



4th edition EMBL - IBEC Conference

Engineering Multicellular Systems

11th - 13th March 2026 · Barcelona



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Welcome

EMBL and IBEC aim to contribute to the discussion on challenges and opportunities in the expanding field of engineered multicellular systems.

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

This biennial series will focus on how engineering such systems is advancing our understanding of tissue and organ function, with applications in disease modeling, developmental biology, and regenerative medicine.

#EMBL_IBECConf

ORGANIZING COMMITTEE

James Sharpe, EMBL

Xavier Trepas, IBEC

Kristina Haase, EMBL

Josep Samitier, IBEC

Vikas Trivedi, EMBL

Zaida Álvarez, IBEC



venue

Barcelona Biomedical Research Park (PRBB)

Carrer Dr. Aiguader, 88
08003 Barcelona





program



11th March

EMBL-IBEC Conference 2026

08:30 – 09:15 Registration

09:15 – 09:45 Opening remarks

Session 1 -Chair: James Sharpe

09:45 – 10:15 **Scott Fraser - Multimodal imaging of complex biological events in their normal context**

10:15 – 10:45 **Nicolas Rivron – Tissue crowding maintains the pool of trophoblast stem cells during mouse and human blastocyst implantation**

10:45 – 11:00 Short Talk: Andrea Iglesias-Ramas – Bioelectric regulation of epithelial tissue organization

11:00 – 11:30 Coffee Break

Session 2 - Chair: Josep Samitier

11:30 – 12:00 **Manuel Salmeron - Engineered hydrogels to capture ECM viscoelasticity**

12:00 – 12:15 Short Talk: Shailaja Seetharaman – Mechanosensitive regulation and prediction of vascular dysfunction in disease

12:15 – 12:45 **Hannah Stuart - Reconstitution of minimal requirements for neural tube self-organisation**



11th March

EMBL-IBEC Conference 2026

12:45 – 13:15

Flash talks

- Miquel Bosch Padrós: Optogenetic Control of Force Transmission in Pluripotent Epithelia
- Florencia Lezcano: Bioinspired 3D bioprinting of functional skeletal muscle constructs with controlled fiber orientation
- David Oriola: Collective fate decisions and cell rearrangements underlie gastruloid symmetry breaking
- Özge Özgüc: Building Mechanically Accessible Models of Early Human Development
- Gal·la Vinyes i Bassols: A versatile 3D bioprinting platform for engineering physiologically relevant and high throughput human blood-brain barrier models
- Tassilo von Trotha: Boosting microtissue growth by tuning the scaffold's cleft angle
- Nigar Abbasova: Height and mass dynamics of MDCK monolayers probed by quantitative phase imaging
- Adrian Candelas: How do malaria cues rewire endothelial mechanics?

13:15 – 14:45

Lunch and Poster session 1

Session 3 - Chair: Vikas Trivedi

14:45 – 15:15

Eduard Hannezo – Robustness of multicellular morphogenesis

15:15 – 15:30

Short Talk: Carlos Pérez – Self-organization of tumor heterogeneity and plasticity



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15:30-16:00	Coffee Break
16:00 – 16:30	Meritxell Rovira – Shedding light on pancreas regeneration and PDAC cell of origin with organoids single cell sequencing
16:30 – 16:45	Short Talk: Viola Introini – Modelling complex host-parasite interactions in 3D microvessels
16:45 – 17:15	Ewa Paluch - Morphogenesis across scales: from cell shape to tissue organisation
17:15	Cockatil



12th March

EMBL-IBEC Conference 2026

Session 4 - Chair: James Sharpe

9:30 – 10:00 **Renske Vrooman: Evolutionary models to understand multicellular systems**

10:00 – 10:15 Short Talk: Jordi Comelles – Emergent 3D morphogenesis of epithelial sheets via mechanically-driven phase transitions on planar matrices

10:15 – 10:45 **Elena Camacho - Dynamic signalling and cell fate transitions in early human development**

10:45 – 11:15 Coffee Break

Session 5 - Chair: Kristina Haase

11:15 – 11:45 **Eduard Batlle - Plasticity and therapy resistance in metastatic colorectal cancer**

11:45 – 12:00 Short Talk: Ricard Alert – Internal durotaxis and asymmetric shapes of cell clusters

12:00 – 12:30 **Mingxia Gu – Unveiling the Mysteries of Vascular Development and Regeneration with Advanced Human Organoid Models**

12:30 – 13:15 Flash talks

- Palash Chandravanshi: Engineering Developmentally Informed ECMs for Human Neuronal Maturation
- Marina Cuenca: Characterizing airway organoid architecture in health and disease
- Valerio Di Carlo: Self-Healing in Skeletal Muscle Bioactuators Triggered by Mechanical Damage



12th March

EMBL-IBEC Conference 2026

- Nadia Vertti-Quintero: Spherical Skin Model: Stratified Co-Culture of Fibroblasts and Keratinocytes on Spherical Beads Toward Compound Screening
- Jeremy Vicencio: Engineering the auxin-inducible degron system for tunable in vivo control of organismal physiology
- Jose Munoz: Morphogen concentration patterns in growing domains

13:15 – 13:30 Group photo

13:30– 14:45 Lunch and Poster session (even numbers)

Session 6 - Chair: Zaida Alvarez

14:45– 15:15 **Barbara Treutlein - "Reconstructing human brain organoid development with single cell technologies"**

15:15 – 15:30 Short Talk: Marion Raich – Multi-cellular rosette formation guides epithelial tissue assembly in pancreatic ductal adenocarcinoma cell organoids

15:30 – 16:00 **Jianping Fu – Bioengineering Human Embryo and Organ Models**

16:00 – 16:30 Coffee break

16:30 – 16:45 Short talk: Daniel Selma Herrador – MorphoChip: A Minimalistic In Vitro Assay To Study Cell Intercalation In Morphogenesis

16:45 – 17:00 Short talk: Paula Belska – Curvature and its role on the morphology of tissues.

17:00 – 17:15 Short Talk: Achyuth Acharya – Tension-Driven Self-Organization as an Engineering Principle for Building Human Skeletal Muscle from iPSCs



13th March

EMBL-IBEC Conference 2026

Session 7 - Chair: Xavier Trepas

- 9:30 – 10:00 **Lorenzo Moroni – Design of 3D scaffolds with intrinsic and controllable mechanical instructions to steer tissue regeneration**
- 10:00 – 10:15 Short Talk: Benoit Sorre – Engineered morphogen gradients applied basally to human embryonic stem cells to control and dissect tissue patterning
- 10:15 – 10:30 Short Talk: Daniel Krueger – Epithelial tension controls cell extrusion
- 10:30 – 10:45 Short Talk: Sandra Petrus – Immune and Mutational Profiling of Low-Immunogenic Human Primary Cholangiocyte Organoids for Bile Duct Disorders
- 10:45 – 11:45 Coffee break and Poster session
- 11:45 – 12:15 **Lauren Saunders – When fates collide: lineage-specific programs in shared tissues revealed by embryo-scale studies of single cells**
- 12:15 – 12:30 Short Talk: Clara Delahousse – Mechanically triggered fate reversion in mouse embryonic stem cells using microfluidics
- 12:30 – 13:00 **Sebastian Streichan - Engineering structured active matter with stem cells**
- 13:00 – 13:15 Closing remarks and farewell





plenary speakers



PLENARY SPEAKER

SCOTT FRASER

Chan Zuckerberg Imaging Institute

**Multimodal imaging of complex
biological events in their normal
context**



PLENARY SPEAKER

NICOLAS RIVRON

IMBA

Tissue crowding maintains the pool of trophoblast stem cells during mouse and human blastocyst implantation



PLENARY SPEAKER

MANUEL SALMERÓN

Institute Bioengineering of Catalonia

Engineered hydrogels to capture ECM viscoelasticity



PLENARY SPEAKER

HANNAH STUART

EMBL Barcelona

**Reconstitution of minimal
requirements for neural tube self-
organisation**



PLENARY SPEAKER

EDOUARD HANNEZO

ISTA, Austria

**Robustness of multicellular
morphogenesis**



PLENARY SPEAKER

MERITXELL ROVIRA

iDIBELL

**Shedding light on pancreas
regeneration and PDAC cell of origin
with organoids single cell sequencing**



PLENARY SPEAKER

EWA PALUCH

Cambridge University

Morphogenesis across scales: from cell shape to tissue organisation



PLENARY SPEAKER

RENSKE VROOMANS

Sainsbury Laboratory

**Evolutionary models to understand
multicellular systems**



PLENARY SPEAKER

ELENA CAMACHO

Centro Andaluz de Biología del Desarrollo

Dynamic signalling and cell fate transitions in early human development



PLENARY SPEAKER

EDUARD BATLLE

Institute for Research in Biomedicine

**Plasticity and therapy resistance in
metastatic colorectal cancer**



PLENARY SPEAKER

MINGXIA GU

UCLA Medical School

**Unveiling the Mysteries of Vascular
Development and Regeneration with
Advanced Human Organoid Models**



PLENARY SPEAKER

BARBARA TREUTLEIN

ETH Zurich

Reconstructing human brain organoid development with single cell technologies



PLENARY SPEAKER

JIANPING FU

University of Michigan

**Bioengineering Human Embryo and
Organ Models**



PLENARY SPEAKER

LORENZO MORONI

Maastricht University

Design of 3D scaffolds with intrinsic and controllable mechanical instructions to steer tissue regeneration



PLENARY SPEAKER

LAUREN SAUNDERS

Heidelberg University

When fates collide: lineage-specific programs in shared tissues revealed by embryo-scale studies of single cells



PLENARY SPEAKER

SEBASTIAN STREICHAN

University of California Santa Barbara

**Engineering structured active matter
with stem cells**



The background image shows the interior of a mosque, featuring several domes and arches. The architecture is illuminated with warm, golden light, highlighting the intricate details of the stone and brickwork. The domes are of varying sizes, and the arches are supported by columns. The overall atmosphere is one of grandeur and historical significance.

oral presen- tations



Bioelectric regulation of epithelial tissue organization

Andrea Iglesias-Ramas¹, Mathieu Coppey¹, Mirna Kramar¹

1. Institut Curie, Physics of Cells and Cancer, UMR 168, 75005 Paris, France

Mammalian cells maintain an electric potential across their plasma membrane, known as the resting transmembrane potential (T_p). This potential arises from localized ionic gradients and the zeta potential (Z_p), as well as from long-range influences generated by extracellular electric fields and tissue-scale bioelectric interactions. While T_p has been studied extensively in excitable cells, its function in non-excitable cells remains comparatively unexplored. Emerging evidence indicates that T_p can directly modulate intracellular signaling pathways, suggesting a role in regulating coordinated behaviors that underlie tissue formation.

In this work, we investigate the contribution of T_p to epithelial assembly, with a particular focus on how cell density influences collective behavior. Using live imaging with Genetically Encoded Voltage Indicators (GEVIs), we obtain non-invasive, long-timescale measurements of T_p in developing epithelial layers. These measurements suggest systematic differences in T_p associated with variations in cell density, as well as with cell type and spatial arrangement. We further assess how electrical coupling between neighboring cells and the emergence of supra-cellular T_p patterns may contribute to the coordination of multicellular organization within epithelial tissues.

Taken together, our results identify **distinctive bioelectric signatures associated with epithelial formation and suggest** that T_p acts as an active regulator of tissue-level coordination.

Mechanosensitive regulation and prediction of vascular dysfunction in disease

Shailaja Seetharaman

Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore
Mechanobiology Institute, National University of Singapore

Abnormalities in blood vessel and blood flow properties are key drivers of severe cardio- and cerebro-vascular pathologies. The remarkable ability of the vasculature to sense and respond to mechanical and biochemical signals across multiple scales presents both a challenge for understanding disease progression and an opportunity for therapeutic intervention. Here, we investigate the mechanisms by which the endothelial tissue lining of the vasculature responds to blood flow using *in vivo* and *in vitro* models of healthy and atherosclerotic tissues. Using bulk RNA sequencing, partial carotid artery ligation, and microfluidics, we find that the transcription as well as protein expression of Four-and-a-half LIM protein 2 (FHL2) are enriched in endothelial cells experiencing atherosclerosis-like flow profiles. We further demonstrate that the FHL2 perturbs actin-microtubule cytoskeletal crosstalk, resulting in aberrant cell junction morphology, heightened contractility and tissue permeability in a force-dependent manner. These results uncover a novel mechano-chemical feedback loop important for driving vascular dysfunction in disease. Next, building on these insights, we have developed machine learning (ML) models for the prediction of tissue-scale endothelial morphology and function in health and disease. In summary, our work highlights an integrated approach which enables the engineering of physiological vascular tissue function, with the ultimate goal of targeting the vasculature in therapies.

Self-organization of tumor heterogeneity and plasticity

Carlos Pérez-González^{1*}, David B. Brückner^{2,3,4*}, Mickael Di-Luoffo⁵, Sophie Richon¹, Ruchi Goswami⁶, Meryem Baghdadi¹, Florence Piastra-Facon¹, Neta Felsenthal¹, Réda Bouras¹, Arianna Fumagalli⁷, Mirjam van der Net⁸, Martijn Gloerich⁸, Salvatore Girardo⁶, Jochen Guck⁶, Jacco van Rheenen⁷, Julie Guillermet-Guibert⁵, Edouard Hannezo⁴, Danijela Matic Vignjevic^{1,9}

Affiliations:

1. *Institut Curie, PSL Research University, CNRS UMR 144; F-75005, Paris, France*
2. *Biozentrum, University of Basel; 4001, Basel, Switzerland*
3. *Department of Physics, University of Basel; 4001, Basel, Switzerland*
4. *Institute of Science and Technology Austria, 3400, Klosterneuburg, Austria*
5. *CRCT, Université de Toulouse, Inserm, CNRS, Centre de Recherches en Cancérologie de Toulouse; 31100, Toulouse, France ; Labex Toucan, Toulouse.*
6. *Max Planck Institute for the Science of Light & Max-Planck-Zentrum für Physik und Medizin; 91058, Erlangen, Germany.*
7. *Department of Molecular Pathology, Oncode Institute, Netherlands Cancer Institute; 1066 CX, Amsterdam, the Netherlands.*
8. *Center for Molecular Medicine, University Medical Center Utrecht and Utrecht University; 3584 CX, Utrecht, the Netherlands.*
9. *Equipe Labellisee LIGUE Contre le Cancer*

* These authors contributed equally to this work.

Abstract

Phenotypic heterogeneity and plasticity drive tumor growth, metastasis, therapy resistance, and relapse. This heterogeneity is mainly interpreted as a response to external signals from the microenvironment. However, here we show that cancer cells also follow intrinsic self-organized programs that are sufficient to coordinate the spatiotemporal patterning of tumor cell states. By combining quantitative measurements in tumors and organoids with theoretical modeling, we reveal emergent mechanical gradients that orchestrate cell state transitions during colorectal tumor growth. Compression at the tumor center induces a transition from a fetal-like state into a cancer stem cell (CSC) state. The CSC compartment exhibits a characteristic size determined by tumor rheological properties. Once this size is surpassed, a translationally arrested apoptotic core emerges, triggering a shift from homogeneous proliferation to a hierarchical cell turnover. These findings uncover stereotyped programs of self-organization that likely cooperate with the microenvironment to shape tumor heterogeneity and plasticity.

Title

Modelling Febrile Host–Parasite Interactions in 3D Human Microvessels

Authors

Viola Introini^{1,2,3}, Rory KM Long¹, Olawunmi R Oyerinde¹, Silvia Sanz Sender¹, Frank Stein⁴, Gyu Min Hwang^{1,5}, Borja Lopez Gutierrez¹, Karl B Seydel^{6,7}, Gretchen Birbeck^{7,8,9}, Maria Bernabeu¹

¹ European Molecular Biology Laboratory (EMBL) Barcelona, Barcelona, Spain

² Max Planck Institute for the Science of Light, Erlangen, Germany

³ Max-Planck-Zentrum für Physik und Medizin, Erlangen, Germany

⁴ European Molecular Biology Laboratory (EMBL) Heidelberg, Proteomics Core Facility, Heidelberg, Germany.

⁵ Heidelberg, University, Faculty of Engineering Sciences, Heidelberg, Germany

⁶ Department of Osteopathic Medical Specialties, College of Osteopathic Medicine, Michigan State University, East Lansing

⁷ Blantyre Malaria Project, Kamuzu University of Health Sciences, Blantyre, Malawi

⁸ Epilepsy Division, Department of Neurology, University of Rochester, Rochester, New York

⁹ University Teaching Hospitals Neurology Research Office, Lusaka, Zambia

Abstract

Fever, a universal host defence mechanism during infection and inflammation, paradoxically contributes to neurological complications in malaria. Although febrile temperatures are known to increase the expression of parasite virulence proteins that mediate vascular adhesion and disease severity, the corresponding effects on the endothelium have remained unclear.

Here, we present a 3D vasculature-on-a-chip model that recapitulates human brain and lung microvessels under febrile conditions. Short febrile episodes at 40 °C—commonly observed in treated cerebral malaria patients—rapidly enhanced the binding of infected red blood cells and immune cells under flow. Mechanistically, we show that this phenotype is driven by endothelial glycocalyx shedding, which exposes the adhesion receptors EPCR and ICAM-1. Preserving glycocalyx integrity with a broad matrix metalloproteinase inhibitor prevented the temperature-induced increase in cytoadhesion.

Together, these findings identify fever as a host-specific amplifier of vascular pathology in malaria and highlight endothelial-protective or antipyretic interventions as potential strategies to mitigate febrile microvascular injury.

Emergent 3D morphogenesis of epithelial sheets via mechanically-driven phase transitions on planar matrices

Enara Larrañaga¹, Aina Abad-Lázaro¹, Mireia Flores-Expósito¹, Pau Canaleta-Vicente¹, Verónica Acevedo¹, Vanesa Fernández-Majada¹, Xavier Hernando-Momblona², Eduard Batlle^{2,3,4}, Jordi Comelles^{1,5,*} and Elena Martínez^{1,5,6,*}

¹ *Biomimetic Systems for Cell Engineering Laboratory, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain*

² *Colorectal Cancer Laboratory, Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology (BIST), Baldiri Reixac 10-12, 08028 Barcelona, Spain*

³ *Centro de Investigación Biomédica en Red (CIBERONC), Barcelona, Spain*

⁴ *ICREA, Passeig Lluís Companys 23, 08010 Barcelona, Spain*

⁵ *Department of Electronics and Biomedical Engineering, University of Barcelona (UB) Barcelona, Spain*

⁶ *Centro de Investigación Biomédica en Red (CIBER), Madrid, Spain*

Epithelial morphogenesis on planar substrates can undergo abrupt, collective transitions, yet the mechanical rules that gate these state changes remain unclear. We show that primary intestinal epithelial cells, seeded as single cells at high density on 2D substrates, undergo a sharp, mechanically-driven 2D to 3D transition controlled by Matrigel surface density and the substrate's deformability/plasticity. Keeping dimensionality constant while tuning Matrigel, tissues organize either as flat monolayers or as transient 3D tubular networks. The critical Matrigel density that triggers tubes generalizes across epithelial cell types, where Caco-2 and MDCK exhibit a parallel monolayer-to-cluster transition. Near the transition, network formation is synchronous across millimeter scales and yields highly ordered topology, consistent with a collective, system-level instability. Mechanistically, network formation requires integrin $\alpha 6$ -mediated traction onto laminin to plastically remodel the matrix, Rac1-dependent apical-in polarity and enriched stemness, as reducing the initial stem-cell fraction progressively erodes network topology and metrics. These indicate a finite window for 3D emergence. Together, these results identify a percolation-like mechanical threshold on planar matrices that controls epithelial shape state and stemness through active cell-ECM coupling and viscoelastic-plastic remodeling.

Internal durotaxis and asymmetric shapes of cell clusters

Groups of migrating cells are usually guided by external cues, such as gradients of chemoattractant (chemotaxis), substrate stiffness (durotaxis), or electrostatic potential (electrotaxis). Here, I will show that cell groups can also be guided by internal cues, i.e., by gradients of their own properties. We found that, when moving from soft to stiff substrate, clusters of neural crest cells exhibit an opposite gradient in their own tissue stiffness, with soft cells at the front and stiff cells at the back. We predict that this internal stiffness gradient is enough to guide collective cell migration — a phenomenon that we call internal durotaxis. Moreover, these cell clusters are taller at the back than at the front. We explain this asymmetric height profile by modeling the cell cluster as an active liquid droplet driven by the motile cells at its base. We speculate that the emergence of internal guidance cues could provide robustness to the migration of cell clusters in noisy environments.

Multi-cellular rosette formation guides epithelial tissue assembly in pancreatic ductal adenocarcinoma cell organoids

Marion K. Raich^{1,2,3}, Fridtjof Brauns^{4,5,6}, Tamara Müller^{1,2,3}, Maximilian Reichert^{2,3,7,8,9}, Andreas R. Bausch^{1,2,3}

¹ Heinz Nixdorf Chair in Biophysical Engineering of Living Matter, Department of Bioscience, Technical University of Munich, 85748 Garching b. München, Germany.

² Center for Functional Protein Assemblies (CPA), Technical University of Munich, 85748 Garching b. München, Germany.

³ Center for Organoid Systems (COS), Technical University of Munich, 85748 Garching b. München, Germany.

⁴ Max Planck Institute for the Physics of Complex Systems, Nöthnitzer Straße 38, 01187 Dresden, Germany.

⁵ Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstraße 108, 01307 Dresden, Germany.

⁶ Center for Systems Biology Dresden, Pfotenhauerstraße 108, 01307 Dresden, Germany.

⁷ Klinik und Poliklinik für Innere Medizin II, Klinikum rechts der Isar der TUM, 81675 Munich, Germany.

⁸ German Cancer Consortium (DKTK), Partner site Munich, 81675 Munich, Germany.

⁹ Translational Pancreatic Cancer Research Center, Medical Clinic and Polyclinic II, Klinikum rechts der Isar der TUM, 81675 Munich, Germany.

