds for tissue engineering. Our particular focus is on near field electrospinning (NFES), which enables patterning of the nanofibers into 2D geometries at a high printing speed (>10 cm/s). We are currently characterizing different processing parameters (such as electric field, printing speed, conductivity, and dielectric constant of solution), which can lead to stacking of the nanofibril towards creating a 3D structure. Till date, we have enabled the NFES technique to fabricate a 3D wall (Figure 1) featuring a maximum height of ~120 µm and a diameter of ~6 µm, using a solution of 10% polyethylene oxide in mixture of water (60%) and methanol (40%) and a rotating drum as the high-speed collector.

Our ongoing work is to optimize the processing parameters to narrow down the diameter of nanofiber and to achieve the straight wall of stacked fibers with high aspect ratio. We plan to use these NFES-driven 3D stacked walls of nanofibers as scaffolds for tissue engineering.

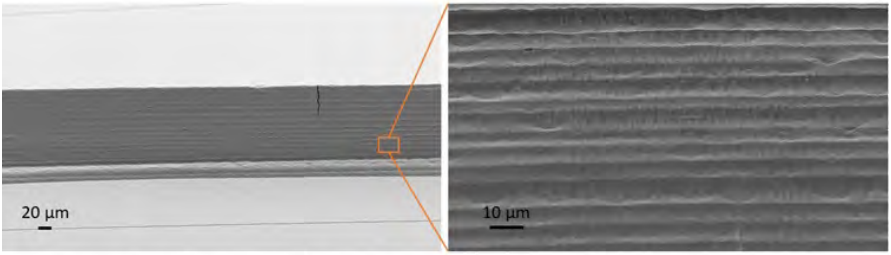



Fig. 1 SEM image of (left) a 3D wall obtained by stacking of nanofibers using NFES, and (right) high magnification image of the wall showing layers of the fiber stacking. The SEM was performed by 60° tilting of the SEM stage.

Reference:

[1] Chew, S.Y., M.A.H. Ruifa and K.W. Leong, 2008. The effect of the alignment of electrospun fibrous scaffolds on Schwann cell maturation [J]. Bio-Materials , 29(6): 653-661.

[2] Feifei Yan, Haiping Chen, Lulu Zheng, Weihua Chen, Yuanyuan Liu and Qingxi Hu, 2008. The Controllable PVA-Chitosan Fiber Prepared by the Near-field Electro Spinning for Tissue Engineering. Advance Journal of Food Science and Technology 5(8): 1073-1078

# High-Resolution, 3D-Printed Cell Niches Mimicking Native Tissue in Composition and Mechanics

**ORAL & POSTER PRESENTATION**

**Amelie Erben,**[1,2] **Stefanie Sudhop,**[1] **Hauke Clausen-Schaumann,**[1] **Michael Heymann**[3]

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The ECM offers pivotal cues guiding cellular behavior and fate *in vivo*, while it its self is deposited, remodeled and resorbed by cells. Apart from biochemical and mechanical factors, the micro-scale ECM architecture has been identified to guide cellular actions inducing tissue development, remodeling and homeostasis. Hence, controlling the 3D micro environment *in-vitro* with (sub-) cellular precision plays a pivotal role in creating instructive cell niches for tissue development.

Here, we adopted two-photon stereolithography (TPS), which is a high-resolution printing technique, to fabricate up to $mm^3$ -sized scaffolds with defined mechanical properties and sub-cellular resolution using protein-based resins such as gelatin methacryloyl. By converting the printing setup to dip-in and by additionally using a custom made objective seal, preventing resin to dry out during prolonged prints, we were able to mimic the ultrastructure of native alveoli. Further, we seeded these micro scaffolds with primary lung fibroblasts and compared them to *ex-vivo* alveolar biopsies. By adjusting printing parameters such as the laser speed, two-pass printing and post-processing, we were able two tune the resulting Young's Modulus in a range within 7 - 300 kPa, which we measured using atomic force microscopy. TPS will allow for a systematic investigation of single-cell and tissue dynamics in response to defined mechanical and bio-molecular cues and is ultimately scalable to full organs[1].


**Figure 1:** An excerpt of a native mouse alveoli was imaged by confocal microscopy and converted to print job instructions. These were printed using two-photon stereolithography with gelatin methacryloyl. After development, cells were seeded and cultivated on micro scaffolds in vitro. (Image adapted from [1])

[1] A. Erben, M. Hörning, B. Hartmann, T. Becke, S. A. Eisler, A. Southan, S. Cranz, O. Hayden, N. Kneidinger, M. Königshoff, et al., **2020**, 2000918, 1.

# A New Technology for the Generation of Perfect Cell Spheroids for Cell-Based Assays

ORAL & POSTER PRESENTATION

**Annamarija Raic,**[1] **Anett Ullrich,**[2] **Nadine Kaiser,**[3] **Véronique Schwartz**[1]

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3 BASF SE, Germany

The usage of 3D *in vitro* model systems in cancer and stem cell research as well as in pharmaceutical applications is becoming more and more relevant due to their potential to resemble the *in vivo* environment and human pharmacokinetics more properly than traditional 2D systems. Unnatural 2D cell cultures restricted to cell-cell contacts in one dimension influencing cell behavior and drug-target interactions.

3D cell spheroids – dense cell aggregates – allow cell-cell contact in all dimensions for more precise validation of cell based assays. For this purpose, we developed a chemically defined, animal-free coating solution which can be easily applied to create a robust and homogeneous coating by simply rinsing the culture surface of choice for a few seconds. The coating is biologically inert and prevents nonspecific binding of proteins and cells. We benchmarked our new technology using different cell lines such as hepatocytes showing a reproducible, rapid generation of round spheroids within 24 h outperforming current competitors.

This new technology allows the development of spheroid-based model systems within a remarkable short time for pharmaceutical as well as medical research.

# Yap Nuclear/Cytoplasmic Relocation In 2D, 2.5D And 3D Constrained Fibroblasts

ORAL & POSTER PRESENTATION

**Enrico Domenico Lemma,**[1] **Marc Hippler,**[1,2] **Sarah Bertels,**[1,3] **Kai Weißenbruch,**[1] **Stephan Keppler,**[1] **Martin Bastmeyer**[1,3]

1 Zoological Institute, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany
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In physiological conditions, cells interact in three-dimensional (3D) environments with other cells and with the extracellular matrix (ECM). Among other techniques, 3D direct laser writing, also known as two-photon lithography (2PL, Fig.1a), has emerged as a promising technology for fabricating tailored 3D scaffolds for cell biology studies.

A number of scaffolds realized via 2PL for biological applications have been described [1-2], but several cells properties which have been deeply studied on 2D substrates still have not been properly investigated in 3D scaffolds, which potentially may better resemble the physiological extracellular matrix (ECM)/cells interactions. Among these, the effects of such 3D microenvironments on the biochemical pathways which regulate mechanotransduction (e.g.Rho/ROCK) and actomyosin contractility have to be explored. In this context, YAP/TAZ proteins have been shown to play a key role in the activation of gene transcription involved in mechanosensing [3].

Here we exploited 2PL to realize microstructures for the investigation of YAP distribution in 3T3 fibroblasts, at single-cell resolution. In particular, 200x200µm 2D fibronectin-functionalized areas (Fig.1b) and 25µm squared 3D scaffolds with four fibronectin-coated spots (Fig.1c) were used to assess the predominant location of YAP in cells spread on flat surfaces and selectively attached to a 3D microenvironment, respectively.

Three hours after seeding on samples, 3T3 cells showed to have fully spread on flat areas and to have adhered only to the fibronectin-coated spots of the 3D scaffolds. Immunohistochemical staining was performed to retrieve YAP distribution within cells, and the ratio between nuclear vs. cytoplasmic YAP fluorescence intensity was calculated (N/C). Interestingly, N/C ratio was higher in the case of spread cells, while fibroblasts confined in 3D microenvironments showed predominantly cytoplasmic YAP (Fig.1d).

The study was then extended to further 2D, 3D and 2.5D (i.e. 3D structures with planar symmetry) patterns of different dimensions (Fig.1e). In these cases, N/C YAP ratio showed to be lowest in all cases in which cells could not spread over wide areas but were instead constrained within the fabricated pattern. Taken together, these results suggested that dimensionality does not induce per se the cytoplasmic relocation of YAP. On the contrary, geometrical constraints on 2D, 2.5D or 3D microstructures may interfere with the mechanosensing pathways responsible for YAP nuclear localization and subsequent genes transcription activation. Further experiments are needed to confirm the meaningfulness of the aforementioned observations.

