2022

EMBL · IBEC Conference

ENGINEERING MULTICELLULAR SYSTEMS

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Welcome to EMBL · IBEC Conference on ENGINEERING MULTICELLULAR SYSTEMS

Recent breakthroughs in stem cell biology, organ-on-chip assays, 3-D bioprinting, and cell mechanobiology have revolutionized our ability to design and assemble multicellular living systems, from organoids to embryos.

This biennial series of will focus on how engineering multicellular living systems is boosting our understanding of tissue and organ function, with applications in disease modelling, drug screening, and tissue engineering.

The 2nd edition conference will take place in PRBB Auditorium (Barcelona Biomedical Research Park), in Barcelona from 8-10th June 2022. We expect to bring together 150 researchers including stem cell biologists, systems biologists, physicists and engineers.

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Programme 8th June

08:15 – 09:00	Registration
09:00 – 09:30	Opening remarks
Session 1	**Chair: James Sharpe**
09:30 – 10:00	Self-organization and symmetry breaking in multicellular systems. *Prisca Liberali, Friedrich Miescher Institute for Biomedical research, Switzerland*
10:00 – 10:30	Bioengineering Motile Hybrid Robots and actuators. *Samuel Sanchez, Institute for Bioengineering of Catalonia, Spain*
10:30 – 10:45	*Short Talk* A stem cell zoo platform to study interspecies differences in developmental tempo. *Jorge Lazaro, European Molecular Biology Laboratory (EMBL), Spain*
10:45 – 11:00	New approaches for the multi-omic analysis in tissue engineering: looking deeper into the organoids. *Aitor González, PhD, Business Line Manager Izasa Scientific*
11:00 – 11:30	Coffee Break
Session 2	**Chair: Josep Samitier**
11:30 – 12:00	Self-organization of flow networks *Karen Alim, Technical University of Munich, Germany*
12:00 – 12:15	*Short Talk* Brain organoids to decipher the neuropathology of COVID-19 *Sandra Acosta, Universitat Pompeu Fabra, Institut de Biologia Evolutiva, Spain*

12:15 – 12:45	*In vitro* morphogenesis of cellular tornadoes *Aurélien Roux, Université de Genève*
12:45 - 13:15	Liver Organoids for Human Biology and Disease *Meritxell Huch, Max Planck Institute of Molecular Cell Biology and Genetics, Germany*
13:15 – 14:45	Lunch and Poster sesión with ODD numbers
Session 3	**Chair: Núria Montserrat**
14:45 – 15:15	*Ethics session* Rescuing Bioengineering Ethics from Bioengineering Ethicists *Matthiew Sample, Leibniz Universität Hannover, Germany*
15:15 – 15:45	*Ethica session* Biotechnology and Human Futures *Amy Hinterberger, King's College London, UK*
15:45 – 16:15	Coffee break
16:15 - 16:30	*Short Talk* Synthetic epithelial morphogenesis through controlled stretching and buckling *Nimesh Chahare, Institute for Bioengineering of Catalonia (IBEC)*
16:30 – 16:45	*Short Talk* Computational Models Guide the Research and Control of Angiogenesis *Tommaso Ristori, Eindhoven University of Technology, the Netherlands*
16:45 – 17:15	3D organotypic cultures from fish: towards an artificial retina *Joachim Wittbrodt, Universität Heidelberg, Germany*

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Programme 9th June

Session 4	**Chair: Tina Haase**
09:00 – 9:30	Cardioids unravel human heart development and disease *Sasha Menjdan, Austrian Academy of Sciences*
9:30 – 10:00	Neuromuscular organoids to study human development and disease *Mina Gouti, Max Delbrück Center for Molecular Medicine*
10:00 – 10:15	*Short Talk* Development of a contractile skeletal muscle organoid for the modelling of muscular dystrophies *Ainoa Tejedera, Institute for Bioengineering of Catalonia (IBEC), Spain*
10:15 – 10:45	From cell generated forces to global tissue pattern and shape (and back) *Pierre François Lenne, Institut de Biologie du développement de Marseille, France*
10:45 – 11:15	Coffee Break
Session 5	Chair: Miki Ebisuya
11:15 – 11:45	Topological defects in Hydra morphogenesis *Kinneret Keren, Technion – Israel Institute of Technology, Israel*
11:45 – 12:00	*Short Talk* Polarised contractile jiggling drives the collective amoeboid migration of cell clusters *Diane-Laure Pagès, INSERM U-1279, Gustave Roussy, France*
12:00 – 12:30	Biophysics of symmetry breaking in a mammalian embryo-like system *Vikas Trivedi, European Molecular Biology Laboratory, Spain*

12:30 – 12:45	*Short Talk* Using bioelectric cues to engineer collective migration, healing, and 3D form in multicellular systems *Daniel Cohen, Princeton University, United States of America*
12:45 – 13:00	*Short Talk* Modeling pancreatic cancer morphogenesis by self-organized branching organoids *Aristeidis Papargyriou, Klinik und Poliklinik für Innere Medizin II, Klinikum rechts der Isar der TUM*
13:00 – 14:30	Lunch and Poster session with EVEN numbers
Session 6	**Chair: Vikas Trivedi**
14:30 – 14:50	High-throughput mechanical screening solutions for cells and biomaterials *Luca Bersanini, Product Specialist Optics 11 Life.*
14:50 – 15:20	Bioengineering vascularized microtissues *Cristina Barrias, i3S – Instituto de Investigação e Inovação em Saúde, Portugal*
15:20 - 15:50	Neurovascular *in vitro* models *Anna Herland, KTH Royal Institute of Technology, Sweden*
15:50 – 16:15	Coffee break
16:15 – 16:45	Cellular proliferation and plasticity in pulmonary neuroendocrine cells and cancer: towards building engineered organoid models of cancer *Tayla Dayton, Hubrecht Institute, the Netherlands*
16:45 – 17:15	Tissue engineering with mechanically induced solid-fluid transitions *Selman Sakar, École polytechnique fédérale de Lausanne (EPFL), Switzerland*
17:15 – 18:00	Networking coctail

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Programme 10th June

Session 7	**Chair: Xavier Trepat**
09:00 – 9:30	From Stem Cells to Embryos – Ex Utero *Jacob Hanna, Weizmann Institute of Science, Israel*
9:30 – 9:45	*Short Talk* Entering the matrix: mapping, and using, the 3D topology of the extracellular matrix to reveal its role as a master cell regulator *Alejandro Mayorca Guiliani, Biotech Research and Innovation Center, Faculty of Health Sciences, University of Copenhagen, Denmark*
9:45 – 10:15	Coordinating cell fate decisions and tissue shape changes during mammalian development *Marta Shahbazi, MrC Laboratory of Molecular Biology, UK*
10:15 – 11:15	Coffee Break and poster session
11:45 – 12:15	Cell growth under mechanical pressure *Morgan Delarue, Laboratoire d'analyse et d'architecture des systèmes, France*
12:15 – 12:30	*Short Talk* Mapping tumor spheroid mechanics in dependence of 3D microenvironment stiffness and degradability by Brillouin microscopy *Vaibhav Mahajan, TU Dresden, Center for Molecular and Cellular Bioengineering (CMCB), Germany*
12:30 – 13:00	Models of neurological disease: Technologies and applications *Roger Kamm, Massachusetts Institute of Technology, USA*
13:00 – 13:15	Closing remarks and awards

Keynote Lectures

Self-organization and symmetry breaking in multicellular systems

Prisca Liberali

Multicellular organisms are composed of cells with identical genomes but different properties and functions. They all develop from one cell to form multicellular structures of astounding complexity. I will present how cellular interactions generate emergent tissue scale properties and drive spatio-temporal coordination during development and regeneration. Moreover, I will present the molecular mechanisms underlying intestinal organoid self-organization and the role of cell-to-cell variability in populations of differentiating cells during symmetry breaking

Prisca Liberali
Friedrich Miescher Institute for Biomedical research, Switzerland

Prisca Liberali is a senior group leader at the Friedrich Miescher Institute for Biomedical Research (FMI) in Basel. She has been trained as a physical organic chemist and then changed fields for her PhD and became a cell biologist. Prisca Liberali made important contributions in fields ranging from fundamental chemistry to in vivo tissue regeneration. Currently, her laboratory uses organoid, 3D mini organs that mimic tissue organization. She continue developing imaging methods and computational pipeline for all different type of organoids, such as intestine and liver. This year she was awarded the Friedrich Miescher price.

Bioengineering Motile Hybrid Robots and actuators

Samuel Sánchez

The combination of biological components and artificial ones emerges into what we call hybrid machines/bots/robots [(1)]. One of the challenges in bioengineering is to fabricate small yet complex living systems which can recapitulate some of the features of *in vivo* counterparts and be used for various applications from medicine to robotics. In our lab, we combine hydrogels, nanoparticles and skeletal muscle tissues to develop 3D actuators and swimmers.

In this talk, I will present the use of 3D bioprinting techniques to fabricate hybrid 3D BIOBOTS which provides flexibility, scalability, rapid prototyping and simplicity. This technique has emerged as a powerful tool for the development of functional three-dimensional tissues and, in particular, skeletal muscle. By electrical stimulation, we studied the adaptability of 3D hybrid Robots after long-term trainings and the force evolution during the trainings together with the dynamic gene expression. Two types of devices are bioengineered in our lab: (i) 3D Actuators which useful force measurement platform for drug screening against dystrophies [(2, 3)] and (ii) 3D BIOBOTS that can be a next generation of living soft robotics systems with swimming capabilities, being the fastest swimmer of its kind [(4)].

References:

(1) Mestre, Patino, Sánchez. WIREs Nanomed Nanobiotechnol, 2021, e01703

(2) Mestre, Patino, et al. Adv. Mat. Tech. 2019, 4 (2), 1800631

(3) Mestre et al. Biofabrication, 2021, 13 (4), 045011

(4) Guix, Mestre, et al. Sci. Robot. 2021 6 (53), eabe7577

Samuel Sánchez Ordóñez
Institute for Bioengineering of Catalonia (IBEC)

Samuel is ICREA Research Professor, Group Leader and Deputy Director at the Institute for Bioengineering of Catalonia. Before that, he worked at the Max Planck Institute for Intelligent Systems Stuttgart, at the Institute for Integrative Nanosciences at IFW Dresden, Germany, and at MANA-NIMS in Japan. He is currently honorary visiting Professor at HIT Harbin in China and Adjunct Professor at POSTECH University in South Korea. He received several awards: MIT TR35 Top Innovator Under 35 Spain 2014, Guinness World Records in 2010 and 2017, the Princess of Girona Scientific Award 2015 and the National Research Award for Young Talent 2016 by the Catalan Research Foundation. He is elected member of the Young Academy of Spain and acted as Young Ambassador of Innovators Under 35 giving a pitch at the EU Parliament in 2017, and invited talks and seminars at the European Research Council. He received the prestigious ERC-Starting grant in 2013 and the ERC-Consolidator Grant in 2019 in addition to two ERC Proof of Concept in 2016 and 2018. Besides extensive public funding (>8Mi€), he has cooperation agreements with the Private sector and hospitals. He has published >150 papers with h-index of 64 and filed 7 patents.

His group's main interests span from self-propelled nanoparticles as intelligent vehicles in biomedicine to the 3D Bioengineering of biohybrid robots and actuators.

Self-organization of flow networks

Karen Alim

Vascular networks constantly reorganize to optimize function. Some veins grow, others shrink and disappear. How can a local adaptation mechanism account for the plethora of vein dynamics observed? Here, we quantify network-wide vein dynamics in the slime mold Physarum polycephalum. We identify that flow shear stress is driving adaptation yet with a time delay. Vein fate, however, depends, beyond shear stress, on the vein's connections to the network and relative position. Finally, as network architecture constantly changes vein fate is dynamic driving overall network reorganization including avalanches of vein disappearance. Addition of external stimuli on top drives vein adaptation dynamics – imprinting the stimuli location into the network architecture. The memory of the stimulus location within the network architecture is retained during network reorganization and impacts overall network function.

Karen Alim
Technical University of Munich, Germany

Karen Alim studied physics in Karlsruhe, Manchester and Munich. She obtained an MSc in Theoretical Physics in 2004 working with Alan J. Bray from Manchester University, U.K., followed by a Diplom (MSc) in Physics and Biophysics at the LMU Munich. During her PhD with Erwin Frey at the LMU in Munich she investigated the form of biological materials like DNA/actin and patterning mechanism during leaf development. As a grad fellow at the KITP in Santa Barbara, United States, she investigated the mechanics of plant growth. After her doctoral degree in 2010 she joined Michael P. Brenner's group at Harvard University where she focused on the adaptation dynamics of the network-like forager Physarum polycephalum. *In 2015 she started as an independent group leader at the Max Planck Institute for Dynamics and Self-Organization. In 2019 she joined the Technical University of Munich as a permanent professor.*

Karen is recipient of the John Birks Award of Manchester University and held an appointment as lecturer in Applied Mathematics at Harvard University. In 2020 Karen was awarded an ERC Starting Grant.

In vitro morphogenesis of cellular tornadoes

Aurélien Roux

Tissues acquire function and shape via differentiation and morphogenesis. Both processes are driven by coordinating cellular forces and shapes at the tissue scale, but general principles governing this interplay remain to be discovered. Here, we report that self-organization of myoblasts around integer topological defects, namely spirals and asters, suffices to establish complex multicellular architectures. In particular, these arrangements can trigger localized cell differentiation or, alternatively, when differentiation is inhibited, they can drive the growth of swirling protrusions. Both localized differentiation and growth of cellular vortices require specific stress patterns. By analyzing the experimental velocity and orientational fields through active gel theory, we show that integer topological defects can generate force gradients that concentrate compressive stresses. We reveal these gradients by assessing spatial changes in nuclear volume and deformations of elastic pillars. Altogether, we propose integer topological defects as mechanical organizing centers controlling differentiation and morphogenesis.

Aurélien Roux
Université de Genève

Since Aug 1st 2019: Full Professor (tenured), Biochemistry Department, University of Geneva, Switzerland.

Reconstituting Tissue Morphogenesis: Recently, my goal of understanding how biological surfaces deform drove my interest towards how cell monolayers – epithelia – deform during development. I have put a strong effort in innovative assays to reconstitute epithelium folding in vitro.

We grew epithelia in alginate tubes to constrain them in a cylindrical geometry, and showed that their final tubular shape critical depends on the initial curvature of the tube, and the contractility of the cells (Maechler et al. J Cell Sci 2019).

My group optimized an encapsulation technology for adhesive cells in hollow spheres of alginate (Alessandri et al. LOC 2016), and we showed that epithelium growing under the spherical confinement of those capsules fold by buckling (Trushko et al. Dev Cell 2020). This process is similar to the one we proposed for ESCRT-III, showing that biological surfaces are deformed through similar mechanisms at different scales.

Following our interest on curvature of epithelia, Caterina Tomba showed that cells transiently swell upon rolling (Tomba et al. Dev Cell 2022). Moreover, Ilaria Di Meglio showed that pressure controls the cell cycle progression of epithelia in a b-catenin dependent manner (Di Meglio et al. Cell Reports 2022)

Our interest in understanding principles of morphogenesis in vitro extended to nematic tissues. Pau Guillamat showed that confining myoblasts on 2D micropatterns forced them to adopt a rotating spiral organisation. While proliferating, cells reorganised into a contractile aster that further grew into a dome, at the top of which cells readopted the spiral configuration. Further grow transformed the dome spiral into a vortex, forming long protrusion (half a millimeter) that rotates, forming cellular tornadoes.

Liver Organoids for Human Biology and Disease

Meritxell Huch

In vitro 3D cultures are emerging as novel systems to study tissue development, organogenesis and stem cell behavior ex-vivo. My lab and I, we have developed organoid cultures from healthy and diseased, human and mouse, adult and embryonic tissues for a range of organs including stomach, liver and pancreas. These have allowed, for the first time, the long-term expansion of adult (stomach, liver and pancreas) and embryonic (liver) tissue into 3D-epithelial structures that we have termed organoids, since these (1) self-assemble and can be clonally expanded, (2) resemble the corresponding tissues-of-origin and (3) allow the study of some aspects of tissue physiology in a dish. Here, I will present our liver organoid work and summarize our findings whereby our organoid culture system recapitulates many aspects of liver regeneration in a dish, especially the cellular plasticity that enables the activation of adult differentiated liver cells into proliferating progenitors. At the molecular scale, we have found that progenitor activation from differentiated cells occurs through a transient, genome-wide remodeling, of the cells' transcriptome and epigenome (DNA methylome/ hydroxymethylome), both during organoid initiation and *in vivo*, following tissue damage. We are studying similar plasticity in cancer using patient-derived liver cancer organoids we recently developed. Our results indicate that adult tissue derived organoid cultures represent novel *in vitro* models that enable the understanding of basic biological principles of tissue regeneration and cancer across different biological scales.

Meritxell Huch
Max Planck Institute of Molecular Cell Biology and Genetics, Germany

Dr Meritxell Huch is a Lise Meitner Max Planck Research Group Leader at the MPI-CBG, in Dresden. She obtained her PhD at the Center for Genomic Regulation in Barcelona, Spain in 2007 and moved to the Netherlands at the Hubrecht Institute, for her postdoc. There, she pioneered the generation of organoids for non-intestinal tissues including stomach, liver and pancreas. Following these studies, she obtained a independent position at the Gurdon Institute, University of Cambridge where she moved her research focus to the understanding of liver tissue both during regeneration and disease and where she has made very important contributions. In 2019, she was awarded the prestigious Lise Meitner Award from the Max Planck Society and moved to Germany to continue her research on tissue regeneration, organoid models and cancer.

Her lab established the first human liver organoid models derived from human liver tissue from both healthy and diseased patients. Also, her lab generated the first liver cancer organoid model and described that liver embryonic progenitors, hepatoblasts, are a functionally heterogenous and bi-potent at the single cell level. In addition, her studies in regeneration unveiled that epigenetic remodelling, in the form of DNA (hydroxy) methylation changes, is crucial to induce cellular plasticity during regeneration. For these achievements, she has received several awards including the Hamdan Award for Medical Excellence, The Women in Cell Science Prize from the British society, the EMBO young investigator award or the BINDER prize.

Biotechnology and Human Futures

Amy Hinterberger

Recent developments in cell and tissue research pose new challenges because they blur legal and ethical distinctions between human beings and other animals, and between human tissues/cells and nonhuman ones. In this talk, I will show how biotechnologies, such as organoids, are not only tools for researching health and disease but are also conduits through which humans realize visions for the future. Specifically, I will discuss the extent to which changes in technology are transforming the pursuit of human-specific models of disease and development, and their attenuating ethical and legal considerations. Using a social sciences approach, I will draw on a series of prompts and responses from researchers' themselves to review how human attributes are modelled and understood in multicellular living systems.

Amy Hinterberger
King's College London, UK

Positions:
2018 - Associate Professor/Reader, Department of Global Health and Social Medicine, King's College London

2017 - 2018 Associate Professor, Sociology Department, University of Warwick

2013 - 2017 Assistant Professor, Sociology Department, University of Warwick

Education:
2010 Ph.D., Sociology, LSE
2005 MSc., LSE, (Distinction)
2003 B.A., Political Science, University of Victoria (First Class Honors)

Research grants:
2020 Wellcome Trust Investigator Award in Humanities and Social Science. PI: Amy Hinterberger, Biomedical Research and the Politics of the Human, £629,823

Publications:
Hinterberger, A and Bea, S. 2021. 'Cells, Animals and Human Subjects: Regulating Interspecies Biomedical Research' Laurie, G.,et al. (Eds.). The Cambridge Handbook of Health Research Regulation Cambridge: Cambridge University Press.

Fitzgerald D, Hinterberger A, Narayan J, Williams R. 2020. 'Brexit as heredity redux: Imperialism, biomedicine and the NHS in Britain'. The Sociological Review. 68 (6):1161-1178.

Hinterberger, A. 2018. 'Marked "h" for Human: Chimeric Life and the Politics of the Human'. BioSocieties, 13(2): 453-469.

Rescuing Bioengineering Ethics from Bioengineering Ethicists

Matthew Sample

Recent work in bioethics has proclaimed a wide range of "ethical issues" associated with the engineering of new living systems. Some issues are common to any new technological object, like the question of who benefits, and others, such as the moral status of bioengineered objects, seem to demand special attention due to the emergent character of multi-cellular living systems. In many countries since the 1970s, the historical response to such issues is to delegate them to select bioethical experts to solve or (worse) to do nothing at all. Here, I will argue that these attempts to identify "ethical issues" or moral challenges in the lab are misguided and self-defeating. Decades of sociological research on science and engineering reveals that lab practice is highly structured by funding agencies' demands, societal ideologies, and internalized norms of practice. Taking for granted these social determinants of practice entails that any list of "ethical issues" that we generate is not only somewhat arbitrary but also is impossible to meaningfully address as such. The core of bioengineering ethics, I conclude, is not a search for answers but a need for decisive responses to our shared social world and its failures. Rather than ethical expertise or "further research," this demands a spirit of collective action, reclaiming democratic agency in spite of the institutions around us.

Matthew Sample
Leibniz Universität Hannover, Germany

Matthew Sample is a philosopher of science and technology and Professor for Responsible Research and Innovation at Leibniz Universität Hannover (Centre for Ethics and Law in the Life Sciences). He studies the intersection of knowledge practices, technological artifacts, and democracy, including most recently the governance of human genome editing. He has previously held positions at McGill University (Institut de recherches cliniques de Montréal) and the Harvard Kennedy School of Government (Program on Science, Technology and Society).

3D organotypic cultures from fish: towards an artificial retina

Joachim Wittbrodt

Lucie Zilova, Venera Weinhardt, Christina Schlagheck, Cassian Afting, Jonathan Schmidt, Tinatini Thavhelidse-Suck, Thomas Thumberger and Joachim Wittbrodt
Centre for Organismal Studies Heidelberg, Germany

Organization of pluripotent stem cells into organ-like structures represents rapidly growing field. However, so far, organoid field has been highly restricted to mammalian species. While human organoids are the most desired, they also take the longest time to develop and lack direct comparison to *in vivo* biological processes.

We have established an approach to derive organoids from rapidly developing teleost species such as medaka and zebrafish, opening up a potential for easier and

quicker ways to address multiple aspects of development and disease, and systematically probe interactions of organoids with variable physical environments.

I will present our results on retinal (and other) organoids in different “environments" and present an approach to tackle the genetics underlying physical constraints in the embryo.

Joachim Wittbrodt
Universität Heidelberg, Germany

Jochen Wittbrodt is working and teaching at Heidelberg University where he has founded the Centre for Organismal Studies (COS Heidelberg) that he was heading as acting director from 2010 to 2014. He has studied Biology and Chemistry at the LMU Munich where he received his PhD for his work in tumour genetics at the MPI for Biochemistry. After a Postdoc at the Biocentre Basle he became junior group leader at the MPI for Biophysical Chemistry in Göttingen. Here he started his work on eye development and evolution that his group has been focussing on ever since. This work was continued and extended after his move to the European Molecular Biology laboratory where he worked from 1998-2009. He became full professor at Heidelberg University in 2006 and was co-appointed as director at the Institute for Toxicology and Genetics at KIT in Karlsruhe. Since 2012 he has focussed his full attention to COS Heidelberg.

His work deals with the mechanisms that control the balance between proliferation and differentiation during development, growth and regeneration and lately again tumourigenesis in fish model systems.

Cardioids unravel human heart development and disease

Sasha Mendjan

Organoids capable of forming tissue-like structures have transformed our ability to model human development and disease. With the notable exception of the human heart, lineage-specific self-organizing organoids have been reported for all major organs. Here, we established self-organizing cardioids from human pluripotent stem cells that intrinsically specify, pattern, and morph into chamber-like structures containing a cavity. Cardioid complexity can be controlled by signaling that instructs the separation of cardiomyocyte and endothelial layers and by directing epicardial spreading, inward migration, and differentiation. We find that cavity morphogenesis is governed by a mesodermal WNT-BMP signaling axis and requires its target HAND1, a transcription factor linked to developmental heart chamber defects. Upon cryoinjury, cardioids initiated a cell-type-dependent accumulation of extracellular matrix, an early hallmark of both regeneration and heart disease. Thus, human cardioids represent a powerful platform to mechanistically dissect self-organization, congenital heart defects and serve as a foundation for future translational research.