Epithelial tissues – ordered and polarized cellular structures – are essential for organ development, homeostasis, and barrier function. A key mechanism underlying epithelial formation is the mesenchymal-to-epithelial transition (MET), in which simple cell sheets and mesenchymal cell clusters assemble into organized layers. Recent studies have identified multi-cellular rosettes as polarized epithelial intermediates during MET. While the molecular components contributing to rosette assembly have been characterized, the underlying physical mechanisms remain unaddressed due to the lack of suitable model systems.

Here, we use murine pancreatic ductal adenocarcinoma (PDAC) cells embedded in collagen I, which self-organize into highly branched, dynamic three-dimensional organoids. During their development, the organoids undergo MET, progressing from invasive, mesenchymal morphologies to a polarized columnar epithelium, accompanied by branch thickening, micro-lumen nucleation, and fusion into a continuous lumen. We find that this transition is associated with the formation of rosettes.

Quantitative analysis reveals that fluctuations in acto-myosin contractility on the developing apical side of high-cell-density branches generate a tug-of-war mechanism, resulting in a regular spacing of rosettes. This spacing scales with the branch diameter and is captured by a minimal theoretical model based on apical constriction, combining active stresses with mechanical yielding at large strains. Rosette resolution ultimately leads to lumen formation through apoptosis, leading to an epithelial layer that lines the cavity.

In summary, by using branched organoid systems, we demonstrate that the rosette architecture is generated through the geometrical confinement and acto-myosin-driven contractility, resulting in the epithelial structure required for lumen formation. This underscores the critical role of mechanical forces in the self-organized assembly of epithelial tissues.

MorphoChip: A Minimalistic *In Vitro* Assay to Study Cell Intercalation in Morphogenesis

Daniel Selma Herrador[†], Vladimir Misiak, Giovanni Cappello, Thomas Boudou, and Martial Balland^{*}

LIPhy, Université Grenoble Alpes, CNRS (UMR5588), F-38000 Grenoble, France

ABSTRACT

Cell intercalation, or T1 transition, is a central morphogenetic process in which cells exchange neighbors to shape tissues while preserving overall integrity. It involves active mechanisms, such as cell crawling, and passive actomyosin pulsations. Traditional *in vivo* studies (e.g., *Drosophila*, zebrafish embryos) complicate analysis of isolated events, whereas *in silico* models require experimental validation.

We introduce an *in vitro* assay using four-cell assemblies (cell quadruplets) that replicate the minimal tissue architecture for T1 transitions. This setup allows real-time imaging and force measurement at single-cell resolution. Micropatterned glass or Polyacrylamide (PAA) gels coated with Extracellular matrix (ECM) proteins define cell boundaries, and Madin-Darby canine kidney (MDCK) cells self-organize into quadruplets, enabling statistical analysis of morphological and mechanical parameters. Cell intercalation rate can be tuned by changing the pattern aspect ratio, substrate stiffness, and imaging height, with each condition generating a distinct double-well energy landscape consistent with cell-vertex models. These results provide a direct link between geometrical constraints, mechanical stress, and intercalation dynamics.

Ongoing experiments may extend the assay to discriminate epithelial versus mesenchymal intercalation modes in the framework of cancer research. Targeted perturbations in human carcinoma, such as FAT1-KO to induce a mesenchymal state, are expected to yield higher transition rates and phenotype-specific energy landscapes. Finally, a recent collaboration with Eric Theveneau's lab with quadruplets derived from *Xenopus* explants will explore the assay as a potential platform to study the epithelial-to-mesenchymal transition (EMT) in a developmental context too.

This assay highlights the critical role of geometry and mechanics in epithelial organization, offering a quantitative framework for tissue development studies and cancer research *in vitro*. It provides novel insights into the mechanochemical principles governing topological rearrangements across molecular and tissue scales.

Curvature and its role on the morphology of tissues.

Belska, Paula, Anoop, Parvathy, Pertl-Obermeyer, Heidi, Dunlop, John W. C., and Roschger, Andreas

In recent years, it is becoming clearer that the surface curvature of a substrate can strongly influence tissue growth of a cell culture and as a consequence, the patterning of cells on a surface. We explore tissue formation of murine pre-osteoblast cell line (MC3T3-E1) on doubly curved surfaces with negative Gaussian curvatures. Cells are seeded onto PDMS scaffolds and tissue growth and actin stress fibres are observed as a function of time. The resultant tissue grows in a way such that the geometry is the same as that expected from a liquid. In other words, surface curvature and tissue area are minimised according to the Laplace Young equation giving rise to rotationally symmetric constant mean curvature surfaces of revolution. In addition, fixed samples, stained for actin, are observed using Light Sheet (LS) microscopy. Actin stress fibres spanning the entire surface together with a consistent twist in alignment could be observed. At shorter growth periods (day 7) the actin orientation had a right twist (spiralling around the surface), whereas later, at day 32 a left-handed alignment is observed. Surprisingly, this twist reverses at long times (day 63), giving rise to a multilayered tissue similar to what occurs in bone. Live cell imaging gives hints as to how cells orient themselves and the tissue along various curvature directions, providing new insights into how macroscopic multiscale tissues are formed *in vivo*.

Tension-Driven Self-Organization as an Engineering Principle for Building Human Skeletal Muscle from iPSCs

Achyuth Acharya, Fabrice Dessolis, Qiyao Mao, Frank Schnorrer

Aix Marseille University, CNRS, IBDM, Turing Centre for Living Systems, LAI

Abstract:

Engineering complex and functional tissues requires understanding the rules that guide cells to assemble into defined higher-order structures. The skeletal muscle is an exemplary system in which molecular, cellular, and tissue-scale architectures must align precisely to produce coordinated contraction-driven movement. Yet the engineering principles governing this multi-scale self-assembly remain poorly understood.

We utilized a minimal human *in vitro* model to investigate how mechanical tension serves as a central self-organizing cue that drives and coordinates pattern formation in skeletal muscle across the molecular, cellular, and tissue scales. Human iPSC-derived myogenic cells are differentiated on simple substrates—with no external patterning, scaffolding, or imposed geometry. Remarkably, these cells autonomously **align, elongate, and self-assemble into coherent myofiber bundles** and exhibit **de novo sarcomere formation**.

Live imaging, quantitative fluorescence analysis, and laser nano-ablation revealed that **tension** builds up progressively during spontaneous bundling, **preceding and sustaining sarcomerogenesis**. Fiber bundles generate a stable mechanical environment that supports the emergence of periodic, titin-based sarcomeres across long myofibrils. Perturbing bundle formation by altering cell density modulates tension build-up and correspondingly shifts the timescale of sarcomere assembly, demonstrating a direct, causal relationship between tissue-level mechanical architecture and molecular-scale patterning.

To directly manipulate tension levels, we are currently further exploring the role of various external mechanical stimuli in the maintenance and maturation of these *in vitro* muscle cultures. Increasing tension in the 2D cultures often results in culture detachment after a certain point. To resolve this issue, we are currently testing a 2.5D culture system. Preliminary data strongly suggest increased long-term viability, maturation and capacity for spontaneous contraction in these 2.5D cultures.

Importantly, we have developed a novel cell stretcher in-house to test the role of direct mechanical stimulation on the elongation, bundling and maturation of the muscle cultures. This stretcher is capable of long-term programmed stretching patterns on cultures in standard incubators and is compatible with imaging chambers for live imaging on high resolution microscopes. In early experiments, we have documented that muscle fibers respond directly to stretch-relax cycles, displaying increased uniform sarcomeric patterning and higher global bundling.

Together, these findings position human iPSC-derived muscle as a minimal engineered system where emergent and controlled mechanical stimuli result in tension-driven self-organization, which replicates key stages of in vivo myogenesis. This platform enables bottom-up reconstruction of human muscle morphology, provides a quantitative framework to test how physical forces drive myogenesis, and offers versatile opportunities for engineering mature, functional muscle tissues.

Engineered morphogen gradients applied basally to human embryonic stem cells to control and dissect tissue patterning

Tom Wyatt, Mingfeng Qiu, Julie Stoufflet, Hassan Omais, Gabriel Thon, Sara Bonavia, Pascal Hersen, Vincent Hakim & Benoit Sorre

Morphogen gradients are used repeatedly during development to pattern embryonic tissues. Absolute concentration, duration or even temporal derivative of morphogen concentration have all been proposed to carry positional information depending on the context. However, establishing the causal relationship between the spatio-temporal profile of the gradient and the resulting cellular diversity and tissue patterning is difficult to address in live embryo because of lack of tools to take control of those variables. This is especially true during mammalian gastrulation, where the primitive streak is patterned by a complex, time evolving signalling landscape of the BMP, WNT and NODAL pathways

Here, using microfluidics devices able to apply well defined morphogen landscapes on the basal side of human embryonic stem cells colonies, thus mimicking how BMP4 is delivered to the pluripotent epiblast during mouse gastrulation, we show that in this configuration absolute concentration of BMP4 provides positional information and the cell identities emerging during differentiation can vary according to the shape of the gradient¹.

As morphogen gradients and signaling centers are ubiquitous during development, our toolbox provides powerful mean to dissect the logic of patterning at all developmental stages and to engineer tissue precisely.

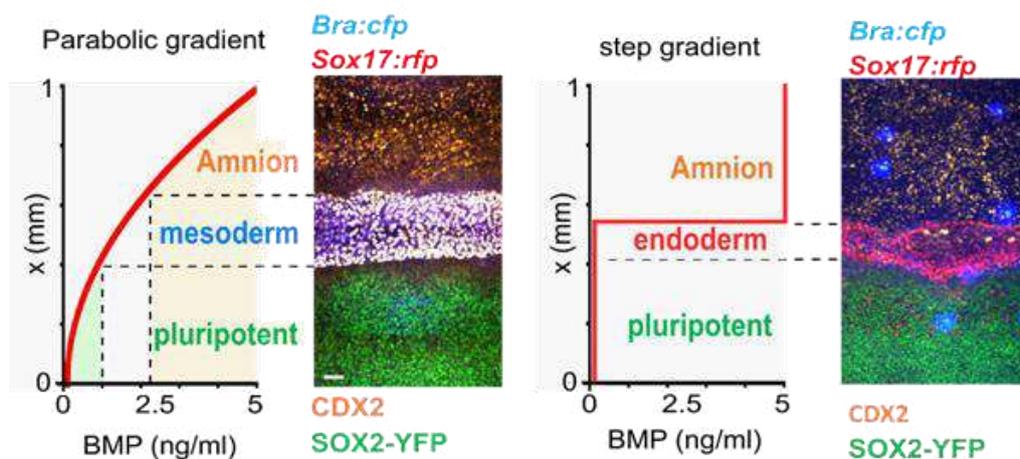


Figure 1: Different cell types are generated in human embryonic Stem Cells colonies depending on the steepness of the applied BMP gradient.

1. Wyatt, T. et al. "Morphogen gradients applied basally to human embryonic stem cells to control and dissect tissue patterning". 2025.10.30.682158 Preprint at <https://doi.org/10.1101/2025.10.30.682158> (2025).

Epithelial tension controls cell extrusion

Daniel Krueger^{†1,5*}, Willem Kasper Spoelstra^{†2}, Dirk Jan Mastebroek¹, Rutger Kok^{2,3,6}, Shanie Wu¹, Mike Nikolaev^{3‡}, Marie Bannier-Hélaouët^{1,5‡}, Nikolche Gjorevski^{3‡}, Matthias Lutolf^{3‡}, Johan van Es^{1,5}, Jeroen van Zon^{2*}, Sander Tans^{2,4*}, Hans Clevers^{1,5*§}

¹Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences (KNAW) and University Medical Centre Utrecht (UMC); Utrecht, the Netherlands.

²AMOLF institute; Amsterdam, The Netherlands.

³École Polytechnique Fédérale de Lausanne/ Institute of Human Biology; Basel, Switzerland.

⁴Bionanoscience Department, Kavli Institute of Nanoscience Delft, Delft University of Technology; Delft, The Netherlands.

⁵Oncode Institute, Hubrecht Institute; Utrecht, the Netherlands.

†These authors contributed equally

* Correspondence

Cell extrusion is essential for homeostatic self-renewal of the intestinal epithelium. Extrusion is thought to be triggered by crowding-induced compression of cells at the villus tip. Here, we found instead that a local "tug-of-war" competition between contractile cells regulates extrusion. We combined quantitative live microscopy, optogenetic induction of tissue tension, genetic perturbation of myosin II activity, and targeted disruption of the basal cortex in mouse intestines and organoids. These approaches reveal that a dynamic actomyosin network generates tension throughout intestinal villi. Cells unable to sustain this tension are mechanically outcompeted and extruded. In a model of congenital tufting enteropathy, myosin II hyperactivation disrupts this balance, leading to excessive extrusion and loss of tissue architecture. This raises the question of what renders individual cells mechanically vulnerable. Our findings reveal that epithelial barrier integrity depends on active intercellular mechanics rather than passive crowding.

Immune and Mutational Profiling of Low-Immunogenic Human Primary Cholangiocyte Organoids for Bile Duct Disorders

Petrus-Reurer, Sandra¹; Baez-Ortega, Adrian²; Martincorena, Iñigo²; Saeb-Parsy, Kourosh¹

¹Department of Surgery, University of Cambridge and NIHR Cambridge Biomedical Research Centre, Cambridge, United Kingdom

²Wellcome Trust Sanger Institute, Cambridge, United Kingdom

Background: Beyond complex surgery or transplantation, there are no current curative therapies for bile duct diseases/cholangiopathies affecting the intra- or extrahepatic biliary tree. We have previously shown that human bile duct epithelial cells can be cultured as 3D organoids to generate mature human primary cholangiocyte organoids (PCOs) for the treatment of cholangiopathies. Since the generation of autologous PCOs is likely to remain logistically and economically prohibitive for the foreseeable future, immune rejection of allogeneic PCOs remains a key outstanding barrier to their clinical translation. We thus aimed to develop and characterize the immune and mutational profiles of engineered low-immunogenic cholangiocyte organoids for regenerative medicine applications.

Methods: Human leukocyte antigen (HLA) I and II double knock out (DKO)-edited PCOs (ePCOs) were generated using CRISPR-Cas9 and sorting of double-negative cells. Assessment comparing to parental wild-type cells was carried out by flow cytometry, functional readouts, co-culture with human peripheral blood mononuclear cells (PBMC) *in vitro*; and by engraftment under kidney capsule of immunodeficient mice subsequently humanized and further analyzed using spatial transcriptomics. Mutational load and CRISPR-driven off-target genetic mutations of parental vs ePCOs was quantified using whole genome sequencing and Nanoseq techniques.

Results: The HLA I and II DKO ePCOs generated maintained a mature PCO phenotype demonstrated by flow cytometry and functional analyses. Off-target analysis and mutation burden of parental vs ePCOs did not show CRISPR-driven off-target sites nor excess mutation in ePCOs. Importantly, our mutational results revealed that passaging in culture is a much more substantial source of mutations than CRISPR-Cas9 edits, but without evident selection for cancer-driver mutations. Immune characterization *in vitro* by co-culture with PBMC experiments showed that ePCOs have reduced PBMC cell activation and a donor-dependent NK cell cytotoxicity. In *in vivo* studies with humanized mice, ePCOs showed better preserved graft survival and a significantly reduced local immune infiltration compared to parental unedited controls, mainly due to evasion of T cell mediated cytotoxic responses and downregulated cell graft stress and extrinsic apoptotic pathways.

Conclusions: Human PCOs lacking HLA I and HLA II can be efficiently generated using a CRISPR-Cas9 approach without CRISPR-driven off-target effects. Additionally, ePCOs retain the phenotypic characteristics of mature PCOs and show reduced immunogenicity when co-cultured with PBMC and in humanized mouse models compared to parental cells. These high-resolution analyses and findings have important implications for the assessment of safety and immunogenicity of future organoid cellular therapies aiming for clinical translation.

Mechanically triggered fate reversion in mouse embryonic stem cells using microfluidics.

Mechanical cues are known to promote differentiation of pluripotent stem cells (PSCs), but their potential to induce phenotypic reversion remains unexplored. Recent studies reveal that hyperosmotic stress can revert mouse embryonic stem cells (mESC) to a state resembling the two cell embryo (2C-like state). This state is associated with an upregulation of totipotency markers (Mervl, Zscan, Dux) along with a downregulation of pluripotency markers (Oct4, Nanog). Such findings suggest that mechanical stimuli might drive phenotypic reversion in PSCs through unexplored mechanotransduction pathways.

To explore this hypothesis, aggregates of a Mervl-reporter mESC line are mechanically actuated and imaged to track the conversion of PSCs to Mervl-positive 2C-like cells. To quantify the mechanical forces associated to phenotypic reversion, a novel microdevice is used to apply uniaxial compressive stress to 3D mESC aggregates. This integrated PDMS based device is used to form, encapsulate in hydrogels and mechanically stimulate mESC aggregates. It is composed of two air cavities framing a fluidically independent chamber. The chamber's ceiling deforms as negative pressure is imposed in the air circuit, thus applying dynamical or static stress to the encapsulated aggregates.

Imaging, qPCR and FACS results as well as functional assays indicate that mechanical compression can induce phenotypic reversion in undifferentiated PSCs. The extent of reversion depends on the magnitude and mode of the forces applied. These findings highlight the role of mechanical stimulation in stem cell reprogramming and suggest the need for further research into how mechanical forces are transmitted within mESC aggregates and the molecular mechanisms linking mechanical cues to stem cell reprogramming.



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Title: Optogenetic Control of Force Transmission in Pluripotent Epithelia

Authors: Miquel Bosch-Padrós, Guillermo Martínez-Ara, Miki Ebisuya, Xavier Trepap

Development relies on the interplay of three fundamental processes: cell proliferation, fate specification and morphogenesis, the acquisition of correct tissue shapes. Apical constriction is a key driver of morphogenesis, acting at the cellular level but influencing tissue-scale shape formation. While apical constriction has been extensively studied within individual cells and is conserved across the animal kingdom, the mechanical forces generated and transmitted through tissues during this process have not been measured and described. To address this gap, we employed a novel optogenetic tool to induce apical constriction in human pluripotent stem cells, combined with traction force microscopy to quantify the mechanical forces involved in the process. Using this approach, we discovered that constriction produces a consistent but small signature in traction maps, compatible with increased apical contractility and volume conservation. Furthermore, when apical constriction was induced in localized regions of a monolayer, the resulting cellular displacement field followed a screened Poisson equation in two dimensions. This finding reveals the existence of a length scale with a rheological origin and enables derivation of the Green's function of the tissue. While spatial and temporal deformation patterns can be precisely controlled, we also observed that jamming transitions cannot be induced through apical contractility, highlighting the inherently unjammed nature of this pluripotent epithelium. Together, these results uncover key rheological properties of human pluripotent stem cells at timescales relevant to morphogenesis, inaccessible through other techniques. As these cells are widely used to generate organoids and embryo models but remain poorly characterized mechanically, our work establishes a foundational framework for future studies requiring shape or force control in stem cell-derived tissues.

Bioinspired 3D bioprinting of functional skeletal muscle constructs with controlled fiber orientation

Florencia Lezcano¹, Marina Rovira¹, Judith Fuentes¹, Serxio Álvarez¹, Samuel Sánchez^{1,2}

¹*Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain.*

²*Catalan Institute for Research and Advanced Studies (ICREA), Barcelona, Spain.*

flezcano@ibebarcelona.eu

3D bioprinting allows the precise arrangement of cells, materials, and functional molecules at desired places within pre-designed structures, enabling the fabrication of reproducible and scalable capable of mimicking the complexity of natural tissue. Despite advancements in bioinks, printing approaches, and established techniques, achieving complex three-dimensional skeletal muscle construct shapes with controlled myofiber orientation have remained challenging. This study aims to obtain bioprinted skeletal muscle constructs inspired by the natural shapes and myofiber organization of human skeletal muscles. Different anchoring systems and different printing path designs were implemented in order to determine the best approach to control myofiber orientation. After method optimization, skeletal muscle constructs with parallel, fusiform, bipennate, and circular shapes were 3D printed using an extrusion bioprinter controlled by a custom-made, high-precision, Python-based tool. The different muscle constructs were bioprinted around their specific, pre-printed, and pre-cured PDMS anchoring systems using a gelatin-fibrinogen-C2C12 cell-based bioink. After 14 days in differentiation media, all constructs demonstrated a cell viability close to 100% and matured myotubes. Parallel- and fusiform-shaped constructs showed fibers aligned in parallel, while bipennate constructs exhibited myofiber alignment in two directions according to the design. Circular constructs showed concentric myofiber alignment. Under electrical pulse stimulation, all constructs contracted along myofiber direction. Our results demonstrate that functional, complex skeletal muscle constructs with controlled myofiber orientation can be obtained through the implementation of dedicated anchoring systems and careful control of the bioprinting process. By recapitulating native muscle architecture, this approach advances the design of bioengineered functional skeletal muscles.

Collective fate decisions and cell rearrangements underlie gastruloid symmetry breaking

David Oriola^{1,2}, Gabriel Torregrosa-Cortés^{1,2,3}, Sotiris Samatas^{1,2}, Krisztina Arató¹, David Fernández-Munuera¹, Elisa Maria Hahn¹, Kerim Anlas¹, Jordi Garcia-Ojalvo^{2,3}, Vikas Trivedi^{1,2,4}

¹EMBL Barcelona, Barcelona Biomedical Research Park, 08003, Barcelona, Spain

²Barcelona Collaboratorium for Modelling and Predictive Biology, 08005 Barcelona, Spain

³Department of Medicine and Life Sciences, Universitat Pompeu Fabra, Barcelona Biomedical Research Park, 08003 Barcelona, Spain

⁴EMBL Heidelberg, Developmental Biology Unit, 69117 Heidelberg, Germany

In the embryo, morphogenetic signals guide regional patterning during body axis formation. Remarkably, pluripotent stem cell aggregates can self-organise and break symmetry *in vitro* without external cues. Gastruloids, three-dimensional stem cell structures, form an anterior-posterior axis via polarised Brachyury/T expression. How cell fate transitions integrate with collective behaviours to generate embryo-like structures remains unclear. By forming gastruloids with varying initial T populations, we show that fate decisions occur collectively: the pluripotent population inhibits differentiation, critically regulating the timing of symmetry breaking. Combining fusion and nanoindentation experiments, we reveal differences in surface tension between T+ and T- tissues, in concordance with radial cell sorting. Finally, integrating cell fate dynamics and mechanics into a computational model recapitulates the sequential steps of gastruloid formation. Our study uncovers a mechanochemical basis for symmetry breaking in gastruloids and provides insights into how multicellular systems self-organise in the absence of external cues.