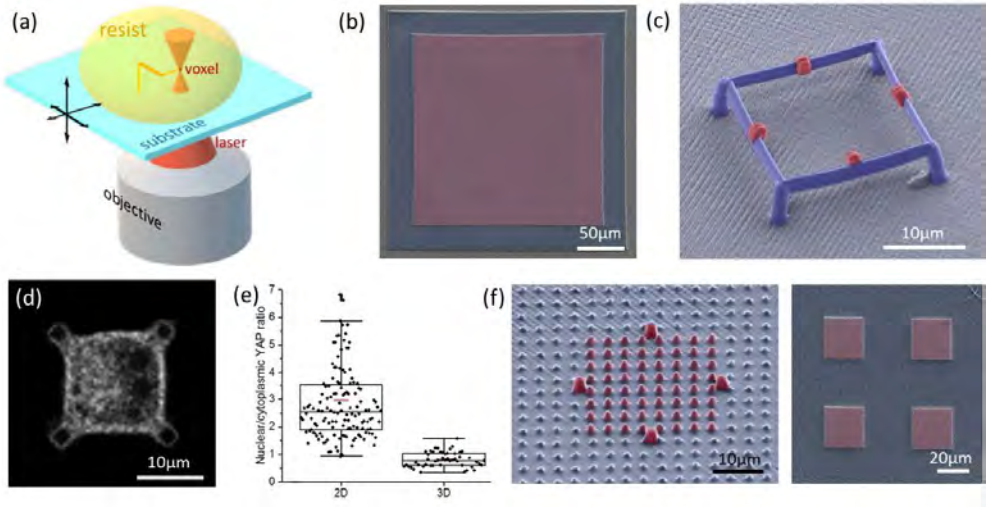


Fig.1. (a): sketch of 2PL; (b) flat squared structures and (c) 3D scaffolds for YAP location assessment; (d) YAP staining of a cell a 3D scaffold; (e) N/C ratio for 2D (left) and 3D (right) structures; (f) other 2PL-based structures for YAP location tests.

References: **[1]** Hippler et al. 2019; **[2]** Lemma et al., 2019; **[3]** Dupont et al. 2011.

# Adhesion Miniproteins for Tissue Engineering

ORAL & POSTER PRESENTATION

**Florian Häge,**[1] **Franziska Thomas**[1]

1 Institute of Organic Chemistry (OCI), Heidelberg University, Heidelberg, Germany

Tissue Engineering requires artificial constructs to support the formation of tissue from cells. These support structures consist of microporous organic or inorganic material that binds the cells of interest.

There are two common strategies to functionalize materials for cell responsiveness: the immobilization of cell adhesion motifs, which bind to a cell receptor, or the immobilization of entire domains of cell binding proteins. However, the small motifs lack a defined three-dimensional structure and thus bioactivity and the large protein fragments are difficult to immobilize and often denature in the attempt.

We aim to unify the bioactivity of a cell binding protein with the chemical modifiability of cell adhesion motifs: the design of adhesion miniproteins based on independently folding peptide scaffolds with a cell binding active site. Extracellular matrix proteins are known to bind the carbohydrate chains of cell receptors with, often calcium-dependent, protein-carbohydrate binding sites. One such active site is found in Laminin G4, which serves as our natural model for the peptide design. The natural model is incorporated into the sequence of a β-sheet peptide scaffold, such as the SH3 or WW domains, using computational modeling to ensure similar-to-native folding. We chose Rosetta as our modeling suite. Suitable sequences are then synthesized by solid-phase peptide synthesis (SPPS) and characterized with respect to structure and binding. We already designed multiple calcium-binding WW-domains that fold independently and will soon be tested for their carbohydrate binding activity.

Our goal is to design a biomimetic, easy to immobilize miniprotein that adds cell responsiveness to a microporous material and thus generate a functionalized material for retina tissue engineering.

Fig.1: Active site of Laminin G4 (left), native SH3 domain (center) and immobilized Adhesion Miniprotein (right).

# Assembly of Multi-spheroid Cellular Architectures by Programmable Droplet Merging

ORAL & POSTER PRESENTATION

**Haijun Cui,**[1] **Xianxian Wang,**[1] **Janine Wesslowski,**[1] **Tina Tronser,**[1] **Jakob Rosenbauer,**[3] **Alexander Schug,**[3,4] **Gary Davidson,**[1] **Anna A. Popova,**[1] **Pavel A. Levkin**[1,2]

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Artificial multicellular systems are gaining importance in the field of tissue engineering and regenerative medicine. Reconstruction of complex tissue architectures in vitro is nevertheless challenging, and methods permitting controllable and high-throughput fabrication of complex multicellular architectures are needed. Here, a facile and high-throughput method is developed based on a tunable droplet-fusion technique, allowing programmed assembly of multiple cell spheroids into complex multicellular architectures. The droplet-fusion technique allows for construction of various multicellular architectures (double-spheroids, multi-spheroids, hetero-spheroids) in a miniaturized high-density array format. As an example of application, the propagation of Wnt signaling is investigated within hetero-spheroids formed from two fused Wnt-releasing and Wnt-reporter cell spheroids. The developed method provides an approach for miniaturized, high-throughput construction of complex 3D multicellular architectures and can be applied for studying various biological processes including cell signaling, cancer invasion, embryogenesis, and neural development.

The established proMAD technology allows for controllable and high-throughput generation and screening of complex multi- and hetero-spheroidal architectures on demand. Hetero-spheroid complexes obtained with the proMAD method can be used to mimic distinct natural cellular compartments, their interactions, and signaling processes, thus enabling high-throughput investigation of biological processes under more controlled, semi-native environments and in a miniaturized way.

# NMR Metabolomic Profiling for Efficient Derivation of Retinal Organoids

ORAL & POSTER PRESENTATION

**Hannah Soergel,**[1] **Lucie Zilova,**[2] **Felix Loosli,**[3] **Claudia Muhle-Goll,**[1] **Joachim Wittbrodt**[2]

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The genetic background of stem cells affects their molecular and physiological traits including differentiation (Suzuki et al., 1999). In addition, it has been shown that reprogramming of induced pluripotent cells is accompanied by metabolomic changes, revealing a new layer of regulation (Panopoulos et al., 2012). Although details are not known, it is highly likely that the genetic background of cells affects the metabolome and both govern pluripotency and differentiation.

Medaka (*Oryzias latipes*) as a vertebrate teleost model offers the molecular genetic resources and tools required for this project (Kirchmaier et al., 2015). A panel of near-isogenic inbred lines has been established, covering a wide range of genetic and phenotypic variants. A pilot study on medaka liver extracts of two model inbred lines already indicated significant metabolic variation both between the lines and sexes. Thus, these lines are an ideal source of genetically diverse pluripotent cells that can be derived from blastula-stage embryos.

We have started a project where the metabolome of these embryos will be analysed using nuclear magnetic resonance (NMR) methodology. NMR spectroscopy offers simultaneous and quantitative detection of all kinds of small metabolites which makes it a perfect tool especially for untargeted analyses where the composition of the metabolome is not *a priori* known. By statistical methods we will search for distinct metabolite features that correlate with the differentiation potential of the cultured cells.

# Peptide-Based Hybrid Hydrogel as Novel Scaffold

ORAL & POSTER PRESENTATION

**J. Luisa J. L. L. Wiechmann,**[1] **Jasmina Gačanin,**[1] **Christopher V. Synatschke,**[1] **Tanja Weil**[1]

1 Max Planck Institute for Polymer Research, Mainz, Germany

Peptide-based hydrogels have appeared as promising biomaterials due to their biocompatible and biodegradable properties as well as their intrinsic ability to facilitate cell-material communication.[1] To address weak mechanical properties of many peptide hydrogels, our group has introduced hybrids by combining classical, covalent polymers with self-assembling peptides (SAP).[2] Gelation occurs due to hydrogen bonds between SAPs acting as supramolecular crosslinkers for covalent polymer backbones. These gels exhibit interesting mechanical properties such as thixotropy and injectability. Peptidic crosslinkers were chosen from so-called depsi peptides, isopeptides with an ester bond in the main chain, which undergo a pH-induced O-N-acyl shift enabling assembly through β-sheet formation (Figure 1 a)). In this study the influence of these peptide cross-linker was systematically studied by investigating the rheological effect of varying the peptide charge individually and conjoint, as well as analysing the impact of the peptide grafting density (Figure 1 b)). Different molecular weights of the backbones are tested to compare their influence on gel formation. Thereby structure-property relationships can be established based on varying hydrogel components. We anticipate these materials to be promising mimics of the ECM as well as suitable candidates for 3D-bioprinting, due to their biocompatible components. Their influence on cell behaviour will be subject of future studies.

