
2020
EMBL · IBEC Winter Conference
ENGINEERING
MULTICELLULAR
SYSTEMS
EMBL
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Institute for Bioengineering of Catalonia

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Myofibroblast-derived mechanical cues direct Epithelial Migration

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Understanding how intestinal migration occurs is crucial to comprehend many aspects of the intestinal physiology and pathology. Under homeostasis, intestinal cells originate at the crypt bottom from where migrate up to the tip-villi while differentiating in all different intestinal cell types. Some pathological conditions cause the disruption of the intestinal epithelium exposing the lamina propria immune system to the lumen's content and causing a detrimental inflammatory condition. A fast and efficient reinstatement of the epithelial barrier integrity, including epithelial proliferation and migration, is crucial to ameliorate the disease.

Intestinal subepithelial myofibroblasts (ISEMF) are a unique group of smooth-muscle-like fibroblast located underneath the intestinal epithelium forming a contractile cellular network via gap junctions. It has been shown that ISEMF play an important role in organogenesis, tumorigenesis, inflammation and repair through the secretion of cytokines, chemokines, growth factors as well as extracellular matrix. However, whether the contractile capacities of ISEMF contribute to epithelial migration has not been explored yet. Here we have designed an experimental set-up to analyse the ISEMF-derived mechanical contribution to epithelial movement.

We have engineered a 3D intestinal mucosal-like model including the basal membrane, the lamina propria and the epithelial compartments in a spatial-relevant manner. Specifically, on top of a thin layer of Matrigel® we have successfully generated a two-layer co-culture of primary mouse derived intestinal epithelial cells and ISEMF. Our results show that both the co-culture and the use of ISEMF-conditioned medium (ISEMF-CM) boosts epithelial proliferation and the spreading of the epithelial monolayer, indicating a paracrine biochemical effect of ISEMF on intestinal epithelial growth. To analyse the effect of ISEMF on epithelial cell migration we performed wound healing assays on our intestinal mucosa-like model. We demonstrated that when ISEMF are present, epithelial migration occurs in a more gap-oriented and efficient manner than in the absence of ISEMF or with the ISEMF-CM, indicating a physical effect of the ISEMF on the epithelial restoration. In agreement with this, our immunostaining analysis revealed that α-smooth muscle actin (α-SMA) positive ISEMF accumulate underneath the healing area in a clearly aligned disposition with the migration direction. Furthermore, we observed that a ribbon of those aligned ISEMF precedes the migration front suggesting that ISEMF movement might be directing epithelial migration.

Our results demonstrate that ISEMF contribute to epithelial restoration upon tissue damage by physically guiding epithelial migration to heal the wounded area.

Efficient Generation of Human Pluripotent Stem Cell-derived Kidney Constructs by 3D Bioprinting of cell-laden Kidney Specific Bioinks.

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Three-dimensional (3D) bioprinting technology provides a promising scenario for the generation of bioinspired tissue constructs with potential for regenerative medicine applications. However, the seek for the proper cell source and biocompatible materials -termed as bioinks- for 3D bioprinting remains still an open question in the field. Here, we describe the fabrication of 3D printed renal constructs using cell-laden kidney specific bioinks. These can mimic the extracellular matrix of the native kidney and are fabricated by decellularization of kidney tissue. Using our previously established differentiation methodology, intermediate mesoderm progenitors are generated from human pluripotent stem cells and combined with kidney bioinks for 3D bioprinting. Printed renal constructs show the development of organized nephron-like structures containing podocyte-like cells, proximal/distal tubuli as well as stromal and endothelial compartments. This 3D bioprinting approach provides a robust methodology to fabricate 3D kidney-like constructs, opening the door to future functional genetic and disease modeling studies.

The role of intermediate filaments in stress resistance in 3D epithelial structures

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The epithelium is a thin cellular layer that covers inner and outer surfaces in mammalian bodies. Thereby, it constantly has to withstand and exert mechanical stresses. The cells within the epithelium must be able to both undergo large deformations and resist large forces to avoid mechanical failure of the tissue. It has been hypothesised that a central role for retaining the structural integrity of cells exposed to large deformations is played by intermediate filaments (IFs). IFs are one of the most abundant cytoskeletal components and they feature unique physical properties at large strains. However, the contribution of IFs to the physical properties of cells and the generation of inter- and intracellular remains elusive.

We aim to conclusively test the hypothesis of IFs acting as a “cellular safety belt”. For this purpose, we developed a model system for stretching cells within a three-dimensional epithelial monolayer. With this system, we imaged IFs and other cytoskeletal components in MDCK cells with super resolution microscopy and measured tissue tension with the help of traction force microscopy. As a result, we formulated a qualitative model to explain how the intercellular IF-networks gets reorganized when cells are exposed to large strains. Disrupting desmosomes by removing the desmosomal keratin-binding domain revealed, that desmosomes are crucial for the structural organization of both intra- and intercellular IF networks. Together, these findings establish a mechanism by which IFs contribute to the mechanical resistance of epithelial structures.

Natural variation in 3D tissue organization

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Natural variation in tissue development is essential for evolution. However, uncontrolled variation can be detrimental to tissue function. Here, we study the stochasticity of 3D cysts growth to understand the causes and consequences of Natural variation in tissue development. To this aim, we use a bioimage processing method to quantify the variation in tissue architecture in 3D cell cultures. We analyse and classify the 3D cyst-like structures following different geometrical and morphological parameters. In each category of cyst morphology, we quantify different features describing the 3D packing of the curved epithelial organization such us the number of cells and neighbours, volume, surface ratio and presence/abundance of scutoids. We will show the limits of the parameters that characterize the physiological tissue packing. These results capture the variation allowed in the tissue in terms of tissue packing and give us an idea of the limits of the Natural variation at the level of epithelial organization. We think that our approach can serve also as a reference to study how geometrical and biophysical constrains drive self-organization of tissues.

Steps towards Mimicking the Lymph Nodes to Enhance T Cell Expansion

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Adoptive cell therapy, which consists of the extraction, manipulation, and administration of ex vivo generated autologous T cells to patients, is an emerging alternative to regular procedures in cancer treatment.[1] Nevertheless, these personalized treatments require laborious and expensive laboratory procedures that should be alleviated to enable their incorporation into the clinics. With the objective to improve the ex vivo expansion of large amounts of specific T cells, we used 2D nanostructured substrates as artificial antigen presenting cells[2,3] as well as 3D scaffolds to mimic the secondary lymphoid organs[4]. The activation, proliferation, and differentiation of human primary T cells were analyzed showing an increase in cell proliferation of relevant phenotypes compared to standard suspension systems. Moreover, new synthetic biomaterials are being investigated with the same purpose.

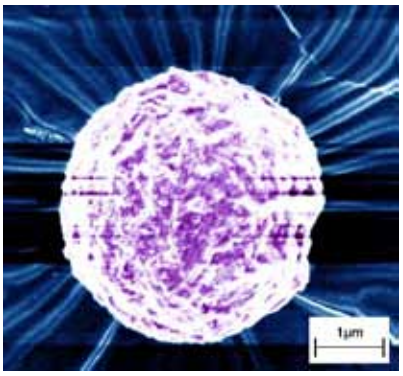

Figure 1. Primary human CD4+ T cell on a nanostructured substrate.

(1) Fesnak, A. D.; June, C. H.; Levine, B. L. Engineered T Cells: The Promise and Challenges of Cancer Immunotherapy. *Nat. Rev. Cancer* **2016**, *16*, 566-581.

(2) Guasch, J.; Hoffmann, M.; Diemer, J.; Riahinezhad, H.; Neubauer, S.; Kessler, H.; Spatz, J. P. Combining Adhesive Nanostructured Surfaces and Costimulatory Signals to Increase T Cell Activation. *Nano Lett.* **2018**, *18*, 5899-5904.

(3) Guasch, J.; Muth, C. A.; Diemer, J.; Riahinezhad, H.; Spatz, J. P. Integrin-Assisted T-Cell Activation on Nanostructured Hydrogels. *Nano Lett.* **2017**, *17*, 6110-6116.

(4) Pérez del Río, E.; Martinez Miguel, M.; Veciana, J.; Ratera, I.; Guasch, J. Artificial 3D Culture Systems for T Cell Expansion. *ACS Omega* **2018**, *3*, 5273-5280.

Elastin-like recombinamers as poly(vinylidene fluoride) membrane coatings, a new approach for mesenchymal stem cell culture and differentiation

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Bone inherent piezoelectricity, produced by collagen fibers that form its extracellular matrix, is a key factor regulating bone growth. As occurs in native tissue, our research is based on the hypothesis that electrical stimulation can influence bone marrow mesenchymal stem cell differentiation towards the osteogenic lineage. The use of piezoelectric polymers in regenerative medicine has increased in the last decades, proving its suitability for tissue engineering applications.

As a first approach, poly (vinylidene fluoride) (PVDF) has been used to manufacture membranes by non-solvent induced phase separation (NIPS), using ethanol at 25ºC as non-solvent. This technique allows the production of skin-less, porous membranes with around 80% of β-phase, its most electroactive crystalline phase.

PVDF is characterized by its hydrophobicity, which hinders cell adhesion and proliferation. This feature requires a coating to improve cell culture conditions. Layer by layer (LbL) has been used for the deposition of elastin-like recombinamers (ELRS), containing RGD sequences, to promote cell adhesion and proliferation. Modified ELRs containing azide and cyclooctine groups have been used as polyelectrolytes enabling the stabilization of the layers by interchain covalent crosslinking. Homogeneous coating deposition has been confirmed by field emission scanning electron microscopy and Fourier-transformed infrared spectroscopy. A remarkable reduction of the contact angle, from 133.2 º to 84.4º, proves a surface hydrophobicity reduction.

To confirm ELRs potential, porcine bone marrow mesenchymal stem cells (pBM-MSCs) were used to study cell adhesion and proliferation in fibronectin adsorbed and ELRs coated membranes at 1, 3 and 7 days. After 24h, cells adhered in every condition, showing a well-developed cytoskeleton. Proliferation study revealed that even if cells are able to adhere, they are not able to proliferate in fibronectin coated PVDF membranes. However, ELRs coated membranes provided a good initial adhesion and enhanced proliferation after 7 days.

These findings lead to new possibilities for electrical stimulation of BM-MSCs on PVDF substrates to study cell differentiation towards the osteogenic linage.

To which extent is digit patterning a reaction-diffusion system?

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Digit patterning is a representative example of a periodic structure in development. Previous studies have shown that reaction–diffusion (Turing) system, in which diffusible activator and inhibitor interact, is responsible for generating such spatial information. The model identifies Bmp, Sox9, and Wnt as components of reaction-diffusion model.

This research focuses on the dynamics of the digit pattern shape changes, upon perturbation of the mouse embryonic limb culture and micromass culture. The goal of the research is to find out to which extent digit patterning is a pure reaction-diffusion system. I analyse digit patterning dynamics in multiple timepoints of 1) Wild type embryonic limb, 2) in limbs with perturbed Turing network consisting of Bmp2, Sox9 and Wnt, and 3) limbs with perturbed tissue mechanics components such as integrin and Notch. I recapitulate all experimental results in a computational model, and characterise the network involved.

Preliminary results suggest that plasticity, the measure of how flexible the cell fate is, largely determines the influence of Turing system on digit patterning. We conclude that in younger, plastic limbs, reaction-diffusion mechanism accounts for highly dynamic reorganization of digits, while less plastic cells are directed by tissue movements.

Fabrication of biphasic microstructures for viscoelastic cell culture substrates

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Abstract: **(378 words)**

In order to study how cells respond to mechanics *in vitro*, it is now know that it is important not only to tune the elastic properties of the substrates but also the viscous ones (Charrier et al. 2018). It has been shown recently that adapting the viscoelastic properties of the substrate to that of the cells is also important, especially in spreading (Gong et al. 2018). Great efforts have been made to both tune the stiffness of the culture substrates and microstructure their surface topology to control constraints boundaries, but usually, “stiffness control” only means a control of the elastic properties (storage modulus) of the material. Dissipative viscous properties (loss modulus) are more difficult to control and tune, although efforts are being made to incorporate un-crosslinked viscous hydrogels inside a crosslinked matrix (Charrier et al. 2018) or with liquids of different viscosities inside an elastic matrix (Cacopardo et al. 2019). However, microstructuring such constructs results much more difficult and less reproducible due to the difficult manipulation of soft gels. This structuring of mechanically tunable materials is also important as the shape (Park et al. 2009), dimensions (Chen et al. 2018) and boundary conditions, including thickness of the substrates (Evans and Gentleman 2014), are key parameters to control.

In this work, we propose a method to use high-resolution two-photon polymerization (2PP) of photoresist with different viscosities to fabricate biphasic microstructures with an elastic (crosslinked) shell with a controlled thickness encapsulating a core of un-crosslinked photoresist. Thanks to a 2PP Photonic Professional GT system (Nanoscribe GmbH), it was possible to control the thickness of the top surface from 2 µm up to 20 µm and different photoresists were used and characterized. After measuring the mechanical properties of the structures using a Femto Tool FT-MTA-03 nanoindenter in the relaxation mode, we extracted the mechanical properties and found that the superficial viscoelastic properties of these highly reproducible cell culture substrates were strongly dependent on the 3D dimensions of the constructs, especially the upper shell thickness and the viscosity of the pristine photoresist. Preliminary cellular culture with 3T3 fibroblasts and epithelial cells is showing different mechanical responses after a few hours, demonstrating the high-resolution biphasic microstructures may be used for mechanobiology assays, allowing both control of the substrates viscoelastic properties and shapes/dimensions.

References:

Cacopardo, Ludovica, Nicole Guazzelli, Roberta Nossa, Giorgio Mattei, and Arti Ahluwalia. 2019. “Engineering Hydrogel Viscoelasticity.” *Journal of the Mechanical Behavior of Biomedical Materials* 89 (January): 162–67.

Charrier, Elisabeth E., Katarzyna Pogoda, Rebecca G. Wells, and Paul A. Janmey. 2018. “Control of Cell Morphology and Differentiation by Substrates with Independently Tunable Elasticity and Viscous Dissipation.” *Nature Communications* 9 (1): 449.

Chen, Tianchi, Thuan Beng Saw, René-Marc Mège, and Benoit Ladoux. 2018. “Mechanical Forces in Cell Monolayers.” *Journal of Cell Science* 131 (24). https://doi.org/10.1242/jcs.218156.

Evans, Nicholas D., and Eileen Gentleman. 2014. “The Role of Material Structure and Mechanical Properties in Cell–matrix Interactions.” *Journal of Materials Chemistry B*. https://doi.org/10.1039/c3tb21604g.

Gong, Ze, Spencer E. Szczesny, Steven R. Caliari, Elisabeth E. Charrier, Ovijit Chaudhuri, Xuan Cao, Yuan Lin, et al. 2018. “Matching Material and Cellular Timescales Maximizes Cell Spreading on Viscoelastic Substrates.” *Proceedings of the National Academy of Sciences of the United States of America* 115 (12): E2686–95.

Park, Joong Yull, Dae Ho Lee, Eun Joong Lee, and Sang-Hoon Lee. 2009. “Study of Cellular Behaviors on Concave and Convex Microstructures Fabricated from Elastic PDMS Membranes.” *Lab on a Chip* 9 (14): 2043–49.

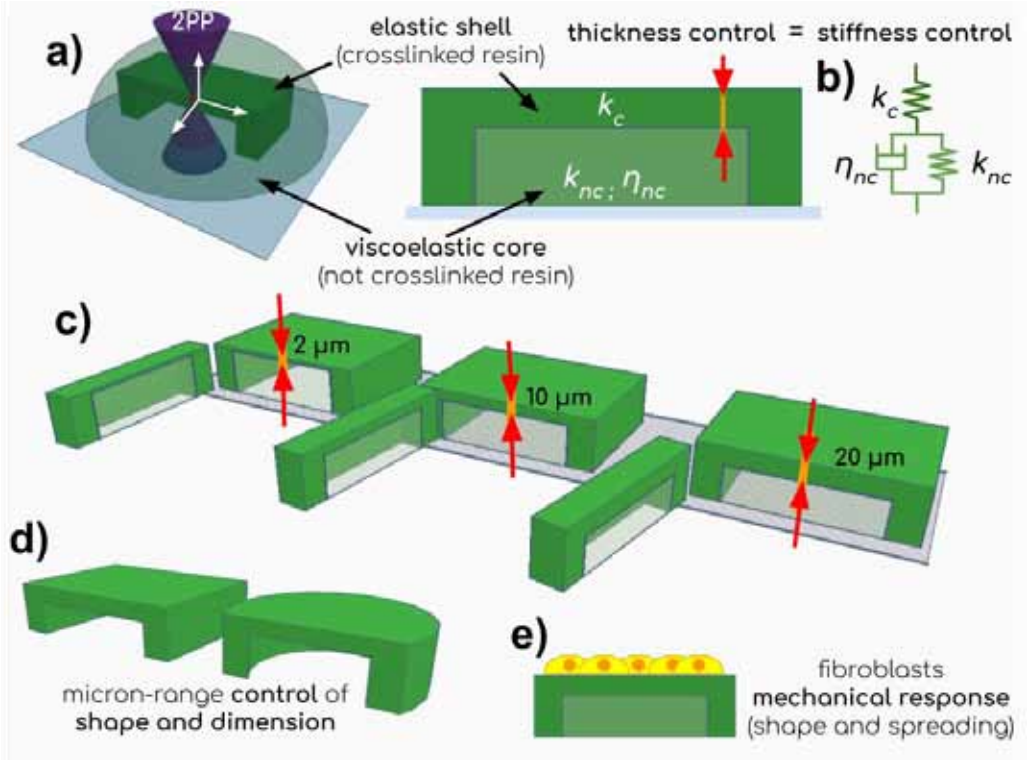

Figure 1: Diagram of the fabrication of microstructure with tunable viscoelastic properties for mechanobiology assays. a) 2PP fabrication of biphasic microstructures. b) Mechanical model of the structures. c) Fabricated structures of different thickness and d) shape/dimensions control. e) Schematic of the cell culture tests on biphasic microstructures.

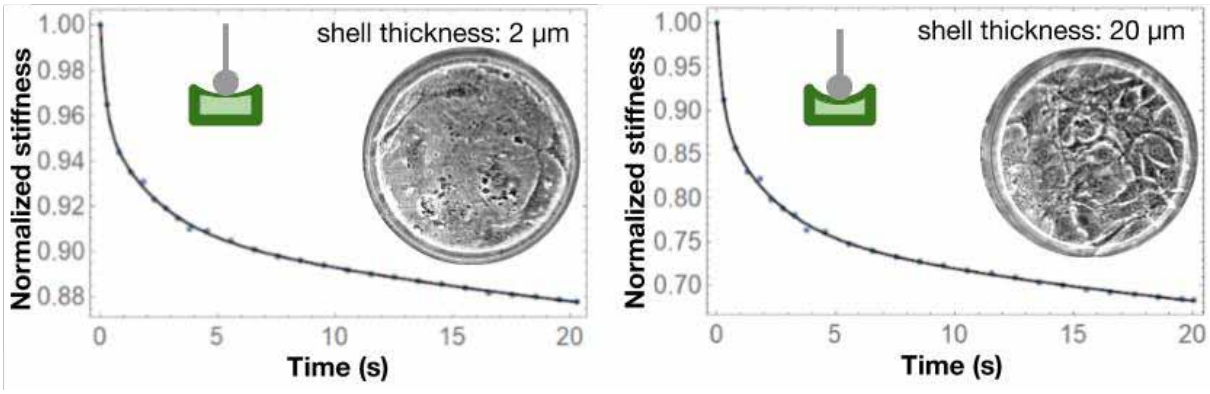

Figure 2: Relaxation tests of two similar circular structures made of the same material (IP-S proprietary resist) and preliminary cell culture results (3T3 after 24h). Fitting was achieved using general Maxwell model to obtain relevant mechanical properties.

Impact of the ECM stiffness and geometry in the collective dynamics of epithelial and mesenchymal lung cells

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Abstract: **(378 words)**

Biological cells suffer from a wide range of mechanical stimuli that strongly influence their phenotype, behavior, fate and function not only at a single cell level but also at a collective level. Some of those stimuli originate from the local intrinsic properties of the materials that constitute the extracellular matrix, such as stiffness, geometry and chemical composition. In addition to this, cell microenvironment is in constant dynamic activity since it is constantly modified by the cells in order to maintain the cell homeostasis or respond to external forces and any disturbance may induce a rapid response of cells. Recently, efforts are being made to understand how alterations in collective behavior may be associated with pathological processes such as cancer metastasis and fibrosis. It is indeed necessary to study and characterize how topological and mechanical intrinsic properties of cell microenvironments influence the collective dynamics such as long range directional order, self-organization and migration patterns of cells that form organs and tissues.

In order to elucidate the impact of the stiffness and geometry of the substrates onto which cells are sitting on the collective dynamics, we prepared polyacrylamide hydrogel substrates with different stiffnesses and cultured alveolar epithelial cells (A549) that were compared with normal human lung fibroblast (NHLF). Then, the system was disturbed by adding the transforming growth factor beta (TGFβ) known to provoke epithelial-mesenchymal transition in A549. The morphology and phenotype of the cells were analyzed by immunofluorescence and western blot assays to verify the transition *in vitro*. Then, collective dynamics of the cultured cells was studied by modelling the confluent epithelium and fibroblasts monolayers as an active nematic liquid crystal that presents a long range directional order. Interestingly, we observed a decrease of the activity in the cells seeded on stiffer hydrogels together with the emergence of topological defects, whereas in soft gels the activity increased, interfering with the collective nematic order. Finally, using soft-lithography, we fabricated alveoli-shaped microstructures in polyacrylamide hydrogels in order to study the influence of geometric constraints on the collective dynamics and cells phenotype. In this case, we also observed an heterogeneous mechanical response of the cell layer associated to the stiffness and geometric constraints of the substrates. These results provide a deeper insight towards the understanding of the cellular response to the combined influence of several external mechanical stimuli in different pulmonary pathologies associated with an increase in lung tissue stiffness and overexpression of TGFβ.

Embryonic Forces: Illuminated by Microlasers

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Embryonic development is a burgeoning area of research due to recent developments in molecular biology and imaging technologies. With this it has been shown that the cellular mechanics of the embryo is important for such events as tissue morphgenesis[1]. Recent advances have shown the viability of using confocal imagery coupled with dye doped oil droplets as metric for measuring stresses within tissue[2–4]. Presented here is a deformable microlaser[5] that can sense mechanical forces within the embryo. The microlaser constitutes a high refractive index (n = 1.67) polyphenyl ether-based oil that has been doped with the laser dye Nile Red. A mode splitting of the laser spectrum is observed when a geometric deformation of the microlaser occurs, caused by the impacting forces of neighboring cells. Assuming a spheroidal geometry, the semi-major and semi-minor axes lengths are obtained by optically modelling the laser spectrum which can be directly related to an applied force using Laplace's law. The use of a laser allows extremely high acquisition rates over confocal imagery with a force resolution of ~pN. Further, the depth at which data can be extracted within a tissue exceeds that of other standard imaging techniques such as confocal or two-photon microscopy[6]. We apply the microlaser to track forces produced by the pole cells of a *Drosophila Melanogaster* embryo. These measurements are combined with 3D confocal images to correlate the stages of development with the mechanical forces. The developmental cycle of the pole cells allows a defined and clear system to demonstrate the versatility and applicability of the microlasers as extracellular force sensors.