Sasha Menjdan
Austrian Academy of Sciences

Education:
1997-2002: Diploma studies in Biology, LMU, Munich, Germany

2001-2002: Diploma in Biochemistry, LMU, Munich, Germany; Rudolf Grosschedl lab

2002-2006: Ph.D. EMBL & University of Heidelberg, Germany; Asifa Akhtar lab

Professional career:
2007-2013: Postdoc, Pedersen lab, LRM, University of Cambridge, UK
2013-2015: Senior postdoc, Vallier lab, Cambridge Stem Cell Institute, UK
Oct. 2015- Group leader, Institute of Molecular Biotechnology (IMBA), Austria

Selected publications:
Cardioids reveal self-organizing principles of human cardiogenesis. Pablo Hofbauer, Stefan Jahnel, Nora Papai, Magdalena Giesshammer, Mirjam Penc, Katherina Tavernini, Nastasja Grdseloff, Christy Meledeth, Alison Deyett, Clara Schmidt, Claudia Ctortecka, Šejla Šalic, Maria Novatchkova, Sasha Mendjan. Cell. 2021 Jun 10;184(12):3299-3317.e22. doi: 10.1016/j.cell.2021.04.034. PMID: 34019794.

NANOG and CDX2 pattern distinct subtypes of human mesoderm during exit from pluripotency. Mendjan S, Mascetti VL, Ortmann D, Ortiz M, Karjosukarso DW, Ng Y, Moreau T, Pedersen RA*. Cell Stem Cell. 2014 Sep 4;15(3):310-25. doi: 10.1016/j.stem.2014.06.006. Epub 2014 Jul 18. PMID: 25042702. (* Co-corresponding).*

Research topics:
Molecular and cell biology of human cardiogenesis
Mechanisms of congenital heart disease
Stem cell-derived self-organization into cardioids

Research achievements in numbers:
928 citations, h-index 12 (Google scholar)
18 publications in Cell, Nature, Molecular Cell, Cell Stem Cell, Genes &Development, Stem Cell Reports and Development
2 pending patent on cardioid generation
> 30 invited talks at national and international conferences or institutions
5 research grants as PI (total budget > 2 million €).

Neuromuscular organoids to study human development and disease.

Mina Gouti

Locomotion results from the interaction between muscles and the nervous system. Dysfunction of such cells results in deadly diseases such as spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS). Neuromuscular diseases often show regional selectivity but the underlying reasons remain obscure due to the lack of a suitable human model system. We have recently used human pluripotent stem cell derived axial stem cells, the building blocks of the posterior body, to simultaneously generate spinal cord neurons and skeletal muscle cells that self-organize in 3D to generate neuromuscular organoids (NMOs). NMOs contain functional neuromuscular junctions supported by terminal Schwann cells. They contract and develop central pattern generator-like neuronal circuits. We are currently applying NMOs to study the early development of the human neuromuscular system and to model neuromuscular diseases. This approach promises to uncover the sequence of events and provide greater insight into the mechanisms that lead to specific diseases by tackling previously inaccessible features of neuromuscular junction biology.

Mina Gouti
Max Delbrück Center for Molecular Medicine

Since 2016 Group Leader, Stem Cell Modeling of Development & Disease Lab,

Max Delbrück Center (MDC) for Molecular Medicine

Mina Gouti is a group leader at the Max Delbrück Center for Molecular Medicine in Berlin. She is a developmental biologist working on stem cell research with a focus on the development and disease of the human neuromuscular system. Her lab has recently pioneered the generation of complex human neuromuscular organoids from pluripotent stem cells.

From cell generated forces to global tissue pattern and shape (and back)

Pierre-François Lenne

How shapes of organs and organisms emerge from the interaction of cells has been a central question of the past decades. This question has been mainly formulated with the strong assumption that cells are assigned with physical properties and rules of interactions that guide how they arrange and grow to form functional structures. However, many multicellular systems self-organize without prior assignment of cell properties. In this context, I will show how collective cell movement and cell-state transitions cooperate to pattern and shape gastruloids, minimal self-organized multicellular systems that mimic early mammalian embryogenesis.

Pierre François Lenne, Institut de Biologie du développement de Marseille, France

Pierre-François studied physics at the University of Paris and Ecole Normale Supérieure of Paris, France, before completing his PhD in soft matter physics at the University of Grenoble, France. After postdoctoral research in the cell biology and biophysics unit of EMBL (Heidelberg, Germany), he joined the National Centre for Scientific Research (CNRS) as a research scientist in the Fresnel Institute of Marseille. Group leader at the Institute for Developmental Biology of Marseilles-Luminy (IBDM) and CNRS research director since 2009, his current research focuses on cell dynamics and mechanics in the context of tissue morphogenesis.

With his group, he aims to understand the physical principles that underpin the morphogenesis of animals. To do so, he develops and applies quantitative approaches to observe, perturb and predict morphogenetic movements. He studies how cells generate and respond to mechanical forces, from supramolecular interactions at cell-cell contacts to the global shape of tissues.

Topological defects in Hydra morphogenesis

Kinneret Keren

We focus on the mechanical aspects of morphogenesis using Hydra, a small multicellular fresh-water animal, as a model system. Hydra has a simple body plan and is famous for its ability to regenerate an entire animal from small tissue pieces, providing a flexible platform to explore how mechanical forces and feedback contribute to the formation and stabilization of the body plan during morphogenesis. I will present our recent results showing that the nematic order of the supra-cellular actin fibers in regenerating Hydra defines a coarse-grained field, whose dynamics provide an effective description of the morphogenesis process. Topological defects in the nematic order, which emerge early in the regeneration process, identify the sites where morphological features develop in the regenerating animal. I will further present our studies on the establishment of body axis polarity in regenerating Hydra under constraints, showing that body axis determination is a dynamic process that arises from the interplay between signaling pathways and mechanical processes. Together our results suggest that the nematic actin fiber orientation field can be considered a mechanical morphogen that interacts with other mechanical and biochemical morphogens, towards the robust formation of functional tissues in regenerating Hydra.

Kinneret Keren
Technion – Israel Institute of Technology, Israel

Education:
The Hebrew University in Jerusalem, Jerusalem, Israel, B.Sc., 1996, Physics and Math.

Weizmann Institute of Science, Rehovot, Israel, M.Sc., 1998, Physics.

Technion- Israel Institute of Technology, Haifa, Israel, Ph.D., 2003, Physics.

Stanford University, Stanford, CA, USA, Postdoc, 2003-2008, Biophysics

Positions and Employment:

2020 - present Full Professor of Physics (tenured), Technion- Israel Institute of Technology, Haifa, Israel

2014 - 2020 Associate Professor of Physics (tenured), Technion- Israel Institute of Technology, Haifa, Israel

2015 - 2016 Visiting Professor, Department of Physics, MIT, Cambridge, MA, USA.

2008 - 2014 Assistant Professor of Physics, Technion- Israel Institute of Technology, Haifa, Israel

Biophysics of symmetry breaking in a mammalian embryo-like system

Vikas Trivedi

Emergence of a coordinate system, in a group of genetically equivalent cells, through asymmetries in gene expression and cell behavior, is a recurrent theme in morphogenesis during metazoan development. Despite more than 100 years of embryology, how networks of genes integrate with the forces and mechanical properties of the cells and come together to self-organize complex structures is still a fundamental challenge at the heart of biology. Pluripotent embryonic stem cells (ESCs) represent a valuable tool for studying this *in vitro* because they allow experimentation in a tractable manner in a well-defined controlled environment. One such example is 3D gastruloid - aggregate of embryonic stem cells that recapitulates the axial organization of post-implantation embryos. Here we focus on the first symmetry breaking event that establishes anteroposterior polarity in 3D mouse gastruloids through a polarised expression of the mesodermal transcription factor Brachyury (Bra/T). By means of mechanical measurements, single-cell sequencing and mathematical modelling, we show that coordinated changes in tissue rheology and cell signalling guide the timescale and the size of the Bra/T pole in the absence of any external signals. We further examine how the initial pluripotency state of the cells might affect the dynamics of this symmetry breaking event in gastruloids and what could these insights imply for aggregates of pluripotent cells from other species.

Vikas Trivedi
European Molecular Biology Laboratory, Spain

Education:
2015 PhD, California Institute of Technology, Pasadena, USA

Thesis: From molecules to organs: Microscopy and multi-scale nature of development

2010 Bachelor of Technology (B.Tech), Indian Institute of Technology Kanpur, India Thesis: Multi-scale model of articular cartilage for mechanical stimuli on chondrocytes

Selected Publications:
Peer-reviewed publications or preprints

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Bioengineering vascularized microtissues

Cristina Barrias

Modular tissue-engineering approaches provide a promising strategy for building complex living structures from the bottom-up, through the co-assembly of microscale tissue units. Using biofabrication tools, multiple modular units of parenchymal, stromal, and vascular tissues can be rationally combined to recreate structurally/functionally different compartments of human organs. Microtissue units present a high surface area, which facilitates the diffusion of oxygen and molecules, and even cell mobility, through interstitial gaps, affording a useful tool to generate densely cellularized 3D constructs. In this talk, we outline different approaches to engineering vascularized microtissues and describe some of their applications in the fields of regenerative medicine and *in vitro* tissue/organ modeling.

Keywords: bottom-up tissue engineering, microtissue, vascularization

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Cristina Barrias
i3S – Institute of Research and Innovation in Health, Portugal

Cristina Barrias is Principal Investigator and Group Leader at i3S/INEB-Institute for Research and Innovation in Health/Instituto de Engenharia Biomédica (University of Porto, www.i3s.up.pt and www.ineb.up.pt) and invited Associate Professor at the Institute of Biomedical Sciences Abel Salazar (ICBAS, University of Porto) and at the Instituto Superior de Engenharia do Porto (ISEP, Polytechnic of Porto). Currently, she is also a member of the Board of Directors of INEB, vice-coordinator of the integrative program Host Interaction and Response at i3S, and a member of the Council of the European Society of Biomaterials (ESB).

She is the head of the Bioengineered 3D Microenvironments group at i3S focusing on (micro)tissue engineering strategies for regenerative therapies and in vitro *modeling of human tissues/organs. Her group designs customizable, biofunctionalized hydrogel-based 3D matrices and uses biofabrication tools for driving multicellular self-organization into micro-sized building blocks for bottom-up tissue engineering. These 3D microsystems recapitulate complex morphogenetic processes, providing key tools for studying cell-to-cell and cell-matrix crosstalk under physiological and pathological contexts, and for uncovering biochemical and biomechanical regulators of cell behavior. This knowledge is currently being translated into the design of advanced* in vitro *3D models of vascularized human tissues/organs and innovative pro-angiogenic cell-based therapies.*

Neurovascular *in vitro* models

Anna Herland

The neurovascular unit (NVU) is the most restrictive barrier of the human body, essential for the function and health of the central nervous system (CNS). Despite being of major importance for evaluating brain targeting of drugs and disease-induced alternations, the established *in vitro* models of the NVU are disappointingly non-predictive. Animal *in vivo* models typically also fail to severe as human NVU and CNS models due to species-specific differences. We have developed a range of micro-engineering approaches to create vascular-mimicking, fluidic Organ-on-Chip models of the NVU. First, we created a 3D microfluidic NVU model to allow direct interaction between the human endothelium and perivascular cells such as astrocytes or pericytes. This configuration resulted in higher barrier function and a more *in vivo* like response to an acute inflammation compared to a traditional culture. For evaluation of blood-brain biodistribution, we developed a compartmentalized NVU-on-Chip system. This system facilitated studies of drug-induced blood-brain-barrier alternations, metabolic and proteomic characterization, and studies of drug efflux and penetration of biopharmaceuticals, including antibodies, nano lipid carriers and viral vectors.
To improve NVU functionalities and to enable patient-specific studies, we have now developed protocols to derive all relevant NVU cells from pluripotent stem cells for on-chip studies. We further increased the system's utility by developing a new chip material, off-stochiometry thiolene-epoxy (OSTE) with impedance sensor integration for barrier studies with high temporal resolution. The whole system material, chip and tubing, was also optimized on 15 neuropsychiatric drugs for minimizing passive compound losses.

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Winkler, T.E. and Herland, A. (2021) Sorption of neuropsychopharmaca in microfluidic materials for in-vitro studies. ACS Applied Materials & Interfaces , doi: 10.1021/acsami.1c07639

Anna Herland
KTH Royal Institute of Technology, Sweden

Anna Herland is an Associate Professor in Nanobiotechnology at SciLifelab, Department of Protein Science at KTH Royal Institute of Technology, Sweden and Department of Neuroscience at Karolinska Institute (Sweden). She is in the management group of AIMES, Center for the Advancement of Integrated Medical and Engineering Science at KTH and Karolinska Institutet and the vice-director of KTH Life Science Platfor. Herland received a Ph.D. in Organic Bioelectronics from Linköping University with Olle Inganäs as a supervisor. She did postdoc fellowships at Karolinska Institutet in stem cell engineering under Ana Teixeira and at Harvard University (USA) in tissue engineering under Donald Ingber. She is a Wallenberg Academy Fellow and has > 50 peer-reviewed journal publications.

Her research group focuses on creating microphysiological models of tissue, especially the central nervous system. She develops human primary and stem cell-derived systems combined with microfluidics and uses organic electronics or bioelectronics stimuli and read-outs for real-time assessment of biological functions.

Cellular proliferation and plasticity in pulmonary neuroendocrine cells and cancer: towards building engineered organoid models of cancer

Tayla Dayton

Pulmonary neuroendocrine (NE) cells are rare cells that secrete bioactive compounds to influence lung physiology, and are a cell of origin for pulmonary NE cancers. Despite their relevance, the study of pulmonary NE cells has been limited by the lack of tractable model systems. Likewise, there are a limited number of models for the study of NE cancers, resulting in a paucity of therapeutic options for these diseases. To overcome these challenges, we have developed tractable organoid models of human pulmonary NE cells and cancers.

We generated airway organoid cultures for the indefinite propagation and expansion of pulmonary NE cells derived from human fetal lung tissue. These organoid cultures also contain all of the other major human airway cell types, enabling the study of epithelial cell-cell interactions. By applying a cocktail of small molecules, we can direct the differentiation of progenitors in these cultures to the pulmonary NE cell fate. Time-resolved analysis of pulmonary NE cell differentiation in this system revealed signaling pathways important for pulmonary NE cell maturation.

To study NE cancers, we developed the first described patient-derived tumor organoids (PDTOs) from low-grade pulmonary NE tumors and also developed PDTOs from an understudied subtype of high-grade NE cancer. Multi-omic analyses of these PDTO lines and the tumor tissue they were derived from demonstrate that they retain the intratumoral heterogeneity and major gene expression patterns observed in their parental tumors. Targeted screening of growth factor components of a fully defined growth media uncovered a subset of pulmonary NE tumors that expresses high levels of EGFR and depends on EGF for their growth in culture. These studies uncover a therapeutically targetable growth dependency in a clinically relevant subset of pulmonary NETs.

In the future, we will use these tractable organoids systems to engineer organoid models of NE cancer and other NE-related diseases.

Tayla Dayton
Hubrecht Institute, the Netherlands

Talya L. Dayton is senior postdoctoral fellow in the lab of Hans Clevers at the Hubrecht Institute in Utrecht, the Netherlands. Her research is focused on understanding cell state transitions that lead to tumor initiation and progression in neuroendocrine cells and tumors. Talya received her Ph.D. from the Massachusetts Institute of Technology (MIT) for her work in the lab of Tyler Jacks using mouse models to study metabolism in development and cancer. For her graduate work, Talya was awarded fellowships from the National Science Foundation and the Department of Defense. For her postdoctoral work, Talya was awarded an EMBO Fellowship, a Marie Curie Individual Postdoctoral Fellowship, and received funding from the Neuroendocrine Tumor Research Foundation (NETRF).

Tissue engineering with mechanically induced solid-fluid transitions

Selman Sakar

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

Selman Sakar
École polytechnique fédérale de Lausanne (EPFL), Switzerland

Mahmut Selman Sakar is a Tenure-Track Assistant Professor in the Institutes of Mechanical Engineering and Bioengineering at EPFL, and the head of the MicroBioRobotic Systems (MICROBS) Laboratory. He obtained his PhD in Electrical and Systems Engineering from the University of Pennsylvania in 2010. He contributed to the development of tissue-engineered biological robots while working as a postdoctoral associate at the Massachusetts Institute of Technology. He was a research scientist at ETH Zurich, exploring advanced manufacturing and magnetic manipulation techniques at microscale, before joining EPFL in 2016.

His current work focuses on the applications of small-scale robotics in life and health sciences including mechanobiology, neuroscience, and minimally invasive medicine. He is a recipient of ERC Starting (2017) and Proof of Concept Grants (2021).

From Stem Cells to Embryos – Ex Utero

Jacob H. Hanna

The identity of somatic and pluripotent cells can be epigenetically reprogrammed and forced to adapt a new functional cell state by different methods and distinct combinations of exogenous factors. The aspiration to utilize such ex vivo reprogrammed pluripotent and somatic cells for therapeutic purposes necessitates understanding of the mechanisms of reprogramming and elucidating the extent of equivalence of the *in vitro* derived cells to their *in vivo* counterparts. In my presentation, I will present my group's recent advances toward understanding these fundamental questions and further detail our ongoing efforts to generate developmentally unrestricted human naive pluripotent cells with embryonic and extra-embryonic developmental potential. I will conclude by highlighting new avenues for utilizing custom made ex utero platforms for growing mammalian embryos ex utero until advanced stages, for better studying of stem cell transitions during embryogenesis and organogenesis. Collectively, I will be highlighting prospects for new platforms for advancing human disease and developmental modelling.

Jacob Hanna
Weizmann Institute of Science, Israel

Born and raised in the Galilee, Israel, Dr. Hanna earned a B.Sc. in Medical Sciences (2001, summa cum laude), an M.Sc. in Microbiology and Immunology (2003), and a PhD/MD in clinical medicine (2007, summa cum laude), all from the Hebrew University of Jerusalem, where he was among the top five percent of all Israeli medical school graduates. For more than four years, he conducted postdoctoral research at the Whitehead Institute for Biomedical Research at the Massachusetts Institute of Technology. Dr. Hanna joined the department of Molecular Genetics at the Weizmann Institute in March 2011.

Dr. Jacob (Yaqub) Hanna is a medical doctor who conducts interdisciplinary research into the properties of embryonic stem cells and induced pluripotent stem cells (IPS) – adult cells induced to behave like embryonic ones. Both embryonic and IPS cells have tremendous therapeutic potential in future regenerative medicine; the advantage of IPS cells is that they can be obtained from any tissue, without using fetal material or an egg. During his postdoctoral studies at the Massachusetts Institute of Technology, Dr. Hanna worked in one of the first labs worldwide to successfully reprogram mouse skin cells into IPS cells. He was the lead researcher in a study that showed how further-modified IPS cells could be used to treat sickle-cell anemia in mice, the first proof of concept of the therapeutic application of IPS. In his lab at the Weizmann Institute, Dr. Hanna combines diverse experimentation methods with computational biology to explore topics in embryonic stem cell biology, early embryonic development and the modeling of human disease. Projects include deciphering the mechanisms by which IPS cells are produced, characterizing various stages in the life of these cells and turning them into cell types relevant for treating a variety of diseases. In addition to helping elucidate the molecular basis of cell reprogramming, this research offers the promise of creating powerful research models for infertility, cancer and tissue degeneration.

During his postdoctoral work, he received a prestigious Novartis Fellowship from the Helen Hay Whitney Foundation. In 2011 he received the Clore prize for distinguished new faculty at the Weizmann Institute and was accepted as an Alon Program Scholar for junior faculty in Israel. His numerous honors include the Rappaport prize in Biomedical Research 2013, the Krill prize by the Wolf foundation, the Kimmel award for innovative scientist at the Weizmann Institute. In 2014 he was selected as a member of the Young Israeli Academy of Sciences and among "40 under 40" most innovative young scientists by the prestigious Cell journal and became an EMBO member in 2017.

Coordinating cell fate decisions and tissue shape changes during mammalian development

Marta Shahbazi

Embryo development entails the generation of diverse cellular identities and tissues morphologies. Cells need to take the right decision, at the right location, at the right time, and this decision needs to be coordinated with concurrent changes in tissue organisation. The mechanisms that ensure the tight coordination between cell fate decisions and tissue shape changes remain poorly explored. To address this question, my group focuses on the development of the mammalian embryo at the time of implantation into the uterus, a developmental stage that involves a global transcriptional and morphological transformation. We have recently developed a new 3D culture platform that maintains mouse pluripotent cells as self-renewing organised epithelial structures. Under these conditions, cells closely recapitulate the organization and transcriptional profile of the embryonic epiblast tissue of early post-implantation mouse embryos. Moreover, we have uncovered a functional relation between epithelial architecture and pluripotency maintenance. Our work establishes these 3D epiblast stem cells as a tractable model of the post-implantation embryo. We are currently applying this system to deconstruct the complexity of mammalian development.

Marta Shahbazi
MrC Laboratory of Molecular Biology, UK

Education and research experience:
2020 - present: Junior group leader. Laboratory of Molecular Biology (LMB), Cambridge, UK.

2014 – 2020: Postdoctoral fellow, University of Cambridge, UK.

2009 – 2014: PhD in Molecular Biology, Spanish National Cancer Research Centre, Spain.

2008 – 2009: MS Degree in Molecular Biomedicine, Autonoma University of Madrid, Spain

2003 – 2008 : Bachelor's Degree in Biology (Class I), Autonoma University of Madrid, Spain

Most relevant research funding and prizes:
2022: Starting ERC grant
2020: International 3Rs prize
2016 : Science popular vote award for the top breakthrough discovery of 2016

Most relevant publications:
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Cell growth under mechanical pressure

Morgan Delarue

Cells act upon the elastic extracellular matrix and against steric constraints when growing in a spatially-limited environment. At the multicellular level, confined cell proliferation results in the emergence of a compressive, growth-induced, mechanical stress. Compressive stresses are ubiquitous to any cell population developing in confinement, such as most solid tumors or microbes, and can deeply impact cell physiology. In this talk, we will mainly be focusing on the impact of growth-induced pressure on a model organism, the budding yeast S. cerevisiae.

We observed that the growth of S. cerevisiae decreased under pressure. Using novel genetically-encoded nanoparticles (GEMs) to assess the rheological properties of a cell, we show that compressive stress alters the motion of macromolecules inside the cell, in a size-specific manner. Under compression, reactions such as protein synthesis can become diffusion-limited, globally decreasing the dynamics of biomass production, and elucidating a mechanism in which growth limitation can be attributed to modifications in the rheological properties of cells.

At the end of the talk, we will explore the possibility that the cytosolic rheological properties are partly conserved across organisms. This result can expand the observations made on S. cerevisiae to other organisms such as bacteria and mammalian cells, and point towards a universally-conserved biophysical mechanism regulating growth under pressure.

Morgan Delarue
Laboratoire d'analyse et d'architecture des systèmes, France

I am a biophysicist working at LAAS-CNRS in Toulouse, France. Our research lies at the interface between physics, engineering, biology and medicine, and aims at the understanding of the physiological response to compressive stress in different organisms, with a particular emphasis on cancer.

Models of neurological disease: Technologies and applications

Roger D. Kamm

In recent years, there has been a tremendous increase in the capabilities of *in vitro* microphysiological systems to recapitulate complex disease processes in the central and peripheral nervous systems. Associated advances have led to new insights into the underlying process of disease. This presentation will focus on two recent models developed in our lab. The first aims to recapitulate the function of a neuromuscular junction utilizing separate but communicating neurons and skeletal muscle in a 3D environment (Osaki, et al., 2020). Application of the model provides new insights into the role of TDP-43 in deterioration of muscle performance. In the second system, we combine previous 3D models of the blood-brain barrier (Hajal et al., 2022) and Alzheimer's disease neurons (Shin et al., 2019) to produce an integrated neurovascular model. We use this to demonstrate the distribution of amyloid-β protein in the brain extracellular matrix and its progressive accumulation at the vascular wall, leading to an increase in vascular permeability, a characteristic of cerebral amyloid angiopathy. These models have potential not only for probing disease mechanisms but also in drug development and discovery.

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Roger Kamm
Massachusetts Institute of Technology, USA

Roger Kamm is currently the Cecil and Ida Green Distinguished Professor of Biological and Mechanical Engineering at MIT, where he has served on the faculty since 1978. Kamm has long been instrumental in developing research activities at the interface of biology and mechanics, formerly in cell and molecular mechanics, and now in engineered living systems. Current interests focus on the development of models for healthy and diseased organ function using microfluidic technologies, with an emphasis on vascularization. Kamm has fostered biomechanics during his career as Chair of the US National Committee on Biomechanics *(2006-2009) and of the* World Council on Biomechanics *(2006-2010). I*

n 2014, Kamm co- chaired the World Congress of Biomechanics and currently directs the NSF Science and Technology Center on Emergent Behaviors of Integrated Cellular Systems. He is the 2010 recipient of the ASME Lissner Medal and the 2015 recipient of the ESB Huiskes Medal, both for lifetime achievements, and in 2018 was the inaugural recipient of the Nerem Medal for mentoring and education. He is a member of the National Academy of Medicine since 2010. Kamm is co-founder of two companies, Cardiovascular Technologies and AIM Biotech.