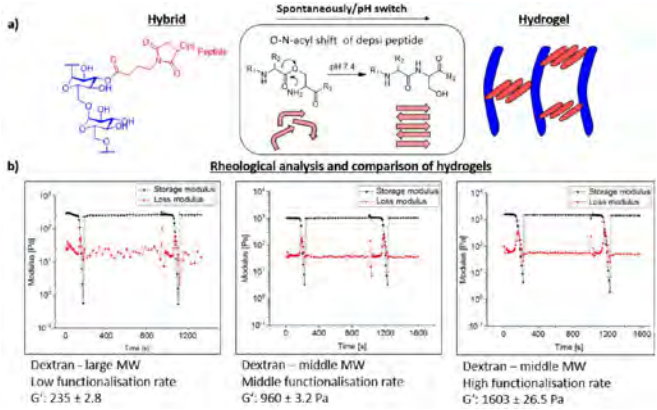


Fig 1:
a) Maleimide-peptide (red) grafted polymer backbone (blue) hybrid stimuli induced forming a hydrogel.
b) Rheological analysis of hydrogels with different compositions, like MW of dextran and peptide functionalisation rates

[1].Koh R.H.; Jin, Y.; Kang, B.J.; Hwang, N.S., Acta Biomater. 2017, 53, 318–328

[2].Gačanin, J., Hedrich, J., Sieste, S., Glaßer, G., Lieberwirth, I., Schilling, C., Fischer, S., Barth, H., Knöll, B., Synatschke, C. V., Weil, T., Adv. Mater. 2019, 31, 1805044.

# Co-Cultivation of mESC-Derived Retinal Pigment Epithelium With Retinal Progenitor Cells

ORAL & POSTER PRESENTATION

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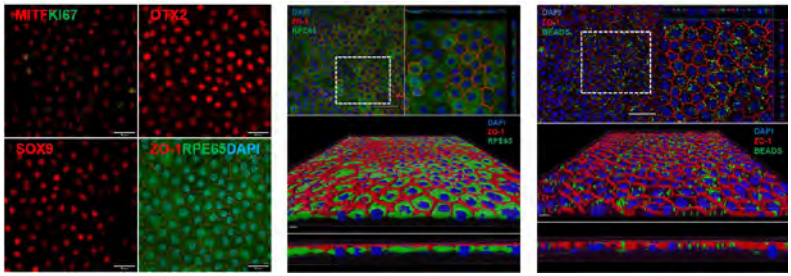
The vertebrate retina is multi-layered neural tissue composed of the neural retina and the retinal pigment epithelium (RPE). The neural retina consists of six neuronal and one glial cell types that modulate and transmit information that is perceived by the light-sensitive photoreceptor cells. Photoreceptors are embedded in the RPE, which has crucial functions in development and maintenance of retinal integrity. In the mature retina the RPE establishes the outer blood-retina barrier and thus has to set up a bi-directional transport between the retina and the choroid. Since photoreceptors cannot regenerate their visual pigment, many enzymatic reactions to maintain the visual cycle occur in RPE cells. Furthermore, shed photoreceptor outer segments are phagocytosed and recycled in the RPE.

Three-dimensional cell culture offers methods for the generation of organ-like structures, termed organoids. By the use of established protocols retina-like structures can be developed by self-organization and differentiation in organoids. However, RPE cells were rare in these organoids and not adjacent to the neuroretinal tissue.

The aim of this work is to establish the differentiation of RPE cells from mouse embryonic stem cells (mESCs). The differentiation protocol is based on a publication by Iwasaki et al. with minor variations. Serum-free floating culture of embryoid body-like aggregates with quick aggregation was used to generate early retinal progenitor-like cells that were then shifted towards RPE cell fate by application of a fibroblast growth factor receptor inhibitor and a WNT-signaling activator. mESCs could be successfully differentiated to RPE cells expressing a set of characteristic RPE marker proteins and showing internalization and secretion activity.

To resemble the in vivo development mESC-derived RPE cells grown on permeable membranes will be co-cultivated with retinal progenitor cells. The RPE cells grown on a porous membrane represents the outer blood-retina barrier. To enhance a pseudostratified self-organized retina-like layering 3D printed structures can be used as scaffolds for the retinal progenitor cells.



# Engineering and Actuation of Synthetic Cells With Light

ORAL & POSTER PRESENTATION

**Kevin Jahnke,**[1,2] **Noah Ritzmann,**[3] **Julius Fichtler,**[1,2] **Tobias Abele,**[1,2] **Rasmus Schröder,**[4] **Joachim P. Spatz,**[5] **Daniel Müller**[3] **and Kerstin Göpfrich**[1,2]

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Bottom-up synthetic biology aims to construct synthetic cells from natural and artificial molecular constituents. However, the creation, combination and manipulation of subcellular structures within synthetic cells towards multifunctional and dynamic systems with high spatiotemporal control remains difficult. To overcome this challenge, light is a suitable external, noninvasive and local trigger to engineer synthetic cells.

Here, we realize a strategic merger of top-down and bottom-up synthetic biology to convert light into proton gradients for the actuation of synthetic cellular systems. We genetically engineer *E. coli* to overexpress the light-driven inward-directed proton pump xenorhodopsin and encapsulate them as organelle mimics in artificial cell-sized compartments. Exposing the compartments to light-dark cycles, we can reversibly switch the pH by almost one pH unit and employ these pH gradients to trigger the attachment of DNA structures to the compartment periphery. For this purpose, a DNA triplex motif serves as a nanomechanical switch responding to the pH-trigger of the E. coli. By attaching a polymerized DNA origami plate to the DNA triplex motif, we obtain a cytoskeleton mimic that considerably deforms lipid vesicles in a pH-responsive manner. We envision that light-triggered actuation and more specifically 3D laser nanoprinting will be a leap forward for the bottom-up construction of synthetic cells.

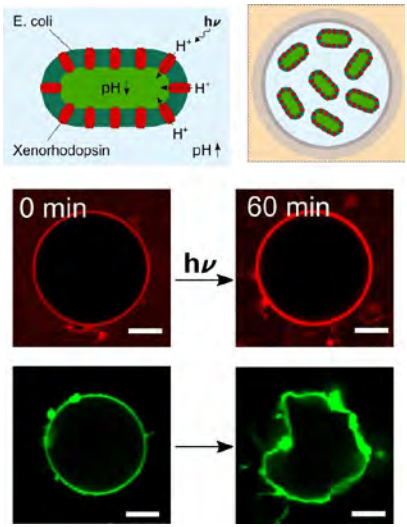


**Figure 1:**
*Top* Genetically engineered xenorhodopsin-expressing *E. coli* generate a pH gradient upon illumination with white light.
*Middle* They can stimulate the attachment of pH-sensitive DNA to the periphery within or around synthetic cells upon light illumination.
*Bottom* The use of pH-sensitive DNA can be expanded with a DNA origami that deforms giant unilamellar vesicles as synthetic cell models upon a pH stimulus.

# Bioprinting by Light Sheet Lithography: Engineering Complex Tissues With High Resolution at High Speed

ORAL & POSTER PRESENTATION

**Louise Breideband,**[1] **Francesco Pampaloni,**[1] **Levin Hafa,**[1] **Gustaf Mårtensson,**[2] **Robert Eklund,**[2] **Helmut Wurst,**[3] **Brigitte Angres,**[3] **Núria Torras,**[4] **Elena Martinez,**[4] **Ruby Shalom-Feuerstein**[5]

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Three-dimensional bioprinting (3D bioprinting) has been at the forefront of tissue engineering research in the past years, with ever more efficient systems reaching the market. While existing 3D bioprinting techniques are numerous and varied, they are limited by long printing times when used at high resolution. The technique described in this work aims at enabling fast and accurate production of monolayered skin constructs.