1. Bailles, A. *et al.* Genetic induction and mechanochemical propagation of a morphogenetic wave. *Nature* **572**, (2019).
2. Campàs, O. *et al.* Quantifying cell-generated mechanical forces within living embryonic tissues. *Nat. Methods* **11**, 183–189 (2014).
3. Serwane, F. *et al.* In vivo quantification of spatially varying mechanical properties in developing tissues. *Nat. Methods* **14**, 181–186 (2017).
4. Mongera, A. *et al.* A fluid-to-solid jamming transition underlies vertebrate body axis elongation. *Nature* **561**, (2018).
5. Humar, M. & Yun, S. H. Intracellular microlasers. *Nat. Photonics* **9**, 572–576 (2015).
6. Schubert, M. *et al.* Monitoring contractility in single cardiomyocytes and whole hearts with bio- integrated microlasers. *bioRxiv* (2019).

A surface geometry of living things induces a topological change of a chemical traveling wave during morphogenesis

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A wide variety of shapes appear in living things. These shapes are reproducibly formed by cells self-organizing using intercellular communications through chemical and mechanical signals. In recent years, these formation processes have become observable in real time and in three dimensions. The observations of these formation processes suggests that the cells communicate by traveling waves of chemical signals. In this case, the changes of shapes during the processes are expected to be sequentially fed back to the cells communication because the geometries of the shapes change the traveling direction of the waves. Especially, discontinuous changes of the traveling waves associated with the shape changes, such as topological changes of the waves, may seriously affect the formation processes. Therefore, the clarification of geometric effects of shapes on the waves is an important issue for understanding of the reproducible formation processes. In this study, we first demonstrated that the curved surface geometry induces a topological change through bending, collision, and splitting of a planar stable wavefront by numerically solving an excitable reaction–diffusion equation on a bell-shaped surface. We determined two necessary conditions for inducing a topological change: the characteristic length of the curved surface (i.e., the height of the bell-shaped structure) should be greater than the width of the wave, and the ratio of the height to the width of the bell shape should be greater than a threshold. As for the geometric effect of the latter, we found that a bifurcation of the geodesics on the curved surface provides the alternative minimal paths of the wavefront, which circumvent the surface region with a high local curvature, thereby resulting in the topological change. These conditions imply that the topological change of the wavefront can be predicted on the basis of the curved surfaces, whose structures are larger than the wave width. This prediction promises to provide an engineering method for designing the shape of organoids during three-dimensional culture.

Studying developmental dynamics and pattern emergence in human cerebral organoids

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Human cerebral organoids have been established as a system to study foetal neocortex development and brain disorders *in vitro*. Starting as a spherical embryoid body with neuroectoderm, the organoids self-pattern by forming several buds of neuroepithelium which mature into different brain regions and generate a mixture of cell types in a single organoid. Large ventricular zones showing dorsal and the ventral forebrain patterning with discrete cell populations such as progenitor neurons, radial glia cells and signaling centers are seen. The spatiotemporal cellular dynamics associated with transitioning of a neuroepithelium bud into dorsal and ventral forebrain regions are not clear. Consequently, methods to generate controlled spatial identity and stereotypic tissue morphology have also not been established. We want to study and identify the link between tissue morphogenesis and spatial pattern establishment during cerebral organoid development by comparing cell behaviours and cell types specification between ventricles in an organoid and between different organoids. For this, we are establishing a lightsheet imaging and image analysis framework for longterm imaging of cerebral organoids. We will generate nuclei lineage trees of growing neuroepithelial buds from organoids generated with reporter cell lines together with effector markers such as tubulin and actin to study cell behaviours. We are combining our live imaging approaches with microfluidic chips to develop cerebral organoids in a controlled environment with patterning cues. By comparing cell behaviours and cell type emergence in self patterning and controlled systems we aim to shed light on the early developmental events in brain organoids. Lastly, we will compare the of wild-type organoid development with instances of aberrant patterning seen in loss-of-function mutations in GLI3 and Periventricular Heterotopia (PH), to shed light on the mechanisms underlying brain malformations linked to mutations in these genes.

The Integrin β4-keratin link impairs mechanosensing by protecting the nucleus form mechanical loading.

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There is ample evidence on the importance of matrix rigidity and composition in triggering cellular responses during morphogenesis and disease progression. However, how these two factors jointly influence cell behavior and gene expression remains unclear. During invasive dissemination of breast cancer cells, for instance, the composition of the extracellular matrix (ECM) changes from a laminin rich basement membrane (BM) to a collagen and fibronectin rich environment with altered mechanical properties. To understand the mechanisms involved in these physiological scenarios, we plated the cells on gels of varying stiffnesses (0.5-30kPa) and coated them with different ECM proteins (laminin-111, collagen 1 and Fibronectin). We found that cells seeded on laminin exerted lower traction forces and exhibited compromised mechanosensitivity as shown by reduced YAP nuclear translocation, and smaller focal adhesions (FAs). Blocking integrin β4, a laminin-specific integrin, led to changes in the organization of the keratin network and its interaction with the actin cytoskeleton, and increased nuclear YAP ratios. By perturbing the interaction between integrin β4 and keratins and stretching cells, we show that the link between laminin, integrin β4, and the keratin cytoskeleton shields the nucleus from mechanical loading, thereby reducing YAP nuclear localization. Finally, interfering with integrin β4 and keratin expression in a mouse model of breast cancer tumoroids, hinders cell invasion in 3D, suggesting a role of integrin β4 and their link to keratins in invasion and metastasis. Overall, we propose a novel mechanism, by which ECM composition can influence gene expression, by protecting the nucleus from mechanical loading.

Asamataxis: Mechanical inhomogeneity driven cell migration leading to long-range self-patterning of cells

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The chemical and mechanical cues drive cell migration in several physiological and pathological conditions. While the effects of these cues are well studied, the role of stiffness inhomogeneity in cell migration and pattern formation is unexplored. Here, we show that a rigid body embedded in homogeneous soft polyacrylamide (PAA) gel directs cell migration; we call it 'Asamataxis' (Asama- uneven in Sanskrit, taxis-migration). Such mechanical inhomogeneity leads to long-range (20-35 cell length) self-organized cellular pattern depending on the shape and size of the embedded rigid structures, elastic modulus of PAA gel and thickness of gel on top of the embedded structure. Interestingly, the pattern formation was lost when we disrupted the bonding between the embedded structure and the gel. Also, this patterning depends on cellular traction and morphology. These observations lead us to the hypothesis that the rigid structure works as an anchor for the gel and thus modifies the strain field caused by cellular traction. This information about the modified strain field is relayed outward. Cells align their axis in response to this relay and migrate towards the embedded structure leading to the observed long-range patterning. We propose a dimensionless number 'f', combining the governing parameters, to predict the possibility of pattern formation. We have also shown that the pattern can be tailor-made by pre-designing sub-surface structures, a potential tool for tissue engineering. This mechanism of directed migration driven long-range pattern formation in response to mechanical inhomogeneity may be involved during several pathophysiological conditions, a proposition that needs further investigation.

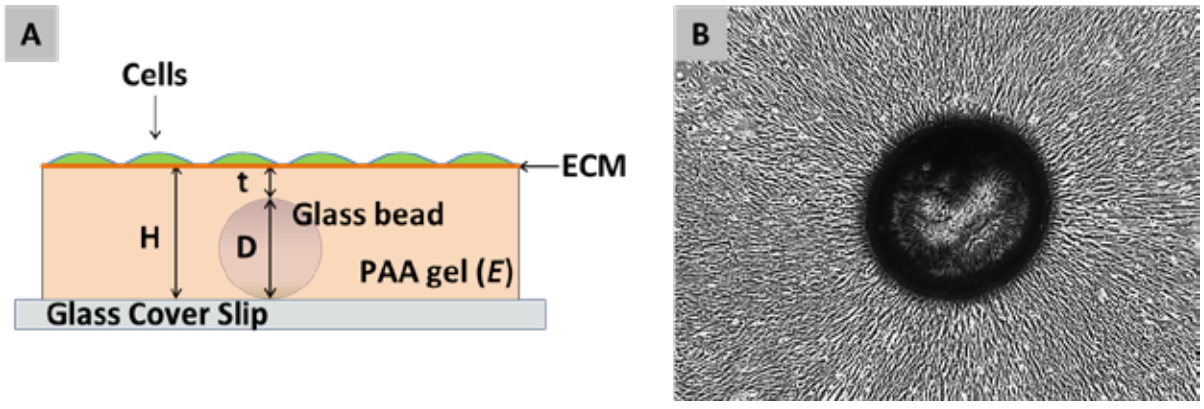

Figure 1: (A) Schematic of mechanically inhomogeneous substrate. H=height of substrate, D=diameter of bead, t=thickness of polyacrylamide (PAA) gel, *E*=elastic modulus of PAA gel, ECM= Extracellular matrix. (B) Radially patterned C2C12, mouse myoblast cells, in response to glass bead embedded in 2 kPa PAA gel.

Anisotropic materials with dual functionality for cardiac tissue engineering

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Myocardial infarction (heart attack) leads to extensive cardiomyocyte death and remodeling at the infarct site. Due to the low proliferative capacity of adult cardiomyocytes, regeneration of heart muscle tissue is poor. Regenerative approaches rely on the ability to design biomaterials supporting cardiac progenitor cell proliferation and differentiation. So far biomaterials for cardiac tissue engineering have included matrix adhesive factors (cell-matrix interactions) and the anisotropic morphology of cardiac native tissue. Cell-cell adhesions, formed by cadherin complexes, are critical for tissue integrity and electrophysiological function of the myocardium. Besides, their strong localization at cardiomyocyte cell-cell contacts is one of the hallmarks of cardiomyocyte differentiation and maturation. So far, no material strategy has targeted these interactions to support cardiac cell function. In this work, we aim to develop 3D biomaterials for cardiac-like tissue engineering by incorporating mechanical anisotropy and a regulated formation of cell-cell and cell-matrix adhesions in spatially and mechanically defined matrices. Cardiac muscle progenitor cell response to defined physical and biochemical cues will systematically be investigated to reveal parameters for functional cardiac-like tissue formation. Such biomimetic, highly tunable microenvironments will not only enable us to develop biomaterials for regenerative medicine but can also serve as biomimetic in vitro tools to study basic questions in cardiac biology and development.

Material properties of pilus mediated cellular aggregates

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Living cellular aggregates are an example of active materials with unconventional rheological properties. Motivated by colonies of *Neisseria gonorrhoeae* bacteria, we develop a continuum theory to study cellular aggregates formed by attractive pilus mediated intercellular interactions. We link the formation of cellular aggregates as an active phase separation process and discuss the activity-induced viscoelastic properties of such aggregates. By studying the behaviour of aggregates under oscillatory shear, we link the loss and storage moduli of the aggregates to the dynamics of the active intercellular forces. Because of the turnover of pili, the aggregates show a liquid-like behaviour at large times and strong shear-thinning effect under the large amplitude oscillatory shear. Our theory provides an important insight on how pilus mediated intercellular forces in cellular aggregates govern their material properties which in the future could be tested experimentally.

Design of a Microphysiological System to Model Ischemic Cardiac Tissue

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One of the overarching goals of cardiac tissue engineering is to create an *in vitro* model that closely resembles native cardiac tissue in order to perform developmental or disease studies (*Tandon et al., Nature Protocols 2009*). Traditional cell culture methods are not reliable models because they lack many of the guiding cues (topographical, electrical,…) that determine the functional properties of cardiac tissue *(Bhatia et al., Nature Biotechnology 2014*). The emergence of microphysiological systems has opened up unprecedented possibilities to create a biomimetic environment that can recapitulate specific environmental changes at the cellular scale that are the onset of pathological conditions.

The goal of this work is to present a preliminary microfluidic cell culture platform in which a physiologically relevant 2D ischemic cardiac model can be obtained. To do so, we combined the use of electrospun PLA fibers as guiding cues to mimic the anisotropy of cardiac tissue, with electrical stimulation using a user-friendly setup based on stainless steel rod electrodes to enhance tissue maturation. HL-1 cells (cardiac muscle cell line) were loaded alone or in co-culture with primary cardiac fibroblasts in neighboring cell culture chambers flanked by two media channels. The main chamber is delimited by two lines of microposts, which are used to confine the cell suspension. After 4 days in culture (stimulation starting at day 2), an oxygen gradient is created for a few hours inside the cell chamber space by using two scavenging channels separated 30 µm from the main ones. Levels between physioxia (6 % O_2) and hypoxia (1-3 % O_2) can be obtained by placing the chips in a hypoxia incubator and using a scavenger (sodium sulfite) to drop the oxygen levels to 0 % in one of the channels.

We confirmed that cardiac cells showed an elongated morphology with the cytoskeleton aligned following the orientation of the nanofibers. Higher expression levels of the tight junction protein Cx-43 were also found upon electrical stimulation, which is generally used as indicator of cardiac cell maturation. The generation of a hypoxic gradient was also confirmed by performing measurements with optical oxygen sensors. Future work will focus on further developing the setup and studying if we are able to replicate the key cell responses of an ischemic cardiac tissue (such as cardiac fibroblasts proliferation, collagen deposition, etc.).

The Role of Surface Stresses in Fibrous Tissue Morphogenesis

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The formation and maintenance of tissue boundaries is vital for morphogenesis and homeostasis as groups of cells with distinct functions must often be kept physically separated. Cells organize into sheets on the tissue surfaces by forming intercellular mechanical connections directly or through the extracellular matrix. The collective dynamics of boundary regulation have been extensively studied with micropatterned epithelial monolayers, whose behavior has been captured using physical models based on nematic liquid crystals. However, less is known about surface effects in three-dimensional fibrous tissues and their contribution to tissue architecture. We developed a high-throughput biomimetic platform for the study of morphological changes in three-dimensional microtissues. We performed local mechanical perturbations using a robotic microsurgery system and selective optochemical manipulations using a programmable projector (Fig. 1). We show both experimentally via time-lapse microscopy and by using computer simulations that cells at the tissue boundary develop surface stresses and, together with contractile activity of cells residing in the core, drive macroscale deformation [1]. By only fitting bulk and surface contractile moduli, the model simulation recapitulates tissue shape evolution after well-defined incisions and implantations. We apply the computational and experimental methods to engineered multilayered three-dimensional microtissues and demonstrate that targeted elimination of cells at the tissue surface induces a local stress gradient that leads to morphogenesis.

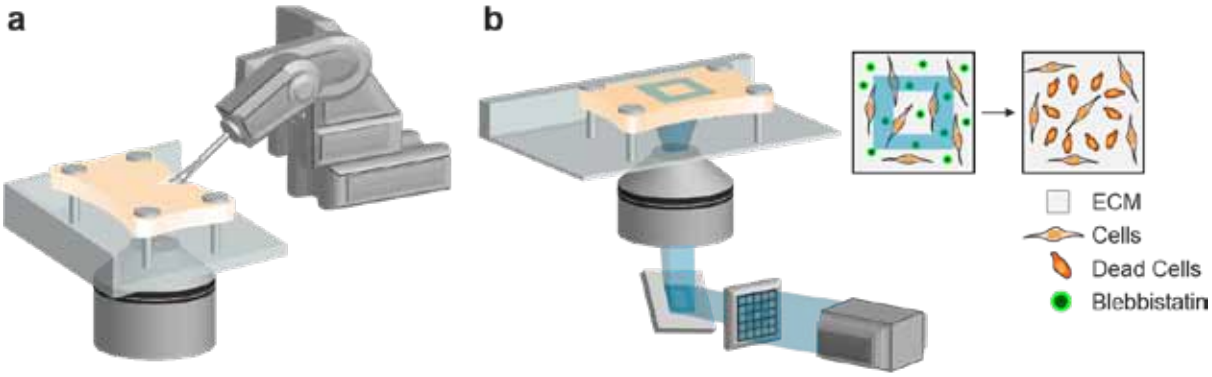

Figure 1. Schematic illustrations of the experimental platform to study tissue morphogenesis. a) Tissues are manipulated using actuated microscissors mounted on a robotic surgery system. The operation is performed on an inverted microscope. b) Spatiotemporally controlled photoconversion of blebbistatin leads to local killing of cells. A programmable digital micromirror display system is used for projecting blue light on predefined regions of the sample. The culture medium is supplemented with blebbistatin before exposure.

1. Mailand, E., Li, B., Eyckmans, J., Bouklas, N. & Sakar, M. S. Surface and bulk stresses drive morphological changes in fibrous microtissues. *Biophysical Journal*, 117:975–986 (2019).

In Vitro Modeling of Micro-Emboli and Their Effect on Microvascular circulation And Endothelium Mechanics

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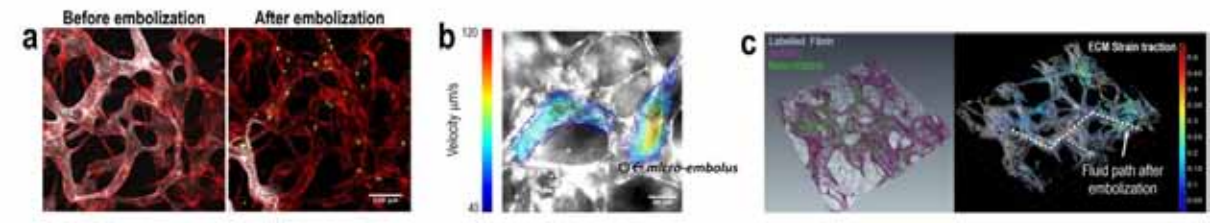
Obstructions in the microvasculature caused by microembolization of blood clots, gas or adipose tissue, are observed in many pathologies such as embolic stroke and acute pulmonary embolism. In the case of the lung vasculature, micro-emboli produce an increase of vascular resistance, which results in acute and/or chronic cardiac failure; despite its physio-pathological importance, little is known about the physical response of the endothelium to emboli-impaired microcirculation. Here, we model embolization in a microvascular model *on a chip*. We hypothesize that the impaired flow caused by the emboli is sufficient to trigger a rapid



biomechanical response. To investigate this question, we use self-organized microvasculature cultured inside microfluidics chips. We use Human Umbilical Vein Endothelial Cells (HUVEC) as a general established cell model of microvasculature, and Human Pulmonary Artery Endothelial Cells, a more specific lung-related cell model. To model emboli, we dilute carboxylated micro-beads of compatible dimensions (i.e. ~5 □m, green dots in **a**) in the cell culture media and perfuse the mixture in the microvascular lumens using a controlled pressure head applied through microfluidic media channel. Throughout the experiment, we also visualize fluid flow in real-time combining nano-tracers of distinct fluorescent emissions diluted in the cell culture media. We perfuse the media and the diluted nano-tracers sequentially, i.e. before and after embolization. We perform this procedure on stage during the 3D confocal image acquisition. We also acquire high-speed time-lapses and use Particle Image Velocimetry (PIV) to quantify fluid velocity of the nano-tracers (**b**). We additionally use the PIV algorithm on the fluorescent signal of labeled fibrin gel to measure extracellular matrix (ECM) mechanical deformations (**c**). Despite observing some variability partly depending on the number of micro-emboli perfused, *in vitro* microembolization effectively decreases up to 75% the perfused area of the examined regions (**a**), accompanied by a flow redirection, with preferential paths of high fluid velocity created after the introduction of the micro-emboli (**b**). Preliminary results from our model show fibrin deformations, with poorly perfused endothelia applying higher forces onto the ECM (**c**), revealing a possible mechanical response in the microvascular network. These observations suggest the possibility of a rapid mechanical adaptation to the presence of micro-obstructions, an outcome that might help understanding the physiopathology of pulmonary embolisms and other embolic disorders. As such, our *in vitro* microembolization model paves the way to integrate the study of endothelium mechanics and fluid perfusion disturbances in 3D using a realistic self-organized microvasculature.

Engineering human Pluripotent Stem Cells (hPSCs) lines with CRISPR/Cas9 for inducible Knock Out in Kidney Organoids.

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The possibility to generate organ-like cultures from human pluripotent stem cells (hPSCs), so called organoids, opens the door to immediate studies in human development and disease. Currently, major drawbacks of this technology are related to the immaturity of these systems when compared to adult organ counterparts, and the lack of complex tissue-tissue interactions (vascularization, innervation, immune system). In this regard, the field is starting to apply cutting-edge approaches aiming to facilitate the maturation of hPSCs derived organoids, (i.e., microfluidics for vascularization, biomaterials for their maturation, among others). Other limitations are related to the technical challenges aiming to target specific *locus* (either for knock-out (KO) or knock-in (KI) applications) determinant for human disease and development in a time dependent fashion. In this regard, our laboratory has recently generated hPSCs lines for the inducible expression of Cas9. In these lines Cas9 is expressed under the control of the TET/ON system upon doxycycline treatment. Through the introduction of the Cas9 module via TALEN mediated targeting in the safe harbour locus *AAVS1* we have generated hPSCs heterozygous lines that express constitutively gRNAs for further knock out in different *loci* determinant for kidney development (PAX2, LHX1, WT1) and disease (WT1 and VHL). This inducible knock out lines (iKO lines) allow for the interrogation of knock-out associated phenotypes upon their differentiation into kidney organoids. Specifically, we have been able to perform stage-specific gene ablation in isogenic backgrounds for the study of gene function during kidney morphogenesis and disease. Importantly, this approach could be expandable to any hPSC-based model system for assessing human genetics in a dish.

Linking epithelial geometry to tension and pressure

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Epithelia are thin cellular layers that act as mechanical and biochemical barriers. They are dynamic tissues that present strong intercellular junctions needed to maintain their integrity while growing and regenerating. During embryogenesis, they fold progressively and give rise to highly reproducible 3D geometries that guide the shape and positioning of organs[1].

The way pressure and tension depend on the size of 3D epithelial structures can help us understand how epithelia fold into determined shapes and are able to maintain them even under the continuous remodelling due to cell division[2]. In this project we study the link of epithelial size and shape with luminal pressure and intercellular tension in fluid-filled MDCK 3D monolayer structures.

To guide the 3D folding of an epithelial monolayer, we create micropatterns with different concentration of adhesion proteins (less and more adherent regions) on top of 3kPa Polydimethylsiloxane (PDMS) gels[3]. We then seed MDCK cells stably expressing a GFP cell membrane marker that allows tracking of the epithelium's curvature. The difference in adhesion between regions permits the delamination of cells to form fluid-filled pressurized structures of different sizes and shapes.

Using Traction Force Microscopy, we can measure the forces exerted by the cells on the substrate, as well as luminal pressure inside of the 3D structures[4], and relate them to cell tension and epithelial curvature. Our findings indicate that luminal pressure decreases with the radius of the curved monolayers and that monolayer tension is independent of size.

1) Guillot C, Lecuit T. **Mechanics of Epithelial Tissue Homeostasis and Morphogenesis**. *Science,* Vol. 340, Issue 6137, pp. 1185-1189 (2013).
2) LeGoff, L. & Lecuit, T. **Mechanical Forces and Growth in Animal Tissues**. *Cold Spring Harb Perspect Biol* 8, a019232 (2015).
3) Strale PO *et al*. **Multiprotein Printing by Light-Induced Molecular Adsorption**. *Adv Mater*, 9;28(10):2024-9 (2016).
4) Latorre E et al. **Active superelasticity in three-dimensional epithelia of controlled shape**. *Nature*, 563, 203–208 (2018).

