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2	Juan Francisco	Abenza Martínez	IBEC	Mechanical control of the mammalian circadian clock via YAP/TAZ
3	Gaia	Amato	IBEC	Developing Human Organoids To Model Genetic And Systemic Conditions During Congenital Anomalies Of The Kidney And Urinary Tract.
4	Maria José	Antunes Sintra	EMBL	In vitro recapitulation of heterochrony during vertebrate anterior?posterior axis development
5	Katia	Barrett	IBDM	Epithelial-mesenchymal coupling drives axis elongation in Xenopus explants
6	Nataliya	Basalova	Lomonosov Moscow State University	Dynamics of fibrotic foci formation during bleomycin-induced pulmonary fibrosis in mice
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8	Dirk	Benzinger	The Francis Crick Institute	Optogenetic engineering of morphogen gradients recapitulates dynamic neural tube patterning
9	Valentin	Bonnet	Institut Pasteur / Ecole Polytechnique	Deciphering the impact of the APC mutation on CAR T cells cytotoxicity using mouse and patient-derived 3D models in microfluidics.
10	Miquel	Bosch Padrós	IBEC	Mechanics of apical constriction: an optogenetic approach
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12	Joan	Casamitjana	UB / IDIBELL	A single-cell atlas of the murine pancreatic ductal tree identifies novel cell populations with potential implications in pancreas regeneration and exocrine pathogenesis.

13	Maria	Costanzo	EMBL Barcelona	Size control of in vitro somites
14	Ibrahim Halilullah	Erbay	University of Galway/ IBEC	Integrated Computational-Experimental Analysis of Shear Impact on Intestinal Crypt Dynamics and Mucus Mechanics
15	Hiromune	Eto	Hubrecht Institute	Microfluidic control of Notch signalling reveals a dynamic communication code for cell fate determination during intestinal homeostasis
16	Laura	Faure	IBEC	3D micropatterned traction force microscopy : a new technique revels that single epithelial cells can exert pushing forces on their environment
17	Ainhoa	Ferret Miñana	Institute for Bioengineering of Catalonia	3D bioengineered liver for the study of acute and chronic hepatic damage
18	Daniel	Garcia-Gonzalez	Universidad Carlos III de Madrid	Mechanical and Functional Responses in Astrocytes under Alternating Deformation Modes Using Magneto-active Substrates
19	Amélie	Godeau	Institute for Bioengineering of Catalonia (IBEC)	Mechanics of Human and Mouse Embryo Implantation
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21	Matt	Govendir	EMBL Barcelona	Biomechanics of blood brain barrier disruption in cerebral malaria
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23	Alice	Gros	IBDM/Aix Marseille Uni/CNRS/Turing Centre for Living Systems	3D quantitative analysis of gastruloid symmetry breaking
24	Judith	Guasch	ICMAB-CSIC	Artificial extracellular matrices based on 3D hybrid hydrogels for immune cell and organoid manufacture
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26	Paula	Guerrero López	Aragon Institute of Engineering Research (I3A)	Unravelling the relationship between nutrient availability in tumor microenvironment and cancer progression.
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29	Masaya	Hagiwara	RIKEN BDR	Engineering In-vitro Microenvironments to Replicate Complex In Vivo Conditions for Organoid Architecture
30	Elisa	Hahn	EMBL Barcelona	Cellular Mechanics and Self-Organization during Axes Formation in Mouse Gastruloids
31	Soraya	Hernández	University of Zaragoza	Engineering of a simplified 3D microfluidic in vitro model for tumour-stroma dynamics of pancreatic ductal adenocarcinoma microenvironment
32	Sarah	Hindle	Blizard Institute, Queen Mary University of London	Replicating Dynamic Immune Responses within a Microfluidic Human Skin Equivalent Model
33	Christine	Ho	University of Southern California	Synthetically guided development of mobile embryoid bodies based on cardiac contractions
34	Alex	Hughes	Department of Bioengineering, University of Pennsylvania	Harnessing the rhythmic biology of early kidney formation for synthetic morphogenesis
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36	Míriam	Javier Torrent	University of Liège, GIGA Neurosciences	Role of mechanotransduction in the control of interneurons migration in the cortex
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39	Sebastian	Kühn	Leibniz-Institut für Polymerforschung Dresden e.V.	µGUIDe ? A precision microgel platform to direct development in vitro
40	Jorge	Lázaro Farré	EMBL	A stem cell zoo to study interspecies differences in developmental tempo
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42	Valentina	Magno	Leibniz Institute for Polymer Research	Sulfated glycosaminoglycan-based microgels for programming VEGF gradients in human kidney organoids
43	Marina	Marchenko	Physics of Life TU Dresden / EMBL Barcelona	Influence of apical constriction on tissue morphology and cell fate in brain organoids
44	Nick	Marschlich	EMBL	Influence of geometry on self-organisation in early zebrafish development
45	Guillermo	Martínez-Ara	Institute for Bioengineering of Catalonia	An optogenetic toolset to understand and control epithelial mechanical balance.
46	Marija	Matejcic	IBEC	Mechanics of cell extrusion in intestinal organoids
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48	Matthias	Merkel	Turing Center for Living Systems, Center for Theoretical Physics, CNRS, Aix-Marseille University	Robustness of oriented tissue deformation
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67	Hanna	Szafranska	Laboratory of Molecular Biology	Scaled-up temporally resolved transcriptomics to uncover species-specific neurodevelopmental regulation
68	Wim	Thiels	KU Leuven	Using mechanical simulation to study early gastrulation movements in C. elegans.
69	Casper	van Bavel	KU Leuven	A Minimal Model for Early C. elegans Embryogenesis
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72	Isabel	Villaoslada	Instituto de Investigación Sanitaria de Aragón	PDXO-on-chip: a novel approach for studying mechanical properties on pancreatic ductal adenocarcinoma.
73	Srivatsava	Viswanadha Venkata Naga Sai	IBEC Barcelona	Cell-matrix force transmission regulates the transition between naïve and primed pluripotency.
74	Kaja Nicole	Wächtershäuser	BMLS, Goethe University Frankfurt a.M.	Modulating ubiquitin signaling to control (non)immunogenic cell death, necroinflammation, and tumor development in patient-derived human mammary organoids
75	Tobias	Walther	Max Planck Institute for Medical Research	DNA microbeads for spatio-temporally controlled morphogen release within organoids
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77	Shafaq	Zahra	Universitat Politècnica de Catalunya	Inference of cytoskeleton and cell stress from TFM

1- Long-range organization of primary intestinal fibroblasts guides in vitro epithelial migration through the secretion of aligned extracellular matrix proteins

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Fibroblasts contribute to epithelial migration in multiple tissues and processes. However, the function of fibroblasts underneath the intestinal epithelium has been mostly restricted to a secretory and supportive role, while epithelial migration has been traditionally attributed to a passive process driven by the mitotic pressure exerted by the cryptal stem cells. Recent studies have shown that intestinal epithelial cells are actually able to actively migrate. Yet, whether interactions between epithelial cells with fibroblasts and with the matrix contribute to epithelial movement remains elusive. Here, we show that intestinal subepithelial fibroblasts play a crucial role in epithelial migration. By using a novel gap closure in vitro model of the intestinal mucosa that includes both the epithelium and the stromal compartments, we demonstrate that the physical presence of fibroblasts in contact with the epithelium leads to epithelial migration both in organoids and monolayers and plays a crucial role in epithelial restoration by enhancing epithelial tissue integrity. Our results demonstrate that fibroblasts undergo long-range organization to align perpendicularly to the epithelial migrating front, and deposit protein paths that act as contact guidance features to direct epithelial migration. The physical presence of fibroblasts greatly accelerates epithelial restoration and reveal subepithelial fibroblasts as new potential therapeutic targets in intestinal pathologies.

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2- Mechanical control of the mammalian circadian clock via YAP/TAZ

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Cells sense and respond to the mechanical properties of their environment. This sensing is accomplished thanks to a diverse set of biochemical pathways which impact gene expression, subsequently affecting key cellular processes like proliferation and differentiation. Very recently, mechanics has been observed to also affect circadian rhythms, further broadening its importance in tissue homeostasis.

Our project aims to clarify the influence of mechanobiological hallmarks on the regulation of the mammalian circadian clock. We have used NIH3T3 fibroblasts expressing Venus fluorescent protein under the promoter of the circadian gene *Rev-erbα* (RevVNP), confocal microscopy and customised computational analysis. Our results indicate that RevVNP expression depends on cell density. By performing gap closure experiments, we observed that basal and circadian RevVNP expression, typically low and rhythmic in dense monolayers, is perturbed upon cell migration.

To disentangle the pathway that influences *Rev-erbα* transcription upon cell density changes, we used fibronectin micropatterning. Confined cells on single cell-sized areas displayed RevVNP circadian oscillations like those of confluent cells. Next, we stopped the migration of cells at low density by altering their actin dynamics with jasplakinolide and latrunculinA and observed the striking emergence of robust circadian oscillations, unlike the case of untreated single cells.

We then checked the intracellular localization of two prototypical mechanosensitive transcriptional regulators, YAP/TAZ and MRTFA, under a set of conditions that affect cell mechanics in different ways. We observed a strong anticorrelation of RevVNP circadian robustness and YAP/TAZ nuclear levels but not with those of MRTFA.

To test if YAP/TAZ regulate the clock directly, we overexpressed dominant positive mutants of YAP/TAZ. This caused a huge impairment of the oscillations of not only *Rev-erbα* but also other

core clock transcripts, like *Bmal1* and *Cry1*, in a TEAD-dependent manner. This demonstrates a novel role of YAP/YAZ as a circadian modulator.

Considering the role of YAP/TAZ as core mechanosensors and the metabolic importance of REV-ERB, our findings provide a fundamental link between the largely disconnected fields of chronobiology, metabolism and mechanobiology. Our next objective is to investigate whether this recently identified regulatory pathway influences intestinal circadian biology. To accomplish this, we are using intestinal epithelium organoids, where our observations reveal higher levels of BMAL1 and REV-ERBα in the stem cell-rich crypts compared to the villus-like regions, coinciding with the expression patterns of YAP/TAZ.

3- Developing Human Organoids To Model Genetic And Systemic Conditions During Congenital Anomalies Of The Kidney And Urinary Tract.

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Congenital anomalies of the kidney and urinary tract (CAKUT) encompass a spectrum of malformations affecting the kidneys, urethra, ureters, and bladder during embryonic development. With an incidence of 4-60 per 10,000 births, CAKUT presents a significant challenge, primarily addressed through invasive treatments like urologic surgery, dialysis, or transplantation. Many of the 40 established monogenic causes of human CAKUT were initially derived as candidate genes from observations in mouse models of CAKUT and subsequently screened for their prevalence in human disease cohorts. However, the insights from mouse models, do not always directly translate to human genetics.

Human kidney organoids have emerged as crucial tools to study morphogenetic processes under healthy and disease states. When developed from human pluripotent stem cells (hPSCs), human kidney organoids represent an unprecedented tool set to study how mutations previously related to CAKUT can explain early disease phenotypes. Here we employed CRISPR/Cas9 to engineer hPSCs, creating reporter cell lines to monitor the endogenous expression of GATA3, a lineage specifier of one of the two stem progenitor cells of the kidney, namely the ureteric progenitor cells. At the same time, GATA3 represents one of the most prevalent genes leading to renal and extra-renal CAKUT manifestations in patients. Similarly, we have further generated knock-out (KO) lines in the background of the fluorescent reporter lines to investigate the impact of PAX2 and HNF1B mutations in the GATA3 lineage.

At the present time, we are assessing successful protein suppression in the KO backgrounds through Western blot and confocal microscopy analyses. Furthermore, we are validating the successful development of our CRISPR/Cas9 engineered lines through their differentiation into nephron-like kidney organoids. Importantly, we will investigate the impact of the different genetic backgrounds in extrarenal complications of CAKUT through the differentiation of these lines into cardiac and retinal organoids. The approach generated here will further allow for the investigation of maternal conditions leading to CAKUT such as gestational diabetes, hypertension, and hyperlipidemia.

4- *In vitro* recapitulation of heterochrony during vertebrate anterior–posterior axis development

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Segmentation and regionalization of the anterior–posterior (AP) axis of the animal body plan are strictly regulated. Coordination of these processes requires a tight and effective regulation of gene expression temporal dynamics so that tissues organize into specific spatial patterns. During vertebrate development, an important regulator of the sequential formation of body segments is the segmentation clock. This “clock” consists of periodic oscillations of gene expression in the pre-somitic mesoderm (PSM) which in turn originate segmental axial structures, known as somites, that give rise to the formation of the vertebral column.

A slowdown in the rate of somitogenesis can be observed during AP axis development within one species. These relative differences in the timing, rate, or duration of developmental events are known as heterochronies. One extreme example is the marsupial development. These species present a strong pattern of heterochrony, meaning they are born with the anterior region and forelimbs in a very advanced stage of development in contrast to the posterior somites and hind limbs. These heterochronies are important for the evolution of species, however, the mechanism by which they happen is still to be understood. How can marsupials modulate their developmental tempo during AP axis development?

With this project, we aim to investigate how the patterns of heterochrony emerge in different species and impact the developmental tempo of somite formation, especially in the marsupial *Monodelphis domestica*. For this, we have developed a stem cell differentiation protocol that allows the temporal and sequential expression of HOX genes from anterior to posterior levels with concomitant differentiation into PSM. By comparing the tempo of anterior and posterior differentiated PSM cells, this protocol will allow us to investigate the emergence of heterochrony along the vertebrate AP axis.

5- Epithelial-mesenchymal coupling drives axis elongation in *Xenopus explants*

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Embryonic morphogenesis is a complex process characterized by coordinated movements of tissues, driven by an interplay of mechanical and biochemical processes. Within this process, morphogenetic patterning leads to regional cell differentiation yielding specific cell behaviours crucial for shaping the developing organism. In addition to regional differences in cell behaviour, coupled neighbouring tissues interact to impose organisational constraints through cell sorting, surface tension effects, tissue-tissue boundary conditions; adding an additional layer of guidance and influence over morphogenesis. In this research, we focus on a phenomenon observed in Xenopus during the formation of the anterior-posterior axis where deep mesodermal cells in the dorsal marginal zone polarise mediolaterally and intercalate; which drives tissue extension of the axial mesoderm. Notably, the initial stages of this process are contingent upon the attachment of the overlying endodermal epithelium, without which tissue extension fails. Equally, when deep mesodermal cells are removed from the environment of the embryo prior to the onset of gastrulation and left to develop in isolation, also fail to elongate. The capacity for tissue extension is recovered when deep mesodermal cells remain coupled to the overlying endodermal epithelium, even outside the environment of the embryo. This implies that epithelial-mesechymal tissue interaction is important for axial extension but the precise role of the overlying ectoderm and how orientational information that drives oriented cell intercalation emerges in the system remains an open question. In this study we find that epithelium is both playing an active role in tissue extension and also sets cell elongation orientation coordinating cell intercalation through boundary condition effects that propagates deep into the internal mesenchymal cells.

6- Dynamics of fibrotic foci formation during bleomycin-induced pulmonary fibrosis in mice

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Fibrotic focus is the main structural unit in developing fibrosis of various tissues, like lungs, liver or mammary glands. The focus consists of the myofibroblast core of misfolded specific extracellular matrix (ECM) proteins (collagens I, III, IV, V, and VI, as well as fibronectin) synthesized by myofibroblasts and activated stromal cells located at the periphery of the core. The process of the fibrotic focus formation, as well as its further development or resolution, remains understudied. However, recreating the structure of the focus *in vitro* may be a good model for assessing the dynamics of fibrosis development and testing antifibrotic drugs. Due to the fact that the reconstruction of this structure *in vitro* may require reproducing *in vivo* conditions, it is important to study the dynamics of formation and the cellular composition of this structure in models of fibrosis *in vivo*.

Materials and methods

A model of bleomycin-induced pulmonary fibrosis in C57BL/6 mice was used. The dynamics of fibrosis development were assessed on days 3, 7, 14, 28 after bleomycin instillation. For a general assessment of the fibrosis level, MRI as well as H&E and picrosirius red staining was used. Location and amount of the main cell types influencing the development of the fibrotic focus including the general population of stromal cells (CD90), activated stromal cells (FAPa), myofibroblasts (aSMA), M2 macrophages (CD206, CD163), epithelium (cytokeratin, HOPX), as well as major ECM proteins (type I collagen, fibronectin) and proliferating cells (PCNA) were evaluated by IHC.

Results and discussion

Based on the data, we can assume the following dynamics of the development of a fibrotic focus in the lungs: activated stromal FAPa+ cells, which are considered the “progenitors” of the focus, are formed already in the early stages of tissue damage (3 days after bleomycin instillation) due to signals received from the damaged epithelium. Activated cells begin to differentiate into aSMA+ CD90+ myofibroblasts on 3-7 days. Subsequently, this process leads to thickening of the alveoli

walls both due to the proliferative activity of FAPa+ cells and their differentiation into myofibroblasts. This process leads to formation of FAPa+aSMA+ CD90+ small fibrotic foci rich in fibronectin and, probably, other extracellular matrix proteins. In the formed fibrotic focus, aSMA+CD90- FAPa- negative cells form the core of the focus, while CD90+ FAPa+ and CD90- FAPa+ cells occupy its periphery. The study was supported by the Russian Science Foundation grant No. 23-15-00198, https://rscf.ru/project/23-15-00198/.