Building Mechanically Accessible Models of Early Human Development

Özge Özgüç¹, Thomas Wilson¹, Xavier Trepat^{1,2,3}

¹ Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute for Science and Technology (BIST), Barcelona, Spain.

² Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Barcelona, Spain

³ Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

Despite its scientific and clinical significance, the dynamics of Human post-implantation development remain poorly understood due to embryo inaccessibility and the scarcity of biological material. Various stem cell-based models of early stages have been developed, recreating aspects of human embryo development in vitro in a controllable and quantitative manner. However, they often lack the ability to measure and manipulate mechanical forces driving development. To address this, we focus on building bottom-up, mechanically accessible models of human amniotic sac development to study tissue mechanics and mechanotransduction in shaping early human development.

The first model is a microfluidic human amniotic sac system where we control the shape and lumen pressure to measure and regulate the mechanics of the amniotic cavity during formation, development, and homeostasis. This system provides a quantitative measure of tissue mechanics and allows us to explore how mechanical forces modulate key fate differentiations, such as the specification of amniotic versus epiblast lineages and the formation of the primitive streak via epithelial-to-mesenchymal transition, within pathways known to be mechanosensitive. By applying isotropic or anisotropic pressure, we aim to determine whether mechanical cues can drive these fate decisions and spatially define where they occur.

The second model investigates the intrinsic mechanics underlying the onset of gastrulation, symmetry breaking, and primitive streak movement. By culturing lumenoids attached to a soft substrate, we obtain a hemispherical structure mimicking the amniotic sac in vivo. Using this model, we measure the forces exerted by cells during morphogenesis through 3D traction force microscopy, tracking bead displacement caused by cellular pushing and pulling. Spatiotemporal quantification of such mechanical forces enables us to explore changes in the tissue mechanics guiding primitive streak initiation and movement.

Together, these models provide a comprehensive framework to investigate how intrinsic mechanical forces, including lumen pressure and traction forces, shape early human development.

A versatile 3D bioprinting platform for engineering physiologically relevant and high throughput human blood-brain barrier models

Gal-la Vinyes-Bassols, Anna Vilche, Oscar Castaño, Anna Lagunas, Josep Samitier

The blood–brain barrier (BBB) is a highly selective interface preserving central nervous system (CNS) homeostasis by regulating molecular exchange and blocking toxins, pathogens, and inflammatory mediators. While essential for protection, the BBB’s restrictive permeability hampers therapeutic delivery for neurodegenerative diseases, highlighting the need for physiologically relevant in vitro models that replicate human neurovascular architecture and cellular dynamics.

We present a versatile three-dimensional (3D) bioprinting platform to generate physiologically relevant, high-throughput human BBB models. This platform employs a novel bioink optimized for rheological properties and biocompatibility, compatible with microvalve-based embedded 3D bioprinting—a technique not previously applied to BBB modeling. The bioink allows precise, low-shear deposition while maintaining excellent cell viability. Using this approach, 48 uniform 3D ring-shaped hydrogel scaffolds are fabricated in nine minutes, demonstrating scalability and reproducibility.

Human brain microvascular endothelial cells (BMECs) bioprinted within fibrin-rich constructs remain viable and proliferative over seven days, organizing into microvascular-like structures. The scaffolds sustain structural integrity and biological functionality, validating the material and printing parameters.

While this study focuses on the endothelial component, the platform’s modular and adaptable design offers future potential for integration with co-cultures of astrocytes and pericytes, as well as incorporation into microfluidic systems and brain organoids. These advancements could further enhance physiological relevance and translational capacity by combining the strengths of multiple contemporary BBB models.

This work represents the first successful application of microvalve-based embedded 3D bioprinting for BBB modeling, establishing a rapid, reproducible, and adaptable platform with broad utility for drug screening, disease modeling, and neuroengineering applications.

Reducing the scaffold cleft angle boosts microtissue growth and broadens the width of the unjammed growth front

Tassilo von Trotha^{1,*}, Konstantin M. P. Wolf^{1,*}, Roman Vetter^{2,3}, Dagmar Iber^{2,3}, Mario C. Benn^{1,#,*}, Viola Vogel^{1,#}

¹Laboratory of Applied Mechanobiology, Institute of Translational Medicine, Department of Health Sciences and Technology, ETH Zurich, Gloriastrasse 37/39, 8092 Zürich, Switzerland

²Department of Biosystems Science and Engineering, ETH Zürich, Schanzenstrasse 44, 4056 Basel, Switzerland

³Swiss Institute of Bioinformatics, Schanzenstrasse 44, 4056 Basel, Switzerland

*These authors contributed equally

#corresponding authors

Biochemical cues are widely used to accelerate tissue growth *in vitro*, but how geometric parameters of engineered scaffolds can be tuned to physically boost tissue growth remains poorly defined. We investigated how scaffold cleft angles, by modulating tissue tension, regulate 3D tissue morphogenesis. Microtissues grown from human dermal fibroblasts or pancreatic stellate cells were cultured in V-shaped clefts of 22.5°, 45°, and 90°. Smaller angles produced enhanced growth and increased curvature of the tissue-liquid interface (growth front). Computational modeling integrating cleft angles and relative surface tensions recapitulated both the curvature of the growth front and the enhanced growth rates in smaller angles. Immunostaining revealed a thin layer of α SMA-positive, proliferative myofibroblasts with nuclear YAP1 at the growth front, while smaller angles also induced nuclear YAP1 and α SMA deeper in the core, indicating tension across a broadened growth front. Live-cell tracking showed rapid, tangential migration in an unjammed growth front zone that widened with decreasing angles, in contrast to the largely immobile core cells. Occasionally, core cells escaped toward the open cleft which correlated with collagen fiber alignment along scaffold walls. These findings are physiologically relevant and offer practical applications in both regenerative medicine and the emerging field of lab-grown food.

Height and mass dynamics of MDCK monolayers probed by quantitative phase imaging

Nigar Abbasova and Amin Doostmohammadi

Niels Bohr Institute

Time-lapse imaging of epithelial monolayers reveals prominent area fluctuations, and recent evidence suggests that these are directly linked to volume dynamics, implicating three-dimensional cell size regulation in collective migration. However, experimental evidence remains limited and conflicting, and a quantitative framework connecting volume fluctuations to tissue-scale motion is still lacking.

Here, we address this gap using quantitative phase imaging (QPI) to obtain direct measurements of monolayer height, volume, and mass dynamics. We introduce novel methods to determine monolayer height from both two-dimensional and three-dimensional QPI data and demonstrate quantitative agreement between the two approaches.

Our measurements reveal substantial spatial and temporal variability in cell height, with variations of 15–25% across a monolayer. Cell height increases by 50–100% as cell density doubles, in contrast to previous reports suggesting constant height. In comparison, the average refractive index and dry mass fraction remain constant across the entire density range. We further show that the dry mass fraction is conserved on both short and long timescales, implying that cell volume is proportional to dry mass volume and that two-dimensional QPI directly measures dry mass and height.

Analysis of the dynamic structure factor of monolayer height uncovers distinct intracellular and intercellular relaxation modes. During collective motion and density fluctuations, cell height, projected area and volume oscillate synchronously with a period of approximately four hours. Together, these results establish a quantitative framework linking three-dimensional cell volume dynamics to tissue-scale motion and position QPI as a powerful tool for probing epithelial monolayer mechanics, growth, and active behaviour.

How do malaria cues rewire endothelial mechanics?

Adrian Candelas, Matt Govendir, Waleed Mirza, Juan Francisco Abenza, Manuel Gómez-González, Borja López Gutiérrez, Martin Bergert, Xavier Trepas, Alejandro Torres-Sánchez, Alba Diz-Muñoz, Maria Bernabeu

Barrier integrity of endothelial tissues relies on a fine balance between intercellular junctional forces and focal adhesion-mediated traction. In the context of cerebral malaria, one of the deadliest complications of *Plasmodium falciparum* infection, the blood-brain barrier (BBB) is compromised, leading to brain swelling and cerebral haemorrhages that cause over 500.000 deaths annually. Although endothelial disruption is well documented in post-mortem samples, the events that initiate barrier failure remain uncharacterized.

Endothelial barrier function is regulated by mechanosensitive adherens junctions, composed of membrane VE-cadherin homodimers, intracellular protein scaffolds, and a cortical actin network that maintains junctional forces and adherens junction stability. Combining cutting-edge biophysical microscopy techniques and high-resolution live imaging with mathematical modelling, we show that human brain endothelial cells are rapidly compromised by exposure to *P. falciparum* products. During the first hour, they transiently accumulate VE-cadherin at junctions, while concurrently recruiting vinculin to focal adhesions. This results in increased tension, preceding an abrupt intercellular junction breakdown and a rapid focal adhesion elongation and stabilisation, indicative of a fast mechanoreponse to parasite exposure. After 8 hours, endothelial cells have undergone a profound morphological remodelling, with enhanced numbers of focal adhesions, transversal stress fibre formation, and the acquisition of a migratory phenotype. Traction force microscopy reveals increased matrix forces during this transition, which coincides with elevated FAK activation, a common sign of pathological pro-migratory states. Altogether, our findings suggest that BBB breakdown upon parasite exposure is driven by an early imbalance between adherens junctions and focal adhesions, leading to mechanical instability, junctional failure, and actin network reorganisation. Importantly, pharmacological inhibition of FAK with PF-573228 preserves endothelial barrier properties, highlighting the importance of endothelial mechanics in cerebral malaria pathogenesis.

Engineering Developmentally Informed ECMs for Human Neuronal Maturation

Palash Chandravanshi¹, Gisele P. Soares^{2,3}, Serafima Beletskaya¹, Francisco J. Lopez-Murcia^{2,3}, Paulina N. Bernal⁴, Kelly Marshall⁵, Thao Nguyen⁵, Leo Garma⁶, Susana Rodriguez¹, Jesús Asín⁷, Alexandra Naba⁸, Ricardo Levatto⁴, Evangelos Kiskinis^{5,9}, J. Alberto Ortega^{2,3}, **Zaida Álvarez**^{1,9,10}

¹Biomaterials for Neural Regeneration Group, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain, ²Department of Pathology and Experimental Therapeutics, Institute of Neurosciences, University of Barcelona, L'Hospitalet de Llobregat, Barcelona, Spain. ³Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet del Llobregat, Spain. ⁴Department of Orthopaedics, University Medical Center Utrecht, Utrecht University, Heidelberglaan 100, 3584 CX, Utrecht, The Netherlands, ⁵The Ken & Ruth Davee Department of Neurology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA; ⁶Department of Neuroscience, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA. ⁶Breast Cancer Clinical Research Unit, Centro Nacional de Investigaciones Oncológicas - CNIO, Madrid, Spain. ⁷Department of Statistical Methods, School of Engineering, University of Zaragoza, Zaragoza 50018, Spain. ⁸Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, Illinois, USA. ⁹Simpson Querrey Institute for BioNanotechnology, Northwestern University, Chicago, Illinois, USA. ¹⁰CIBER en Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, Spain.

Human iPSC-derived neurons fail to attain adult-like maturity *in vitro*, limiting their utility for modeling neurological development and disease.¹ Neuronal maturation *in vivo* is orchestrated by dynamic extracellular matrix (ECM) remodeling, yet the developmental ECM cues driving this process remain largely unexplored.²

Here, we define a developmental map of ECM signatures across key stages of spinal cord maturation and identify perinatal-stage matrisome components as critical regulators of neuronal maturity. This developmental window coincides with the emergence of perineuronal nets (PNNs), specialized ECM structures enriched in chondroitin sulfate proteoglycans, link proteins, and tenascins, which are known to modulate neuronal plasticity and stabilize mature synaptic networks. Leveraging these developmental insights, we engineered a synthetic PNN composed of Tenascin-R, Versican V3, and HAPLN1 along with Hyaluronic acid to recapitulate key features of the perinatal ECM microenvironment.

This defined ECM environment accelerates maturation of human iPSC-derived neurons, marked by increased neurite complexity, enhanced synaptic activity, and spontaneous electrophysiological firing. These effects are observed across both two-dimensional and complex three-dimensional culture systems, underscoring the broad applicability of this approach. Together, our findings establish perinatal PNN components as instructive cues for neuronal maturation and provide a developmentally informed ECM-based strategy to overcome neuronal immaturity, advancing the fidelity of human neuronal models for basic research and disease modeling.

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Characterizing airway organoid architecture in health and disease

Marina B. Cuenca, Andrés Marco-Gimenez, Vikas Trivedi, Talya Dayton, EMBL Barcelona

Pulmonary neuroendocrine cells (PNECs) are a rare (~0.5%) epithelial population that reside either as solitary cells or as clusters at airway branch points. In vivo studies indicate that these clusters arise through active migration, suggesting that PNECs sense and respond to chemical, mechanical or geometric cues within the airway epithelium. PNECs contribute to epithelial repair following injury, and their dysregulation is implicated in diseases such as cystic fibrosis, chronic obstructive pulmonary disease and small-cell lung cancer (SCLC). These observations point toward a mechanosensitive and highly plastic cell type whose behavior is tightly coupled to the mechanical and architectural state of the surrounding tissue.

To investigate these relationships, our lab has established two complementary human organoid systems: fetal airway organoids enriched in PNECs and patient-derived tumor organoids (PDTOs) that preserve neuroendocrine identity. These models reveal striking morphological variability, ranging from organized, lumen-forming epithelia to compact, mechanically rigid structures in PDTOs. These architectural changes are reflected in actin organization, tight-junction proteins such as ZO-1 and redistribution of β -catenin. Such cytoskeletal and junctional remodeling likely influences local tissue mechanics and PNEC positioning, providing an entry point to study how mechanical architecture shapes PNEC behavior in health and malignancy.

We present a dual-scale imaging strategy combining high-throughput single-organoid imaging with long-term volumetric light-sheet microscopy to quantify tissue architecture, cytoskeletal dynamics and PNEC distribution over time. This enables us to correlate mechanical features such as epithelial compaction, curvature and actin remodeling with PNEC clustering and malignant phenotypes. Together, these approaches establish a framework for dissecting how tissue geometry and mechanosensation contribute to PNEC organization, morphogenetic transitions and early steps of SCLC initiation.

Self-Healing in Skeletal Muscle Bioactuators Triggered by Mechanical Damage

Valerio Di Carlo*, Judith Fuentes*, Maria Guix, Florencia Lezcano, Serxio Álvares Olcina, Marina Rovira, Nil Fontanals, Samuel Sánchez

Abstract

Self-healing is a key functional property for engineered multicellular systems that aim to operate reliably over extended periods. Skeletal muscle is an attractive building block for biohybrid machines due to its intrinsic adaptability and force generation, yet its capacity to recover from mechanical damage in engineered bioactuators remains insufficiently characterized (Guix et al., 2021; Webster-Wood et al., 2023). Here, we investigate the self-healing behavior of 3D bioprinted skeletal muscle bioactuators subjected to distinct types of mechanical damage (Dumont et al., 2015; Sakar et al., 2016; Tiburcy et al., 2019). C2C12 myoblasts were embedded in a gelatin/fibrin-based bioink and bioprinted into anchored muscle constructs, which matured into aligned and contractile tissues. After maturation, bioactuators were subjected to either localized cut or localized crush injuries. Tissue recovery was assessed over time through morphological characterization and quantitative force measurements. Crush-injured bioactuators exhibited a pronounced self-healing response, with force progressively increasing and approaching control levels by day 10 post-injury. In contrast, cut-injured constructs showed limited recovery, reaching approximately 50% of their control bioactuators within the same timeframe, indicating more severe starting damage. At the structural level, evaluation of myotube formation and alignment over time via F-actin staining revealed progressive tissue remodeling, with crush injuries exhibiting slower repopulation of the damaged area and only partial restoration of actin organization, whereas cut injuries showed rapid structural recovery through the formation of aligned myotubes. The faster recovery in cut injuries may be facilitated by widening or stretching of the cuts due to sustained mechanical tension. These findings demonstrate that 3D bioprinted C2C12 skeletal muscle bioactuators possess an intrinsic, injury-dependent self-healing capacity, driven by tissue reorganization and the presence of myogenic cell populations within the construct. Overall, this work highlights self-healing muscle bioactuators as resilient multicellular systems for future biohybrid and soft robotic applications.

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Spherical Skin Model: Stratified Co-Culture of Fibroblasts and Keratinocytes on Spherical Beads Toward Compound Screening

Elisa Lenzi¹, Nadia Vertti-Quintero^{2*#}, Julien Husson³, Anne-Laure Bulteau², Carine Nizard², Karl Pays², Sébastien Sart¹, Charles N. Baroud^{1,3*}

¹ Institut Pasteur, Université Paris Cité, Physical Microfluidics and Bioengineering Unit, 25-28 Rue du Dr. Roux, 75015 Paris, France

² LVMH Recherche, 185 avenue de Verdun, 45804 Saint Jean de Braye, France

³ Laboratoire d'Hydrodynamique (LadHyX), CNRS, Ecole Polytechnique, Institut Polytechnique de Paris, 91128 Palaiseau, France

* Corresponding authors

Presenting author

Advanced skin models are critical for pursuing non-animal approaches in drug and cosmetic testing. However, existing 3D models remain complex and time-consuming, which limits their adoption. Spherical skin model (SSM) is presented, a platform that balances biological fidelity with experimental robustness. The SSM is based on a core-shell structure where the dermal core is modeled by embedding human fibroblasts into collagen microcarriers (150 μm), while the epidermal shell is formed by outer layers of immortalized keratinocytes. The collagen beads are generated using droplet microfluidics to enable rapid and reproducible production. The biological relevance of SSM is revealed through elevated expression of epidermal differentiation markers (loricrin, involucrin, keratin 1, keratin 10) and the dermal-epidermal junction marker collagen VII. The barrier function is validated by permeability assays that show strong exclusion of fluorescent dextran above 4 kDa. Moreover, their usefulness for screening is shown by identifying a dose-dependent effect of vitamins in reducing oxidative stress and apoptosis against tert-butyl hydroperoxide. As such, this 3D microphysiological model recapitulates key structural, molecular, and functional features of human skin while offering rapid generation, scalability, and compatibility with high-throughput applications in dermatological and cosmetic research.

Engineering the auxin-inducible degron system for tunable in vivo control of organismal physiology

Jeremy Vicencio¹, Daisuke Chihara¹, Matthias Eder¹, Lucia Sedlackova¹, Julie Ahringer², Nicholas Stroustrup^{1,3}

¹ Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Dr. Aiguader 88, Barcelona 08003, Spain

² The Gurdon Institute and Department of Genetics, University of Cambridge, Cambridge, UK

³ Universitat Pompeu Fabra (UPF), Barcelona, Spain

ABSTRACT

The auxin-inducible degron (AID) is designed for the rapid and near-complete degradation of a specific target protein in vivo. However, to understand the dynamics of complex physiological networks, researchers often need methods that produce graded, quantitative changes in degradation rates for multiple proteins simultaneously. Here, we develop the AID system for in vivo, quantitative control over the abundance of multiple proteins simultaneously. First, by measuring and modeling the on- and off-target activities of different AID system variants in *Caenorhabditis elegans*, we characterize a variant of the TIR1 E3-ubiquitin ligase enzyme with improved degradation activity compared to the original AID and AID2 systems. Then, we develop a TIR1 expression construct that enables simultaneous pan-somatic and germline protein degradation. Finally, we expand the AID toolkit to allow independent, simultaneous degradation of two distinct tissue-specific proteins. Together, these technologies enable new in vivo approaches for studying quantitative cellular biology and organismal dynamics.

Morphogen concentration patterns in growing domains

Alba Tacoronte¹, Soha Ben Tahar², Ester Comellas^{1,3}, Sandra Shefelbine², Jose Muñoz^{1,3,4}

¹Universitat Politècnica de Catalunya, Barcelona, Spain.

²Northeastern University, College of Engineering, Boston, USA.

³Centre Internacional de Mètodes Numèrics en Enginyeria, Barcelona Spain.

⁴Institut de Matemàtiques de la UPC-BarcelnaTech, Barcelona, Spain.

Abstract Text:

Embryo development and organogenesis are governed by robust inhomogeneous growth distributions that are controlled by morphogen concentration patterns [1,2]. Changes in organ size and growth rates can in turn also influence pattern evolution, resulting in feedback that provides the necessary robustness during organogenesis.

The modelling of this feedback loop between growth and (Turing) patterns is traditionally accomplished through reaction-diffusion equations [3], which provide the base for mathematically analysing the stability of the concentration patterns as a function of model parameters. Extension of this analyses have been also been developed on growing domains [4].

In this work, we show how growth rate controls the emergence or inhibition of patterns through two combined effects: the modulation of admissible modes as a function of dilution effects, and the reduction of diffusion velocity as a function of domain velocity. We show that for some models and parameters, these effects may be relevant and determine mode switching. As a general rule, slow rates may ease the emergence of new pattern, while fast growth tends to stretch the pattern, providing a memory effect on the concentration of morphogens. We demonstrate our results mathematically and illustrate these effects on one- and two-dimensional domains.