To achieve shorter production times, a digital scanned light-sheet is used to produce patterns of polymerized hydrogel, which enables the printing of a full three-dimensional plane in a matter of a few hundred milliseconds. The high resolution resides in the properties of the light sheet itself – the width of the light sheet represents the z-axial resolution of the system (as low as 10 µm) and the x-axial resolution is determined by the intensity profile of the gaussian beam (around 50 µm). In order to fully exploit this system, the hydrogel used to encapsulate the cells must therefore be tailor-made for photoactivated cross-linking.

As a proof of concept, a light sheet microscope is used as a polymerization source for novel photosensitive hydrogels. The upcoming hardware, software, chemical and biological improvements needed to reach the full potential of this system are expected to eventually be sufficient to print a complete skin construct, which could be used in the drug development industry, or as a graft for regenerative medicine therapy. Additionally, the constructs can be used to reduce and even replace animal testing for drug or cosmetic testing.

# Sensitive Multi-Photon Photoresists for Rapid Parallelized 3D Laser Nanoprinting

ORAL & POSTER PRESENTATION

**Pascal Kiefer,**[1] **Vincent Hahn,**[1,2] **Martina Nardi,**[2,3] **Liang Yang,**[1,2] **Eva Blasco,**[2,3,4] **Christopher Barner-Kowollik,**[2,3,5,6] **Martin Wegener**[1,2]

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Recent applications of 3D laser nanoprinting in biology, optics, and material sciences demand for high quantities of increasingly complex and bigger structures. To meet these requirements, faster 3D laser lithography systems are mandatory. The fabrication process involves the curing of a liquid photoresist by scanning of a laser beam. Thus the obvious approach is to increase the scan speed of this laser.

However, this option is limited by the current technological state of the art. In our approach, we split the laser beam into nine separate laser foci while maintaining the high scan speed to enable parallelized manufacturing of multiple structures. This decreases the production time tremendously. However, if one increases the number of foci further, the necessary average total laser power of femtosecond laser oscillators exceeds the Watt level. By developing more sensitive multi-photon photoresists, we are able to decrease the necessary total laser power and enable yet faster printing rates.

To draw a fair comparison between more than 70 published multi-photon photoresists, we introduce a dimensionless photoresist-sensitivity figure-of-merit (FOM). Using this data, already efficient photoresist systems can be identified and improved by either adding co-initiators or modifying comprised photoinitiators. Enhancing the commonly used composition of 7-diethylamino-3-thenoylcoumarin (DETC) in pentaerythritol triacrylate (PETA) by adding co-initiators increases the sensitivity only at low scanning speeds.

Therefore, we have developed a new photoresist system based on a modified benzylidene ketone photoinitiator. While sharing advantageous characteristics at high scanning speeds with DETC, the new systems shows a very large FOM, making it a promising candidate for highly sensitive photoresists.

# Niche-Guided Tissue Patterning by Chemomechanical Flow Lithography

ORAL & POSTER PRESENTATION

**Peter L. H. Newman,**[1] **Pierre Osteil,**[2,3] **Tim Anderson,**[1] **Jane Q. J. Sun,**[2,3] **Daryan Kempe,**[4] **Maté Biro,**[4] **Patrick P.L. Tam,**[2,3] **Jae-Won Shin,**[5] **Hala Zreiqat**[1]

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4 EMBL Australia, Single Molecule Science Node, School of Medical Sciences, UNSW, Sydney, Australia
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Pluripotent-stem-cell-derived tissue-models have been established with increasingly physiological shape, size and function, however, the histo-and-morphogenetic processes present in these models proceeds stochastically. This reflects an absence of technologies able to produce complex supportive cell niches that can reproducibly guide tissue patterning and generate welldefined tissue structures.

To address this, have developed chemomechanical flow lithography (CMFL), a printing technology that delivers orthogonally programmable chemical and mechanical properties to microstructured niches that drive the differentiation of selective cell types and spatial emplacement of these cells in amicropattern.

We print microstructured niches with conjugated of peptides, proteins and macromolecular morphogens across a range of Young's Modulus. Using such niches, we generated tissue patterned constructs from a single cell source with regionalised cell differentiation, including a bone-fat osteoid from stromal mesenchyme, and a patterned assembly of germ-layer tissues derived from pluripotent stem cells.

Thus, CMFL is a valuable tool for generating orthogonally and chemomechanically defined niches able to guide spatially defined tissue patterns.

This approach enables studies to better understand how extrinsic niche factors regulate histogenic and morphogenetic processes, towards engineering complex structured tissue and organ systems with higher-level emergent function.

# Cultivation and Differentiation of Retinal Cells in 3D Scaffolds

ORAL & POSTER PRESENTATION

**Stephan Keppler**[1]

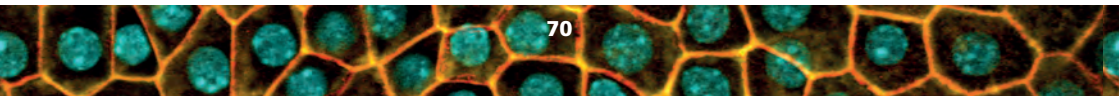
1 Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

The retina consists of six neuronal and one glial cell type and is enclosed by a pigmented epithelial layer. Most knowledge about the retina has been gained from animal models. In recent years, a cell culture method named organoids has emerged as an *in vitro* alternative. Organoids are obtained by aggregating pluripotent stem cells, which further differentiate to a retina-like tissue. They consist of the major retinal cell types and show a layering and organization similar to the *in vivo* situation. Accordingly, organoids are a well suited starting point for an *in vitro* culture system. However, there are two major drawbacks:

1. Organoids are limited in size due to insufficient nutrient supply,
2. in mouse organoids, retinal cells are not fully matured and resemble early postnatal cells.

In order to address these drawbacks, we combine established organoid cell culture with geometrically defined 3D growth substrates, thereby achieving a higher level of nutrient supply. This will result in extended cultivation times and, correspondingly, more matured tissue. We utilize 3D laser nanoprinting to fabricate highly defined, tailored 3D substrates in the sub-micrometer scale. After functionalization with extracellular matrix proteins, the substrates offer a growth-promoting environment for the retinal cells. By dissociating organoids at early stages of development, retinal progenitor-like cells are obtained and seeded into these substrates, allowing proliferation and differentiation in a defined environment. We aim to establish a correctly layered organotypic culture system with all major retinal cell types, allowing easy accessibility for microscopic observations and the combination with tissues like the retinal pigmented epithelium.

# Poster Presentations



# In Scheduled Order – Overview:

| | |
|---|---|
| **Characterization and Staging of Outer Plexiform Layer Development in Human Fetal Retina and Retinal Organoids** | Sumitha Prameela Bharathan, Angela Ferrario, Kayla Stepanian, G. Esteban Fernandez, Mark W. Reid, Justin S. Kim, Narine Harutyunyan, Carolyn Marks, Matthew E. Thornton, Brendan H. Grubbs, David Cobrinik, Jennifer G. Aparicio, Aaron Nagiel |
| **Apico-Basal Polarity in Stem Cells** | Elisa Josephina Genthner |
| **3D Vascularized Human Liver Model Establishment** | Hannah Buntz |
| **Fish Primary Embryonic Stem Cells Self-Assemble Into Retinal Tissue Mirroring *In Vivo* Early Eye Development** | Lucie Zilova, Venera Weinhardt, Tinatini Tavhelidse, Thomas Thumberger, Joachim Wittbrodt |
| **Differentiation of Mouse and Human Stem Cells into Müller Glia Cells Using 3D Printed Scaffolds** | Martina Deimling |
| **Microarchitectured Carbon Structures as Innovative Scaffolds for Tissue Engineering** | Monsur Islam, Andrés Díaz Lantada, Milagros Ramos Gómez, Dario Mager, Jan G. Korvink |
| **Development of 3D Vascularized Barrier Models for Disease Investigation** | Nicole Pleschka |
| **Live Imaging of Retinal Cell Growth and Differentiation in Organoids** | Nihad Softic, Bahtiyar Kurtulmus, Gislene Pereira |
| **Mechanoactivation of c-Abl Kinase** | Svenja de Buhr |
| **Reconstruction of the Blood-Retinal Interface by Combining New Hydrogel Materials and Stem Cell Technologies** | Tamara Molitor |
| **Magnetoelectric Nanoelectrodes for Wireless Deep Brain Stimulation in Freely Moving Mice** | Kristen L. Kozielski, Hunter Gilbert, Ali Jahanshahianvar, Yan Yu, Önder Erin, David Francisco, Faisal Alosaimi, Yasin Temel, Metin Sitti |
| **Superfast Curing and Crosslinker-Free Gelatin-Based Hydrogel Using Thiol-Ene Chemistry** | Xenia Kempter, Sonja Haase, Tobias Göckler |