7- The contribution of the innate immune response to blood-brain barrier breakdown in Cerebral Malaria

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Cerebral Malaria (CM) is a severe neurovascular pediatric complication of Plasmodium falciparum infections characterized by vasogenic edema and brain swelling caused by the disruption of the blood-brain barrier (BBB). Evidence of immune cells accumulating in the microvasculature of CM patients suggests proinflammatory immune response as an important factor in CM pathogenesis. However, it is not completely understood whether immune cell activation and accumulation is an important contributing cause or mainly a consequence of BBB breakdown. As studies on post-mortem patient samples do not provide information about causation we are using in-vitro approaches, including an engineered 3D-BBB model seeded with endothelial cells, pericytes, and astrocytes to study the interaction between immune cells and brain microvasculature in CM. Preliminary studies in 2D monolayers of human brain microvascular endothelial cells (HBMEC) have shown a disruption of the endothelial barrier after addition of P. falciparum-stimulated leukocytes (Pf.-leukocytes). The endothelial disruptive mechanisms include disruption of adherens junctions, increased apoptosis of HBMEC, along with NFkB nuclear translocation and endothelial activation. When perfused through the 3D-BBB model, Pf.-leukocytes adhere within the microvessels and induce inflammatory activation and stress fiber formation in HBMEC. We are currently performing single-cell RNA sequencing of the 3D-BBB model perfused with Pf.-leukocytes in order to characterize the activation of Pf.-leukocytes, the leukocyte populations predominantly adhering within the 3D-BBB model, and the transcriptomic mechanisms of BBB disruption. Taken together, our results show that Pf.-leukocytes induce BBB disruption in-vitro, suggesting that the innate immune response is a key player in CM pathology.

8- Optogenetic engineering of morphogen gradients recapitulates dynamic neural tube patterning

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Morphogen gradients dictate tissue patterning during development, but mimicking this process with in vitro differentiation systems remains challenging. Existing approaches lack the precise spatiotemporal control required for recapitulating robust patterning, limiting their usefulness for studying the dynamic interplay between morphogen gradient formation and cellular responses.

We present an optimized optogenetic system for tight regulation of morphogen expression in space and time, enabling in vitro recapitulation of neural tube patterning. Spatially restricted production of sonic hedgehog (SHH) in micropatterned colonies resulted in a long-range morphogen gradient that instructed the progressive formation of spatially distinct neural progenitor domains. By dynamically regulating SHH production, we show that the level and duration of morphogen exposure are crucial for the acquisition and maintenance of positional identity. We show how the post-translational processing of SHH and the expression of extracellular morphogen-interacting proteins affect the size of progenitor domains. Finally, we demonstrate how dynamic perturbations can be used to quantify rates of morphogen secretion and degradation, which define gradient dynamics and length scale.

Our work highlights the application of optogenetically induced signalling centres to gain quantitative insight into morphogen gradient formation and interpretation in vitro. This positions optogenetics as a powerful tool for synthetic biology, paving the way for more accurate and reproducible engineering of complex multicellular systems.

9- Deciphering the impact of the APC mutation on CAR T cells cytotoxicity using mouse and patient-derived 3D models in microfluidics.

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Adenomatous polyposis coli (APC) is a gene frequently involved in the development of colorectal cancer as it can induce accumulation of beta-catenin in the cells and therefore promote the activation of oncogenic genes. However, very little is known about whether mutations of APC either in cancer cells or in immune cells can affect the activity of cytotoxic T lymphocytes (CTLs) against solid tumors. Here we address this question using a droplet microfluidic platform recently developed in our team [1], in which tumor spheroids are challenged with CTLs within stationary droplets. The CTL behavior is obtained by tracking their history as they explore the extra-tumoral space, find the cancer spheroid, accumulate on it, and kill the cancer cells.

We first compare CTLs originating from wildtype or $APC_{Min/+}$ mice and show that the APC mutation reduces the ability of CTLs to destroy the tumor spheroids. Quantitative analysis of the CTL dynamics on and around the spheroids indicates that APC mutants migrate in the extra-tumoral space and accumulate on the spheroids as efficiently as control cells. Once in contact with the tumor however, mutated CTLs display reduced engagement with the cancer cells, as measured by a new metric that distinguishes different modes of CTL migration. We also identify localized killing cascades, in which several cancer cells go through apoptosis in quick succession. Then aligning the CTL trajectories around these spatio-temporal events reveals that all CTLs transition to high engagement in the two hours preceding the cascades, which confirms that the low engagement is the cause of reduced cytotoxicity [2].

Our current experiments are exploring the impact of the APC mutation in the cancer cells, using patient-derived organoids in the same microfluidic platform. For this, we engineered human patient-derived-organoids (PDOs) from colorectal cancer to make them sensitive to CAR-T cells, while also generating knock-out lines for the APC gene. This toolbox will allow us to investigate how the CTL-cancer interactions are modified by molecular or physical properties of the PDOs.

In addition to addressing this fundamental question, this technological platform and quantitative approach can be used to investigate a wide range of questions concerning immuno-oncology, both for fundamental studies, pharma applications, and personalized medicine.

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10-Mechanics of apical constriction: an optogenetic approach

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Developing an animal embryo is a multidisciplinary task. To successfully achieve morphogenesis, biochemical signaling and physical forces are tightly coordinated, giving rise to events such as gastrulation or organ formation. Apical constriction has been identified as a process that can drive morphogenesis, but the role of cellular forces during it is not identified. By using a novel optogenetic tool named OptoShroom3 we gain spatiotemporal control of apical constriction in human pluripotent stem cells and measure how forces evolve during the process. Our results detail the effects of apical contractility, reporting pivotal out-of-plane deformations required for three-dimensional morphogenesis, as well as enabling long-range transmission and generating tissue scale effects. These results provide a quantitative mechanical framework for apical constriction that can be applied to the rational engineering of multicellular systems.

11-Upgrading a Consumer Stereolithographic 3D Printer to Produce Physiologically Relevant Cancer Models

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Keywords — Bioprinting, cancer models, organoids, spheroids

A widespread application of 3D bioprinting in basic and translational research requires accessibility to affordable printers able to produce physiologically relevant tissue models. To facilitate the use of bioprinting as a standard technique in biology, an open-source device based on a consumer-grade 3D stereolithography apparatus (SLA) printer was developed. To showcase the capability of the bioprinter, constructs consisting of cancer cells (patient-derived cholangiocarcinoma (CCA) organoids or breast cancer cell lines) encapsulated in a gelatin methacrylate (GelMA)/polyethylene glycol diacrylate (PEGDA) hydrogel are produced. Several hydrogel concentrations were prepared and their rheology measured, and it was shown that higher concentrations of GelMA and PEGDA led to stiffer hydrogels with smaller pore sizes. After 3D bioprinting and cultivating patient-derived CCA organoids in GelMA and PEGDA, the cells remained highly viable. Immunofluorescence staining and RT-qPCR showed that the morphological, physiological and genetic expression of the CCA organoids were representative of the patients' original biopsy. Next, a breast cancer model was 3D bioprinted: T47D spheroids were encapsulated in increasingly stiff hydrogels to mimic a fibrotic environment, leading to an aggressive phenotype. When introduced to simulated microgravity, the previously described phenotype was reversed. However, when increasing the stiffness of the extracellular matrix (ECM) of the hydrogel encapsulating the spheroids cultured in simulated microgravity, the aggressive phenotype was reestablished, indicating the crucial role of the ECM in breast tumor progression. This work shows that using an easily customizable and affordable bioprinter; complex cancer models can be produced that could enable personalized medicine and further complex research in both basic and applied biology.

12- A single-cell atlas of the murine pancreatic ductal tree identifies novel cell populations with potential implications in pancreas regeneration and exocrine pathogenesis.

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Background and aims: Pancreatic ducts form an intricate network of tubules that secrete bicarbonate and drive acinar secretions into the duodenum. This network is formed by centroacinar cells, terminal, intercalated, intracalated ducts, and the main pancreatic duct. Ductal heterogeneity at the single-cell level has been poorly characterized; therefore, our understanding of the role of ductal cells in pancreas regeneration and exocrine pathogenesis has been hampered by the limited knowledge and unexplained diversity within the ductal network.

Methods: We used scRNA-seq to comprehensively characterize mouse ductal heterogeneity at single-cell resolution of the entire ductal epithelium from centroacinar cells to the main duct. Moreover, we used organoid cultures, injury models and pancreatic tumor samples to interrogate the role of novel ductal populations in pancreas regeneration and exocrine pathogenesis.

Results: We have identified the coexistence of 15 ductal populations within the healthy pancreas and characterized their organoid formation capacity and endocrine differentiation potential. Cluster isolation and subsequent culturing let us identify ductal cell populations with high organoid formation capacity and endocrine and exocrine differentiation potential *in vitro*, including Wnt-responsive-population, ciliated-population and FLRT3^{+} cells. Moreover, we have characterized the location of these novel ductal populations in healthy pancreas, chronic pancreatitis, and tumor samples, highlighting a putative role of WNT-responsive, IFN-responsive and EMT-populations in pancreatic exocrine pathogenesis as their expression increases in chronic pancreatitis and PanIN lesions.

Conclusions: In light of our discovery of previously unidentified ductal populations, we unmask the potential roles of specific ductal populations in pancreas regeneration and exocrine pathogenesis.

KEYWORDS: ductal cells; scRNA-seq; organoids; exocrine pathologies; pancreas regeneration.

13- Size control of *in vitro* somites

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Somites, the precursors of our body vertebrae, ribs, and skeletal muscles, emerge as repetitive structures lining either side of the neural tube during the post-gastrulating embryo development. Different models have been proposed so far to explain somite size determination, with classical ones pointing to patterning and global positional information as a possible answer (e.g. the clock and wavefront model), and more recent ones emphasizing the role of local cell-cell interactions in self-organizing the somite unit. Recently, a 3D in vitro model of human somitogenesis has been established in our lab, starting from human induced pluripotent stem cells (iPSCs): the somitoids. Interestingly, when we increase the number of cells to aggregate to make the somitoids, the size of the somites remains constant despite the overall size of the organoid getting bigger. This non-scaling behavior of somites makes human somitoids a suitable model to investigate which factors control somite size in vitro. Quantifying the tissue proportions in somitoids of different sizes, we found out that another tissue displays similar behavior as the somites: the presomitic mesoderm, which is the source of somites themselves. We hypothesize that the size of in vitro somites is controlled because a size control mechanism is acting upstream, on the presomitic mesoderm. Our current focus is therefore trying to understand how the size of presomitic mesoderm is controlled and how the regulation of this tissue proportion can change during somitoids development.

14- Integrated Computational-Experimental Analysis of Shear Impact on Intestinal Crypt Dynamics and Mucus Mechanics

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The motor functions of the intestine including rhythmic contractions and relaxations allow transferring and processing of chyme. Each of these functions generates a shear stress on the epithelium that varies in magnitude. How such shear stress affects epithelial processes such as crypt fission and fusion, and tissue compartmentalization as well as the mechanical role of mucus is not well understood. To address these questions, here we developed a computational fluid dynamics model that integrates intestinal mucus, chyme, intraluminal pressure, and crypt geometry to predict the time-space mosaic of shear stress. We combine this model with an organ-on-a-chip device that could allow traction force microscopy to map cellular forces. Our computational data show that the intestinal mucus may significantly reduce the amount of shear stress applied to the crypts with significant apico-basal variation and increase chyme velocity. Using our on-chip model we aim to explore the interplay among shear stress, crypt dynamics, and mucus.

15-Microfluidic control of Notch signalling reveals a dynamic communication code for cell fate determination during intestinal homeostasis

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Notch signalling plays a crucial role in maintaining cell-type patterning in the intestine. Notch signalling is active during stem cell maintenance, and is also a decisive factor in their differentiation into the absorptive/secretory lineage. During homeostasis, however, the timing of these processes must be dynamically coordinated to preserve intestinal patterning, and exactly how Notch strikes this balance is far from understood.

In this project, we generated a mouse line with a fluorescent reporter for Notch pathway activity, from which we derived intestinal organoids for live-cell imaging. We then carried out single-cell tracking, which revealed that Notch signalling is oscillatory in certain cell types of the epithelium. We also developed a microfluidics system to perturb signaling dynamics in real-time, which showed a dynamics-dependent regulation of specific cell types. Taken together, our study demonstrates that these dynamics play a functional role in tissue-wide cell fate specification as has been seen in model systems of embryonic development.

16-3D micropatterned traction force microscopy : a new technique revels that single epithelial cells can exert pushing forces on their environment

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From the tiny sperm cells to the star shaped neurons, how cells size and shape are link to their functions have not cease to question and amazed scientists. More recently, cell morphology have been correlated to their response to mechanical signal with cell spreading being associated with the mechanical activity of cells. However interesting, most studies use 2 dimensional (2D) systems and thus do not recapitulate to the full extent 3 dimensional (3D) cell shape, especially in the case of epithelial cells, though we know the importance of mechanical homeostasis inside the epithelium and its role in wound healing.

In this work, we have developed a system of structured hydrogel that enables us to measure, in 3D, the amount of forces exerted by a single-cell of controlled morphology. With it, we report a novel phenomenon in which breast epithelial cells exert pushing forces on their environment, and not only pulling forces as previously described. Moreover, we demonstrated that the shift from pulling to pushing is correlated with a diminution in cell volume and that, though, the actin cytoskeleton plays a role in both behaviors, the myosinII is only implicated in the contractile activity. More generally, this raises the question of the importance of such a phenomenon in an epithelium where the cell volume and mechanical homeostasis need to be constantly maintain through cell division and cell death.

17-3D bioengineered liver for the study of acute and chronic hepatic damage

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The liver, a vital organ, faces acute and chronic insults that disrupt its normal function. Acute damage, caused by toxins or infections, triggers inflammation and necrosis. Chronic insults, such as alcohol abuse or viral hepatitis, lead to fibrosis, cirrhosis, and hepatocellular carcinoma, posing significant clinical challenges. Fibrosis is a hallmark of liver damage driven by the activation of hepatic stellate cells (HSCs). Understanding the mechanisms underlying acute and chronic liver damage is crucial for developing effective treatments. Traditional liver models face several limitations. 2D cultures cannot maintain liver phenotype and functions for extended periods, making it difficult to model chronic exposure. Additionally, replicating fibrosis in 2D cultures is challenging due to HSC activation on plastic or glass surfaces. As a result, 3D models have emerged as a more physiologically relevant cellular microenvironment for investigating disease progression, identifying potential therapeutic targets, and developing new drugs.

We developed a 3D liver using human hepatocytes (HepaRG), HSCs (LX-2), and monocytes (THP-1). The cells were encapsulated in a mixture of gelatin methacryloyl and carboxymethyl cellulose methacrylate, and lithium phenyl(2,4,6-trimethylbenzoyl)phosphonate as a photo-initiator. The 3D livers were kept in culture for up to 30 days in serum-free medium. They were challenged with acetaminophen and LPS (APAP-LPS), known hepatotoxic compounds, to recreate the pathophysiological phenotype of liver damage in vitro. Dexamethasone was used as an anti-inflammatory drug to test the ability of 3D livers to predict drug efficacy.

Extensive liver damage characterized by hepatic stellate cell (HSC) activation and proliferation was observed upon challenge with APAP-LPS. In vivo, these cells exhibited the myofibroblast phenotype typical of activated HSCs. Additionally, impaired gene expression of hepatocyte functionality markers was observed. The transition from monocytes to proinflammatory cytokine-releasing macrophages measured the inflammation level. Notably, dexamethasone demonstrated potent beneficial effects, reducing hepatocyte damage, inhibiting HSC activation, and decreasing collagen production. These results were observed in both acute (high APAP-LPS concentration/3 days) and chronic (low APAP-LPS concentration/30 days) models.

The 3D model presented here demonstrates its value as a versatile platform for drug screening in both acute and chronic liver damage scenarios. Its ability to reproduce critical features of liver pathophysiology, including hepatocyte functionality impairment, HSC activation, and inflammation, makes it a valuable tool for studying liver diseases and evaluating potential therapeutic interventions. Furthermore, the adaptability of this model for high-throughput screening provides an opportunity to accelerate the drug discovery process and improve patient outcomes in liver damage-related conditions.

Disclosures

Conflict of interest. E.A., R.H. are full time employees of Grifols. This study is supported by Grifols.

18- Mechanical and Functional Responses in Astrocytes under Alternating Deformation Modes Using Magneto-active Substrates

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Key Words: *Mechanobiology, Brain mechanics, Magnetorheological elastomers, Astrocytes, Piezo1*

This work introduces NeoMag, a system designed to enhance cell mechanics assays in substrate deformation studies. NeoMag uses multidomain magneto-active materials and external magnetic fields to mechanically actuate the substrate, transmitting reversible mechanical cues to cells. The system boasts full flexibility in alternating loading substrate deformation modes, seamlessly adapting to both upright and inverted microscopes. The multidomain substrates facilitate mechanobiology assays on 2D and 3D cultures. In addition, the integration of the system with nanoindenters allows for precise evaluation of cellular mechanical properties under varying substrate deformation modes. The system's efficacy is demonstrated by studying the impact of substrate deformation on astrocytes, simulating mechanical conditions akin to traumatic brain injury and ischaemic stroke. The results reveal local heterogeneous changes in astrocyte stiffness, strongly influenced by the orientation of subcellular regions relative to substrate strain. These stiffness variations, exceeding 50% in both stiffening and softening, and local deformations significantly alter calcium dynamics. Furthermore, sustained deformations induce actin network reorganization and activate Piezo1 channels, leading to sustained calcium influx that inhibits calcium events. Conversely, fast and dynamic deformations transiently activate Piezo1 channels and disrupt the actin network, causing cell softening over 24 hours. These findings unveil mechanical and functional alterations in astrocytes during substrate deformation, illustrating the multiple opportunities this technology offers.

19-Mechanics of Human and Mouse Embryo Implantation

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During implantation, the mammalian embryo establishes attachment to the endometrium, the lining of the maternal uterus, followed by invasion into the underlying tissue. To understand how embryos penetrate the collagen-rich endometrial stroma, we have developed an innovative hydrogel-based ex-vivo platform supporting traction force microscopy. We reveal the forces applied by human and mouse embryos and recapitulate implantation specificities of both species in our platform. Mouse embryos exhibit limited penetration depth whereas human embryos integrate into the matrix. Nevertheless, both types of embryos apply forces during implantation resulting in the remodelling of the collagen matrix. Interestingly, the applied forces lead to distinct displacement patterns: Isotropic radial displacement for human and anisotropic with main displacement axes for mouse embryos.