Self-organized intestinal epithelial monolayers in crypt and villus-like domains show effective barrier function

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The surface of the small intestine is lined by a monolayer of tightly packed, polarized epithelial cells organized into invaginations called crypts, and finger-like protrusions called villi[1]. The intestinal epithelium is a rapidly renewing tissue sustained by the highly proliferative $Lgr5^+$ intestinal stem cells (ISCs) that reside at the crypt bases[2]. ISCs undergo self-renewal and generate transit amplifying cells, which migrate up the crypt-villus axis and differentiate into the different intestinal cell types.

Technological advances in epithelial cell culture methods have permitted the long-term culture of intestinal stem cells (ISCs) with self-renewal and differentiation capacities. When ISCs are embedded in Matrigel® and cultured with biochemical factors mimicking the *in vivo* ISCs niche[3] they form intestinal organoids which resemble the *in vivo* tissue at the structural and functional levels. A major drawback of organoids is their 3D closed geometry which impedes direct access to the apical region of the epithelium, hampering their use in studies of nutrient transportation, drug absorption and delivery, and microbe-epithelium interactions.

Here, we describe a simple method that allows the formation of intestinal epithelial monolayers that recapitulates the *in vivo*-like cell type composition and organization and that is suitable for functional tissue barrier assays. Our results show that epithelial monolayer spreading in 2D is driven by substrate stiffness, while tissue barrier function is achieved by basolateral delivery of medium enriched with stem cell niche and myofibroblast-derived factors.

Using intestinal cells derived from *Lgr5-EGFP-ires-CreERT2* organoids[3], expressing GFP under the control of the stem cell marker *Lgr5*, we monitored, by live-imaging microscopy, the stem cell behaviour during the 2D monolayer formation. Our results show that when grown on thin Matrigel® films, organoid-derived stem cells self-organize into crypt-like domains, from which cells migrate out to form non-proliferating and differentiated villus-like regions, closely resembling the *in vivo* cell distribution. Asymmetric administration of stem cell niche molecules and myofibroblast-derived factors allowed for the full growth of the epithelial monolayers on Transwells® inserts. As a unique characteristic, these epithelial monolayers form functional epithelial barriers with an accessible apical surface and physiologically relevant transepithelial electrical resistance and apparent permeability values.

Our technology offers an up-to-date and novel culture method for intestinal epithelium, providing an *in vivo*-like cell composition and distribution in a tissue culture format compatible with high-throughput drug absorption or microbe-epithelium interaction studies.

1. Clevers, H. The intestinal crypt, a prototype stem cell compartment. *Cell* **154**, 274–84 (2013).
2. Barker, N. *et al.* Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* **449**, 1003–7 (2007).
3. Sato, T. *et al.* Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature **459**, 262–5 (2009).

Recapitulating the somitogenesis *in vitro* to identify novel causative genes for congenital bone diseases

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Somites are periodically formed though the segmentation of anterior parts of presomitic mesoderm (PSM). In mouse embryos, this periodicity is controlled by the segmentation clock gene *Hes7*, which exhibits a wave-like oscillatory expression in the PSM. The periodical somite formation is a crucial event for body segment formation and abnormal somitogenesis leads to congenital bone diseases.

Spondylocostal dysostosis (SCD) is a group of bone malformation disease which is characterized by morphological abnormalities of vertebrae and ribs. Mutations in several somitogenesis-related genes, including *HES7*, are already known as the cause of SCD. As for 75% of SCD patients, however, the causative gene and at what stage of bone development the abnormality occurs are still unclear.

Thus, the aim of this study is to establish the method to recapitulate the somitogenesis *in vitro* and to identify novel causative genes of SCD.

To recapitulate the somitogenesis *in vitro*, we previously reported a simple and efficient method to generate mouse embryonic stem (ES) cell-derived PSM-like tissues (Matsumiya *et al*., Development, 2018). In these tissues, *Hes7* oscillation was synchronized among neighboring cells, the anterior-posterior axis was self-organized, and somite-like structures were observed. We are currently developing a similar method to recapitulate the human somitogenesis by using human induced pluripotent stem (iPS) cells instead mouse ES cells. Furthermore, by using human iPS cell lines that lack the candidate gene of SCD for the *in vitro* somitogenesis, we are trying to identify a novel causative gene of SCD.

LIMBNET: online image-based computational modelling of limb development

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A key challenge for the future of computational modelling is how to easily share complex image-driven simulations between a diverse community of theoreticians and experimentalists. This is essential so that different researchers can test, compare, and reproduce each other's hypotheses, and to allow their new results to build on the previous results of others. We present our new modelling platform LIMBNET, an openly accessible online simulator for intuitive image-based computational modelling, simulation, and visualisation of gene expression patterns crucial to limb development.

In LIMBNET, 2D gene expression patterns taken from mesoscopic scale images (e.g., from WMISH, immunostaining, or other data) are mapped into a standard morphological framework, the 'morphomovie', covering the developmental stages E9.5 to E12.5 of mouse hindlimb bud. LIMBNET's customisable models are based on a reaction-diffusion framework simulated using finite-volume techniques on a moving finite element mesh. The models can use the experimental data stored in morphomovies as inputs. We simulate regulatory networks throughout the mesoderm, but also across the ectoderm, with tunable coupling and communication between multiple compartments with their own signaling dynamics. Our interface allows rapid prototyping of model networks; users can quickly perturb parameters and visualize the results of a simulation within seconds. Crucially, we will also allow for subsequent offline optimization of model networks, to infer parameters and network motifs that give rise to given spatio-temporal patterns.

Expression patterns predicted by a given computational model can be visualised in the same morphomovie framework as experimentally acquired data, permitting a direct comparison to be made between the two, or indeed between outputs of different models. Most importantly, all this functionality is accessible through a standard web browser, avoiding the need for any special software, and thus opening the field of image-driven modelling to the full diversity of the scientific community.

Quantitative characterization of adhesion and cytomechnics of living cells on biomaterials and tissues

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The versatility and refinement of biomaterials and tissues engineered for regenerative medicine is growing, as is the need to characterize their properties and host interactions. The topography and mechanical properties of biomaterials are crucial parameters that influence cell adhesion/motility, morphology and mechanics as well as the fate of stem and progenitor cells [1, 2, 3].

AFM (atomic force microscopy) is an advanced multiparametric imaging technique which delivers 3D profiles of the surfaces of molecules and cells in the nm-range. It also enables the characterization of nanomechanical properties (adhesion, elasticity etc.) as well as the visualization of dynamic processes using high-speed Imaging.

In order to perform AFM experiments in the range of mm to nm resolution, we have developed a versatile solution – the HybridStage™. Three main components can move the tip/sample respectively. The AFM head houses the xyz tip scanner, which has a scan range of 100x100x15 μm^3. The HybridStage houses a xyz sample scanner (optional with 100, 200 or 300 µm for each axis) which allows the appropriate AFM operations. It also houses a motorized unit for large sample movements (20x20 mm^2). The wide choice of scanners, together with optical tiling and multi-region AFM probing, enables multiparametric characterization of soft samples such as tissues over a large area and provides additional optical data sets.

Fast AFM imaging of several frames per second can be seamlessly combined with methods such as epi-fluorescence, confocal, TIRF, STED microscopy, and many more.

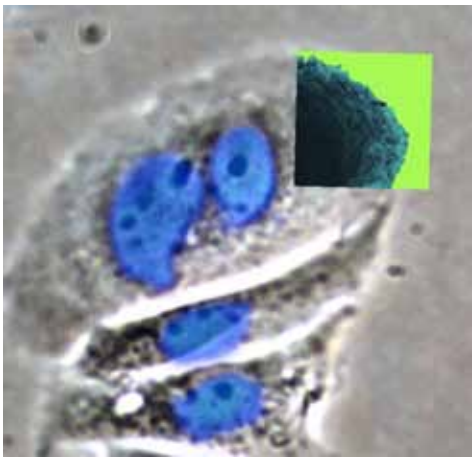

Fig: Combined optical image of phase contrast and fluorescence of mammalian cells correlated with a local Young's Modulus distribution.

Using AFM, mechanical properties like the Young's modulus of biomaterials, tissues or cells can be determined. Furthermore, the nanostructure of biomaterials like aligned collagen matrices and cell alignment on such structures have been resolved [3]. Using Single Cell Force Spectroscopy (SCFS), cell-substrate or cell-cell/tissue interactions can be measured down to single protein unbinding. The nano-mechanical analysis of cells is increasingly gaining in importance in different fields in cell biology like cancer research and developmental biology.

We will present how the latest advances in the High-Speed AFM are being applied to study a wide range of biological specimen, from individual biomolecules to collagen type I fibrillogenesis to mammalian cells and tissues.

[1] Elter et al., Eur Biophys J 2011: 40: 317-327.

[2] Engler et al., Cell 2006: 126:677-689

[3] Cisneros et al., Small 2007: 3:956-63.

Stability and oscillatory evolution of Drosophila Central Nervous System depends on tissue rigidity and adhesive contributions

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Experimental measurements of the length evolution of the central nervous system (CNS) in Drosophila fly reveals that it contracts in an oscillatory manner during its condensation. We make use of the observed displacement field in order to numerically simulate the oscillatory phases using a viscoelastic model. The *in silico* model is able to mimic the segmented structure of the CNS, and also show that compressive stresses are concentrated within each segment.

In order to reproduce the time-oscillations, we resort to a specific rheological model where rest-length of the cells are able to dynamically adapt with a given delay with respect to the stress field. We also include force contributions for the surrounding tissue of the CNS. The analytical results show that the oscillatory regime and the stability of the deformations depends on the tissue stiffness and adhesion with the epithelia, in agreement with experimental measurements of tissue rigidity.

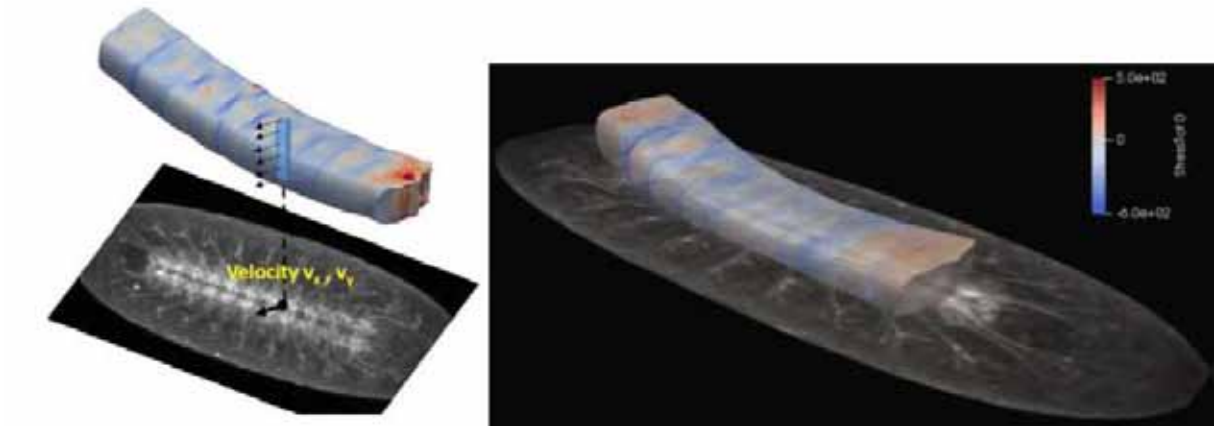

Figure 1. (a) Mapping of the two-dimensional measured velocity field onto the three-dimensional finite element model. (b) Simulated condensation process and contour map of the stress field on CNS tissue.

In Vitro Vascular Networks for Bone Tissue Engineering

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Functional microvascular systems become an important component of advanced *in vitro* models enabling to recapitulate human tissue. Prevascularization is also essential for engineering tissue constructs for *in vivo* implantation improving oxygen and nutrients supply to the construct and increasing integration with the host tissue. Vasculature formation could be achieved by including endothelial cells into a multi-culture system that requires careful consideration of media and seeding methodology to achieve sufficient functionality of each cell type. Therefore, the aim of the current work was to define media composition suitable to simultaneously support vasculogenesis and osteogenesis for bone tissue engineering.

We studied vascularization in monoculture of human umbilical vein endothelial cells (HUVECs) and in co-cultures of HUVECs and bone marrow mesenchymal stromal/stem cells (BMSCs) on fibronectin coatings and in hydrogels. We used microfluidic chips as a 3D co-culture platform enabling perfusable vascular network formation. We studied effect of osteogenic differentiation medium (OM, commonly used for BMSCs osteogenesis and mineralization induction) and mixture of endothelial growth medium (EGM-2, Lonza) with OM (EGM-2+OM 1:1) on vascular network formation by assessing the expression of vascular (CD31, vWF) and pericytic (α-SMA) markers. Osteogenesis was assessed by hydroxyapatite staining. Vascular network properties such as vessel density and branching index were analyzed with AngioTool software.

The study confirmed that tubular network formation by HUVECs is dependent on the co-culture with BMSCs. We found that HUVECs-BMSCs at ratios of 10:2 and 10:1 generated branched interconnected vascular networks in collagen and collagen/fibrin (40:60) hydrogels over the course of 4-5 days. The BMSCs localized close to the vessels and differentiated into perivascular cells, marked by expression of α-SMA. The results showed that HUVECs don't tolerate OM and form sparse vascular network in EGM-2+OM 1:1 medium when compared to EGM 2. Strong osteogenic induction lead to matrix mineralization while prevented the formation of a vascular network in co-culture system.

Overall, we showed vasculature formation in 3D hydrogels using co-cultures of HUVECs and BMSCs. The culture conditions need to be further optimized to simultaneously support bone mineralization and vasculature formation.

Physical and chemical basement membrane disruption modes synergistically promote the invasiveness of MCF10A breast acini.

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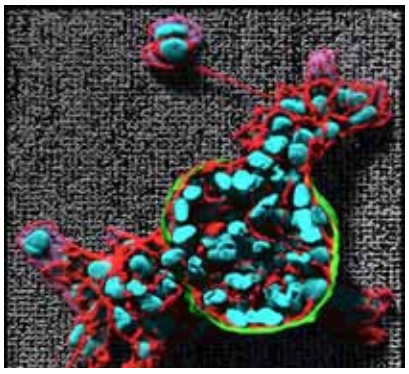
Mechano-reciprocity between cells and their microenvironment is a fundamental gatekeeper for tissue homeostasis and impaired during malignant transformation of breast gland tissue: Stromal stiffening, misbalanced tensile stresses and tumor cell forces induce basement membrane (BM) disruption and invasive progression that is further modulated by aberrant growth factor signaling and MMP-proteolysis[1]. How this complex reciprocal triangle of BM stability, physical and biochemical cues could mutually trigger cell invasion is under vivid debate[2].

The present study addressed this still less understood paradigm of cell-force driven BM weakening and disruption mechanisms. Traction force microscopy (TFM)[3] and elastic resonator interference stress microscopy (ERISM)[4] techniques were adapted to a newly designed *in vitro* BM-invasion assay that allowed to analyze cell force-mediated BM-stress generation and the mechanical activity of living pre-invasive breast acini, respectively. We applied MCF10A breast acini with BM scaffolds of tunable mechanical strength[5] to tumor-like substrate stiffening and oncogenic epidermal growth factor stimulation to resemble progressing tumor tissue states. Hundreds of breast acini were measured to determine the invasion time course and incidence, potentially triggered by those altered conditions.



BM-Invasion *in vitro* assay: An invasive MCF10A derived breast acini on a TFM surface (fiducial beads, grey) undergoes collective cell migration (F-actin, red; nuclei, cyan) through the locally disrupted BM shell (collagen IV, green). Top view on a cross section through a 3D rendered confocal image stack.

Our results demonstrate the pro-invasive effects of ECM stiffening, oncogenic EGF signaling, PI3-Kinase activity, and increased proteolytic and mechanical BM stresses. Moreover, pre-invasive breast acini form mechanically active and BM-traversing F-actin rich protrusions that could probe ECM rigidity thereby reinforce cell contractility and hence contribute to solid BM-weakening stress. In summary, the present study highlights the major anti-invasion barrier function of mechanically strengthened BM-scaffolds and. Our data revealed the synergistic action of soluble and mechanical tissue parameters that ease both chemical and physical modes of BM-disruption and finally facilitate collective cell invasion.

References:

1.Paszek, M.J. & Weaver, V.M. The tension mounts: mechanics meets morphogenesis and malignancy. *Journal of mammary gland biology and neoplasia* **9**, 325-342 (2004).

2.Rowe, R.G. & Weiss, S.J. Breaching the basement membrane: who, when and how? *Trends in cell biology* **18**, 560-574 (2008).

3.Merkel, R., Kirchgessner, N., Cesa, C.M. & Hoffmann, B. Cell force microscopy on elastic layers of finite thickness. *Biophysical journal* **93**, 3314-3323 (2007).

4.Kronenberg, N.M. *et al.* Long-term imaging of cellular forces with high precision by elastic resonator interference stress microscopy. *Nat Cell Biol* **19**, 864-872 (2017).

5.Gaiko-Shcherbak, A. *et al.* The Acinar Cage: Basement Membranes Determine Molecule Exchange and Mechanical Stability of Human Breast Cell Acini. *PloS one* **10**, e0145174 (2015).

Inferring the network topology driving mesoderm differentiation from single cell time-course data

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During early embryonic development multiple signaling pathways specify cells to adopt different cell fates. Understanding the topology of these biochemical networks is crucial for engineering cells and tissues. *In vitro* systems such as embryonic organoids mimic the first events in early development and constitute an ideal system to study differentiation and patterning in a controllable and reproducible manner. Despite most of the molecular players in early development being known, the underlying topology of the networks is far from understood. One approach to solve this problem is to use statistical inference methods on single-cell time-course data. Here we focus on the expression of the canonical mesodermal marker T/Brachyury (T/Bra), a transcription factor involved in anterior-posterior axis determination during early gastrulation.

T/Bra has been recently shown to appear as a polarized domain in 3D cell aggregates such as *embryoid bodies* and *gastruloids*, marking a symmetry-breaking event in a homogenous cell population. Using a mouse transgenic line with a GFP reporter, we quantified single-cell fluorescence using flow cytometry under the action of temporally controlled external biochemical signals in 2D cell cultures and 3D cell aggregates. In both cases, T/Bra + and T/Bra - populations were identified, hinting towards the presence of bistability in the underlying biochemical network. We propose a mathematical model to explain bistability in the system and present a statistical inference method to characterize the network topology from the single-cell data. Our study lays the groundwork to understand self-organized symmetry breaking in early development.

Upscaling active gel models of the actin cortex to epithelial mechanics

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Epithelial tissues are cohesive sheets of cells that line free surfaces and cavities of animals. During development and adult life, epithelia maintain their functionality in dynamic mechanical environments where they undergo deformations of varied magnitudes and loading rates. Recent *in vitro* and *in vivo* observations across various species have revealed a rich phenomenology of epithelial mechanics originating from the tightly coupled mechanics of adherent epithelial cells, which is in turn governed by the active-viscoelasticity of the actomyosin cortex. However, a link between the subcellular cortical dynamics and the tissue scale response has been lacking in theoretical models of epithelia. For instance, in classical vertex models, a phenomenologically motivated work function governing the vertex dynamics [1] often lacks a direct connection to the microscopic subcellular physics. We address this gap by developing a formalism (Fig. 1 a) allowing us to bridge active-gel models of the cortex [2] and vertex-like models at a tissue scale. We show that this modeling approach provides a unified framework capturing numerous seemingly disconnected epithelial phenomenologies (Fig. 1 b-d), in which junctional network topological rearrangements are minimal, over a wide range of loading rates. These include stress relaxation following stretch/unstretch maneuvers [3,4], solid-like or fluid-like creep behavior [5] , buckling and transient buckling upon compression [6], pulsatile contractions during dorsal closure in Drosophila [7] or active superelasticity [8] . Overall, the proposed framework provides a systematic procedure to examine the effect at the epithelial scale of sub-cellular cortical dynamics and, in the process, ties a diverse epithelial phenomenology to a common subcellular origin.

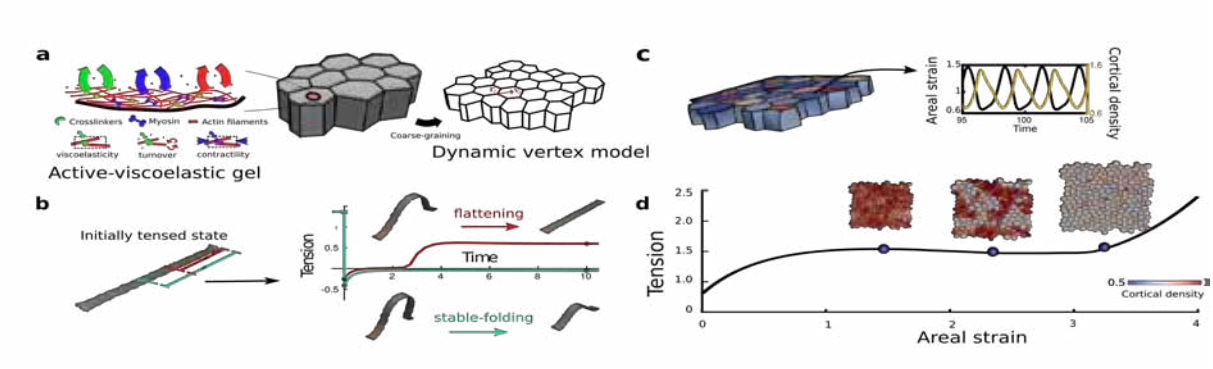

Figure: (a) Schematic of the framework. (b)Transient or stable fold upon tissue compression. (c) Cell pulsatile contractions. (d) Active-superelasticity of an epithelium under stretch.

References

[1] S. Alt, *et al*., *Philos. T. Roy. Soc. B*, 372:20150520, 2017

[2] J. Prost, *et al*., *Nat. Phys.*, 11:111–117, 2015

[3] L. Casares, *et al*., *Nat. Mat.*, 14:343–351, 2015

[4] N. Khalilgharibi, *et al*., *Nat. Phys.*, 15:839-847, 2019

[5] A. Harris, *et al*., *PNAS*, 109:16449-16454 2012

[6] T. Wyatt, *et al*., *BioRxiv*, 2018

[7] J. Solon, *et al*., *Cell*, 137:1331–1342, 2009

[8] E. Latorre, *et al*. *Nature*, 563:203–208, 2018

Platelet and Thrombus Biomechanics: The Need for Vasculature-on-Chip

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Background:

Platelets are discoidal, anucleate cellular fragments (2-5µm in diameter). Generated by bone marrow megakaryocytes and released into blood circulation, platelets primarily contribute to the formation of stable haemostatic plug at the site of vascular injury thus preventing excessive blood loss. In the absence of bleeding, platelets contribute to thrombosis - a process of pathological clot formation resulting from excessive activation of coagulation cascade. Pathological manifestation of thrombosis and clot embolism lead to life-threatening complications when they occur in heart (e.g. heart attack), in brain (e.g. ischemic stroke), or in lungs (e.g. deep vein thrombosis). Clinically, these conditions are managed through anti-platelet, fibrinolytic and anti-fibrinolytic drugs. Although the biochemical and cell biological aspects of thrombosis, its resolution and pharmacokinetics of drugs used for treatments are well characterized, little is known about the underlying biophysical and biomechanical principles governing these processes.