Short talks

A stem cell zoo platform to study interspecies differences in developmental tempo

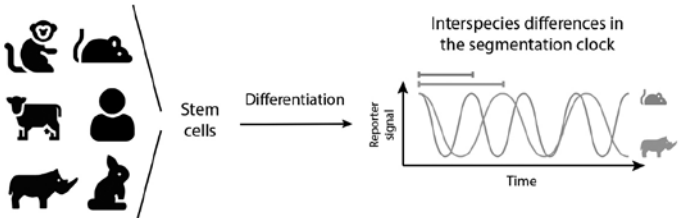
Jorge Lazaro Farre, Maria Costanzo, Mitsuhiro Matsuda, Vikas Trivedi, Miki Ebisuya

European Molecular Biology Laboratory (EMBL), Barcelona, Spain

During embryogenesis, different mammalian species present differences in their developmental speed. These controlled differences in the tempo or duration of developmental processes are thought to influence animal size and morphology, being an important mechanism of evolutionary change. The goal of this project is to investigate how different mammalian species, despite using conserved molecular toolkits, develop with different speeds. For this, we have focused on investigating the segmentation clock, the oscillatory gene expression regulating the sequential formation of body segments.

The period of the segmentation clock oscillations changes dramatically across mammals, ensuring the development of organisms with properly sized body segments. However, a systematic study of the mechanism behind these temporal differences has remained challenging due to the difficulties in obtaining embryos from many mammalian species. To overcome this limitation, we have used stem cells from different mammalian species, a stem cell zoo, to develop a 2D *in vitro* model of the segmentation clock. This platform is able to recapitulate developmental gene oscillations in six mammalian species that span through a wide range of body sizes and morphologies, including human, mice, rabbit, cow, rhinoceros and marmoset.

Quantification of the segmentation clock oscillations revealed that their period scales with the duration of embryogenesis but not with the animal body size. This suggests that larger species do not necessarily have a slower early developmental progression as previously thought. Additionally, we observed that the rate of protein degradation and mRNA production scales with the segmentation clock period, indicating that the speed of biochemical reactions might underlie developmental time. To better understand how biochemical kinetics change across species, we are now using our cell zoo to explore species-specific differences in metabolism and other cellular properties. Through this work, we aim to better understand how is developmental tempo regulated and its role in shaping the final organism.



Brain organoids to decipher the neuropathology of COVID-19

Maria C Puertas[1], Isabel Turpin[2,4], Angel Bayón[1,4], Jakub Chojnacki[1], Patricia Resa-Infante[1], Javier Martínez-Picado[1,3], **Sandra Acosta**[2,4]

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the coronavirus disease 2019 (COVID-19) pandemic. In addition to pneumonia and acute respiratory distress, COVID-19 is associated with a host of symptoms that relate to the CNS in about 30% of hospitalized COVID-19 patients, ranging from anosmia to severe epileptic encephalitis and delirium. It has been postulated that some of the symptoms of COVID-19 may be due to direct actions of the virus invading the respiratory centers of the brain. Severe neurological symptoms could also be a result of viral particle or proteins having entered the brain. SARS-CoV-2 RNA has been recovered from the cerebro-spinal fluid (CSF) and detected in brains from deceased patients, suggesting it can cross the blood–brain barrier (BBB).

Here, we infected brain organoids with the human 2019-nCoV reference viral isolate strain BavPat1/2020. Brain organoids derived from human embryonic stem cells (hESC) were differentiated for 90 days following the Lancaster protocols. Only organoids that have passed the quality control based on their morphological traits during the differentiation milestones and reach day 90 with the expected size with approximately 2.5-5x105 cells were included in the study. Upon selection, these organoids are directly inoculated with SARS-CoV-2 and letting the infection for 4 to 7 days upon washing the culture. No significant low-magnification morphological changes were observed in the infected organoids independently of the multiplicity of infection (MOI=1; 0.1 and 0.01). The amount of viral RNA in the culture supernatant increased along time upon infection, suggesting viral replication in the organoids. Brain organoids show ACE2 expression in neurons, further supporting the observation of viral infection in neurons. Organoid neural network was analyzed with the axonal tracer Cellbrite, and a decrease in the neural network was observed upon infection. Immunofluorescence staining for the viral nucleocapsid protein along with the cellular subtype markers for neurons (MAP2 and Tubulin-β3), neural progenitors (nestin) and astroglia (GFAP) indicated the capacity of the virus to infect neurons and astrocytes. Together, these data demonstrate that SARS-CoV-2 is able to infect different cell types in the brain structure, paving the way for using organoids as a tool to characterize viral pathogenesis and also as a platform to test drugs against COVID-19.

Synthetic epithelial morphogenesis through controlled stretching and buckling

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Epithelial sheets form specialized 3D structures suited to their physiological roles, such as branched alveoli in the lungs, tubes in the kidney, and villi in the intestine. To generate and maintain these structures, epithelia must undergo complex 3D deformations across length and time scales. How epithelial shape arises from active stresses, viscoelasticity and luminal pressure remains poorly understood. To address this question, here we developed a microfluidic setup and a digital twin to engineer 3D epithelial tissues with controlled shape and pressure. In the experimental setup, an epithelial monolayer is grown on a porous surface with circular low adhesion zones (footprint). On applying hydrostatic pressure, the monolayer delaminates into a spherical cap (dome) from the circular footprint. This simple shape allows us to calculate epithelial tension using Laplace's law. Through this approach, we subject MDCK epithelial cells to a range of lumen pressures at different rates and hence probe the relation between strain and tension in different regimes, while computationally tracking actin dynamics and their mechanical effect at the tissue scale. Slow pressure changes relative to the time-scales of actin dynamics allow the tissue to accommodate large strain variations. However, under sudden pressure reductions, the tissue develops buckling patterns and folds with different degrees of symmetry-breaking to store excess tissue area. These insights from experiments and the digital twin allow us to pattern epithelial folds by rationally directed buckling. Our study establishes a new approach for engineering epithelial morphogenetic events.

Computational Models Guide the Research and Control of Angiogenesis

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Controlling angiogenesis is crucial for the treatment of several diseases and to achieve functionality of large engineered tissues. This process is regulated by several cross-talking signaling pathways, such as Notch and vascular endothelial growth factor (Vegf) [1]. Recent studies have shown that spatiotemporal manipulations of these pathways may enable the development of new strategies to control angiogenesis, towards novel therapies and engineered tissues [2]. However, due to the complexity of the angiogenic process, understanding the spatiotemporal dynamics of the signaling pathways underlying angiogenesis is extremely challenging with a trial and error approach. Here, we combined experiments with computational models of cell-cell signaling to uncover that the temporal dynamics of Notch signaling is a key parameter for the spatial control of angiogenesis, and that Notch temporal dynamics in angiogenesis is regulated by the bone morphogenetic protein 9 (BMP9).

First, we coupled *in vitro* and in silico models to analyze the potential of micropatterns of different Notch ligands (Dll4 and Jag1) to control the location of endothelial sprouts. The *in vitro* experiments showed that only Dll4 micropatterns can control the sprout location, differently than Jag1. The in silico simulations suggested that this different control arises from distinct timings of Notch activation elicited by Dll4 versus Jag1. Further *in silico* simulations suggested that this timing of Notch activation can be regulated by BMP9. These new computational findings were validated via *in vitro* experiments uncovering that BMP9 upregulates the expression of Lunatic Fringe (LFng), known to enhance Notch ligand-receptor binding affinity [3]. Further coupling between experiments and simulations confirmed that the effects of BMP9 on endothelial cells and angiogenesis are partially mediated by the BMP9-induced enhancement of LFng. Finally, the simulations suggested that BMP9 and LFng regulate angiogenesis by influencing the temporal dynamics of Notch and endothelial cell fate selection. These computational findings were validated via *in vivo* experiments in zebrafish.

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In conclusion, we demonstrated that computational models are a game-changer in the context of angiogenesis research and control; in combination with *in vitro* and *in vivo* experiments, they can unravel the spatiotemporal interactions of different pathways, with the potential to optimize regenerative medicine techniques.

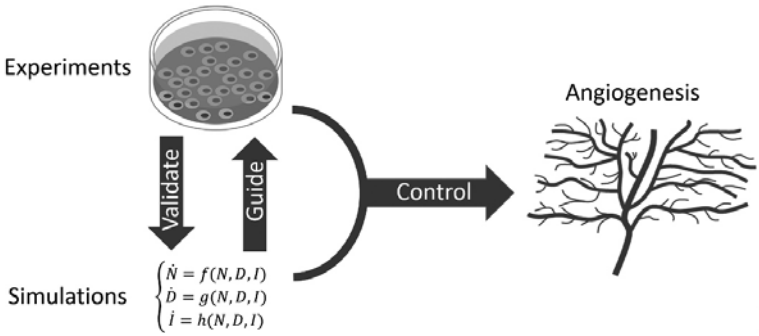

Fig. 1: Coupling of experiments and simulations unravels the underlying mechanisms of angiogenesis and provides tools to optimize its control.

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Development of a contractile skeletal muscle organoid for the modelling of muscular dystrophies

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Muscular dystrophies are a complex and diverse subset of degenerative genetic disorders characterized by muscle weakness and wasting. Individually, they are considered rare diseases, but their prevalence together represents a great impact on society. Although there are several molecules in drug development for some muscular dystrophies, considerable efforts are needed to reach the patients. To overcome the limitations of traditional 2D culture and animal models, new approaches, such as human bioengineered skeletal muscle organoids, are emerging as new tools for preclinical research. These advanced models would allow the study of both pathological processes and discovery of new potential drugs. However, given the importance of the structure of myofibers for their correct functionality, skeletal muscle represents a complex and challenging tissue for its *in vitro* generation. In this work, we developed a functional skeletal muscle organoid for the study of muscular dystrophies. Specifically, we used patient-derived cells from Duchenne muscular dystrophy (DMD), the most prevalent neuromuscular disease diagnosed during childhood. By using a 3D-printed casting mold, we encapsulated patient-derived myogenic precursor cells in a fibrin-composite matrix. This platform incorporates two flexible T-shaped pillars that provide continuous tension to the tissue, thus allowing the orientation of the muscle fibers. These posts served as anchoring points and allowed an easy evaluation of the contractibility of the muscles. After seven days of differentiation, DMD organoids expressed mature muscle markers and showed functional phenotypes as they responded to electrical pulse stimulation (EPS) by contracting. Using this strategy, we identified DMD-related functional phenotypes similarly present in DMD patients. Moreover, loss of myotube integrity, typically observed in DMD, was evaluated in skeletal muscle organoids after EPS. Finally, the applicability of this dystrophic skeletal muscle model in evaluating therapeutic compounds was explored using anti-DMD drug candidates. Taking all these considerations together, it appears that our technology has excellent potential to be especially valuable in the context of current and future discovery and development of drugs to treat DMD and other muscular dystrophies.

Polarised contractile jiggling drives the collective amoeboid migration of cell clusters

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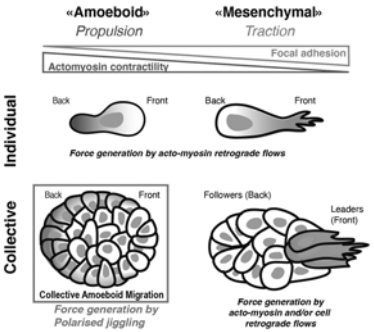
Migration is a key step in many biological processes, including the metastatic progression of cancers which accounts for most patient's deaths. As far as we know, cell locomotion occurs through three distinct mechanisms. In a few words, single cells can migrate via two modes, mesenchymal (adhesive, traction-based) or amoeboid (non-adhesive, propulsion-based). Cell cohorts are generally led by protrusive leaders, towing the collective through adhesion to the substrate.

We have been able to demonstrate the existence of an undescribed mode of collective migration. We study tumour cell clusters' migration, transformed and non-transformed, in non-adherent microfabricated channels. This collective migration is independent of focal-adhesions and traction but is dependent on integrin-mediated friction to the substrate. Moreover, cell clusters display an actomyosin cortex that is polarised to the rear of clusters, proportionally to migration speed. Inhibiting ROCK and myosin activity decreases migration, while optogenetic activation of RhoA dictates directionality, demonstrating that this migration relies on actomyosin contractility[2]. However, such migration is not driven by a sustained cell or myosin flow. Instead, we observed fluctuating cell and myosin displacements that are correlated with clusters' speed. We then demonstrate analytically that, together with friction with the substrate

and myosin polarisation, this behaviour leads to migration. Our results suggest that cell clusters can use a unique mode of collective migration, based on “polarised jiggling”, that may explain the metastatic potential of these tumour intermediates. We call this new mode of migration “collective amoeboid migration”, by analogy with single cell amoeboid migration.



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Using bioelectric cues to engineer collective migration, healing, and 3D form in multicellular systems

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Imagine the potential of 'herding' or programming large-scale cell migration over thousands of cells to either create or alter a multicellular tissue. Such control could enable sculpting of morphogenesis, accelerated wound healing, and inhibition of invasive diseases—all processes where coordinated cell migration is essential. However, realizing this potential requires a 'sheepdog' stimulus capable of inducing a desired approach in a continuous tissue. Bioelectric cues offer an interactive and broadly compatible solution via 'electrotaxis'—directed cell migration along naturally occurring direct current (DC) electrochemical fields. Such fields naturally arise in many multicellular systems when and where ion gradients are disrupted—development, healing, pathologies—and most cell types migrate '+' to '-'. Ionic fields can induce forces on membrane receptors and activate ion channels, ultimately controlling cell directionality and speed in proportion to field strength. Our group works to program large-scale collective cell changes in tissues by literally herding cell migration, using approaches such as our SCHEEPDOG electrobioreactor. We have now used this interface to drive millimeter-scale migration of intact skin monolayers, accelerate *in vitro* healing of monolayers, and actuate growth in cysts and organoids.

Key challenges are understanding and optimizing how different, complex multicellular processes respond to bioelectric programming. For example, we recently demonstrated that cells time-average ionic gradient information (~1 min timescale), allowing us to use rapidly changing stimulation patterns to program any arbitrary 2D maneuver into epithelia. Critically, we have also found that programming multicellular migration patterns can set up a 'tug-of-war' within the tissue between the external command and the pre-existing collective dynamics of the tissue. We found that transiently modulating relative cell-cell adhesion strength can circumvent this conflict and make a multicellular group far more susceptible to external control. This approach allowed us to electrically increase *in vitro* 'healing' speeds in scratched skin layers by 2-fold. These data suggest mechanical changes to the tissue, and we have now demonstrated that the collective dynamics of a tissue exhibit an hours-long memory of even short-term electrical stimulation. Building on these findings, we are now exploring electrical programming of 3D multicellular organization with organotypic models from skin, immunological migration, and the gut. In recent work with hollow, 3D vessels such as cysts and intestinal organoids we have found that the same stimuli that trigger 2D collective migration instead trigger rapid and programmable swelling of lumenized structures mediated by ion channel activation and a time-dependent mechanical relaxation.

Modeling pancreatic cancer morphogenesis by self-organized branching organoids

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[‡] *These authors jointly supervised this work*

The biophysical dynamics occurring at the self-organizing glandular epithelial during branching morphogenesis are pivotal for deciphering various processes ranging from normal tissue growth to cancer formation. A prominent example of such complex tubular structure formation is the pancreas and pancreatic ductal adenocarcinoma (PDAC). Organoids growing in a protein mixture such as matrigel form only spherical structured organoids with hollow lumen and a polarized outer epithelium, thus providing with limited morphological information and highlighting the necessity of novel model systems.

We have developed a novel PDAC organoid morphogenesis assay in which single-cell derived organoids from primary murine pancreatic ductal adenocarcinoma tumours, growing inside a 3D floating collagen matrix are able to self-organize into multicellular highly branched structures with a seamless lumen connecting terminal end-buds thus, replicating the tubular *in vivo* architecture of PDAC. In this morphogenesis process, we identified four distinct developmental phases each characterized by a unique pattern of cell proliferation, invasion, matrix deformation and respective molecular dependencies. Branching PDAC morphogenesis starts by establishing an elongated proliferative structure, followed by a second phase where migratory cells continuously invade the matrix, thus forming the tree-like architecture. In the next phase the branches thicken before entering the fourth phase, where microlumens will coalesce into one single continuous duct. We propose that the spatiotemporal synchronized processes of cell proliferation, matrix remodeling, contraction and ion channel flux are key-events of the different morphogenic phases and lead to the formation of these complex structures. Importantly, these dynamic processes are accompanied by strong transcriptional profile changes, with the organoids undergoing de-differentiation during branch elongation and later on activating an epithelial gene expression program upon maturation. Taken together, these results illustrate the ability of tumour cells to self-organize in multicellular complex structures and provide with a novel system to study branching morphogenesis and tumour biology *in vitro*.

Entering the matrix: mapping, and using, the 3D topology of the extracellular matrix to reveal its role as a master cell regulator

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Normal organ function is critically dependent on an intact three-dimensional architecture. Structural stability is ensured by the extracellular matrix (ECM), a complex biomaterial made of proteins and glycans that shapes all tissues while regulating cell behavior. Under pathological situations, remodeled ECM compels cells to behave abnormally, disrupting homeostasis and promoting disease progression, oftentimes leading to organ failure and patient death. Understanding ECM structure during health and disease is a fundamental biological challenge. Here, we develop methods to map ECM architecture and then use it as a culture substrate to reveal its role as a cell regulator. First, we generate 3D maps of the human lung ECM during health, emphysema, usual interstitial pneumonia, pulmonary sarcoidosis, and COVID-19, revealing major structural abnormalities previously unseen by existing methodologies. Using Machine Learning (ML), we identify disease-specific patterns of structural remodeling, including the altered spatial relationship between ECM proteins. Second, we design an ECM-based perfusion bioreactor to model metastatic cancer, showing that lung ECM coerces cells into mimicking *in vivo* tumor signaling, while allowing them to refashion normal ECM into a cancer-like niche. In summary, our ECM mapping and ML analysis can guide the design of future diagnostic tools and explain the detrimental effects of lung disease, including long COVID. Similarly, we reveal new roles of ECM in metastatic progression, using a novel, ECM-based disease model.

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Mapping tumor spheroid mechanics in dependence of 3D microenvironment stiffness and degradability by Brillouin microscopy

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Altered biophysical properties of cancer cells and of their microenvironment contribute to cancer progression. While the relationship between microenvironmental stiffness and cancer cell mechanical properties and responses has been previously studied using two dimensional (2D) systems, much less is known about it in a physiologically more relevant 3D context and in particular for multicellular systems. To investigate the influence of microenvironment stiffness on tumor spheroid mechanics, we first generated MCF-7 tumor spheroids within matrix metalloproteinase (MMP)- degradable 3D polyethylene glycol (PEG)- heparin hydrogels, where spheroids showed reduced growth in stiffer hydrogels. We then quantitatively mapped the mechanical properties of tumor spheroids in situ using Brillouin microscopy. Maps acquired for tumor spheroids grown within stiff hydrogels showed elevated Brillouin frequency shifts (hence increased longitudinal elastic moduli) with increasing hydrogel stiffness. Maps furthermore revealed spatial variations of the mechanical properties across the spheroids' cross-sections. When hydrogel degradability was blocked, comparable Brillouin frequency shifts of the MCF-7 spheroids were found in both compliant and stiff hydrogels, along with similar levels of growth-induced compressive stress. Under low compressive stress, single cells or free multicellular aggregates showed consistently lower Brillouin frequency shifts compared to spheroids growing within hydrogels. Thus, the spheroids' mechanical properties were modulated by matrix stiffness and degradability as well as multicellularity, and also to the associated level of compressive stress felt by tumor spheroids. Spheroids generated from a panel of invasive breast, prostate and pancreatic cancer cell lines within degradable stiff hydrogels, showed higher Brillouin frequency shifts and less cell invasion compared to those in compliant hydrogels. Taken together, our findings contribute to a better understanding of the interplay between cancer cells and microenvironment mechanics and degradability, which is relevant to better understand cancer progression.

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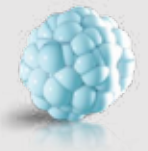

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2	Jordi Alcaraz	Institute for Bioengineering of Catalonia (IBEC)	SMAD3 in tumor associated fibroblasts promotes early cancer cell invasion in lung adenocarcinoma consistent with clinical observations
3	Feyza Nur Arslan	IST Austria	Regulation of E-cadherin-mediated contacts via cortical F-actin flows
4	Thomas Bessy	INSERM	Engineering the bone marrow niche at different scales
5	Vladyslav Bondarenko	European Molecular Biology Laboratory, Heidelberg, Germany	Ex Vivo Engineering Uterine Environment For Peri-Implantation Mouse Development
6	Louise Breideband	Goethe University Frankfurt	Democratizing stereolithography 3D bioprinting: modeling the liver cancer microenvironment using a commercially available 3D printer
7	Marta Cherubini	EMBL Barcelona	Study of flow impact on the formation of fetal placental vascular network on-chip
8	Giovannni Dalmasso	EMBL Barcelona	The interplay between SOX9 and endothelial cells in vasculature formation
9	Simone de Jong	Eindhoven University of Technology	Supramolecular hydrogels for hiPSC culture and differentiation towards kidney organoids

10	Bregje de Wildt	Eindhoven University of Technology	Towards an *In Vitro* Platform to Evaluate Material-driven Human Bone Regeneration
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15	Ainhoa Ferret Miñana	Institute for Bioengineering of Catalonia (IBEC)	Fatty Hepatocytes Induce Skeletal Muscle Atrophy *In Vitro*: A New 3D Platform To Study The Protective Effect Of Albumin In Non-Alcoholic Fatty Liver Disease
16	Giulia Fornabaio	Institute for Bioengineering of Catalonia (IBEC)	Biomechanics of the progression of hypermethylated colorectal carcinomas
17	Victor Fournie	LAAS, Toulouse, France	Opto-Fluidic 3D printing platform for micro-environment and tissue engineering
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19	Maria Gallo	Institute for Bioengineering of Catalonia (IBEC)	Generation of reporter human pluripotent stem cell lines to study cardiac and renal development and disease
20	Maria Guix	Institute for Bioengineering of Catalonia (IBEC)	Motion performance and shape-shifting of 3D bioengineered living robots
21	Levin Hafa	Goethe University Frankfurt am Main	Bioprinting by light sheet lithography: engineering complex tissues with high resolution at high speed
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24	Ignasi Jorba	Eindhoven University of Technology (TU/e)	Methacrylated recombinant collagen peptide as human collagen mimicking 3D hydrogel model for cardiac tissue engineering
25	Cansu Karakaya	Eindhoven University of Technology	Mechanosensitive Notch signaling regulates strain-mediated phenotypic changes in vascular smooth muscle cells
26	Jiřina Kroupová	Department of Chemical Engineering, University of Chemistry and Technology, Prague,	3D Cell Cultures Revealed Different Efficacy of Two Anticancer Agents with Comparable Cytotoxicity in 2D Cell Cultures
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45	Melika Parchehbaf Kashani	Institute for bioengineering of Catalonia (IBEC)	Bioprinted hydrogel-based microfluidic chip to mimic the tumor metastatic microenvironment
46	Inês Pereira	Institute for bioengineering of Catalonia (IBEC)	Towards a 3D Brain-on-a-chip for Organotypic Culture and Differentiation of Neuroprogenitor Cells
47	Livia Piatti	European Molecular Biology Laboratory	Recreating the blood-brain barrier complexity *in vitro*: a three-dimensional microvascular model to investigate cerebral malaria
48	Aref Saberi	Eindhoven university of Technology	In-vitro Engineered Human Cerebral Tissues Mimic Pathological Circuit Disturbances In 3D
49	Kabilan Sakthivel	Lund University	Deciphering the Effect of Mechanical Signals in Tumorigenesis with a Dynamically Tunable 3D Mammary Epithelial Microenvironment

51	Yvenn Sermeus	Mebios, KU Leuven	Identification of mechanical cues during early limb bud development using individual cell based modeling
52	Pawel Sikorski	Norwegian University of Science and Technology, Dept of Physics	Model System to Study Bone Mineralization in 3D
53	Josephine Solowiej-Wedderburn	University of Surrey	Pulling on Springs: Exploring Feedback Mechanisms In Cellular Mechanosensation
54	Charisios Tsiairis	Friedrich Miescher Institute for Biomedical Research	Cellular Synchronization through Unidirectional and Phase-Gated Signaling
55	Elise Van Breedaml	Laboratory of Experimental Hematology, University of Antwerp	Luminescent Human iPSC-derived Neurospheroids Enable Modelling of Neurotoxicity after Oxygen-glucose deprivation
56	Cas van der Putten	Eindhoven University of Technology	Understanding cell adhesion on the microscale, from contact guidance to substrate curvature
57	Jef Vangheel	KU Leuven	Epithelial dynamics and rheological properties in the framework of the jamming phase transition
58	Daniel Vera	Institute for bioengineering of Catalonia (IBEC)	Bioprinted hydrogel-based gut-on-chip model
59	Michelle Vis	Eindhoven University of Technology	Towards bone-remodeling-on-a-chip
60	Byung Ho Lee	Max Planck Institute of Molecular Cell Biology and Genetics. Dresden, Germany	The interplay between lumen and tissue branching in pancreas morphogenesis

Engineering a 3D small intestine mucosa model by light-based bioprinting

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Intestinal organoids are nowadays extensively used as a model to study gut pathophysiology; they organize into proliferative and differentiated regions partially retrieving the 3D architecture of the native epithelium. However, their standard culture implies short-spanned experiments as the matrix they grow within quickly degrades and dead cells accumulate in the lumen because of their enclosed structure. More importantly, these limitations also prevent experiments involving non-epithelial cells, such as those forming part of the mucosal stroma.