# Characterization and Staging of Outer Plexiform Layer Development in Human Fetal Retina and Retinal Organoids

**POSTER PRESENTATION**

**Sumitha Prameela Bharathan,[1,2] Angela Ferrario,[1] Kayla Stepanian,[1] G. Esteban Fernandez,[2] Mark W. Reid,[1] Justin S. Kim,[1] Narine Harutyunyan,[1,2] Carolyn Marks,[3] Matthew E. Thornton,[4] Brendan H. Grubbs,[4] David Cobrinik,[1,2,5,6,7] Jennifer G. Aparicio,[1,2] Aaron Nagiel[1,2,5]**

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3 Core Center of Excellence in Nano Imaging, University of Southern California, Los Angeles,CA, USA
4 Maternal-Fetal Medicine Division, Department of Obstetrics and Gynecology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA
5 USC Roski Eye Institute, Department of Ophthalmology, Keck School of Medicine, Universityof Southern California, Los Angeles, CA, USA
6 Department of Biochemistry and Molecular Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA
7 Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

The first synapse of the visual system between photoreceptors and bipolar cells in the outer plexiform layer (OPL) of the retina is critical for visual processing. Development of this synaptic layer in the human retina is poorly understood. Using the human fetal retina, we establish a staging system for OPL development defined by OPL-Stages 0 to 4. In addition to cell type maturation and lamination, we demonstrate increasingly precise subcellular localization of the ribbon synapse proteins, Bassoon and Ribeye, with each stage.

This trend was reflected in decreasing interquartile range (IQR) values for Bassoon puncta intensity distribution along the apical-basal axis from OPL-Stages 0 to 4 (IQR = 27.76, 23.69, 13.46, 2.10, and 1.30 for OPL-Stages 0 to 4, respectively).

We next analyzed induced pluripotent and embryonic stem cell-derived human retinal organoids (HROs) to ascertain their ability to model these developmental stages. HROs formed an OPL by day 160 in culture with further maturation by day 220. Although development by cell type was dyssynchronous in the HROs, we applied OPL staging criteria for each cell type to HROs, revealing comparable progression through OPL-Stages 0 to 3 in the HROs from day 100 to day 220. Bassoon and Ribeye targeting to a thin strip along the OPL evolved in a similar fashion up to human fetal retina OPL-Stage 3.

We also identified regions resembling OPL-Stage 3 in day 160 HROs with presynaptic Ribeye apposed to the postsynaptic bipolar receptor mGluR6. Overall, this study defines early stages of human OPL development until mid-gestation and establishes HROs as a model system that recapitulates key aspects of human photoreceptor-bipolar cell synaptogenesis.

# Apico-Basal Polarity in Stem Cells

POSTER PRESENTATION

**Elisa Josephina Genthner**[1]

1 Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

The establishment of cell polarity is a key event for cell fate decision during embryogenesis and a prerequisite for differentiation. Thus, fundamental knowledge of this process is essential for understanding the formation of tissue consisting of multiple cell types, e.g., the retina. One of the earliest described polarity event linked to cell lineage specification occurs during the compaction of the pre-implanted eight-cell mouse embryo. Here, mouse embryonic stem cells exhibit an apical domain at the contact-free surface defined by an enrichment of the apical protein Ezrin and encircled by a contractile actomyosin ring.

The aim of this work is to investigate the apical polarization in stem cells. In preliminary work cytoskeletal structures associated with the apical marker protein Ezrin were identified in human induced pluripotent stem cells (hiPSCs). Single hiPSCs exhibit an actomyosinring that encircles an Ezrin enriched domain which we termed the apical cap. When cultivated as colonies, assemblies of hiPSCs display an intracellular structure that resembles the apical cap in molecular composition. It is characterized by a cell-spanning actomyosin ring which encircles an inner population of hiPSCs. This population is defined by an Ezrin enrichment with varying degrees from cell to cell and a network of pronounced actomyosin fibres on the apical side. We termed this structure the overarching apical structure. Interestingly, these self-organized apically located structures exhibited by hiPSCs *in vitro* resemble the apical domain occurring in early mouse embryonic development. Thus, highlighting a potential use of hiPSCs to acquire *in vitro* models for early human development.

To support the resemblance between the apical domain and the apical cap, as well as to determine if hiPSCs retain features of early blastocyst formation, further characterization of the presented apical structures need to be performed. In addition, the transfer to a 3D culture system, such as 3D micro-cavities, might be valuable. The scaffolds, together with a defined protein coating, allow small aggregate formation of hiPSCs, which mimics the *in vivo* situation better than 2D surfaces.

# 3D Vascularized Human Liver Model Establishment

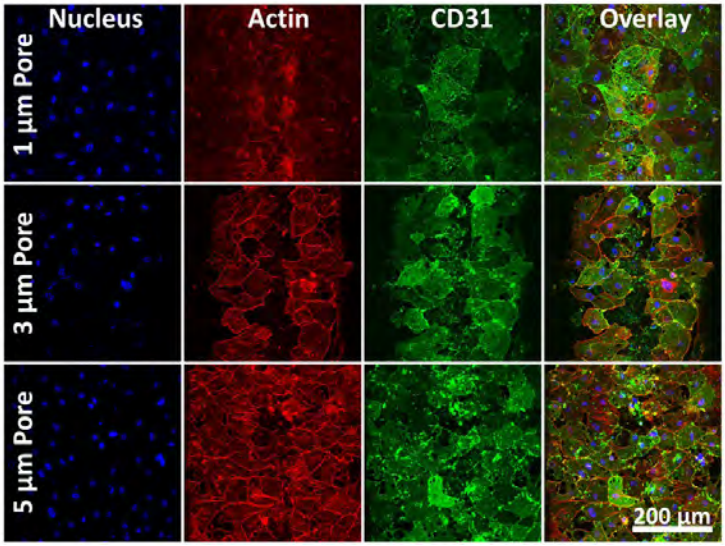
POSTER PRESENTATION

**Hannah Buntz**[1]

1 Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

The liver plays a crucial role in the human body and impairment, diseases or damage can be fatal. The liver is also a major threshold in drug development. The right metabolization, passage or interaction in the liver is crucial for the desired effect of drugs in the human body. In vitro liver models mimicking the human liver to study diseases or to develop drugs is the next step in liver research. The species-specific systems could simulate liver diseases like hepatitis or cirrhosis and in combination with human induced pluripotent stem cells (hiPSC) personified models are possible.

The aim of this project is to establish the sinusoid, the functional entity of the liver in a vascularized 3D organ-on-a-chip system. Therefore, human like conditions need to be replicated in which liver cells can be cultivated *in vitro*, in a microfluidic device. The optimal coating and fluidic conditions must be found for the liver sinusoidal endothelial cells (LSEC) and for the hepatocytes the right 3D scaffold must be established. Furthermore, the cell type functionalities need to be verified. Another goal is to differentiate LSEC from hiPSC and to apply them to the microfluidic system.



# Fish Primary Embryonic Stem Cells Self-Assemble Into Retinal Tissue Mirroring *In Vivo* Early Eye Development

**POSTER PRESENTATION**

**Lucie Zilova,**[1] **Venera Weinhardt,**[1] **Tinatini Tavhelidse,**[1] **Thomas Thumberger,**[1] **Joachim Wittbrodt**[1]

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Organization of pluripotent stem cells into organ-like structures (organoids) represents rapidly growing field providing a unique platform to study normal development as well as mechanistic and genetic roots of pathologies in a controlled environment. While human organoids are the most desired, they also take the longest time to develop, lack direct comparison to the *in vivo* biological processes and their systematic analysis is limited by technical challenges in genetic manipulations. To overcome these limitations, we turned to rapidly developing teleost species medaka and zebrafish. Although fish embryonic stem cells have been established, their ability to self-organize into high complexity tissues has not been elucidated.