Currently used method(s) and their limitations.

To understand the biophysical characteristics of human platelet thrombus, we combine *in vitro* platelet adhesion and platelet thrombus formation assays under hydrodynamic shear in microfluidic systems on purified extracellular matrix (ECM) proteins followed by force spectroscopy. This simplified approach is useful in revealing previously unknown biomechanical peculiarities of platelet thrombus structure and its stability. However, it fails to emulate the complexity of vascular microenvironment and architecture. Specifically, the contribution of cellular heterogeneity, nano/-microstructure of exposed ECM and geometrical constraints affecting hemodynamics such as stenosis and blood vessel bifurcations on thrombus biomechanics are least characterized.

Why are we attending the EMBL-IBEC Winter Conference?

We are seeking collaboration to establish a 3D-vasculature-on-chip platform which would allow us to overcome most of the current limitations. Ideally, the experimental platform should be compatible with light microscopy imaging modalities, facilitate perturbation of cellular layers in order to expose underlying ECM, mechanically stable enough to tolerate arterial and venous shear flows. In addition, it is desirable to excise the region of interest for further biophysical characterization by force spectroscopy, light-sheet and super resolution microscopy.

What are the potential applications areas?

Our lab specializes in platelet biology, platelet biomechanics, diagnostics of human platelet function defects and translational transfusion medicine.

We would like to apply 3D-vasculature-on-chip platform to investigate

1. Effect of clinically relevant fibrinolytic (e.g. tissue plasminogen factor), anti-fibrinolytic drugs (e.g. tranexamic acid) and anti-platelet drugs (e.g. clopidogrel, prasugrel).
2. Effect of hematocrit values on thrombus biomechanics.
3. Effects of blood product and platelet concentrate storage conditions (e.g. temperature, duration, additives).

Differentiation of Human Pluripotent Stem Cells into Ureteric Bud-like Cells and Assessment of their Renal Potential by the Use of ex vivo Kidney Reconstruction Assays.

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Human pluripotent stem cells (hPSCs), due to their unlimited self-renewal capacity and their ability to give rise to cells of all three embryonic germ layers *in vitro* and *in vivo*, represent an ideal tool for the study of human development and disease modelling *in vitro.* Exploiting these inherent properties has resulted in the definition of several approaches for kidney-like cells generation through the exposure of undifferentiated hPSCs to specific growth factors, chemicals and cytokines guiding renal development. Importantly, hPSCs have been differentiated towards the metanephric mesenchyme (MM) and ureteric bud (UB) lineages, the two progenitor cell populations that give rise to the nephrons and the collecting duct of the adult kidney, respectively. We have recently reported the definition of a methodology to efficiently differentiate hPSCs into kidney organoids containing nephron-like structures, reducing the time needed to generate them by 30% compared to previous works. Notably, the generated kidney organoids transcriptionally resembled second-trimester human fetal kidneys. However, as also seen by others, the presence of a proper UB branching structure within kidney organoids has been not achieved yet. Here, we have established a UB differentiation protocol by directing hPSCs into the anterior intermediate mesoderm lineage, leading to the generation of UB progenitor cells after 6 days of differentiation in 2D monolayer culture. The effective acquisition of the UB fate was assessed by the expression of UB markers by immunofluorescence, and by quantitative PCR (qPCR). Moreover, taking advantage of the established disaggregation-reaggregation methods that allow the reconstruction of mouse kidney rudiments, we could asses the UB identity and branching capacity of the differentiated cells. Overall, we have performed different *ex vivo* reaggregation assays with mouse MM and UB cells together; or with isolated mouse MM cells or UB tips in order to further study the interaction between these different cell types for the reconstruction of chimeric kidney organoids *ex vivo*. This approach may lead to expand our knowledge on the early steps defining both MM and UB differentiation and also to define a single procedure for the generation of UB and MM derivatives in the organoid setting.

CDK8/19 inhibition stabilizes human naïve pluripotency

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In mice, naïve pluripotent stem cells (PSCs) constitute the optimal starting state for subsequent developmental applications, providing high quality cells with full differentiation capacity. However, in human naïve PSCs, recent evidence reveals widespread karyotypic abnormalities, cancer-associated mutations, aberrant DNA methylation patterns, and severely compromised developmental potency. Towards a solution, we have developed a novel culture method which promotes naïve human pluripotent features during self-renewal. Using CDK8-kinase inhibition (CDK8i), we have identified a way to chemically-induce hyperactivation of enhancers, and we show that this stabilizes cell identity in the naïve state. Importantly, we find that the CDK8i-naïve human PSCs display normal karyotype and DNA methylation pattern at imprinted loci. Moreover, they have the capacity to form teratomas showing differentiation in all three germ layers, which is severely compromised in 2i-naïve human PSCs. We now investigate the developmental potency of CDK8i-naïve human PSCs using a micro-pattern-directed differentiation protocol. Based on preliminary data, we anticipate that CDK8i-mediated naïve human PSCs may offer a stable and more homogeneous alternative to primed human PSCs.

Biofunctionalized Hydrogels based on Benzene-1,3,5-tricarboxamides (BTAs) for Kidney Regeneration

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The development of renal organoids by *in vitro* culturing will aid in understanding nephrogenesis and could be useful in disease modelling. The current state-of-the-art hydrogel used for cell culturing is Matrigel, which has serious drawbacks, like batch-to-batch variations and is of pathogenic origin. Hence, there is an urgent need to develop a synthetic version of Matrigel. One way to do so, is by developing a mimic of the 3D micro-environment, the extracellular matrix (ECM), of human cells. The ECM is a multicomponent network that provides both structural support and biochemical information to its surrounding cells. To successfully reproduce nature's ECM with synthetic methods, both should be imitated accurately.

Aqueous synthetic supramolecular assemblies have great promise to mimic the ECM owing to their water-compatibility and dynamic nature. Besides, the monomeric building blocks could be functionalized with bio-active cues to easily introduce function using a modular approach. In this contribution, we propose a supramolecular hydrogel system based on a mixture of a monofunctional BTA (mBTA) and a bifunctional BTA (bBTA) building block (Figure 1). Herein, the monovalent BTAs can form one-dimensional fiber-like structures, while the bBTA could act as a crosslinker between the mBTAs to create a network. Mixing these components in desired ratio yields a hydrogel system with tunable mechanical properties.

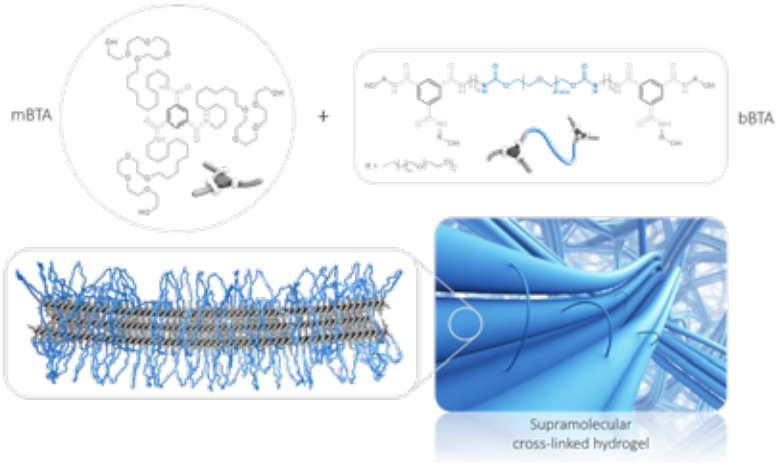

Figure 1: Chemical structures and illustration of mBTA and bBTA, which can form a supramolecular cross-linked hydrogel.

Ultimately, we envision to develop a BTA-based hydrogel system capable of steering nephrogenesis. To realize this, we plan to functionalize the supramolecular BTA hydrogels with bio-active signals such as peptides, proteins and carbohydrates.

SMARt engineering: Generation of isogenic Stem Cells and transgenic models utilising a non-integrative and autonomously replicating DNA vector system

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The capability of Stem Cells to differentiate into all cell types holds great promise for the future of gene therapy, disease modelling, drug screening and tissue engineering. Typically, the modification of Stem Cells is done by using integrating viral vectors. Although unquestionably the most effective gene delivery system in use today, their efficacy at gene transfer is tempered by their potential integration-mediated genotoxicity and their typical silencing, either directly at the pluripotent stage or during differentiation. An ideal vector for the genetic modification of cells should deliver sustainable levels of gene expression without compromising the viability of the host cell or its progeny in any way. Permanently maintained, episomal and autonomously replicating DNA vectors, which comprise entirely human elements, might provide the most suitable method for achieving these goals.

Here we propose a non-viral, non-integrating and autonomously replicating DNA vector system based on the use of Scaffold/Matrix Attachment Regions (S/MARs) as a novel technology to persistently genetically modify pluripotent and primary differentiated cells without causing any molecular or genetic damage.

For the first time, we demonstrate that non-viral episomal DNA vectors based on mammalian chromosomal elements can persistently genetically modify both murine and human pluripotent cells, while providing robust levels of transgene expression during random and directed differentiation, whilst avoiding vector loss or differentiation-mediated transgene silencing.

The vectors remained episomal and did not modify the stem cells' pluripotent properties, as demonstrated by the expression of pluripotency markers and the capability of the cells to differentiate *in vitro* into representatives of the three germ layers. Additionally, microarray data showing the minimal impact of the vector in the cells' transcriptome supports the generation of isogenic engineered cells.

As an ultimate demonstration, the vectors were challenged *in vivo* and were able to generate transgenic mice, whose transgenic organs – including highly regenerative hematopoietic tissues – showed high levels of transgene expression. Our data demonstrate that S/MAR DNA vectors can sustain episomal transgene expression from embryonic stem cells to fully differentiated tissues, organs and organisms, without molecular damage, silencing, vector loss or integration. This DNA vector system has multiple applications and is currently being used in our lab for cell therapy of hematopoietic, renal and degenerative ocular diseases. Further applications include the vectors' use to generate engineered organoids, as well as the generation of genetically modified iPSC for disease modelling and drug screening.

Generation of an in vitro neuromuscular junction at the cellular resolution

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The neuromuscular junction (NMJ) is a specialized cell-cell interface compartment essential for muscle contraction. Signal transmission occurs through the release of acetylcholine from the neuronal pre-synaptic cleft to its receptors on the muscular post-synaptic cleft 30 nm away. Perturbation to this intercellular communication system can have dramatic consequences as can be observed in many disorders such as ALS, Myasthenia Gravis and Spinal Muscular Atrophy all leading to a gradual loss of muscle motor capacity. Proper NMJ function relies on a highly organized architecture acquired through the interplay between neuron and muscle during development and homeostasis to strengthen axonal stability and muscle width. Deciphering the communication channels between these two cell types will reveal ubiquitous cellular cross-talk mechanisms necessary for our understanding of diseases.

In vitro systems represent optimal tools to study this intercellular communication if both neuronal and muscle component can be sufficiently matured at the single cell level. Currently, NMJ in vitro systems are limited to neurospheres and muscle bundles that are poorly differentiated and thus do not fully recapitulate in vivo architecture and function. Using microdevices/fluidics, hiPSCs and biomatrices, we developed an NMJ in vitro system with single cell resolution that is observable by fixed and live imaging and can be manipulated using all the current cell and molecular biology techniques. Moreover, the cellular maturity and architecture of both motor neurons and muscle cells mimic that of in vivo. This system allows us to monitor the developmental interplay occurring at the NMJ such as incorporations of receptors, accumulation of myonuclei at the NMJ and axonal projections.

Topological defects organize morphogenetic stresses

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During development, morphogenetic movements and forces are generated by collective actions of cells strongly adhering to each other. While some of the cellular events involved in generating those shape changes are well understood, such as conversion-extension or apical constriction, larger scale coordination is much less well understood. Recent description of epithelial layer using a nematic framework led to the hypothesis that topological defects that are present in cell layers can organize morphogenetic movements and forces to shape the embryo. Here, by confining C1C12 myoblast cells in small circular patterns, we create cell spirals, which have a +1 topological defect in their center. These spirals are rotating because of cell contractility, and at higher densities, transition to asters, another pattern with +1 topological defect. Thus, we can organize cell fluxes around +1 topological defects. In asters, stresses concentrate in the center, and drive protrusions of the cells into cylindrical protrusions of a few hundreds of microns. We thus show that by controlling the formation of topological defects, we can control the stress field in cell monolayers, and their protrusion.

Development of an *in vitro* three-dimensional colorectal tumor model for drug screening

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KEYWORDS: Tumor model, Cell-derived matrices, PLA Microparticles.

INTRODUCTION: The use of cell-derived matrices (CDM) is a promising alternative to decellularized tissues/organs as these are bioactive and biocompatible materials consisting of a complex assembly of proteins, growth factors and matrix macromolecules. 3D cell-cultured poly-lactic acid microparticles combined with macromolecular crowding (MMC) effect, offers the possibility to tailor-made bioactive materials for tissue engineering applications. We propose CDMs as potential colorectal tumor models for personalized medicine by mimicking tissue microenvironment properties [1].

METHODS: Poly-lactic acid (PLA) microcarriers were made by jetting this polymer through a coaxial needle into a coagulation bath [2] and coating them with Fibronectin to enhance cell adhesion.

Human adipose mesenchymal stem cells are seeded at 100,000 cells/mg of PLA microparticles [2] in 24 well plates and cultured for 10 days under stirring conditions. Obtained CDMs are characterized by quantifying total protein and DNA; topography by SEM; gene and protein expression by qRT-PCR, semi-quantitative immunofluorescence staining of key components of CDMs and mass spectrometry; and mechanical properties by nanoindentation. Besides, tissues are decellularized and particles are removed to be then recellularized in a perfusion bioreactor with colon cancer cells and cancer associated fibroblasts (CAFs) [3] to further characterize cell-cell interactions and their CDMs remodeling potential.

RESULTS: Addition of MMCs enhances protein deposition in CDMs. Fibrillary proteins collagen types I, III and fibronectin, which are highly present in colon tumor extracellular matrix (ECM), are over expressed after 10 days of culture (Figure 1A, B). Tissues density and size is greater, and final tissue stiffness is increased. Cells (Figure 1B) and microparticles were successfully removed from CDMs, and their recellularization (Figure 2A, B) and cancer CDM characterization are taking place to finally produce an *in vitro* tumor model to understand cancer promoting mechanisms, to develop a patient-specific drug screening platform and to identify potential therapeutic targets.

DISCUSSION & CONCLUSIONS: CDMs composition, like expression of fibrillar proteins of the ECM, and the tunable matrix stiffness provides reproducible tissue microenvironment. By repopulating the tissue microenvironment with cancer and stroma cells, we pretend to mimic native tissue structure (Figure 2C) to obtain promising platform for *in vitro* tumor model generation.

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REFERENCES

[1] Crotti S, et al. J Cell Phys, 2017, 232(5), 967–975

[2] Levato R, et al. Macromol Biosci, 2012, 12, 557–566

[3] Balkwill FR, et al. J Cell Sci, 2012, 125, 5591–5596

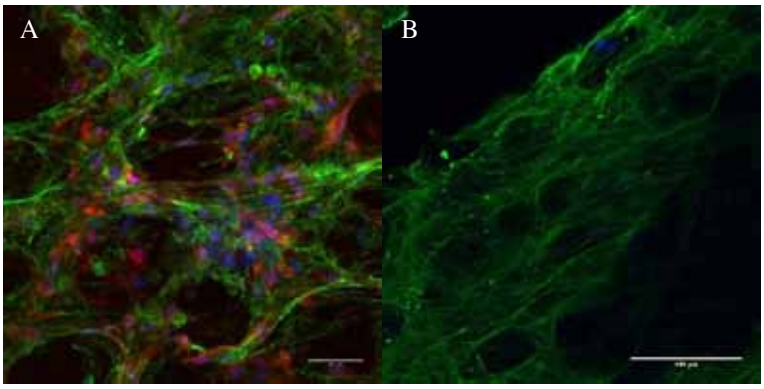

Figure 1. CDMs immunofluorescent staining. A) hAMSCs CDMs, immunofluorescent staining of collagen types I (red) and III (green) and nuclei (blue). B) Decellularized and microparticles free CDMs, immunofluorescent staining of cell cytoskeleton (red) fibronectin (green) and nuclei (blue). Scale bars = 100μm.

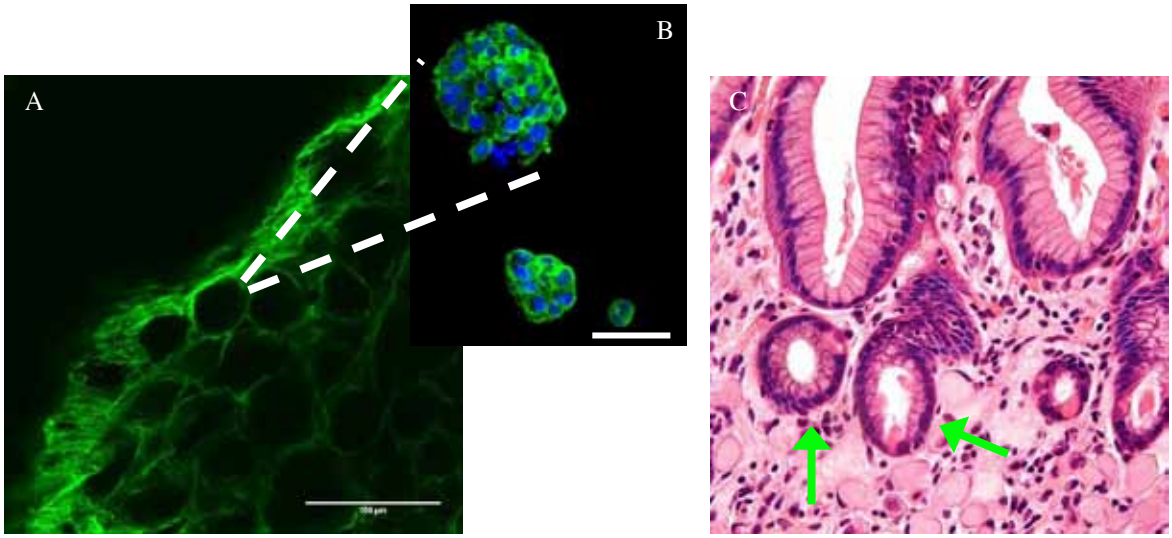

Figure 2. Colorectal cancer cells CDMs recellularization. A) CDM immunofluorescent staining of fibronectin B) Spheroids immunofluorescent staining of cytokeratin-20 (green) and nuclei (blue). C) Histological cut of Signet ring cell carcinoma. Scale bars = 100μm.

Generation and maintenance of robust cell fate proportions by FGF/ERK signaling

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The formation of complex multicellular systems requires the specification of specific proportions of differentiated cell types from uniform precursor populations. Current theories that emphasize the role of intracellular gene regulatory networks for cell fate decisions cannot explain how specific proportions of cell types are robustly established given unpredictable initial conditions in the precursor cells, and how these proportions can be maintained upon perturbations. We present an embryonic stem cell model for a cell fate decision of the mammalian preimplantation embryo, in which robust proportions of cells with epiblast-like and primitive-endoderm-like identity are specified from a wide range of experimentally controlled initial conditions. Using mutant analysis, we demonstrate how this population-level behavior of robust fate proportioning emerges from intercellular communication via fibroblast growth factor (FGF) signaling. The topology of the global intercellular regulatory network in our system consists of single-cell mutual repression circuits that are coupled in an inhibitory manner. This network architecture maps onto a generic class of models for which we have identified a population-based heterogeneous attractor as a dynamical mechanism of functional differentiation in multicellular systems. We experimentally verify a central prediction of this theory, that cell fate proportions will be re-established upon removal of one cell type. Collectively, we provide a new theoretical framework for robust fate proportioning in cell populations and show how it is implemented molecularly via FGF signaling. We expect that these concepts extend to a larger range of developmental fate decisions beyond those of preimplantation development, and that they will be informative for engineering cell fate proportioning in synthetic systems.

Studying Wilms' Tumor 1 (WT1) function in human kidney development and disease using human pluripotent stem cells-derived organoids and genome editing

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The Wilms' tumor 1 (*WT1*) gene was first characterized in the context of the childhood kidney cancer, Wilms' tumor, and was rapidly shown to play a role in the development of several organs. In disease, WT1 can be inactivated by mutations, acting as a tumor suppressor gene as in Wilms' tumors. So far, most of what is known about WT1 function in development and disease comes from mice models. Mice have many advantages as model organisms, but also shortcuts. For instance, the function of a protein and its regulation described can differ between mice and humans. Therefore, it is necessary to have models closer to humans in order to properly understand kidney development and disease.

Nowadays it is possible to have "human-like" models thanks to human pluripotent stem cells (hPSCs). These cells can be differentiated towards any kidney like-cells, recapitulating early events of human development thanks to the establishment of fast and efficient organoid differentiation protocols. In addition, they are an amenable source for genome editing, allowing us to use tools like CRISPR-Cas9 technology to study the role of WT1 in human kidney development and disease.

Therefore, in the lab we have generated *WT1* knock-out hPSC clones, heterozygote and homozygote, to study the impact of the lack of WT1 in early kidney development. Moreover, we have also generated *WT1* knock-in clones carrying a hotspot mutation, R394W, which has been described in patients with Wilms' tumors. These clones will give us insight on the effect of a specific patient-related *WT1* mutation in kidney development. In order to characterize the resulting phenotypes when these cell lines are differentiated towards kidney organoids, we have analyzed the mRNA and protein expression levels of renal development markers during renal vesicle stage and nephron structure stage. Preliminary data show that both the stable WT1 knock-out and knock-in mutations lead to an impairment of renal development.

In parallel, we have generated and characterized a WT1-GFP reporter cell line, which will enable us to easily isolate the different WT1+ cells that occur during kidney development, ranging from cells of the metanephric mesenchyme to podocytes, and further investigate the molecular mechanisms in which WT1 is involved depending on the cellular context by means of ChIP-Seq and RNA-Seq. Among other applications, the WT1-GFP reporter cell line will also allow us to perform straightforward time-lapse imaging studies and organotypic cultures with mouse embryonic kidney rudiments for better understanding of human kidney development.

Individual cell based modeling of limb bud outgrowth to predict shape and mechanical properties

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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. Here, condensation occurs simultaneously with the outgrowth and widening that shapes the limb bud. This distinct paddle shape results from the interplay between active developmental processes, individual cellular mechanical properties, cellular interactions and ectodermal molding [2]. These four factors are hypothesized to influence the cells mechanical state thereby controlling the condensation process, particularly to reveal mechanical stress. We propose an individual center-based cell model where mesenchymal cells are represented as individual agents that are mechanically characterized by a surface tension and interaction energy. The ectoderm surrounding the mesenchyme functions as a boundary condition, and is modeled as a visco-elastic material. Using this model, we are investigating how underlying biological processes, e.g. polarized proliferation or migration, help to achieve a paddle shaped limb bud and create mechanical stress. Additionally, we use AFM measurements to calibrate model parameters such as cellular stiffness. Furthermore, to validate the model, we compare measured tissue stiffness with model predicted values.