Blocking force transmission through integrins, specifically β5 and β3 integrins with a cyclic pentapeptide or Src kinase with dasatinib, reduces the size of mouse embryos and their matrix displacement. Notably, when placed pairwise, embryos form tension-bearing mechanical bridges between them, leading to collagen densification and directed matrix displacement along the connecting axis.

Furthermore, both human and mouse embryos exhibit mechanosensitive responses to an external mechanical stimulus: The mouse embryo either orients its growth direction or aligns its axis relative to the external force cue. The human embryo recruits phosphorylated myosin basally and forms a cellular protrusion towards the external force cue.

In conclusion, our findings highlight the intricate mechanical interactions between embryos and their environments and mechanosensitive capacity of embryos during implantation. We suggest that mechanical forces may play an important role in guiding the invasion of the extracellular matrix during implantation.

20- Adipose Prototissues: biomaterial-based synthetic tissues to investigate cancer metastasis

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Ovarian cancer cells commonly metastasise to adipose tissues such as the omentum and the peritoneum. As ovarian cancer cells colonise adipose tissues, adipocyte size and content diminish whilst extracellular matrix (ECM) and stromal cell content increases. These alterations greatly impact the biochemical and biophysical features of these tissues. Cancer cell invasion has classically been investigated in ECM-rich environments. However, how the specific characteristics of ECM-poor adipose tissues and their evolving features regulate ovarian cancer cell invasion is not well understood.

To investigate the process of ovarian cancer invasion into adipose tissues, we developed adipose prototissues comprising biomaterial-based synthetic proto-adipocytes and natural or synthetic ECM matrices. These adipose prototissues present remarkable structural and mechanical similarities with native human adipose tissues. Moreover, by modifying the distinct components of adipose prototissues, we modelled the evolving characteristics observed in ovarian cancer metastases.

Using the adipose prototissues, we found that the invasive response and mechanisms of ovarian cancer cell invasion into adipose prototissues mirror those observed in human adipose tissue explants. Conversely, these invasive responses were not recapitulated in commonly used ECM hydrogels. Additionally, we found that the biophysical characteristics of adipose prototissues, including proto-adipocyte size, volume fraction, and mechanical characteristics, or ECM adhesion but not its degradation, regulate ovarian cancer cell invasion.

Altogether, these results show that adipose prototissues may be used as adipose tissue mimetics and that the biophysical characteristics of adipocytes are major regulators of ovarian cancer cell invasion.

21-Biomechanics of blood brain barrier disruption in cerebral malaria

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A hallmark of cerebral malaria is *Plasmodium falciparum*-infected red blood cell (iRBC) sequestration in the brain vasculature leading to blood brain barrier (BBB) dysfunction and fatal brain swelling. While, iRBCs have been found to sequester throughout the entire brain vasculature, post-mortem samples display increased evidence of haemorrhaging in white matter compared to grey matter. Mechanically speaking, these two tissues are very different with white matter being approximately twice as stiff as grey matter. We hypothesize that these changes are, at least, partially caused by brain differences in mechanical properties. In this study we visualise endothelial breakdown in real-time upon exposure to iRBC egress products in human primary brain microvascular endothelial cells. By customising matrix composition, we can precisely tune the mechanics of our system to observe its role in endothelial cell dysfunction in cerebral malaria. We reveal that when brain endothelial cells are cultured with iRBC egress products they demonstrate a significant reduction in barrier function alongside increases in actomyosin contractility and changes to cell morphology and migration. Additionally, we show that changes in barrier function can be partially rescued by changing the mechanics of the substrate or after inhibition of signalling through focal adhesions. We are currently using mechanobiological tools such as atomic force and Brillouin microscopy to measure how endothelial cell mechanics change in response to parasite products and to get deeper insights in the mechanisms behind cerebral malaria pathogenesis.

22-Reconstitution of the cellular microenvironment using decellularized extracellular matrix activates cell differentiation in vitro

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The extracellular matrix (ECM) is a multicomponent mixture of fibrillar and adhesive proteins, glycoproteins and proteoglycans, as well as signaling molecules and matrix-associated vesicles. The ECM creates tissue structure and generates mechanical forces that influence differential gene expression in associated cells. In addition, the tissue-specific composition of the ECM, which is different in maintaining homeostasis and in pathology, influences the processes of cell differentiation. When studying cell differentiation in vitro, modeling conditions often include the addition of soluble factors, but overlook such a component of the microenvironment as ECM. Modeling the microenvironment using individual ECM components (collagens, fibronectin, etc.) has obvious limitations, since it does not reproduce the multicomponent composition and stiffness of ECM tissue. We used the technology of decellularization of multilayer cell sheets obtained from different types of human stromal cells, including mesenchymal stromal/stem cells (MSCs) and fibroblasts from different tissues. The resulting dECM retained a multicomponent composition (type I collagen, fibronectin, EDA-fibronectin) and complex architecture, which was studied by scanning electron microscopy. We have previously shown that ECM obtained from human adipose tissue MSCs leads to increase of induced differentiation of multipotent stem cells into osteocytes, adipocytes and chondrocytes. Presumably, dECM, through an ERK-dependent mechanism, stimulated the proliferation of progenitor cells and potentiated differentiation upon the addition of appropriate stimuli. At the same time, dECM obtained from MSCs isolated from human dental pulp had the ability to accelerate the induced differentiation of multipotent cells in the osteogenic direction, compared with dECM obtained from MSCs of adipose tissue and skin fibroblasts. This observed effect could be associated with an increase in the basal expression of the RUNX2 gene, the master gene of osteogenic differentiation, in multipotent cells cultured on dental pulp MSCs dECM. Moreover, in each case, dECM itself did not stimulate differentiation without additional induction. In profibrotic in vitro model, cultivation only on dECM obtained from human skin fibroblasts led to rapid (within 12 hours) growth of the fibroblast activation protein (FAPa), but did not stimulate the formation of myofibroblasts, which indicates the transition of fibroblasts to an activated state on dECM. Myofibroblast formation was observed only 72 hours

after TGF-β1 induction of fibroblasts. Thus, in vitro modeling of the cellular microenvironment using dECM obtained by decellularization of cell sheets can recreate more relevant conditions for cell differentiation. The data presented indicate that cultivation on dECM puts cells in a state susceptible to further differentiation stimuli, which is an integral part of the differentiation process.

23- 3D quantitative analysis of gastruloid symmetry breaking

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The early development of mammalian embryos after implantation remains largely a mystery in biology. In particular, the initial formation of the body during gastrulation is a fundamental process that is not well characterized due to its inaccessibility. To overcome this limitation, recent works have shown that mouse embryonic stem cells can aggregate and self-organize *in vitro* in structured tissues that share similarities with the mouse embryo during gastrulation. This multicellular system, called gastruloid, develops from a spherical and homogeneous stem cells aggregate to a 3D tissue breaking its symmetry in both morphology (tissue elongation) and gene expression (tissue polarization). My project is to understand the contribution of cell division to the gastruloid symmetry breaking process. My working hypotheses are (i) that oriented cell divisions/anisotropic growth could participate to tissue elongation and (ii) that differences in growth rates between cell types could contribute to polarization.

However, the 3D geometry and complex dynamics of the system require specific tools for deep tissue imaging, temporal registration of 3D movies, segmentation and analysis. Experimentally, I image with two-photon microscopy both live and fixed gastruloids, to quantify cell position, tissue collective movements and gene expression patterns. From my images, I have developed a deep learning-based pipeline to segment cells and perform quantitative measurements (cells shape and size but also coarse-grained metrics reflecting cell density, position or orientation of the divisions).

Together, this high-throughput analysis allows me to quantify how gene expression interplays with physical properties at multiple scales during gastruloid development.

24- Artificial extracellular matrices based on 3D hybrid hydrogels for immune cell and organoid manufacture

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Artificial extracellular matrices (ECM) based on 3D hydrogels consisting of covalently crosslinked polyethylene(glycol) and heparin have been developed, which can easily be loaded with positively charged biomolecules through electrostatic interactions.[1] To finely control both the structural and mechanical properties of these 3D hydrogels, we have used different manufacturing procedures, such us the inverse opal technique or 3D printing.[2]

These PEG-heparin hydrogels were designed to mimic the ECM of healthy secondary lymphoid organs, in particular the lymph nodes, with the objective of improving the current T cell expansion technologies. In particular, our goal was to obtain in vivo persistent CAR T cells; a current limitation of the adoptive cell (immuno)therapies. Indeed, we have been able to increase the proliferation of primary human CD4+ T cells, when compared with state-of-the-art expansion systems, while maintaining therapeutically desired phenotypes.[1-2] Additionally, we tailored our 3D hydrogels to mimic the ECM of malignant tissues with the aim of creating well-controlled and reproducible patient-derived tumoroids.

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25- Targeting hypersialylation in pancreatic ductal adenocarcinoma models generated with microfluidic devices reverses its malignant phenotype

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Aberrant glycosylation such as hypersialylation, is a common feature of pancreatic ductal adenocarcinoma (PDA). Glycan changes during oncogenesis are closely related to tumor malignancy and immune suppression among others. For this reason, altered glycosylation represents a dynamic target for new treatment development efforts.

In previous works, we evaluated using 2D cell models, the potential of inhibiting α2,3-Sialyltransferases (α2,3-STs) ST3GAL3 and ST3GAL4. Theses enzymes add sialic acid (SA) with an α2,3 linkage to galactose of type-1 or 2 glycan structures and are overexpressed in PDA tissues. Knockdown of ST3GAL3/4 in PDA cells led to a significant decrease in cell migration, invasion, adhesion and rolling to E-selectin, which represent key steps in metastasis1. In an attempt to corroborate if a decrease in tumor sialoglycans with pharmacological targets could be useful to revert the aggressive phenotype of PDA, we also treated PDA cell lines with a sialyltransferase inhibitor. We observed that Ac53FaxNeu5Ac treatment decreased α2,3-SA and sialyl-Lewisx, which resulted once more in a significantly impaired cell migration, invasion and E-selectin adhesion. In an attempt to improve current immunotherapy strategies against solid tumors, PDA tumors were generated in syngeneic mice and treated with Ac53FaxNeu5Ac. We observed that subcutaneous murine tumors treated with Ac53FaxNeu5Ac reduced their volume, their SA expression, and modified their immune component, with an increase in CD8+ T-lymphocytes and NK cells2.

In addition to its altered glycosylation, PDA is also characterized by the presence of a dense and complex microenvironment formed by an abundant stroma, that favors the survival of the tumor and contributes to its wide resistance against current antitumor therapies. Thus, the final aim of this work was to better understand the functional impact of decreasing tumor sialoglycans using Ac53FaxNeu5Ac treatment onto a tumor 3D cell model that takes into account the complex extracellular matrix that surrounds pancreatic tumors. For that we designed an in vitro technique based on microfluidic devices that allow the development of multicellular clusters of PDA cells in a 3D extracellular matrix that mimics PDA stroma using collagen-I hydrogels. This novel approach allows us to study cell proliferation and evaluate the decreased of α2,3-SA in the PDA spheroids

by imaging with lightsheet microscopy. We also performed time-lapse imaging in 3D hydrogels and with 2D microchannel-based devices to corroborate the great inhibitory effect of Ac53FaxNeu5Ac in cell migration for both conditions.

Altogether, these results show the positive impact of reducing SA expression by inhibiting cell sialyltransferases and opens the way to use sialyltransferase inhibitors to improve immunotherapy in solid tumors and target PDA metastasis, which is one of the reasons for PDA dismal prognosis.

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26- Unravelling The Relationship Between Nutrient Availability In Tumor Microenvironment And Cancer Progression.

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Introduction

Cancer is one of the leading causes of death in the world and the search for its cure is the main goal of a large percentage of researchers worldwide1. There are different approaches for cancer research and in this study, we have focused on three-dimensional (3D) cell cultures and the effect of the microenvironment over tumor formation.

3D cell cultures allow a better recreation of cancer biology and represents more faithfully the physiological conditions of tumor formation2. Tumors are characterized by being highly heterogeneous, with each cell showing a different metabolite consumption according to its location within the tumor3. Our aim is to recreate the process of tumor formation in vitro under different glucose and oxygen conditions, as main nutrients for cancer cells4, to analyzed their effects over spheroid growing and structure. To determine metabolite dynamics inside the tumor spheroid, we used a microfluidic system, which will allow us to perform 3D cell cultures on a small scale with reasonable control of the environment5.

Materials and methods

A variety of microfluidic, microscopy and image analysis and 3D cell culture techniques were used in this work.

The microfluidic devices were made of PDMS, with a central chamber containing a hydrogel based on collagen type I that mimics matrix for cell culture and two side channels through which nutrients are introduced6. The cells used were A549 lung tumor and were monitored with a phase contrast microscope, which was used to take pictures of the progress of the cells. These pictures were then analyzed with ImageJ and Matlab software to obtain the growth curves. In addition, different stains were developed to study some parameters of the spheroids formed such as structure, cell junctions and viability. These stains were visualized by confocal and Lattice Lightsheet microscopy where Z-stacks allowed us to reconstruct the spheroids obtained in 3D.

Results

In this study, we analyzed the growth capacity of A549 cancer cells under different glucose and oxygen conditions, ranging from the most favorable microenvironment with high glucose and normoxia to the most unfavorable one without glucose in hypoxia. In this way, interesting differences in tumor evolution were observed. Under standard conditions, cells were able to form many large, compact and well-structured spheroids. However, when glucose, oxygen or both were removed from the culture, the few spheroids formed were small and disorganized. In addition, we tested the effect of removing some of these nutrients when the spheroids were developed and alterations over their structure were observed, indicating serious damage over cells viability.

Conclusion

Most existing 3D models of lung cancer are based on cell clusters already formed by the accumulation of cells7. However, this work proposes a novel model that encompasses the entire process of tumor morphogenesis, growth and maintenance guided by the surrounding microenvironment, allowing all stages to be studied. The results obtained highlight the importance of the microenvironment, establishing a relationship between nutrient availability and tumor progression. This may provide new insights of how glucose and oxygen can regulate cancer development and progression, which can be used to design new therapies.

Acknowledgements

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27-Nematically-guided morphogenesis

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Tissue morphogenesis relies on the orchestration of subcellular contractility into supracellular force patternsby multicellular assemblies,governed by an interplayof chemical and physical cues1. Despite the numerous scientific opportunities associated with the creation of synthetic tissues, both in fundamental and applied contexts, the precise control of tissue reshaping in vitrocontinues to pose a significant challenge2.To address this, it is crucial to developexperimental systems thatleveragethe inherent self-organization mechanisms ofliving tissuesto promote force patternsleadingto specificmorphogenetic transformations. For instance, in tissuescomposed of elongated cells,force organization is dominated by the orientationof cells in nematically-ordered domainsand the presence of topological defects,regions where the order is lost3.Here, we harness these characteristics, known toprovide unique mechanicalcues crucial fortissue remodelling4–7, toinducepre-definedtissuedeformations. In particular,by directly controlling cellular orientation and topological defects, weobtaincellularmonolayers that featurenematically-guided tension patterns, whichcan be released via out-of-plane deformationsintoreproducible three-dimensionaltissueshapes. By enabling the mapping of morphogenetic eventswithin living tissues, this strategy has the potential to open doors to applications across diverse fields, ranging from tissue engineering to soft robotics.

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28- Laser patterning bioprinting using a light sheet-based system equipped with light sheet imaging produces long-term viable full-thickness skin constructs

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Keywords — Bioengineering, Bioprinting, Light sheet imaging, full thickness skin model

INTRODUCTION

Light-based 3D bioprinting has emerged as a pioneering technique in tissue engineering and regenerative medicine, offering the promise of fabricating complex, functional three-dimensional (3D) tissue constructs with precise control over cellular organization and spatial distribution. This research introduces a new 3D bioprinter that incorporates live imaging of the bioprinted tissue with high resolution and high-speed capabilities. The printer employs a light sheet-based system to photocrosslink polymers into hydrogels at a printing speed of up to 0.66 mm^3/s with a resolution of 15.7 µm. A significant advancement of this bioprinter is its ability to track cells and bioink during crosslinking, which enables real-time evaluation of the 3D-bioprinted structure's quality.

MATERIALS AND METHODS

The light sheet bioprinter was built by means of optical and electrical engineering on top of an optical breadboard. Custom designed cuvettes were thermoformed and filled with photocrosslinkable hydrogels and human Hs27 and HaCaT cells. Bioprinting took place at 37° C and constructs were extracted afterwards into a well plate for further investigation. Cell viability was assessed at several timepoints and immunofluorescence staining of markers were conducted. Fluorescence recovery after photobleaching was carried out to determine the crosslinked hydrogel's properties. Image processing was done in Fiji by ImageJ. The statistical analysis and plotting were conducted on Python 3.9. Normality was tested with a Shapiro-Wilk test ($p>0.01$). Statistical comparison between two groups was tested with Welch t-test ($p<0.01$).

RESULTS AND DISCUSSION

The custom-made light sheet bioprinter reads common G-code files used in 3D printing, hence any 3D structure can be bioprinted. For proof of concept, a wheel of resolution, with spokes ranging from 1 to 120 µm, a liver lobule and a torus were 3D printed. Then, fibroblast cells (Hs27) were encapsulated using this method, and the viability was evaluated directly after bioprinting and seven days after encapsulation, which was found to be high (83% ± 4.34%). Furthermore, a full-thickness skin construct (with Hs 27 and HaCaT cells) was bioprinted and maintained in culture for 6 weeks, demonstrating the long-term viability and physiological relevance of the bioprinted tissue in terms of stratification and gene expression.

CONCLUSIONS

This novel, light based bioprinting technique enables fast and high resolution biofabrication, coupled with the possibility to capture cells and hydrogel before, during and after the bioprinting procedure via light sheet fluorescence imaging. High viability and physiological human full thickness skin constructs were bioprinted and cultivated over the course of 42 days.