Here we have fabricated microstructured 3D hydrogels with embedded stromal cells mimicking the lamina propria and an apically accessible epithelial monolayer outlining the microstructures. For the hydrogel' fabrication, we relied on light-based 3D bioprinting, as it allows to produce complex geometries such as the crypt-villus architecture of the small intestine. Digital light processing - stereolithography (DLP-SLA), which is based on the photocrosslinking of polymer chains, offers high speed and spatial resolution. In this work, we have adapted a commercial low-cost DLP-SLA system to fabricate 3D cell-laden hydrogel scaffolds using visible light (Torras et al, *in prep*). The bioink employed combined Gelatin Methacryloyl (GelMA) with poly(ethylene glycol) diacrylate (PEGDA), thereby providing cells with adhesion and biodegradation sites while being mechanically stable. By adjusting the GelMA-PEGDA blend composition and the printing parameters, we could achieve hydrogels with villous-like structures.

To produce cell-laden hydrogels, we mixed NIH-3T3 fibroblasts with the GelMA-PEGDA bioink. The embedded fibroblasts not only remained viable for long periods of time, but they also elongated and migrated within the hydrogels as they do in the native lamina propria. To reproduce the epithelial tissue, we used primary cells derived from intestinal organoids, since, compared to commonly employed Caco-2 cell line, they retain native tissue features such as most of the different cellular kinds of the epithelium and the ability to self-organize. We digested the organoids into single cells, and we seeded them on top of the villous-like GelMA-PEGDA hydrogels

containing mesenchymal cells. The organoid-derived cells attached and grew on the hydrogels forming epithelial clusters while the stromal cells were radially disposing around them. With culture time, the clusters expanded on the surface of the hydrogels possibly physically aided by the stromal cells. In conclusion, we have constituted a new 3D intestinal surrogate with villous-like microstructures that includes both a primary epithelial layer and a stromal compartment. Our more physiologically relevant *in vitro* system will further enable the study of intestine physiology, in particular the crosstalk between epithelial and mesenchymal cells.

SMAD3 in tumor associated fibroblasts promotes early cancer cell invasion in lung adenocarcinoma consistent with clinical observations

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Lung cancer is the leading cause of cancer death worldwide, and lung adenocarcinoma (ADC) and squamous cell carcinoma (SCC) are their most common histologic subtypes. Although both ADC and SCC are epithelial in origin, it is now clear that the fibrotic tumor microenvironment rich in tumor-associated fibroblasts (TAFs) plays a critical role in virtually all steps of tumor progression, including migration, invasion and metastasis. In addition to a distinct tumor architecture, there is clinical evidence of different patterns of dissemination in which ADC tumors tend to disseminate earlier than SCC tumors, although the underlying mechanisms remain unknown. Intriguingly, we recently reported that the important pro-fibrotic TGF-β1 transcription factor SMAD3 was epigenetically repressed in SCC-TAFs compared to ADC-TAFs, which elicited a lower SMAD3 expression and activity that was compensated by a larger expression and activity of its close homolog SMAD2 selectively in SCC-TAFs (Ikemori et al, Cancer Res 2020). Since there is growing evidence that SMAD2/3 regulate cell migration, we analyzed the impact of altered SMAD2/3 expression in fibroblasts in their ability to lead cancer cell invasion. For this purpose, we knocked-down either SMAD2 or SMAD3 by shRNA in primary pulmonary fibroblasts, and used them as ADC-like or SCC-like fibroblast models, respectively. To study collective cell invasion we formed 3D tumor spheroids by mixing fluorescently labelled ADC cancer cells and fibroblasts within a mixture of collagen and basement membrane (Matrigel). Confocal microscopy revealed more invasive events in tumor spheroids containing shSMAD2 (ADC-like) fibroblasts compared to shSMAD3 (SCC-like) fibroblasts. To validate these distinct invasion patterns *in vivo*, we coinjected ADC cells with fibroblasts into immunodeficient mice and monitored tumor growth up to 3 weeks. Notably, tumor xenografts bearing shSMAD2 (ADC-like) fibroblasts exhibited larger tumor growth and developed more invasive structures than

tumors bearing shSMAD3 (SCC-like) fibroblasts. Mechanistically, shSMAD2 fibroblasts exhibited larger expression of N-cadherin than shSMAD3 fibroblasts, which we previously implicated in collective cancer cell invasion led by TAFs through enhanced force transmission (Labernadie et al, *Nat Cell Biol* 2017), supporting that the larger N-cadherin of ADC-like fibroblasts may promote cancer cell invasion through a similar process. Our results reveal that SMAD3 in fibroblasts drives tumor growth and invasion of ADC cells, which may contribute to the early dissemination that is frequently observed in the clinic selectively in ADC.

Regulation of E-cadherin-mediated contacts via cortical F-actin flows

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The regulation of intercellular adhesion is a key process in the development and physiology of multicellular organisms. At a molecular scale, regulation of cadherin-mediated cell-cell adhesion entails the reorganization of cadherins and the associated actomyosin cortex locally at the contacts. This reorganization in many systems manifests itself in clustering of cadherins at the contact rim, where they function to stabilize the contact. However, how this stereotypical clustering is achieved remains unexplored due to technical limitations in high-resolution live imaging of cell-cell contacts. Here, we engineered supported lipid bilayers functionalized with E-cadherin ectodomains as a cell surface model, and showed that centrifugal flows of F-actin and E-cadherin clusters contribute to the enrichment of these molecules at the contact rim of cells adhering to the bilayers. The cortical flows arise due to a gradient of Myosin-II activity that peaks at the contact rim, carrying E-cadherin clusters along and dilating the F-actin network. The Myosin-II gradient forms due to the depletion of Myosin-II at the contact center during contact formation, which again is regulated by E-cadherin engaged in *trans* binding over the contact, reducing RhoA GTPase activity in the contact center. Consistent with this, we found that manipulation of Myosin-II activity affects the translocation and accumulation of F-actin and E-cadherin at the contact rim, and associated dilation of the F-actin network density at the contact disc. Thus, using a biomimetic assay, we identify contractility-driven cortical flows as a novel mechanosensitive mechanism regulating E-cadherin and F-actin reorganization at cell-cell contacts.

Engineering the bone marrow niche at different scales

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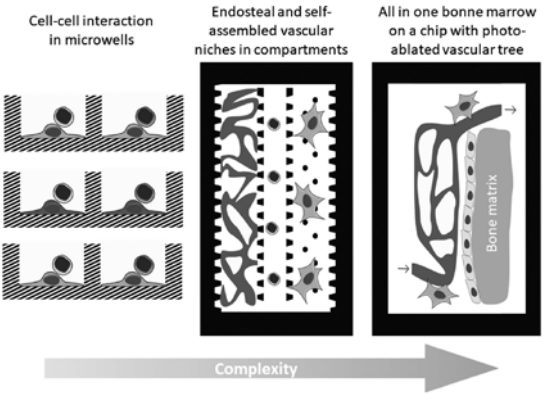
The bone marrow (BM) tissue is the main physiological site for adult hematopoiesis. Bioengineering a functional BM multicellular unit is of great interest for both fundamental and translational research. The dual nature of the local environment made of both hard (the bone) and soft (the marrow) matrix makes it particularly difficult to bioengineer. Specific manufacturing approaches are thus required to reproduce the complex cellular and mechanical properties of the tissue. To guarantee the choice of the best model for different applications, we suggest a scaling approach to bone marrow engineering going from two-cells microwells for heterotypic interaction observation to a complex multi-material and multi-cellular tissue-on-chip.

First, we designed a microwell technology to study specific hematopoietic progenitors' interactions with niche cells. This first order of engineering decomposes the marrow in simple element to decipher key cellular interactions between the main actors of the tissue.

In the second order of organization, we compartmentalized the endosteal and vascular niche cells within a microfluidic chip, separating self-assembled vessels from osteoblast in a 3D hydrogel. The aforementioned interactions can thus be observed in a 3D environment, with competition between the endosteal and vascular cells to study hematopoietic engraftment, migration and cell adhesion.

Lastly, we propose the generation of an all in one BM-on-chip with fully controlled cell deposition, with photo-ablated vessels highly resembling the native BM in terms of size and morphology, in a BM-like matrix and in close proximity with bone-like structures. This chip connected with a microfluidics system can recapitulate the native vascular architecture, the differentially perfused and oxygenated regions as well as the heterogeneity in materials and physical properties. The system is currently used to study vascular aberration in leukaemia.

In conclusion, our multi-scale bioengineering approach offers a variety of models with applications spanning from single cell interactions to complex tissue observations.


Cell-cell interaction in microwells
Endosteal and self-assembled vascular niches in compartments
All in one bonne marrow on a chip with photo-ablated vascular tree
Bone matrix
Complexity

Ex Vivo Engineering Uterine Environment For Peri-Implantation Mouse Development

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Mouse embryo development requires a direct interaction between the extraembryonic and the uterine tissues during implantation. However, our mechanistic understanding of its role in development is limited by the inaccessibility *in vivo* and the remaining challenges to recapitulate feto-maternal interaction *ex vivo*. Here, we applied biomaterial engineering to reconstitute geometrical and physicochemical cues of the intrauterine environment with unprecedented precision and reproducibility. In our method, 3E-uterus (*Ex vivo* Engineered uterine Environment), the topographically structured hydrogel supports mouse blastocysts through implantation and the peri-implantation embryonic morphogenesis: epiblast patterning and the egg cylinder formation. It promotes the development of the Reichert's membrane and all extraembryonic tissues, including extraembryonic ectoderm and the giant trophoblast, which directly interacts with the uterus *in vivo*. To enable live imaging of the in toto multicellular dynamics, we integrated 3E-uterus with the multiview light-sheet microscopy (MuVi-SPIM) and the deep learning-based single-cell segmentation. We find that the uterine geometry and the attachment of the trophoblast upon implantation provide the mechanism for the extraembryonic ectoderm cell shape-driven transition of the mouse blastocyst to the egg cylinder. Thus, our method enables mechanistic studies of the multicellular crosstalk between the uterine environment, embryonic and extraembryonic tissues.

Democratizing stereolithography 3D bioprinting: modeling the liver cancer microenvironment using a commercially available 3D printer

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Three-dimensional (3D) bioprinting has been at the forefront of tissue engineering research as a versatile technique that allows the production of models mimicking *in vivo* tissues. Indeed, bioprinters print now faster and with higher resolution[1]. However, the accessibility of those bioprinters is still limited in the research and medical field due to the high price tag. Additionally, the current 3D bioprinters that are financially affordable are based on extrusion bioprinting[2,3,4] which has been shown to generate shear stress damaging the cells5. A further 3D bioprinting technique using layer-by-layer light polymerization, called stereolithography, shows promising results in terms of printing speed, resolution and cell viability[5].

In this work, we used a commercially available consumer stereolithographic 3D printer and modified it to a functioning 3D bioprinter. As a proof of concept, liver cholangiocarcinoma organoids were bio-printed in gelatin methacrylate (GelMA) and polyethylene glycol (PEG) hydrogel and allowed to grow for seven days. After analyzing the hydrogel in terms of material characterization, the time-dependent morphology and growth of the organoids was analyzed. Next, the expression of specific markers was verified using RT qPCR and immunofluorescent staining. We conclude that it is possible to culture the organoids in the printed constructs and their morphology is closer to the *in vivo* conditions. Additionally, we determined a differential expression of markers between the controls (cultured in Matrigel) and the bio-printed constructs. In conclusion, we show that using accessible 3D bioprinting, a viable model for cholangiocarcinoma suitable for drug discovery and basic research is straightforwardly accessible.

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Study of flow impact on the formation of fetal placental vascular network on-chip

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The placenta is a highly vascularized organ – one that encompasses the maternal-fetal interface and regulates the exchange of oxygen and nutrients required for a successful pregnancy. To achieve sufficient exchange from maternal to fetal circulations, the placenta houses villous trees and a network of dense blood vessels. Thus, vasculogenesis, angiogenesis and regulation of vascular function in placental development are vital. Dysfunction of placental vasculature is associated with common pregnancy-related complications, such as intrauterine growth restriction and pre-eclampsia. The underlying mechanisms of these pathologies remain poorly understood due to the inaccessibility of the placenta, particularly in early pregnancy. Thus, *in vitro* models are required for investigating dysregulation of placental hemodynamics and vascular function. Herein, we focused on generating perfusable fetal microvessels using an improved *in vitro* triculture of human umbilical vein endothelial cells, placental fibroblasts and pericytes. Using a PDMS-based microfluidic model, combined fluidics, and advanced microscopy techniques, we monitored growth and remodeling of these live placental vessels. Given the complex structural and mechanical environment of the placenta, our main aim was to elucidate the role of hemodynamics in fetal vascular development and remodeling. Specifically, we demonstrate the influence of interstitial flow and continuous flow on microvessel formation and remodeling, as well as endothelial barrier function (permeability to solutes) and corresponding changes (diffusivity) in the extravascular matrix properties. Vessels exposed to interstitial flow form connected networks earlier than in static conditions. Within 3 days following initial culture, interstitial flow led to significant increases in a number of morphologic parameters including: vessel area, branch length, and diameter. Interstitial flow transitioned to complete intraluminal flow by day 5 in these vessels. Importantly, we found that the presence of flow is essential to maintain a viable and fully perfusable vascular network, observed after 7 and 14 days of culture, while static culture conditions resulted in regressive remodeling and few to no perfused vessels. Overall, our model reveals important insight into the role of fluid dynamics in regulating the development and remodeling behavior of fetal vessels, which is a necessary, yet missing component in a complete *in vitro* model of the vascularized human placenta.

The interplay between SOX9 and endothelial cells in vasculature formation

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The growth of an embryo, after it reaches a size of ca. 2mm, heavily depends on a functional vascular system. Unlike other organs, the vascular system needs to be fully functional and constantly remodeling itself to support all tissues from an early stage in embryo development. A crucial question - still not totally understood - is how the 3D architecture of blood vessel networks is controlled during organogenesis. Specifically, in the case of limb development, vasculature is not only essential to supply nutrients but is also fundamental in limb patterning. It has been shown that blood vessels, after creating a functional network, regress from the region where the formation of cartilage condensation begins, while they are maintained in the other parts. Since is not yet entirely possible to observe in real time the full growth of a developing limb, to understand the coordination between vascular patterning and skeleton formation we adopted an *in vitro* approach using cells from limb of mouse embryos in micromass cultures. With this experimental set up, we were able to see proliferation of endothelial cells and quantify the difference of the network's extent in several culture media, such as those designed for endothelial cell growth (e.g., EGM-2, ECCM) and with different amount of Sox9 expression. Specifically, having VEGF in the culture media, as in EGM2 or DMEM+VGEF, causes a higher proliferation and longer connections of endothelial cells, which is not seen with pure DMEM. However, the network formed by endothelial cells appears always in Sox9 negative regions and it slowly gets disrupted by the increase of Sox9 pattern establishment. To further investigate this phenomenon and consequently the relation between Sox9 and endothelial cells, we used Wint3a to perturbate Sox9's pattern. As a result, in micromass culture with an impaired pattern of Sox9, a higher connectivity of endothelial cells was established, and the network appeared to be in phase with the Sox9 pattern in some part of the micromass, suggesting that Sox9 could represent one principal factor in the process of vasculature regression. These findings represent a first step in understanding the relation between skeletal patterning and vasculature network formation improving our knowledge on how blood vessels orchestrate organogenesis.

Supramolecular hydrogels for hiPSC culture and differentiation towards kidney organoids

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In most studies induced pluripotent stem cells (iPSCs) are cultured on polystyrene surfaces coated with Matrigel or other ECM proteins for the differentiation towards kidney organoids. It has recently been shown, however, that surface stiffness influences differentiation and eventual kidney organoid formation. (Garreta, Nat. Mat., 2019) Supramolecular biomaterials can be used to exactly control the bioactivity and stiffness of a material, offering an interesting platform for the culture and differentiation of iPSCs towards kidney organoids. In this study, the culture of human iPSCs on ureido-pyrimidinone (UPy)-based hydrogels (Diba, Adv. Mat., 2021) was investigated. Both the culture in 2D and 3D, and the effect of stiffness on hiPSC behavior and pluripotency was explored. Later, the differentiation of hiPSCs towards kidney organoids on these materials will be examined.

hiPSCs were seeded on top of (2D) and inside (3D) UPy-hydrogels. UPy-cRGD peptides were incorporated to introduce bioactivity in these matrices, and the weight percentage (wt%) of the polymers was varied to control the stiffness of the matrix. One day after seeding, in 2D, the cells formed small spread-out clusters on all materials. Over time the cells changed morphology and became more compact, forming tightly packed round spheroids after 3 days. On the softest material (0.5 wt%) this was most pronounced. E-cadherin (Ecad) and F-actin stainings show that the cells became polarized, and that lumens had formed inside the spheroids. This lumenogenesis is less evident than on Matrigel hydrogels, although this is expected to form further over time. The cells remained Oct4+ on all materials, demonstrating pluripotency maintenance. In 3D, small seeded cell clumps grew out into large, polarized structures in all conditions. Also here the cells remained Ecad+ and Oct4+. No clear differences were found between the different conditions, indicating that all hydrogels were able to support hiPSC 3D culture.

It is hypothesized that these soft materials allowed the formation of epiblast-like spheroids, as opposed to the monolayer morphology on stiff polystyrene plates. Although this effect was slightly stronger in Matrigel, all UPy-hydrogels resulted in similar structures as well, indicating that these hydrogels offer sufficient bioactivity and mechanical cues for hiPSC survival, proliferation and pluripotency maintenance. No clear differences were found regarding the variations in stiffness, but this will be further evaluated via YAP staining in the next experiments. In addition, long-term cell passage on these materials will be studied, as well as the differentiation towards renal lineage.

Towards an *In Vitro* Platform to Evaluate Material-driven Human Bone Regeneration

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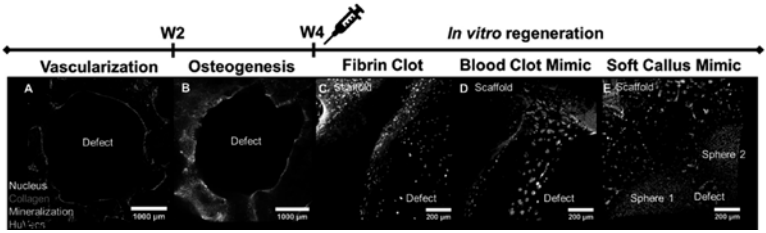
Introduction: In situ tissue engineering is a potential treatment approach for large bone defects, where implanted smart materials make use of the bone's innate capacity to regenerate upon implantation. Such treatments are routinely studied in animal models with a clinical success rate of only 10%, possibly caused by the lack of resemblance of these models to the human physiology [1, 2]. To enable the investigation of human bone regeneration upon biomaterial implantation, we aimed at developing a 3D *in vitro* human bone defect model. Such models will allow studying a material's potential to induce cell migration and vascularization, which are critical processes in the early stages of bone regeneration.

Materials and Methods: Silk fibroin scaffolds were produced with 3 mm diameter defects, representing the bone model. Scaffolds (N = 4) were seeded with human mesenchymal stromal cells (MSCs) and GFP transfected human umbilical vein endothelial cells (HUVECs). Scaffolds were cultured for a period of 2 weeks supplemented with endothelial growth medium, followed by a period of 2 weeks supplemented with osteogenic medium. After 4 weeks, a fibrin hydrogel (control), a blood clot mimic (ELAREM™ Matrix, PL Bioscience, Aachen, Germany), and a soft callus mimic (devitalized tissue-engineered hypertrophic cartilage spheres) were implanted into the scaffold defects as regenerative materials, aiming at mimicking physiological bone regeneration for an additional 2 weeks.

Results: After 2 weeks, vascular-like structures were visible throughout the scaffold (Fig. 1A). After 4 weeks, scaffold pores were filled with collagen and minerals indicating bone-like tissue formation (Fig. 1B). Two weeks after material implantation, MSCs had migrated into the fibrin clot (Fig. 1C). The blood clot mimic was found to stimulate the migration of the HUVECs into the clot (Fig. 1D). The soft callus mimic induced the migration of both MSCs and HUVECs into the clot surrounding the spheres (Fig. 1E). HUVECs appeared to be attached to the soft callus mimic.

Discussion and Conclusion: To enable the investigation of material-driven human bone regeneration *in vitro*, we developed a 3D *in vitro* human bone defect model that allows the evaluation of a material's potential to induce cell migration and



vascularization. To validate this model, physiologically relevant materials were used. Our initial results show that the soft callus mimic induced MSC migration and HUVEC migration and attachment, indicating the potential of the soft callus mimic to attract vascularization.

Figure 1. Confocal microscopy results indicating vascular-like structures at week 2 (A), bone-like tissue formation at week 4 (B), and migration of HUVECs (green) and MSCs (white) 2 weeks after implantation of the materials (C-E).

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Murine microglia-containing brain organoids as a tool to study CNS inflammatory and degenerative processes

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Introduction: Brain organoids are composed of neural stem/progenitor cells, as well as neurons and astrocytes. They are considered as emerging *in vitro* models that may offer more realistic insights in neuro-developmental, -degenerative and -regenerative processes as compared to traditional monolayer (co-)cultures. One of the main advantages of brain organoids lies in their ability to display multiple characteristics of brain tissue, such as morphology, signaling and gene expression patterns. More recently, immune-competent brain organoids that also contain a microglial cell population have become of high interest to extend the current brain organoid toolbox towards the study of neuro-inflammatory responses in a 3D cell environment. In this study, we report on the optimization and functional characterization of murine immune competent brain organoids.

Methods: Murine microglia progenitors were differentiated from CX3CR1eGFP/+CCR2RFP/+ murine iPSC and subsequently mixed with murine embryonic brain-derived neural stem cells in 96well plates and cultured for 4 weeks. Immunocytochemical analyses were performed to investigate the maturation of neural stem cells into neurons and astrocytes, as well as the presence of microglia. To achieve microglia activation, multiple pro-inflammatory stimuli were added to the cell cultures: LPS + IFNγ, polydAT or polydIC. Flow cytometry was performed to assess viability of the generated brain organoids. Finally, we evaluated a different type of inflammatory stimulation, incubating the organoids under glucose- and oxygen-deficient conditions for a time span of 2, 4 and 6 hours.

Results: We here demonstrate the possibility to generate murine iPSC-derived brain organoids that consist of neurons, astrocytes and microglia, as demonstrated by immunocytochemistry. In addition, we quantified the presence of microglia in the organoids to be around 4-5%, resembling the composition of mouse cortex *in vivo*. Following pro-inflammatory stimulation, flow cytometry showed a decrease in viability with all the different stimuli tested.

Conclusions: In this study we have shown the possibility to induce an inflammatory or traumatic event in microglia-containing brain organoids, which are subsequently subjected to neuro-degeneration. Interestingly, the observed neurodegeneration is milder than what observed in monolayer cultures under similar experimental conditions. Nevertheless, it might be that this model is more representative of the *in vivo* situation.

Organoid culture and 3D bioprinting of pluripotent stem cell-derived pancreatic progenitors

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Recently the establishment of 3D cell culture allows pluripotent stem cells to recapitulate many aspects of their differentiation programs and development in vitro. Specifically, embryonic stem cells (ESCs) can be coaxed into specific structures resembling *in vivo* tissues and organs which termed organoids. In our study we focus on differentiating mouse ESCs to pancreas identity. We developed an efficient differentiation protocol of ESCs to pancreas progenitors (PPs) which we then either aggregate to pancreatic organoids or print to a predesign 3D structure. In our 3D culture we do not use Matrigel, but well-defined hydrogels and we incorporate endothelial and mesenchymal support cells that make blood vessels due to their importance for functional maturation in vitro. In the organoid culture we aggregate PPs with mesodermal progenitors (MPs) differentiated from ESCs. The MPs serve as a source for the mesenchymal and endothelial cells which establish the blood vessel network in the organoid. The 3D bioprinter is used to print PPs, primer pancreas endothelial cells and mesenchymal support cells. In the organoid culture we utilize the cells' self-organization, whereas in bioprinting we control the location of the different cell types. In both culture techniques we find endocrine, acinar and duct cells together with vessel-like network, mimicking the complex structure of the developing pancreas.