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Epithelial Tissue Response Under Solid Shear Stress

Narmin Abasova, PULS Group, Institute for Theoretical Physics I, FAU Erlangen

Annemarie Wirth, PULS Group, Institute for Theoretical Physics I, FAU Erlangen

Madhura Ramani, PULS Group, Institute for Theoretical Physics I, FAU Erlangen

Rudolf Merkel, ICS-7: Biomechanics, Forschungszentrum Jülich

Ana-Sunčana Smith, PULS Group, Institute for Theoretical Physics I, FAU Erlangen, Division of Physical Chemistry, IRB Zagreb

Tissues are often subjected to mechanical stresses that are either internally generated or externally imposed. Some theoretical arguments say that the response to stress, and in particular solid shear, should depend on the state of the tissue, which can be either fluid-like or jammed. Unfortunately, there are very few experimental studies addressing this response. We research this issue by applying controlled shear forces to MDCK II epithelial monolayers cultured on polydimethylsiloxane (PDMS) substrates using a custom-built shear device. According to our results, the whole cluster elongates post-shear, but its area remains constant. We observe that adjacent cells rarely show junctional fluctuations after they reach the homeostatic state, and T1 transitions are not observed in either the test or control samples. The overall motility of cells is restricted in the jammed state of the monolayer, before and after shear. The results show that plain shear stress does not substantially disrupt cellular morphology, proliferation, or cell-cell rearrangements in a low-density or homeostatic epithelial monolayer. Irrespective of the state of the tissue, the response on the macroscopic and cellular level is that of an elastic solid. Moreover, a detailed analysis of the cellular response shows that the elongation of individual cells is preserved, which points to a cooperative, more complex regulation of cell shapes than initially anticipated. The mechanism of this response is yet to be fully examined.

Height and mass dynamics of MDCK monolayers probed by quantitative phase imaging

Nigar Abbasova and Amin Doostmohammadi

Niels Bohr Institute

Time-lapse imaging of epithelial monolayers reveals prominent area fluctuations, and recent evidence suggests that these are directly linked to volume dynamics, implicating three-dimensional cell size regulation in collective migration. However, experimental evidence remains limited and conflicting, and a quantitative framework connecting volume fluctuations to tissue-scale motion is still lacking.

Here, we address this gap using quantitative phase imaging (QPI) to obtain direct measurements of monolayer height, volume, and mass dynamics. We introduce novel methods to determine monolayer height from both two-dimensional and three-dimensional QPI data and demonstrate quantitative agreement between the two approaches.

Our measurements reveal substantial spatial and temporal variability in cell height, with variations of 15–25% across a monolayer. Cell height increases by 50–100% as cell density doubles, in contrast to previous reports suggesting constant height. In comparison, the average refractive index and dry mass fraction remain constant across the entire density range. We further show that the dry mass fraction is conserved on both short and long timescales, implying that cell volume is proportional to dry mass volume and that two-dimensional QPI directly measures dry mass and height.

Analysis of the dynamic structure factor of monolayer height uncovers distinct intracellular and intercellular relaxation modes. During collective motion and density fluctuations, cell height, projected area and volume oscillate synchronously with a period of approximately four hours. Together, these results establish a quantitative framework linking three-dimensional cell volume dynamics to tissue-scale motion and position QPI as a powerful tool for probing epithelial monolayer mechanics, growth, and active behaviour.

The mechanical control of the mammalian circadian clock

Juan F. Abenza¹, Leone Rossetti¹, Malèke Mouelhi¹, Stijn van der Klei¹, Javier Burgués¹, Ion Andreu¹, Keith Kennedy³, Pere Roca-Cusachs^{1, 4}, Santiago Marco^{1, 5}, Jordi García-Ojalvo³ and Xavier Trepat^{1, 2, 4, 6}

¹ *Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute for Science and Technology (BIST), 08028 Barcelona, Spain.*

² *Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), 08028 Barcelona, Spain.*

³ *Department of Experimental and Health Sciences, Universitat Pompeu Fabra, 08003 Barcelona, Spain.*

⁴ *Facultat de Medicina, Universitat de Barcelona, 08036 Barcelona, Spain.*

⁵ *Department of Electronics and Biomedical Engineering, Universitat de Barcelona, 08028 Barcelona, Spain.*

⁶ *Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.*

Cells sense and respond to the mechanical properties of their environment through diverse pathways that impact gene expression and affect key processes such as migration, proliferation, and differentiation. Recently, mechanics has also been shown to influence circadian rhythms, broadening its importance in tissue homeostasis. This project aims to understand how the circadian clock in individual mammalian cells is influenced by their physical context.

We used a transcriptional fluorescent reporter of the circadian clock gene *Rev-erba* (RevVNP), confocal microscopy, computational analysis, and microfabrication to study the clock in different mammalian cell types. In a first phase, using NIH3T3 mouse fibroblasts, we observed that basal and circadian RevVNP expression, typically low and rhythmic in dense, jammed monolayers, is perturbed at low density, when cells are allowed to freely migrate.

We then confined single cells in islands of different sizes using fibronectin micropatterning. As in dense monolayers, we found that the robustness of RevVNP circadian oscillations in isolated cells correlated with the level of confinement. By examining the intracellular localisation of the mechanosensitive transcriptional regulators YAP/TAZ under a series of mechanical perturbations, we observed a strong anticorrelation between RevVNP circadian robustness and YAP/TAZ nuclear levels. Overexpression of YAP/TAZ severely impaired the oscillations of the main core clock transcripts (*Bmal1*, *Rev-erba*, and *Cry1*), which demonstrates a direct role for YAP/TAZ as circadian modulators.

Given the broad importance of YAP/TAZ as mechanoregulators, our next goal is to determine whether they can also regulate the clock in more complex tissues, like the small intestinal epithelium. This tissue is highly subjected to mechanical forces and its activity is regulated by the circadian clock.

We have developed and characterised new fluorescent reporters to track *Bmal1* expression in individual cells within mouse intestinal organoids grown in 2D. Our preliminary results indicate that YAP/TAZ levels, high in the stem cell niche and low in differentiated enterocytes, correlate with both *Bmal1* and *Rev-erba* basal expression. Although both stem cells and enterocytes display robust *Bmal1* oscillations, we have found intriguing differences in phase and amplitude. This reflects complex regulation based on distinct time interpretations by the different cell types that compose the intestinal epithelium.

Tension-Driven Self-Organization as an Engineering Principle for Building Human Skeletal Muscle from iPSCs

Achyuth Acharya, Fabrice Dessolis, Qiyao Mao, Frank Schnorrer

Aix Marseille University, CNRS, IBDM, Turing Centre for Living Systems, LAI

Abstract:

Engineering complex and functional tissues requires understanding the rules that guide cells to assemble into defined higher-order structures. The skeletal muscle is an exemplary system in which molecular, cellular, and tissue-scale architectures must align precisely to produce coordinated contraction-driven movement. Yet the engineering principles governing this multi-scale self-assembly remain poorly understood.

We utilized a minimal human *in vitro* model to investigate how mechanical tension serves as a central self-organizing cue that drives and coordinates pattern formation in skeletal muscle across the molecular, cellular, and tissue scales. Human iPSC-derived myogenic cells are differentiated on simple substrates—with no external patterning, scaffolding, or imposed geometry. Remarkably, these cells autonomously **align, elongate, and self-assemble into coherent myofiber bundles** and exhibit **de novo sarcomere formation**.

Live imaging, quantitative fluorescence analysis, and laser nano-ablation revealed that **tension** builds up progressively during spontaneous bundling, **preceding and sustaining sarcomerogenesis**. Fiber bundles generate a stable mechanical environment that supports the emergence of periodic, titin-based sarcomeres across long myofibrils. Perturbing bundle formation by altering cell density modulates tension build-up and correspondingly shifts the timescale of sarcomere assembly, demonstrating a direct, causal relationship between tissue-level mechanical architecture and molecular-scale patterning.

To directly manipulate tension levels, we are currently further exploring the role of various external mechanical stimuli in the maintenance and maturation of these *in vitro* muscle cultures. Increasing tension in the 2D cultures often results in culture detachment after a certain point. To resolve this issue, we are currently testing a 2.5D culture system. Preliminary data strongly suggest increased long-term viability, maturation and capacity for spontaneous contraction in these 2.5D cultures.

Importantly, we have developed a novel cell stretcher in-house to test the role of direct mechanical stimulation on the elongation, bundling and maturation of the muscle cultures. This stretcher is capable of long-term programmed stretching patterns on cultures in standard incubators and is compatible with imaging chambers for live imaging on high resolution microscopes. In early experiments, we have documented that muscle fibers respond directly to stretch-relax cycles, displaying increased uniform sarcomeric patterning and higher global bundling.

Together, these findings position human iPSC-derived muscle as a minimal engineered system where emergent and controlled mechanical stimuli result in tension-driven self-organization, which replicates key stages of in vivo myogenesis. This platform enables bottom-up reconstruction of human muscle morphology, provides a quantitative framework to test how physical forces drive myogenesis, and offers versatile opportunities for engineering mature, functional muscle tissues.

Self-sustaining long-term 3D epithelioid cultures reveal drivers of clonal expansion in esophageal epithelium

Albert Herms, David Fernandez-Antoran, Maria P. Alcolea, Argyro Kalogeropoulou, Ujjwal Banerjee, Gabriel Piedrafita, Emilie Abby, Jose Antonio Valverde-Lopez, Inês S.

Aging epithelia are colonized by somatic mutations, which are subjected to selection influenced by intrinsic and extrinsic factors. The lack of suitable culture systems has slowed the study of this and other long-term biological processes. Here, we describe epithelioids, a facile, cost-effective method of culturing multiple mouse and human epithelia. Esophageal epithelioids self-maintain without passaging for at least 1 year, maintaining a three-dimensional structure with proliferative basal cells that differentiate into suprabasal cells, which eventually shed and retain genomic stability. Live imaging over 5 months showed that epithelioids replicate in vivo cell dynamics. Epithelioids support genetic manipulation and enable the study of mutant cell competition and selection in three-dimensional epithelia, and show how anti-cancer treatments modulate competition between transformed and wild-type cells. Finally, a targeted CRISPR–Cas9 screen shows that epithelioids recapitulate mutant gene selection in aging human esophagus and identifies additional drivers of clonal expansion, resolving the genetic networks underpinning competitive fitness.

Internal durotaxis and asymmetric shapes of cell clusters

Groups of migrating cells are usually guided by external cues, such as gradients of chemoattractant (chemotaxis), substrate stiffness (durotaxis), or electrostatic potential (electrotaxis). Here, I will show that cell groups can also be guided by internal cues, i.e., by gradients of their own properties. We found that, when moving from soft to stiff substrate, clusters of neural crest cells exhibit an opposite gradient in their own tissue stiffness, with soft cells at the front and stiff cells at the back. We predict that this internal stiffness gradient is enough to guide collective cell migration — a phenomenon that we call internal durotaxis. Moreover, these cell clusters are taller at the back than at the front. We explain this asymmetric height profile by modeling the cell cluster as an active liquid droplet driven by the motile cells at its base. We speculate that the emergence of internal guidance cues could provide robustness to the migration of cell clusters in noisy environments.

Biofabrication of the osteochondral unit through biphasic layering of wild-type and RUNX2 KO primary human MSCs

Anna Puiggali-Jou, Simone Ponta, Siyi Chen, Martin J. Stoddart, Marcy Zenobi-Wong

The osteochondral (OC) unit is a layered system composed primarily of hyaline cartilage, calcified cartilage, and the underlying subchondral bone. Its primary function is to provide load-bearing capacity and stability to the skeletal system. The ability of this unit to maintain distinct oxygenation, vascularization, mechanical properties, transport dynamics, and regenerative capacity in such proximity remains not fully understood. This fact makes it difficult to reproduce in the lab. Many attempts have involved using inorganic materials, such as ceramics or metals, to recreate the bone component, or employing microfluidics to dispense different media across distinct zones. In contrast, in this study we aim to employ a single material and uniform culture conditions, while generating two distinct mesenchymal stem cell (MSC) populations through genetic modification: one capable of undergoing spontaneous endochondral ossification (EO) and one that does not. This strategy is intended to recapitulate the layer-specific cellular phenotypes of the native tissue.

MSCs are an attractive cell source due to their accessibility, immunomodulatory features, and dual osteogenic and chondrogenic differentiation potential. However, MSC-derived cartilage frequently undergoes undesired EO driven by Runt-Related Transcription Factor 2 (RUNX2), resulting in instability of the hyaline cartilage template and progressive remodelling into bone *in vivo*. To overcome this limitation, we designed biphasic OC constructs containing CRISPR/Cas9 RUNX2-knockout (KO) primary human MSCs in the cartilage layer and wild-type (WT) MSCs in the bone layer. Furthermore, to spatially distribute cells and generate a 3D shape resembling the OC unit, we used a light-based 3D printing technique known as Filamented Light (FLight), which enables the fabrication of anisotropic structures within seconds (Figure 1a). As a resin, we used a mixture of hyaluronic acid and decellularized extracellular matrix.

First, we investigated the effect of KO MSCs in single-layer grafts. KO MSCs exhibited significantly enhanced chondrogenic differentiation compared with WT MSCs, with increased glycosaminoglycan (GAG) deposition and mechanical properties (1204 ± 296 kPa) comparable to native cartilage (~ 1000 kPa). Moreover, KO MSC grafts were resistant to calcification, maintaining full GAG content under hypertrophic stimulation, displaying low expression of hypertrophic markers (e.g., Collagen X, Alkaline Phosphatase, and Osteocalcin), and forming only a negligible calcified volume (0.29 ± 0.26 mm³) compared with WT constructs (2.70 ± 1.73 mm³). Across *in vitro* and *in vivo* studies, we consistently observed that KO cells combined superior chondrogenic performance with sustained resistance to extracellular matrix mineralization. Once we achieved the generation of stable hyaline cartilage even in hypertrophic conditions, we tested the bioprinted bilayer OC grafts. Successfully, these retained a sharply defined interface characterized by a localized calcified WT MSC region and a distinct KO MSC hyaline cartilage region, as confirmed by histology (Figure 1b), micro-computed tomography (μ CT), mechanical testing, and scanning electron microscopy (SEM).

Their unstable cartilaginous maturation has long hindered the use of MSCs for treating OC defects, and this remains an unresolved challenge. Here, we demonstrated that RUNX2 KO MSCs exhibit markedly enhanced chondrogenic potential that is maintained under hypertrophic stimulation during prolonged *in vitro* culture. Furthermore, biphasic constructs comprising WT and KO MSCs maintained a clear separation between a calcified and an uncalcified layer, making our strategy suitable for future applications in the treatment of OC defects.

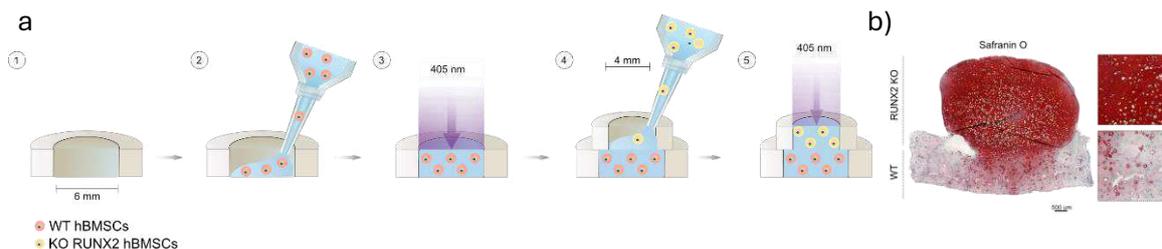


Figure 1. a) Illustration of the osteochondral unit biofabrication process. B) Safranin O of the construct cultured for 4 weeks in chondrogenic media followed by 4 weeks in hypertrophic media.

Cell response to curvature gradients

Parvathy Anoop, Paula Belska, Diogo Almas, Heidi Pertl-Obermeyer, Andreas Roschger, John Dunlop

Cells actively sense and respond to their surroundings. These responses can be triggered by biochemical signals as well as physical. Physical signals include substrate stiffness, compressive forces from neighbouring cells, and shear stress from fluid flow. Recent research has highlighted the significance of another key physical factor: substrate curvature. Previous studies in the group reveal that tissues of pre-osteoblast MC3T3-E1 cells grown on 3D capillary bridges lead to the emergence of a chiral tissue alignment. Additionally, it is known that cells grown on constrained regions of flat surfaces also demonstrate a chiral alignment. In this work, 2D micropatterning techniques and 3D printing is used to create systems that enable a comparison of tissue alignment of MC3T3-E1 cells in 2D and 3D curved environments. Additionally, techniques are explored to combine elements of 2D patterning on 3D substrates to constrain cell paths on 3D surfaces.

Covalently Crosslinked DNA Hydrogel Platforms: Enabling Tunable Viscoelasticity for 3D Cell Culture

Arena, D.^a; Rodrigo-Navarro^a; A.; Salmeron-Sánchez, M.^{a,b,c}

a. Institute for Bioengineering of Catalonia (IBEC)The Barcelona Institute for Science and Technology (BIST)Barcelona 08028, Spain

b. Institució Catalana de Recerca i Estudis Avançats (ICREA)Barcelona, Spain

c. Centre for the Cellular Microenvironment University of Glasgow Advanced Research Centre11 Chapel Lane, GlasgowG11 6EW, UK

Abstract

The extracellular matrix (ECM) provides cells with a dynamic mechanical landscape, where viscoelasticity critically regulates processes such as migration, differentiation, and mechanotransduction. The mechanical response probed by cells is, indeed, time-dependent, with the ECM stress-relaxation being a key factor in dictating the perceived mechanical properties driving cell behavior and fate.[1] The dissipation with time of the mechanical stress exerted by cells can be interpreted as a direct consequence of ECM viscoelasticity, which has been associated to the dynamic breaking and restoration of constitutive weak inter-polymer bonds, polymer entanglements and their unfolding. Replicating the time-dependent mechanical cues with synthetic matrices remains a major challenge for pushing forward 3D cell culture and mechanobiology studies. As in the case of Matrigel, which is currently the most employed ECM mimetic material, existing hydrogel systems often lack tunability of their elastic and viscous components, or either rely on chemistries that limit functionalization and structural precision.

Although DNA, as the genetic molecule of living systems, possesses the capability to precisely regulate biological outputs, it is not only a powerful genetic molecule. DNA can also serve a generic polymer of outstanding properties, that is versatile, biodegradable, and programmable. With its remarkable biological and polymeric features, DNA has been regarded as a universal building block for the construction of diverse materials and through various approaches -spanning from ligation, polymerization, chemical and physical crosslinking- both pure and hybrid DNA gels have been developed for a plethora of different applications, including hydrogel for 3D culture and tissue engineering. [2,3]

Herein, we present a modular platform in which DNA strands are covalently crosslinked for the fabrication of hydrogels with finely tunable viscoelastic properties. By taking advantage of the intrinsic reactivity of DNA towards a tailor-made polyethylene glycol (PEG)-based cross-linker (**P1**, Figure 1), we readily transform biomass-derived DNA into stable covalent networks of varied mechanical properties. By altering either, the molecular weight of the DNA polymeric precursor or the crosslinker density, we achieved robust, DNA-based hybrid bulk materials whose mechanical behavior can be systematically modulated, and that can be cast as continuous bulk surfaces for 2D mechanobiology studies. Specifically, we formulated a series of different covalently crosslinked DNA hybrid hydrogels of adjustable Young's modulus (E) in the range of 0.5 kPa up to 20 kPa, as revealed by nanoindentation measurements. Building on this bulk system, we demonstrated that a complementary microgel formulation can be achieved by exploiting the same covalent crosslinking chemistry between DNA and **P1**, further expanding the platform to 3D culture applications. Hydrogels with dimensions within the range of 0.5 and 2000 μm are produced *via* an emulsion-in-oil technique, enabling good control over size and internal crosslinking for mechanically consistent microenvironments engineering.

DNA-based hydrogel design inherently presents the capacity for orthogonal functionalization. As after the crosslinking reaction the backbone DNA remained an active

substrate of DNA processing enzymes including restriction endonucleases and ligases, the microgels can be decorated with aptamers, growth-factor-binding motifs, and other chemically active groups without perturbing the underlying mechanical framework (Figure 1). We believe that this feature could enable localized presentation or sequestration of signaling molecules, which would be of pivotal interest for the facilitation of studies of, *e.g.*, how biochemical gradients and dynamic ligand availability interplay with mechanical cues to regulate cell behavior and fate.

Overall, we expect that this covalently crosslinked, DNA-based hydrogels platform provides a versatile toolkit for mechanobiology, contributing to bridge the gap between tunable viscoelastic mechanics, high-precision biochemical functionalization and sustainable fabrication costs. The dual implementation -bulk gels for surface investigations and functional microgels for 3D cell culture- offers a unified approach for dissecting cell-matrix interactions across scales, paving the way for next-generation ECM mimetics with potential resonance in both fundamental and translational research.

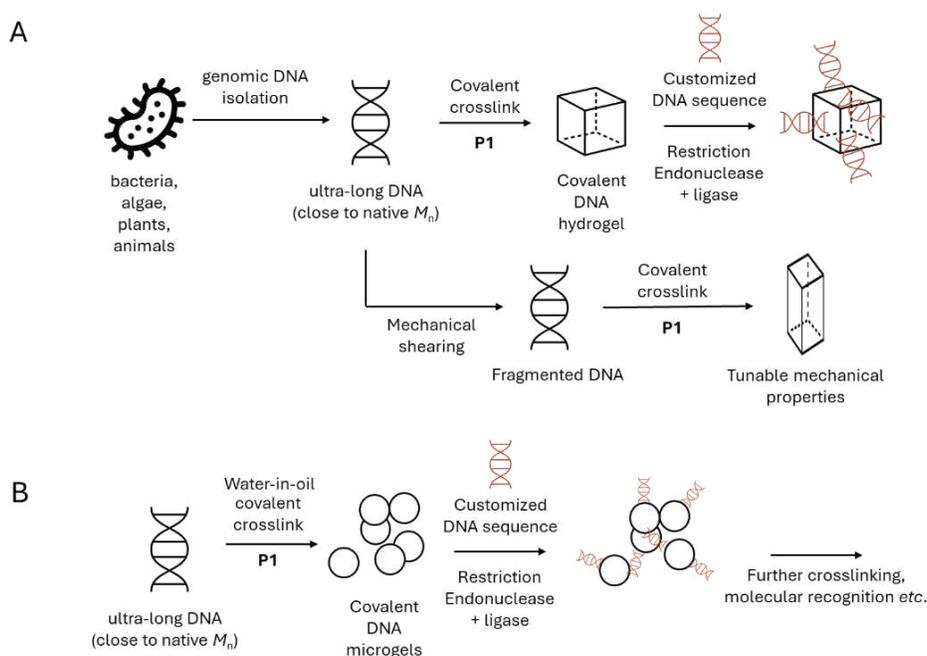


Figure 1. Pictorial representation of the covalently crosslinked, DNA-based hydrogels platform for 3D cell culture and tissue engineering. By the same chemistry, both bulk hydrogels (A) and microgels (B) are yielded, enabling a multi scale control of the mechanical properties of the medium. The intrinsic bioactivity of DNA strands is further exploited though decorating the gels with customized DNA sequences (*e.g.* aptamers), both for enabling further crosslinking and providing enhanced affinity towards small molecules of interest such as relevant growth factors.