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29-Engineering In-vitro Microenvironments to Replicate Complex In Vivo Conditions for Organoid Architecture

Masaya Hagiwara,

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In vitro 3D and organoid culture methods that emulate the intricate complexity of cell populations and extracellular matrix (ECM) components in vivo significantly contribute to advancing our understanding of various biological phenomena. However, achieving precise control over the complex shapes, architectures, and interactions among different tissues within cultured organoids remains a challenge. Current organoid development heavily relies on cellular self-organization, yet the uniform culture conditions in vitro fall short of providing accurate spatial cues to cells. Conversely, leveraging engineering principles offers a promising avenue to customize the design, composition, and construction of organoids based on specific research objectives.

We have developed an in vitro experimental platform for the organoid culture to design and control microenvironment. The simple cube device, which comprises a polycarbonate frame with rigid agarose walls and an inner ECM hydrogel, can be used as a carrier of organoid to (i) control the spatial distribution of cells by employing 3D-printed carbohydrate moulds to create cell seeding pockets in the ECM hydrogel, (ii) design tissues with localized ECM by isolating ECM hydrogels of varying the composition or stiffness in separate compartments, (iii) facilitate integration with microfluidics to generate the concentration gradient of morphogens to direct cell growth and differentiation, (iv) assemble multi-CUBE with organoids or tissues to express tissue-tissue interactions. By employing above technologies, we were able to replicate the notochord signal during the development of the neural tube. The Shh gradient, facilitated through a 100 μm slit on a CUBE device, was applied to the neural plate, resulting in the generation of a localized expression pattern on the neural tube organoid.

30-Cellular Mechanics and Self-Organization during Axes Formation in Mouse Gastruloids

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Early development has been studied from genetic and biochemical perspectives for decades. However, gene regulatory networks (GRNs) and signaling pathways alone cannot fully explain how a coordinated body plan arises from a homogeneous pool of pluripotent cells in the early mammalian embryo. In our work, we want to add a biophysical perspective to this classical view of developmental dynamics.

We aim at understanding the role of forces, material properties and tissue mechanics during early mammalian development, using mouse gastruloids. These multi-cellular aggregates of mouse embryonic stem cells self-organize into embryo-like structures, comprising the three major germ layers and a coordinate body plan including anterior-posterior, dorso-ventral and medio-lateral directionality. This allows us to study the formation of a global coordinate system in a minimal in vitro system without extraembryonic tissues. We therefore consider our findings relevant to the inherent properties of mammalian cells to self-organize in the absence of external clues, rather than being species-specific to mouse development.

Combining physics and engineering approaches, our team has characterized anterior-posterior axis development and identified a link between gene expression state and visco-elastic properties of differentiating gastruloids. Furthermore, we have shown that the ground state of cells (naive vs primed pluripotency) can determine germ layer proportions and shape of differentiated gastruloids. Perturbation of essential signaling pathways (Wnt/Mek/Erk or Tgf-β) during differentiation influences these properties, which hints at a connection between GRNs/signaling and cellular behaviors such as migration and multicellular rearrangements. In the following, we want to examine more deeply how mechanics and signaling influence each other to promote symmetry breaking, elongation and primary body axis formation on a molecular, cellular and tissue level.

31- Engineering of a simplified 3D microfluidic *in vitro* model for tumour-stroma dynamics of pancreatic ductal adenocarcinoma microenvironment

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Pancreatic cancer, specifically pancreatic ductal adenocarcinoma (PDAC), has a poor prognosis and is one of the deadliest cancers with a 5-year relative survival rate of only 12% [1]. Late diagnosis and limited treatment options contribute to the high mortality rate associated with this invasive neoplasm [1]. Despite significant advancements in our understanding of this disease, its complex microenvironment plays a crucial role in tumour progression and therapy resistance [2]. The microenvironment of PDAC is characterised by the interactions between cancer cells and the extracellular matrix (ECM), also known as stroma. The stroma can represent up to 90% of the tumour volume. This complex tumour-associated stroma, originating from pancreatic stellate cells, presents a challenge for effective treatment strategies, making it essential to explore innovative approaches that target both cancer cells and their supportive stromal elements in the pursuit of more successful outcomes for pancreatic cancer patients [3], [4]. This study aims to develop a simplified and reproducible *in vitro* 3D model of the PDAC microenvironment by incorporating key components such as collagen I hydrogels to mimic the extracellular matrix, pancreatic stellate cells (PSCs), and tumour cells. Utilizing a humanized microfluidic method, we have successfully engineered a multicellular setting that closely mimics the complexities of the PDAC microenvironment, incorporating a spatially defined 3D architecture. In our model, tumour cells autonomously organize into three-dimensional tumour spheroids, driven by biomechanical cues from their environment. This self-organization is initiated from single cells, rather than relying on pre-formed multicellular aggregates. Advanced imaging techniques are used to investigate the biochemical and mechanical effects of human pancreatic stellate cells (HPSCs) on processes such as cancer cell proliferation and migration. This study investigated the growth of tumour spheroids from four PDAC cell lines that represent different tumour subtypes (classical and squamous). Additionally, the migratory capacity of these representative PDAC cell lines was

examined under different microenvironments. Matrix stiffness and the co-culture of PDAC cell lines with HPSCs reveals how matrix density and HPSCs presence positively correlate with the formation and growth of tumour spheroids. Furthermore, we explore how HPSCs-conditioned medium influences PDAC tumour cell migration, indicating

that ECM remodelling processes and soluble factors secreted by HPSCs activate signalling pathways associated with cancer cell survival, growth, migration, and invasiveness. These findings demonstrate the crucial role of stellate cells in supporting and promoting pancreatic cancer. In addition, our results demonstrate the ability of our 3D microfluidic model to recapitulate key *in vivo* processes in PDAC tumour development, making it a valuable tool for the advancement of novel therapeutic and diagnostic approaches in PDAC and other solid tumours.

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32-Replicating Dynamic Immune Responses within a Microfluidic Human Skin Equivalent Model

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Dynamic communication between tissue resident cells and circulating immune cells, including monocytes, orchestrates the skin's responses to infection and plays a role in tissue repair and regeneration. However, the factors that drive monocyte recruitment into human skin during inflammatory events and the signals that direct monocyte fate are poorly understood. Current 3D in vitro models recapitulate the structure of skin by fabricating dermal and epidermal-like layers. However, only a limited number of in vitro skin models have incorporated immune cells, and these do not effectively model the complex and dynamic interactions between the tissue and immune system. This research aimed to gain new insights into human-specific inflammatory responses within skin, through the development of a novel immune-responsive in vitro model, using 3D bioprinting technology. A microfluidic human skin equivalent (HSE) was constructed by 3D printing a sacrificial gelatin microchannel template within a fibroblast embedded fibrin hydrogel. The microchannel template was then selectively removed by melting the gelatin at 37°C. The hollow microchannel was then lined with human endothelial cells, mimicking a vascularised dermis. Human keratinocytes were cultured on the surface of the construct to create mimic the epidermis.

Dynamic immune responses in the HSE were investigated by exposing the epidermal layer to lipopolysaccharide and nigericin, activating the inflammasome and inducing the secretion of cytokines, including IL-1β and IL-18. The vascular microchannel was then employed as a conduit for the delivery of primary CD14+ monocytes, and monocyte trafficking into the tissue was investigated by live confocal microscopy. Monocyte fate within the microfluidic HSE was first investigated using whole mount immunofluorescence staining for tissue resident macrophages. CD68+ and CD163+ cells could be identified within the dermal and epidermal compartments in both control and treated day 1 and day 6 conditions, with significantly increased numbers (2-3 fold) of recruited cells in the treated conditions, compared to controls.

Single cell transcriptomic analysis (10X Genomics) of the microfluidic HSE revealed dynamic transcriptional responses activated by inflammation in the resident skin cell populations and monocytes. Monocyte-derived cells displayed early inflammatory and migratory gene signatures at day 1, which resolved by day 6. Three distinct monocyte-derived clusters were identified in the day 6 samples, and results demonstrated the plasticity and differential potential of recruited monocytes within the in vitro model. Additionally, the gene signatures of these populations closely aligned with the profiles of resident myeloid cells in existing in vivo human skin datasets. Further analysis of putative inter-cellular signalling networks (CellPhoneDB) identified not only expected chemokine and cytokine interactions in the inflamed conditions, but also integrin, semaphorin, Wnt and Notch signalling between resident cell populations and monocytes. Furthermore, potentially novel nectin-nectin interactions were identified between keratinocytes and monocyte-derived cells.

Overall, the microfluidic HSE developed here accurately modelled dynamic immune responses in human skin and could be a powerful tool in drug development and discovery research.

33-Synthetically guided development of mobile embryoid bodies based on cardiac contractions.

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Organoids provide excellent models to study complex mechanisms of tissue formation, physiology, and associated diseases, driven by self-organizing stem cells resulting in complex tissues. While many studies focus on inducing specific differentiation states to mimic in vivo organogenesis using soluble factors-based protocols; controlling embryonic stem cell fate for tissue formation remains a challenge. Indeed, recent advancements in the field have not yet fully addressed how to epigenetically instruct stem cells for customized spatial patterns using encoded genetic information.

Therefore, one of the main captivating challenges in stem cell engineering is to develop the ability to synthetically guide the developmental trajectories of cells, leading to tissue patterning and ultimately enabling the implementation of novel functions for therapeutic purposes. My postdoctoral project aims to conduct proof-of-principle research on synthetic guidance to develop embryonic tissue in 3D. As a functional outcome, we have chosen to implement a 3D mouse stem cell aggregate with a cardiac contraction-based motility feature, which is easy to observe and measure. Numerous studies have shown that motility features can be attained through the utilization of cardiomyocytes as a propelling force. Through a synthetic developmental biology approach, our objective is to genetically drive a sequential cell fate decision that culminates in the creation of mobile embryoid bodies propelled by cardiomyocytes.

This study will leverage embryonic stem cell-based tissue engineering using synthetic biology tools to explore novel developmental trajectories.

34- Harnessing the rhythmic biology of early kidney formation for synthetic morphogenesis

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In this work we discover rhythmic mechanical and differentiation 'pace-making' in nephron-forming kidney niches using live imaging, packing theory, organoids, and spatial sequencing. We apply these new rhythmic biology principles to human kidney organoid engineering for regenerative medicine.

The kidney develops through branching of ureteric bud epithelial tubules (the future urinary collecting ducts), stroma, and nephron progenitors in the cap mesenchyme that surrounds each tubule tip. Dynamic interactions between these tissues set nephron numbers for life, impacting adult disease. How then are the rates of nephron formation and ureteric tubule branching balanced? Here we study the consequences of tubule tip packing at the embryonic kidney surface for tip organization and nephron formation. Over developmental time, kidney curvature reduces and 'tip domains' pack more closely, creating a semi-crystalline tip geometry at the kidney surface. This causes a rigidity transition to more solid-like tissue properties at later developmental stages, confirmed by micromechanical measurements. We then define a tip 'lifecycle' between branching events and find that nephrogenesis rate varies over this life-cycle. We show that tip domains experience a cyclical mechanical transient over each life-cycle. We then hypothesized that tip duplication periodically creates a mechanical microenvironment permissive to nephrogenesis. Indeed, mimicking a mechanical transient in human iPSC-derived nephron progenitor organoids increased Wnt-driven commitment to early nephron cell aggregates. The data suggest that temporal waves of mechanical stress within nephron progenitor populations could constitute a clock that synchronizes nephron formation and ureteric tubule duplication. We went on to find that the avalanche-like commitment of nephron progenitors to early nephrons reflects rhythmic transcriptional priming associated with the ureteric bud branch life-cycle using spatial sequencing. This acts to peg nephron formation rate to the ureteric bud branching rate. This rhythm shares features with the somitogenesis clock, an intriguing observation for future study. We next observe

significant changes in renewal vs. differentiation and subsequent nephron segmentation upon mimicking rhythmic YAP and retinoic acid signaling in nephron progenitor organoids. This new rhythmic biology principle presents the opportunity to create self-sustaining nephrogenic niches in vitro by mimicking cycles of alternating differentiation and renewal cues. Ongoing work will clarify variation in nephron endowment between kidneys and advance engineered replacement kidney tissues for regenerative medicine.

35- Effect of febrile temperatures on cerebral malaria in a 3D *in vitro* microvascular model

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Fever is the main symptomatic response to infection. In patients affected by malaria, febrile episodes synchronise with the release of parasites into the circulation every 48 hours. Pathogenesis and severity of the malaria disease occur when infected red blood cells sequester in the brain microvasculature, causing vessel occlusion, blood-brain barrier disruption, and haemorrhages. This study investigates how fever affects endothelial functions and parasite accumulation in the brain microvasculature, leading to one of the most severe consequences of malaria infection, cerebral malaria.

We developed a 3D engineered human brain microvessel model grown in a collagen hydrogel. Microchannels are patterned on a microfluidic grid geometry designed to span multiple flow velocities, thus mimicking properties of a branching microcirculatory postcapillary network. Our results show that febrile temperatures up to 40 oC increase binding of human red blood cells infected with multiple strains of parasites to primary brain microvascular endothelial cells in a receptor-dependent fashion. This suggests specific molecular rearrangement and surface expression of endothelial receptors such as EPCR and ICAM-1, as well as the possible contribution of unknown co-receptors. Parasite lines binding to both EPCR and ICAM-1 show a significant increase in binding at all flow rates tested, while parasites mainly interacting with brain endothelial receptors EPCR, bind preferentially at high wall shear stress (> 2 dyn/cm2). Furthermore, we found that hyperthermia changes endothelial cell morphology and vascular permeability, and that the blood-brain barrier is more vulnerable to parasite breakdown at 40 oC.

We are investigating the mechanism of increased binding and endothelial dysfunction at febrile temperatures, which alter flow mechanics and parasite attachment dynamics, as well as endothelial signaling pathways, and membrane lipid and protein compositions. Our results reveal that temperature is a critical factor for malaria pathogenesis that could be exploited for therapeutic intervention through antipyretic treatments.

36-Role of mechanotransduction in the control of interneurons migration in the cortex

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Cortical interneurons (cINs) are born in the ganglionic eminences (GE) and enter into multiple tangential routes to reach the cortex. While migrating, cINs are subjected to mechanical forces arising from cellular interactions and the extracellular matrix. Here we aim to understand how mechanotransduction events may shape cINs behaviour during cortical development. By combining atomic force microscopy (AFM) with time-lapse recordings we found that the intermediate zone gets stiffer at E16.5 as compared to E13.5, which also correlates with cINs migrating slower and with a reduced nuclear translocation frequency. These migration differences were also seen between E13.5 and E16.5 cINs cultured within a viscous 3D environment. Using heterochronic organotypic slices, we showed that the migration of E16.5 cINs within heterochronic cortex (*i.e.* E13.5) induced an increase of speed and frequency as compared to the controls. By performing single cell AFM we found that E16.5 cINs exhibit softer somas and display higher nuclear deformations while migrating. Finally, transcriptomic data from cINs at both stages indicated differences in expression of key nuclear and mechanotransduction genes during development. Our findings suggest that while migrating, E16.5 cINs might be more sensitive to environmental changes in part due to their viscoelastic properties which would allow them to integrate shifts of substrate stiffness and adapt their migratory behavior.

37-Génera — a Tissue Engineering Machine

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The quest to create complex multicellular systems has been a driving force behind major advances in the methodologies and tools used by researchers and engineers in tissue engineering and regenerative medicine (TERM). In recent decades, the field of TERM has witnessed a significant surge in the development of such systems to meet various medical and fundamental research needs. However, as more methods have been developed, it has become increasingly apparent that their initial promises were only partially fulfilled, falling short of their ultimate goals.

This scenario of a **constant and interrupted revolution in tissue engineering** is a common theme for many promising approaches. While technologies like bioprinting and organoid technologies have profoundly impacted areas such as prosthesis creation, organ replacements, artificial meat production, and drug testing, their development trajectory has notably deviated from the initial promise of addressing the organ shortage issue.

Against this backdrop of technological evolution, my research introduces a novel philosophical perspective, bridging the gap between current methodologies and their untapped potential. In my work, I will demonstrate how **philosophical deconstruction** of tissue engineering methods and the identification of key **philosophical distinctions** can lead to a better understanding of the organ creation issue.

The first distinction will be drawn between the concept of **tissue** *per se* **and tissue engineering**. Tissue engineering is portrayed as a **departure from various centrisms** (gene centrism, cell centrism, biophysical centrism) and aligns more closely with Developmental Systems Theory. Along this line of inquiry, **three fundamental modes** of tissue fabrication will be identified: **morphic** (dominance of form), **hylomorphic** (equitable interaction of form and matter), and **hylic** (complete dominance of matter and its self-organization). This leads to the construction of the '**Tissue Engineering Triangle**,' elucidating fundamental methodological differences. This will culminate in the conceptualization of a '**periodic table of Tissue Engineering**' for discovering new methods and enhancing existing ones. This domain of '**engineering the engineers**' will, in turn, open up new possibilities in the construction of tissue engineering machines, first machines to weave real biological tissues by the rules of tissue.

Finally, I will demonstrate the feasibility of a universal tissue engineering machine (**Génera**), which likely possesses a weaving nature and will fill a vacant niche in this periodic system. It will ultimately **conclude the tissue engineering revolution**, resolving the longstanding enigma of genuine tissue weaving.