Our study has enormous potential in the fields of regenerative medicine and developmental biology and provides new approaches that relays either on the inherent self-organization of ESCs, or on engineering cells in a tissue-like structure to better resemble the mammalian developing pancreas.

Diet during tissue regeneration and engineering

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Background. Tissue reconstruction after chronic diseases depends on the cellular scaffolds and energy sources to support the generation of new cells as well as to induce and maintain the cell-to-cell and cell-to-extracellular matrix interactions. Artificial tissue engineering is also in need of cellular supplies that maintain the tissue homeostasis of the new organ. Hence, successful tissue regeneration as well as generation and implantation of artificial organs will depend on the macro- and micronutrient composition of the diet. In addition, the nutritional background of the host recipient will also play an essential role on the adequate homeostasis of the tissue (during organ regeneration) and systemic microenvironment to accept or reject the new tissue.

Objectives. In our laboratory we investigate the nutritional requirements needed to build back the tissue mass damage during diseases and mechanical mechanisms during organ regeneration. In relation to these needs, we provide unknown insights regarding the molecular mechanisms underlying the cell-type specific epigenetic modifications, metabolic adaptations and landscape of signaling cascades occurring during the successful overcome of tissue damage. Moreover, we have significant interest in how cells acquire cell plasticity while maintaining tissue specific functions

in order to restore damaged tissue. Hence, we also study the inflammatory signals within the regenerating tissue including the organ composition of immune cell populations.

Methods and results. We used mouse models of liver disease to decipher how specific nutritional interventions could assist to restore the regeneration capacity of the cells. We also use liver organoids to study how these nutritional interventions could be applied in the generation of artificial –exogenous- livers. Here we show how dietary amino acids could restore liver regeneration in a liver pathology-specific manner by mechanisms that involve epigenetic modifications and metaboloma imbalance.

Conclusions. We conclude that it is possible to restore back the regenerative capacity of the liver. However, environmental factors limit this capacity.

A multi-organ-on-a-chip device to study the metabolic crosstalk between muscle and pancreatic islets

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Diabetes mellitus is a chronic disease that represents a major public health problem worldwide. Type 2 diabetes (T2D) is the most common form of this disease, accounting for 90–95% of cases of diabetes and is characterized by hyperinsulinemia and insulin resistance. T2D is a complex metabolic disorder that comprises several organs. The pancreas is the organ with a critical role in T2D since in the pancreatic islets, beta-cells produce, store and release insulin. Skeletal muscle is one of the major tissues targeted by insulin and is responsible for maintaining whole-body glucose homeostasis. Understanding the mechanisms that control the metabolic control between skeletal muscle and pancreatic islets is fundamental to developing new molecular drugs to prevent and control this disease. Organ-on-a-Chip (OOC) devices offer new approaches to studying human diseases. In this work, we engineer a new *in vitro* model to study skeletal muscle and pancreas. To this aim, muscle tissues and pancreatic islets have been fabricated and combined in a multi-OOC approach. Moreover, the multi-OOC device was integrated with a Localized Surface Plasmon Resonance (LSPR) sensing module to monitor the insulin and interleukin-6 (IL-6) secretion online and label-free. Using this *in vitro* platform, we have monitored insulin secretion dynamics by the pancreatic islets in response to the skeletal muscle contraction induced by electric pulse stimulation. These results point that this multi-OOC is an important enabling step for diabetes modeling, the study of insulin resistance, and the investigation of drug candidates for therapy, usually performed by long-time and expensive animal experiments. It would open new areas of research on human diabetes disease.

Fatty Hepatocytes Induce Skeletal Muscle Atrophy *In Vitro*: A New 3D Platform To Study The Protective Effect Of Albumin In Non-Alcoholic Fatty Liver Disease

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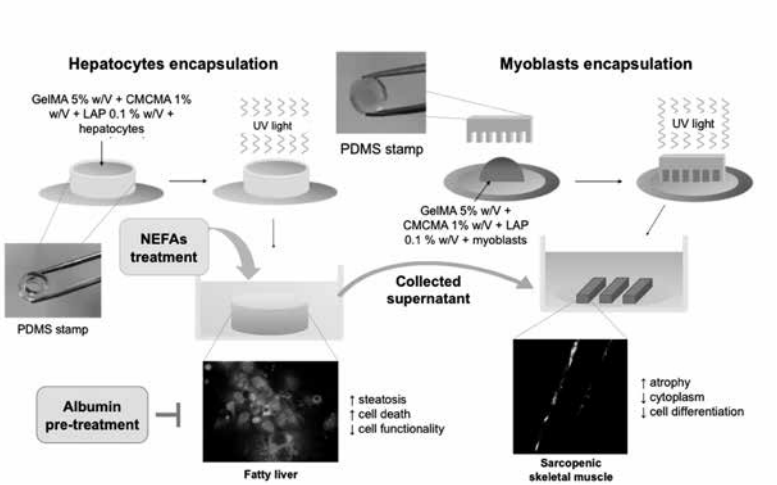
Non-alcoholic fatty liver disease (NAFLD), as the new silent disease of the 21st century, affects 1 in 4 people worldwide. It ranges from simple steatosis to non-alcoholic steatohepatitis, which may progress to cirrhosis and hepatocellular carcinoma. Many evidences show that the liver damage propagates to the skeletal muscle, leading to loss of mass and physical performance, known as sarcopenia. However, why fatty liver influences the development of sarcopenia in NAFLD is still not completely elucidated. Infusion of human serum albumin (HSA) has been demonstrated to reduce renal dysfunction, hospital readmissions and mortality in patients with acutely decompensated cirrhosis. HSA, synthesized in the liver, is the main fatty acids transporter and plays major roles in the binding of drugs. In a previous work, we have demonstrated that hepatocytes cultured *in vitro* release albumin in response to lipids challenge (Lopez-Muñoz et al. 2020). Our hypothesis is that in NAFLD, the fatty liver induces gradual atrophy of skeletal muscle tissues accelerating the progression of liver disease, that might be mitigated by administration of albumin. To test this hypothesis, we developed a 3D platform for hepatocytes and skeletal muscle cells crosstalk under non-esterified fatty acids (NEFAs) regimen. In this project, hepatocytes and myoblasts were encapsulated in a solution of gelatine-methacryloyl and carboxymethylcellulose at concentration of 5% and 1%, respectively. The photo-initiator LAP was then added at concentration of 0.1% and the polymer exposed at UV light for 30 seconds. The 3D tissues were fabricated using PDMS as moulds (Figure 1). 3D hepatocytes showed the typical signs of NAFLD such as lipid accumulation, metabolic activity impairment and apoptosis after 72h of culture with NEFAs. The 3D myotubes incubated with supernatant from fatty hepatocytes displayed loss of cytoplasmatic mass, impaired metabolic activity, and altered genes expression involved in the maturation of myotubes. In the following set of experiments, we pre-treated healthy hepatocytes with albumin prior to incubation with NEFAs,

then we collected the supernatant and treated the myotubes. We saw reduced hepatocytes' lipids accumulation and signs of cell death, lower level of ammonia in the supernatant and improved muscle mass in the myotubes. In conclusion, this study establishes the direct connection between liver and skeletal muscle in



NAFLD, and the beneficial effect of albumin treatment on both liver and skeletal muscle in an *in vitro* model of NAFLD. The tool herein presented can be employed as a customizable 3D *in vitro* platform for drug screening.

Figure 1. Schematic representation of the 3D in vitro experimental model employed in this study.

Biomechanics of the progression of hypermethylated colorectal carcinomas

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According to the World Health Organization, cancer is one of the main causes of death worldwide, with colorectal carcinoma (CRC) being the second-leading cause of tumour related-death. The high rate of mortality of CRCs is principally attributed to the metastasis of neoplastic cells from the primary tumour to secondary organs such as the liver, the lung and the peritoneum. These cells can disseminate either as single isolated cells or as collective clusters, undergoing a series of molecular and cellular changes commonly known as Epithelial to Mesenchymal Transition (EMT).

However, in 2018, our laboratory described a novel modality of peritoneal metastatic spread characterized by the presence of large clusters of cancer cells, which maintain their epithelial properties and display an outward apical polarity. These clusters of cells, termed tumour spheres with inverted polarity (TSIPs), were found in peritoneal effusions of CRCs patients showing early KRAS mutation and hypermethylation of CpG Islands.

TSIPs originate through a series of morphological changes: the first event is the sprouting of hypermethylated epithelia, followed by their apical budding, leading to the formation of rounded spherical clusters of cells called buds, and the subsequent cleavage of the newly formed spheres. How cell and tissue mechanics drive this process is still unclear. To provide novel insights into this metastatic cascade, our project aims at deciphering the biomechanical and cellular events regulating the formation of buds in colorectal cancer cell lines.

Employing a combination between cellular and molecular biology techniques with biophysical methods, we showed that this process is characterized by over-proliferation, local changes in cell adhesion and by increased contractility of epithelia, coupled with the formation of topological defects at the level of the monolayer underneath the buds. Our study demonstrates that buds development in colorectal carcinomas epithelia is governed by morphological transitions occurring entirely at multicellular level, rather than by single cells aggregation or cell extrusion. By understanding the formation of TSIPs and buds, we wish to enlarge our knowledge about collective dissemination in CRCs and to identify new potential markers of colorectal cancer progression.

Opto-Fluidic 3D printing platform for micro-environment and tissue engineering

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The cell behavior in a tissue is governed by the 3D microenvironment and involves a dynamic interplay between biochemical and mechanical factors. Current 2D culture approaches have yielded important insights in the fields of biology but cannot reproduce certain key physiological features of the in vivo 3D environment. Thus, the development of techniques for 3D heterogeneous models of tissue is critical. Most commonly approaches for 3D microfabrication, such as µ-extrusion, inkjet and photolithography [1] have shown the potential of bioprinting to create models of living tissues [2-3]. However, these techniques generally lack the resolution or the ability to print variations of physico-chemical (stiffness, topography, molecular gradient, etc...) or biological cues (cells positioning or heterogeneity) to reproduce the specificities on tissues [4][5].

In this work, we combined the advantages of microfluidics and laser-lithography to develop a novel 3D printing concept adapted to liquid photosensitive polymers (Fig 1). The 3D FlowPrint technology is a microfluidic 3D-printing device consisting of two channels, injection and aspiration, to locally deliver liquid material. This system benefits from the parallelization capabilities of microfluidics to rapidly deliver sequences of materials. A microfluidic set-up, including Fluigent Flow EZ and M-switch, provides sequential injection of fluids to print multi-material structures (Fig 3C). The integration a collimated laser-fiber to the printing head enables the precise polymerization of voxels. Decoupling the materials injection mechanism from the polymerization process is advantageous as the writing resolution is independent from materials properties. The technology also includes a home-made slicer, capable of multi-material and multi-resolution slicing, and a fully commanded instrumental set-up, so that experimenters can work with STL 3D files.

The printing was itself fabricated 3D-printed of hard photoresist for more flexibility (Fig 2A-B). It combines a microfluidics circuitry and optical vias. It operates directly in immersion in the culture medium to maintain cell viability. The head is placed so that only a thin layer of material is present between it and the previous slice. The microfluidic guiding structures present at the head surface provide, together with the control of the inlet and outlet pressure, an efficient delivery and recovery of polymerizable material in the polymerization area. Computer

simulations were carried out to optimize the flows and we found correlations with experimental work (Fig 2C-D). This system is particularly adapted to low viscosity material and low young modulus tissue (0.5-10 kPa). It thus enables a low material consumption (0-10 µl/min).

We used hydrogels (PEG-DA) solutions with photo-initiators (LAP) to print on MAPTMS- functionalized glass. We employed several additives to create adhesion contrast on the hydrogel surface to promote either culture of intestinal cancer cell (Fig 3D) or create cellular anti-adhesive areas (Fig 3E), enabling the localization of cells. The writing velocity (0.1-1 mm/s) and laser power (405 nm, 10-1000 kW/m2) have been optimized to reach either 20 µm resolution (Fig 3A) for details or 100 µm for high-speed printing. The XY resolution is mainly given by the laser spot size (≈ 20 µm) and the interaction with the liquid material. The structure thickness is controlled by the distance between the previous slice and a layer of PDMS at the head apex, which prevents the adhesion of hydrogel on the head (Fig 3A-B).

From the cell biology point of view, the 3D FlowPrint bioprinting platform opens intriguing perspective for the automated and reproducible fabrication of multi-material 3D scaffolds, in particular to create architectures modeling both the topography and heterogeneity of the physico-chemical properties of in- vivo cell microenvironment.

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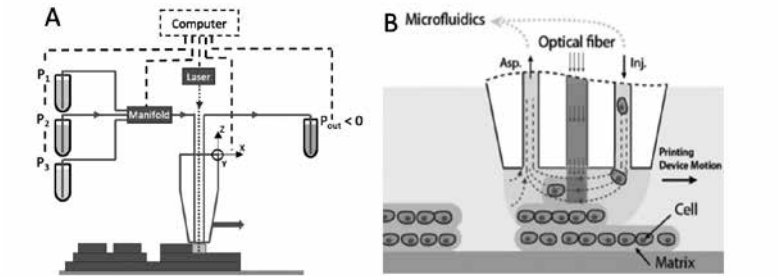

Figure 1. (A) Overview of the LAMP technology, including microfluidic and optical aspects. (B) Illustration of the printing head working principle for material injection, recovery and polymerization

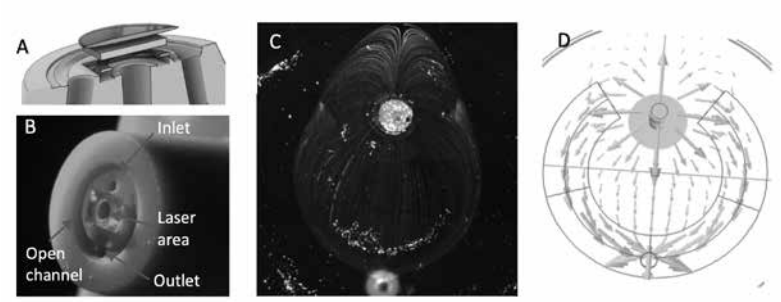

Figure 2. (A, B) Example of assembly, design and micrograph of the printing head. (C) Experimental image obtained with fluorescent particles showing the hydrodynamic flow at the head vicinity. (D)- Corresponding hydrodynamic simulation obtained with COMSOL. showing the theoretical material displacement.

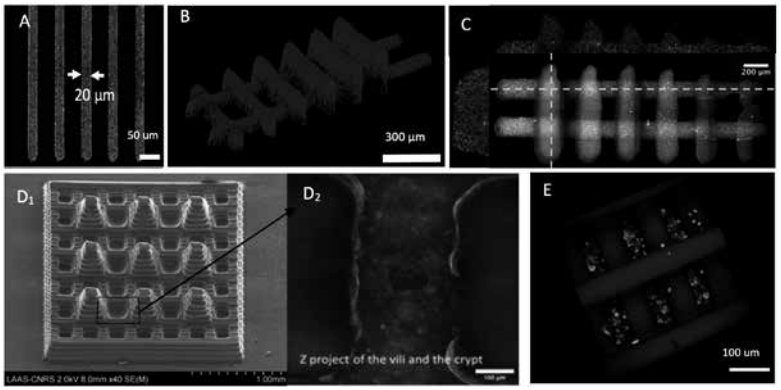

Figure 3. Printing examples. (A) Hydrogels lines showing a 20 µm lateral resolution. (B) 3D Ladder structure used to demonstrate Z accuracy. (C) Bi-color ladder: Horizontal line and vertical were printed with two different PEGDA solutions with different staining (Rhodamine/Fluoresceine). (D) Intestinal gut model, SEM image of the printed structure (D1) and cell culture covering both villi and crypts (D2). (E) 3D structure showing adhesion contrast (PC3 cell culture)

3D-bioprinting for Biomimetic Multifiber Skeletal Muscle-based Bioactuators

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Recent advances in three-dimensional (3D) bioprinting and tissue engineering have opened new possibilities in the fabrication of bioengineered muscle models to mimic the complex hierarchical organization and functional properties from the native tissues [1]. The combination of skeletal muscle tissue and artificial elements has led to a wide variety of innovative solutions for the creation of bio-hybrid robotics and bioactuators [2]. The fabrication of a biomimetic muscle tissue by using 3D bioprinting is key to better understand the physiological conditions of native muscle in order to integrate some of their unique properties to the bioactuator platform, such as self-healing, adaptability, or response to external stimuli. Moreover, these muscle-based bioactuators offer the opportunity to study other processes of interest in the biomedical field, such as muscle development, regeneration, and diseases in a biomimetic environment that resembles the native tissue.

In our work, we developed a 3D-bioprinted bioengineered skeletal muscle bioactuator that shows a bundle-like structure and contraction patterns similar to the ones present in the native muscular tissue. We explored two extrusion-based 3D-bioprinting techniques: the conventional one and a patented co-axial 3D-printing approach [3] (Figure 1). In conventional 3D-bioprinting, a single syringe containing the cell-laden bioink is used. During the crosslinking process, the bioprinted layers fuse together resulting in a single fiber of larger diameter. Alternatively, in co-axial 3D-bioprinting, commonly used for the fabrication of vascular systems [4], an outer layer of sacrificial material (pluronic acid in this study) allows a physical confinement on the inner layer (i.e bioink), obtaining thin independent printed fibers that can be hierarchically organized. Matured and functional bioengineered muscle-based bioactuators with contractile behavior were obtained using both bioprinting approaches. However, the formation of thinner and individual fibers obtained by co-axial 3D-printing allowed an enhanced nutrient diffusion during the muscle maturation process, improving cell differentiation and resulting in stronger bioactuators which present an increased force output in comparison with the actuators fabricated with conventional printing.

After exploring the potential of 3D bioprinting for fabricating 3D bioengineered skeletal muscle bioactuators, our interests are currently focused on exploiting the regenerative capabilities of muscle tissue to integrate self-healing properties to living actuators [5] and create more biomimetic in vitro muscle models for biomedical applications.

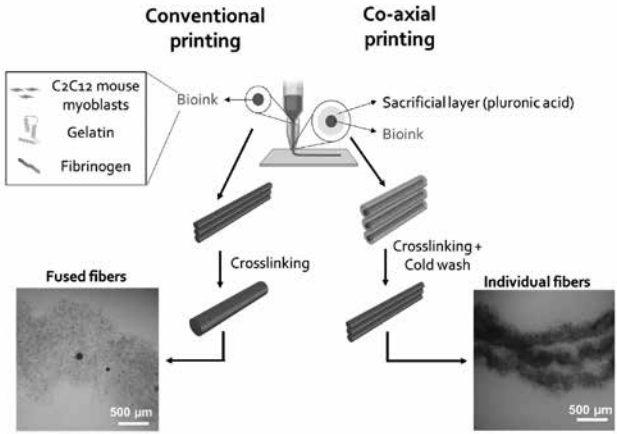

Figure 1. Schematic of the two 3D bioprinting techniques used: conventional printing and co-axial printing [3].

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Generation Of Reporter Human Pluripotent Stem Cell Lines To Study Cardiac And Renal Development And Disease.

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The development and use of human pluripotent stem cells (hPSCs), represent an effective tool to recapitulate characteristics related to human disease, tissue differentiation and morphogenesis. This is due to their capacity to generate three dimensional (3D) self-organized organ-like structures, called organoids, that can constitute an effective alternative to animal models. Until recently, the genome of hPSCs was difficult to manipulate, limiting considerably their use for functional genetics. Thanks to the development of the CRISPR-Cas9 technology, it is now possible to enable precise, intentional, and permanent changes in the DNA of living organisms and cells. Our laboratory has developed a cellular platform, named iCRISPR2 (iC2), that allows to perform highly efficient genome editing in hPSCs, through TALEN-mediated gene targeting that introduces a doxycycline-inducible Cas9 expression cassette at the AAVS1 locus. This editing platform enables a myriad of applications such as the generation of reporter, knock-out and knock-in cell lines. We focus our attention on the generation of cardiac and renal reporter cell lines. To this aim we designed highly active and specific sgRNAs, to make Cas9-mediated site-specific double strand breaks (DSB). Each specific sgRNA was co-nucleofected in iC2 line, together with a specific double strand DNA donor sequence that matches the broken ends of the genomic DNA. In the middle section of the donor DNA we introduce the sequence of a specific reporter gene. After the Cas9 mediated double strain break, the DNA repair machinery catalyzes the homology-directed repair (HDR), using the donor DNA as template to fill the gap. As consequence of this repair event, the sequence of the reporter gene is introduced into the genome. Nucleofected cells are plated as single cells and cultivated until clones appear. Clonal lines are established by manual colony picking. By genotyping analysis, we identified positive clones, in which after the DSB, the DNA repair machinery introduces the sequence of the

reporter gene in the genome. Following this strategy we have been able to start generating cardiac (MYH6, MYL2, SIRPA) and renal reporter cell lines (MEIS-1 and GATA3) for applications on organoid derivation. Currently, our efforts are guided towards the definition of cell culture conditions sustaining cardiac and renal differentiation exploiting these cell sources. Combining these reporter cell lines with cell culture conditions mirroring systemic conditions compromising organ function already available in the laboratory (i.e., Diabetes) will lead to the generation of advanced biomodels to study cardiac and kidney diabetic disease.

Motion performance and shape-shifting of 3D bioengineered living robots

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Biohybrid robotic systems are designed based on the combination of synthetic materials and biological entities aiming to acquire improved performance or properties that are difficult to mimic with artificial materials [1]. The integration of biological components (i.e. skeletal muscle cells) in robotic systems provides some of the desired capabilities from such living entities, including linear contraction, self-organization, self-healing, energy efficiency, high power-to-weight ratio, adaptability, or bio-sensing [2]. Three-dimensional (3D) printing is a versatile technique that has opened new possibilities for the fabrication of bio-hybrid robots, permitting the easy integration of 3D structured biological and synthetic elements to obtain advanced actuation modes.

In this work, we develop a skeletal muscle-based swimming biobot formed by a 3D-printed serpentine spring integrated into a 3D cell laden scaffold [3]. The spring skeleton provides dynamic mechanical self-stimulation during the cell differentiation process, promoting a greater maturation and alignment of the muscle fibers that results in a higher force output (Figure 1A). Upon electrical stimulation, the biobot exerts a directional swimming motion at the liquid-air interface. We achieved a maximum velocity of 800 μm/s, 791 orders of magnitude higher than the fastest skeletal muscle-based swimming biobot up to date. Additionally, alternative skeletal-muscle based biobot configurations arising from a self-assembly process of the 3D cell-laden scaffold are currently under study. They present a characteristic infinity shape that results in a crawling motion by either spontaneous contraction or controlled electrical pulse stimulation (Figure 1B). The infinity biobots guidance has also been explored by using topographical and external magnetic fields. Both the self-stimulating skeleton and the self-assembly process demonstrated in our studies are of great interest to the 3D bioengineering robotic community, providing useful tools to create advanced robotic systems with programmable actuation.

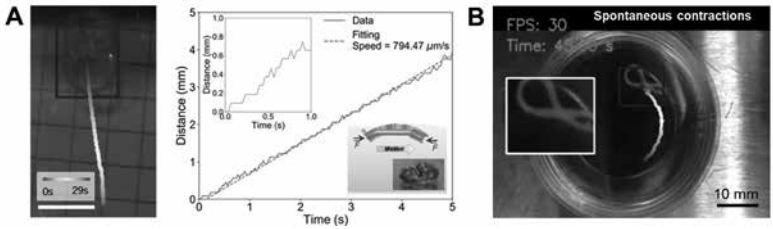

Figure 1. Asymmetric biobot swimming motion evaluation. (A) Motion tracking of an asymmetric swimming biobot over time under an electrical stimulation frequency of 5 Hz. Scale bar, 10 mm. (B) Motion by spontaneous contraction, no external stimulation (electric/magnetic) is imposed.