[1] N. Mahmoudifar (2012) Trends Biotechnol, 30(3): 166-176

[2] S. Hopyan (2017) Dev Biol, 429(2): 429-433

A sound-induced technology for spatial orchestration of functional large-scale microvascular networks

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Cell patterns are important for studying morphogenesis, unravelling biophysical mechanisms, and in the development of novel therapeutic approaches. Surface acoustic wave (SAW) technologies enable the generation of highly-complex spatially-orchestrated particulate systems (cells, organoids, inorganic aggregates). Patterns shape can be tuned on demand by varying a set of parameters, such as sound frequency, amplitude, chamber shape.

Here we propose the use of a SAW-based technology, based on Faraday waves principle, named 3D sound induced morphogenesis (3D-SIM)[1], that allows the generation of morphologically-relevant tissues through acoustic patterning. The method is fast, contactless, mild and cytocompatible, and can be used to assemble large numbers of spheroids in large volumes of fluid which can be crosslinked to stabilize the cellular patterns. In particular, we will use 3D-SIM to create precise and reproducible microvascular networks formed by interconnected and perfusable vessels.

To do that, spheroids formed by human umbilical vein endothelial (HUVECs) and human mesenchymal stem cells (hMSCs) are patterned in few seconds within an extracellular matrix-like hydrogel. Then, HUVECs sprouting from the patterned spheroids and self-organization into micro-vessels will finish the work. Hierarchically shaped vessels with a multiscale organization (micro-meso scale) can be integrated into fluidic chip where perfusion can be performed in a reproducible manner with a controlled flow rate. Additionally, to demonstrate the influence of cell local-density-enhancement mechanism in self-assembly process, acoustic patterning of single-cell populations (at different ratio HUVECs:hMSCs) will be used to generate gradients of vessels with tunable anisotropy in extracellular matrices. Results achieved through sound patterned vessels will be compared with vascular networks obtained via state-of-the-art microfluidics systems.

Like a music director, 3D-SIM orchestrate the cells by playing the music of mild physiological conditions that recapitulate natural complexity of living tissues.

Bulk Actin Dynamics Drive Phase Segregation in Zebrafish Oocytes

Shayan Shamipour, Roland Kardos, Shi-Lei Xue, Björn Hof, Edouard Hannezo, and Carl-Philipp Heisenberg

Segregation of maternal determinants within the oocyte constitutes the first step in embryo patterning. In zebrafish oocytes, extensive ooplasmic streaming leads to the segregation of ooplasm from yolk granules along the animal-vegetal axis of the oocyte. We show that this process does not rely on cortical actin reorganization, as previously thought, but instead on a cell-cycle-dependent bulk actin polymerization wave traveling from the animal to the vegetal pole of the oocyte. This wave functions in segregation by both pulling ooplasm animally and pushing yolk granules vegetally. Using biophysical experimentation and theory, we show that ooplasm pulling is mediated by bulk actin network flows exerting friction forces on the ooplasm, while yolk granule pushing is achieved by a mechanism closely resembling actin comet formation on yolk granules. Our study defines a novel role of cell-cycle-controlled bulk actin polymerization waves in oocyte polarization via ooplasmic segregation.

Quantifying invasion and 3D traction forces during multicellular angiogenic sprouting upon CCM-2 loss shows a ROCK-dependent increase in contractility and invasiveness

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The relevance of angiogenesis, the process of growing new blood vessels, for tissue engineering, cancer, and vascular disease is beyond doubt. Cerebral Cavernous Malformation (CCM) is one such disease characterized by lesions presenting structural defects similar to tumor blood vessels. ROCK-1 dependent intracellular tension is one factor responsible for CCM lesions. Further, cellular force generation is now widely known to underlie angiogenesis and is mediated by both chemical and physical (mechanical) cues.

We report here on a three-dimensional (3D) live invasion assay utilizing natural collagen and synthetic modular polyethylene glycol (PEG) matrices to characterize the relationship between cellular traction-induced deformations and angiogenic invasion by CCM-2 depleted human umbilical vein endothelial (HUVEC) cells. HUVECs were cultured in a monolayer on substrates containing 200 nm fluorescent fiducial markers for live imaging of matrix deformations. Confocal fluorescence microscopy imaging was performed during overnight invasion and image stacks were registered to quantify 3D deformations and invasion dynamics. Our novel nonlinear inverse methodology that uses a physics-based regularization term was used to compute 3D traction forces around invading angiogenic sprouts for the first time. Angiogenic invasion was quantified across naïve, CCM depleted, CCM+ROCK-1 depleted, and CCM+ROCK-2 depleted endothelial cell types. Chimera invasion assays combining these silenced cell types with naïve HUVECs were studied to understand recruitment by diseased cells during CCM lesion formation.

Our results show a clear increase in invasive capacity of HUVECs upon CCM depletion in degradable PEG matrices. CCM lacking HUVECs are also able to recruit naïve cells towards a significant increase in invasiveness. These effects on invasiveness upon CCM loss are partially recovered through ROCK-1 silencing, but not ROCK-2. Recovered preliminary 3D traction forces around invading sprouts additionally suggest an increase in force generation during invasion upon CCM loss, which is reduced upon ROCK-1 depletion. Our assays enabled us to study invasion in combination with force generation, thereby highlighting the role of ROCK-dependent contractility on CCM. We demonstrate for the first time that our novel 3D TFM workflows allow for a mechanical characterization of the angiogenic and invasive response to pathogenic cell types as a screening tool for improved disease modelling.

Examination of proliferative activity and cell viability during neurogenic differentiation from iPSCs in 3D culture of cerebral organoids.

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3D cultivation in the organoids composition is a modern method that allows reproducing the stages of early ontogenesis most approximate to processes *in vivo*. This method is relevant in application to the induced pluripotent stem cells (iPSCs) cultivation, which is a main source of neurogenesis *in vitro*.

The cultivation of iPSCs and differentiation into organoids was performed according to the protocol (Eremeev et al., 2019) in the laboratory of cell biology of the Federal Research and Clinical Center for Physical-Chemical Medicine (Moscow, Head of department M.A. Lagarkova). In the experiment, 3 groups of iPSC lines derived from donors were examined: with spinocerebellar ataxia (SCA, group 1), with Huntington's disease (HD, group 2) and from healthy patients (group 3, control).

Performing analysis of organoids included routine histological methods (hematoxylin and eosin), immunocytochemical and transmission electron microscopy. In the process of studying organoids was revealed the formation of primitive neural tubes - “rosettes” formed by meduloblasts or ependym-like cells with dense intercellular contacts.

The received index ki67 of the examined groups: 1 - 61.29%; 2 - 57.37 ± 1.1%; 3 (control) - 35.4 ± 22.78%. In the organoids (inside groups themselves) with d> 900 μm, cells dead in the central part and survived at the periphery, where $GFAP^+$ (some $SOX1^+$) and $Ki67^+$ cells prevailed in the cortical zone. For organoids d<900 μm, there is a diffuse expression of cell markers over the entire section area.

Thus, the possibility of proliferation and divergent neuro- and gliocytic differentiation in the model conditions of 3D cultivation *in vitro* is shown. Cell death in the central part of large spheroids is most likely associated with metabolic deficiency in this area, which may be an objective limitation of the model used. Metabolic deficiency is probably associated with the size of cell aggregates caused by the timing of cultivation. All organoids retain proliferative activity, regardless of origin.

Superresolution architecture of cornerstone focal adhesions in human pluripotent stem cells.

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Pluripotency is maintained by tight control of transcriptional programmes guided upstream by external cues such as growth factors and extracellular matrix (ECM). However, the role of cell adhesion remains poorly defined. Previously we have observed that human pluripotent stem cell (hPSC) colonies adhere to ECM via large and stable cornerstone focal adhesions. In this project, we elucidate the three-dimensional architecture of the of cornerstone adhesions in hPSCs by using interferometric photo-activated localisation microscopy. We report vertical and horizontal compartmentalisation of focal adhesion proteins previously unreported in other cell types: 1) integrin β5 and talin are occupying with higher abundancy the edges of focal adhesions compared to the centers, 2) vinculin is localised on higher z-plane and displaying inverted orientation compared to the previously reported and 3) actin and α-actinin are present in two separate layers on top of the focal adhesions. Finally, we report the z-localisations of two focal adhesion linked proteins kank1 and kank2. In addition, we show that the depletion of kanks leads to reorganisation of the focal adhesions in hPSC colonies.

Interacting Cellular Meshes: A new theoretical and computational framework to investigate tissue reorganisation during morphogenesis

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We propose a novel theoretical and computational framework to help answer a fundamental question in developmental biology, namely how does the interplay between cell mechanics, cell-cell adhesion and cell-fate decision lead to the shaping and organisation of a tissue? Several models in the past were introduced to partly tackle this problem. For instance, Pott's models reproduce tissue reorganisation and predict tissue layering in an embryo. More recently, vertex models, first developed to describe 2D epithelia and later generalised to general 3D confluent tissues, have been successfully employed to describe tissue growth, to infer mechanical properties of cells, or to characterise tissue rheology. Here, motivated by key processes in morphogenesis where cells have to separate from one another, such as blastulation or gastrulation, or where cell shape cannot longer be described by a polyhedron, we introduce a novel approach based on the description of cells as independent 3D deformable surfaces interacting with one another. In our framework, these surfaces are subject to active, elastic and viscous stresses, representing the inner cytoskeletal dynamics, and bear a number of internal variables characterising cell fate or polarity. Cells interact with one another through adhesive forces, whose strength and space distribution depend on cell fate and polarity. Computationally, we describe each cell as a finite element mesh interacting with neighbouring meshes. We also describe cell division by splitting the cell mesh into two, with a direction that may depend on inner variables such as polarity. The interplay between cell fate and polarity with cell mechanics and adhesion forces as well as cell division determines the dynamics and final organisation of the tissue. Here, we will show preliminary results on the types of tissue shape and organisation that can be obtained by modulating this interplay in different ways. We will focus on small organoids based on the division of a single stem cell.

Non-viral episomal S/MAR DNA vectors for the persistent genetic engineering of hiPSCs and their progenies

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The use of patient-derived induced pluripotent stem cells (iPSCs) is a promising tool for cell therapies, tissue engineering and drug screenings. They combine the advantages of both, a decreased immune reaction due to the derivation from the individual patient, and the characteristics of stem cells with their self-renewing capacity and competence to differentiate into all kinds of cell types.

However, there is often the need to genetically alter or repair these cells, in order to obtain functional tissues or before they can be reintroduced to the patient. Modification of cells in this context is mainly done using integrating viral vectors or Crispr/Cas9. While being highly efficient at modifying cells, they harbour the risk of integration-mediated genotoxicity or off target effects.

Our lab has developed an alternative DNA vector system based on a Scaffold Matrix Attachment Region (S/MAR) and a minimally sized Nanoplasmid™ as a novel technology to persistently genetically engineer human cells without causing any molecular or genetic damage. This powerful tool offers the functionality of viral vectors while providing advantages such as easy and economical production, no limitation in transgene size and minimal impact on the host cell. We have previously shown that S/MAR DNA vectors can provide sustained transgene expression while remaining episomal and have utilised them to genetically modify a variety of cell types.

Human induced pluripotent stem cells (hiPSCs) are typically refractory to genetic modifications due to the stringent control mechanisms that their nature as progenitor cells entails. Here, we show that we can reprogram both fibroblasts and cells isolated from the urine to hiPSCs, efficiently deliver our S/MAR DNA vectors to these hiPSCs and generate stable cell lines without altering their stem cell properties. These stable genetically modified hiPSCs maintain transgene expression throughout expansion, passaging and freeze-thawing cycles. Differentiation of these modified hiPSCs into progenies of the three germ layers demonstrates the sustained transgene expression throughout and without hindering this process. Finally, we show the use of S/MAR DNA vectors in patient derived hiPSCs for long-term genetic correction of a therapeutic gene and are currently working on the engineering of functional kidney organoids. Together, this work shows for that our improved S/MAR DNA vector platform can be applied to genetically engineer hiPSCs from different sources without altering their characteristics, opening possibilities for their application in tissue engineering.

Supramolecular Hydrogels For Kidney Organoid Development

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Over the last decade, numerous protocols have been developed for generating kidney organoids from human-induced pluripotent stem cells (hiPSCs).[1,2] The majority of these kidney organoids lack in macroscale organization, extensive vasculature, and mature nephron segmentation. These limitations prevent organoids from properly modelling the adult kidney. One major reason for these shortcomings may be that these organoids are cultured in suspension-culture or on non-bioactive polymeric substrates. These environments do not properly recapitulate the native extracellular matrix, which plays a major role in hall-mark developmental processes, such as nephrogenesis.

Here, we developed a synthetic extracellular matrix based on ureido-pyrimidinone (UPy)-molecules to create a more relevant environment for kidney organoids. These UPy-molecules are capable of self-assembling into fibrous aggregates due to quadruple hydrogen bonding and π-π interactions. The non-covalent nature of these interactions allows for the formation of a hydrogel network with modular properties in terms of biological complexity and dynamics.[3] Tuning of these hydrogels is possible by adding different UPy-based molecules with various properties, that seamlessly incorporate into the network in a stable manner.[4]

To demonstrate the merit of introducing bioactive UPy-additives, two material conditions are used: (1) UPy-hydrogels without bioactive UPy-additives, and (2) UPy-hydrogels with UPy-cRGD additives. Renal progenitor cells were produced based on the Takasato et al. protocol[2], and cultured on top of UPy-hydrogels. While renal progenitor cells had a tendency to cluster, it was evident they were better capable of interacting and spreading on UPy-hydrogels when UPy-cRGD additives were incorporated. As such, UPy-hydrogels with UPy-cRGD additives were used to encapsulate kidney organoids based on the Takasato et al. protocol.[2] Organoids remained viable in all conditions and showed evidence of tubule formation, which indicates nephrogenesis. Further characterization was performed using immunofluorescent staining of E-cadherin, LTL, and NHPS1, confirming nephron formation and evidence of tubule segmentation in organoids encapsulated in hydrogels.

References

1. Morizane, R. *et al.* Nephron organoids derived from human pluripotent stem cells model kidney development and injury. *Nat. Biotechnol.* **33,** 1193–1200 (2015).
2. Takasato, M. *et al.* Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature* **526,** 564–568 (2015).
3. Hendrikse, S. I. S. *et al.* Controlling and tuning the dynamic nature of supramolecular polymers in aqueous solutions. *Chem. Commun.* **53,** 2279–2282 (2017).
4. Hendrikse, S. I. S., Spaans, S., Meijer, E. W. & Dankers, P. Y. W. Supramolecular Platform Stabilizing Growth Factors. *Biomacromolecules* **19,** 2610–2617 (2018).

Exploring the Cell Jamming Phase Diagram Using a 3D Deformable Cell Model

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In many biological processes such as wound healing, embryonic morphogenesis, gastrulation and cancer metastasis, a transition occurs where the tissue changes from a solid-like to a fluid-like state, in which cells migrate collectively. This transition resembles the granular jamming transition and the closely related glass transition, which led to the proposal of a cell jamming phase diagram controlled by mechanical cell parameters [1]. Recently, it was shown that upon loss of cell-cell adhesion and tissue confluence, cells unjam [2], while other research suggests the importance of cell-cell contact dynamics [3].

Computational studies using vertex models have improved our understanding concerning the jamming transition rapidly. These studies have shown that cell jamming is dependent on the balance between adhesion and cortical tension, the cell's active motility, and cell-cell alignment [4, 5]. However, current models fail to accurately represent cell-cell contact dynamics and do not naturally account for non-confluent tissue densities and open boundaries. In this work, we address these limitations using a 3D deformable cell model, which represents the shape of each individual cell rather than the cell-cell interface, and captures cell-cell interactions by explicit adhesive forces (Fig. 1) [6].

Using this computational framework we study, for the first time, the effect of tissue non-confluency and cell-cell contact dynamics on the jamming transition, and the influence of this transition on cells at the tissue boundary. Our results are compared against results in literature. We show that our model can replicate natural gap closing ability of cells related to wound closure by introducing an active cell orientation term.

Figure 1: Cells confined in a cylinder simulated using the 3D deformable cell model.

References

[1] Sadati et al. (2013). *Differentiation*, 86(3):121-125. [2] Petridou et al. (2019). *Nat. Cell. Biol.*, 21(2):169-178. [3] Doyle et al. (2015). *Nat Comm*, 6(1): 8720. [4] Bi et al. (2016). *Phys. Rev. X*, 6(2):021011. [5] Giavazzi et al. (2018). *Soft Matt.*, 14(18):3471-3477. [6] Odenthal et al. (2013). *PLoS Comp. Biol.*, 9(10): 1-14

A generalized clutch model to explain cell adhesion mechanics

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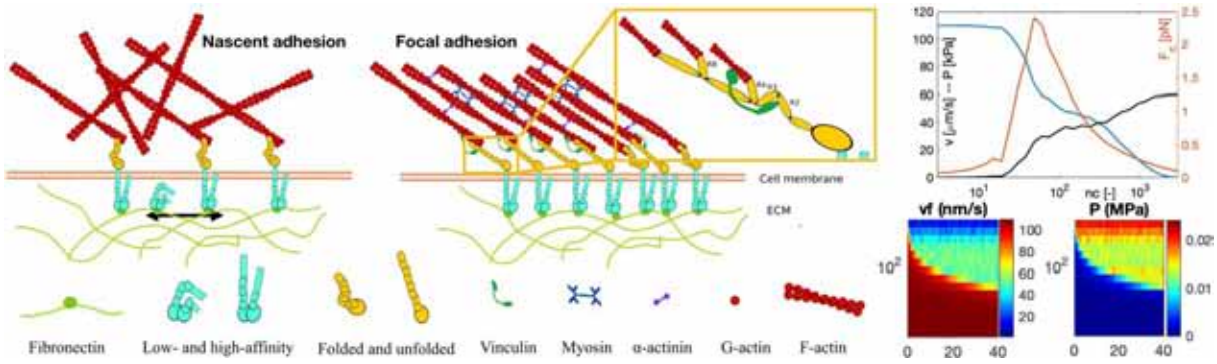
Integrin-based cell adhesion is a key mechanism in a large number of physiological processes and diseases. The composition and nanoscale organization of adhesion complexes have shown that the integrin-talin-actin chain plays a fundamental role in the formation of small nascent adhesions and further maturation into focal adhesions. The role of ligand spacing and substrate rigidity has been also clearly demonstrated. The clutch model has been widely used to describe how cell adhesion works. However, they have not been capable of rationalizing many of the aspects described above; probably, because current clutch models are built under a number of simplifications of the adhesion's mechanisms. Here, we extend the classical clutch model with a detailed description of the talin rod as well as a space-dependent ligand distribution. We will show the minimal building block and adhesion length of focal adhesion. Following the same computational model, we will also show that focal adhesions form for stiff substrates for low spacing while they form in soft substrates for large spacing, replicating previous experimental results and proposing a model that unifies most aspects disused in cell adhesion architecture and turnover.



Lung-on-a-chip microphysiological systems for studies of host-pathogen interactions in Tuberculosis

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Introduction

Most host-*Mycobacterium tuberculosis* (*Mtb*) encounters lead to latent infections in which the bacteria exist in a poorly characterized host-pathogen equilibrium [1]. The widely-used i*n vitro* model of batch culture infections of macrophages with *Mtb* captures neither the effects of cellular individuality nor the complexity of the lung environment including the role of other cell types such as alveolar epithelial cells (AECs) and the diversity of possible host-pathogen interactions, especially at later stages of infection.

Methods

Here we report on experiments that successfully reconstitute the murine alveolar interface entirely from primary cells in a lung-on-a-chip microfluidic device. This builds upon the previously reported human lung-on-a-chip system [2] by incorporating aspects of the innate immune system including alveolar and bone-marrow derived macrophages and monocytes. This system is then inoculated at low multiplicity of infection (MOI) with *Mtb*, and the progression of the infection is monitored in real-time through a combination of live-cell time-lapse microscopy (7-10 days) followed by confocal microscopy.

Results & Discussion

This “bottom-up” approach allows for imaging and temporal tracking of the dynamics of immune cells and their interactions with apoptotic tissue cells and pathogens and enables us to closely follow the chain of events from initial infection in a manner currently impossible in infection studies in animal models. It has revealed several insights into the roles of AECs in orchestrating the response to first contact with *Mtb*. First, we observe first-contact infection of AECs even in the presence of macrophages. If infected, they provide a permissive environment for a more rapid initial growth of single bacteria into clumps or long intracellular cords and do not restrict Mtb growth even when pre-activated with Interferon-gamma. However, pulmonary surfactant secreted from type II AECs can significantly attenuate initial Mtb growth and improve the ability of macrophages to kill first-contact macrophages. This phenotype can be partially recapitulated using artificial surfactant mixtures for neonatal infants, suggesting a role for hydrophobic proteins SP-B and SP-C in mediating this interaction. In contrast, single Mtb bacteria grow *intracellularly* into serpentine cords at the Air-Liquid Interface (ALI), growth is rapid especially in the absence of surfactant. We find that these cords can also grow *intercellularly* between AECs where they are effectively masked from patrolling immune cells. This is also a likely mechanism for bacterial aggregates to penetrate deeper into the lung interstitium These observations underlie a key role for AECs in the early stages of *Mtb* infection and informed a set of animal experiments where we observed similar corded structures in immune cells extracted from mice infected with Mtb via the aerosol route at day 7 post infection.

Outlook

With further development, this is an ideal system to study how epithelial/stromal cells and tissue-resident memory cells interact with and influence the innate immune system in the distal lung in both homeostasis and in response to infections.

References

1. C.J. Cambier *et al. Cell*, **157**, 1497-1509 (2014).

2. D. Huh *et al*. Science **328**, 1662-1668 (2010).

Development of an in vitro microfluidic model of thymocyte extravasation

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The thymus is the site of T-cell development and is therefore a key organ in the development of a functional immune system. However our understanding of the functional role of the human thymus microenvironment in the various steps to T-cell selection and maturation remains fragmented. In order to develop into fully functional T-cells, hematopoietic stem cells (HSC) are recruited from the bone marrow to the thymus. While studies have shown the importance of the thymic stroma in thymocyte recruitment and development, most research has focused on rodent thymi. Microfluidic devices offer a unique system to investigate key biological questions using human cells. Their flexible and tunable nature give the user a greater level of control than traditional 2D or 3D culture systems. Here, we utilized current microfluidic methods to investigate the role of the thymus microenvironment on T-cell progenitor migration using primary human cells. Briefly, microfluidic devices were seeded with human umbilical vein endothelial cells (HUVEC) to form an endothelial monolayer. Bulk thymic stromal cells (TSC) were then added to a separate channel with fibrin gel separating the HUVEC and TSC populations. HSCs were added to the endothelial channel and were allowed to extravasate over a 24-hour period before imaging with confocal microscopy. Preliminary results indicate that HSC extravasation was increased when co-cultured with TSCs as compared to controls, demonstrating the importance of signals from the thymus microenvironment for thymocyte seeding. Separating the bulk TSCs into the constituent epithelial and mesenchymal populations showed that these populations have decreased recruitment potential when grown on their own compared to when co-cultured. Further development of this microfluidic model will allow us to better understand the biology of the human thymus microenvironment and the role that these cell types play in the development of the immune system.