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EMBL-IBEC abstract (231/300 words)

Mechanotransduction of soft matrix viscoelasticity: a molecular clutch perspective

Mariana A. G. Oliva, Manuel Salmeron-Sanchez

The extracellular matrix (ECM) is increasingly recognized to mechanically behave as a viscoelastic solid, exhibiting fluid-like energy loss (viscosity) and solid-like energy storage (elasticity) in response to applied deformations. ECM viscoelasticity has been shown to profoundly influence cell behavior, including proliferation, differentiation as well as disease progression. Notably, the brain is among the most viscoelastic tissues in the body, and alterations in its mechanical properties are hallmarks of neurodegeneration ¹. However, most in vitro brain models rely on unphysiologically stiff, purely elastic substrates that fail to capture the dynamic mechanics of native tissue ².

Our work has previously demonstrated the importance of the concerted action of Piezo1 and the molecular clutch complex in transducing viscoelasticity ³. For this, we employed polyacrylamide (PAAm) hydrogels with tuneable elastic and viscous moduli as well as a modified viscoelastic molecular clutch model that incorporated the crosstalk of Piezo1 and integrin complexes. With our experimental set-up we demonstrated integrin–Piezo1 concerted action is a key regulator of the mechanotransduction of viscoelasticity in soft matrices. Building on these findings, we are now extending this approach to neuronal systems to decode how viscoelasticity shapes neuronal mechanosensing, activity, and communication. Using induced pluripotent stem cells (iPSCs) derived neurons (iNeurons), we investigate cell response to physiological and pathological viscoelasticity and assess how and whether these changes influence neuronal communication, investigating the key regulators of circuitry development. By integrating advanced biomaterials, live-cell imaging, and computational modeling, we aim to reveal how the dynamic mechanical properties of the brain microenvironment modulate synaptic and cytoskeletal dynamics.

¹ Chaudhuri et al., “Effects of Extracellular Matrix Viscoelasticity on Cellular Behaviour.”

² Lantoine et al., “Matrix Stiffness Modulates Formation and Activity of Neuronal Networks of Controlled Architectures”; Rabadan et al., “An in Vitro Model of Neuronal Ensembles.”

³ A. G. Oliva et al., “Piezo1 Regulates the Mechanotransduction of Soft Matrix Viscoelasticity.”

Nuclear-dependent and integrin-dependent mechanotransduction mechanisms integrate chemical and physical factors to regulate EMT in pancreatic cancer cells

Ona Baguer ^{1,2}, Laura M. Faure ¹, Mikhail Chesnokov ³, Gotthold Fläschner ¹, Sladjana Zagorac ³, Érika Ventoso ¹, Francisco X. Real ^{3,4}, Pere Roca-Cusachs ^{1,2}

¹ Institute for Bioengineering of Catalonia (IBEC), Spain

² University of Barcelona (UB), Spain

³ Spanish National Cancer Research Centre (CNIO), Spain

⁴ Pompeu Fabra University (UPF), Spain

The epithelial-to-mesenchymal transition (EMT) is an important event that regulates the progression of solid tumours. EMT is known to be triggered by both chemical and physical factors, but how cells integrate these factors is not well understood. To address this question, we induced EMT in a pancreatic cancer cell model chemically with TGF- β and physically by controlling substrate stiffness and cell spatial positioning. Specifically, we patterned circular monolayers onto hydrogels of different rigidities and treated them with or without TGF- β . We found that substrate stiffness and TGF- β synergistically induce a mesenchymal phenotype at the periphery of the monolayer, and that this effect is driven by nuclear mechanics and deformation. In contrast, the effects on cell migration are predominantly driven by integrin-mediated mechanotransduction through FAK signalling and the AP-1 complex. Altogether, these results demonstrate a fine-tuned mechanochemical integration that drives different EMT phenotypes, with relevance to pathologies exhibiting mechanical alterations, such as pancreatic cancer.

Keywords: nuclear mechanics, EMT, nuclear lamina, pancreatic cancer.

The contribution of the innate immune response to cerebral malaria vascular pathogenesis in a 3D blood-brain barrier model

Alina Batzilla^{1,2}, Pia Dernick^{1,3}, Jon Bezney⁴, Andreas R. Gschwind⁴, Fumio Nakaki¹, Mireia Altadill⁵, Hannah Fleckenstein^{1,6}, Livia Piatti¹, Silvia Sanz Sender¹, Manuel Fiegl¹, Olawunmi Rashidat Oyerinde¹, Borja Lopez Gutierrez¹, Miguel Lopez-Botet^{5,7}, James Sharpe^{1,8}, Lars Steinmetz^{4,9,10}, Gemma Moncunill^{11,12}, Maria Bernabeu^{1,13}

¹ European Molecular Biology Laboratory (EMBL) Barcelona, Barcelona, Spain

² Collaboration for joint PhD degree between EMBL and Heidelberg University, Faculty of Biosciences, Heidelberg, Germany

³ Humboldt University, Faculty of Life Sciences, Berlin, Germany

⁴ Department of Genetics, Stanford University School of Medicine, Stanford, CA, USA.

⁵ University Pompeu Fabra, Barcelona, Spain

⁶ European Molecular Biology Laboratory (EMBL), Cell Biology and Biophysics Unit, Heidelberg, Germany

⁷ Hospital del Mar Research Institute, Barcelona, Spain

⁸ Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

⁹ European Molecular Biology Laboratory (EMBL), Genome Biology Unit, Heidelberg, Germany

¹⁰ Stanford Genome Technology Center, Palo Alto, CA, USA

¹¹ ISGlobal, Barcelona, Catalonia, Spain

¹² CIBER Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Barcelona, Spain

Cerebral Malaria (CM) is a severe neurovascular complication of *Plasmodium falciparum* infections, in children characterized by brain swelling due to blood-brain barrier (BBB) disruption. Postmortem samples provide evidence of immune cells accumulating in the microvasculature of CM patients, suggesting a role of proinflammatory immune response in disease pathogenesis. However, it remains unclear whether leukocyte activation and accumulation are primary contributors to BBB breakdown or merely a consequence of BBB damage. We used a systems immunology approach comprised of an engineered 3D-BBB model and single-cell RNA sequencing (scRNAseq) to study leukocyte interactions with the BBB in CM pathogenesis. After stimulating peripheral blood mononuclear cells (PBMC) from healthy donors with *P. falciparum* (*Pf*-PBMC), we perfused them through the 3D-BBB model. scRNAseq analysis confirmed activation of T-cells, monocytes, and NK cells, and revealed increased binding of *Pf*-PBMC to resting brain endothelial cells. This increased binding was mostly driven by CD8+ and innate-like T-cells and associated with a conformational change of the binding receptor LFA1, quantified by FACS. We identified three transcriptional modules in BBB cells that were differentially expressed upon *Pf*-PBMC exposure. One inflammatory module was shared between endothelial cells and pericytes and could mostly be attributed to TNF- α and IFN- γ released by monocytes, T-cells, and NK cells. The two remaining, endothelial-specific modules included genes associated with cytoskeletal processes and apoptosis suggestive

of barrier dysfunction. Permeability measurements in the 3D-BBB confirmed an increased permeability caused by *Pf*-PBMC. Vascular disruption was dependent on cytoadhesion of *Pf*-PBMC as barrier function could be restored by blocking endothelial-leukocyte interactions using an anti-ICAM1 monoclonal antibody. Additionally, we demonstrate how the presented data can be used to disentangle which pathogenic features observed in human CM are likely to be caused by the innate immune response or *P. falciparum*-infected red blood cells respectively.

Intercellular vs. multicellular contributions to spheroids' mechanics

Hiba Belkadi^{1,2}, Enara Larrañaga¹, Clara Delahousse^{1,2}, Sébastien Sart¹, Charles Baroud^{1,2}

¹Institut Pasteur, Université Paris Cité, Physical Microfluidics and Bioengineering, 25-28 Rue du Dr Roux, 75015 Paris, France

²Laboratoire d' Hydrodynamique (LadHyX), CNRS, Ecole Polytechnique, Institut Polytechnique de Paris, 91128 Palaiseau, France

The mechanical properties of 3D tissues arise from a hierarchy of processes, with mechanical energy being stored both at the intracellular level, via cortical tension, and at the multicellular scale, through cell–cell adhesion and tissue topology. Here we explore the contributions of the different scales by measuring the recovery of multicellular spheroids after they are compressed in a microfluidic device [1]. The experiments are performed on both epithelial (MCF10A) and mesenchymal (MSC) spheroids, using three compression durations (10s, 5min, 30min) in order to isolate the effect of the intracellular vs. multicellular remodeling under compression.

The recovery dynamics of the spheroids upon release follows a double exponential characterized by two timescales: A short time scale (τ_1) on the order of 10s and a longer time scale (τ_2) on the order of 100s. For both cell types τ_1 increases with increased compression duration and it shows a strong dependence on cytoskeletal drugs for compression durations longer than 5min: spheroids recover slower when cortical contractility is decreased (using blebbistatin, both cell types) and faster when it is increased (using calyculin A on MCF10A). These measurements confirm that τ_1 represents the dynamics of the active intracellular mechanical response.

In contrast, the value of τ_2 displays strong differences between the epithelial and mesenchymal cell types: while it increases for increasing compression duration in the epithelial case, it is independent of the duration in the mesenchymal case. Moreover, τ_2 is not modified by the cytoskeletal drugs for either cell type. These observations indicate that τ_2 reflects the dynamics of the spheroid's 3D organization at the aggregate scale. This relationship is further confirmed using image correlation analysis, which reveals structural rearrangements occurring over this timescale.

Our current experiments are focused on measuring cellular rearrangements during the recovery phase, in order to distinguish the dynamics of epithelial vs. mesenchymal cells, in parallel with the development of a mathematical model of the hierarchical dynamics of the spheroid response.

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Curvature and its role on the morphology of tissues.

Belska, Paula, Anoop, Parvathy, Pertl-Obermeyer, Heidi, Dunlop, John W. C., and Roschger, Andreas

In recent years, it is becoming clearer that the surface curvature of a substrate can strongly influence tissue growth of a cell culture and as a consequence, the patterning of cells on a surface. We explore tissue formation of murine pre-osteoblast cell line (MC3T3-E1) on doubly curved surfaces with negative Gaussian curvatures. Cells are seeded onto PDMS scaffolds and tissue growth and actin stress fibres are observed as a function of time. The resultant tissue grows in a way such that the geometry is the same as that expected from a liquid. In other words, surface curvature and tissue area are minimised according to the Laplace Young equation giving rise to rotationally symmetric constant mean curvature surfaces of revolution. In addition, fixed samples, stained for actin, are observed using Light Sheet (LS) microscopy. Actin stress fibres spanning the entire surface together with a consistent twist in alignment could be observed. At shorter growth periods (day 7) the actin orientation had a right twist (spiralling around the surface), whereas later, at day 32 a left-handed alignment is observed. Surprisingly, this twist reverses at long times (day 63), giving rise to a multilayered tissue similar to what occurs in bone. Live cell imaging gives hints as to how cells orient themselves and the tissue along various curvature directions, providing new insights into how macroscopic multiscale tissues are formed *in vivo*.

Temporal requirements of Nodal signaling in mammalian epiblast development

Francesca Boffa¹, Ramiro Alberio², Domenico Iuso¹, Marta Czernik¹

¹Dipartimento di Medicina Veterinaria, Università degli Studi di Teramo,

²School of Biosciences, University of Nottingham, Sutton Bonington Campus, LE12 5RD – UK

The TGF- β /Nodal/ActivinA signaling pathway regulates Nanog expression through SMAD2 and is critical for the maintenance of pluripotency in embryonic stem cells. However, its role during the in vivo establishment of pluripotency remains incompletely understood. Early studies using the inhibitor SB431542 reported impaired epiblast development following TGF- β /Activin/Nodal inhibition, but more recent evidence suggests that these effects may be influenced by concentration-dependent toxicity or off-target activity. In human embryos, for instance, low doses of SB431542 have been associated with an increase in NANOG-positive cells, whereas higher concentrations reduce both NANOG and SOX17 expression. Comparable dose-sensitive responses have also been described in bovine and murine systems.

Here, we investigate the requirement for Nodal activity in NANOG induction and epiblast expansion using the ALK4/5/7 inhibitor A83-01. Compared to SB431542, A83-01 is a more selective and better-tolerated compound, providing a suitable alternative for probing Nodal function during early development. Embryos were treated across two distinct developmental windows—morula to early blastocyst (E4–E7) and early to late blastocyst (E6–E7)—and subsequently analyzed by immunostaining for NANOG, SOX2, and SOX17 to assess stage-specific dependencies on Nodal signaling. This approach aims to clarify the temporal and functional contribution of Nodal signaling to epiblast development and its relationship with pluripotency and hypoblast-associated pathways, with implications for the design of robust and reproducible in vitro lineage specification protocols across mammalian species.

Title: Optogenetic Control of Force Transmission in Pluripotent Epithelia

Authors: Miquel Bosch-Padrós, Guillermo Martínez-Ara, Miki Ebisuya, Xavier Trepap

Development relies on the interplay of three fundamental processes: cell proliferation, fate specification and morphogenesis, the acquisition of correct tissue shapes. Apical constriction is a key driver of morphogenesis, acting at the cellular level but influencing tissue-scale shape formation. While apical constriction has been extensively studied within individual cells and is conserved across the animal kingdom, the mechanical forces generated and transmitted through tissues during this process have not been measured and described. To address this gap, we employed a novel optogenetic tool to induce apical constriction in human pluripotent stem cells, combined with traction force microscopy to quantify the mechanical forces involved in the process. Using this approach, we discovered that constriction produces a consistent but small signature in traction maps, compatible with increased apical contractility and volume conservation. Furthermore, when apical constriction was induced in localized regions of a monolayer, the resulting cellular displacement field followed a screened Poisson equation in two dimensions. This finding reveals the existence of a length scale with a rheological origin and enables derivation of the Green's function of the tissue. While spatial and temporal deformation patterns can be precisely controlled, we also observed that jamming transitions cannot be induced through apical contractility, highlighting the inherently unjammed nature of this pluripotent epithelium. Together, these results uncover key rheological properties of human pluripotent stem cells at timescales relevant to morphogenesis, inaccessible through other techniques. As these cells are widely used to generate organoids and embryo models but remain poorly characterized mechanically, our work establishes a foundational framework for future studies requiring shape or force control in stem cell-derived tissues.

How do malaria cues rewire endothelial mechanics?

Adrian Candelas, Matt Govendir, Waleed Mirza, Juan Francisco Abenza, Manuel Gómez-González, Borja López Gutiérrez, Martin Bergert, Xavier Trepas, Alejandro Torres-Sánchez, Alba Diz-Muñoz, Maria Bernabeu

Barrier integrity of endothelial tissues relies on a fine balance between intercellular junctional forces and focal adhesion-mediated traction. In the context of cerebral malaria, one of the deadliest complications of *Plasmodium falciparum* infection, the blood-brain barrier (BBB) is compromised, leading to brain swelling and cerebral haemorrhages that cause over 500.000 deaths annually. Although endothelial disruption is well documented in post-mortem samples, the events that initiate barrier failure remain uncharacterized.

Endothelial barrier function is regulated by mechanosensitive adherens junctions, composed of membrane VE-cadherin homodimers, intracellular protein scaffolds, and a cortical actin network that maintains junctional forces and adherens junction stability. Combining cutting-edge biophysical microscopy techniques and high-resolution live imaging with mathematical modelling, we show that human brain endothelial cells are rapidly compromised by exposure to *P. falciparum* products. During the first hour, they transiently accumulate VE-cadherin at junctions, while concurrently recruiting vinculin to focal adhesions. This results in increased tension, preceding an abrupt intercellular junction breakdown and a rapid focal adhesion elongation and stabilisation, indicative of a fast mechanoreponse to parasite exposure. After 8 hours, endothelial cells have undergone a profound morphological remodelling, with enhanced numbers of focal adhesions, transversal stress fibre formation, and the acquisition of a migratory phenotype. Traction force microscopy reveals increased matrix forces during this transition, which coincides with elevated FAK activation, a common sign of pathological pro-migratory states. Altogether, our findings suggest that BBB breakdown upon parasite exposure is driven by an early imbalance between adherens junctions and focal adhesions, leading to mechanical instability, junctional failure, and actin network reorganisation. Importantly, pharmacological inhibition of FAK with PF-573228 preserves endothelial barrier properties, highlighting the importance of endothelial mechanics in cerebral malaria pathogenesis.

In situ tracking of clonal evolution and phenotypic heterogeneity in tumors by spatial epitope barcoding

Jaime Casado García-Consuegra; Alexandros Drainas; Antonio Delgado-González; Garry Nolan; Julien Sage; Xavier Rovira-Clavé

Understanding how cancer cell subclones adapt to the changing tumor microenvironment is key to designing successful cancer therapies. Combinatorial tagging combined with spatial omics readouts to track barcodes in situ within tissue samples has allowed analyzing the spatial organization of cell lineages and phenotypes in xenograft models of cancer, uncovering emergent behaviors from mixed clones and the selective growth of clonal regions. However, it is still unclear how genetic or chemical perturbations influence subclonal evolution and phenotypic heterogeneity. Here, we incorporated genome editing tools and chemotherapy treatment to a barcoded xenograft model of small cell lung cancer, allowing us to investigate how the interplay between genetic and chemical modifications affect clonal behavior and phenotypic diversity within the tumor microenvironment. We observed that knocking out certain genes involved in small cell lung cancer progression exhibited distinct growth behaviors, suggesting gene-specific effects on clonal expansion. We identified clear patterns of clonal advantage that varied depending on treatment conditions: in untreated tumors, clones lacking NF2, a tumor suppressor involved in contact inhibition, showed enhanced expansion. Conversely, under cisplatin treatment, clones with SLFN11 knockouts, known to mediate sensitivity to DNA-damaging agents, expanded preferentially. In this context, we observed a structured spatial distribution of clones across both treatment-exposed and phenotypically distinct regions of the tumor, which may play a role in shaping tumor progression. Furthermore, our results revealed diverse interactions between clones under different treatment conditions pointing to a potential role of spatial context and interclonal relationships in modulating clonal behavior in small cell lung cancer. This study underscores a connection between genetic alterations, subclonal dynamics, spatial distribution and chemotherapy response of cancer cells in the tumor microenvironment. The results exemplify the utility of this method in providing new perspectives on the processes driving tumor growth and therapeutic resistance, offering valuable insights for the advancement of personalized cancer treatments.

MECHANOBIOLOGY OF MICROPATTERNED hiPSC COLONIES

Ricard Casanovas-Zapata, Özge Özgüc, Miquel Bosch-Adrós, Steffen Grosser, Aina Ventura-Porcar, Xavier Trepal-Guixer

Over the last decade, the emergence of hPSC-based models has provided significant insight into early human developmental events that are otherwise difficult to study due to both ethical constraints and physical inaccessibility. While phenomena such as gene patterning and axis specification have been widely investigated, the mechanical processes underlying these events remain comparatively understudied, despite evidence of their key role. We use micropatterned hPSC colonies, including the 2D gastruloid model whose biochemical evolution is well characterized, to investigate the mechanobiology of the same cell types that make up the post-implantation human embryo.

To interrogate how cells jointly integrate mechanics and signalling during differentiation, we combine 2.5D traction force microscopy to extract spatiotemporal force patterns with immunostaining-based readouts of signalling and lineage markers, while live imaging and single-cell tracking capture the evolving collective dynamics of the colonies. This multimodal approach enables us to follow not only the lineages cells adopt, but also how they mechanically and dynamically change their fate in real time.

The co-evolution of biomechanics and biochemistry in such a controllable system opens the door to addressing classically inaccessible questions in human development, including how initially homogeneous cell populations undergo symmetry breaking and generate organized multicellular behaviours. For example, we are now able to track the mechanical signature of differentiating colonies as they establish the three germ layer-like structure. Ultimately, our system provides a tractable platform with which to explore fundamental principles guiding early human embryogenesis.

Engineering Developmentally Informed ECMs for Human Neuronal Maturation

Palash Chandravanshi¹, Gisele P. Soares^{2,3}, Serafima Beletskaya¹, Francisco J. Lopez-Murcia^{2,3}, Paulina N. Bernal⁴, Kelly Marshall⁵, Thao Nguyen⁵, Leo Garma⁶, Susana Rodriguez¹, Jesús Asín⁷, Alexandra Naba⁸, Ricardo Levatto⁴, Evangelos Kiskinis^{5,9}, J. Alberto Ortega^{2,3}, **Zaida Álvarez**^{1,9,10}

¹Biomaterials for Neural Regeneration Group, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain, ²Department of Pathology and Experimental Therapeutics, Institute of Neurosciences, University of Barcelona, L'Hospitalet de Llobregat, Barcelona, Spain, ³Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet del Llobregat, Spain, ⁴Department of Orthopaedics, University Medical Center Utrecht, Utrecht University, Heidelberglaan 100, 3584 CX, Utrecht, The Netherlands, ⁵The Ken & Ruth Davee Department of Neurology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA; ⁶Department of Neuroscience, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA, ⁷Breast Cancer Clinical Research Unit, Centro Nacional de Investigaciones Oncológicas - CNIO, Madrid, Spain, ⁸Department of Statistical Methods, School of Engineering, University of Zaragoza, Zaragoza 50018, Spain, ⁹Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, Illinois, USA, ¹⁰Simpson Querrey Institute for BioNanotechnology, Northwestern University, Chicago, Illinois, USA. ¹⁰CIBER en Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, Spain.