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Bioprinting by light sheet lithography: engineering complex tissues with high resolution at high speed

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Three-dimensional bioprinting is constantly reaching new horizons in the field of tissue engineering, with various applications reaching the market every year. Nevertheless, systems are limited in resolution when required to print in a fast manner (e.g., extrusion-based) or limited in speed when required to print at high resolution (e.g., 2-photon-polymerization). Here, we present a bioprinting technique, which produces millimeter-sized objects in less than 3 minutes, using a digitally scanned light sheet. Hence, the resolution is dictated by the light sheet dimensions, as low as 20 µm for the z-axis and about 50 µm for the x-axis, also depending on the used photo-crosslinkable hydrogel. In this study, a novel hydrogel was developed to fully exploit the light sheet resolution. Additionally, light sheet bioprinter enables live acquisition of the printing process in high resolution for real-time observation of the cell-laden hydrogel. Initial testing with human cell lines shows promising results in terms of cell outgrowth and interconnectivity. The goal will be to produce human skin models and provide the pharmaceutical and cosmetics industry with an alternative to animal testing.

Engineering an Immune Niche for T Cells

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T cells are instrumental in fighting cancers and infectious diseases, but it is still not feasible to effectively tailor the T cell phenotype and TCR repertoire for immunotherapeutic purposes. In the body, T cell priming, differentiation and activation occurs in immune niches like the T cell zone of lymph nodes and sometimes in ectopic tertiary lymphoid structures found in association with tumors. We are building multicellular assemblies to recapitulate the physiology of T cell-focused immune niches and have recently been awarded an EU-funded EIC Pathfinder project named INCITE (#964955), Immune Niches for Cancer Immunotherapy Enhancement, to further explore the path towards targeted immunotherapy by T cell manipulation. Here I will present the project and some preliminary results.

The immune niches are multicellular, harboring supporting stromal cells, antigen presenting dendritic cells and T cells of diverse maturation stages and antigen specificity. The T cells undergo a complex differentiation process which *in vivo* is dependent on the biological microenvironment, and of the dynamics of the central stimulators. The approach for building this niche will be outlined.

Methacrylated recombinant collagen peptide as human collagen mimicking 3D hydrogel model for cardiac tissue engineering

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Collagen is the most abundant extracellular matrix (ECM) protein in the myocardium. It is responsible for providing cell and tissue support and mediates a myriad of cell biological process. Usually 3D *in vitro* models makes use of animal-derived collagen for cardiac disease modeling and tissue engineering purposes. However, increasing concerns associated with animal-derived materials, batch-to-batch variations, and the inability to tune the mechanical properties in a well-controlled manner result in a need to find alternative materials. Methacrylated Recombinant Collagen Peptide (RCP-MA, Fujifilm) is a human collagen I mimicking peptide containing no animal-derived material. The main advantages of the RCP-MA are that (1) is highly reproducible, allowing for more accurate *in vitro* experiments; (2) methacrylic group modifications of the peptide backbone enable the tuning of its mechanical properties from 1kPa to ~300 kPa with UV illumination; and (3) enrichment with arginine-glycine-aspartic acid (RGD) sequences allow for the control over its cell-adhesive properties.

In this study, we explored RCP-MA as a 3D hydrogel *in vitro* platform for cardiac tissue engineering. We demonstrated that, using RCP-MA of different degrees of methacrylation (20, 50 and 100%), we could fabricate hydrogels that mimic the stiffness of normal and pathological myocardium, respectively. At any methacrlyation degree and UV illumination time, cells showed a high cell viability (>90%) after 10 days of culture inside the RCP-MA. Moreover, electromechanically active human embryonic stem cell derived cardiomyocytes (hESC-CMs) were found, indicating the biocompatibility and suitability of RCP-MA. Next, we investigated how changes in ECM stiffness affect co-cultures of hESC-CMs and primary cardiac fibroblasts (cFb). These cocultures showed a gradual altered phenotype and function with increasing ECM stiffness, suggesting that the injured myocardium guides cellular function *in vivo* and might hamper the regenerative potential of newly injected or recruited cells. Altogether, these result show how RCP-MA could serve as a valuable alternative to animal-derived collagen for cardiac disease modeling and tissue engineering applications.

Mechanosensitive Notch signaling regulates strain-mediated phenotypic changes in vascular smooth muscle cells

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Introduction:

In situ vascular tissue engineering, which aims to transform biodegradable scaffolds into living tissues at the implantation site, has the potential to meet the clinical need for small-diameter vascular replacements. Despite the advances in strategies over the years, the long-term functionality of tissue-engineered vessels, strongly correlated with their biomechanical properties and tissue organization, still needs improvement. To address this challenge, a mechanistic understanding of the processes mediating functional growth and remodeling of tissue-engineered vessels is needed.

Vascular smooth muscle cells (VSMCs) are the regulators of growth and remodeling in vessels, with the ability to switch from a quiescent contractile phenotype to a more synthetic phenotype. This plasticity is regulated by mechanical cues [1] and cell-cell signaling pathways such as Notch [2]. Increasing evidence confirms the mechanosensitive characteristics of Notch signaling [3,4]. Thus, Notch signaling can be a link between mechanical cues and phenotypic changes of VSMCs. Fully unraveling this interplay could open new possibilities in controlling VSMC behavior via manipulating mechanical cues or cell-cell signaling to induce functional growth and remodeling in tissue-engineered vessels.

Methods:

Human coronary artery smooth muscle cells were grown and differentiated in vitro to obtain synthetic and contractile VSMCs, respectively. Notch signaling was either inhibited via treating the cells with DAPT or activated by seeding the cells on immobilized Jagged1 ligands. The cells were equibiaxially stretched (10% strain) at 1Hz for 48 hours. The displacement of the underlying membranes was tracked with a camera and quantified to calculate the corresponding strain. Immunofluorescence imaging and quantitative PCR were performed to characterize the changes in cell phenotype, extracellular matrix production and Notch signaling activity.

Results:

Contractile VSMCs demonstrated different characteristics than synthetic VSMCs by obtaining alpha smooth muscle actin stress fibers and having a higher Notch signaling activity. The application of strain resulted in a transition in contractile VSMCs towards the synthetic phenotype, with an accompanying decrease in Notch signaling. The inhibition of Notch signaling during stretching didn't result in a significant change compared to the stretched controls. On the other hand, the constant activation of Notch signaling rescued the phenotype of stretched contractile VSMCs.

Discussion:

This study suggests that Notch signaling has a key role in regulating strain-mediated changes in VSMC phenotype. Understanding this interaction is an important step towards steering and controlling functional growth and remodeling in tissue-engineered vessels.

Acknowledgements:

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (Grant agreement No. [802967]).

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3D Cell Cultures Revealed Different Efficacy of Two Anticancer Agents with Comparable Cytotoxicity in 2D Cell Cultures

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Three-dimensional cell culture systems are increasingly used for biological and anticancer drug screening as they mimic the structure and microenvironment of tumors more closely than conventional two-dimensional cell models. Herein a method for formation of colon adenocarcinoma-derived spheroids of defined size is presented. Additionally, the importance of complex spheroid architecture for drug screening studies is drawn by analyzing the cytotoxic effect of two anticancer agents, 5-fluorouracil and irinotecan hydrochloride. Results revealed that the cytotoxicity of 5-fluorouracil and irinotecan on cells grown in a 2D monolayer after 24-hour exposure is comparable (5-fluorouracil IC_{50} = 41.5 ± 11.6 μM, irinotecan IC50 = 30.1 ± 5.3 μM), however, their ability to reduce the viability of cells in spheroids differs considerably. A 20-fold higher concentration of 5-fluorouracil (2,500 μM) had to be applied to spheroids to achieve a similar cytotoxic effect as the 125μM irinotecan solution. After 48 hours of incubation with tested compounds, the inhibitory concentration IC50 for 5-fluorouracil was established to be 11.3 ± 3.7 μM for a 2D cell culture and 707.7 ± 20.9 μM for a 3D spheroid.

In the case of irinotecan, IC_{50} increased from 24.9 ± 4.4 μM to 77.8 ± 5.3 μM. While 5-fluorouracil mainly caused suppression of spheroid growth from the outside, irinotecan affected the entire spheroid and caused its originally compact structure to disintegrate. The acquired results indicate that 3D tumor models pose a barrier for compounds to efficiently penetrate through the microtissue, that this barrier's effect on cytotoxicity differs for different compounds, and thus corroborate the need to test cancer chemotherapeutics and potential anticancer agents on complex 3D tumor models.

Keywords: 3D cell cultures, tumor spheroids, 5-fluorouracil, irinotecan hydrochloride

Microfluidic platform for continuous perfusion of transwell-based barrier models

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We present a microfluidic platform for establishing continuous perfusion in transwell-based in-vitro barrier models. We developed a poly(methylmethacrylate) (PMMA)-based microfluidic device, which allows for dynamic control of culture microenvironments in both, apical and basolateral compartments of cell-covered transwell inserts.

The proposed platform consists of a PMMA-based microfluidic chip, fabricated by a CNC milling process (Fig. 1A). The bottom surface of the chip contains a micro-milled channel of 200 µm height with circular openings in the center, in which the cell-coated transwells are inserted. The channel is sealed from the bottom with a glass coverslip using UV-curable adhesive bonding. The stable and leakage-free insertion of the transwell into the micro-milled PMMA-chip is realized with an elastic silicon gasket, which tightly seals the transwell insert. The basolateral side of the transwell is closed with a custom-designed polylactic acid (PLA)-based lid. Perfusion control in the apical and basolateral compartments of the transwells is realized by using fluidic connections at the inlet and outlet ports of the PMMA chip and PLA lid. The chip features standard microscope slide dimensions (72 x 25 mm^2) and contains four individual fluidic units for transwell insertion and culturing (Fig. 1B).

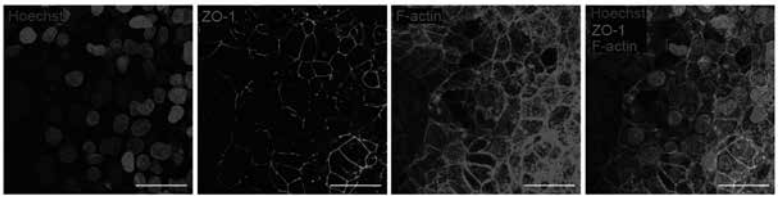
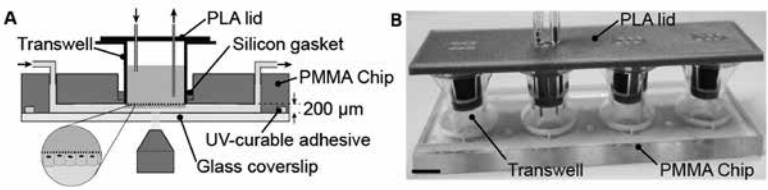


Figure 1: A) Schematic illustration of the microfluidic platform. B) Photograph of the fully assembled PMMA chip that contains four transwell inserts and the PLA lid that features inlet and outlet connections for perfusion of the basolateral compartments (scale bar = 5 mm).



In a proof-of-concept application of our device, we demonstrated its usability for high-resolution confocal imaging of a placental-trophoblast barrier (Fig. 2). We performed immunofluorescent stainings of cell nuclei, the tight junction protein ZO-1, and the cytoskeleton marker F-actin, all of which confirmed the formation of a tight and confluent placental-trophoblast barrier on the apical side of the transwell insert.

Figure 2: Immunofluorescence staining of cell nuclei (Hoechst), tight-junctions (ZO-1), and cytoskeleton (F-actin) of the placental-trophoblast barrier after insertion of the transwell into the microfluidic device (scale bar = 50 µm).

Acknowledgements

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A high-throughput approach for the application of FRET-based tension sensors to pancreas-derived organoids

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Pancreatic ductal adenocarcinoma (PDAC) remains a particularly aggressive and lethal form of cancer, being mostly resistant to conventional treatments. In decades of cancer research, the primary role of the extracellular matrix (ECM) mechanics was underestimated, which is now known to be crucial for the initiation of malignant phenotype.

Organoids are a promising in vitro model system for the investigation of the role of forces in the characteristic branching morphogenesis of PDAC under controlled and tunable mechanobiological conditions *like ECM stiffness and cellular curvature*.

We show, that genetically encoded FRET-based tension sensor modules (TSMs) integrated into vinculin and alpha-catenin can be transferred from cell monolayers to complex multicellular systems, representing a powerful tool for the measurement of the spatiotemporal distribution and the quantification of forces in adherens junctions and cell-ECM interaction regions of PDAC organoids.

The successful application of TSMs to 3D structures, requires the imaging and analysis of multiple organoid replicates. For that, a high-throughput approach for the microfabrication of PDAC organoids of defined geometry and position in the ECM is applied. Bioengineered PDAC microenvironments are utilized to specify the initial geometry of PDAC organoids, that controls their patterning. By controlling organoid growth through a variety of geometries, the underlying mechanisms of epithelial patterning and the spatiotemporal distribution of forces across adherens junction proteins can be investigated. These highly controlled settings help to understand the role of ECM mechanics in specifying mechanosensitive pathways and thus open up new prospects in therapeutic development.

Switch in extracellular matrix dynamics mediates morphodynamical luminal transition in malignant ovarian spheroids

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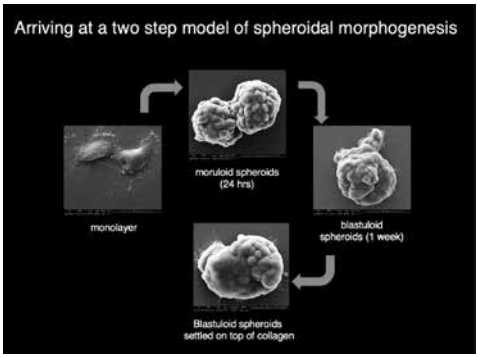
Ovarian cancer metastasizes into the peritoneum through dissemination of transformed epithelia in the form of multicellular spheroids. Harvested from the malignant ascites of patients, such spheroids exhibit startling features of organization typical to homeostatic glandular tissues: lumen surrounded by tightly apposed and adhered epithelia. We demonstrate that cells of specific ovarian cancer lines in suspension, aggregate into dysmorphic solid "moruloid" clusters that permit intercellular movement, cell penetration, and interspheroidal coalescence. Moruloid clusters subsequently mature into "blastuloid" spheroids with smooth contours, a temporally dynamic lumen, and immotile cells. Blastuloid spheroids neither coalesce nor allow cell penetration. Ultrastructural examination reveals a basement membrane-like extracellular matrix coat on the surface of blastuloid, but not in the moruloid, spheroids. Quantitative proteomics reveals down-regulation in ECM protein Fibronectin-1 associated with the moruloid-blastuloid transition; immunocytochemistry also confirms the relocalization of basement membrane ECM proteins: collagen IV and laminin to the surface of blastuloid spheroids. Fibronectin depletion accelerates, and enzymatic basement membrane debridement impairs, lumen formation, respectively. Further, our investigation using a small molecule inhibitor screen targeted at teasing apart actin cytoskeletal dynamics in spheroidal morphogenesis suggests the role of Arp2/3 in aggregation and Rho-ROCK signalling in lumen formation and maintenance. An interplay between cell aggregation and polarity driven through basement membrane thus seems crucial to the patterning of circulatory ovarian cancer cells in a hostile fluid milieu.



Long-range organization of intestinal 2D-crypts using micropatterning

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Intestinal epithelial cells *in vivo* are spatially segregated in proliferative crypts and differentiated villi. Such organization is achieved through a set of biomolecular gradients which set these two compartments. Several signaling pathways such as Wnt, Notch, BMP or Eph/ephrin are involved in inducing these differential patterns of cell types along the crypt-villus axis. Among them, Wnt plays a crucial role in regulating the proliferation and organization of epithelial cells. While Paneth cells are the main source of epithelial Wnt signaling, *in vivo* compartmentalization can also be set by exogenous Wnt signals originated in non-epithelial cells residing in the lamina propria. On the other hand, Eph/ephrin repulsive interactions control cell positioning along the crypt-villus axis mediating the communication signaling between cells. *In vitro*, primary intestinal epithelial cells also organize in proliferative crypts and villi, both in 3D organoids and in 2D monolayers. However, while the importance of signaling pathways such as Wnt and Eph/ephrin both *in vitro* and *in vivo* has been proven, the role of their spatial distribution in crypt-villus organization is difficult to address both *in vitro* and *in vivo*.

Here, we show that long-range organization of proliferative crypts *in vitro* can be controlled by the spatial distribution of either exogenous Wnt3a or ephrinB1 ligands. We made use of 2D intestinal epithelial monolayers and micropatterned substrates to bioengineer a system that allows us to precisely tune both the molecular signaling and its localization. We created arrays of either Wnt3a dots or ephrinB1-depleted circular regions. Our results demonstrate that by adding Wnt3a ligands in an exogenous manner, the size and shape of the crypt-like domain areas can be controlled. Furthermore, we show that exogenous Wnt3a and ephrinB1 signaling patterns regulate crypt positioning. Then, we used arrays of different

sizes to vary the distances between crypts. By doing so, we observed that distance between crypts is limited by the size of the proliferative regions, suggesting that crypt- and villus-like domains are arranged following a scaling law.

We believe that our results show that the spatial distribution of exogenous signals can synergize with the intrinsic self-organization of intestinal epithelial cells to control tissue architecture. Altogether, we have designed and established a tool to effectively investigate the self-organization of organoid-derived intestinal epithelial cells and systematically study the effects and mechanisms of individual ligands on this process.

SMAD3 in tumor associated fibroblasts drives enhanced fibroblast accumulation in lung adenocarcinoma through increased migration

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A hallmark of lung cancer and other solid tumors is a stiff fibrotic microenvironment rich in activated tumor-associated fibroblasts (TAFs). Because TAFs have been implicated in all steps of tumor progression, there is growing interest in understanding their aberrant mechanobiology. TGF-β1 is an efficient fibroblast activator frequently upregulated in lung cancer. Intriguingly, we previously reported a larger accumulation of TAFs in lung adenocarcinoma (ADC) compared to lung squamous cell carcinoma (SCC) (Puig et al, Mol Cancer Res 2015), which are the two most frequent lung cancer subtypes, although the underlying mechanisms remain elusive. TAF accumulation is largely contributed by the proliferation and/or migration of resident fibroblasts. Notably, SMAD3 is an important transcription factor of the TGF-β1 pathway that has been implicated in the regulation of proliferation and migration of different cell types. Moreover, we recently showed that SMAD3 is epigenetically repressed in SCC-TAFs compared to ADC-TAFs owing to an excessive exposure to cigarette smoke particles, which elicited a compensatory increase in its closely related homolog SMAD2 in SCC-TAFs (Ikemori et al, Cancer Res 2020). However, it remains unknown whether the differential SMAD2/3 expression between ADC- and SCC-TAFs contributes to the larger accumulation of TAFs in ADC. To address this question, we knocked-down SMAD2 or SMAD3 in control pulmonary fibroblasts by shRNA and used them as ADC-like or SCC-like models. To assess proliferation we examined cell number density and found that shSMAD2 (ADC-like) fibroblasts exhibited a significantly lower number density in basal conditions (i.e. absence of TGF-β1) compared to shSMAD3 (SCC-like) fibroblasts, which was confirmed in TAFs. In the presence of TGF-β1, number density increased and attained similar values in shSMAD2 and shSMAD3 fibroblasts as well as in ADC- and SCC-TAFs. To assess fibroblast migration we used a microfluidic device to quantify biophysical descriptors of protrusions and subsequent migration within a 3D collagen culture. Notably, both protrusions and migration descriptors were

increased in basal conditions selectively in shSMAD2 (ADC-like) fibroblasts, whereas TGF-β1 globally impaired migration. Consistent results were observed in ADC-TAFs upon knocking-down SMAD3. These results reveal that altered SMAD2/3 expression provide growth and migration advantages only in the absence of TGF-β1, although in opposing directions, since growth advantage was observed in high SMAD2 conditions (as in SCC), whereas migration advantage was found in high SMAD3 conditions (as in ADC). These findings strongly support that the larger TAF accumulation in ADC occurs at early stages (under low TGF-β1) and is driven by the enhanced migration of ADC-TAFs due to their high SMAD3. These findings may also shed light in other processes involving fibroblast accumulation, such as repair and fibrosis.

Active mucus-cilia hydrodynamic coupling drives self-organization of human bronchial epithelium

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The respiratory tract is protected by mucus, a complex fluid transported along the epithelial surface by the coordinated beating of millions of microscopic cilia, hence the name of mucociliary clearance. Its impairment is associated with all severe chronic respiratory diseases. Yet, the relationship between ciliary density and the spatial scale of mucus transport, as well as the mechanisms that drive ciliary-beat orientations are much debated. Here, we address these questions by combining experiments on in vitro reconstituted human bronchial epithelia and a hydrodynamic model. We observe and quantify the dynamics of formation and growth of swirly patterns of mucus flows during ciliogenesis. We show that at a physiological cilia density, a macroscopic swirl of mucus develops associated with a circular coordination of ciliary-beat directions and a circular pattern of the planar cell polarity of the tissue. By establishing that the macroscopic ciliary-beat order is lost and recovered by removing and adding mucus, respectively, we demonstrate that cilia–mucus hydrodynamic interactions drive the collective dynamics of ciliary-beat directions. We developed a two-dimensional hydrodynamic model that highlights two relevant physical parameters of the epithelium, the density of cilia and mucus viscosity. The model predicts a phase diagram of mucus transport in accordance with the experiments. This paves the way to a predictive in silico modelling of bronchial mucus transport in health and disease.

Exploring the role of hypoxia and Plasmodium falciparum pathogenesis in a 3D brain microvascular model

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Cerebral malaria is a severe form of malaria responsible for most of 400,000 malaria-related deaths each year. Cerebral malaria is characterized by patient coma and cytoadhesion of *Plasmodium falciparum*-infected red blood cells to the brain microvasculature. Recent magnetic resonance imaging has revealed differences in the cause of death in adults and children with cerebral malaria. While children present brain swelling and blood brain barrier breakdown, adults present decreased brain volume associated with ischemia and hypoxia. However, the pathogenic mechanisms that lead to such differences remain unknown. This is due to a lack of animal models that recapitulate the main pathogenic events associated with human cerebral malaria, as well as, the inability of 2D *in vitro* models to replicate the 3D environment of the brain microvasculature. To investigate this question, we are developing a pre-patterned microfluidics-based 3D *in vitro* model that possesses 100 µm-sized microvessels that are simultaneously seeded with both primary human brain endothelial cells and brain pericytes. The resultant microvessels show self-organization of endothelial cells to the luminal interface and pericytes to the abluminal side, wrapping around the endothelium. Increased pericyte to endothelial cell ratio is associated with vessel shrinkage and negatively associated with expression of two microRNA hypoxia biomarkers, miR-150-5p and miR-3158-3p (Figure 1). Notably, increased plasma concentration levels of both microRNAs have been shown to be associated with cerebral malaria with miR-3158-3p additionally being correlated with decreased brain volume and hypoxia on patient admission. Future studies in the presence of *P. falciparum*-infected red blood cells and hypoxic conditions will be presented. Likewise, other pathways associated with cerebral malaria and hypoxia, such as the Angiopoietin-Tie2 axis, will be investigated. Overall, we aim to develop and utilize a 3D microfluidics-based *in vitro* brain microvasculature model to mimic hypoxia and vascular pathway disruption during cerebral malaria, which could inform on future diagnostics or therapeutics to reduce mortality in CM patients.

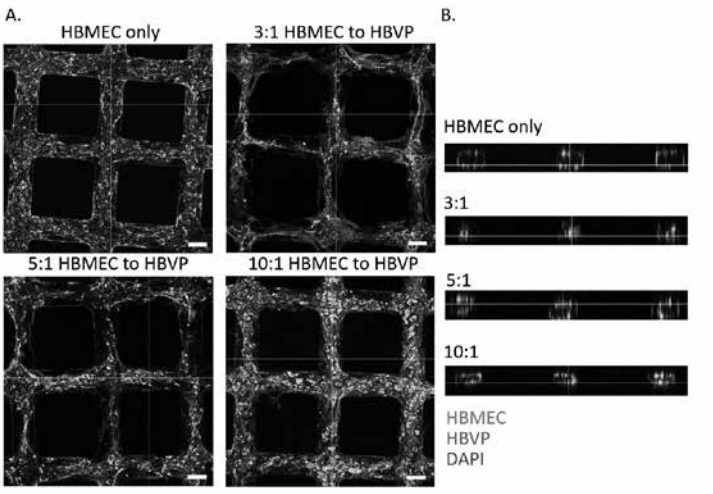

Figure 1: Vessel shrinkage is increased in lower HBMEC to HBVP seeding ratios. (A) Primary human brain microvascular endothelial cells (HBMEC, green) and primary human brain vascular pericytes (HBVP, red) are seeded into the pre-patterned 3D *in vitro* model at different ratios (3:1, 5:1, 10:1 or HBMEC only). (B) 3D orthogonal views of three microvessels from each seeding ratio display pericyte wrapping around the endothelium and decreased lumen size upon increased pericyte ratios. Scale bar= 100 um.