38-Engineering adhesion to identify design principles for robust cell-cell aggregation

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Engineering multicellularity provides many advantages that are useful even in model microbial systems. For example, the intrinsic protection within an aggregate can be useful for deploying engineered microbes in harsh or toxic growth conditions, while the increased ease of metabolite sharing can potentiate division of labor. However, aggregation is not always a stable phenotype, as producing adhesion molecules can be costly or diffusion limitations and competition for shared nutrients within an aggregate reduce cell fitness. Therefore, we require a better understanding of the conditions that support robust cell aggregation, which can both guide designs of engineered aggregates and shed light on minimal requirements for multicellularity. To do this, we used a bottom-up approach to engineer Saccharomyces cerevisiae aggregates of diverse sizes by controlling expression of FLO1, a yeast adhesion gene. Using hardware for automated continuous culture, we found that even our largest aggregates, at least 100µm in diameter, were stably maintained across hundreds of generations. We then competed aggregating and non-aggregating strains against each other at different culture densities, revealing that their relative fitness can depend on frequency, media type, and aggregate size. We hypothesized that these phenomena are in part a consequence of the non-specificity of FLO1, where even “non-aggregating” strains are incorporated in aggregates, which we further tested with engineered aggregates using specific adhesion mechanisms. Together, these data highlight key quantitative controls of aggregate stability, as well as highlighting a potentially long-lived chassis for deploying engineered functions in a microbial aggregate.

39-µGUIDe – A precision microgel platform to direct development *in vitro*

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The development and self-organization of tissues is tightly regulated by localized gradients of morphogens emanating from distinct clusters of cells that act as signaling centers. While morphogen-soaked microgels/beads have shown promise to recapitulate this process in engineered tissue constructs, their capacity to finely control spatial and temporal morphogen distribution remains limited. Here, we introduce a library of sulfated glycosaminoglycan (sGAG)-based microgels that offer unprecedented control over morphogen affinity (µGUIDe, µGel Units to Instruct Development), thus enabling precise formation of short-term (hours) and long-term (days) concentration gradients in tissue cultures. Multiparametric adjustment of the microgel charge patterns resulting from sGAG ionization is key to programmable morphogen release, a concept that extends to a variety of GAG-binding morphogens (e.g. SHH, BMPs, TGF-β, FGFs, VEGF, etc.). Owing to the rational holistic material design, the full power of mathematical modeling can be unlocked to predict gradient formation, offering helpful guidance for the experimental design of complex tissue models. We demonstrate the potential of our microgel system to guide tissue morphogenesis through the local administration of VEGF gradients from single µGUIDe in a *microgel-in-gel in vitro* vasculogenesis model and in hiPSC-derived kidney organoid cultures, as well as SHH gradients in a hiPSC-derived neural tube model. Our micromaterials-based methodology offers valuable new options to mimic and modulate morphogen signaling centers, providing a well-equipped toolbox to carefully direct the self-organization of tissues.

40-A stem cell zoo to study interspecies differences in developmental tempo

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During embryogenesis, different mammalian species present differences in their developmental speed. These controlled differences in the tempo or duration of developmental processes are known to influence animal size and morphology, being an important mechanism of evolutionary change. However, the mechanisms regulating developmental tempo have remained elusive due to difficulties in performing direct interspecies comparisons. In this study, we used in vitro differentiation of pluripotent stem cells to recapitulate the segmentation clocks of diverse mammalian species varying in body weight and taxa: mouse, marmoset, rabbit, human, cattle, and rhinoceros. Quantification of the segmentation clock oscillations across species revealed that their period did not scale with the animal body weight, but with the embryogenesis length. The biochemical kinetics of the core clock gene HES7 displayed clear scaling with the species-specific segmentation clock period. However, the cellular metabolic rates did not show an evident correlation. Instead, genes involving biochemical reactions showed an expression pattern that scales with the segmentation clock period, providing evidence of the potential transcriptional regulation of developmental tempo. We are now using gain-of-function screens to characterize the transcriptional signature of developmental tempo, aiming to accelerate the human segmentation clock. Investigating the genetic control of developmental tempo will help us understand how the species-specific phenotypes are determined at the cellular level and how they changed during evolution.

41-The gastrulating zebrafish under cold spells.

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Development of ectotherms occurs over a wide temperature range. Based on macroscopic descriptions, past studies have generally assumed an identical sequence of morphological developmental states that differ only in the rate of progression as temperature changes. Although this presumption may hold true at the systems level, it is unknown how and/or if the mechanical and biochemical events are coordinated to slow down development robustly at lower temperatures.

Zebrafish, being able to develop normally between 23°C and 32°C, is an ideal model organism in which the effect of temperature on development can be probed systematically at the cellular level. Previous literature have revealed early cleavage defects in zygotes under cold temperatures. However, these early stages preclude an understanding of multi-cellular coordination and the failure of it during development outside of the embryos' optimal temperature range. Thus, we have focused on studying gastrulation in zebrafish at different temperatures. Time-lapse light-sheet imaging of gastrulating embryos reveals different dynamics of actomyosin ring assembly and progression with varying temperatures. At suboptimal low temperatures (20°C), the cells in the enveloping layer (EVL) show the most striking response to temperature variations. Around dome stage, the EVL cells become multi-nucleated and their size scales with the number of nuclei, despite no apparent defects in EVL specification. Furthermore, we find that it is cytokinesis, and not nuclear division, that is adversely affected by the cold. Possible molecular candidates involved in this cytokinetic failure were thereafter identified through mass spectrometry. As epiboly progresses, size of EVL cells at 20°C can be up to five times the size of EVL cells at 28.5°C, with apoptosis of EVL cells being responsible for the rupture of embryos before completion of epiboly. Our study gives clues as to why development fails under sub-optimal temperatures and indicates how gastrulation can still occur successfully despite errors and irregularities in the embryo.

42-Sulfated glycosaminoglycan-based microgels for programming VEGF gradients in human kidney organoids

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Artificial signalling centres able to secrete morphogens in precise local concentrations can be a valuable tool to guide and decipher relevant mechanism of tissue development *in vitro*. To this aim, our group has developed a microgel library based on sulfated glycosaminoglycans (sGAG) that allows the precisely programmed generation of morphogen gradients (µGUIDe, µGel Units to Instruct Development). Here, we present the µGUIDe-mediated administration of vascular endothelial growth factor (VEGF) gradients for the localized control of vascular networks in human induced pluripotent stem cell (iPSC)-derived kidney organoids. Guided by a reaction-diffusion model to simulate the formation of the gradients, we applied single VEGF-loaded µGUIDe in the periphery of the organoids, where the majority of PAX2+ renal vesicles (RVs) develop and a dense population of KDR+CD31- endothelial progenitors was observed. An enhanced CD31+ vascular network formation was found locally around the VEGF-loaded µGUIDe by day 7. Notably, exposure to VEGF gradients led to enhanced colocalization of PAX2+ RVs with the CD31+ vascular network, thus more closely mimicking early patterning events of renal vascular development. Our results confirm the significance of localized VEGF gradients in facilitating vascularization of kidney organoids, but also showcase the capability of our sGAG-based µGUIDe to effectively guide region-specific morphogenetic processes in engineered multicellular systems at a local level.

43- Influence of apical constriction on tissue morphology and cell fate in brain organoids

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During tissue development the apical constriction of cells is a key mechanical process, which facilitates cell shape change and differentiation. On the model of brain organoids, it was shown that human organoids undergo apical constriction later compared to other great apes. That leads to a delay in the change of the cell morphology and to the formation of bigger neuroepithelial buds in human. The mechanical and molecular mechanisms of how cell shape change influences their state are still to be discovered. To address these questions, we decided to artificially induce apical constriction in human cerebral organoids with expression of Shroom3, which causes apical constriction. In our system earlier expression of Shroom3 compared to wild-type organoids led to the formation of smaller and more spherical lumens and thickening of neuroepithelia resembling the architecture of nonhuman ape brain organoids. Furthermore, the wild-type brain organoids were 2 times larger. Our current objective is to study if the artificial modification of the organoid shape through apical constriction can lead to changes in cell differentiation. Thus, inner mechanical forces, particularly apical constriction, can be the drivers of developmental processes and lead to tissue morphological and cell functional changes.

44- Influence of geometry on self-organisation in early zebrafish development

Nick Marschlich

EMBL

Embryonic development involves the intricate interplay of genetics and mechanics. Cells in tissues subjected to mechanical forces activate mechanosensitive pathways leading to signal transduction and changes in transcriptional state of the cells. For example, mechanical tension promotes nuclear translocation of ß-catenin leading to mesoderm specification in human embryonic stem cells. Epiboly movement in zebrafish presents a scenario where changes in geometry and changing mechanical stresses drive cellular movements, rearrangements and differentiation concomitantly in a spatially confined environment. Systematic perturbation of geometry including the 3D confinement is limited in vivo. Here we describe an in vitro system consisting of cells derived from the zebrafish blastula before differentiation (termed pescoid) and confined in a systematic manner to perform defined shape changes for understanding self-organization and the emergence of mesoderm under varying geometry and mechanical stresses. We explore how 3D confinement affects cell behavior, packing density and differentiation (expression of the mesendodermal marker mezzo). We developed a novel device that can confine multiple samples in a high-throughput manner and is compatible with standard imaging. Our results show that 'weak' confinement of 300 to 200 µm minimally affects symmetry breaking and elongation. Remarkably, ‘strong’ confinement at 100 µm, shrinking pescoids to 30% of their original height, increases mesendodermal signaling intensity and timing from a single up to multiple mezzo-poles. Elongation is reduced while a wave-like phenotype in the periphery emerges from the earliest expressed mezzo-pole.

Our findings provide insights into the complex mechanisms by which mechanical forces and geometric constraints influence embryonic body axis establishment and tissue differentiation. Through the use of this in vitro model for systematic changes we can understand the relationship between signaling and mechanics during early embryonic development.

45- An optogenetic toolset to understand and control epithelial mechanical balance.

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Cells form tissue structures through mechanical forces[1]. Synthetic mechanobiology proposes the control of such forces to improve our understanding of how tissue structures arise[2]. In addition, optogenetics has opened the possibility of gaining spatio-temporal control of mechanical forces with light[3]. These approaches have proven to be useful for the study of epithelial morphogenesis[4,5]. However, the experimental control achieved does not account yet for all the forces proposed in physical models of tissue morphogenesis, which propose an epithelial mechanical balance between apical, lateral, and basal contractility[6]. In this project, we make use of optogenetic and synthetic approaches to gain control over this set of forces (apical, basal, and lateral contractility) to test whether they are sufficient to understand and control the shape of different epithelial cell types.

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46- Mechanics of cell extrusion in flat intestinal organoid systems

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In homeostasis, development and disease, health of an epithelial tissue is maintained by eliminating unnecessary or damaged cells through a process of cell extrusion. Cell extrusion is especially important to maintain the function of tissues that turn over rapidly. A schoolbook example here is the epithelial tissue that builds the intestinal villi. It is immersed in the uniquely aggressive environment of the small intestine and, to guard against tissue gaps or overgrowth, cell extrusion in the villi needs to be tightly regulated and well executed. Despite the crucial role it plays in the healthy function of the intestine, the mechanism of intestinal cell extrusion is not known. Using open-lumen, flat monolayers of intestinal organoids, I show that villus cells are extruded while still living, contrary to the widely held belief. By measuring deformations of a synthetic substrate that these organoids grow on, I measured the forces that extrude an intestinal cell. Through this quantitative analysis, I also show that a broad tissue neighborhood participates in successfully eliminating an intestinal cell. Overall, by using the emerging, powerful system of flat open-lumen intestinal organoids, I provide a detailed mechanical description of the live cell extrusion process in three dimensions.

47-LimbNET: modelling and simulation of limb developmental patterning in an online platform

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A key challenge for the future of computational modelling is how to easily share complex data-driven simulations amongst a diverse community of theoreticians and experimentalists. This is essential so that different researchers can test and compare each others' hypotheses, building new results upon those previously published across the field, ideally within a common frame of reference.

Our new modelling platform LimbNET is a key step towards this goal within the limb development community. An openly accessible online platform, LimbNET allows remote users to define and simulate arbitrary gene network (GRN) models of 2D spatiotemporal developmental patterning processes.

LimbNET's core incorporates four key functionalities: 1) straightforward formulation and simulation of computational models of GRNs, as reaction-diffusion systems; 2) a database of previously created models, accessible for the user to simulate, explore and build upon; 3) a database of existing 2D gene expression pattern images across space and time; 4) a database of users' own scanned gene expression patterns, e.g., from WMISH or immunostaining, mapped into our modelling framework.

Researchers may share models on LimbNET, thus having the intrinsic potential to build on each others' body of work throughout the community. Crucially, all functionality is accessible through a 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.

Furthermore, users can upload and share spatiotemporally-varying expression patterns of genes relevant to limb development throughout different stages of morphogenesis, and map them onto a previously published standardised computational description of limb growth. These data can be directly compared to each other, and to simulation outputs, closing the feedback loop between experiments and simulation via parameter optimisation.

48-Robustness of oriented tissue deformation

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Oriented tissue deformation is a fundamental process omnipresent during animal development. However, it is so far unclear how exactly tissue-scale robustness is ensured during this process. Indeed, from a physics perspective, deforming tissues can be described as oriented active materials, and it is known that oriented active materials inherently exhibit instabilities. Such instabilities can destroy the homogeneously deforming state of active materials. We want to understand: How is this instability prevented during animal development? In particular, we ask whether the presence of a chemical signaling gradient (e.g. a morphogen gradient) can help stabilize oriented tissue deformation. Using a combination of vertex and hydrodynamic models, we find that stability depends on whether the signaling gradient acts to extend or contract the tissue along the gradient direction. In particular, gradient-extensile coupling can be stable, while gradient-contractile coupling is generally unstable. Intriguingly, developing tissues seem to exclusively use the gradient-extensile and not the unstable gradient-contractile coupling. This suggests that the active matter instability acts as an evolutionary selection criterion. Thus, our work points to a potential developmental principle that is directly rooted in active matter physics.

49- From morphology patterns to epithelial morphogenesis: exploring topology and natural variation

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Through the process of animal development, multiple factors such as cell-cell interactions, cell proliferation and environmental variables, actively contribute to organogenesis. To gain a deeper understanding of tissue organization and elucidate the mechanisms driving developmental and pathological variations, it is essential to conduct a comprehensive analysis of how cells organize and interact to form the same 3D structure under different conditions. This analysis is key to understand biological processes, particularly in the context of epithelial morphogenesis.

In this study, we investigate the impact of diverse environmental conditions on the development of Madin-Darby Canine Kidney cysts, employing a high-content image analysis tool known as CartoCell. CartoCell allows us to extract distinct characteristics from this model, that can unveil the natural variation in tissue organization and identify morphological and connectivity patterns within individual cysts, even at the cellular level.

Here, we focus on the analysis of cystogenesis under two specific conditions: nutrient starvation, achieved by reducing Fetal Bovine Serum (FBS) in the culture media, and oxygen deprivation (induced hypoxia). These conditions serve as artificially induced stressors when compared to the normal cystogenesis environment, which occurs under normoxia with the appropriate concentration of FBS. We will present data illustrating how these conditions can impact global cyst features and cell parameters during cyst development. Our particular emphasis is on the examination of cell packing, topological organization, and morphology patterns. In conclusion, understanding how cysts can adapt to stress conditions by adjusting specific parameters while adhering to essential constraints may provide valuable insights into the boundaries of natural variation.

Keywords: cystogenesis, natural variation, morphology patterns, cell packing.

50-Computation of growth distribution in organogenesis

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Embryo development and organogenesis are governed by robust inhomogeneous growth distributions and contractile forces, which are in general hard to quantify. The analysis of those spatial distributions is commonly based on experimental techniques that allow visualizing the deformation of the tissue at the macroscale [1] or cellular level [2]. Despite recent advances in spatial resolution and reduction in the of time lapses, growth patterns are in elusive, and can be in general only computationally estimated. Importantly, imaging techniques can capture the positions and proliferation rates in partial domains of the whole organism, raising uncertainties on the forces at the boundary and growth distribution at interior regions of the while organ.

In this work we propose a computational method that infers the best matching growth according to elasticity assumptions and material properties. The inverse method resorts to a dynamic iterative regularization algorithm, and is applied to synthetic cases and heart looping process [3]. We show that the known values of the reaction forces at the boundary and inhomogeneous growth can be recovered for some simple synthetic cases, and that plausible growth patterns can be estimated for control and abnormal heart development. Current work aims at applying the method to other well-known developmental processes such as limb growth, Drosophila invagination or wing disk folding [4].

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51-Uncovering the maternal-fetal crosstalk during implantation by live-imaging

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The process of blastocyst implantation in humans is very inefficient, leading to around 70% of pregnancy attempts being unsuccessful. Failure of implantation could be caused by the endometrium or by the embryo and can occur during early or late implantation. However, we have very little knowledge about when and why implantation fails, as it is impossible to study this process *in vivo*. To address this issue, I combine endometrial organoids with blastoids, *in vitro* models of the human blastocyst. We can start to understand how the blastocyst and the endometrium establish their first communication to obtain a successful implantation, and what could go wrong. I will identify key signalling pathways involved in the interaction between endometrium and blastocyst using single-cell RNA sequencing. I will then study the function of key players by high-resolution live-imaging on the single cell level. To this end, we have set up a blastoid implantation assay in the lightsheet microscope which allows for long-term high-resolution imaging. By understanding how these signals play a role in implantation, we aim to improve the efficiency of this process.

52-A positive feedback loop controls the onset of gastruloid symmetry-breaking

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In the embryo, exogenous morphogentic signals guide tissue polarization thereby defining the body axes of the future animal. In the absence of such signals, embryonic stem cell aggregates can still spontaneously polarise in vitro, a process known as symmetry-breaking. How cell fate dynamics control tissue polarisation keeping robust cell proportions is still not understood. By combining experiments and mathematical modelling in mouse embryonic stem cell aggregates, we uncover a positive feedback loop that controls the onset of symmetry-breaking. We find that the expression dynamics of the mesodermal gene Bra/T is critically affected by the initial fraction of Bra/T+ cells and demonstrate that a minimal cell fate model including feedback captures the observed Bra/T dynamics. Our model suggests that primed pluripotent cells inhibit mesoderm differentiation in the aggregates and that cell-cell signalling controls cell fate proportions. In addition, we identify differences in Bra/T expression between the core and the periphery of the aggregates as an early signature of symmetry-breaking prior to polarisation. Mechanical measurements reveal an increase in viscosity and surface tension upon Bra/T expression. We thus propose differential mechanics as the main driver of spatial symmetry breaking in the aggregates. Our work paves the way to understand how symmetry-breaking emerges in embryo-like structures.