Human iPSC-derived neurons fail to attain adult-like maturity *in vitro*, limiting their utility for modeling neurological development and disease.¹ Neuronal maturation *in vivo* is orchestrated by dynamic extracellular matrix (ECM) remodeling, yet the developmental ECM cues driving this process remain largely unexplored.²

Here, we define a developmental map of ECM signatures across key stages of spinal cord maturation and identify perinatal-stage matrisome components as critical regulators of neuronal maturity. This developmental window coincides with the emergence of perineuronal nets (PNNs), specialized ECM structures enriched in chondroitin sulfate proteoglycans, link proteins, and tenascins, which are known to modulate neuronal plasticity and stabilize mature synaptic networks. Leveraging these developmental insights, we engineered a synthetic PNN composed of Tenascin-R, Versican V3, and HAPLN1 along with Hyaluronic acid to recapitulate key features of the perinatal ECM microenvironment.

This defined ECM environment accelerates maturation of human iPSC-derived neurons, marked by increased neurite complexity, enhanced synaptic activity, and spontaneous electrophysiological firing. These effects are observed across both two-dimensional and complex three-dimensional culture systems, underscoring the broad applicability of this approach. Together, our findings establish perinatal PNN components as instructive cues for neuronal maturation and provide a developmentally informed ECM-based strategy to overcome neuronal immaturity, advancing the fidelity of human neuronal models for basic research and disease modeling.

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Emergent 3D morphogenesis of epithelial sheets via mechanically-driven phase transitions on planar matrices

Enara Larrañaga¹, Aina Abad-Lázaro¹, Mireia Flores-Expósito¹, Pau Canaleta-Vicente¹, Verónica Acevedo¹, Vanesa Fernández-Majada¹, Xavier Hernando-Momblona², Eduard Batlle^{2,3,4}, Jordi Comelles^{1,5,*} and Elena Martínez^{1,5,6,*}

¹ *Biomimetic Systems for Cell Engineering Laboratory, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain*

² *Colorectal Cancer Laboratory, Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology (BIST), Baldiri Reixac 10-12, 08028 Barcelona, Spain*

³ *Centro de Investigación Biomédica en Red (CIBERONC), Barcelona, Spain*

⁴ *ICREA, Passeig Lluís Companys 23, 08010 Barcelona, Spain*

⁵ *Department of Electronics and Biomedical Engineering, University of Barcelona (UB) Barcelona, Spain*

⁶ *Centro de Investigación Biomédica en Red (CIBER), Madrid, Spain*

Epithelial morphogenesis on planar substrates can undergo abrupt, collective transitions, yet the mechanical rules that gate these state changes remain unclear. We show that primary intestinal epithelial cells, seeded as single cells at high density on 2D substrates, undergo a sharp, mechanically-driven 2D to 3D transition controlled by Matrigel surface density and the substrate's deformability/plasticity. Keeping dimensionality constant while tuning Matrigel, tissues organize either as flat monolayers or as transient 3D tubular networks. The critical Matrigel density that triggers tubes generalizes across epithelial cell types, where Caco-2 and MDCK exhibit a parallel monolayer-to-cluster transition. Near the transition, network formation is synchronous across millimeter scales and yields highly ordered topology, consistent with a collective, system-level instability. Mechanistically, network formation requires integrin $\alpha 6$ -mediated traction onto laminin to plastically remodel the matrix, Rac1-dependent apical-in polarity and enriched stemness, as reducing the initial stem-cell fraction progressively erodes network topology and metrics. These indicate a finite window for 3D emergence. Together, these results identify a percolation-like mechanical threshold on planar matrices that controls epithelial shape state and stemness through active cell-ECM coupling and viscoelastic-plastic remodeling.

Characterizing airway organoid architecture in health and disease

Marina B. Cuenca, Andrés Marco-Gimenez, Vikas Trivedi, Talya Dayton, EMBL Barcelona

Pulmonary neuroendocrine cells (PNECs) are a rare (~0.5%) epithelial population that reside either as solitary cells or as clusters at airway branch points. In vivo studies indicate that these clusters arise through active migration, suggesting that PNECs sense and respond to chemical, mechanical or geometric cues within the airway epithelium. PNECs contribute to epithelial repair following injury, and their dysregulation is implicated in diseases such as cystic fibrosis, chronic obstructive pulmonary disease and small-cell lung cancer (SCLC). These observations point toward a mechanosensitive and highly plastic cell type whose behavior is tightly coupled to the mechanical and architectural state of the surrounding tissue.

To investigate these relationships, our lab has established two complementary human organoid systems: fetal airway organoids enriched in PNECs and patient-derived tumor organoids (PDTOs) that preserve neuroendocrine identity. These models reveal striking morphological variability, ranging from organized, lumen-forming epithelia to compact, mechanically rigid structures in PDTOs. These architectural changes are reflected in actin organization, tight-junction proteins such as ZO-1 and redistribution of β -catenin. Such cytoskeletal and junctional remodeling likely influences local tissue mechanics and PNEC positioning, providing an entry point to study how mechanical architecture shapes PNEC behavior in health and malignancy.

We present a dual-scale imaging strategy combining high-throughput single-organoid imaging with long-term volumetric light-sheet microscopy to quantify tissue architecture, cytoskeletal dynamics and PNEC distribution over time. This enables us to correlate mechanical features such as epithelial compaction, curvature and actin remodeling with PNEC clustering and malignant phenotypes. Together, these approaches establish a framework for dissecting how tissue geometry and mechanosensation contribute to PNEC organization, morphogenetic transitions and early steps of SCLC initiation.

Mechanically triggered fate reversion in mouse embryonic stem cells using microfluidics.

Mechanical cues are known to promote differentiation of pluripotent stem cells (PSCs), but their potential to induce phenotypic reversion remains unexplored. Recent studies reveal that hyperosmotic stress can revert mouse embryonic stem cells (mESC) to a state resembling the two cell embryo (2C-like state). This state is associated with an upregulation of totipotency markers (Mervl, Zscan, Dux) along with a downregulation of pluripotency markers (Oct4, Nanog). Such findings suggest that mechanical stimuli might drive phenotypic reversion in PSCs through unexplored mechanotransduction pathways.

To explore this hypothesis, aggregates of a Mervl-reporter mESC line are mechanically actuated and imaged to track the conversion of PSCs to Mervl-positive 2C-like cells. To quantify the mechanical forces associated to phenotypic reversion, a novel microdevice is used to apply uniaxial compressive stress to 3D mESC aggregates. This integrated PDMS based device is used to form, encapsulate in hydrogels and mechanically stimulate mESC aggregates. It is composed of two air cavities framing a fluidically independent chamber. The chamber's ceiling deforms as negative pressure is imposed in the air circuit, thus applying dynamical or static stress to the encapsulated aggregates.

Imaging, qPCR and FACS results as well as functional assays indicate that mechanical compression can induce phenotypic reversion in undifferentiated PSCs. The extent of reversion depends on the magnitude and mode of the forces applied. These findings highlight the role of mechanical stimulation in stem cell reprogramming and suggest the need for further research into how mechanical forces are transmitted within mESC aggregates and the molecular mechanisms linking mechanical cues to stem cell reprogramming.

Self-Healing in Skeletal Muscle Bioactuators Triggered by Mechanical Damage

Valerio Di Carlo*, Judith Fuentes*, Maria Guix, Florencia Lezcano, Serxio Álvares Olcina, Marina Rovira, Nil Fontanals, Samuel Sánchez

Abstract

Self-healing is a key functional property for engineered multicellular systems that aim to operate reliably over extended periods. Skeletal muscle is an attractive building block for biohybrid machines due to its intrinsic adaptability and force generation, yet its capacity to recover from mechanical damage in engineered bioactuators remains insufficiently characterized (Guix et al., 2021; Webster-Wood et al., 2023). Here, we investigate the self-healing behavior of 3D bioprinted skeletal muscle bioactuators subjected to distinct types of mechanical damage (Dumont et al., 2015; Sakar et al., 2016; Tiburcy et al., 2019). C2C12 myoblasts were embedded in a gelatin/fibrin-based bioink and bioprinted into anchored muscle constructs, which matured into aligned and contractile tissues. After maturation, bioactuators were subjected to either localized cut or localized crush injuries. Tissue recovery was assessed over time through morphological characterization and quantitative force measurements. Crush-injured bioactuators exhibited a pronounced self-healing response, with force progressively increasing and approaching control levels by day 10 post-injury. In contrast, cut-injured constructs showed limited recovery, reaching approximately 50% of their control bioactuators within the same timeframe, indicating more severe starting damage. At the structural level, evaluation of myotube formation and alignment over time via F-actin staining revealed progressive tissue remodeling, with crush injuries exhibiting slower repopulation of the damaged area and only partial restoration of actin organization, whereas cut injuries showed rapid structural recovery through the formation of aligned myotubes. The faster recovery in cut injuries may be facilitated by widening or stretching of the cuts due to sustained mechanical tension. These findings demonstrate that 3D bioprinted C2C12 skeletal muscle bioactuators possess an intrinsic, injury-dependent self-healing capacity, driven by tissue reorganization and the presence of myogenic cell populations within the construct. Overall, this work highlights self-healing muscle bioactuators as resilient multicellular systems for future biohybrid and soft robotic applications.

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Vascularized lymphoma model enhances B cell viability and tumor crosstalk

Marina Fortea¹, Rubén Gimenez², Noelia Sandoval², Farid Seral¹, Patricia Pérez-Galán^{2*}, Kristina Haase^{1*}

¹ EMBL Barcelona, Parc de Recerca Biomèdica de Barcelona, Barcelona, Spain

² Department of Hemato-Oncology, IDIBAPS, Barcelona,

³ Centro de Investigación Biomédica en Red-Oncología (CIBERONC), Madrid, Spain.

Follicular lymphoma (FL) is an indolent but relapsing B-cell malignancy, which constitutes ~20-30% of non-Hodgkin lymphomas. Although patient-derived follicular lymphoma tumoroid models recapitulate key lymph-node tumor-immune signaling, they are short-lived (days-to~1 week) and lack a perfused vascular niche, there is a need to develop a vascularized FL-on-chip. A vascularized model of FL will enable vascular/ECM remodeling and assessment of angiogenic potential and enable more physiologic, longer-term studies of drug delivery, immune-cell trafficking, and transformation risk. Herein, patient-derived FL tumoroids (PDLTs) are combined with functional (perfusable) tonsil-like microvasculature in a lymphoma on-chip model – employed to investigate patient-specific effects on vascular remodeling. Tonsil-like microvasculature significantly improves lymphoma B-cell survival compared to conventional PDLT cultures in ultra-low attachment (ULA) plates. Vascularization enhances cell viability in both FL tumoroids and reactive lymph node controls. Vascularized FL tumoroids induce vascular barrier disruption ($p < 0.0001$), inferring that PDLTs compromise endothelial junctions, while vascular network morphology is not strongly altered. Ongoing single-cell sequencing analysis may reveal FL-induced endothelial transcriptomic changes. Overall, a novel microfluidic vascularized lymphoma-on-chip platform has been developed and will allow us to unravel FL-environmental crosstalk, in addition to perfusion and assessment of relevant therapeutics.

Joel Girón-Hernández^{abc}, Sofia Mares Bou^b, Andrea Martelli^d, Gloria Gallego Ferrer^{bc}, Piergiorgio Gentile^{bc}

^a *Department of Applied Sciences, Faculty of Health and Life Sciences, Northumbria University, NE1 8ST Newcastle Upon Tyne, United Kingdom*

^b *Centre for Biomaterials and Tissue Engineering (CBIT), Universitat Politècnica de València, 46022 Valencia, Spain*

^c *Biomedical Research Networking Centre on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Carlos III Health Institute, 46022 Valencia, Spain*

^d *Dipartimento di Ingegneria Enzo Ferrari, Università degli Studi di Modena e Reggio Emilia, 41125 Modena, Italy*

Garlic Extracts and Probiotics in a Lipopolysaccharide-Stimulated Intestinal Multicellular Model

Intestinal inflammation and impaired epithelial barrier function are characteristic features of several functional gastrointestinal disorders. Phenolic compounds with antioxidant and anti-inflammatory properties, as well as probiotics, have been proposed as complementary approaches to modulate these alterations. This study evaluated the individual and combined effects of probiotics and white or black garlic extracts on inflammatory responses and barrier integrity using an intestinal epithelial model based on a Caco-2/HT29 (9:1) co-culture differentiated for 21 days. Inflammation was induced by lipopolysaccharide (LPS) stimulation for 72 h, followed by exposure to probiotics and *in vitro*-digested garlic extracts.

Barrier function was assessed by transepithelial electrical resistance (TEER), ZO-1 tight junction localization, and Lucifer Yellow (LY) permeability, while cytokine levels (TNF- α , IL-1 β , IL-6) were quantified by ELISA. LPS stimulation resulted in a significant reduction in TEER compared with non-stimulated controls (924 ± 126 vs. 1129 ± 74 $\Omega \cdot \text{cm}^2$), although LY permeability remained below 3% across all conditions, indicating largely preserved barrier integrity. The highest permeability (2.1%) was observed in tissues treated with LPS and probiotics. ZO-1 analysis revealed greater tight junction disruption in white garlic-treated tissues, whereas black garlic-treated monolayers more closely resembled non-inflamed controls.

Among the cytokines analyzed, IL-6 exhibited the most pronounced increase following LPS stimulation. Treatments containing white or black garlic significantly reduced IL-6 levels, with white garlic decreasing expression to below baseline reference values. No significant changes were observed for TNF- α or IL-1 β . Overall, garlic extracts, particularly white garlic, attenuated IL-6-mediated inflammatory responses, while probiotics alone did not show a clear protective effect and appeared to increase epithelial stress and permeability, except when combined with white garlic, suggesting a potential synergistic antimicrobial or protective interaction.

Bioengineering vascularized human microlivers for malaria infection

Ines Geraldés, Judit González-Gallego, Eliana Coelho Real, Jerome Wong, Silvia Sender, Dennis Crusius, Samy Gobaa, Liliana Mancio-Silva, Maria Bernabeu

After transmission through the bite of an infected mosquito, malaria-causing *Plasmodium* sporozoites enter the host bloodstream and travel to the liver. There, they arrest in the liver microvasculature, cross the liver sinusoidal endothelial cells (LSECs), and infect hepatocytes. Despite decades of research, the cellular and molecular mechanisms that enable the parasite to arrest in the liver microvasculature and invade hepatocytes remain unclear, partly due to the lack of adequate human infection models.

Here, we have generated a 3D liver sinusoidal microvascular model that recapitulates key structural and cellular features of the human liver vasculature. LSECs are characterized by surface expression of markers such as CD36, the presence of irregular and dynamic fenestrations, as well as the establishment of discontinuous adherens junctions, the latter characteristics that confer the high permeability typical of liver sinusoids. The initial screening of primary human LSECs revealed rapid loss of endothelial identity and function while in culture. As an alternative, we generated iPSC-derived liver endothelial cells (iLECs). Notably, iLECs express and maintain CD36 expression and fenestrations like structures, as confirmed by scanning electron microscopy. By growing iLECs in a pre-patterned collagen hydrogel, we have generated a bioengineered liver 3D microvessel model, creating 100–150 μm microvessels. We have coupled this engineering method with photoablation and created 20 μm sinusoidal capillary vessels that better recapitulate the dimensions of liver sinusoidal capillaries, mimicking the unique structure and phenotype of liver endothelium.

We are currently characterizing the parasite interactions with human liver microvessels to dissect early events that could prime and boost hepatocyte infection. To achieve this goal, we are currently focusing on the co-culture of 3D liver microvessels with hepatocytes to fully recapitulate the liver infection cycle. This understanding could provide valuable insights for malaria prevention.

Modeling of Pulmonary Fibrosis: Towards Recreating Tissue Specificity

Olga Grigorieva, Natalia Basalova, Uliana Diachkova, Anastasia Smirnova, Anastasia Tolstoluzhinskaya, Maksim Vigovskiy, Petr Rogachev, Roman Eremichev, Anastasia Efimenko

Fibrosis remains an unsolved medical problem, accounting for 50% of deaths in developed countries. The tissue-specific nature of fibrosis development should be considered when studying its mechanisms and developing drugs, as each organ has a unique set of cell types interacting with each other and the extracellular matrix (ECM) in specific ways. The development of *in vitro* and *in vivo* models that account for the tissue-specific nature of pulmonary fibrosis provides an opportunity to test potential new approaches to therapeutics and develop new models of specific activity for quality control assessment. Treatment of cultured pulmonary fibroblasts with TGF β is used to assess myofibroblast differentiation, but does not reflect the *in vivo* picture. In our study, we culture cells on decellularized ECM derived from stromal cells cultured as cell sheets (2.5D) and spheroids (3D), allowing us to create a model of fibrotic foci in the lung. These conditions reflect the deposition of excess matrix with a complex chemical composition, allowing us to assess the contribution of the ECM to cellular differentiation.

A classic *in vivo* model of fibrosis is bleomycin-induced pulmonary fibrosis in mice. Induction of fibrosis in PTHrP-CreERT mice with inducible expression of tdTomato revealed, contrary to existing literature data, that cells expressing parathormone-related protein (PTHrP) are predominantly located in the bronchiolar epithelium. Upon induction of fibrosis, PTHrP⁺ cells partially migrate into lung parenchyma, which may indicate the contribution of this cell subpopulation of bronchiolar epithelium to the epithelial-to-mesenchymal transition.

These models allow us to evaluate the effects of potential drugs on the development of fibrosis. Thus, our group demonstrated that extracellular vesicles (EV) from mesenchymal stromal cells carry microRNAs -21, -29c, and -129, which stimulate myofibroblast dedifferentiation in *in vitro* models and reduce the severity of pulmonary fibrosis in mice with bleomycin-induced pulmonary fibrosis. Analysis of the biodistribution of fluorescently labeled PKH26/DiR EVs in the *in vitro* model revealed that a large proportion of them are taken up by alveolar macrophages, while results from three-dimensional contactless coculture of macrophages and lung fibroblasts suggest that the effect of EVs is mediated by their action on the M2c subtype of macrophages.

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Engineering Neural Tube–Like Cytoarchitecture in Human Spinal Cord Organoids Using 3D-Printed Geometric Cues

Rita Grimalt-Mirada¹, Francisco M. Sáez¹, Xavier Barceló¹, J. Alberto Ortega^{2,3}, Zaida Álvarez^{1,4,5}

¹Biomaterials for Neural Regeneration Group, Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain. ²Department of Pathology and Experimental Therapeutics, Institute of Neurosciences, University of Barcelona, L'Hospitalet de Llobregat, Barcelona, Spain.

³Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet de Llobregat, Spain. ⁴CIBER en Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, Spain. ⁵Center for Regenerative Medicine (CRN), Northwestern University, Chicago, Illinois, USA.

Abstract:

The ability of central nervous system (CNS) organoids to model human development and disease is fundamentally limited by their inability to recapitulate key morphogenetic events that drive tissue patterning *in vivo*¹. Current neural organoids rely on stochastic self-organization, leading to the emergence of multiple, repetitive microstructures within a single construct and a lack of global tissue polarity^{2,3}. In the case of the spinal cord, this limitation is particularly evident in the failure to generate a defined neural tube–like architecture, including a central canal that acts as a developmental organizer for the spatial distribution of neural progenitors and differentiated cell types^{4,5}. Here, we propose a strategy to impose early morphogenetic constraints on human spinal cord organoids (hSCOs) by engineering their initial geometry using a high-resolution 3D-printed platform. Rather than allowing cells to aggregate freely, human induced pluripotent stem cells (hiPSCs) are guided to self-assemble within toroidal microarchitectures that promote the formation of a continuous, tube-like structure reminiscent of the early neural tube. This geometrically instructed organization induces a fundamentally different mode of tissue expansion and cellular reorganization compared to conventional aggregate-based organoids, resulting in a more coherent and reproducible cytoarchitecture. We demonstrate that this platform supports neural induction, tissue viability, and lumenized organization, and can be readily combined with biochemical functionalization to further direct rostrocaudal and dorsoventral patterning. By shifting organoid formation from purely stochastic self-assembly to geometry-guided morphogenesis, this approach establishes a biomimetic framework for generating hSCOs with enhanced structural fidelity. Ultimately, this system provides a powerful foundation for studying human spinal cord development, injury mechanisms, and the evaluation of regenerative therapies in a physiologically relevant context.

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Bioelectric regulation of epithelial tissue organization

Andrea Iglesias-Ramas¹, Mathieu Coppey¹, Mirna Kramar¹

1. Institut Curie, Physics of Cells and Cancer, UMR 168, 75005 Paris, France

Mammalian cells maintain an electric potential across their plasma membrane, known as the resting transmembrane potential (T_p). This potential arises from localized ionic gradients and the zeta potential (Z_p), as well as from long-range influences generated by extracellular electric fields and tissue-scale bioelectric interactions. While T_p has been studied extensively in excitable cells, its function in non-excitable cells remains comparatively unexplored. Emerging evidence indicates that T_p can directly modulate intracellular signaling pathways, suggesting a role in regulating coordinated behaviors that underlie tissue formation.

In this work, we investigate the contribution of T_p to epithelial assembly, with a particular focus on how cell density influences collective behavior. Using live imaging with Genetically Encoded Voltage Indicators (GEVIs), we obtain non-invasive, long-timescale measurements of T_p in developing epithelial layers. These measurements suggest systematic differences in T_p associated with variations in cell density, as well as with cell type and spatial arrangement. We further assess how electrical coupling between neighboring cells and the emergence of supra-cellular T_p patterns may contribute to the coordination of multicellular organization within epithelial tissues.