In our manuscript, we used blastula stage medaka and zebrafish embryos as a source of pluripotent stem cells and established the conditions to generate anterior neural structures, particularly the retina. Within four days, blastula-stage cell aggregates reproducibly execute key steps of eye development: retinal specification, optic vesicle morphogenesis and the onset of differentiation. The number of aggregated cells as well as genetic factors crucially impacted on concomitant morphological changes that were intriguingly similar to *in vivo* situation.

We anticipate that fast development in combination with advanced techniques of genome editing will establish fish-derived organoids as a versatile tool enabling quicker ways to address multiple aspects of development and disease, and systematically probe interactions of organoids with variable physical environments.

Furthermore, derivation of organoids from a wide range of evolutionarily diverse species provides the opportunity to systematically address and distinguish intrinsic and extrinsic constraints contributing to organ morphogenesis and self-organization to ultimately tackle the question of the "conservation" of cellular self-organization.

# Differentiation of Mouse and Human Stem Cells into Müller Glia Cells Using 3D Printed Scaffolds

**POSTER PRESENTATION**

**Martina Deimling**[1]

1 Zoological Institute, Cell- and Neurobiology, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

To differentiate pluripotent stem cells (PSCs) into retinal cell types either organoid culture or direct differentiation into one specific retinal cell type is used. In my project, I will focus on the differentiation of human induced pluripotent stem cells (hiPSCs) and mouse embryonic stem cells (mESCs). In order to control and improve differentiation efficiency, it is important to understand how PSC aggregates or colonies self-organize during differentiation. It was found that upon differentiation cells of a pluripotent population gain different degrees of priming. Interestingly, cells within the colony that are located more peripheral have a higher probability to differentiate.

In a first step, I will analyze how the self-organization of hiPSCs affects differentiation or is affected by it. In a second step, I want to apply this knowledge to induce differentiation of pluripotent cells into one specific cell type, the Müller glia cells.

Since Müller glia cells have a rod-like morphology with many branches, I want to investigate whether a framework of tailor-made 3D scaffolds that support this morphology can improve the differentiation efficiency and maturation of the cells.

After differentiating PSCs into Müller glia cells within 3D scaffolds it would be possible to culture and co-cultivate the neural retinal cell types within the same scaffold, enabling them to establish contacts to the supporting structure of the Müller glia cells.

# Microarchitectured Carbon Structures as Innovative Scaffolds for Tissue Engineering

**POSTER PRESENTATION**

**Monsur Islam,**[1] **Andrés Díaz Lantada,**[2] **Milagros Ramos Gómez,**[3] **Dario Mager,**[1] **Jan G. Korvink**[1]

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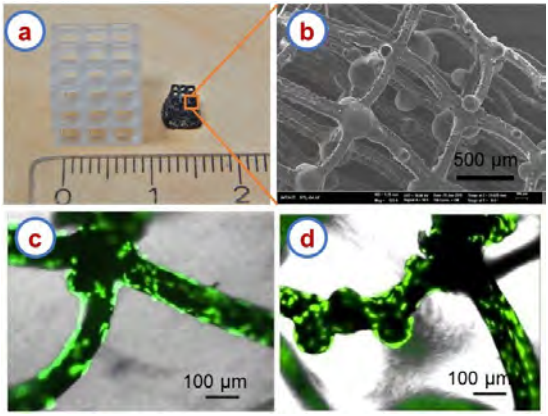
Carbon has been proven to be a promising material for tissue engineering due to its interesting bioactivity, and mechanical and electrical properties. However, control of geometrical features of carbon scaffolds is still a major issue. In this work, we postulate fabrication of design-controlled 3D carbon scaffolds using stereolithography, followed by carbonization. Using this approach, we aim to fabricate 3D carbon architectures mimicking the structural functionalities of human bones towards personalized tissue repair.

Till date, we successfully fabricated different 3D architectures of carbon microlattices by combining stereolithography and carbonization, as shown in Figure 1a. Around 60%-80% shrinkage occurred during the carbonization, resulting in a minimum carbon lattice thickness of 103.22±22.84 µm. Albeit overall structural integrity, the microlattices featured several microstructural deformations including wavy and bent lattices (Figure 1b), and hollow bulges along the microlattices, which yielded a low elastic modulus of the architectures (~2.28 MPa). However, the elastic modulus is within the range for application in human tissue repair. The *in vitro* cytocompatibility of the carbon materials was analyzed by culturing osteoblast-like murine MC3T3-E1 cells for three days on the fabricated architecture. The results showed that cells were able to adhere and survived on the carbon microlattices with a high viability (figure 1c and d). These results are promising for future applicability of the carbon architectures for personalized scaffolds for tissue engineering.




The ongoing work includes (i) tuning up the carbonization process to obtain carbon lattices with minimal deformities, (ii) fabrication of 3D structures mimicking bone structures and (iii) culturing of osteoblasts and chondrocytes on similar structures analyze additional types of cell-material interactions.

# Development of 3D Vascularized Barrier Models for Disease Investigation

POSTER PRESENTATION

**Nicole Pleschka**[1]

1 Institute of Functional Interfaces (IFG), Karlsruhe Institute of Technology (KIT), Eggenstein-Leopoldshafen, Germany

The human body can be considered as a collection of gas and liquid-filled compartments. For the correct physiological function of these compartments, a mutual delimitation by biological barriers is of great importance. These biological barriers are located at the interface between blood vessels and the surrounding organ tissue and are tight depending on the organ. Due to regulated molecule transport, biological barriers are only permeable to selective substances in a defined direction. In addition to providing the tissue with important nutrients, biological barriers protect individual organs from potentially toxic substances. Both the blood-brain barrier (BBB) in the brain and the inner blood-retinal barrier (iBRB) in the eye represent strong barriers in the human body. For this reason, the treatment of barrier dependent diseases is complicated by medication via blood flow to the organ target site. Besides, the review of new potential drugs as the direct investigation of barrier dependent diseases such as Parkinson´s disease or diabetic retinopathy in humans is limited. Animal experiments are used as an alternative, but due to species-specific differences, they are often not transferable to humans and ethically difficult to justify. Therefore, the focus of research today is on so-called Organ-on-a-chip systems. These are microfluidic test systems that can be used to simulate microfluidic organ models or for an in vitro reconstruction of clinical diseases. By connecting to a microfluidic pump system, the in vivo conditions can be better mimicked. Such models often base on human cells and have the potential to test new medical drugs with better efficiency and contribute to the development of new treatment options for medical purposes. For this reason, two barrier models (brain, retina) are being developed in this doctoral thesis in a 3D microfluidic Organ-on-a-chip system based on human cells. The establishing of these models involves the characterization of a suitable support matrix to mimic the natural cellular environment in the retina as well as in the brain. To generate three-dimensional cellular networks, human stem cells are integrated into the support matrix. These established models will be modified in the last step so that the disease reconstruction of diabetic retinopathy and Parkinson's are mapped in the Organ on a-chip system. These successful established barrier models with the required demand has not yet been generated and could lead to a more effectively drug development and new therapeutic options for barrier dependent diseases.

# Live Imaging of Retinal Cell Growth and Differentiation in Organoids

POSTER PRESENTATION

**Nihad Softic,**[1] **Bahtiyar Kurtulmus,**[1] **Gislene Pereira**[1]

1 University Heidelberg, Heidelberg, Germany

In the last decade, several groups have been working towards establishing a protocol to develop retinal organoids from murine and human embryonic stem cells, capable of fully maturing into all cell types found in the retina. Thanks to this research, many aspects of cellular behavior during retinal development started to become elucidated. For example, the signaling cascades responsible for the morphogenesis of optic vesicle and optic-cup, as well as, the effect of Wnt inhibitors on accumulation of cones and rods. Additionally, during the development of existing imaging approaches, markers for the majority of the cell types found in the retina have been identified.

However, despite the progress in field of retinal organoids, limitations to producing a functional tissue in an *in vitro* setting still exist. Most significantly, organoids reported so far start degrading before the maturation of photoreceptors takes place. Furthermore, most imaging has been done on fixed cells, via immunofluorescence for example. The aim of my project is thus to observe the maturation process via modern live imaging techniques in order to determine cellular behavior as a way to increase our understanding of this process. To achieve this, I aim to produce cell lines expressing fluorescent proteins under markers specific for each of the cell types found in the retina. More precisely, I am currently optimizing the recently developed CRISPR/Cas12a system to integrate expression cassettes into safe harbor loci, such as Rosa26, in murine stem cells. Moreover, I will produce lines expressing FUCCI cell cycle sensor combined with genomic markers, capable of monitoring changes in cell proliferation, cell death and chromosome instability. This is of particular relevance, as understanding the changes in the cell cycle length, programmed apoptosis, as well as oriented cell division is essential in mimicking the *in vivo* developmental process.