Biomechanics of the progression of hypermethylated colorectal carcinomas

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According to the World Health Organization, cancer is one of the main causes of death worldwide, with colorectal carcinoma (CRC) being the second-leading cause of tumour related-death. The high rate of mortality of CRCs is principally attributed to the metastasis of neoplastic cells from the primary tumour to secondary organs such as the liver, the lung and the peritoneum. These cells can disseminate either as single isolated cells or as collective clusters, undergoing a series of molecular and cellular changes commonly known as Epithelial to Mesenchymal Transition (EMT).

However, in 2018, our laboratory described a novel modality of peritoneal metastatic spread characterized by the presence of large clusters of cancer cells, which maintain their epithelial properties and display an outward apical polarity. These clusters of cells, termed tumour spheres with inverted polarity (TSIPs), were found in peritoneal effusions of CRCs patients showing early KRAS mutation and hypermethylation of CpG Islands.

TSIPs originate through a series of morphological changes: the first event is the sprouting of hypermethylated epithelia, followed by their apical budding, leading to the formation of rounded spherical clusters of cells called buds, and the subsequent cleavage of the newly formed spheres. How cell and tissue mechanics drive this process is still unclear. To provide novel insights into this metastatic cascade, our project aims at deciphering the biomechanical and cellular events regulating the formation of buds in colorectal cancer cell lines.

Employing a combination between cellular and molecular biology techniques with biophysical methods, we showed that this process is characterized by over-proliferation, local changes in cell adhesion and by increased contractility of epithelia, coupled with the formation of topological defects at the level of the monolayer underneath the buds.

Our study demonstrates that buds development in colorectal carcinomas epithelia is governed by morphological transitions occurring entirely at multicellular level, rather than by single cells aggregation or cell extrusion. By understanding the formation of TSIPs and buds, we wish to enlarge our knowledge about collective dissemination in CRCs and to identify new potential markers of colorectal cancer progression.

Altering Organoid Shape Through Optogenetic Control Of Apical Constriction

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During the last years, formation of 3D tissues in vitro has been achieved through either tissue engineering techniques or organoid self-organization. While tissue engineering has focused on the use of structures that will direct cells to acquire different tissue morphologies, the organoid field relies on the self-organizing properties of pluripotent stem cells to form complex tissue architectures. To this date, we still lack control over the cellular processes that drive tissue morphogenesis. Acquiring such control would allow us to improve our understanding of how tissue architecture is developed and how it impacts cell fate and function.

With the aim of filling this gap, our lab recently developed an optogenetic tool to induce apical constriction in mammalian systems: OptoShroom3[1]. Apical constriction is an essential morphogenetic process involved in the acquisition of curvature in epithelial tissues. It is recurrently used in the early stages of mammalian embryo development to establish the shape of different organ primordia. With OptoShroom3, spatio-temporal control of apical constriction can be achieved through light illumination, which permitted us to induce morphological changes from the cell to the tissue level.

In our latest work, we make use of OptoShroom3 to modify the shape cerebral organoids. It has recently been shown that Shroom3 is differentially expressed during the development of human cerebral organoids, when compared with Gorilla organoids[2]. Our aim is to take advantage of OptoShroom3 to study how these changes in shape can impact cell fate and the rate of differentiation. We expect that the development of new tools that allow us to alter tissue shape will help us further investigate the feedback between shape and function.

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Title: “Mechanistic insights on the regulation of the period gradient in the PSM”

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One of the most important questions in the biology of multicellular systems is how cells exploit intrinsic and extrinsic cues to form patterns. The pre-somitic mesoderm is the far most posterior part of a developing embryo and its physiological output is to generate the somites. These epithelial blocks of tissue are periodically deposited and they pattern the antero-posterior axis of an embryo. This pattern is the result of the interaction of a spatial with a temporal component. Spatially the tissue is characterized by molecular gradients of Wnt, FGF and Retinoic acid. The temporal component is a clock that ticks within each cell of the tissue and induces the oscillatory expression of a large network of genes. The autoregulatory negative feedback loop of *Hes7* is at the core of the engine that generates those oscillations. An important metric of the oscillations is the period, which corresponds to the time needed for a full cycle to be completed. In the tissue, the period is graded from posterior to anterior but the mechanism through which this is achieved is not known. In my research, I investigate the role of Wnt signaling in the regulation of the period gradient by focusing on Wnt associated regulatory elements upstream of *Hes7*. I combine genetic and live imaging tools as well as single cell RNAseq approaches to test the functionality of those regulatory elements. The data indicate the importance of those genomic regions for the period gradient along the tissue and provide mechanistic insights on how cells can make use of an extrinsic signal to tune their gene activity dynamics.

Three Dimensional Mechanical Constraint Model to Understand Remodeling in Beating Cardiac Microtissues

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The mechanically active myocardial microenvironment functions through a complex interplay between structural tissue organization, cardiac beating, and extracellular matrix (ECM) mechanics. Mechanical forces generated by cells propagate through the myocardium and in turn regulate the phenotype and function of cells and tissue. Upon cardiac injury, ischemia results in a massive loss of beating cardiomyocytes (CMs) and initiates adverse remodeling of the anisotropic structure, typically found in healthy myocardium, into disorganized fibrotic tissue. Disruption of the highly organized structure does not only result in impaired coordinated contraction but also in compromised differentiation, matrix remodeling and mechanotransduction of resident and newly injected or recruited cardiac cells [1]. Understanding the remodeling processes in beating cardiac tissues is therefore crucial to provide new insights to (re) engineer structural organization in living cardiac tissues *in vivo* and *in vitro*.

In this study, we aim to investigate the interplay between coordinated contractility and remodeling of aligned *in vitro* 3D engineered cardiac microtissues. To do so, we generated microscale constructs of cFBs and hPSC-CMs encapsulated within 3D matrices, inspired by micro tissue gauges (μTUGs) [2], called cardiac microtissues. Micropillars of polydimethylsiloxane (PDMS) were used to constrain and direct the remodeling of collagenous 3D matrices while simultaneously quantifying tissue contractility. In order to study the interplay between cardiac beating and remodeling towards anisotropy three types of microtissues are formed: 1) cardiac fibroblast (cFB) tissues, 2) co-culture tissues consisting of cFBs and non-beating hPSC-derived CMs, and 3) co-culture tissues consisting of cFBs and beating hPSC-CMs. After biaxially constrained tissue formation, the micropillars were released in one direction, allowing tissue remodeling towards anisotropy because of the resulting uniaxial constraints. Additionally, after model validation and *in vitro* result replication, we use *in silico* modeling to identify key factors that determine tissue remodeling and coordinated contractility.

Collectively, this study highlights the dynamic relationship between structural organization, coordinated contractility, and ECM mechanics. Our preliminary data indicate agreement between the *in vitro* and *in silico* results for cFB monocultures, which next can be exploited to understand the interplay between remodeling and coordinated contractility in co-cultures containing non-beating and beating CMs, and how to modulate this interplay for the formation of functionally engineered cardiac microtissues.

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Role of tight junctions on lumen size and shape in MDCK cysts

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A hallmark of higher multicellular organisms is the compartmentalization of tissue into distinct organs with specific shapes and functions. During organogenesis these structures initially form as fluid filled cavities, so called lumen surrounded by epithelial cells. How epithelial cells control the chemical and physical properties underlying lumen morphogenesis is not completely understood. Here we investigate the role of tight junctions (TJ) in lumen formation in a 3D tissue culture system (MDCK cysts). TJ regulate tissue permeability for solutes via external diffusion barriers (Claudins) and are involved in controlling mechanical properties of cells via cortical scaffolding proteins (ZOs).

Combining genetic, pharmacologic and mechanical perturbations we quantified tissue permeability, tissue tension and hydrostatic lumen pressure of WT and TJ mutant tissues. Our results reveal that deletion scaffolding proteins (ZO1/2) lead to a strong reduction in lumen volume accompanied by a strong increase in tissue tension. Perturbations of tissue tension (myosin) suggest that the TJ-scaffold in WT tissue is able to reduce tissue tension and thus facilitate lumen opening at low hydrostatic pressure.

Taken together, this work revealed that tight junctions play a central role in coupling hydrostatic pressure and tissue tension and that TJs regulate the force balance between hydrostatic lumen pressure and tissue adhesion/tension and thereby control lumen morphology

Cytoskeletal reconstruction from Traction Force Microscopy

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Traction Force Microscopy (TFM) has emerged as an essential tool for quantifying the forces that cells exert on elastic or viscoelastic substrates [1,2]. After experimentally measuring the displacement field on a substrate, the traction field may be computed by resorting to inverse or convolution methods [1,3]. TFM has allowed researchers to track the forces exerted by monolayers during collective migration [4], wound healing [5], dome formation or cell extrusion [6].

However, at the cell scale, the traction field may in turn exploited to infer the cytoskeletal structure that is mechanically compatible with the set of computed forces on the surface of the substrate. We present a methodology to infer an approximated geometry of the cytoskeleton.

The technique aims to construct a mesh of dipoles of increasing size that minimizes the equilibrium error at each traction pixel. An iterative process filters the non-contributing dipoles while adds those that best minimize the resulting error. The approach does not require assuming any constitutive law, and as such is applicable to viscous and elastic substrates and arbitrary cell rheology.

Current efforts focus on the reconstruction of the cytoskeleton in time evolving processes. In this case, the method is potentially able to track the remodeling process of the actin network.

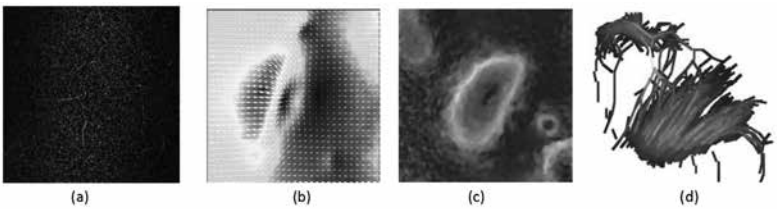

Figure 1. TFM and cytoskeletal reconstruction. (a) Substrate beads tracking, (b) Norm of displacement field. (c) Norm of traction field, (c) Reconstructed cytoskeleton.

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Divergence and Convergence of Morphogenetic Paths in Embryo-like Models

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Embryos of a given species follow a single morphogenetic trajectory, from zygote to fully developed organs. Variability is largely quantitative (length of limbs, number of cells per organ) with no qualitative differences. For example, all mouse embryos develop a single gut tube, stretching along their antero-posterior axis, and a fixed number of bilaterally arranged somites arranged symmetrically along this axis. This robustness stands in contrast to in-vitro embryo-like models, which, like most organoids, display a high degree of variability. What makes embryonic morphogenesis that robust is unclear.

We use extended gastruloids, or Trunk Like Structures, to study the morphogenetic progression of definitive endoderm (DE) and its divergence. We first catalog the different morphologies and characterize their statistics. We then learn predictive models for the lineage morphotype based on earlier expression and morphology measurements. Finally, we analyze these models to identify key drivers of morphotype variability, and devise “personalized” (organoid-specific) interventions that will lower this variability and steer morphotype choice.

An important aspect of morphogenetic robustness is the question of morphogenetic convergence. Alternative protocols in embryo-like models can converge on similar morphologies and spatial expression patterns. In such cases, does the future developmental potential of the organoid depend on its history, or only on its current state? Such convergence of future developmental potential may point to another mechanism driving developmental robustness - “canalization” at the level of morphogenesis. We study this question focusing on pre-somitic mesoderm progression.

We expect the insights obtained here will improve the quality and usability of 3D embryo like models, chart a methodology extendable to other organoids for controlling variability, and will also shed light on the factors that provide the embryo its morphogenetic robustness.

Dissecting early nephron patterning in kidney organoids derived from hPSCs

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Introduction. The formation of organs during development is a complex and orchestrated process. Human pluripotent stem cells (hPSCs) represent unique model systems to dissect early steps related to tissue specification and differentiation. Within the last decades the inherent capacity of hPSCs to self-organize and differentiate into tissue-like derivatives has been exploited to underscore morphogenetic-related processes.

During embryogenesis, kidney development depends on reciprocal interactions between the ureteric bud (UB) and metanephric mesenchyme (MM), which give rise to the collecting duct system and nephrons, respectively. The MM undergoes mesenchymal to epithelial transition (MET), giving rise to epithelial renal vesicles (RVs) that further undergo structural changes and shift towards comma shaped and s-shaped bodies (CSBs/SSBs), which eventually develop into nephron like structures. In this regard, animal studies have helped identify Wnt/b-catenin and Notch signalling pathways as key players in nephron patterning and specification (proximal, medial, distal segments).

During nephron formation, like in any other morphogenetic process, RV emergence and transition towards segmented and patterned nephrons may arise from constant movements of cells and changes in cell force transmission. In this regard, the biophysical cues governing these processes are totally unknown. Moreover, if these processes work in concert with classical biochemical constrains remains to be investigated.

Objective. Here, we aim to use hPSC derived nephron progenitor cells to dissect morphogenetic changes occuring during RV emergence and nephron formation.

Methodology. Human PSCs are guided towards the renal fate on compliant polyacrylamide (PAA) hydrogels with controlled rigidities (mimicking embryonic microenvironment) in a 2D culture system. Polyacrylamide hydrogels of 1 kPa (soft) and 60 kPa (rigid) are generated by adapting the compositional ratio of acrylamide to bis-acrylamide and are further functionalized using acrylic acid NHS. Using this system, we will spatiotemporally characterise early steps of nephrogenesis by immunofluorescence and confocal analysis, time-lapse imaging and traction force microscopy (TFM). These analyses will be performed at different stages during renal differentiation including nephron progenitor cells (NPCs) induction, RV emergence, proximal-distal RV polarization and formation of the nephron-like segments.

Conclusion. The current techniques will permit quantitative and qualitative observations of multicellular behaviours at key stages of 2D renal differentiation. Furthermore, this system will allow us to spatiotemporally map cell-cell and cell-ECM forces and evaluate their evolution throughout renal fate specification.

Dynamics of tissue spheroid fusion: perspectives from the individual cell

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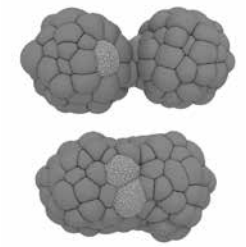
In bottom-up tissue engineering individual subunits, called tissue spheroids, are assembled to form larger structures. To make coherent tissues the spheroids have to fuse together. Although fusion of spheroids has become a standard assay, the relationships between fusion dynamics, tissue rheological properties and cell mechanics remains unclear. Up to recently, fusion dynamics were analysed by a liquid framework which states that the decrease in surface tension energy was balanced by viscous dissipation. However, this resulted in some inconsistencies. For example, Kosheleva et al. observed a faster fusion i.e. higher surface tension, while measuring lower surface tension by nano-indentation [1]. Furthermore, the liquid model predicts that fusion is always complete due to minimization of surface area. Grosser et al observed that fusion could be halted, which has been called arrested coalescence [2]. To account for this observation, we expanded the liquid framework by adding an elastic energy term [3]. Moreover, we performed computational simulation of tissue spheroid fusion using a centre-based model to map individual cell parameters to tissue rheology. In accordance with the experimental results of Steffen Grosser, we identified that the level of cell locomotion is an important predictor for arrested coalescence. On the other hand, we show that when cell-cell repulsion was high more cell locomotion is needed to result in complete fusion. However, in the centre-based model approach, complete fusion coincided with full spheroid mixing, which is not observed experimentally. This unexpected behaviour could be explained by the spherical description of the cell. Therefore, we are currently studying the fusion using a deformable cell model where cell shape is represented more accurately and contact mechanics are explicitly computed. Our preliminary data shows that a high level of cell activity as well as a high ratio of cell adhesion to cortical tension promote spheroid fusion. Furthermore, complete fusion can occur without simultaneously mixing. With this modelling approach we hope to elucidate the relationship between tissue rheology and cell mechanics, which will serve as a powerful tool to better understand *in vitro* culture growth and differentiation.



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Arrested coalescence as a method to explore the mechanics of 3D multicellular aggregates

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Multicellular aggregates are known to exhibit liquid-like properties. The fusion process of two cell aggregates is commonly studied as the coalescence of two viscous drops. However, tissues are complex materials, which usually exhibit viscoelastic behaviour. It is known that elastic effects can prevent the complete fusion of two drops, a phenomenon known as arrested coalescence. Here we show that this phenomenon can be exploited to infer the mechanical properties of 3D multicellular aggregates. By analyzing the dynamics of the fusion process and combining it with nanoindentation measurements, we obtain the effective viscosity, elastic modulus and surface tension of the aggregates. We illustrate our method by studying how the expression of the mesodermal gene Bra/T affects the mechanical properties of mouse embryonic stem cell aggregates. Additionally, agent-based simulations suggest that arrested coalescence can be found in the vicinity of an unjamming phase transition. Our work provides a simple, fast and inexpensive method to characterize the mechanical properties of 3D multicellular aggregates and sheds light on the impact of cellular activity on tissue mechanics.

Bioprinted hydrogel-based microfluidic chip to mimic the tumor metastatic microenvironment

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Metastasis remains the leading cause of cancer-related deaths. In colorectal cancer, the second most common cancer in humans, approximately 50% of patients develop metastasis [1]. Metastasis triggers when cells from the primary tumor intravasate into the blood circulation, circulate in the bloodstream, and eventually extravasate into a metastatic region by migrating through the vessel walls [2]. This cascade is driven by many factors such as the tumor microenvironment (TME) and fluid shear forces, among others. This complex scenario requires in vitro models that account for the contribution of all the players. Thus, we have developed a microfluidic model based on a bioprinted hydrogel channel that mimics the TME of the primary tumor and the endothelial barrier to study tumor cell intravasation during metastasis.

The vessel-like hydrogel channel was bioprinted using as bioink a co-network of poly(ethylene) glycol diacrylate (PEGDA) and gelatin methacryloyl (GelMA). We applied an innovative set-up based on stereolithography bioprinting that uses visible light photopolymerization. The polymer composition and the fabrication conditions were optimized to match hydrogel physicochemical properties with the native extracellular matrix (ECM). The printing parameters were optimized to obtain a microfluidic channel of 0.5 mm in diameter, mimicking a human blood vessel with a hollow lumen lined by endothelial cells and surrounded by stromal tissue. Additional lateral channels ensured nutrient diffusion to the stromal microenvironment. The hydrogel channel was then assembled into a microfluidic chip.

To optimize the printing parameters and bioink composition, we bioprinted hydrogel discs on porous polymeric membranes and mounted into modified Transwell® inserts. Intestinal fibroblasts were embedded in the hydrogel discs to mimic the intestinal stromal microenvironment. They showed high cellular viability

after 7 days' post encapsulation. Moreover, primary endothelial HUVEC cells formed a continuous monolayer on top of these cell-laden hydrogel discs that was preserved after 7 days as confirmed by the TEER values and the expression of VE-cadherin and CD31 markers.

To complete the TME model, GFP-labeled HT29 cells were grown into spheroids and encapsulated inside the bioprinted hydrogels. The growth and interaction with the stromal cells were monitored over time, as well as their transepithelial mesenchymal transition with the differential expression of E-cadherin and vimentin markers.

The proposed hydrogel-based microfluidic technology is considered a technological breakthrough innovation that will allow the study of key events in the metastasis cascade such as the tumor cell intravasation and the tumor-endothelial and tumor-stromal interactions in flow conditions.

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Towards a 3D Brain-on-a-chip for Organotypic Culture and Differentiation of Neuroprogenitor Cells

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Current 2D in vitro and animal models are not sufficient to fully understand the brain and its diseases mainly because of their lack of complexity, ethical concerns, and differences in species. New engineered models, such as 3D culture and microfluidic devices, have been proved to be interesting choices for better resembling the brain [1]. Microfluidic devices even have a tunable architecture that can include 3D cultures [2]. This work aims to mimic the brain microenvironment using a hydrogel embedded in a microfluidic device to study the differentiation process of neuroprogenitor cells in 3D. The scaffold material used for this 3D culture is composed of different polymers (methacrylated gelatin, methacrylated alginate, and hyaluronic acid). The physical characterization of the biomaterial was performed by swelling analysis and compression test where results showed that this material has a good water intake and a low compressive Young's Modulus matching nerve tissue's stiffness range. Neuroprogenitor cells were seeded in the hydrogel and placed in the microfluidic device for up to 15 days. The viability and differentiation into neurons were tested through a LIVE/DEAD assay and immunostaining. Results showed high cellular viability, proliferation, and differentiation into early neurons, with an increased connectivity and potentially functional between cells, evidencing that this model can be used as a further platform with human cells for future studies on neurodegenerative diseases.

Keywords: 3D models, neuronal culture, microfluidic devices, biomaterials, hydrogels.

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Recreating the blood-brain barrier complexity *in vitro*: a three-dimensional microvascular model to investigate cerebral malaria

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Cerebral malaria is the deadliest complication of *P. falciparum* infections, carrying 15-20% mortality rates. *P. falciparum*-infected red blood cell (iRBC) sequestration in the brain microvasculature is considered one of the main pathogenic events leading to brain microvascular dysfunction. As rodent malaria models present modest iRBC sequestration rates, the pathogenic mechanisms of cerebral malaria remain unknown. Here, we aim to study malaria pathogenesis in a three-dimensional blood-brain barrier (3D-BBB) *in vitro* model. Our model is composed of a collagen hydrogel pre-patterned with microfluidic networks that recreate the wide range of flow conditions present in the brain vasculature. Here we have generated two models composed of either human primary brain microvascular endothelial cells or induced pluripotent stem cell-derived endothelial cells (iPSC-EC) in combination with primary human astrocytes and pericytes. While the primary endothelial model presents the highest transcriptional expression of BBB markers at day 7 of culture, the iPSC-EC model peaks at day 3. Direct comparison of the models reveals that the iPSC-EC provide improved barrier properties, as shown by increased junctional expression of claudin-5 and zonula occludens-1 (ZO-1), and permeability assays performed using low molecular weight fluorescent Dextran. Nevertheless, the main caveat of iPSC-EC models is mixed expression of epithelial and endothelial markers. Therefore, we are using both models to surveil BBB alterations following exposure to *P. falciparum*-iRBC, including the assessment of changes in barrier integrity, junctional organization or cell activation (Figure 1). Overall, the use of novel 3D-BBB microvascular *in vitro* models will elucidate the dynamics of parasite-BBB interactions, and could shed light on novel malaria adjunctive therapies to decrease patient mortality.

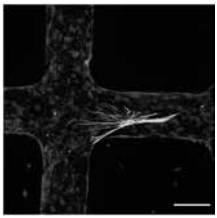

Figure 1. Representative image showing a glial fibrillary acidic protein (GFAP)-positive astrocyte (green) contacting a microvessel after exposure to *P. falciparum*-infected red blood cells (iRBC). Primary human microvascular endothelial cells show expression of tight junction protein zonula occludens-1 (ZO-1) (magenta) and iRBC can be detected as smaller nuclei (DAPI). Scale bar: 100 µm.

In-vitro Engineered Human Cerebral Tissues Mimic Pathological Circuit Disturbances In 3D

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Understanding and treating neuropathological network disorders such as epilepsy has remained a challenge as existing *in-vitro* models lack the complex and intact neuronal circuitry found in the human brain. Generation of current brain organoid models originates from classic dissociation-reaggregation paradigms, often relying on mechanically-enforced quick aggregation of pluripotent stem cells. Here we describe an alternative method that promotes matrix-supported active (migrative) reaggregation of cells (MARC), reminiscent of *in-vivo* developmental morphing processes, to engineer multi-regional brain tissues *in-vitro*. Measurements of neuronal activity in intact 3D tissues revealed functional interconnectivity, characteristic of cerebral neuronal networks. Combining our interconnected cerebral tissues with a multi-chambered tissue-culture chip, we demonstrate induction of propagation of epileptiform discharges, thereby establishing an *in-vitro*, clinically relevant model of an epileptic seizure.