Taken together, our results identify **distinctive bioelectric signatures associated with epithelial formation and suggest** that T_p acts as an active regulator of tissue-level coordination.

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Title

Modelling Febrile Host–Parasite Interactions in 3D Human Microvessels

Authors

Viola Introini^{1,2,3}, Rory KM Long¹, Olawunmi R Oyerinde¹, Silvia Sanz Sender¹, Frank Stein⁴, Gyu Min Hwang^{1,5}, Borja Lopez Gutierrez¹, Karl B Seydel^{6,7}, Gretchen Birbeck^{7,8,9}, Maria Bernabeu¹

¹ European Molecular Biology Laboratory (EMBL) Barcelona, Barcelona, Spain

² Max Planck Institute for the Science of Light, Erlangen, Germany

³ Max-Planck-Zentrum für Physik und Medizin, Erlangen, Germany

⁴ European Molecular Biology Laboratory (EMBL) Heidelberg, Proteomics Core Facility, Heidelberg, Germany.

⁵ Heidelberg, University, Faculty of Engineering Sciences, Heidelberg, Germany

⁶ Department of Osteopathic Medical Specialties, College of Osteopathic Medicine, Michigan State University, East Lansing

⁷ Blantyre Malaria Project, Kamuzu University of Health Sciences, Blantyre, Malawi

⁸ Epilepsy Division, Department of Neurology, University of Rochester, Rochester, New York

⁹ University Teaching Hospitals Neurology Research Office, Lusaka, Zambia

Abstract

Fever, a universal host defence mechanism during infection and inflammation, paradoxically contributes to neurological complications in malaria. Although febrile temperatures are known to increase the expression of parasite virulence proteins that mediate vascular adhesion and disease severity, the corresponding effects on the endothelium have remained unclear.

Here, we present a 3D vasculature-on-a-chip model that recapitulates human brain and lung microvessels under febrile conditions. Short febrile episodes at 40 °C—commonly observed in treated cerebral malaria patients—rapidly enhanced the binding of infected red blood cells and immune cells under flow. Mechanistically, we show that this phenotype is driven by endothelial glycocalyx shedding, which exposes the adhesion receptors EPCR and ICAM-1. Preserving glycocalyx integrity with a broad matrix metalloproteinase inhibitor prevented the temperature-induced increase in cytoadhesion.

Together, these findings identify fever as a host-specific amplifier of vascular pathology in malaria and highlight endothelial-protective or antipyretic interventions as potential strategies to mitigate febrile microvascular injury.

The spatial organization of cancer cell subclones is a critical determinant of tumor evolution. Although combinatorial tagging and spatial omics allow for subclonal tracking in situ, these current 2D imaging methods struggle to quantify how 3D volumetric constraints influence subclonal evolution and phenotypic heterogeneity. To investigate these volumetric relationships, we generated barcoded tumor spheroids with distinct genetic perturbations, imaged serial sections by Multiplex Ion Beam Imaging (MIBI), and built a computational pipeline specifically developed to reconstruct the virtual 3D model of these spheroids from serial data. The serial sections' images of the spheroids are first aligned using a pairwise PCA-based registration system. The former can be extended with a non-rigid deformation alignment with the Demons algorithm in order to account for subtle deformations during sample processing. Unlike conventional methods that rely on interpolation, which distorts marker distributions and masks subtle clonal boundaries, our approach preserves the integrity of the raw data. We utilize the Hungarian algorithm to resolve cross-slice cell continuities, minimizing global error while defining 3D cell volumes based on neighbor constraints. Preliminary results demonstrate that this tool successfully reconstructs realistic 3D spheroid architectures. This enables the quantification of clonal growth patterns within their native volumetric environment, providing a tool to investigate how genetic perturbations shape 3D spatial organization in solid tumors.

Shaping cortical interneuron migration through mechanical signaling during brain development

M. Javier Torrent¹, S. Helgueta-Romero^{1,2}, S. Derclaye³, X. Li⁴, P. van Delft¹, L. Ergot⁵, A.-S. Duwez⁴, S. Gabriele⁵, Lasse Sinkkonen², D. Alsteens³, L. Nguyen¹

¹Laboratory of Molecular Regulation of Neurogenesis, GIGA institute, University of Liège, University of Liège, C.H.U. Sart Tilman, Liège 4000, Belgium

²Department of Life Sciences and Medicine (DLSM), University of Luxembourg, Luxembourg

³Louvain Institute of Biomolecular Science and Technology, Université Catholique de Louvain, 1348 Louvain-la-Neuve, Belgium

⁴UR Molecular Systems, Department of Chemistry, University of Liège, 4000 Liège, Belgium

⁵Mechanobiology & Biomaterials group, University of Mons, 7000 Mons, Belgium

Cortical interneurons (cINs) are generated in the ganglionic eminences (GE) and migrate tangentially to populate the developing cortex. During migration, cINs encounter mechanical forces arising from cell–cell interactions and the extracellular matrix, yet how mechanotransduction influences their behavior remains poorly understood. Here we aim to investigate how mechanotransduction events may shape cINs behaviour during cortical development. By combining atomic force microscopy (AFM) with time-lapse imaging, we found that the cortical intermediate zone becomes stiffer at embryonic day (E)16.5 compared to E13.5, correlating with reduced migratory speed and decreased nuclear translocation frequency of cINs. Using heterochronic organotypic slice cultures, we showed that E16.5 cINs migrating within a younger (E13.5) cortical environment exhibit increased speed and nuclear translocation frequency compared to age-matched controls. Similar stage-dependent differences were observed when E13.5 and E16.5 cINs were cultured in a viscous 3D matrix. Single-cell AFM measurements revealed that E16.5 cINs display softer somas and increased nuclear deformation during migration. Consistently, transcriptomic data from cINs at both stages indicated differences in expression of key nuclear and mechanotransduction genes during development. Together, 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. Ongoing single-cell multiomics analyses of migrating cINs cultured in hydrogels of increasing stiffness will further elucidate molecular mechanisms linking mechanical cues to cIN maturation and identity.

Epithelial tension controls cell extrusion

Daniel Krueger^{†1,5*}, Willem Kasper Spoelstra^{†2}, Dirk Jan Mastebroek¹, Rutger Kok^{2,3,6}, Shanie Wu¹, Mike Nikolaev^{3‡}, Marie Bannier-Hélaouët^{1,5‡}, Nikolche Gjorevski^{3‡}, Matthias Lutolf^{3‡}, Johan van Es^{1,5}, Jeroen van Zon^{2*}, Sander Tans^{2,4*}, Hans Clevers^{1,5*§}

¹Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences (KNAW) and University Medical Centre Utrecht (UMC); Utrecht, the Netherlands.

²AMOLF institute; Amsterdam, The Netherlands.

³École Polytechnique Fédérale de Lausanne/ Institute of Human Biology; Basel, Switzerland.

⁴Bionanoscience Department, Kavli Institute of Nanoscience Delft, Delft University of Technology; Delft, The Netherlands.

⁵Oncode Institute, Hubrecht Institute; Utrecht, the Netherlands.

†These authors contributed equally

* Correspondence

Cell extrusion is essential for homeostatic self-renewal of the intestinal epithelium. Extrusion is thought to be triggered by crowding-induced compression of cells at the villus tip. Here, we found instead that a local "tug-of-war" competition between contractile cells regulates extrusion. We combined quantitative live microscopy, optogenetic induction of tissue tension, genetic perturbation of myosin II activity, and targeted disruption of the basal cortex in mouse intestines and organoids. These approaches reveal that a dynamic actomyosin network generates tension throughout intestinal villi. Cells unable to sustain this tension are mechanically outcompeted and extruded. In a model of congenital tufting enteropathy, myosin II hyperactivation disrupts this balance, leading to excessive extrusion and loss of tissue architecture. This raises the question of what renders individual cells mechanically vulnerable. Our findings reveal that epithelial barrier integrity depends on active intercellular mechanics rather than passive crowding.

A comparative evaluation of Multiplex Ion Beam Imaging (MIBI) and Co-Detection by indEXing (CODEX) multiplex imaging for targeted spatial proteomics

Michela Lain Contato^{1,2}, Maximilian Haist^{3,4}, Jaime Casado García-Consuegra^{1,2}, Enric Ariza Ortiz^{1,2}, Antonio Delgado-González^{3,4}, Garry Nolan³, Xavier Rovira-Clavé^{1,3,4}

¹ Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute of Science and Technology (BIST), Barcelona, Spain

² Department of Biomedicine, University of Barcelona, Barcelona, Spain

³ Department of Pathology, Stanford University, Stanford, United States

⁴ Department of Microbiology and Immunology, Stanford University, Stanford, United States

Solid tumors are composed of a diverse mixture of cancer, immune, and stromal cells. Understanding their spatial organization within the tumor microenvironment (TME) is critical for deciphering mechanisms of tumor progression and response to therapy. Targeted spatial proteomics has emerged as a powerful extension of immunohistochemistry, enabling high-dimensional, spatially resolved characterization of these systems in intact tissues. However, direct comparisons that evaluate the relative capabilities and trade-offs of the major available targeted spatial proteomics platforms are currently scarce. In this study, we systematically evaluate two leading targeted spatial proteomics platforms: Co-Detection by Indexing (CODEX), a cyclic immunofluorescence technology, and Multiplexed Ion Beam Imaging (MIBI), an imaging mass spectrometry technique. Using a tissue microarray from a clinical cohort of 85 patient-derived head and neck squamous cell carcinoma samples, we processed a section from the same tissue blocks on each platform. To enable a side-by-side evaluation, we applied a comparable Python pipeline for image pre-processing, segmentation, and iterative clustering. Our results demonstrate that despite inherent differences in sample preparation and imaging approaches, both technologies can yield consistent and spatially relevant biological conclusions. For example, using antibody markers such as CD4, FOXP3, γ H2AX, and cytokeratin, we successfully differentiated and localized CD4+ T cells, regulatory T cells, and cancer cells while simultaneously assessing cellular states like DNA damage. Our workflow identified a broad range of cell phenotypes with high resolution, including those tightly packed or morphologically ambiguous. In conclusion, both MIBI and CODEX enable robust multiplexed, spatially resolved characterization of cellular architecture and heterogeneity in the TME. Future work will focus on a deeper spatial characterization of the dataset, alongside a direct comparison of the resolution and dynamic range of the two platforms. Once completed, this study will provide a robust framework for understanding the respective strengths and limitations of each technology, helping researchers select the optimal platform to apply not only for observational studies in a clinical setting, but for engineered in vitro tumor models such as spheroids and organoids. Applying these technologies to three-dimensional model systems would allow for perturbational studies while preserving key features of multicellular architecture, for a deeper understanding of the complex and heterogeneous cellular organization in solid tumor ecosystems.

Bioinspired 3D bioprinting of functional skeletal muscle constructs with controlled fiber orientation

Florencia Lezcano¹, Marina Rovira¹, Judith Fuentes¹, Serxio Álvarez¹, Samuel Sánchez^{1,2}

¹*Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain.*

²*Catalan Institute for Research and Advanced Studies (ICREA), Barcelona, Spain.*

flezcano@ibebarcelona.eu

3D bioprinting allows the precise arrangement of cells, materials, and functional molecules at desired places within pre-designed structures, enabling the fabrication of reproducible and scalable capable of mimicking the complexity of natural tissue. Despite advancements in bioinks, printing approaches, and established techniques, achieving complex three-dimensional skeletal muscle construct shapes with controlled myofiber orientation have remained challenging. This study aims to obtain bioprinted skeletal muscle constructs inspired by the natural shapes and myofiber organization of human skeletal muscles. Different anchoring systems and different printing path designs were implemented in order to determine the best approach to control myofiber orientation. After method optimization, skeletal muscle constructs with parallel, fusiform, bipennate, and circular shapes were 3D printed using an extrusion bioprinter controlled by a custom-made, high-precision, Python-based tool. The different muscle constructs were bioprinted around their specific, pre-printed, and pre-cured PDMS anchoring systems using a gelatin-fibrinogen-C2C12 cell-based bioink. After 14 days in differentiation media, all constructs demonstrated a cell viability close to 100% and matured myotubes. Parallel- and fusiform-shaped constructs showed fibers aligned in parallel, while bipennate constructs exhibited myofiber alignment in two directions according to the design. Circular constructs showed concentric myofiber alignment. Under electrical pulse stimulation, all constructs contracted along myofiber direction. Our results demonstrate that functional, complex skeletal muscle constructs with controlled myofiber orientation can be obtained through the implementation of dedicated anchoring systems and careful control of the bioprinting process. By recapitulating native muscle architecture, this approach advances the design of bioengineered functional skeletal muscles.

Data Driven Modelling of Limb Bud Growth and Morphogenesis

Tim Liebisch, James Sharpe

Understanding the complex choreography performed by thousands of cells to create tissues and organs is still a major scientific challenge and will require computational modelling as a key tool.

During limb morphogenesis, a process that has been a focus of study in developmental biology for many decades, chemical information, mechanical processes, and their interactions lead to tissue growth and growth of the organ. Different morphogen gradients and gene-regulatory networks yielding limb patterning are well characterized. Additionally, recent studies provide experimental evidence of mesenchymal cell behavior underlining limb morphogenesis, e.g., for the influence of oriented cell motion, cell intercalations, and controlled cell proliferation. However, the mechanistic basis of how cell orientation and motility are spatially distributed to result in a proper limb bud shape remains an open question.

To address the latter, we pursue a cell-based computational modelling of the mouse limb morphogenesis, in which cell proliferation, orientation, and motility are spatially controlled by morphogen gradients in the form of model parameters. Applying restrictions to the parameters using empirical data and performing a global parameter optimization, such that the model grows into wild type limb bud shapes, we provide a computational model that will help us to pinpoint the influence of distributions of molecular cues on mechanical processes and vice versa.

Finally, the model is used to generate predictions on how limb bud morphogens like FGF8 and Wnt5a may influence cell orientation and motility, and to explain the morphology of certain knock out phenotypes. This will shed some light onto the mechanistic basis of limb bud morphogenesis and generate predictions that may guide future experiments.

Chronic liver diseases lead to approximately 2 million deaths each year. Fibrosis—driven by the excessive deposition of extracellular matrix produced by activated hepatic stellate cells—is the primary risk factor for hepatocellular carcinoma. Despite its clinical relevance, effective antifibrotic treatments remain limited and often burdened by significant side effects. Immune-based cell therapies, especially those involving macrophages, offer a promising avenue for liver regeneration; however, their therapeutic impact has so far been modest, likely because current strategies fail to induce a durable, antifibrotic macrophage state.

This work aims to overcome this limitation by developing a new therapeutic approach that “primes” macrophages through mechanical stimulation. The strategy exploits **mechanical memory**, a long-lasting cellular program that persists even after mechanical cues are removed, providing a safer, more scalable, and more cost-effective alternative to genetic manipulation.

To achieve this, we engineered a light-responsive hydrogel composed of bacterial nanocellulose (BNC), a highly biocompatible natural polymer, functionalized with azobenzene groups to enable on-demand modulation of stiffness. This platform delivers precisely controlled spatiotemporal mechanical cues to encapsulated macrophages, steering them toward an anti-inflammatory phenotype and ultimately offering a promising route to restore liver functionality.

Midline crisis - deciphering mediolateral organisation in human trunk-like structures

Gareth Moore, Vikas Trivedi

Institute for Bioengineering of Catalonia (IBEC)

The establishment of a set of orthogonal body axes, to act as a coordinate system for patterning and morphogenesis, is a fundamental step in bilaterian development. While *in vivo* studies have identified extraembryonic cues directing coordination of the body axes, *in vitro* stem-cell based embryo models show the capacity to break symmetry and organise these axes absent the *in vivo* cues. Human trunk-like structures (hTLS) establish anteroposterior and mediolateral axes through spontaneous symmetry breaking and self-organisation. Yet how the secondary (mediolateral) axis is robustly aligned orthogonally to the primary (anteroposterior) axis remains unclear. Here we use an hTLS model to investigate the intrinsic and extrinsic cues necessary for robust multiaxial organisation. Chemical and environmental perturbations suggest balancing neuromesodermal progenitor fate is key aspect for mediolateral axis establishment *in vitro*. Furthermore, modulation of aggregate size and boundary conditions begins to uncover the mechanical cues necessary for midline neural and mesodermal tissue organisation. Understanding how two body axes can be robustly organised orthogonally is of interest beyond developmental biology with implications for regenerative biology and tissue engineering.

3D-Bioprinting of functional β -cell spheroids for Type 1 Diabetes treatment

Samantha Morón-Ros¹; Laurian de Vries¹; Laura Clua-Ferré¹; Natalia Castro¹; Javier Ramón-Azcón^{1,2}.

1. Institut de Bioenginyeria de Catalunya (IBEC). Barcelona, Spain.

2. Institució Catalana de Recerca i Estudis Avançats (ICREA). Barcelona, Spain.

Type 1 Diabetes Mellitus (T1DM) is characterized by an autoimmune-mediated destruction of pancreatic β -cells, resulting in absolute insulin deficiency. Patients depend on lifelong exogenous insulin therapy and must continually monitor their blood glucose to prevent serious complications. Current treatments cannot truly replicate the physiological insulin secretion of healthy β -cells. Cell-based therapies represent an emerging therapeutic frontier. Our innovative platform, UNIINK, addresses these unmet needs in cell therapy for T1DM. UNIINK enables rapid, sterile, and operator-independent fabrication of 3D bioprinted micro-spheroids, allowing normoglycemia restoration in T1DM patients. Our system allows scalable production using clinically validated materials: collagen I crosslinked with tannic acid (ColTA). This unique biomaterial overcomes the main limitations of existing cell therapies: enhanced structural integrity and resistance to enzymatic degradation, effective containment of cells within spheroids, optimal diffusion of nutrients and oxygen, immune protection of embedded cells, enabling insulin secretion while obviating the need for systemic immunosuppression. We successfully produced ColTA spheroids and demonstrated their long-term viability and functionality in vitro, maintaining β -cell survival and insulin secretion capacity for up to two months. In vivo studies further confirmed the translational potential of our technology. Spheroid transplantation into immunocompetent mice enabled the retrieval of intact spheroids without eliciting inflammatory response, as demonstrated through cytokine profiling and flow cytometry analyses. These results suggest that the biomaterial composition confers a degree of immune invisibility, supporting its use without systemic immunosuppression. To assess therapeutic efficacy, we tested the spheroids in hyperglycaemic mice. Notably, transplantation of INS1-E β -cell-laden spheroids led to substantial and sustained reductions in blood glucose levels. In treated animals, glycaemia decreased from severely hyperglycemic levels (>600 mg/dL) to near-physiological values (200–300 mg/dL). These promising results highlight the robustness, biofunctionality, and immunomodulatory properties of our UNIINK platform and support its further development toward clinical translation in T1DM therapy.

Morphogen concentration patterns in growing domains

Alba Tacoronte¹, Soha Ben Tahar², Ester Comellas^{1,3}, Sandra Shefelbine², Jose Muñoz^{1,3,4}

¹Universitat Politècnica de Catalunya, Barcelona, Spain.

²Northeastern University, College of Engineering, Boston, USA.

³Centre Internacional de Mètodes Numèrics en Enginyeria, Barcelona Spain.

⁴Institut de Matemàtiques de la UPC-BarcelnaTech, Barcelona, Spain.

Abstract Text:

Embryo development and organogenesis are governed by robust inhomogeneous growth distributions that are controlled by morphogen concentration patterns [1,2]. Changes in organ size and growth rates can in turn also influence pattern evolution, resulting in feedback that provides the necessary robustness during organogenesis.

The modelling of this feedback loop between growth and (Turing) patterns is traditionally accomplished through reaction-diffusion equations [3], which provide the base for mathematically analysing the stability of the concentration patterns as a function of model parameters. Extension of this analyses have been also been developed on growing domains [4].

In this work, we show how growth rate controls the emergence or inhibition of patterns through two combined effects: the modulation of admissible modes as a function of dilution effects, and the reduction of diffusion velocity as a function of domain velocity. We show that for some models and parameters, these effects may be relevant and determine mode switching. As a general rule, slow rates may ease the emergence of new pattern, while fast growth tends to stretch the pattern, providing a memory effect on the concentration of morphogens. We demonstrate our results mathematically and illustrate these effects on one- and two-dimensional domains.

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Collective fate decisions and cell rearrangements underlie gastruloid symmetry breaking

David Oriola^{1,2}, Gabriel Torregrosa-Cortés^{1,2,3}, Sotiris Samatas^{1,2}, Krisztina Arató¹, David Fernández-Munuera¹, Elisa Maria Hahn¹, Kerim Anlas¹, Jordi Garcia-Ojalvo^{2,3}, Vikas Trivedi^{1,2,4}

¹EMBL Barcelona, Barcelona Biomedical Research Park, 08003, Barcelona, Spain

²Barcelona Collaboratorium for Modelling and Predictive Biology, 08005 Barcelona, Spain

³Department of Medicine and Life Sciences, Universitat Pompeu Fabra, Barcelona Biomedical Research Park, 08003 Barcelona, Spain

⁴EMBL Heidelberg, Developmental Biology Unit, 69117 Heidelberg, Germany

In the embryo, morphogenetic signals guide regional patterning during body axis formation. Remarkably, pluripotent stem cell aggregates can self-organise and break symmetry *in vitro* without external cues. Gastruloids, three-dimensional stem cell structures, form an anterior-posterior axis via polarised Brachyury/T expression. How cell fate transitions integrate with collective behaviours to generate embryo-like structures remains unclear. By forming gastruloids with varying initial T populations, we show that fate decisions occur collectively: the pluripotent population inhibits differentiation, critically regulating the timing of symmetry breaking. Combining fusion and nanoindentation experiments, we reveal differences in surface tension between T+ and T- tissues, in concordance with radial cell sorting. Finally, integrating cell fate dynamics and mechanics into a computational model recapitulates the sequential steps of gastruloid formation. Our study uncovers a mechanochemical basis for symmetry breaking in gastruloids and provides insights into how multicellular systems self-organise in the absence of external cues.