Word count: 293

Spatial Regulation of Proneural Gene Expression in the Hindbrain

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The precise control of tissue patterning is essential for the diversification of cell fates. This is particularly important at later stage of neural development to ensure the production of diverse neuronal and glial subtypes from a rapidly depleting pool of progenitors. In our lab, the zebrafish hindbrain is used as a model to discover mechanisms involved in the patterning of complex tissue. One interesting characteristic of its development is the presence of anterior-posterior (AP) patterning of proneural genes within each hindbrain segments, independent of the more commonly described dorsal-ventral patterning that is observed throughout in the neural tube. Previous works have identified that Fgf20-signalling produced by subsets of matured neurons in each of the segment is required for the inhibition of proneural genes upregulation in the segment centre (Gonzalez-Quevedo et al., 2010). However, the mechanism by which the Fgf20-signalling regulates the patterning remains obscure.

By using fluorescent in-situ hybridisation chain reaction (HCR) in combination with 3D image analysis and quantification, we analysed the spatial relationship between Fgf20-neurons, the expression of etv5b - a direct transcriptional target of Fgfr-signalling - and the expression of the proneural gene neurog1 in both wildtype and mutant embryos. Here show that Fgf20 is a short-range signal in the hindbrain that activates Fgfr-signalling pathway only in progenitors that are in the proximity of the source. To further investigate how this property of the ligand together with the positioning of the signalling source influence the patterning of the proneural genes, we are generating mutants to manipulate the range of Fgf20-signalling and the position of the signalling source. Interestingly, preliminary analysis from temporally-controlled homogenous activation of Fgfr suggests that specific, local sources of Fgf-signalling are necessary for the differential upregulation of proneural gene among progenitors in hindbrain segments.

Integrated *in vitro* model of tumour vasculogenesis and cancer cell intravasation

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Angiogenesis is known to enhance tumour growth and metastasis spread by supplying nutrients and waste removal, as well as providing a route for cancer cells to disseminate. As a result, several anti-angiogenic therapies aim at starving the tumours by suppressing tumour vascularisation and abrogating local tumour angiogenesis. However, the treated tumours have been shown to relapse faster suggesting that the tumours rely on other vasculogenesis pathways for its regrowth. There are ongoing efforts to replicate the physiology of human tumour tissue using three-dimensional *in vitro* culture models; however, few systems can capture the full range of authentic, complex *in vivo* events such as neovascularisation and intravasation. With the advancement in the generation of 3D functional microvascular networks *in vitro* that has the capability to recapitulate biological complexity, we aim to create an integrated *in vitro* model of tumour vasculogenesis and cancer cell intravasation. This is achieved by employing three-channel 3D microfluidic devices fabricated in-house. To form 3D tumour microvascular model, cancer spheroid, normal human fibroblasts and human endothelial cells were resuspended in an extracellular matrix (ECM). We observed tumour vascularisation and cancer cell intravasation only in ECM which is a hybrid of fibrin, collagen and matrigel hydrogels. As a next step, we would characterise both morphologically and mechanically the hydrogels, and quantify cancer cell migration. This is an attempt to mimic the complexity of tumour microenvironment *in vitro* that could be used to advance our understanding of cancer metastasis, which burgeons from a vascularised tumour.

Engineered cell-derived matrices as 3D tumor stroma models

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Native tissue microenvironments display three-dimensional architectures that control tissue function and pathological alteration (for example, tumor-associated collagen signatures [1]). Numerous efforts have been deployed to develop simple, cost-effective and tunable materials that can replicate in vitro the high structural and molecular complexity of the natural ECM and its pathological presentation. Native-like ECM models of natural composition can be obtained in vitro from culture of sacrificial cells. We apply engineering approaches to control cell derived matrices (named engineered cell-derived matrix, eCDM) deposition as aligned, ordered and random fibers, mimicking native pathophysiological stroma [2].

Sacrificial NIH3T3 fibroblasts are seeded at high confluence on top of guiding templates (arrays of micrometric ridges) to define eCDM growth. Characteristic fiber distributions can be produced to mimic patients' ECM histological appearance and tumor associated collagen signatures. After culture for 8 days, mild decellularization generates cell free structures rich in collagen and fibronectin. Finally, the liberated matrix is repopulated with cancer cells, seeking two main objectives. First, we aim to characterize the effect of eCDM ultrastructure on cell morphology, motility and adhesion, comparing different distribution of fibers (isotropic, anisotropic). Second, we intend to sustain native cell morphologies to investigate cancer progression. Employing live microscopy and fluorescent cell membrane labeling (PKH67 from Sigma Aldrich), we assess cell position and morphology and measure cell morphodynamics with particle tracking tools in ImageJ (Fiji) [3]. Using multiphoton excitation, we also characterize eCDM collagenous structure without prior fixation or labeling by second harmonic generation microscopy. Fiber recognition algorithm CT-FIRE is employed to measure single fiber metric and distribution [4].

Overall, we propose an approachable technique to obtain in vitro ECM models of complex composition, high biomimicry and tunable geometry, which elicit a native-like response in cultured target cells. Moreover, exploiting primary cells as matrix generating and target cultures, it is possible to generate patients' specific models to be employed for drug screening applications.

Keywords: Extracellular matrix, cell-derived matrix, 3D in vitro model, biomimetic, cancer.

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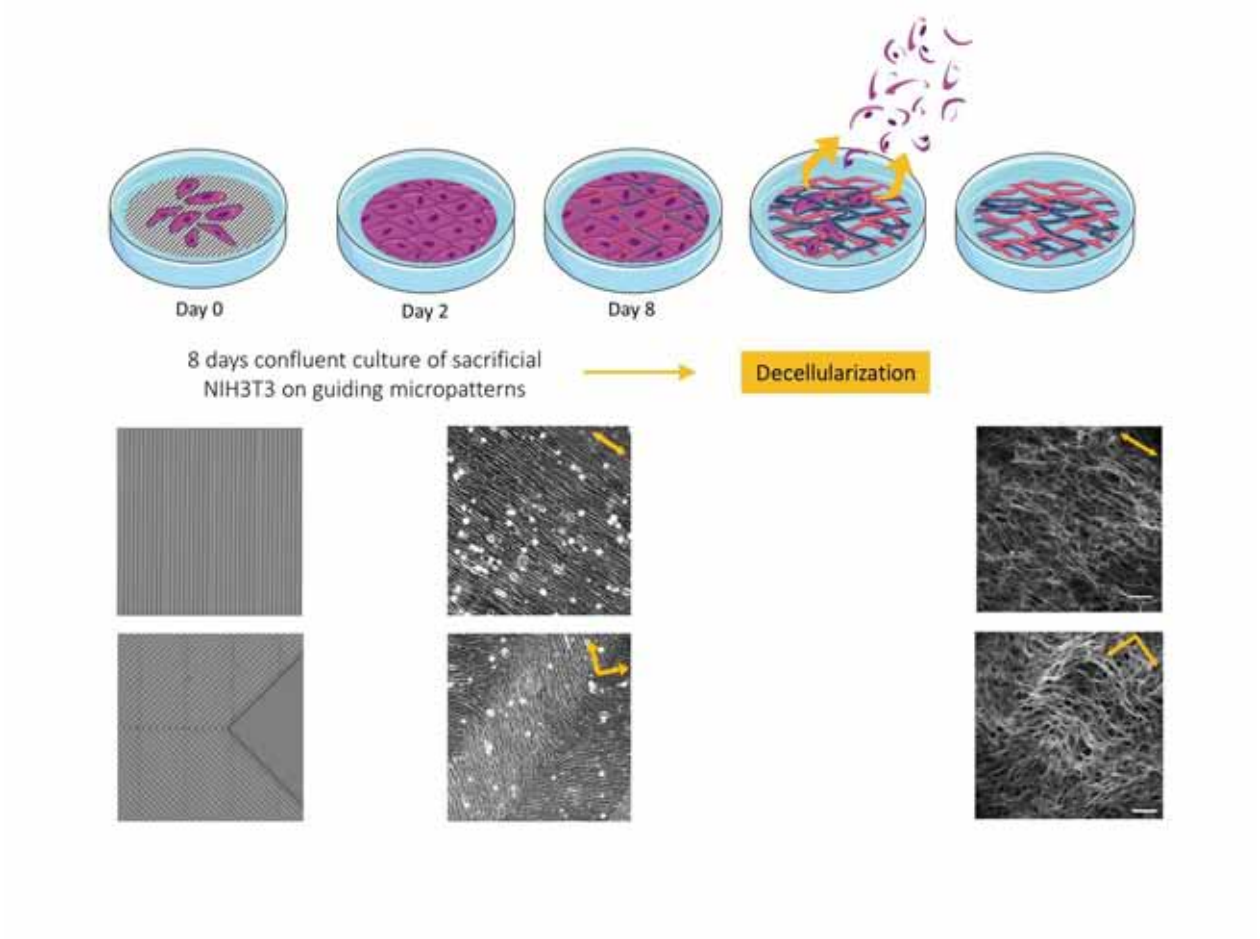
References:

[1] K. Eliceiri, M. Conklin, J. Bredfeldt, T. Mackie, Y. Liu, and P. Keely, “Automated quantification of aligned collagen for human breast carcinoma prognosis,” *J. Pathol. Inform.*, vol. 5, no. 1, p. 28, 2014.

[2] D. Caballero and J. Samitier, “Topological Control of Extracellular Matrix Growth: A Native-Like Model for Cell Morphodynamics Studies,” *ACS Appl. Mater. Interfaces*, vol. 9, no. 4, pp. 4159–4170, Feb. 2017.

[3] J. Schindelin *et al.*, “Fiji: An open-source platform for biological-image analysis,” *Nature Methods*, vol. 9, no. 7. pp. 676–682, Jul-2012.

[4] J. S. Bredfeldt *et al.*, "Computational segmentation of collagen fibers from second-harmonic generation images of breast cancer," *J. Biomed. Opt.*, vol. 19, no. 1, p. 016007, Jan. 2014.



A Study on Actomyosin Influencing the Formation of Spatial Asymmetries in Early Embryonic Organoids

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Biomechanical properties of actomyosin are central to understanding the process of multicellular morphogenesis as they coordinate local cellular activities as well as global tissue movements during embryogenesis. During gastrulation, actomyosin-dependent cell-cortex tension directs cell sorting and germ layer organization at cellular level. Driving forces are generated by myosin contractility, which can tense and deform a group of cells and is modulated by the geometry of the embryo. The tissue behaviour, in turn, is highly dependent on the material properties of actin, on how strongly components are connected and on the influence of neighbouring tissues. Emergence of a coordinate system within the developing embryo can thus be construed as a coordination of actomyosin activities within an embryo-specific geometrical arrangement of cells.

We have recently shown that 3D cell aggregates from different species (mouse embryonic stem cells and zebrafish blastula cells) generate the major body axes even in the absence of any extra-embryonic information. In these embryonic organoids, cells undergo actomyosin mediated sorting and movements, still germ layer organization is accomplished. Using these organoids, as a minimal alternate system, we aim to understand early development in embryos as an emergent phenomenon of the self-organization of cells.

Combining light-sheet imaging with germ layer-specific transgenic lines we are now gaining some insights into how the activities of actin and myosin are translated into these 3D structures in space and time and to which extent they influence the formation of spatial asymmetries within otherwise equivalent groups of cells.

Lacrimal Gland on Chip Derived from Induced Pluripotent Stem Cells

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Lacrimal gland (LG) is an exocrine gland that produces the aqueous layer of the tear film. The LG secretion offers protection from infections, contains growth factors and lubricates and moistens the ocular surface. Dry eye disease due to LG dysfunction leads to discomfort, visual problems, inflammation, infections and eventually loss of vision. Treatment options such as artificial tears focus on relieving the symptoms however fail to solve the underlying problem. Human induced pluripotent stem cells (iPSCs) have the capacity to differentiate into functional specialized cell types, and can be employed to construct 3D functional LG tissues *in vitro*. The use of microfluidics in tissue engineering enhances the recapitulation of native microenvironment by allowing specific control of physical and biochemical parameters. This study aims to develop a 3D LG construct from iPSCs in a microfluidic organ-on-a-chip system. In human LG development, the periocular mesenchyme (POM) secretes FGF10, which results in thickening of the conjunctival epithelium (CE). CE invades the POM and BMP7 induces further branching. Here we focus on mimicking the native development of the gland by differentiating iPSCs separately into POM and CE cells. POM cells are derived from iPSCs by inhibiting the WNT and TGFβ pathways. Multi zonal ocular cells (MZOCs) derived from 2D matrigel cultures of iPSCs consist of four zones of ocular cells and can give rise to CE cells. These CE cells are isolated from the MZOCs with fluorescence activated cell sorting (FACS). Emergence of POM and CE cells is confirmed with immunostaining and gene expression analysis of the marker proteins. The differentiated cells are distinctly encapsulated in a hydrogel and placed into a custom developed microfluidic chip where they are fed with different media and induced with FGF10 and BMP7 to promote interaction and branching. The tear secretion is assessed with enzyme linked immunosorbent assay (ELISA). The ultimate goal is to generate a functional tear-secreting gland.

Magnetic bead-based immunosensing platform for *in-situ* detection of secreted cytokines in response to oligonucleotide treatment

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Abstract

Nowadays, *in vivo* animal models and *in vitro* two-dimensional (2D) cell cultures are the basis for the development of new therapeutic strategies. These, however, entail ethical problems such as experimentation with animals and difficulties in extrapolating the results to human conditions because of the differences between species (Ronaldson-bouchard, K., & Vunjak-novakovic, G., 2018). These configurations do not consider the complexity of the physiological microenvironment in which cells grow, nor faithfully reproduce the biological intricacy of human diseases. Instead, the combination of three-dimensional (3D) tissues with biosensors allows to detect cellular response to external stimuli mimicking the real environment of the tissue *in vivo* (Hernández-Albors et al., 2019).

In the present work, we have integrated 3D skeletal muscle constructs from murine myoblasts treated with antisense oligonucleotides (AON) with immunosensors based on magnetic beads (MB) for the detection of different pro-inflamatory biomarkers related with neuromuscular diseases as the Interleukin 6 (IL-6), the Keratinocyte chemoattractant (KC) and the Monocyte chemotactic protein 1 (MPC-1) (Peake & Della, 2015), using a transwell membrane setup.

In order to obtain a 3D engineered muscle, C2C12 cells have been encapsulated in Gelatin Methacryloyl (GelMA)-based hydrogel combined with non-degradable carboxymethyl cellulose (CMCMA) and the photoinitiator lithium phenyl (2,4,6-trimethylbenzoyl)phosphinate (LAP) resulting in highly aligned myotubes inside the scaffold.

The development of the biosensors has involved the optimization of an immunoassay on the superparamagnetic microbeads surface for a selective and specific detection of the target cytokines. After a capture step, MBs have been incubated with a detection antibody followed by an enzymatic conjugation. Finally, colorimetric signal has been achieved by substrate addition. The intensity of the acquired signal is directly related with the amount of the biomarker secreted by the tissue.

To this end, the optimal concentration of the primary antibody, the second biotinylated antibody and the enzymatic tracer have been established, reaching results with a LOD in the range of ng mL^{-1}.

The technology described, enables the continuous monitoring of the tissue response against the treatment with different AON and expands our knowledge about muscle metabolism.

References:

Zhang, B., Korolj, A., Lai, B. F. L., & Radisic, M. (2018). Advances in organ-on-a-chip engineering. Nature Reviews Materials, 3(8), 257–278. https://doi.org/10.1038/s41578-018-0034-7

Hernández-Albors, A., Castaño, A. G., Fernández-Garibay, X., Ortega, M. A., Balaguer, J., & Ramón-Azcón, J. (2019). Microphysiological sensing platform for an in-situ detection of tissue-secreted cytokines. Biosensors and Bioelectronics: X, 2(July), 100025. https://doi.org/10.1016/j.biosx.2019.100025

Peake, J. M., & Della, P. (2015). cytokine expression and secretion by skeletal muscle cells : regulatory mechanisms and exercise effects, (32), 8–25

Decellularized multicellular fibroblast sheets for mimicking the profibrotic microenvironment in vitro

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The signals from the microenvironment play a crucial role in the development of fibrosis. In particular, the involvement of the extracellular matrix (ECM) is considered as a key modulator of this process. However, there is no relevant in vitro model that would reproduce ECM-based profibrotic conditions to elucidate the role of the microenvironment during fibrogenic process. The aim of the current research was to develop functional and biomarker-related fibrotic ECM in vitro using the decellularization approach.

Material and methods.

Human dermal fibroblasts were used to form the cell sheets for 2 weeks in the presence of ascorbic acid, fetal bovine serum (FBS) or platelet lysate as well as with or without transforming growth factor beta (TGFb). Then the obtained multicellular structures were decellularized using the previously developed protocol (CHAPS and DNAse I) and characterized by SEM. ECM protein deposition as markers of fibrotic microenvironment like collagen I and III, EDA-fibronectin (EDA-FN) was evaluated by immunofluorescent analysis and dot-blot. To validate decellularized ECM-based profibrotic microenvironment human adipose-derived mesenchymal stromal cells (MSC) were cultured on the modelled matrix and then analyzed by expression of alpha smooth muscle actin (aSMA), EDA-FN, collagen I and fibrosis-specific miRNA profile.

Results and conclusions.

Several protocols to the development of multicellular fibroblast sheets were examined to resemble the profibrotic microenvironment. Decellularized ECM had three-dimensional meshy and branched structure, it also mimicked ECM protein orientation in multicellular sheets. The obtained fibrotic ECM conserved increased level of EDA-FN and collagen I to III ratio. Interestingly, adding of TGFb did not demonstrate a significant effect on deposition of fibrotic ECM indicating the predominant role of ECM cell source (dermal fibroblast cell sheets) compared to culture condition. MSC seeded on fibrotic ECM demonstrated increased expression of markers associated with fibrosis (increased expression of aSMA, collagen I, EDA-FN). Moreover, miRNA profile in MSC also shifted toward the profibrotic pattern. Importantly, MSC cultured on decellularized ECM kept the spindle-shaped morphology similar to their shape within stromal tissues compared to cells cultured on plastic.

Taken together, we demonstrated the promise of using decellularized ECM produced by fibroblasts within multicellular sheets to mimic profibrotic microenvironment in vitro. The obtained results could be used for further studies investigating the impact of microenvironment on tissue repair and regeneration.

The study were conducted using biomaterials obtained within the project of Lomonosov Moscow State University "Noah's Ark" and supported by the Russian Scientific Foundation (#19-75-30007).

An *in vitro* system for the mouse Epiblast to investigate the establishment of the antero-posterior polarity

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The development of an embryo is an interplay of phenomena, involving morphogenetic rearrangements, collective migration and cell differentiation. How a complex shape, made of many different tissues, arises from a symmetric pool of identical cells is still not fully unveiled. Here, we are interested in understanding one of the first events that breaks the symmetry of the embryo proper: the establishment of the Antero-Posterior polarity (A-P). We propose to use mouse Embryonic Stem cells to build a synthetic *in vitro* system, that mimics the early Epiblast in morphology and gene expression, to allow the study of minimal conditions to observe symmetry breaking in gene expression, reminiscent of the antero-posterior polarity. We observe how this system reacts under homogeneous stimulation with morphogens such as BMP and we compare the results obtained, to a situation where the symmetry of the stimulus is broken by means of microfluidics gradient generating device. Our device relying on continuous flow to maintain the gradient, we observed a loss of Nodal expression upon stimulation with BMP, compared to static stimulation. We hypothesised the continuous flow to be accountable for washing out some secreted signalling from the cells, necessary to sustain Nodal expression. By modifying the device to induce a gradient of secreted molecules, we were able to observe a polarity of Nodal and Brachyury in the organoids in a more consistent way than when stimulated with uniform and static BMP. These experiments hint to the existence of an epiblast-autonomous mechanism to establish an A/P polarity, but that this mechanism co-operate with others to ensure the robustness of the polarisation, and that a localised source of activator and inhibitor molecules could be relevant to increase the frequency of observation of polarity in Epiblast-like organoids.

Modeling Fragile X syndrome with iPSC-derived neurons in 2D and 3D tissue culture conditions.

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Fragile X syndrome (FXS) is the most common inherited form of human mental retardation and it is caused by the expansion of CGG repeat in the FMR1 gene. The resulting epigenetic silencing causes the loss of the fragile X mental retardation protein (FMRP) with defects in the regulation of dendritic spine morphology and synaptogenesis.

Our study aims to create an in-vitro 2D and 3D model based on human induced pluripotent stem cells (hiPSCs) with the purpose of deciphering the neurobiological phenotypes associated with FXS.

We modified a 2D differentiation protocol [1] for obtaining a mixed cortical neuron culture in order to study the electrophysiological properties of FXS-iPSC derived neurons in-vitro.

Patch-clamp experiments confirmed that similarly to the WT hiPSC line, the FXS patient-derived neuronal cultures were capable to generate large Na+ and K+ currents as well as to trigger action potentials. Nevertheless, calcium imaging analysis showed that the FXS lines appear to display a reduced frequency of spontaneous calcium events, with significantly reduced synchronicity at day 68, thus suggesting an alteration in the neuronal network development, which will be analyzed in further experiments challenging neuronal culture with different plasticity protocols.

Moreover, we set up iPSC-derived 3D cell cultures using whole-brain organoids protocols [2] [3]. Currently, we report the efficient production of cortical organoids from two control hiPSC lines already available in our laboratory and we aim to further study how the absence of FMRP can morphologically and functionally invalidate the formation of cortical plates in this 3D model of neurodevelopment. For these reasons, we propose to generate cortical organoids starting from a control WT line and its isogenic FMR1 knockout (FMR1 KO) iPSC line generated in-house by CRISPR gene-editing. Such approach would minimize every possible bias due to the different genetic background inherited by each iPSC line.

Since self-organizing cerebral organoids excel at recapitulating early developmental events, this approach may be used to study Fragile X syndrome in a relevant time frame, understanding its mechanisms and allowing potential drug screening in in-vitro models that might carry the genetic background of each patient.

[1] - Shi Y, Kirwan P, Livesey FJ. Directed differentiation of human pluripotent stem cells to cerebral cortex neurons and neural networks. Nat Protoc. 2012 Oct;7(10):1836-46.

[2] - Lancaster MA, Knoblic JA (2014) Generation of cerebral organoids from human pluripotent stem cells. Nat Protoc. 2014 Oct; 9 (10): 2329-40.

[3] - Lancaster MA, Corsini NS, Wolfinger S, et al (2017) Guided self-organization and cortical plate formation in human brain organoids. Nat Biotechnol. 2017 Jul; 35 (7): 659-666.