Deciphering the Effect of Mechanical Signals in Tumorigenesis with a Dynamically Tunable 3D Mammary Epithelial Microenvironment

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To find better cancer treatments, it is important to understand the mechanisms that drive metastasis and the process of tumorigenic transformation of normal epithelial tissue. It has been well established that the microenvironment is the key component that drives tumor growth and proliferation. Lately, mechanical changes to the microenvironment have been identified as key drivers of transformation and metastasis in a number of different cancers including breast cancer. However, currently, no 3D physiological model system of the normal mammary epithelial microenvironment (nMEME) exists that allows dynamic tuning of mechanical properties of its components and allows long-term phenotyping and genotyping during the tumorigenesis. To this end, we present a high throughput (HT) platform capable of introducing dynamic mechanical strain to an array of cell-laden hydrogels with tunable stiffnesses. The platform was built by inkjet printing different concentrations of cell-laden gelatin methacrylate (GelMA) microgels in alternate columns on an elastic composite membrane substrate made of poly(dimethylsiloxane) and polyacrylamide. The composite substrate printed with 3D cell-laden GelMA microarray was then subjected to 10% cyclic strain at 1 Hz for 3 days using a customized uniaxial stretcher device and the 3D cellular response was analyzed. In addition, we will also present our recent efforts in developing an HT nMEME platform where we modify the design of the substrate to introduce strain gradient and replace GelMA with mammary acini-laden interpenetrating (IPN) hydrogels, composed of alginate and basement membrane extract. The use of IPN allows for tuning of the mechanical properties of the hydrogels which similar to previous reports, we find alters the architecture and phenotype of the mammary acini. Combining the IPN platform with our stretchable HT platform will enable us in the future to perform combinatorial mechanical screening coupled with either high-resolution microscopy or single-cell transcriptomics to understand the underlying molecular mechanisms of cancer and other diseases associated with the pathophysiological mechanical conditions.

Identification of mechanical cues during early limb bud development using individual cell based modeling

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The initial stages of bone tissue development are marked by condensation of mesenchymal cells, which is critical for the resulting biological structure. Experiments reveal that mimicking both the chemical and mechanical environment of in vivo development typically increases in vitro engineered tissue quality [1]. Despite progress in defining underlying biochemical signaling pathways driving condensation, the effect and origin of mechanical cues are poorly understood. Thus, to accurately replicate the mechanical environment a better understanding of in vivo mechanical condensation is needed.

In this work, the developing chick limb is studied as a model-system for mesenchymal condensation. The mechanical cues that influence the condensation are a direct result of the mechanisms that drive outgrowth the limb bud. Therefor a better understanding of limb bud outgrowth is needed. We propose an individual cell-based model where mesenchymal cells are represented as individual agents. Interactions between the cells are modeled using two types of forces. Passive attraction-repulsion forces are used to represent cell-cell adhesion and cortical tension. The effect of active cell protrusions is represented as a directed migratory force. The ectoderm surrounding the mesenchyme functions as a boundary condition, and is modeled as a visco-elastic material. The computational model is used to investigate the mechanical regulation of limb bud elongation and condensation. Our simulations indicate that graded proliferation mediated by FGF and directed intercalations directed by a WNT3a gradient are needed to ensure limb bud elongation.

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Model System to Study Bone Mineralization in 3D

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Natural bone tissue is a complex, hierarchically structured composite material, mainly comprised of highly mineralised collagen and synthesised cells under stringent biological control. A thorough understanding of how this complex material is formed and remodelled in vivo is far from complete [1,2]. In the context of biomineralisation, unresolved challenges include understanding of the transport of mineral precursors and the role of mineral containing matrix vesicles; the role of mineral precursor phases such as amorphous calcium phosphate (ACP); the role of mineral-protein complexes and molecules modulating the formation hydroxyapatite. On the cellular level, the bone is created by osteoblasts, which lay down collagenous extracellular matrix and control mineralization of that matrix. The exact mechanism by which the mineralization takes place is still debated. The classical description of crystal nucleation and growth fails to fully define the complex process of in vivo biomineralization and several new hypotheses have been proposed. However, due to the complexity of living systems, these theories are challenging to test. Current lack of experimental methods and suitable model systems hampers progress in several important fields, including bone tissue engineering and the design of optimal scaffolds for bone regeneration. 3D spheroids are a promising platform to study cellular processes in an artificial human tissue environment in vitro, as well as develop new cell based tissue engineering therapies [3]. We show that spheroids made from Mesenchymal Stem Cells (MSCs) or primary human osteoblasts (hOBs) can be used as a model system to study biomineralisation of bone. MSCs spheroids were encapsulated in an inert alginate hydrogel (Fig 1A). Such constructs provided a convenient culture platform, allowed easy sample handling, as well as provided a geometrical constraint which contains cell produced ECM and extracellular vesicles (Fig 1B,C). Spheroids and produced ECM can be studied with optical and electron microscopy. We show that in thin sections, mineralised ECM can be detected and characterized using bright field transmission electron microscopy and scanning electron diffraction (Fig 1D-F). Overall, spheroids-based model systems are suitable for studies ECM deposition and mineralisation, processes that are not easily investigated in other 3D model systems.

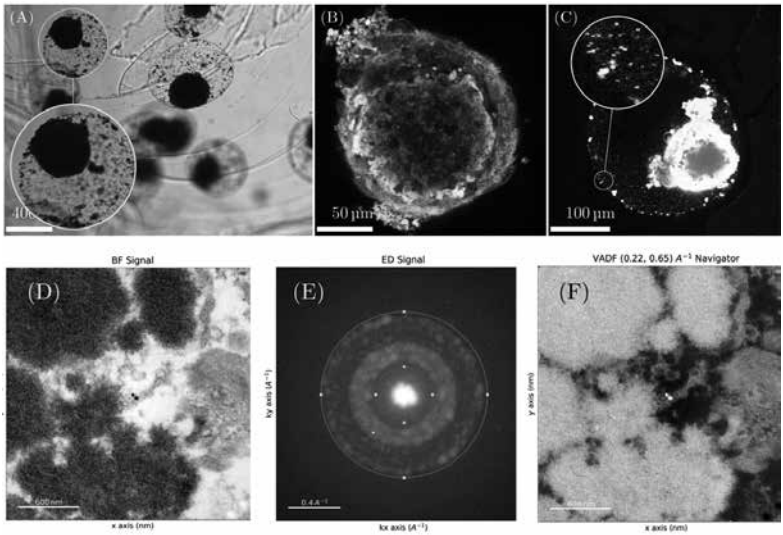

Figure 1: Phase contrast (A) and confocal laser scanning micrographs (B, C) of MSCs spheroids encapsulated in alginate hydrogels. Pockets formed around the spheroids are clearly visible. Membrane is labelled in green, cell nucleus in blue. Red signal is a reflection microscopy signal that originates from cells and the ECM. Scanning electron diffraction data for hOBs spheroid samples (D-F) that shows distribution of HAp within the ECM.

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Pulling on Springs: Exploring Feedback Mechanisms In Cellular Mechanosensation

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Cells are active systems that respond to their environment. In particular, it is becoming increasingly apparent that physical force and the mechanical properties of their microenvironment play a crucial role in determining cell behaviour. Gaining a better appreciation of how these systems function may have significant implications for both tissue engineering applications and in understanding how mechanical factors affect the development and progression of a broad range of diseases such as cancer, osteoporosis, or cardiomyopathies. Here, we explore cellular contractility as a mechanism for mechanosensation. We take a theoretical continuum-mechanics approach, modelling cellular contractility as an active stress. The mathematical model is analysed and solved using both analytical approaches (exploiting approximations and symmetry arguments) and Finite Element Methods. We use this model to investigate energy constraints as a potential feedback mechanism, guiding how the cell may regulate its contractility in response to different environmental cues. We focus our model in the context of the most common biophysical experiments for mechanosensation where cells adhere to flat substrates with known mechanical properties. In such experiments the shape, size and pattern of adhesions between the cell and substrate, and distribution of contractility throughout the cell have significant implications for cellular mechanosensation. Hence, we also investigate the implications of these factors on cellular contractility, subject to our energy constraints.

Cellular Synchronization through Unidirectional and Phase-Gated Signaling

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Synchronization is a temporal self-organization phenomenon where oscillators exchange information and adjust their pace to align their temporal dynamics. Presomitic mesoderm (PSM) is an embryonic tissue whose cells operate as genetic oscillators, and its functionality requires that the periodic gene expression is coordinated between the neighboring cells. Notch signaling is employed by these cells to efficiently communicate and achieve synchronization. However, the exact coupling mechanism describing the type of information that is exchanged between cells and the way they react to the incoming information remains unknown. Currently the Kuramoto model, which was originally developed as an approximation for weakly coupled oscillators, is widely used but it may conceal the true mechanism underlying PSM cell synchronization. Our approach was to develop a coupling mechanism that captures key properties of Notch signaling in the PSM, namely the unidirectionality of the signal from a Delta presenting to a Notch presenting cell as well as the periodic nature of this interaction. A cell declares the oscillation phase where it is when it presents the ligand Delta and can impose on a cell on a different phase, expressing the receptor Notch, to halt its pace and wait until they can cycle in unison. Mathematical formulation of the model enabled us to explore the conditions under which such a coupling scheme can be successful and make predictions about the behavior of the PSM cells as they get synchronized. We were able to show that upon coupling the response of the cells is by slowing down their pace and this happens only to the reacting cells while another group remains unaffected. Moreover, we could show that separate groups of cells synchronize always reaching a specific phase of the cycle. These phenomena contrast the predictions of the Kuramoto model and are compatible with our proposed mechanism. This mechanism is based on simple rules and offers not only a framework for further study of synchronization in PSM but can also inspire synthetic coupling mechanisms for engineered cellular systems.

Luminescent Human iPSC-derived Neurospheroids Enable Modelling of Neurotoxicity after Oxygen-glucose deprivation

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Despite the considerable impact of stroke on both the individual and on society, a neuroprotective therapy for stroke patients is missing. This is partially due to a current lack of a physiologically relevant human *in vitro* stroke model. To address this problem, we have developed luminescent human iPSC-derived neurospheroids that enable the real-time read-out of neural viability after ischemia-like conditions. We subjected 1- and 4-week-old neurospheroids to 6 hours of oxygen-glucose deprivation (OGD) and measured neurospheroid luminescence. At both stages, we detected a decrease in luminescent signal due to ensuing neurotoxicity, as confirmed by conventional LDH assay and flow cytometric viability analysis. Remarkably, 1-week-old, but not 4-week-old neurospheroids recovered from OGD-induced injury, as evidenced by their reduced but overall increasing luminescence over time. This underscores the need for more mature neurospheroids, more faithfully recapitulating the *in vivo* situation. Treatment of oxygen- and glucose-deprived neurospheroids with the pan-caspase inhibitor Z-VAD-FMK did not increase neural survival in a human-based 3D environment. Nevertheless, owing to its three-dimensional organisation and real-time viability reporting potential, the luminescent neurospheroids may become readily adopted in high-throughput screens aimed at identification of new therapeutic agents to treat acute ischemic stroke patients.

Understanding cell adhesion on the microscale, from contact guidance to substrate curvature

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In vivo, the extracellular matrix (ECM) provides cells with a plethora of physical, (bio)chemical and mechanical cues. To understand cell behavior in this complex environment, cells are often studied in ECM mimics containing isolated single cues *in vitro*. Although this approach gives useful insights, the environment is oversimplified, as the *in vivo* environment presents multiple cues at the same time. Unfortunately, recapitulating such a rich environment *in vitro* is challenging due to numerous technical challenges. Here we present a novel experimental platform that can be used to explore cell behavior to a combination of geometric and contact-dependent cues. This is obtained by applying microscale contact-guidance cues using UV-photopatterning of ECM proteins to a 2.5D cell culture chip containing a library of concave and convex curvatures mimicking tissue geometry (see Figure 1)[1].

Characterization of a range of protein patterns (collagen type I, fibronectin and gelatin) on various structured, polymeric materials (polydimethylsiloxane, polystyrene, polycarbonate) showed superior pattern homogeneity and resolution compared to conventional methods that can only be used on planar substrates. Next, we found that multiple adherent cell types such as human bone marrow stromal cells, human myofibroblasts and umbilical vein endothelial cells showed a curvature- and contact guidance dependent orientation behavior when cultured on patterned semi-cylinders using the multi-cue platform. On concave, patterned structures, myofibroblasts and endothelial cells predominantly oriented along the direction of the protein pattern, obeying the principle of contact guidance. In contrast, the majority of human myofibroblasts on micropatterned convex substrates with higher curvatures ($\kappa \geq 1/1000$ μm-1) reorient in the longitudinal direction of the 2.5D semi-cylinders, indicating that cells followed the structural cues from the substrate curvature instead. These findings exemplify the potential of this approach for systematic investigation of cellular responses to multiple microenvironmental cues. Additionally, we show the possibility to examine the effect of these environmental cues on long-term ECM production by fibroblasts, to evaluate the possibility of steering tissue growth using biophysical extracellular cues in the context of tissue engineering.

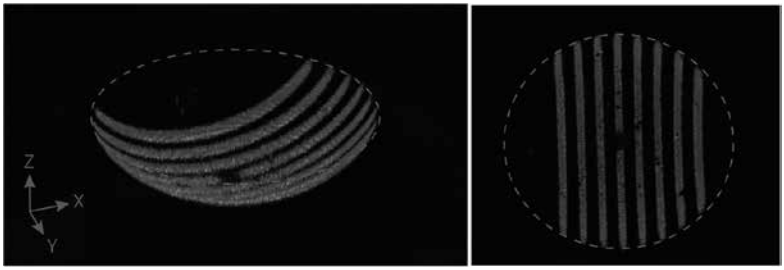

Figure 1: 3-Dimensional (left) and top down (right) visualization of a spherical pit (κ = 1/250 µm-1) containing a line pattern (width: 20 µm, gaps: 20 µm) made of gelatin-fluorescin (green)

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Epithelial dynamics and rheological properties in the framework of the jamming phase transition

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In many biological processes such as wound healing, embryonic morphogenesis, and pathological processes like cancer metastasis, a fluid-like to solid-like (or vice-versa) phase transition occurs, known as the (un)jamming transition. Vertex and voronoi models have provided crucial insights into how this transition is controlled. They show how the jamming transition emerges from the interactions between a large number of cells dependent on the balance between adhesion and cortical tension[1], and cell active motility[2]. However, Vertex models describe confluent tissue whereas tissue often contains pores. Therefore, Kim et al.[3] developed a model that considers both porous and confluent tissues, and showed that cell-cell adhesion favours fluidisation in the confluent state, as predicted by vertex models, while cell-cell adhesion promotes solidification in porous tissue.

The majority of these computational models represent tissue as 2D. However, cell and tissue shape changes during morphogenesis or metastasis are essentially 3D processes. As a solution, we developed a deformable cell model (DCM), where the 3D shape of each individual cell is accurately represented, and cell-cell interactions are captured by explicit interaction forces. This way, the DCM naturally captures changes in cell shape (fig. 1a), and describes the behaviour and properties of both porous and confluent tissue[4].

In this work, we study the jamming phase transition in an epithelial layer in function of cell-cell adhesion, cell motility, and cell density. Particularly, we study cell dynamics and tissue visco-elastic properties (fig 1). We show that cell motility induces tissue fluidisation, and cell-cell adhesion fluidises tissue in the confluent regime, while it promotes solidfication in porous tissue.

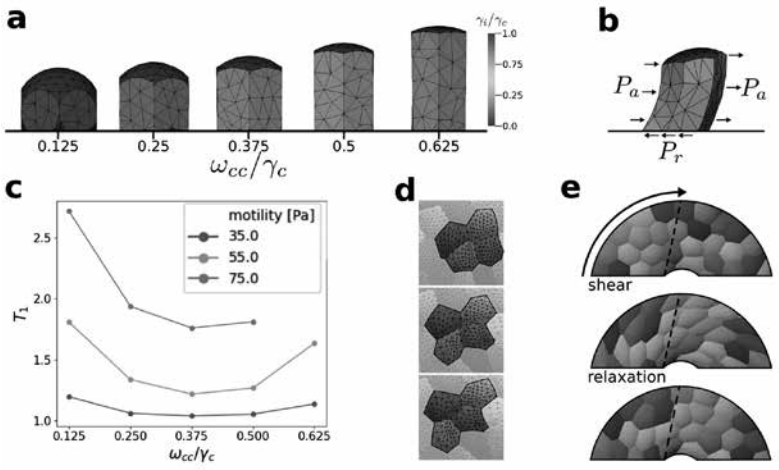

Figure 1: a) Changes in adhesive-to-cortical tension ratio affect cell shape. b) Cell motility is modeled by a protusive and retracting pressure P_a at cell' leading and trailing end, and traction P_r at the cell basal side. c) Cell rearrangement rate T^1 in function of cell motility and cell-cell adhesion. d) example of a T1-transition. e) shear step followed by tissue relaxation.

Acknowledgments: This research received funding from the Research Foundation of Flanders, Grant Nr: 11D9921N.

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“Bioprinted hydrogel-based gut-on-chip model”

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Intestinal barriers play a key role in the uptake of nutrients and the protection against pathogens. Their multicellular and three-dimensional architecture, where stromal cells interact with the epithelium, are essential for their proper function. However, in vitro intestinal models often consist in flat cell monolayers in static conditions, lacking important cues from peristaltic flow that are also required for barrier functioning. In this context, gut-on-chip models have been developed to overcome these limitations by combining microfluidic flow with cell culture[1]. But such dynamic models still not recapitulate the complex cell-ECM interactions nor the compartmentalized 3D structure of the intestinal mucosa. To accurately recapitulate these in vivo-like conditions, hydrogels have been used as suitable cell substrates, as they can be mechanically tuned to match the properties of the tissue and support barrier formation and cell embedding[2,3]. Considering these benefits, hydrogels have been integrated within microfluidic devices to increase the physiological relevance of gut-on-chips[4].

In this work, the integration of a biomimetic hydrogel channel within a microfluidic device is proposed to develop a novel gut-on-chip model. This model consists in a perfusable hydrogel channel with lateral villi and crypt-like structures that reproduces the spatial configuration of the intestinal lumen (Figure 1A). Two lateral channels of the chip are defined to better provide nutrient and oxygen supplies for the different intestinal cellular compartments (Figure 1B). A state-of-the-art light-based 3D bioprinting technology was used to fabricate the hydrogel, which were subsequently integrated into a microfluidic system manufactured by rapid prototyping. The photocroslinkable pre-polymer solutions contain a synthetic polymer, poly(ethylene glycol) diacrylate (PEGDA), and a natural one, gelatin methacryloyl (GelMA), along with a photoinitiator, and a photoabsorber to better control the hydrogel formation. Such hydrogels allowed the embedding of fibroblasts during the printing process to mimic the stromal compartment of the intestinal tissue and the growth of intestinal epithelial cells. Preliminary results have shown high cell viability of embedded 3T3 fibroblasts under perfusion for 4 days and the formation of an epithelial monolayer on the tested hydrogel formulation. It is expected that this novel set up, which includes the architecture, the relevant cellular population and the soft mechanical properties of the intestinal mucosa can better represent the functionality of the in vivo tissue.

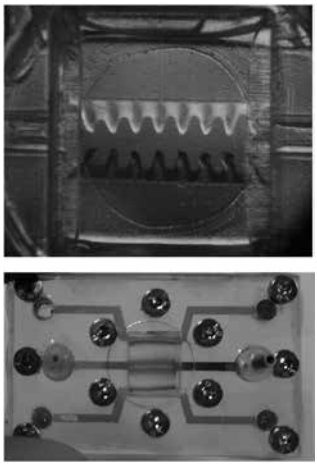

Figure 1 (A) Image of the villi-laden hydrogel channel. (B) Image of the tri-channel microfluidic chip with the integrated hydrogel.

Acknowledgement: Marie Skodowska-Curie DOC-FAM program (grant agreement No 754397)

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Towards bone-remodeling-on-a-chip

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Bone remodeling is the combined process of bone resorption by osteoclasts and bone formation by osteoblasts. This process is regulated by mechanosensing osteocytes. It is the most fundamental physiological process that defines living bone. An imbalance in this process can cause metabolic bone diseases such as osteoporosis. Currently, no complete *in vitro* bone remodeling model is available. Such models could have the potential to increase our knowledge on the physiological and pathological processes underling bone remodeling and could potentially improve drug development processes. Bone-on-a-chip technology has the great potential to advance bone research, allowing for the study of low cell numbers in high temporal and/or spatial resolution. In this study, microfluidic chip technology is used to create a bone remodeling model that contains the interaction between osteoblasts, osteocytes and osteoclasts.

A bone-on-a-chip microfluidic device that facilitates three-dimensional (3D) *in vitro* bone-like tissue formation was developed. A polydimethylsiloxane (PDMS) microfluidic device was fabricated by means of photo- and soft-lithography. The device contained rectangular-shaped cell culture channels that were coated with fibronectin and seeded with human bone marrow derived mesenchymal stromal cells (MSCs). The MSCs were dynamically cultured for a period of 21 days by applying medium flow, resulting in shear stresses acting on the cells. Osteogenic medium was used to differentiate the MSCs towards the osteogenic lineage.

Time-lapse brightfield imaging revealed self-assembly into 3D constructs within the channel. At the end of the 21-day culture period, brightfield imaging visualized the deposition of calcium (Alizarin Red staining) and collagen (Picrosirius Red staining) in the extracellular matrix produced by the cells. Confocal microscopy revealed the formation of 3D bone-like struts through self-assembly. Immunohistochemical staining confirmed the formation of collagen type 1 and revealed the expression of the markers osteopontin and DMP-1, confirming the differentiation of the MSCs into the osteogenic lineage (Figure 1).

Overall, the results revealed mineralized bone-like struts that match the size and shape of human bone trabeculae. With this, the developed bone-on-a-chip microfluidic device showed the first step towards a 3D *in vitro* bone remodeling model, exhibiting 3D bone-like tissue formation. In future research, osteoclasts will be added to add bone resorption.

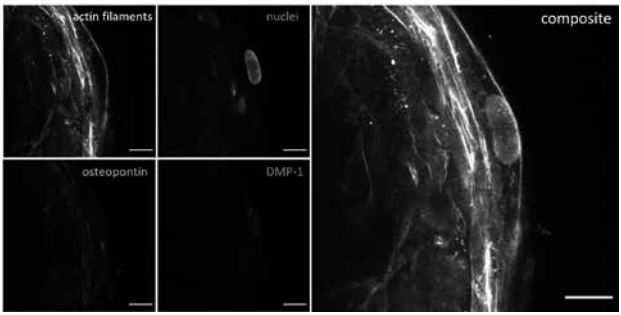

Figure 1 Microscopic image of a cell on the outside of a bone-like strut, after 21 days of culture inside the microfluidic chip. The cell expresses the osteoblast marker osteopontin (yellow) in the actin skeleton (phalloidin stain - white) and the osteocyte marker DMP-1 (green) in the nucleus (cyan). Scale bar is 20 µm.

The interplay between lumen and tissue branching in pancreas morphogenesis

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Branching morphogenesis is a phenomenon exemplified in numerous organs (i.e. the lung, kidney, mammary gland, and pancreas) where the developing epithelium directionally extends into the neighboring mesenchyme, forming tree-like topologies. In parallel to branching, the lumen evolves into a ductal-network that imitates the tree-like epithelium. Although many studies have reported the molecular and biophysical perspectives of branching and lumen morphogenesis, not much has been revealed on the interplay between lumen and branching.

To overcome the inaccessible and invasive challenges of biophysical experiment on in vivo organs, we use *in vitro* pancreatic organoids (hereby called pan-organoids) embedded in 3D Matrigel which also branches and forms a ductal network. We find that pan-organoids initially grow spherical in shape and transitions into branched pan-organoids. When we perturb lumen growth during the transition we observed that the pan-organoids do not branch, while preventing branching via compression (with 2MDa dextran) does not impact lumenogenesis. Furthermore, we observe that pan-organoid lumen occupancy and inter-lumen connectivity increases before branching commences.

Although more experimentation is required, these preliminary observations allow us to hypothesize that lumenogenesis promotes branching by overcoming compaction forces of the spherical pan-organoids. However, whether or not this is achieved by changing other tissue-scale mechanical processes (i.e. mechanical buckling, clefting, etc.) has yet to be confirmed. Further biophysical and biological assays will be carried out, to dissect the role of the mentioned observations in the mechanics of pancreas branching morphogenesis.

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ENERGY

- Turn off lab equipment and switch off computers/screens and light.
- Shut the Sash of the fume hood.
- Reduce the frequency and duration of opening the doors in freezer/ultrafreezers.
- Keep the freezer organized so you can find your samples quickly.
- Throw away all the frozen samples that you are not going to use anymore
- Avoid using air conditioning or heat.

WATER

- Limit the use of distilled and deionized water.
- Reduce single-pass cooling.
- Use auto service autoclaves efficiently.
- Use tap water strainers
- Report leaks promptly.

REDUCE, REUSE AND RECYCLE WASTE

- Label and dispose hazardous waste according to PCB guidelines.
- Reduce the use of disposable plastics by considering the use of glassware.
- Reduce the volume of disposable plastic purchased.
- Find ways to treat and reuse some disposable plastics items.
- Reduce the use of paper. Be conscious of printing practices.
- Separate clean plastic and paper and take them to the yellow and blue containers respectively to recycle.

PURCHASING

- Consolidate orders.
- Reduce the quantities of purchased chemicals.
- Purchase green.

TRAVEL AND COMMUTING

- Commute to work in a sustainable way.
- Reduce flight frequency: travel by train if possible.

EMBL

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