53- Engineering Shape Changing Tissues to Understand Morphogenesis

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Tissues acquire their shapes during morphogenesis. Shape changes in individual cells are required to collectively give rise to large scale tissue shape changes. Cell mechanics during this process is controlled by the actomyosin cytoskeleton, downstream of signaling. However, it is not well understood how individual roles of different signaling pathways control cell shapes. Signaling can be spatiotemporally controlled by combining activators of actomyosin contractility with light sensitive proteins. Here we use optogenetics to recruit signaling domains to different parts of the cell, giving rise to cell shape changes due to cortical contractility.

54- Mechanobiology and Morphogenesis: New (Vertically Integrated) Tools for an Old Problem.

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To understand mechanobiology and morphogenesis, we aim to characterize and model the complex interactions between cells and the extracellular matrix. To achieve this, we developed three innovative tools(Fig. 1): TEMPO, a cell line with advanced sensors for phenotypic screening; HYDRA, an automated method for fabricating hydrogels in high-throughput microplates; and SEM2, a computational framework for cell and tissue mechanics.

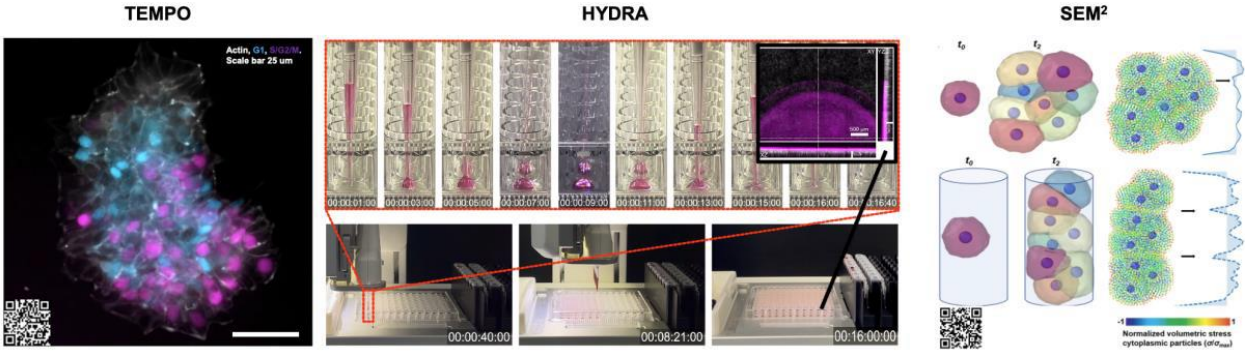

Figure 1: TEMPO hiPSC clone (left), HYDRA 96-well plate fabrication (center), and SEM2 modeling of cell proliferation (right) in conditions mimicking organoids (top) and organs-on-chips (bottom).

TEMPO(timing early and mature morphogenesis) is a suite of genetically encoded fluorescent sensors for cell structure (LifeAct), function (GCaMP6f), and cell cycle (a novel cell cycle indicator called FUCCIplex). We initially prototyped TEMPO in human epithelial cells (HaCaT) and eventually expressed it stably in human induced pluripotent stem cells (hiPSCs). To demonstrate that TEMPO design facilitates complex imaging experiments and cell cycle stratification, we used HaCaT and hiPSCs in a battery of live cell imaging experiments, including migration, proliferation, and smart microscopy routines in 2D preparations and 3D organoids. Integrating structural and functional sensorswith the new cell cycle indicatorallowed for precise cell cycle stratification and detailed phenotypic screening.Moreover, the TEMPO hiPSCs can be used to study simultaneously the contribution of cell structure, function, and cell cycle progression to morphogenetic events in organoids.

In HYDRA(hydrogel dispensing with robotic automation), we utilized liquid handling robotsto automate the production of fish gelatin hydrogels cross-linked with microbial transglutaminase in multiwell plates, ensuring physiological relevance for cell cultures. To demonstrate that HYDRAcould produce hydrogels with physiological stiffness suitable for imaging-based screening experiments, we prepared 96 well plates seeded with TEMPO HaCaT cells to test various imaging modalities. Using quantitative phase microscopy, we demonstrate the chronic effect of cancer drugs such as nocodazole on cell proliferation. Then, we used confocal microscopy to validate the drug mechanism of action with cells grown on hydrogels or traditional cell culture plastic. This compatibility enhances HYDRA's utility in imaging-based analyses and high-throughput screening, including the screening of the many thousands of extracellular matrix components that contribute to morphogenesis.

Finally, SEM_2(subcellular element modeling and mechanics) extends a discrete computational modeling framework called subcellular element modeling by incorporating particle-level stress and strain analyses. This tool simulates cell behaviors in diverse culture environments, focusing on multi-scale mechanics essential for understanding cellular responses to mechanical stimuli. We used SEM_2 to demonstrate that these new tools can be incorporated into computational modeling frameworks well-suited to model morphogenesis. We simulated cell behaviors, such as division, migration, and proliferation, in both classical and engineered cell culture platforms such as organoids and organ-chips. Then, we studied the stress and strain arising in these simulations at subcellular, cellular, and tissue levels. Its application in cell creep experiments and proliferation modeling in constrained environments showcased its versatility and significance in understanding mechanics and morphogenesis.

With the vertically integrated development of TEMPO, HYDRA, and SEM_2we seek to tackle aspects of mechanobiology and morphogenesisthat would be difficult to address using only engineered cells, or engineered platforms, or computational models. Together, these tools enhance our capacity to characterize and model cell-material interactions, contributing to both theoretical and practical applications in the field. We have developed these tools to be available to the academic community via open-science channels and foster collaborative progress inmechanobiology and morphogenesis.

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55- Multi-cellular rosette formation guides cellular rearrangement initiating lumen opening in PDAC organoids

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Morphogenetic shape changes and tissue growth determine organ development and disease. However, the mechanisms that control these changes are not entirely understood and difficult to analyze *in vivo*. Therefore, we use organoid systems to model this complex behavior *in vitro* and reveal underlying processes by investigating key regulators of force generation within tissues, like motor and adhesion proteins, as well as the influence of the extracellular matrix.

We conducted experiments using murine pancreatic ductal adenocarcinoma (PDAC) cells which form highly branched, three-dimensional and dynamic organoids when embedded into a collagen I matrix, resembling the *in vivo* structure of pancreatic cancer. During the development of these PDAC organoids, we observed a process wherein the cellular rearrangement resulted in the transformation of cells from a mesenchymal to an epithelial cell type. This transition is mediated by the formation of three-dimensional rosettes, which result from a combination of geometrical constraints and actomyosin contractions. The constraints are given by the viscoelastic properties of the cell nuclei as well as the branch diameter, being restricted by the surrounding collagen cage. The interaction of these factors leads to a periodic pattern of rosettes along the center of the branches, which spacing is determined by the branch diameter. Concomitant intercalation contributes to the development of the epithelial structures required for lumen opening.

In summary, by using branching organoid systems, we have identified the mechanism underlying rosette formation, leading to the epithelial cellular structure required for lumen formation in PDAC.

56- Exploring Embryonic Development in Simulated Microgravity: Insights from Gastruloid Cultures

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Recent studies aboard the International Space Station (ISS) and in simulated microgravity environments have significantly advanced our understanding of cellular behavior in space, including critical changes in the cytoskeleton, extracellular matrix, and stem cell dynamics (Sarkar, Pampaloni 2022). These insights are crucial for addressing the challenges posed by long-term space travel. Our research contributes to this field by investigating the development of gastruloids—embryonic organoids that emulate early mammalian development (Anlas et al. 2021)—particularly focusing on the formation of the Anterior-Posterior (AP) axis under simulated microgravity conditions.

Utilizing a 2D clinostat and custom-modified protocol to ensure stability, we explored how microgravity affects cellular behavior and gene expression in gastruloids. This involved the use of a specially designed segmented tube with custom silicon sealings, produced using advanced 3D printing and molding techniques. These modifications were critical in accurately simulating microgravity conditions. To analyze the effects, we employed a range of imaging methods, transcript quantification, and nanopore sequencing techniques.

Our preliminary experiments under these conditions revealed distinctive expression patterns in pluripotency markers (Sox2, Nanog, Oct4) and differentiation genes (Brachyury, GATA6), particularly impacting Nanog distribution. This suggests that genes crucial to axis formation and germ layer development in gastruloids are sensitive to changes in gravity.

In conclusion, this study not only enhances our understanding of gastruloid development in microgravity but also provides a valuable model for investigating early mammalian development in space. The results have broad implications for multicellular system engineering and highlight the need for innovative approaches in embryonic research, particularly in the context of space exploration.

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57- Circulation-on-a-Chip: Cell Survival Under Pro-Apoptotic Mechanical Cues in Metastasis

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Lung adenocarcinoma (ADC) is a major contributor to brain metastasis, which is a major cause of cancer death. Conventional treatments and studies have focused on primary and secondary tumors, leaving the circulating tumor cells (CTCs) understudied. Likewise, how CTCs overcome the pro-apoptotic cues posed by the hydrodynamic conditions within the circulation (shear stress, hydrostatic pressure) in their journey to the brain remains largely unknown. To address this gap of knowledge, we designed a microfluidic based circulation-on-a-chip model that reproduces key physiological hydrodynamic features of the middle cerebral artery to investigate how disseminated cancer cells, survive within this hostile mechanical environment. Using this system, we examined the survival of different lung cancer cell lines exhibiting low (H441) or high (H460) metastatic potential Cell lines with traits of monocytes (THP-1) and T-cells (Jurkat) were used as positive controls. Our results demonstrate that the aggressive H460 cell line survives significantly more than non-aggressive H441 cells, yet all cancer cells consistently exhibited lower survival than both THP-1 and Jurkat cells at all times examined. In all conditions, cell viability as a function of time could be modeled with a simple biophysical model based on an exponential decay and an activated process corresponding to the energetic barrier that cells need to overcome to activate death response during the hydrodynamics posed by the circulation. These results provide a proof-of-principle of a novel circulation-on-a-chip model, which provides a suitable tool to study CTCs by identifying the mechanisms underlying the enhanced survival of cancer cells with high metastatic potential, by screening drugs against their aberrant survival as well as to generate models of residual disease by enriching a population of cells with enhanced survival within the circulation.

58- Biofabrication of Structurally Organised Cartilage Through the Integration of Melt Electrowriting and Photocrosslinkable Decellularized ECM Hydrogels

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Introduction

Articular cartilage has a limited capacity for self-repair. Current therapeutic approaches are ineffective at regenerating the damaged tissue, particularly its zonal structural organization (1). Melt electrowriting (MEW) is a promising biofabrication technique for engineering soft tissues, as it allows for the fabrication of highly porous scaffolds with fibre diameters in the micrometer - submicrometer range (2). Decellularized extracellular matrix (dECM) has been shown to provide both biological and structural cues to support tissue repair (3). In our study, we synergistically utilized MEW scaffolds with varying pore sizes and aspect ratios in combination with photocrosslinkable dECM hydrogels to precisely guide collagen orientation, resulting in the development of highly organized tissue structures.

Materials and Methods

We manufactured three distinct MEW scaffolds with different pore aspect ratios (0.4 x 1.6 mm, 0.8 x 0.8 mm, and 0.4 x 0.4 mm). Cartilage ECM obtained from porcine joints was decellularized with a series Triton-X washes and DNAse treatment and subsequently modified with methacrylic anhydride to create photocrosslinkable ECM hydrogels. Mesenchymal stem/stromal cells (MSCs) were cultured for three weeks within the MEW-hydrogel composites and the hydrogel alone, followed by biochemical, histological and polarized light microscopy analyses.

Results

MSCs deposited a neo-tissue directed by the physical boundaries provided by the different MEW designs. Picrosirius red staining demonstrated robust collagen deposition throughout the composites. Polarized light microscopy (figure 1) unveiled preferential collagen alignment within the 0.4×1.6 mm and 0.4x0.4 mm groups, while no specific collagen orientation was observed in the 0.8×0.8 mm constructs. Notably, collagen fibres preferentially aligned parallel to the scaffold long axis in the 0.4x1.6 mm constructs, while no preferential alignment in one direction was

observed in the 0.4x0.4 mm scaffolds. Currently, we are developing multi-layer MEW scaffolds combining zonally defined pore aspect ratios to create scaffolds that better mimic the depth-dependant structural organization of native articular cartilage.

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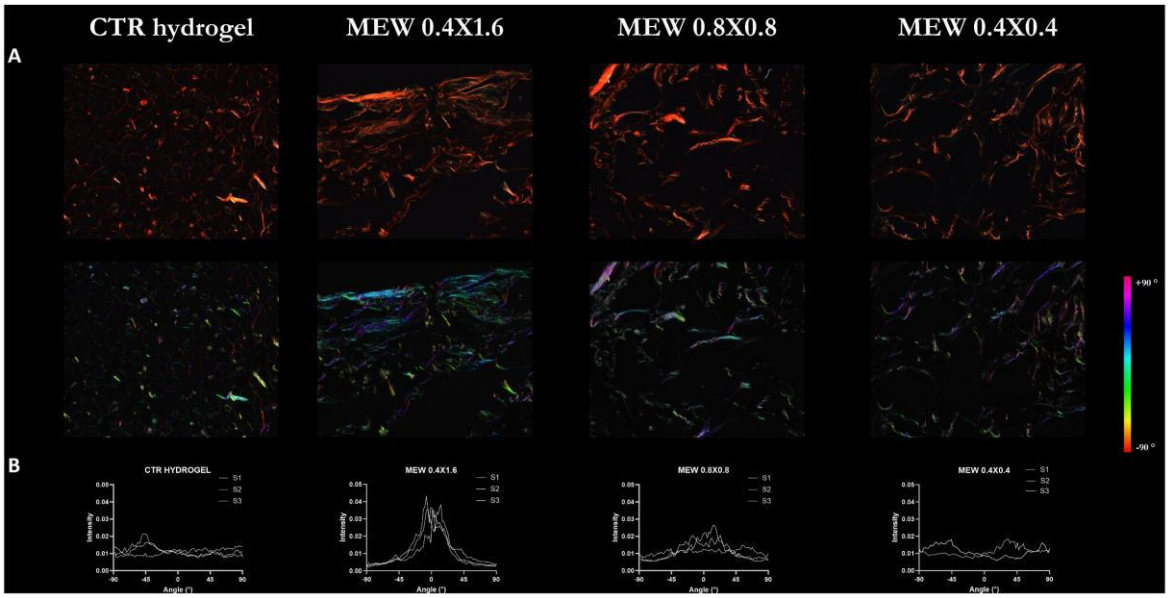

Figure 1 Collagen fibre orientation for different scaffold architectures. (A) colour map imaging of the collagen fibres distributions. Here, cyan/blue colours denote fibres oriented at 0 degrees while purple/red denote fibres oriented at 90 degrees. (B) Quantification of the fibre orientation within different scaffold architecture

59- Investigating the role of exercise on neuromuscular health and disease in a multi-tissue *in vitro* model

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Massachusetts Institute of Technology

By enabling voluntary movement, the musculoskeletal system is a major contributor to the autonomy and life quality of individuals. Unfortunately, motor control can be impaired with aging, often correlated with the onset of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). ALS is typically characterized by the degeneration of motor neurons in the spinal cord and brain stem, accompanied by denervation of neuromuscular junctions and muscle atrophy. Because of its broad clinical presentation and unknown genetic driver in most cases, ALS still lacks a clear etiology.

Repeated muscle contractility, or strenuous exercise, is frequently listed as a potential risk factor for ALS, as it may lead to microinjuries and increased levels of reactive oxygen species. On the other hand, in mice, we have reported exercise-induced benefits for the nervous system, such as upregulation of signaling pathways related to neurite growth and synapse formation. In ALS patients, exercise has also been shown to improve motor function after symptom onset. As illustrated by these contrasted findings, the mechanisms by which exercise training influences signaling between muscles and the nervous system in ALS patients remains unclear. To elucidate this, we are developing a multi-tissue in vitro model comprising muscular and peripheral nervous components derived from human induced pluripotent stem cells (hiPSCs).

We will start by studying how different regimes of exercise influence muscle fiber type and maturation. To meet this goal, we have established a novel method of culturing aligned skeletal muscle on micro-grooved extracellular matrix hydrogels. This 2.5D culture format provides mechanical and biochemical cues that mimic the native environment and enables high-throughput imaging of millimeter-scale muscle tissues with single-fiber precision. Electrical stimulation of the 2.5D platforms enables exercising tissues in a minimally invasive manner, and our custom computational frameworks enable converting videos of muscle contraction into spatial maps of force production. Furthermore, since the micro-grooved substrate promotes alignment of the muscle fibers, they generate forces along a pre-defined direction, thus enabling quantitative comparisons of contractility in different conditions.

We will use our platform to train healthy hiPSC-derived muscle with different frequencies and duration of electrical stimulation, mimicking various intensities of exercise. We will compare

whether and how exercise alters fiber type distribution and maturation by analyzing the expression of different myosin isoforms (MYH1, MYH2, MYH3, MYH7), as well as the muscle stem cell marker PAX7. In addition, we aim to integrate our 2.5D culture platform into a meso-fluidic chip comprising compartments for muscles and motor neurons. This co-culture chip will allow us to investigate exercise-mediated neuromuscular crosstalk in healthy tissues, such as a potential enhancement of neurite growth and synapse formation. In the future, we will extend these methods to hiPSC-derived neuromuscular tissues from ALS patients. Overall, we anticipate that our in vitro multi-tissue platform will be a valuable tool to study the effects of exercise on neuromuscular tissues in health and disease.