Visualization of Mediator complexes in naive and primed embryonic pluripotent cells

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Our understanding of the transcriptional control of cellular identity is rapidly emerging. The Mediator complex enriches at active enhancers, forms a protein bridge with the promoter region of their target genes, and recruits RNA Polymerase II (Pol II). Thus, Mediator plays an essential role in transcriptional regulation. However, precisely how the 30-subunit Mediator complex completes these critical steps remains unclear. **We have investigated the interactions between Mediator and putative new regulators of transcription, in order to explore how they behave during transitions between alternate cell identities.** We have used mouse embryonic stem cells (mES cells), as they represent a prototypical model of how cells can change their identity *in vitro* between the naïve and the primed state by re-directing their transcriptional program. We have also established and employed a **proximity-ligation assay (PLA) to characterize protein interactions in the nucleus of mES cells by immunofluorescence and quantitative imaging**. Using PLA, **we detect changes in Mediator-Pol II complexes** between ES cells in the naïve or primed state. Moreover, we show that small molecule manipulation of the Mediator-Pol II interaction correlates with subsequent changes in cell identity. Taken together, our data provide **novel insights about how the Mediator-Pol II axis controls cell identity**.

Cell-matrix nanoscale adherence continually modulates intercellular communication in morphogenesis

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Mesenchymal condensation is a prevalent morphogenetic transition, regulated by cell adhesion, in which mesenchymal stem cells (MSCs) gather together. In osteochondral development, this is concurrent to the formation of an extensive gap junctional intercellular communication (GJIC) network. Little is known about the way the environment modulates the formation of this network and its implications in tissue architecture and function. Here we used substrates nanocoated with the cell adhesive arginine-glycine-aspartic acid (RGD) peptide to tailor local surface adhesiveness at the nanoscale. Substrates were characterized with Atomic Force Microscopy, and local areas with a mean interparticle distance shorter than 70 nm were considered as adherent.

hMSCs were cultured on the substrates in chondrogenesis-inducing medium, prompting the formation of pre-cartilage cell condensates. We studied the influence of local ligand density on mesenchymal condensation and the establishment of a functional GJIC network, by assessing expression and spatial disposition of GJ protein Cx43 and by a tracer intake assay in condensates.

Substrates with a high percentage of cell-adherent area (90%) promote stable cell condensation, differentiation and Cx43 expression. Cx43 expressed in these condensates forms a tighter communication network than in other substrates of lower ligand density. The architecture of Cx43 has repercussions in establishing a more functional GJIC network, as shown by the tracer intake assay.

To understand the effect of ligand density on tissue formation after condensation, formed condensates were transplanted to new substrates of either the same or a different ligand density, and Cx43 expression was quantified. Transplantation of formed condensates to fresh optimal substrates further increased Cx43 expression, which does not occur in condensates transplanted to low ligand density substrates.

We therefore conclude that nanoscale ligand density regulates not only the process of mesenchymal condensation, but also concurrent protein expression and its spatial disposition during differentiation, affecting tissue functionality. Matrix local adherence regulates morphogenesis pre- and post-mesenchymal condensation.

This knowledge sheds new light on cell- and tissue-matrix interactions during morphogenesis, revealing nanoscale local adherence as a factor that continually modulates the architecture and functionality of protein networks in developing tissue.

We are currently performing assays in which Cx43 expression is evaluated in the presence of blebbistatin, an inhibitor of actin contractility, to investigate the mechanism of GJIC regulation.

References/Acknowledgements:

1. Lagunas et al. (2014) Nano Res. DOI: 10.1007/s12274-014-0406-2

2. Lagunas et al. (2017) Nano Res. DOI: 10.1007/s12274-016-1382-5

3. Casanellas et al. (2018) J. Vis. Exp. DOI: 10.3791/56347

From 2D to 3D cell culture with biomimetic microgels

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The last challenge of Tissue Engineering lies in the transformation of conventional biomaterials into 3D frameworks that mimic the biological, chemical and mechanical features of native tissues[1]. In the cellular niche, the extracellular matrix (ECM) serves not only as an structural support, but also as source of three dimensional biochemical and biophysical cues that trigger and regulate cell behaviour[2]. There is an increasing evidence that this 3D and multifunctional character of the microenvironment is required for the development, under *in vitro* systems, of many critical cell responses that take place *in vivo*. The ability to develop biomaterials biologically specific, temporally dynamic, and patternable greatly augments the development of these biomimetic models.

Cells are now recognized to be strongly controlled by the highly structured and heterogeneous mix of neighbouring cells, soluble factors, ECM and biophysical cues that comprise their 3D microenvironment[2]. The inclusion of all these actors in an *in vitro* approach remains still challenging, however we have worked on a novel cell culture platform, easily tunable for different scopes, based on microgels, consisting in a high-density dispersion of functionalized microspheres in a liquid medium. Grafting diferent biomolecules of the ECM on the microspheres surface allows mimicking the natural environment regulating cell adhesion, distribution and behaviour and, indirectly, the mobility and avaliability of soluble factors.

A 3D environment is achieved without the geometrical restraints of conventional scaffolds, thus decreasing stress and limitations for cell growth and behaviour. Microspheres can be easily produced in various dimensions (from one to hundreds microns), by different polymers (alginate, poly(vinylidene fluoride), polyacrylates) and techniques (emulsion polymerization, microfluidics). Functionality (like ability to provide magnetic or mechanical stimuli to cells) can be added through incorporation of functional particles in the polymeric matrix. Biological response is enhanced through surface deposition of a variety of ECM biomolecules or peptides (fibronectin, collagen, hyaluronic acid) using different technics (layer by layer, covalent graft, physisorption). This highlights the versatility of the platform regarding materials, components, techniques and applications, and all the materials exhibited good biocompatibility when tested with different applications[3,4].

Multiple myeloma is an hematologic neoplasia in which ECM components are major players in the development of drug resistance, the main clinical problem nowadays[5]. Specially designed microsphere's platform has been used for the study of proliferation and viability of myeloma cell lines under biomimetic culture conditions.

1. Nguyen, M. A., Camci-Unal, G. Trends Biotechnol. 1–13 (2019). doi:10.1016/j.tibtech.2019.07.014
2. Huang, G. et al. Chem. Rev. 117, 12764–12850 (2017). doi:10.1021/acs.chemrev.7b00094.
3. Clara-Trujillo, S. et al. Colloids Surf B Biointerfaces 177, 68–76 (2019). doi: 10.1016/j.colsurfb.2019.01.050
4. Sobreiro-Almeida, R. et al. Int. J. Mol. Sci. 18 (11):2391, (2017). doi:10.3390/ijms18112391
5. Di Marzo, L. et al. Oncotarget 7, 60698–60711 (2016). doi: 10.18632/oncotarget.10849

Micro-Spheroids For β-like Cell Encapsulation

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The transplantation of pancreatic insulin-secreting β-cells could restore glycaemic control in patients with type 1 diabetes (T1D). Despite recent advances in the development of in vitro differentiation of human pluripotent stem cells into functional β-like cells under 2D or 3D cultures, rodent models transplanted with cell-laden microspheres have experienced only a transient graft function [1].

The encapsulated b-like cells are not remaining viable following transplantation due to due to the central necrosis and to the immune system response which leads to a foreign-body response and, ultimately, to beta-cell death [2].

Here, we report a novel high-throughput methodology to encapsulate β-like cells in the form of spheroids using a 3D bioprinting approach. Our strategy consists on the encapsulation of in vitro generated β-like cells within a collagen-based hydrogel which enhances functional cell survival and favours insulin-producing cell commitment. Using this technology, it is possible to fabricate large amounts of functionally robust, long-lived and reproducible spheroids with tuneable sizes.

Subsequently, to keep the insulin secreting cells immuno-isolated, the spheroids are reinforced with a second crosslinking which consist on the spheroid immersion into a chemical cross-linking solution. This secondary treatment functions as a collagen crosslinking agent binding to collagen fibres via hydrogen bonds and hydrophobic interactions. The two layered semi-permeable spheroids provide a core nucleus with similar structure to the extracellular matrix while providing an outer surface that allows an efficient protection of cells and reduced capsule degradation while allowing metabolites exchange.

In conclusion, collagen-based formulation may enable the long-term transplantation of in vitro β-like cells for the correction of insulin deficiency in T1D patients.

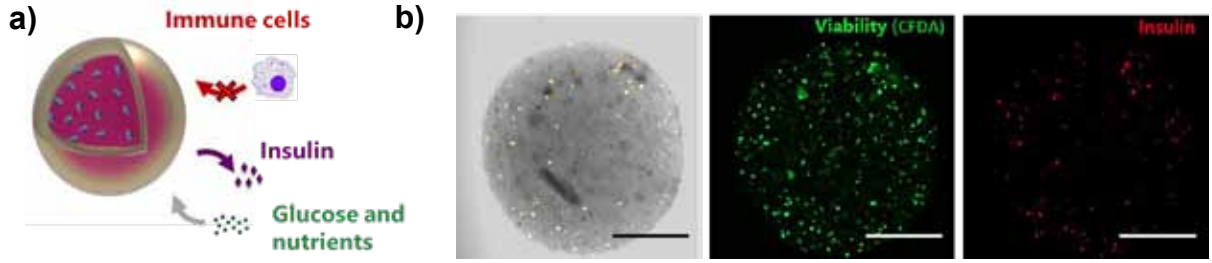

Fig. 1.a) Core-shell hydrogel microcapsule design for improvedβ-like cells encapsulation.

b) Fluorescence images showing β-like cell microspheres.

[1] Bochenek, M. A., Veiseh, O., Vegas, A. J., Mcgarrigle, J. J., Ghani, S., Li, J., … Langer, R. (2018). protection of allogeneic pancreatic islet cells. *Nature Biomedical Engineering*, *2*(November). https://doi.org/10.1038/s41551-018-0275-1

[2] Pagliuca, F. W., Millman, J. R., Gürtler, M., Segel, M., Van Dervort, A., Ryu, J. H., … Melton, D. A. (2014). Generation of functional human pancreatic β cells in vitro. *Cell*, *159*(2), 428–439. https://doi.org/10.1016/j.cell.2014.09.040

Cellular force generation during sprouting angiogenesis

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Abstract

Most, if not all, organs are dependent on the formation of a functional blood circulatory network for development, growth and regeneration. The mechanisms that control the ingrowth of vessels during angiogenesis have gathered much attention given its importance in the pathogenesis of various disease conditions such as ischemia, cancer and diabetes [1, 2].

Mechanical forces are fundamental in angiogenesis, as they drive a wide range of related Endothelial Cell (EC) processes, such as cell adhesion and migration. Previous in vitro work has shown that vascular network formation can be either enhanced or reduced by either upregulating or downregulating myosin activity [3]. However, no one has ever quantified traction fields during angiogenesis so far.

In order to investigate this, we adapted an existing *in vitro* model of endothelial invasion [4] into collagen type I hydrogels for Traction Force Microscopy (TFM) procedures. TFM is performed by using second harmonic generation time-lapse imaging of both invasive endothelial sprouts and collagen fibers to measure 3-D collagen deformation fields, which are used for the recovery of cellular tractions during sprouting angiogenesis.

To compute cellular forces, the mechanical characterization of collagen gels has been performed by means of shear rheology and extensional rheology [5]. By only examining collagen deformations, we have observed how endothelial cells mechanically interact with the collagen network mainly at the sprout tip and base region. Protrusions at the sprout tip were mostly applying pulling and not pushing forces, suggesting a prominent role of tip cell pulling for sprout elongation.

Finally, to understand the role of collagen rigidity during sprouting angiogenesis, differences on forces generated during sprouting angiogenesis will be discussed for two different collagen concentrations, 1.2 and 2.4 mg/ml.

References

[1] Folkman, J., Angiogenesis in Cancer, Vascular, Rheumatoid and Other Disease. Nature Medicine, 1995. 1(1): p. 27-31.

[2] Potente, M., H. Gerhardt, and P. Carmeliet, Basic and Therapeutic Aspects of Angiogenesis. Cell, 2011. 146(6): p. 873-887.

[3] Kniazeva, E., et al. 2012. Quantification of local matrix deformations and mechanical properties during capillary morphogenesis in 3D. Integr Biol-Uk 4(4):431-439.

[4] Bayless, K.J., H.I. Kwak, and S.C. Su, Investigating endothelial invasion and sprouting behavior in three-dimensional collagen matrices. Nature Protocols, 2009. 4(12): p. 1888-1898.

[5] Steinwachs, J., et al., Three-dimensional force microscopy of cells in biopolymer networks. Nature Methods, 2016. 13(2): p. 171-+.

A Local, Self-Organizing Reaction-Diffusion Model Can Explain Somite Patterning in Embryos.

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During somitogenesis in embryos, a posteriorly moving differentiation front arrests the oscillations of "segmentation clock" genes, leaving behind a frozen, periodic pattern of expression stripes. Both mathematical theories and experimental observations have invoked a "clock and wavefront" model to explain this phenomenon, in which long-range molecular gradients control the movement of the front and therefore the placement of the stripes in the embryo. Here, we present a fundamentally different model-a progressive oscillatory reaction-diffusion (PORD) system driven by short-range interactions. In this model, posterior movement of the front is a local, emergent phenomenon that, in contrast to the clock and wavefront model, is not controlled by global positional information. The PORD model explains important features of somitogenesis, such as size regulation, that previous reaction-diffusion models could not explain. Moreover, the PORD and clock and wavefront models make different experimental predictions and here we present evidence that favours the PORD mechanism.

Quantifying biomechanical stresses in 3D cellular environments via micro-droplet laser spectroscopy

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Biomechanical stresses are known to play important roles in processes related to tissue development and shape[1], but measurements of stress in 3D environments remain challenging. In this research, we explored micro-droplet laser spectroscopy for the measurement of biological stresses within 3D cellular environments. Dye-doped polymer spheres and oil droplets represent a simple yet extremely efficient laser architecture. The laser modes supported by the refractive index contrast between the sphere or droplet and their surroundings achieve impressive quality factors. At the same time, they can be used as labels or sensors as the lasing spectrum depends on the refractive index of the surroundings and the geometry of the resonator. Dye-doped polymer spheres have been used for single cell tracking of macrophages[2] and for investigating the contractility of single cardiomyocytes and zebrafish hearts[3]. Recently, it was proposed to use dye-doped oil droplets to measure stresses within the cytoplasm of single cells[4]. Here we expand on this proposal, making use of the fact that the lasing wavelength is sensitive to mechanically induced deformation of the droplet. By relating changes in the droplet laser spectrum to its geometry, Laplace's law was used to infer the stress applied to the droplet. Micro-droplet lasers were characterized and proof-of-principle measurements of cell-cell compressive stresses within an UMSCC1 tumor spheroid were demonstrated *in vitro*. Stress levels on the order of pN/μm^2 were recorded at different positions within the spheroid and compared to results obtained with alternative stress sensing modalities. Compared to the currently widely used method of imaging the deformation of fluorescent droplets via confocal microscopy, micro-droplet laser spectroscopy promises shorter acquisition times and greater stress sensitivity. This research demonstrates the general viability of droplet laser sensors for bio-mechanical stress quantification and provides scope for further biomechanical studies of 3D cellular environments.

1. Roca-Cusachs, P., Conte, V. & Trepat, X. Quantifying forces in cell biology. *Nat. Cell Biol.* **19**, 742–751 (2017).

2. Schubert, M. *et al.* Lasing within Live Cells Containing Intracellular Optical Microresonators for Barcode-Type Cell Tagging and Tracking. *Nano Lett.* **15**, 5647–5652 (2015).

3. Schubert, M. *et al.* Monitoring contractility in single cardiomyocytes and whole hearts with bio-integrated microlasers. *bioRxiv.* 605444 (2019).

4. Humar, M. & Yun, S. H. Intracellular microlasers. *Nat. Photonics* **9**, 572–576 (2015).

Measurement of invadopodia forces in 2D and 3D environments

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Abstract

Mechanical forces exerted by cells are known to regulate many cellular functions, with cell invasion and metastasis being of particular importance in cancer. This study uses Elastic Resonator Interference Stress Microscopy (ERISM), a novel optical technique for force mapping, to measure – to our knowledge for the first time – the forces exerted by invadopodia, using the head and neck squamous cell carcinoma line UM-SCC-1 as model. Invadopodia are actin-rich protrusions formed by cancer cells to invade into neighbouring tissue via extracellular matrix (ECM) degradation. By combining ERISM with a fluorescent gelatine assay, we were able to distinguish mature (degrading) from immature (non-degrading) invadopodia and measure their mechanical forces separately. The mean force exerted by mature invadopodia (5.8 ± 0.4 pN) was significantly larger than the force of generated by the immature invadopodia (3.4 ± 0.4 pN). This suggests that invadopodia forces can indicate the degree of invasiveness of individual UM-SCC-1 cells. Our study also shows that invadopodia force exertion is periodic and highly dynamic. Continuous force imaging revealed a two-mode oscillation; a slow force variation with a period of (7.2 ± 4.1) min, accompanied by a faster force oscillation of (43.3 ± 1.7) sec. Perturbation of invadopodia forces was caused by overexpression of miR-375, which reduced invadopodia forces and oscillatory behaviour.

Forces exerted by 3D tumour spheroids were also imaged with ERISM, showing that forces increase over time in an oscillatory manner, with a period of about 5 min. In addition, small, pushing protrusions were observed on the basal surface of the cancer spheroid, sharing similar force amplitude and dynamics with the invadopodia protrusions seen at the single-cell level. Lastly, the forces exerted by single cells during their escape from the spheroid were also recorded over time. Our study provides a new, physical perspective on cancer invasion and metastasis.

The role of SOX9 in vasculature re-modelling: an *in vitro* study

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The growth of an embryo, after it reaches a size of ca. 2mm, heavily depends on a functional vascular system. The constant and progressive remodeling of the vasculature efficiently provides oxygen and nutrients to all the cells allowing them to grow, form tissues and, later on, organs. 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. Preliminary literature findings suggest that the transcription factor Sox9 is involved in the regulation of VEGF expression responsible for this phenomenon. 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, preliminary results show that 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. Additionally, it is known that endothelial cells are able to convert the mechanical stimuli resulting from the shear stress of blood flow into intracellular signals that affect cellular functions. We tested the influence of a constant flow of medium over the micromass previously placed into a micro-fluidic device. These findings represent a first step in understanding how the vasculature network forms inside the limb and how it orchestrates organogenesis.

Vertex Modeling and Three-dimensional Tissue Reorganization

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During morphogenetic processes, epithelial monolayers exhibit drastic deformations as a result of not only deforming the constitutive cells, but also due to cell rearrangements, and reformation of cell-cell junctions due to intercalation or proliferation events. Vertex models have been one of the most successful tools to describe the mechanistic behavior of cell monolayers. However, the extension of such a frame work to three-dimensions is still very limited, due to the challenges that arise from reorganization of cells.

Here, we present a three-dimensional vertex model, and we focus on reproducing the topological changes of monolayers observed experimentally. We incorporate independent intercalation events between at the apical and basal surfaces, as shown in Figure 1. We study tissue remodeling during wound healing considering various concentration of contractile forces (apical, basal and lateral) with different intercalation rates (see Figure 2). Furthermore, we simulate cluster formation and the transition from single-cells monolayers to 3D cells aggregations through out-of-plane cell extrusion and proliferations (Figure 3), analyzing the effective parameters which characterize the topological transition of epithelial monolayers. The model is also employed for the analysis of the necessary forces in folding tissues that may induce cell extrusion in monolayers (see Figure 4).

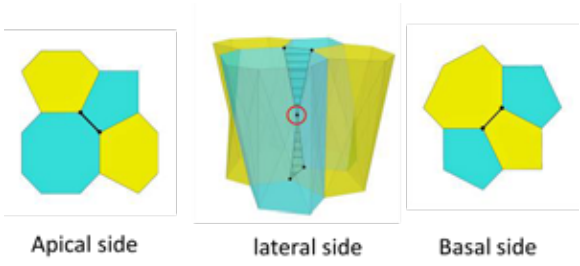

Figure 1. Three-dimensional construction of intercalation

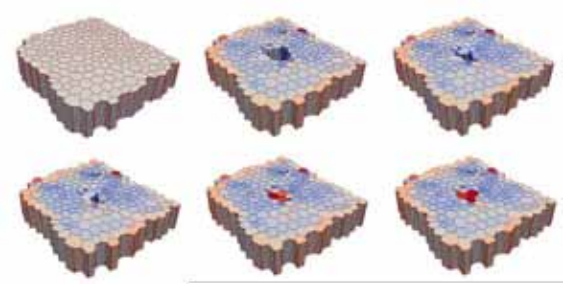

Figure 2. Time series of wound closure simulation

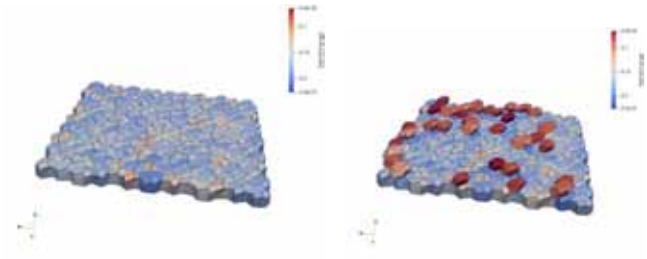

Figure 3. Simulation of bilayer formation through cell extrusion.

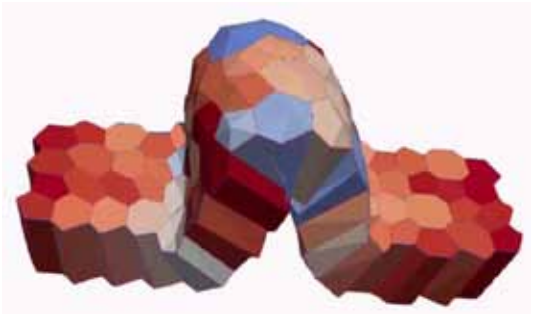

Figure 4. Folding of 3D vertex, without extrusion or delamination.

The Introduction of Fc-Fusion Bioactive Proteins in Supramolecular Biomaterials

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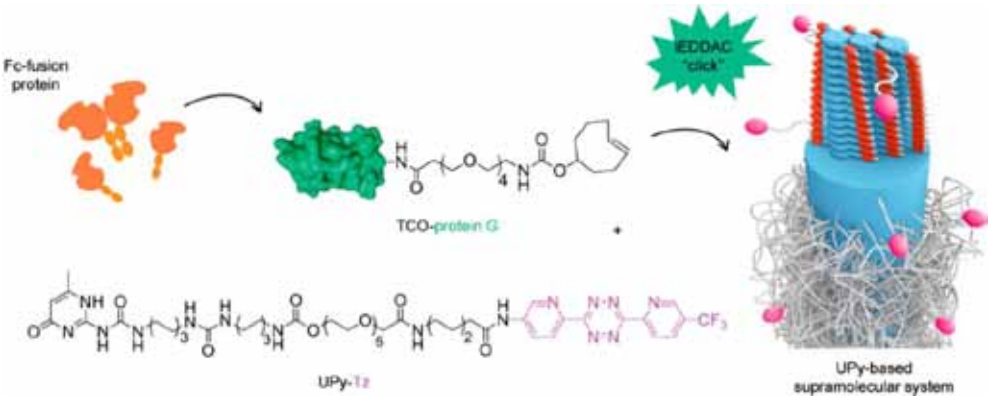
Synthetic biomaterials can be used as three-dimensional extracellular microenvironments to mimic the natural extracellular matrix and therefore offer great possibilities for regenerative medicine. Also for in-vitro organoid culture development, synthetic materials can support organoid survival, growth and can induce differentiation. However, the incorporation of functional proteins in these materials, such as growth factors or antibodies, can be difficult as these materials often undergo harsh treatments while being processed. Post-modification strategies using biorthogonal conjugation chemistries can offer a solution for this issue. The inverse electron demand Diels-Alder cycloaddition click reaction between tetrazine (Tz) and *trans*-cyclooctene (TCO), the fastest biorthogonal reaction known so far, is suitable for targeting biomolecules such as proteins. Recombinant protein G presents two immunoglobulin (IgG)-binding domains, making it useful for the immobilization of antibodies and Fc-fusion proteins. The immobilization of protein G in synthetic materials using Tz/TCO click chemistry therefore offers an effective way to incorporate full-length proteins.