These insights into cellular behavior during retinal development have the potential of contributing to the optimization of 3D printed scaffolds by providing further insight into internal and external cues driving the development process. In particular, I believe that the incorporation of precise scaffolding with the insights obtained from retinal organoid development could produce a more functional retinal tissue, potentially capable of forming mature photoreceptors. Current scaffolds do not consider the developmental cues provided by lineage, like individual cell expressome and polarity. I believe that the effectiveness of the synthesis process could be improved by optimizing the scaffolding to the pre-existing developmental program. More precisely, by developing a temporal and spatial scaffolding schedule directed by the retinal development program, which can be observed in real time via characteristic fluorescent proteins indicative of cell types.

# Mechanoactivation of c-Abl Kinase

POSTER PRESENTATION

**Svenja de Buhr**[1,2]

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2 Interdisciplinary Center for Scientific Computing (IWR), Heidelberg University, Heidelberg, Germany

Stem cell differentiation is, in addition to soluble factors, influenced by physical properties such as matrix elasticity or shear forces. How do cells react to these cues and translate them into biochemical signals and behavioral changes?

Next to scaffold proteins, which become accessible for their downstream binding partners when subjected to force, signalling proteins can also directly sense forces.

The non-receptor tyrosine kinase c-Abl is involved in the regulation of development, cell proliferation and survival. Moreover, it has been shown to have enhanced activity in stretched cells, making it an interesting candidate for force sensing proteins. The kinase domain of Abl is tightly bound by its two Src homology domains SH2 and SH3, keeping it in an autoinhibited conformation. The kinase domain is C-terminally followed by a disordered region and an actin binding domain. A myristic acid moiety is covalently attached to the N-terminus of the SH3 domain through an 80 amino acids long linker. Since protein myristoylation is usually connected to membrane targeting, we reasoned that Abl can sense tension between the cellular membrane and the actin cytoskeleton.

Using force-probe molecular dynamics simulations we mimicked forces acting on the N-terminus of the SH3 domain and C-terminus of the kinase domain, which lead to the release of autoinhibitory contacts of the two Src homology domains from the kinase domain.

# Reconstruction of the Blood-Retinal Interface by Combining New Hydrogel Materials and Stem Cell Technologies

**POSTER PRESENTATION**

**Tamara Molitor**[1]

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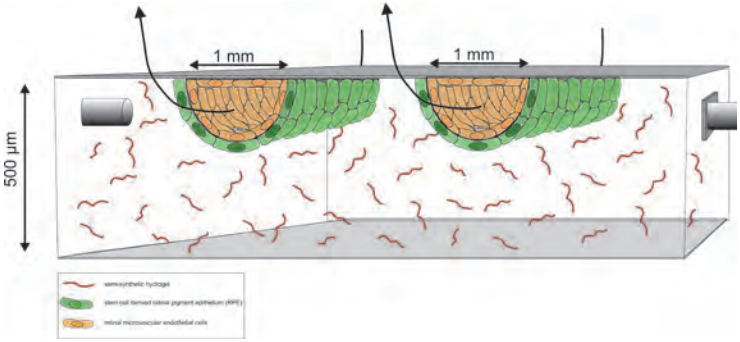
The blood-retinal barrier (BRB) is essential for normal visual function and a particularly tight, restric-tive physiological barrier regulating the bidirectional transport in the retina. The outer BRB (oBRB) is formed at the retinal pigment epithel (RPE), which is a single polarized cell-monolayer. The tight junctions between the apical lateral membranes of the RPE cells mediate the highly selective diffu-sion of solutes and nutrients from the choroid to the sub-retinal space to maintain retinal homeosta-sis. For a 3D *in-vivo*-like reconstruction of the oBRB *in-vitro* under controllable conditions, currently organ-on-chip systems like the vasQchip are used. Mouse embryonal stem cells (mESC) can be differentiated into functional retinal pigment epithelium (RPE) cells and cultured in the vasQchip above a vascularized microchannel with a porous membrane. This allows the interplay of the RPE with the endothelial cells inside the artificial blood vessel. Furthermore, the formation of new blood vessels by extending the existing vasculature, which is called angiogenesis, enables an optimized supply of nutrients and gas to the surrounding tissue. By using angiogenic stimulators like VEGF, tube formation and network assembly of endothelial cells can be artificially induced.

For an optimal growth of the cells during the RPE differentiation Matrigel is required, which is a com-plex protein mixture containing a lot of variables. That's why a possible replacement material has to be found, which allows the stimulated directed growth of mESC into RPE for the resembly of a na-ture like 3D oBRB. Semisynthetic gelatin-based hydrogel scaffolds can represent possible alterna-tives as extracellular matrix analog to maintain a 3D environment for the cells. In future, differentiat-ed RPE precursors and endothelial cells should be controllably dispensed in a biocompatible hydro-gel scaffold to form the oBRB as a complex 3D functional living tissues.

The reconstructed BRB model addresses the need for tissues suitable for drug testing systems as well as for transplantation.



# Magnetoelectric Nanoelectrodes for Wireless Deep Brain Stimulation in Freely Moving Mice

**POSTER PRESENTATION**

**Kristen L. Kozielski,**[1,3] **Hunter Gilbert,**[1] **Ali Jahanshahianvar,**[2] **Yan Yu,**[1] **Önder Erin,**[1] **David Francisco,**[1] **Faisal Alosaimi,**[2] **Yasin Temel,**[2] **and Metin Sitti**[1]

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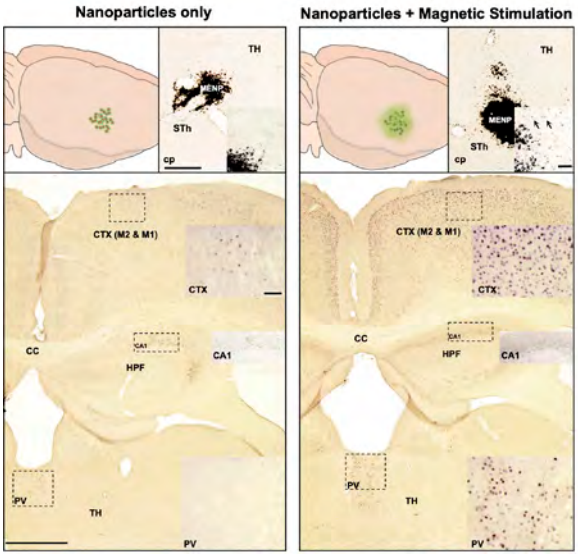
3 Institute of Functional Interfaces, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany

Deep brain stimulation (DBS), a clinical procedure that uses electrodes wired into the deep brain, has provided patients with symptom relief from Parkinson's disease and other disorders for more than 30 years. Recently, efforts to make neural intervention less invasive and safer have generated interest in smaller and wireless devices.

Our goal was to wirelessly modulate the deep brain of mice using injectable materials. We used magnetoelectric nanoelectrodes, which couple magnetic and electric fields, in order to generate electric signals in the brain. Magnetoelectric nanoparticles (MENPs) were synthesized using magnetostrictive $CoFe_2O_4$ nanoparticles strain-coupled to piezoelectric material $BaTiO_3$. MENPs were injected into the subthalamic region of mice, and magnetic stimulation was carried out while mice were awake and freely moving. We found significantly more activated (cFos positive) cells when MENPs were stimulated with a magnetic field versus MENPs only. cFos expression was also significantly higher in the motor cortex and limbic thalamus versus MENPs alone. Importantly, there was not a global change in cFos expression, such as in the CA1 region of the hippocampus.

This suggests that the cFos increases were due to local subthalamic stimulation of basal ganglia circuitry, and not a nonspecific, global modulation of neuronal activity via the magnetic field. Subthalamic modulation in mice led to modulation of the motor cortex and limbic thalamus, and subsequently, behavioral change. This work demonstrates MENPs as a versatile platform technology for less invasive, deep brain modulation.