60-Structure-Function Relationships of Mucociliary Clearance in the Human Airways as Benchmark for Organotypic Lung Tissue Engineering

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Mucociliary clearance (MCC) is a key mechanical defense mechanism of the human airways. MCC failure is linked to major respiratory diseases, making it an important physiological component of disease modeling and drug development. While single-cell transcriptomics and histology have unveiled the cellular complexity of the human airway epithelium, our insights into the mechanical structure-function relationships that link tissue organization to MCC are limited. Currently, our insights are primarily derived from animal models, with unknown relevance to humans and of little guidance to engineering and evaluating tissue-engineered in vitro models of the human airways. In this first ever study of this kind, we address this knowledge gap by directly measuring cellular architecture, ciliary beat mechanics, and cilia-driven particle clearance in human and rat ex vivo airway trees. The comparison of human to rat lungs identifies vast species-to-species structural and functional differences, emphasizing the need for human benchmarks for organotypic tissue engineering. Therefore, using a physics-based model and comparative analysis, we elucidate the mechanical structure-function relationships of human MCC and establish human-specific benchmarks for *in vitro* respiratory cultures, allowing us to evaluate the organotypic degree of the mucociliary machinery of different model systems, *in vitro* culture conditions, and disease states.

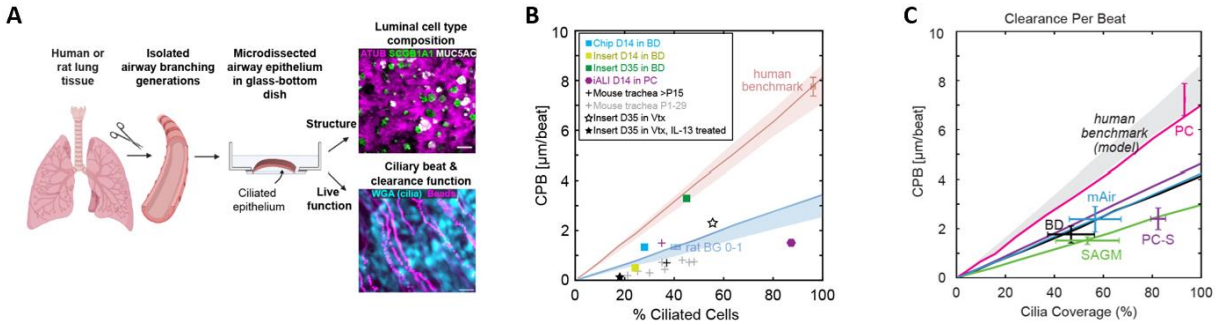

Figure 1 A *Graphical depiction of live ex vivo functional and subsequent structural imaging* ***B*** *Mapping of clearance distance per beat cycle data of different model systems (Lung Chip, human induced pluripotent derived airway epithelial cells and human primary airway epithelial cells) and a proof-of-concept disease state (IL-13 induced chronic airway inflammation in primary airway epithelial cells) to human and rat benchmark curves.* ***C*** *Mapping of clearance distance per beat cycle data of primary airway epithelial cells differentiated in different culture media to the human benchmark curve.*

61- SHAPE: Investigating innate immunity in real microgravity aboard the International Space Station using advanced human bone marrow organoids.

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In 2025, NASA through their Artemis program aims to send humans beyond Low-Earth Orbit for the first time since 1972. As focus on space exploration shifts from the International Space Station (ISS) to targets farther away, the impact of long-duration space travel on the human body and the ability to withstand these effects are becoming the primary factors precluding further expansion of humanity's reach into space. The space environment poses numerous unique challenges to astronauts, with typical examples including immune dysfunction and the loss of bone density.

Advanced bone marrow organoids comprised of primary human mesenchymal and hematopoietic stem cells were developed to investigate links between those two phenomena in real microgravity aboard the ISS. The unique spatial requirements necessitated development of a novel system, termed Hydrowells, to facilitate the long-term culture and subsequent fixation of many organoids in a small volume, remotely. Validating Hydrowells as a suitable system for 3D in vitro experiments in space allows for future experiments with diverse model systems.

The SHAPE project flew over 2,000 organoids to the ISS to investigate innate immunity in vitro in real microgravity. Morphological analysis demonstrated compact organoids with a hematopoietic stem cell niche under earth gravity while space led to looser cell aggregates. The ISS data will be compared to samples cultured in simulated microgravity, centrifuge controls, and earth gravity. Furthermore, MACE-sequencing (Massive Analysis of cDNA Ends) is currently underway to provide insight into the effects of different gravitational conditions on gene expression regarding innate immunity.

Technical insight from SHAPE and the Hydrowells facilitates future 3D in vitro experiments in real microgravity with culture systems as diverse as gastruloids. The results from SHAPE will increase understanding of the interplay between innate immunity and bone morphology in space, enabling the mitigation of health risks to astronauts and freeing humanity to explore our galaxy further.

62- Microfluidic Droplets for Mapping and Regulating Self-Organization of Organoids

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Cell manipulation in droplets has emerged as one of the great successes of microfluidic technologies, with the development of single-cell screening. However, the droplet format can also serve to go beyond single-cell studies, namely by providing confined spaces for studying interactions among different cells or between cells and their physical or chemical environment. The miniaturization of 3D stem cell culture in a droplet format allows high throughput quantification of cellular behavior and the regulation of stem cell microenvironment through mechanical/biochemical confinement. Here, we show a droplet microfluidic platform that allows to resolve spatial heterogeneities within cellular aggregates to link organization and functional properties. The platform is used to investigate the mechanisms determining the formation of organoids by human mesenchymal progenitor cells that recapitulate the early steps of condensation initiating bone repair in vivo. Heterogeneous mesenchymal progenitor cells self-organize in 3D in a developmentally hierarchical manner. We will then demonstrate a link between structural organization and local regulation of specific molecular signaling pathways functions, such as actin organization. Next, I will show that the droplet microfluidic platform sustains the long-term culture of mouse embryonic stem cells (mESCs) at the undifferentiated state and the formation of embryoid bodies (EBs) or cardiac organoids, while regulating cells' fate decision. Moreover, the culture of mESCs into anchored microfluidic droplets enables the self-patterning of gastruloids and embryo-like structures (ELSs), in the absence of any morphogens. ELSs display a unique head-trunk structure, which demonstrates high degree of similarity with the stage E8.5 of the mouse embryonic development. The process of generation of ELSs using droplet microfluidics proved high degree of reproducibility, with more than 75% of generated structures displaying a head-and-trunk structure. As such, the 3D culture of stem cells into microfluidics droplets provides a novel approach to regulate and quantify self-organization towards the derivation of functional organoids.

63-Hormonal Regulation of Germ Layer Specification in Micropatterned Gastruloids

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Hormones play a crucial role in successful embryo implantation and pregnancy outcomes, and have been identified as key players in early differentiation processes. For instance, progesterone stimulation has increased the number of somites in mouse embryos and human chorionic gonadotropin induced neural rosettes in embryoid bodies. However, the direct impact of hormones on early embryonic development remains poorly understood given the limited knowledge of the underlying cellular mechanisms and difficulty to study them from both technical and ethical perspectives in vivo. To bridge this gap in knowledge, we employ an in vitro technique using human pluripotent stem cell (hPSC) micropatterns to investigate the role of pregnancy-related hormones in germ layer specification. Specifically, we investigate the role of progesterone, estrogen, and human chorionic gonadotropin during a gastrulation-like process.

Receptors for estrogen and progesterone are highly expressed in hPSC micropatterns during pluripotency. Thus, we hypothesised hormones may regulate differentiation and the formation of germ layers. Surprisingly, exogenous hormonal stimulation did not disrupt the differentiation of micropatterned gastruloids into ectoderm, mesoderm, endoderm, or the extra-embryonic trophectoderm. Inhibiting estrogen signalling, at the aromatase or receptor levels, suppresses mesendoderm emergence, resulting in the absence of Brachyury and Sox17 expression. Rescue with exogenous estrogen was not successful; however, WNT/Nodal activation reestablishes mesendoderm formation.

Overall, germ layer formation progresses normally in hPSC micropatterns in the presence of pregnancy-related hormones, yet, disrupting estrogen signalling suppresses mesendoderm emergence. These findings highlight a possible interaction between WNT/Nodal and estrogen in germ layer formation during early development. We are also exploring whether impaired hormone signalling in mouse 3D gastruloids similarly affects germ layer specification, which may have wide-implications for the role of hormones in early developing mammals.

64- Active foam behavior of tissue coalescence in biofabrication

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Tissue spheroids, small self-organized cell aggregates resembling tissues or micro-tumors, offer a versatile platform for exploring cellular behavior in a 3D context. As miniature multicellular structures, they play an important role in drug discovery, disease modeling, and various applications in regenerative medicine. In biofabrication, they serve as building blocks for the construction of larger tissue constructs or for grafts that are suitable for transplantation. The fusion of two spheroids resembles the coalescence of two viscous droplets under surface tension. However, fusion may also arrest due to an effective internal elasticity that arises due to jamming that occurs at the cellular scale, which renders cells unable to exchange neighbors as a result of energy barriers due to adhesion or repulsion. However, the relationship between macroscopic visco-elastic material properties of the tissue and microscopic jamming/fluidization in three-dimensional tissues is not yet understood. Furthermore, the role of cell activity in the spheroid fusion remains unclear. In this study, we investigate the fusion of chondrogenic spheroids of human periosteum-derived in various treatment conditions, measuring fusion dynamics and cell-scale mechanical features such as relative cell-cell tension and relative motility. These experiments are then compared to simulations of a novel active foam model of tissue spheroid fusion. In this model, cells are represented as adhesive, viscous shells that migrate due to active protrusive forces. Using simulations of this model, we construct a phase diagram of spheroid fusion to elucidate the interplay between microscopic tissue fluidity and the visco-elastic time-scales of fusion. Moreover, by varying both cell-cell tension and cell motility, these simulations predict the experimentally observed effect of modified cell mechanical properties on fusion kinetics.

65- Metabolic control of germ layer proportions through regulation of Nodal and Wnt signalling

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Metabolic pathways can influence cell fate decisions by modulating the epigenetic, transcriptional, and signalling states of cells, but their regulative role during embryonic development remains poorly understood. Here, we demonstrate an instructive role of glycolytic activity in regulating signalling pathways involved in mesoderm and endoderm specification. Using an mESC-based in vitro model for gastrulation, we found that glycolysis inhibition increases ectodermal cell fates at the expense of mesodermal and endodermal lineages. We demonstrate that this relationship is dose-dependent, enabling metabolic control of germ layer proportions through exogenous glucose levels. We further show that glycolysis acts as an upstream regulator of Nodal and Wnt signalling and that its influence on cell fate specification can be decoupled from its effects on growth. Finally, we confirm the universality of our findings using a human gastrulation model. Our work underscores the dependence of specific signalling pathways on metabolic conditions and provides mechanistic insight into the nutritional regulation of cell fate decision making.

66-Engineering innate immunology in a humanized, functional, in vitro model of healthy myocardium

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INTRODUCTION: The myocardium is a contractile tissue that hosts multiple cell types that work in unison to support a steady supply of oxygen and nutrients throughout the body. Research over the past decade has significantly improved our understanding of the interaction between cardiomyocytes and immune cells, underlining the critical role of cardiac resident macrophages in maintaining homeostasis within the myocardium. In particular, resident macrophages in the heart perform a number of critical functions such as facilitating electrical conduction, capture and elimination of cardiac exophers and routine immunosurveillance[1]. Here, we **engineer a model of healthy myocardium**, integrating an innate immune response for patient-specific drug screening, regenerative medicine and drug discovery applications.

METHODS: Induced pluripotent stem cells (iPSCs) were expanded and differentiated to obtain macrophages (iMacs), cardiomyocytes (iCMs) and cardiac fibroblasts (iCFs). The response of iMacs to a 2D cardiac environment was assessed using co-cultures of iMacs and iCMs, on tissue culture plastic. Similarly, the functional enhancement of iCMs in the presence of iMacs was also analysed. Next, a custom-made bioreactor was used to develop an electrical stimulation (ES) regime, to facilitate iCM maturation. Finally, the information collected from 2D studies was translated into a 3D environment by fabricating engineered heart tissues (EHT), developed with the support of type I collagen and Matrigel™. This was performed by designing and engineering a multi-part setup that facilitates the fabrication of EHTs that can spontaneously twitch, while also fitting into the ES bioreactor. Custom-made MATLAB codes were used for analysing the twitch parameters of iCMs.

RESULTS: In co-culture with iCMs, iMacs were highly pro-regenerative, with increased gap junction activity and improved expression of cardiac developmental markers. Similarly, in co-culture with iMacs, iCMs were found to beat in synchrony and exhibit better response to known drugs. Furthermore, the optimized ES regime was found to facilitate maturation of iCMs, in addition to strengthening the pro-regenerative phenotype of iMacs. Finally, EHTs were

successfully fabricated and were observed to spontaneously twitch and last in culture for 14 days. EHTs incorporated with iMacs displayed robust compaction, indicating improved remodelling.

DISCUSSION & CONCLUSIONS: We demonstrate successful generation and characterisation of iMacs, iCMs and iCFs from the same iPSC parent line. We report excellent integration of iMacs and iCMs in co-culture, with profound functional enhancement. The translation of our 2D findings together with the design and fabrication of the multi-part EHT setup culminates in a robust, functional and innovative model of healthy myocardium. The integration of immune cells not only adds physiological relevance but also provides a platform for studying cardiac diseases, immune responses, and potential therapeutic interventions in a highly controlled and physiologically accurate environment. Future work includes further assessment of the EHTs and understanding the effects of iMacs on improving the maturity of the engineered cardiac tissue.

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67-Scaled-up temporally resolved transcriptomics to uncover species-specific neurodevelopmental regulation

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Humans are distinguished from the animal kingdom by certain cognitive abilities. These are likely attributed to quantitative differences of our brains compared to other species including: larger size, more cell diversity, and increased numbers of neurons and connections. However, how these differences arise is not fully understood. Particularly the differences between human brain development and that of our closest non-human ape relatives have been largely unknown. While the timing of certain developmental transitions have been observed to be different between human and apes a broader characterization of developmental progression, and the mechanisms that lead to differences in timing remain to be determined. To examine this, we plan to perform a comparative single nuclei RNA sequencing experiment in iPS-derived brain organoids examining a large number of time points and species, covering key neurogenic transitions. This study will involve collections from multiple batches of organoids, across multiple individuals, representing five species: mouse, macaque, gorilla, chimpanzee and human. Our preliminary results demonstrate the feasibility of the approach which will involve multiplexed cell mixtures of different species, that can be demultiplexed bioinformatically. Such an approach allows for an unprecedented number of samples across many species and time points, providing an approach for characterization of developmental transitions with much higher temporal resolution than has previously been achieved.

68-Using mechanical simulation to study early gastrulation movements in *C. elegans.*

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KU Leuven

The internalization of two endodermal precursor cells during early gastrulation in C. elegans provides a simplified context to study the physical mechanism of cellular ingression in detail. While apical constriction has been recognized as the primary mechanical driver, the potential contributions of other mechanisms, such as force generation in the covering cells and coordinated cell divisions, have been largely overlooked.

Our study combines a large number of full embryo 3D cell segmentations spanning early gastrulation with a cellular force model, allowing us to perform mechanical simulations. By simulating gastrulation under various scenarios and comparing the results to measured cell shapes, we can test the apical constriction mechanism and explore additional hypotheses. This involves evaluating whether concurrent cell divisions facilitate ingression by directed force generation, and if there is a role for differential cortical tension and adhesion. Additionally, we compare our computational analyses to experimental data, including a comprehensive characterization of cell shapes, cellular movements and cortical protein concentrations, such as myosin and cadherin. Hypotheses that are not rejected due to this comparison, will finally be validated by directed experiments, including cell ablations, and perturbation of adhesion by RNAi experiments.

Our extensive dataset of cell shapes and protein data, in combination with a validated force model, enables us to simulate gastrulation in a realistic in-vivo context. This approach will provide a mechanical dissection of this archetypical example of cellular ingression.

69-A Minimal Model for Early C. elegans Embryogenesis

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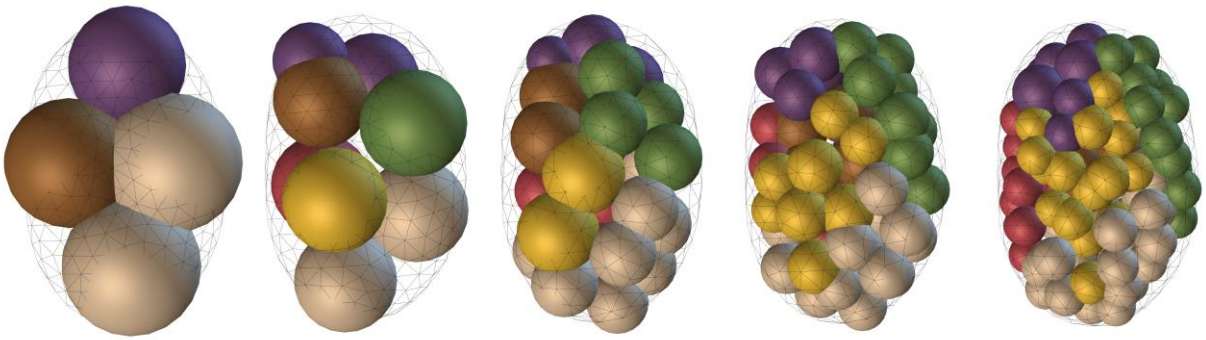
During embryogenesis, cells dynamically navigate to their correct positions. Understanding the systems that gives rise to these robust cellular movements remains a central question in developmental biology. In this work we use a simplified physical model, assuming cells behave as soft spheres, to decompose the complexity of development into a minimal set of behaviors.

Building on prior research on the establishment of the 4-cell stage in C. elegans [1, 2], we extend existing physical models to accurately account for later developmental stages. Our model includes terms for physical constraints imposed by the eggshell, asymmetric cell divisions, cell-specific adhesion forces, and the influence of active cellular movement.

Employing a parallel search strategy, we perform millions of simulations to identify a minimal model that can reconstruct the observed cell positions in live embryos.