As a proof-of-principle, protein G (pG) was immobilized on the surface of a supramolecular ureido-pyrimidinone (UPy)-based material scaffold using Tz/TCO click chemistry. As a model for a bioactive protein, we introduced Fc-Jagged1 (a Notch ligand important in cardiovascular development and homeostasis) to induce Notch signaling activity on the material.



The results show that protein G can successfully be functionalized to obtain a TCO-pG conjugate that could react to the UPy-Tz (5 mol%) mixed into the supramolecular UPy-scaffold. Importantly, on electrospun scaffolds TCO-pG was immobilized in significantly higher quantities (7.2-fold) than physically adsorbed protein G, which resulted in a 9.6-fold increase in Fc-Jagged1 binding. Nonetheless, an increased but not a significant difference in Notch signaling intensity was detected in CASMCs and HEK293FLN1 cells.

Altogether, our results represent the first evidence of successful postprocessing bioactivation of a supramolecular UPy-based material for the immobilization of Fc-fusion ligands. For future studies, it would be interesting to apply this for the development of biomaterials for organoid culture as well, offering a promising way to immobilize growth factors, antibodies and other proteins.

Cell and Nuclear Growth Dynamics in Developing *Drosophila* Germline Cysts

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Across species, from insects to mammals, the future fertilizable egg develops within a cluster of sister germ cells that are interconnected through cytoplasmic bridges. To meet the biosynthetic demands of the developing oocyte and future embryo, germ cells within these clusters grow dramatically, and do so in a differential, yet coordinated manner. As opposed to the known growth scaling laws that govern single cells, the dynamics that direct the coordinated growth of these developing cell clusters remains poorly understood. Here, we use the *Drosophila* egg chamber, a germline cluster of sixteen cells connected in a highly reproducible fashion, to explore the collective growth dynamics of individual cells and organelles over multiple orders of magnitude of size increase. Through a supervised learning algorithm for the automatic reconstruction of cell clusters from 3D stacks of images, we have created a quantitative high-throughput pipeline for systematically isolating and measuring relevant properties of individual cells within each cluster. This work establishes the framework for applying machine learning techniques for the analysis of differential growth rates in other multicellular systems and highlights new approaches for analyzing large datasets of information to address longstanding questions in biology.

Morphogenesis control of dermal tissues produced by Laser-Assisted Bioprinting (LAB)

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Introduction:

Dealing with anatomy complexity to reproduce functional anisotropy of human tissues remains a puzzling challenge for tissue engineers. Specific biological functions arise from dynamic interactions between cells, and with extracellular matrix at different scales and times within a defined structure. Latest Laser-Assisted Bioprinting (LAB) technology demonstrates promising capabilities to organize living cells and biomaterials with a micron-scale resolution and high cell viability. We harness this high cell printing resolution to better understand, model and control *in vitro* cellular behaviour, collective self-organization, and develop numerical models of morphogenesis.

Methods:

Simplified bioprinted models were considered to investigate particular morphogenesis mechanisms in dermis. Human primary dermal fibroblasts were cultured and bioprinted by LAB on collagen I matrices. Patterns of cells were designed and characterized before and after printing with in-house proprietary CAD and Printing Analysis softwares. Cell motility and contractility were appreciated with Time-lapse microscopy. Local matrix deformations were traced with collagen-embedded fluorescent microbeads for analysis with particle tracking algorithms. Whole cellular pattern maturation over time and final matrix remodelling were assessed with bright field microscopy and immunofluorescence. The results are being compared with in-house multi-agent models.

Preliminary results:

Self-organizations of patterned cells enable to identify and leverage various cellular behaviors. Figure 1.A. illustrates different maturation outcomes: culture conditions can lead to distinct post-printing collective behaviors such as fibroblast layer formation or clustering. Fitting bioprinted patterns to specific cellular properties can result in controlled tissue maturation features. Figure 1.B. and 1.C. display how cellular patterns can be tailored to obtain reproducible collective cell orientation.

Discussion:

These 2D preliminary studies highlight that high-resolution LAB patterning is a promising tool to characterize but also trigger specific collective cell behaviors. Current work with time-lapse microscopy and further characterisation of cellular interactions and maturation outcomes will allow us to better generate, isolate and understand key morphogenesis mechanisms.

Conclusion

Studying different patterns in 2D and 3D will help understand cell responses to local micro-environments and resulting tissue-scale collective behaviours. Such reproducible maturation processes also pave the way to data collection and numerical model implementations. Altogether, iterative exchanges back and forth between biological experimentations and numerical tools will lead to better predictions and control of 4D bioprinted tissue maturations.

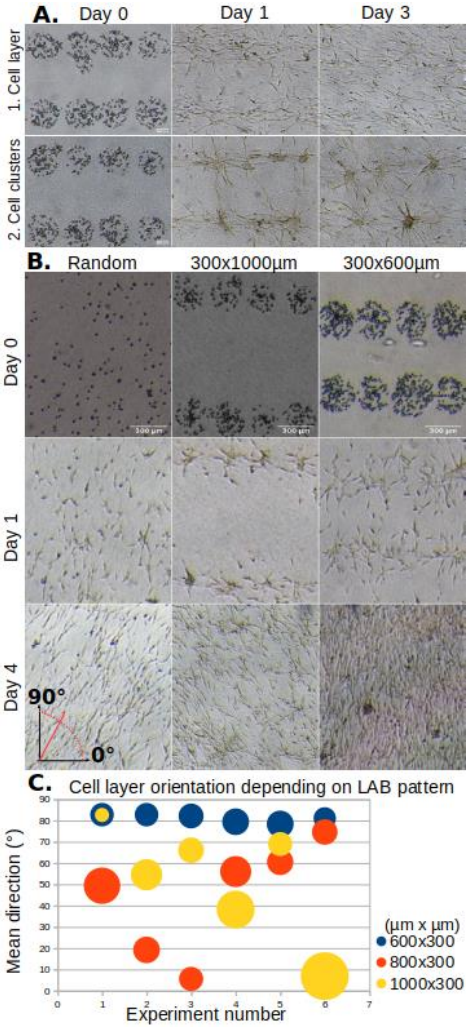

Figure 1: A. Fibroblasts cultured under different conditions result in distinct maturations after LAB patterning: cell homogeneous layer (1) or clusters (2).
B. Tailoring pattern dimensions to fibroblast properties with LAB enables control of cell orientation.
C. Influence of LAB pattern dimensions (µm) on collective cell orientations at day 4 of maturation.

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An Agent-Based Modeling Framework To Predict Bioprinted Tissue Morphogenesis

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Controlled bio-fabrication of biological tissues requires the consideration of complex mechanobiochemical mechanisms involved in cell self-organization. Despite the progress in unraveling the principles that underlie morphogenesis at the organ level, we have yet to understand it at the cell level. We aim to develop a computational framework to study and predict tissue morphogenesis through a large spectrum of cell and extracellular matrix (ECM) interactions in two or three dimensions, with a micrometer resolution. In vitro experimental results from cell and ECM bioprinting can be used to implement and validate the various models.

Laser (Guillotin et al. 2010) and extrusion bioprinting are used to deposit fibroblasts with a micrometer resolution on collagen gels with fluorescent micro-beads. High spatial and temporal resolution data are generated with time-lapse microscopy. Beads tracking are performed to study local ECM deformations and an in-house software based on neural networks is used to track cell clusters.

The software SimCells (Ballet 2018), optimized to run on GPU with highly parallelized execution, was enhanced to mix its agent-based system with a mass-spring system (MSS) representing the ECM. The framework integrates the biochemical and biomechanical cell activities in a spatially and temporally discrete environment. Simulated cells have a three-dimensional deformable membrane, can migrate, divide, die and impact and sense their local environment. The fibrous ECM is represented by an MSS that exhibits non-linear viscoplastic behaviours under cells remodelling mechanisms.

We aim to model hundreds of thousands of cells in interaction with a 3D gel of up to ~1 cm3. Already available frameworks allow us to simulate large environments with a high number of virtual cells (Swat et al. 2013; Kang et al. 2014) but present several limitations to study biomechanical mechanisms at the tissue scale. MSS models have been shown to successfully predict large ECM deformations (Hughes et al. 2018), and we think it is possible to link this kind of model to cell-scale mechanisms. The first experimental data helped us to build a two-dimensional prototype of simulation based on qualitative analysis. Preliminary results are promising to predict tissue deformations between several bioprinted cell clusters.

Summarizing, we present a framework able to include the relevant chemical and mechanical mechanisms needed to explore new insights at cell and tissue levels.

Efficiency evaluation of iPSCs neurogenic and myogenic differentiation in organoids composition

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Histogenesis elements evaluation in model systems *in vitro* during the cultivation of induced pluripotent stem cells (iPSCs) is a new relevant objective in histology. A modern method of working in this stream is 3D cultivation in the composition of spheroids, which allows reproducing volumetric histogenesis processes and simulating conditions close to the *in vivo*.

The cultivation of iPSCs and differentiation into organoids was performed according to the protocol (Eremeev et al., 2019) in the laboratory of cell biology of the Federal Research and Clinical Center for Physical-Chemical Medicine (Moscow, Head of department M.A. Lagarkova). In the experiment, 3 groups of iPSC lines derived from donors were examined: with spinocerebellar ataxia (SCA, group 1), with Huntington's disease (HD, group 2) and from healthy patients (group 3, HUVEC, control).

Performing analysis of organoids included immunofluorescence methods. Cells expressed neuron-specific markers: β-III-tubulin and MAP2 (were detected in all neurospheres); ChAT (marker of cholinergic neurons); GFAP (marker of neuroglia); SOX1 (marker of early progenitor cells). The glia-neuron ratio (GNR) was used as a measure to evaluate the efficiency of neurogenic differentiation. $GFAP^+$ cells were attributed as glial and β-$III\text{-tubulin}^+$ / $MAP2^+$ cells as neurons.

In the 1st group, GNR = 4.29 ± 2.52: 1; in the 2nd group, GNR = 0.98: 1; in the 3rd group (control) GNR = 1.01 ± 0.1: 1. The received data indicated a correlation between the detectable GNR in 3D systems *in vitro* and the pathomorphological picture of the studied diseases in vivo, which are: 1) the prevalence of glial cells in the 1st group - gliosis of patients with SCA 2) insufficiency, functional impairments of glia in patients with HD 3) indicators of the control group are similar to the picture of early ontogenesis (1: 1). The created in vitro 3D model can be a reproducible testing system for studying the pathomorphogenesis of neurodegenerative diseases.

Formation of tumor spheroid models using 3D-printed micromolds

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Currently, the success rate of new drug approval is at unsatisfactory levels due to the lack of an efficient biological system that mimics *in vivo* environments (Ledford, 2011). In 2D monolayer cell culture systems, cell-cell interactions do not reflect the interactions occurring within three-dimensional tissues, and therefore cannot truly mimic cell-drug interactions either. Thus, there is significant research going in defining 3D *in vitro* systems that better mimic the *in vivo* environment. In this context, spheroids, which are self-assembled aggregates of cells emerged as an attractive opportunity for more effective drug screening. In spheroids, signal proteins, which are essential for intercellular communication, are overexpressed. This leads to a more realistic interaction between the cells and, consequently, enhances the meaning of the response of the cells for drug testing (Li and Kumacheva, 2018). The penetration rate of drugs into spheroids is a challenging process and advanced microscopy techniques are required to measure this rate. The present abstract reports on a static microchip towards a *cancer-on-a-chip* concept that will allow the formation of multicellular tumor spheroids and their visualization using two-photon microscopy. 3 different static micromolds with different diameters were fabricated with stereolithography (SLA) technique using 3D printer (Formlabs Form 2). Each micromold had 109 μwells and was designed using Autodesk Inventor 2018 to perfectly fit in a 12-well plate. Then, PDMS-based molds were obtained by soft lithography. The μmolds were placed in each well of the culture plate. GFP-expressing Human Embryonic Kidney (HEK) cells were seeded with densities of 500, 1000, and 1500 cells/μwell on the micromolds and left in the incubator for the formation of spheroids. The size and circularity of the spheroids were analyzed using fluorescence microscopy, whilst cell density and viability were determined using the NucleoCounter® NC-200™ Automated Cell Counter. The necrotic and hypoxic layers of micro-tissues were confirmed with SP8 lightning confocal microscope and Leica TCS SP8 multiphoton (MP) system. Spheroids started to form after 24 h (Day 1) from first cell seeding (Day 0), however, the spheroids in day 1 were found to be very weak. The estimated diameter of spheroids at day 1, 2, 3, 4, and 5 were 228±26, 270±14, 325±8, 352±27, and 389±33 μm, respectively. To determine the cell density in each spheroid, the spheroids were dissociated using an enzyme solution. The cell viability inside the spheroids during 5 days was over 90%. The average cell number of cells in each spheroid in day 2, 3, 4, and 5 was 4835±863, 7857±2161, 8178±2648, and 13975±1917 cells/spheroid, respectively. For multi-photon microscopy analysis, the spheroids were stained with propidium iodide as a dead cell dye and visualized to see the necrotic zone of the spheroids. The scanning depth was significantly affected by the density of the spheroids.

References

Ledford, H. (2011). 4 ways to fix the clinical trial. Nature, 477(7366), 526.

Li, Y., & Kumacheva, E. (2018). Hydrogel microenvironments for cancer spheroid growth and drug screening. Science advances, 4(4), eaas8998.

Substrate Stiffness Impacts the Differentiation of Kidney Organoids Derived from Human Pluripotent Stem Cells.

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Mechanical cues can regulate self-renewal and differentiation of human pluripotent stem cells (hPSCs). However, the role of the mechanical microenvironment on the derivation of specific renal lineages has been not explored. We have recently shown that hPSC-derived kidney organoids revealed important kidney-related features of maturity after implantation into the chick chorioallantoic membrane (CAM). The CAM approach provides an *in vivo* soft microenvironment that can be mimicked *in vitro* by the fabrication of polyacrylamide (PAA) hydrogels. By using rigid and soft PAA hydrogels, we study the impact of substrate stiffness on the differentiation of kidney organoids. Through deep characterization by immunofluorescence and qPCR analyses we observe that nephron differentiation rate is increased in soft versus rigid microenvironments. By the realization of ChIP sequencing and RNA sequencing analyses we seek to identify novel markers and pathways determinant for nephron differentiation from hPSCs.

Organ-on-chip monitoring

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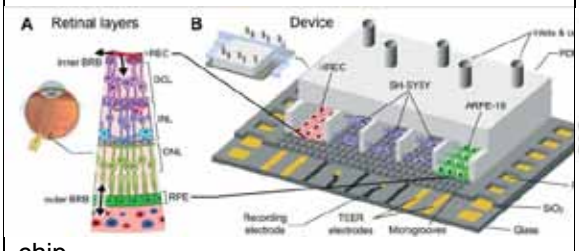
Organ-on-chip (OOC) is the term used to define a microfluidic 3D culture model that contains continuously perfused chambers inhabited by living cells. The development of the OOC technology has been possible thanks to the advancement in the micro and nanotechnologies. The engineered cellular microenvironments reproduce more accurately the in vivo structure and physiological conditions, and allow simulating the activities, mechanics and physiological response of tissues and organs. OOC are considered as very promising tools for investigating many aspects of human physiology and pathophysiology as well as drug testing platforms with future progressions to be used for precision medicine.

As the complexity of OOC systems increases, the necessity to integrate relevant assessment methods to provide information about cell physiology, secreted metabolites as well as pharmacodynamics drug responses also increases.

In this talk, I will focus on the different engineering approaches that we have used to develop physical and chemical sensors that can be integrated into OOC. I will describe our recent works on biological barrier models, including blood-retinal barrier[1], renal tubule[2] and kidney sinusoid. In particular, I will talk about compartmentalization strategies and integration of transepithelial electrical resistance electrodes into these models, fabricated by standard photolithographic processes, for the on-line quantification of ion permeability and continuous evaluation of the barrier functioning. A novel microfluidic device shown in Figure 1 (right) where cells are arranged in parallel compartments and are highly interconnected through a grid of microgrooves, which facilitates paracrine signaling and heterotypic cell–cell contact between multiple tissues, will be presented. I will as well describe the integration of inkjet-printed electrodes into the culture porous membrane for the real-time monitoring of dissolved oxygen levels in a liver-on-a-chip system[3]. Three electrochemical dissolved oxygen (DO) sensors were inkjet-printed along the microfluidic channel allowing local online monitoring of oxygen concentrations as shown in Figure 1 right. I will also talk about our latest developments in the field of gut on a chip for the microbiome study, where new sensors for cytokines and short chain fatty acids (specifically butyrate and acetate) are being develop for their in-line integration with the final device.

TEER and neural recording in a Retina-on-a-chip



Oxygen monitoring in a Liver-on-a-chip.

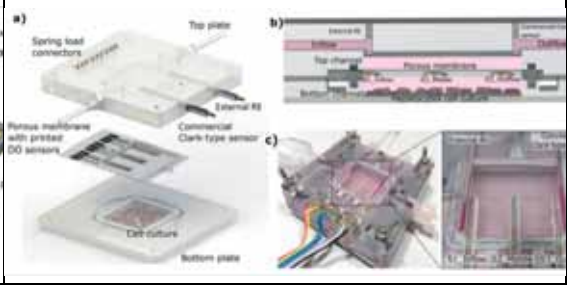


Figure. 1 Left) Recapitulating the cell structure of the retina. (A) Cell layers of the retina. (B) Schematic representation of the presented microfluidic cell culture device, including cell arrangement used in the experimental setup. Endothelial (HREC) and epithelial (ARPE-19) cells are located at the end compartments where there are TEER measurement electrodes, and SH-SY5Y cells are located in the middle compartments where there are extracellular potential recording electrodes. Right) a) Schematic of the OOC system with the modifications to incorporate the control with external elements b) cross-section of the bioreactor showing the position of the three DO printed sensors, and c) real picture of the ExoLiver system with all the fluidic and electrical connections with the three printed sensors along the microfluidic channel

References

[1] Yeste J, García-Ramírez M, Illa X, Guimerà A, Hernández C, Simó R, Villa R, "A compartmentalized microfluidic chip with crisscross microgrooves and electrophysiological electrodes for modeling the blood–retinal barrier" Lab on a Chip 18 (2018) 95-105

[2] Yeste J, Martínez-Gimeno L, Illa X, Laborda P, Guimerà A, Sánchez-Marín JP, Villa R, Giménez I "A perfusion chamber for monitoring transepithelial NaCl transport in an in vitro model of the renal tubule ", Biotechnology and Bioengineering 115 (2018) 1604-1613

[3] Moya A, Ortega-Ribera M, Guimerà X, Sowade E, Zea M, Illa X, Ramon E, Villa R, Gracia-Sancho J, Gabriel G., "Online oxygen monitoring using integrated inkjet-printed sensors in a Liver-On-a-Chip system" Lab on a Chip (2018),18, 2023-2035

Developing kidney-specific bioinks for human pluripotent stem cells 3D Bioprinting into kidney organoids

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Human pluripotent stem cells (hPSCs) inherent properties of self-assembly and symmetry breaking allow for their differentiation in vitro into cells of three germ layers of the embryo. Experimentally, the field has largely relayed in the use of biochemical cues mirroring different steps of human embryonic stem cells (hESCs) specification, commitment and further differentiation into the desired cell types during development for the generation of differentiated cultures. Collectively, the field has put in place, different procedures for the generation of fundamental knowledge with regards to the use of hPSCs as unprecedented tools for understanding human development and disease. Remarkably, through the exposure of three dimensional hPSCs cultures into these biochemical signals the field has started to define protocols for the generation of organ-like cultures containing multiple cell types, so called, organoids. These complex culture systems contain different cell types that are physically and functionally mirroring the organ of interest. Nevertheless, hPSCs derived organoids are still resembling embryonic stages during organ formation imposing the development of new approaches allowing for their maturation. Similarly, the high variability in terms of the extent of differentiation among the same batch of hPSCs derived organoids has also resulted in the repurposing of three-dimensional (3D) bioprinting technology for their derivation. Currently, the application of 3D bioprinting in the organoid field is still in its infancy and faces important questions related to the definition of new methodologies when identifying biocompatible hydrogels sustaining the printing process and allowing for hPSCs differentiation into complex organoid structures.

Our laboratory has recently published a procedure for the successful differentiation of hPSCs into nephron-like structures transcriptomically matching second trimester gestational kidney. Based on this knowledge we have defined a new approach for the derivation of renal constructs from hPSCs via 3D bioprinting. To this aim we have first redefined our procedure of kidney organoid differentiation in the two-dimensional (2D) setting for the generation of renal progenitor cells that show the capability to further generate segmented and patterned nephron-like structures. In parallel, we have developed a new procedure for the fabrication of biomimetic hydrogels from human kidney through organ decellularization. In this manner, decellularized extracellular matrix (dECM) was further combined with different proportions of gelatin and fibrinogen leading the formulation of a kidney-specific bioink. The best composition was further used in combination with nephron progenitor cells that showed the capability differentiate into kidney organoids containing multiple nephron-like structures that express higher levels of ECM-related molecules during the onset of differentiation. Furthermore, the renal constructs have been extensively characterized for the expression of different markers of differentiation, including markers of the proximal, medial and distal segments of the nephron. Overall, the approach detailed here shows the feasibility of this approach for further applications in drug testing and disease modeling.

Mechanical impact on stem cell niche morphogenesis

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The cells that make up an organ are constantly interacting with their environment and responding to it accordingly by controlling important cellular processes such as migration, differentiation, division or apoptosis. Cell contractility in tissues is known to be a key element in developing early embryo, as well in the tissue homeostasis throughout life by regulating adhesive contacts and cellular turnover. Here, we focus on the morphogenesis and organization of the intestinal stem cell niche and the processes involved in maintaining its integrity as it is one of the most dynamic tissues in the body. We question whether mechanical forces generated by the activity of Myosin-II molecular motors shape the stem cell niche and maintain its integrity. To test this hypothesis, we use the mouse gut organoid model which *in vitro* nicely recapitulates the morphogenesis of the intestinal crypt compartment and stem cell behavior. Live imaging of Myosin-II dynamics during crypt development revealed the anisotropic distribution of the contractile apparatus during crypt budding. Basal Myosin-II pulses locally occur at the niche level during crypt expansion. Interestingly, zones that exhibit strong basal pulses of actomyosin corresponded to dividing cells. These encounter apical migration and are being pushed out of the monolayer plane to divide before being reintegrated into the plane of the monolayer, in a pre-extrusion-like manner. Modulation of actomyosin activity show that correct force patterning is required for correct mitosis shaping and polarity cues arrangement to guide planar cell division in stem cell niche.