# Superfast Curing and Crosslinker-Free Gelatin-Based Hydrogel Using Thiol-Ene Chemistry

**POSTER PRESENTATION**

**Xenia Kempter,**[1] **Sonja Haase,**[1] **Tobias Göckler**[1]

1 Institute of Functional Interfaces (IFG), Karlsruhe Institute of Technology (KIT), Eggenstein-Leopoldshafen, Germany

The *in vitro*-replication of tissue can make an immense contribution to the understanding of diseases, but also poses major challenges for researchers. One of these challenges is finding a suitable material for the generation of tissue. Photocurable gelatin-based hydrogels have established themselves as promising bioinks, including tissue engineering and 3D bioprinting.[1] The choice of photoreactive group, crosslinking mechanism and photoinitiator has a crucial impact on curing time and biocompatibility.[2] While the well-known gelatin methacryloyl (GelMA) is criticized for its chain-growth photopolymerization mechanism, heterogeneous network structure and reactivity with cellular components, norbornene-functionalized gelatin (GelNB) gains more and more importance as a suitable substitute.[3] The bioink formulation GelNB/GelS, based on two differently functionalized gelatin molecules, exploits the fast thiol-ene reaction between the norbornene and thiol group. The hydrogel precursor solution consists of GelNB and GelS and is crosslinked by a step-growth photopolymerization mechanism. GelNB and GelS were synthesized by modification of gelatin. Adjusting the synthesis parameters allowed a control over the degree of functionalization (DoF) resulting in GelNB/GelS Low, Medium and High with different rheological properties. Compared to other gelatin-based hydrogels, GelNB/GelS provides the fastest curing time, requires less toxic photoinitiator and shows the highest biocompatibility. Furthrmore, the hydrogel promotes fast cell spreading within the hydrogel. These superior properties renders the GelNB/GelS bioink formulation a promising bioink for light-based biofabrication techniques.[4]

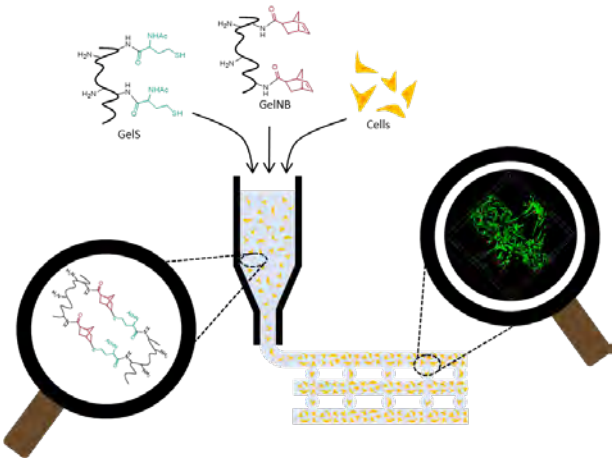



Figure 1: Modified by Göckler et al.[4]

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2. Malda, J., et al., 2013, DOI: 10.1002/adma.201302042
3. Lin, C.-C., C.S. Ki, and H. Shih, 2015, DOI: 10.1002/app.41563
4. Goeckler, T., Haase, S., Kempter, X., Tuning superfast-curing Thiol-Norbornene Functionalized Gelatin Hydrogel for 3D Bioprinting. Advanced Healthcare Materials, submitted.

# THANK YOU

## 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.

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 which made this year's Poster Award possible.

**Martin Bastmeyer** and **Joachim Wittbrodt,** Organizers

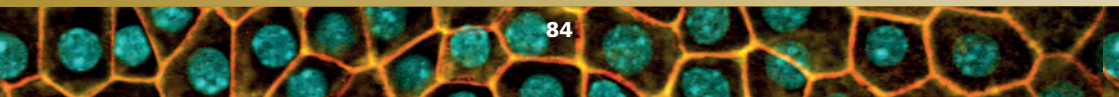
We hope to see you again at Future 3D Additive Manufacturing 2022!

## Upcoming Conferences – Save the Date!

**Future 3D Additive Manufacturing |** The 3DMM2O Conference 2022

**April 3-8, 2022**

**3D Nano- and Micro-Manufacturing: Technology and Technical Applications**

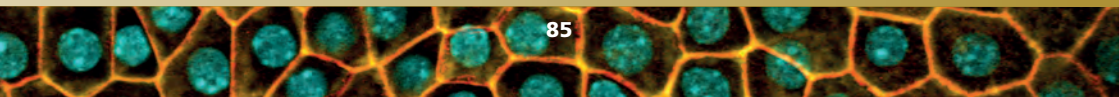


# Save the Date

**Future 3D Additive Manufacturing |** The 3DMM2O Conference 2023

**April 12-17, 2023 |** Working Title:

## 3D Molecular Systems



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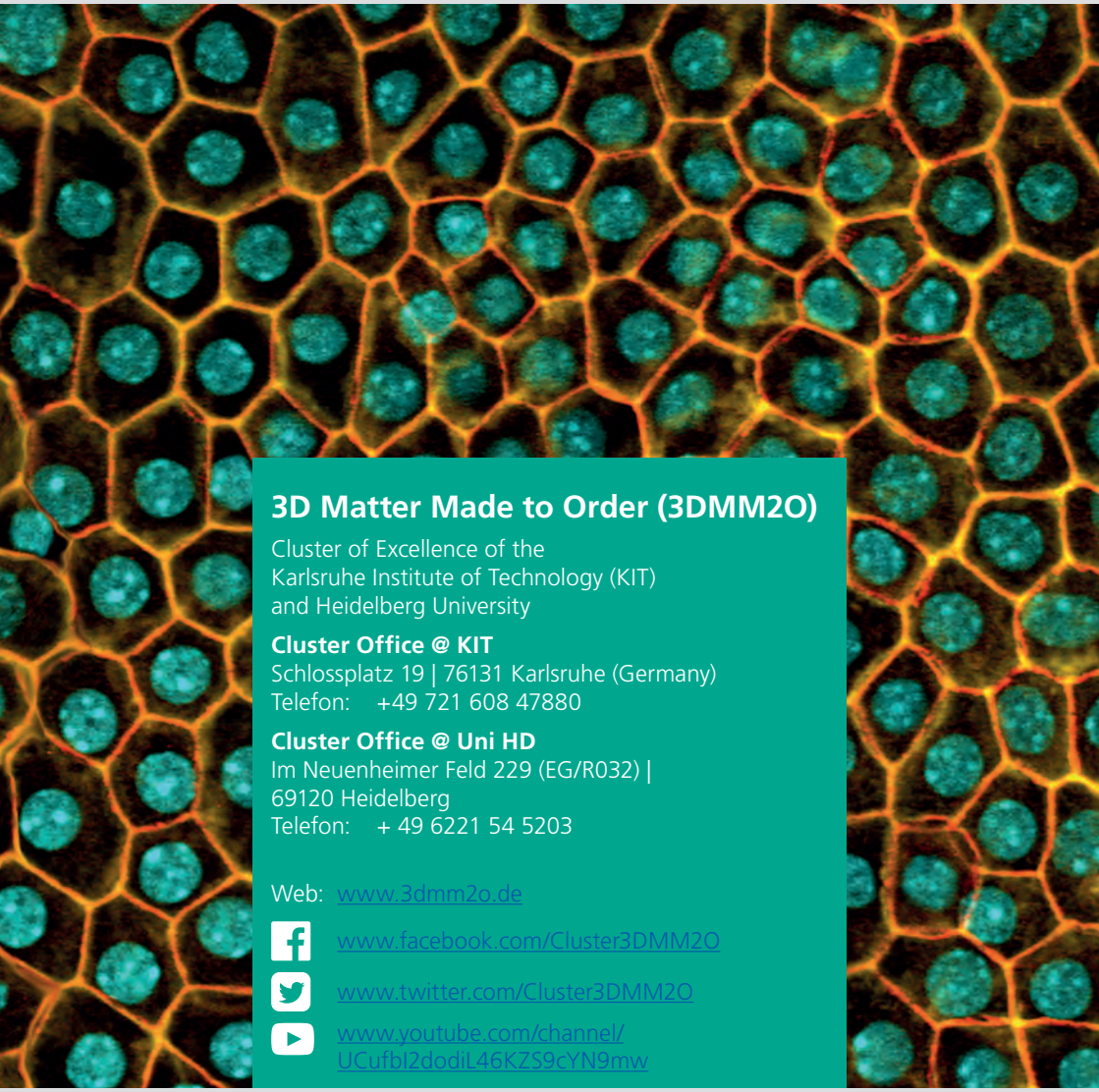

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