Preliminary findings show that already during the 8-cell stage, active movements are necessary to establish correct developmental axes. After that, specific cells need to ingress in order to carry out gastrulation. Finally, we apply the method to the 100-cell stage, and show that a complex set of movements can be efficiently captured by a small number of actively moving groups.



A simulated C. elegans embryo from 4 to 100 cells. Colors correspond to founder cell lineages. The eggshell is shown as a triangular mesh.

70- Image-based force inference by biomechanical simulation

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Further reference: https://www.biorxiv.org/content/10.1101/2023.12.01.569682v2

Tightly controlled changes in cell shape underlie cellular motion and self-organization in processes as diverse as wound healing and embryogenesis. Cell shape arises through contractility of the actomyosin cortex, interactions with the environment like adhesion, and active dynamic processes like cell division and protrusions. Quantifying these forces is a major challenge.

We propose an innovative approach to infer cellular forces from cell shape. We start with confocal fluorescence microscopy time-lapses of C. elegans embryos. After segmentation, the cell shapes are introduced into a numerical simulation that employs a biophysical model of cell shape. We then optimize the system by running simulations until a force landscape is found that explains the cell shapes. The cell models include cytoskeletal force-generating events like protrusions (Figure 1A) and cytokinesis-associated contractile rings (Figure 1B). The framework also allows us to include an eggshell in our model, which plays an important role in constraining the cell shapes. To experimentally validate our inferences, we performed cortical laser ablation experiments on early embryonic cells.

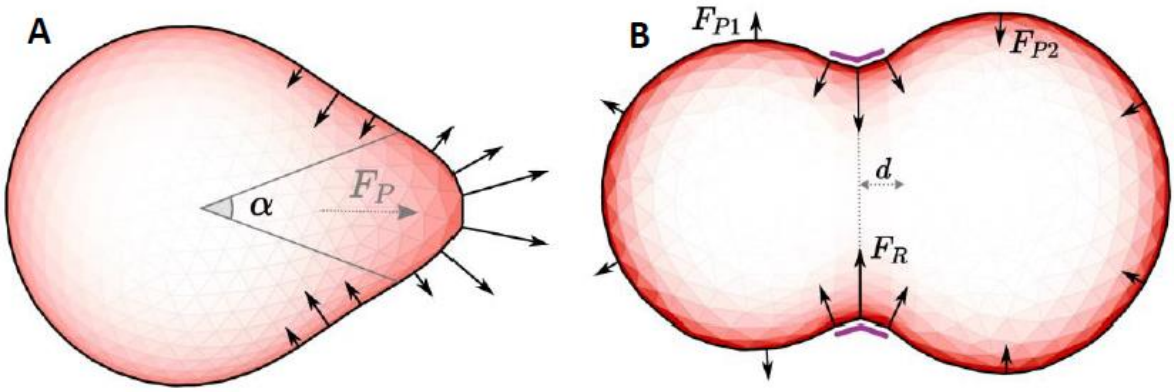

Figure 1: Models for local force generating events. (A) Protrusion. (B) Cytokinetic ring.

By applying this method, we could construct a timeline of force generation based on many embryos without invasive experimental measurements (Figure 2). This pipeline facilitates

generating large amounts of data to analyze morphogenesis, the cellular effects of gene knockouts and to associate protein localization with force generation.

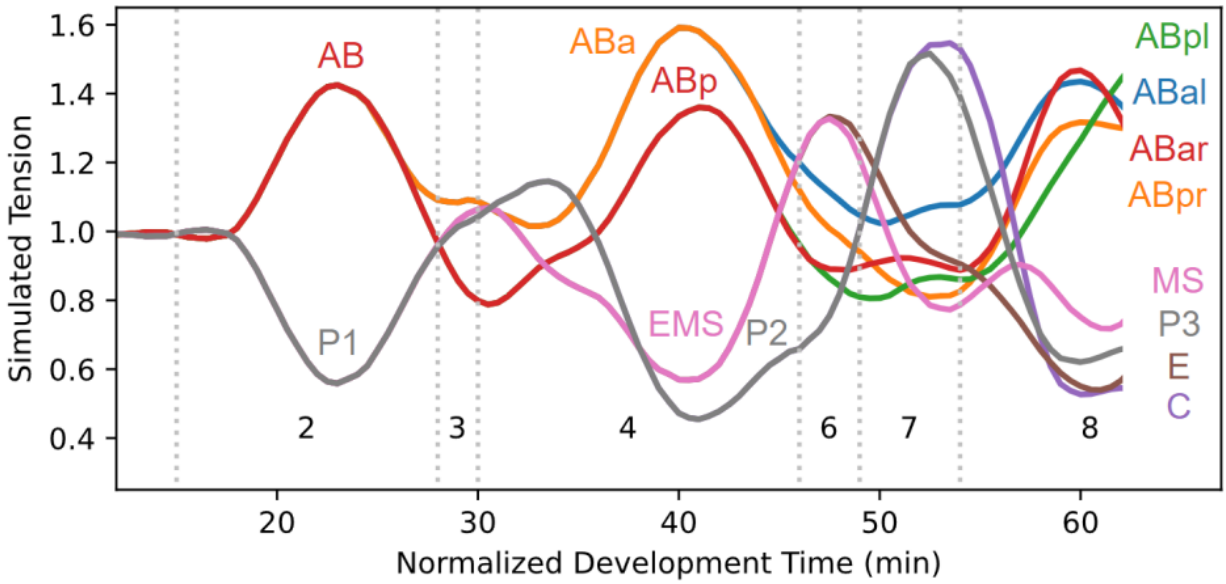

Figure 2: Timeline of relative cortical tension estimations from 1- to 8-cell stage.

71-Bioengineering Intrahepatic bile duct tubulogenesis from hIPSC using ligand-bound colloidal scaffolds

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Development of the intra-hepatic bile duct occurs within a highly orchestrated microenvironment in response to signals emanating from the surrounding portal endothelium and mesenchyme. Defects in their development can lead to an array of congenital conditions that require liver transplantation. It is thus imperative to establish novel in vitro models to better understand these developmental stages and their diseased manifestations. The knowledge on its tubulogenesis process mostly stems from the in vivo studies on animal models that lacks human relevance. In vitro bile duct models derived from stem cells form spherical lumen lacking the complexity of tissue organization observed in the liver that drives the guided tubulogenesis along the portal vein.

Here, using novel bioengineering strategies to impart instructive developmental cues by functionalizing growth factors on to cylindrical scaffolds, we achieved precise control over tissue patterning within 3D bile duct organoids in High content screening format. We devised the scaffolds to mimick the paracrine signaling from the mesenchymal er sourrounding the portal vein. The results demonstrate the spatial organization of cholangiocytes, with differentiation occurring proximal to the signaling centre surrounding their undifferentiated stem cell counterparts, namely the hepatoblast. Notably, under the influence of geometric cues, the differentiated cells self-organized to form bile duct tubules along the two principal axis of the rod, mimicking the different morphogenetic events of bile duct developmental process. Moreover, the combined presentation of Notch with TGFβ signaling in our bioengineering approach served as a potent differentiation trigger, promoting enhanced differentiation at rod interface. This subsequently led to the increased lumen anastomosis resulting in the formation of elongated tubules. Besides having morphological resemblance, the developed organoids exhibited phenotypic and transcriptional relevance to cholangiocyte lineage as evidenced by immunostaining and RNA sequencing data. Furthermore, our sequencing data revealed the presence of diverse liver specific cell types, including hepatocytes, hepatoblast, endothelial cells and transitioning populations towards cholangiocytes,

highlighting the cellular diversity of the engineered organoids. Additionally, we established and validated a high-content screening and image analysis pipeline, leveraging optical coherence tomography and machine learning tools, for quantitative assessment of tubular morphological organization at distinct developmental stages. Our work demonstrated a new class of approach to bioengineer organoids and in particular bile duct organoids with precise spatial control, offering insights into bile duct tubulogenesis process and its regulatory mechanisms.

72- PDXO-on-chip: a novel approach for studying mechanical properties on pancreatic ductal adenocarcinoma.

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Introduction: Pancreatic adenocarcinoma (PDAC) is a highly aggressive and deadly malignancy notorious for its late-stage diagnosis and limited treatment options, as well as its rapid progression and propensity to metastasize to other organs. Consequently, PDAC prognosis in patients has not improved over the years, leading to an overall five-year survival rate below 10%[1].

PDAC is characterized by a dense and complex stromal microenvironment, including cancer-associated fibroblasts, immune cells and a thick extracellular matrix, that take part not only in the disease's progression but also in chemo-resistance[2]. Accordingly, *in vitro* models development recreating PDAC microenvironment is essential for preclinical evaluation of novel therapies. Tumor-on-chip is an advanced platform for growing 3D models of tumors in controlled settings. It incorporates tumor cells and surrounding components in a microfluidic system[3]. We have focused on 'organoid-on-chip' (OOC), a cost-saving technology, that enable the examination of various parameters like matrix stiffness, pore size, and oxygen supply. OOC mimics organ architecture and function, offering a powerful tool for studying complex biological processes, disease modeling, drug screening, and personalized medicine.

Materials/Methods: Biological material: PDAC patient-derived xenograft organoids (PDXOs: 253, 215, 997). Microfluidic devices: single-chamber microchips for cells embedded in different matrices (BME, Collagen type I) and two medium channels. Quantitative PCR (qPCR). CK19 and H&E staining. Immunofluorescence. *In vivo*: orthotopic tumours and metastasis assay.

Results: H&E, CK19 staining and RNA analysis (SOX9, CFRT and KRT19) were performed to verify the correct generation of PDAC PDXOs. H&E revealed a classical cystic morphology with a lumen. RNA levels of CK19, SOX9 and CFRT were stable during the different passages. In contrast with CK19 staining results in one of our PDXOs (253), which decreases in each passage, leading to the generation of two different organoid phenotypes. For this specific phenotype, H&E revealed a pseudostratified cell layer and compact complex inside. Apart from that, *in vivo* experiments were performed to assess PDXOs capacity to generate orthotopic tumours or

metastasis compared to same 2D-cultered PDX. PDXOs gave rise to tumours faster (4 weeks vs 13 weeks) and produce liver macrometastasis in 2/4 contrary to 0/4 from 2D cultures.

After PDXOs characterization, we evaluated several microenvironmental conditions. Inside of our OOC, PDXO grows faster and bigger in hypoxia conditions. Nevertheless, when embedded in matrices, like BME or collagen type I, their behaviour strongly varies depending on the concentration. Higher collagen concentrations, with smaller pore size and increased stiffness, reduce the capacity of our organoids to grow. Importantly, stiffer conditions increase 253 compact organoids generation, which accumulate secretion products inside yet to be identified, due to stress response.

Besides mechanical variables, other of our main aims is chemotherapy treatment. Organoid-on-chip combined three-dimensional and mechanical variables, which can influence response to therapy, mimicking better the tumour microenvironment. Indeed, sensitivity to the chemotherapeutic agent gemcitabine is greatly reduced in our PDXO-on-chip models when compared to 2D cultures.

Conclusion: Although further work is needed, our PDXO-on-chip model greatly reduces the experimental cost, allowing for real-time follow-up of therapy response in strictly controlled mechanical and metabolic conditions.

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73-Cell-matrix force transmission regulates the transition between naïve and primed pluripotency.

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The first step in the differentiation of mouse embryonic stem cells (mESCs) is the transition between naïve and primed pluripotency. This transition involves a major reshaping of cells from a rounded to a spread, adhesive phenotype. Whereas such reshaping is typically associated with increased force transmission to the extracellular matrix (ECM), the magnitude and role of cell-matrix forces in the naïve-to-primed transition is unknown. Here, we show that cell-matrix forces increase during, and are required for, the naïve-to-primed pluripotency transition in mESCs. Using traction force microscopy, we show that mESCs progressively increase cell-traction forces and mechanotransduction markers as naïve pluripotency dissolves. Modulating force transmission through myosin inhibition, substrate stiffness, or spatial differences within mESC colonies regulates the process. Increased force is triggered by β-catenin signalling, and its effect is mediated by its transmission all the way to the cell nucleus. Our work unveils a major role of cell-ECM forces in pluripotency dissolution, adding an important aspect to the interplay between biochemical and biophysical cues that drive this process.

74- Modulating ubiquitin signaling to control (non)immunogenic cell death, necroinflammation, and tumor development in patient-derived human mammary organoids

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Breast cancer (BC) is the most common form of invasive cancer in women and leads to the second most cancer-related deaths worldwide. In luminal BC, high constitutive NF-kB signaling underlies programmed cell death (PCD) resistance as well as tumor survival. Ubiquitin chains and chain-binding proteins, E3 ligases and deubiquitinating enzymes are important drivers of oncogenic NF-kB signaling and influence therapeutic outcomes and drug responses.

Commonly used 2D cultured cell lines cannot reliably remodel the complexity of the tumor microenvironment *in vitro* and are therefore limited to predict clinical outcomes. Furthermore, BC cell lines are insufficient models for drug screening as they are highly PCD resistant.

Here, we apply healthy and cancerous patient-derived human mammary organoids (hMOs) as clinically relevant 3D *in vitro* systems for compound testing. By reprogramming ubiquitin signaling through targeting E3 ligases, chronic NF-kB signaling in hMOs is modulated and drives apoptotic and necroptotic forms of PCD without the need of ectopic TNF-a. For the first time, we applied Smac mimetics in hMOs and thereby induced PCD. We characterized the molecular and cellular pathways with advanced light microscopy applying live time lapse imaging, live-dead and caspase activity assays as well as antibody stainings. Moreover, we could confirm apoptotic and necroptotic cell death using Western Blot and show activation of inflammatory pathways using Cytometric Bead Array solutions and RT-qPCR. scRNA sequencing investigates the influence of diverse cell clusters and identities. Co-cultures with PBMC-derived monocytes will shed further light into the importance and influence of tumor-associated macrophages on tumor sensitivities and immunities.

Together, our results demonstrate that hMOs can be used for *in vitro* drug screening to investigate ubiquitin-based interventions to modulate PCD sensitivities. Patient-derived hMOs will direct

research into personalized therapies and open intriguing possibilities to study how immune cells and TME interplay shape tumor immunity and therapy resistance bridging *in vitro* research and clinical outcome.

75-DNA microbeads for spatio-temporally controlled morphogen release within organoids

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Organoids have proven to be powerful in vitro model systems that mimic features of the corresponding tissue in vivo. However, across tissue types and species, organoids still often fail to reach full maturity and function, because biochemical cues cannot be provided from within the organoid to guide their development. The establishment of such tools has been identified as a major goal of the field. Here, we introduce DNA microbeads as a novel tool for implementing spatio-temporally controlled morphogen gradients inside of organoids at any point in their life cycle. The DNA microbeads are formed in a simple one-pot process, they can be stored for a year and their viscoelastic behavior and surface modification is tunable to mimic the corresponding tissue. Employing medaka retinal organoids and early embryos, we show that DNA microbeads can be integrated into embryos and organoids by microinjection and erased in a non-invasive manner with light. Coupling a recombinant surrogate Wnt to the DNA microbeads we demonstrate the spatio-temporally controlled release of the morphogen from the microinjection site, which leads to the formation of retinal pigmented epithelium while maintaining neuroretinal ganglion cells. We were thus able to bioengineer retinal organoids to more closely mirror the cell type diversity of in vivo retinas. The DNA microbead technology can easily be adapted to other organoid applications for improved tissue mimicry.

76-Unveiling the 3D Mechanics of Tubular Epithelial Structures for Biohybrid Devices

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All surfaces of our body, both internal and external, are covered by thin cellular layers called epithelia. Epithelia are responsible for fundamental physiological functions such as morphogenesis, compartmentalization, filtration, transport, environmental sensing, and protection against pathogens. These functions are determined by the three-dimensional (3D) shape and mechanics of epithelia. One commonly formed shape are 3D tubular structures, such as blood vessels, lung bronchioles, and kidney renal tubules. However, the mechanisms behind how epithelial tubes behave under differing flows and geometric conditions remains poorly understood. We aim to address this question by developing a technology to engineer the elementary building blocks of epithelial morphogenesis and to reverse-engineer their mechanics. With a combination of micropatterning, sacrificial matrices, and microfluidics, we will implement a new experimental platform to sculpt epithelial tubes of a controlled geometry. We apply these engineering principles to build biohybrid devices based on 3D epithelia and create a microfluidic channel composed of epithelial tissue that can be imaged with high spatial-temporal resolution. Through this approach, we will map the stress and strain tensors and luminal pressure, and then to control these variables from the subcellular to the tissue levels. We aim to perform full experimental study of the 3D mechanics of tubular epithelial channels, and to unveil the mechanical principles and underlying forces by which these tissues adopt and sustain their shape. Our study establishes a new approach for engineering epithelial biohybrid microfluidic devices.

77-Inference of cytoskeleton and cell stress from TFM

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Traction force Microscopy (TFM) has become an essential technique for computing forces exerted by cells on hydrogels substrates and matrices. The traction field is computed from a set of measured displacements resorting to analytical and numerical solutions on viscoelastic materials, either homogeneous or with stiffness or shape gradients [1,2].

We here propose a novel technique for inferring a plausible cytoskeletal structure and contractility distribution mechanically compatible with the tractions extracted from TFM results. In contrast to previous results [3], no cell rheology is needed, since the method is solely based on equilibrium conditions, and on an iterative minimisation process, where a set of contractile dipoles are generated. From the mismatch of the TFM traction field and the traction generated by the mesh of dipoles, we design a filtering and reconstruction process for different dipoles sizes and connectivities in an efficient manner. The process is also complemented from the contractility and stress profile obtained assuming a continuous elastic media adhered to the substrate.

We test our methodology to 2D examples, with synthetic and in vitro experiments. Some analytical results allow us to ensure the existence of optimal dipole patterns. In order to ensure unique solutions, we regularise the optimisation problem with respect to the unknown contractility. The preliminary results, which yield a set of optimal cytoskeletal structures with respect to the measured tractions, prompt us to suggest that the method will help scientists and researchers to correlate cell tractions with cytoskeletal dynamics in different conditions

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