Building Mechanically Accessible Models of Early Human Development

Özge Özgüç¹, Thomas Wilson¹, Xavier Trepat^{1,2,3}

¹ Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute for Science and Technology (BIST), Barcelona, Spain.

² Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Barcelona, Spain

³ Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

Despite its scientific and clinical significance, the dynamics of Human post-implantation development remain poorly understood due to embryo inaccessibility and the scarcity of biological material. Various stem cell-based models of early stages have been developed, recreating aspects of human embryo development in vitro in a controllable and quantitative manner. However, they often lack the ability to measure and manipulate mechanical forces driving development. To address this, we focus on building bottom-up, mechanically accessible models of human amniotic sac development to study tissue mechanics and mechanotransduction in shaping early human development.

The first model is a microfluidic human amniotic sac system where we control the shape and lumen pressure to measure and regulate the mechanics of the amniotic cavity during formation, development, and homeostasis. This system provides a quantitative measure of tissue mechanics and allows us to explore how mechanical forces modulate key fate differentiations, such as the specification of amniotic versus epiblast lineages and the formation of the primitive streak via epithelial-to-mesenchymal transition, within pathways known to be mechanosensitive. By applying isotropic or anisotropic pressure, we aim to determine whether mechanical cues can drive these fate decisions and spatially define where they occur.

The second model investigates the intrinsic mechanics underlying the onset of gastrulation, symmetry breaking, and primitive streak movement. By culturing lumenoids attached to a soft substrate, we obtain a hemispherical structure mimicking the amniotic sac in vivo. Using this model, we measure the forces exerted by cells during morphogenesis through 3D traction force microscopy, tracking bead displacement caused by cellular pushing and pulling. Spatiotemporal quantification of such mechanical forces enables us to explore changes in the tissue mechanics guiding primitive streak initiation and movement.

Together, these models provide a comprehensive framework to investigate how intrinsic mechanical forces, including lumen pressure and traction forces, shape early human development.

Self-organization of tumor heterogeneity and plasticity

Carlos Pérez-González^{1*}, David B. Brückner^{2,3,4*}, Mickael Di-Luoffo⁵, Sophie Richon¹, Ruchi Goswami⁶, Meryem Baghdadi¹, Florence Piastra-Facon¹, Neta Felsenthal¹, Réda Bouras¹, Arianna Fumagalli⁷, Mirjam van der Net⁸, Martijn Gloerich⁸, Salvatore Girardo⁶, Jochen Guck⁶, Jacco van Rheenen⁷, Julie Guillermet-Guibert⁵, Edouard Hannezo⁴, Danijela Matic Vignjevic^{1,9}

Affiliations:

1. *Institut Curie, PSL Research University, CNRS UMR 144; F-75005, Paris, France*
2. *Biozentrum, University of Basel; 4001, Basel, Switzerland*
3. *Department of Physics, University of Basel; 4001, Basel, Switzerland*
4. *Institute of Science and Technology Austria, 3400, Klosterneuburg, Austria*
5. *CRCT, Université de Toulouse, Inserm, CNRS, Centre de Recherches en Cancérologie de Toulouse; 31100, Toulouse, France ; Labex Toucan, Toulouse.*
6. *Max Planck Institute for the Science of Light & Max-Planck-Zentrum für Physik und Medizin; 91058, Erlangen, Germany.*
7. *Department of Molecular Pathology, Oncode Institute, Netherlands Cancer Institute; 1066 CX, Amsterdam, the Netherlands.*
8. *Center for Molecular Medicine, University Medical Center Utrecht and Utrecht University; 3584 CX, Utrecht, the Netherlands.*
9. *Equipe Labellisee LIGUE Contre le Cancer*

* These authors contributed equally to this work.

Abstract

Phenotypic heterogeneity and plasticity drive tumor growth, metastasis, therapy resistance, and relapse. This heterogeneity is mainly interpreted as a response to external signals from the microenvironment. However, here we show that cancer cells also follow intrinsic self-organized programs that are sufficient to coordinate the spatiotemporal patterning of tumor cell states. By combining quantitative measurements in tumors and organoids with theoretical modeling, we reveal emergent mechanical gradients that orchestrate cell state transitions during colorectal tumor growth. Compression at the tumor center induces a transition from a fetal-like state into a cancer stem cell (CSC) state. The CSC compartment exhibits a characteristic size determined by tumor rheological properties. Once this size is surpassed, a translationally arrested apoptotic core emerges, triggering a shift from homogeneous proliferation to a hierarchical cell turnover. These findings uncover stereotyped programs of self-organization that likely cooperate with the microenvironment to shape tumor heterogeneity and plasticity.

Immune and Mutational Profiling of Low-Immunogenic Human Primary Cholangiocyte Organoids for Bile Duct Disorders

Petrus-Reurer, Sandra¹; Baez-Ortega, Adrian²; Martincorena, Iñigo²; Saeb-Parsy, Kourosh¹

¹Department of Surgery, University of Cambridge and NIHR Cambridge Biomedical Research Centre, Cambridge, United Kingdom

²Wellcome Trust Sanger Institute, Cambridge, United Kingdom

Background: Beyond complex surgery or transplantation, there are no current curative therapies for bile duct diseases/cholangiopathies affecting the intra- or extrahepatic biliary tree. We have previously shown that human bile duct epithelial cells can be cultured as 3D organoids to generate mature human primary cholangiocyte organoids (PCOs) for the treatment of cholangiopathies. Since the generation of autologous PCOs is likely to remain logistically and economically prohibitive for the foreseeable future, immune rejection of allogeneic PCOs remains a key outstanding barrier to their clinical translation. We thus aimed to develop and characterize the immune and mutational profiles of engineered low-immunogenic cholangiocyte organoids for regenerative medicine applications.

Methods: Human leukocyte antigen (HLA) I and II double knock out (DKO)-edited PCOs (ePCOs) were generated using CRISPR-Cas9 and sorting of double-negative cells. Assessment comparing to parental wild-type cells was carried out by flow cytometry, functional readouts, co-culture with human peripheral blood mononuclear cells (PBMC) *in vitro*; and by engraftment under kidney capsule of immunodeficient mice subsequently humanized and further analyzed using spatial transcriptomics. Mutational load and CRISPR-driven off-target genetic mutations of parental vs ePCOs was quantified using whole genome sequencing and Nanoseq techniques.

Results: The HLA I and II DKO ePCOs generated maintained a mature PCO phenotype demonstrated by flow cytometry and functional analyses. Off-target analysis and mutation burden of parental vs ePCOs did not show CRISPR-driven off-target sites nor excess mutation in ePCOs. Importantly, our mutational results revealed that passaging in culture is a much more substantial source of mutations than CRISPR-Cas9 edits, but without evident selection for cancer-driver mutations. Immune characterization *in vitro* by co-culture with PBMC experiments showed that ePCOs have reduced PBMC cell activation and a donor-dependent NK cell cytotoxicity. In *in vivo* studies with humanized mice, ePCOs showed better preserved graft survival and a significantly reduced local immune infiltration compared to parental unedited controls, mainly due to evasion of T cell mediated cytotoxic responses and downregulated cell graft stress and extrinsic apoptotic pathways.

Conclusions: Human PCOs lacking HLA I and HLA II can be efficiently generated using a CRISPR-Cas9 approach without CRISPR-driven off-target effects. Additionally, ePCOs retain the phenotypic characteristics of mature PCOs and show reduced immunogenicity when co-cultured with PBMC and in humanized mouse models compared to parental cells. These high-resolution analyses and findings have important implications for the assessment of safety and immunogenicity of future organoid cellular therapies aiming for clinical translation.

Electric field intensity modulates keratocyte migration without altering turning dynamics

Niloofer Pishkari, Eloina Corradi, Gawoon Shim, Daniel Cohen , Marine Luciano, Sylvain Gabriele, Giovanni Cappello, Thomas Boudou , Martial Balland

Cell migration is a cornerstone of biological systems, enabling organisms to adapt to environmental stimuli and maintain homeostasis. Disruptions in this process can lead to functional impairment or system failure. In many cases, cells do not move randomly; instead, they migrate directionally in response to external cues, allowing them to perform essential biological functions. This directed movement is especially important in processes such as morphogenesis, cancer invasion, and wound healing. To unravel the complexities of directional cell migration, investigating natural guiding stimuli is crucial. Among these, electrical fields stand out as precise and physiologically relevant stimulus. Using a platform designed to apply programmable electric fields, the SCHEEPDOG device; we applied controlled electric field of varying intensities to keratocytes and quantitatively analyzed their migratory behavior. Our findings reveal that electric field stimulation not only induces robust directional migration but also enhances migration speed in an intensity-dependent manner. Additionally, cells initially moving in random directions gradually align with the field vector, with higher intensities accelerating the alignment. Intriguingly, while both speed and alignment time can be modulated through stimulation, the overall shape of migration trajectories remains unchanged. In other terms, for cells initially moving to the opposite direction of the field, the alignment is accompanied by making a turn and the size and shape of this turn is not affected by the magnitude of the electrical stimulation. Together, these results demonstrate that electrical stimulation can tune the speed and directional alignment of keratocyte migration without altering turning dynamics. These findings contribute to a deeper understanding of electrotaxis and offers new insights into how biophysical cues regulate cell migration in both physiological and pathological contexts.

Poster communication for 4th ed. EMBL – IBEC conference: *Engineering Multicellular Systems*

Title: Heterotypic coalescence of biological viscoelastic drops

Authors: Martí Planasdemunt-Hospital*, Elisa Maria Hahn, Vikas Trivedi and David Oriola

Abstract: Collections of interacting biological units can self-organise into drops, constituting a form of entangled active matter. Biological drops are found across scales, from micron-sized biomolecular condensates or cellular aggregates to centimetre-sized ant colonies [1]. Entanglement allows these biological drops to flow like a fluid and spring back like an elastic solid. In the context of cellular aggregates, for instance, these structures self-assemble through the action of transmembrane proteins such as cadherins, while the extracellular matrix confers them a certain solidity that may not be negligible. Despite multiphase droplet architecture is well understood for the case of liquid-like droplets, little is known about the role of elastic effects on their final configuration. Here, we extend the work in Ref. [2] to study the heterotypic fusion of two different viscoelastic drops by considering an interfacial surface tension between them. Inspired by the energy minimisation approach in Ref. [3] for heterotypic cell doublets, we derive the contact angle dynamics between the different interfaces of two viscoelastic drops through the minimisation of a Rayleighian function. We find that elasticity prevents drop engulfment in a size-dependent manner, revealing bistable behaviour. Finally, we fit our model to experimental data of heterotypic fusion of tissue spheroids and we infer the interfacial tension. We envision drop coalescence as a high-throughput method to characterise the mechanics of soft biological materials.

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*Author for correspondence: marti.planasdemunt@upc.edu

Multi-cellular rosette formation guides epithelial tissue assembly in pancreatic ductal adenocarcinoma cell organoids

Marion K. Raich^{1,2,3}, Fridtjof Brauns^{4,5,6}, Tamara Müller^{1,2,3}, Maximilian Reichert^{2,3,7,8,9}, Andreas R. Bausch^{1,2,3}

¹ Heinz Nixdorf Chair in Biophysical Engineering of Living Matter, Department of Bioscience, Technical University of Munich, 85748 Garching b. München, Germany.

² Center for Functional Protein Assemblies (CPA), Technical University of Munich, 85748 Garching b. München, Germany.

³ Center for Organoid Systems (COS), Technical University of Munich, 85748 Garching b. München, Germany.

⁴ Max Planck Institute for the Physics of Complex Systems, Nöthnitzer Straße 38, 01187 Dresden, Germany.

⁵ Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstraße 108, 01307 Dresden, Germany.

⁶ Center for Systems Biology Dresden, Pfotenhauerstraße 108, 01307 Dresden, Germany.

⁷ Klinik und Poliklinik für Innere Medizin II, Klinikum rechts der Isar der TUM, 81675 Munich, Germany.

⁸ German Cancer Consortium (DKTK), Partner site Munich, 81675 Munich, Germany.

⁹ Translational Pancreatic Cancer Research Center, Medical Clinic and Polyclinic II, Klinikum rechts der Isar der TUM, 81675 Munich, Germany.

Epithelial tissues – ordered and polarized cellular structures – are essential for organ development, homeostasis, and barrier function. A key mechanism underlying epithelial formation is the mesenchymal-to-epithelial transition (MET), in which simple cell sheets and mesenchymal cell clusters assemble into organized layers. Recent studies have identified multi-cellular rosettes as polarized epithelial intermediates during MET. While the molecular components contributing to rosette assembly have been characterized, the underlying physical mechanisms remain unaddressed due to the lack of suitable model systems.

Here, we use murine pancreatic ductal adenocarcinoma (PDAC) cells embedded in collagen I, which self-organize into highly branched, dynamic three-dimensional organoids. During their development, the organoids undergo MET, progressing from invasive, mesenchymal morphologies to a polarized columnar epithelium, accompanied by branch thickening, micro-lumen nucleation, and fusion into a continuous lumen. We find that this transition is associated with the formation of rosettes.

Quantitative analysis reveals that fluctuations in acto-myosin contractility on the developing apical side of high-cell-density branches generate a tug-of-war mechanism, resulting in a regular spacing of rosettes. This spacing scales with the branch diameter and is captured by a minimal theoretical model based on apical constriction, combining active stresses with mechanical yielding at large strains. Rosette resolution ultimately leads to lumen formation through apoptosis, leading to an epithelial layer that lines the cavity.

In summary, by using branched organoid systems, we demonstrate that the rosette architecture is generated through the geometrical confinement and acto-myosin-driven contractility, resulting in the epithelial structure required for lumen formation. This underscores the critical role of mechanical forces in the self-organized assembly of epithelial tissues.

Chemokine driven cell migratory behaviour of breast cancer on a 3D Tumour-on-chip platform using custom developed migration tracker

Keerthy Reghunandan^{a, c}, Jyothsna Vasudevan^b, Javier. G. Fernandez^{c, *}

^a Engineering Product Development (EPD) Pillar, Singapore University of Technology and Design, 8 Somapah Road, Singapore 487372

^b Mechanobiology Institute (MBI), National University of Singapore, 5A Engineering Drive 1, Singapore 117411, Singapore

^c Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain

ABSTRACT

Cell migration guided by chemical gradients (chemotaxis) is a fundamental process from embryonic development immune responses, and cancer metastasis. In cancer, different tumour types exhibit organ-specific patterns of metastasis, suggesting that dissemination is not random, but a pre-programmed event influenced by biochemical cues within the tumour microenvironment. The transition of tumour cells toward a metastatic phenotype is tightly regulated by chemokine gradients and their interactions with surrounding stromal and immune cells. Therefore, elucidating the chemotactic pathways that drive preferential cancer cell migration is essential for identifying therapeutic strategies that interrupt early metastatic events. By simulating this interaction in tumour-on-chip systems, medical professionals can learn about other approaches to treatment that can block the chemical sources that cause cancer invasion.

Here, we propose an integrated approach combining a flow-free, 3D micro-physiological chemotaxis platform with a custom developed cell migration tracker capturing migration behaviour of breast cancer cells under different controlled chemokine stimuli. The microfabricated device generates stable, long-lived chemokine gradients within a 3D ECM-like matrix without inducing shear stress, enabling physiologically relevant, long-term, high-resolution imaging of cell dynamics. Studying cell migration in 3D microenvironments requires not only physiologically relevant models but also powerful analytical tools to handle the large volumes of time-resolved imaging data generated during migration assays. To address the challenges posed by large, time-resolved imaging datasets, we developed a computer vision-based analysis workflow incorporating deep-learning segmentation and robust tracking algorithms. This automated pipeline extracts quantitative migration metrics with reduced user bias and improved reproducibility compared to conventional manual or semi-manual methods. Overall, the study aims to improve our ability to predict metastatic potential and evaluate anti-metastatic treatments by observing not just where and how fast individual cells move, but *how* they move together.

Optogenetic gene expression control in *Lactococcus lactis*.

Lactococcus lactis is a gram-positive bacterium widely used in biotechnological and industrial applications due to its suitability to produce high-value chemicals and recombinant proteins. Multiple chemically inducible gene and protein expression systems have been developed. Here we present a novel inducible system that relies solely blue light, using a combination of small engineered Vivid photoreceptors from *Neurospora crassa* (Magnets or eMags) and a split mutant T7 RNA polymerase.

Our findings demonstrate that the RNA polymerase is active and non-cytotoxic in *Lactococcus lactis*, and when fused to the Magnets/eMags, it drives gene expression in a light intensity- and time-dependent manner. This marks the first time that a split T7 RNA polymerase fused to photoreceptors has been described in a gram-positive bacterium. This system provides an attractive alternative to chemically inducible systems since it can be activated by physical stimulus alone, thus avoiding the need to add inducers into the medium and adding a reversibility dimension, enabling the stopping of the expression without the need to remove the inducer from the medium. Furthermore, this system can be applied in various biotechnological applications where precise spatiotemporal control of gene expression is required, especially in the context of living biomaterials where chemical stimuli are diffusion-controlled, difficult to switch off and thus cannot be applied locally in a reversible way, a role where light excels. We foresee potential applications in tissue engineering and industrial protein production.

Magnetic torque-based stretching platform for 3D human functional engineered skeletal muscle tissues

Carolina Rodríguez-Gallo, Maria Sabater Arcís, Xiomara Fernandez-Garibay, Juanma Fernandez Costa, Javier Ramon Azcon

IBEC, Barcelona, Barcelona, Spain

Abstract

Three-dimensional (3D) tissue bioengineering has recently emerged as a powerful approach for developing more physiologically relevant biological models. The ability to manipulate the cellular microenvironment has already been shown to promote tissue morphogenesis, cell differentiation, and functionality [1]. The most widely used 3D model of human skeletal muscle tissue consists of human myoblasts encapsulated in a biomaterial that compacts around two flexible, biocompatible pillars. Contraction of the matrix drives cell alignment along the axis connecting the two pillars, leading to the formation of elongated, multinucleated myofibers. This 3D architecture enables the quantification of muscle-generated force by measuring the displacement of the flexible pillars following electrical stimulation. Such systems have successfully recapitulated protein expression profiles associated with myogenic differentiation and sarcomere organization, modeled pathophysiological processes in neuromuscular diseases [2], and identified potential therapeutic targets.

Despite these advances, several potential functionalities of the system remain unexplored. For example, environmental stiffness is typically fixed, and tissues are limited to electrical stimulation.

In this project, we developed a new platform that enables dynamic modulation of environmental stiffness and the induction of both stretching and contraction in microtissues. The effects of these two mechanical stimuli on muscle tissue have not yet been investigated in the literature. Our approach embeds ferromagnetic nanoparticles within the flexible pillars supporting the tissue. By aligning the magnetic moments of these particles, we can induce pillar bending through magnetic torque in response to an external magnetic field [3]. Moreover, by varying the field strength, we can finely control the applied force and thereby define distinct stretching regimes. This tunable system allows us to study how different mechanical conditions influence muscle differentiation, gene expression, and fiber-type formation.

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Localised Electrical Stimulation of Engineered Skeletal Muscle Constructs Using rGO Microelectrodes

Marina Rovira¹, Florencia Lezcano¹, Miquel Madrid², Oriol Jutglar¹, Inass Himmiche¹, Elena Del Corro², Samuel Sánchez^{1,3}

¹ Institute for Bioengineering of Catalonia (IBEC), ² Catalan Institute of Nanoscience and Nanotechnology (ICN2) and Barcelona Institute of Science and Technology (BIST), ³ ICREA

Tissue-engineered skeletal muscle models have been successfully developed *in vitro* to actuate biohybrid machines. Electrical stimulation of these actuators is applied to induce controlled movement. Usually, this is achieved by using conventional electrodes directly immersed in the culture medium, stimulating the whole tissue at once. Effective and selective actuation of these tissues is fundamental to the correct functionality of the biohybrid machines [1]. However, controlled localized stimulation of specific tissue areas has not been reported in the literature. The aim of this study is to locally stimulate these skeletal muscle actuators with reduced graphene oxide (rGO) based microelectrodes. To assess the biocompatibility of the microelectrodes, C2C12-based skeletal muscle rings were fabricated by the mold casting method and were incubated in contact with microelectrodes, with a live-dead staining being performed at differentiation day 0, 7 and 14. For stimulation purposes, skeletal muscle rings were fabricated and matured for 14 days. The rings were then electrically stimulated using rGO microelectrodes for 5, 15, 30 and 60 minutes. Contractile force was assessed by measuring the bending of cylindrical PDMS pillars [2]. An immunostaining of the rings was performed to evaluate any change in the fiber alignment and integrity, including Filamin C staining to quantify the mechanical stress-induced damage. rGO microelectrodes showed exceptional biocompatibility during myotube maturation, with the portion of the microelectrode in contact with the tissue being covered by newly formed tissue. Localised contraction was successfully induced using rGO microelectrodes; with the contraction force increasing gradually throughout the whole stimulation period. After 60 minutes of stimulation, myotube alignment was preserved, although signs of structural disruption were observed. These findings demonstrate that rGO microelectrodes can be effectively used to induce controlled stimulation in engineered muscle constructs, offering new opportunities for the precise control of bioactuator systems.

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Building Humanized Neurovascular Models of the Spinal Cord for Injury and Repair

Francisco M. Sáez¹, Marta Cuenca^{2,3}, Eloi Montañez^{3,4}, J. Alberto Ortega^{2,3}, Zaida Álvarez^{1,5,6}

1. Biomaterials for Neural Regeneration Group, Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain

2. Department of Pathology and Experimental Therapeutics, Institute of Neurosciences, University of Barcelona, L'Hospitalet del Llobregat, Barcelona, Spain

3. Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet del Llobregat, Spain

4. Department of Physiological Sciences, Faculty of Medicine and Health Sciences, University of Barcelona, L'Hospitalet del Llobregat, Spain

5. CIBER en Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, Spain

6. Center for Regenerative Nanomedicine (CRN), Northwestern University, 303 E. Superior St, 60611, Chicago, USA

Human organoid technologies offer unprecedented opportunities to model human development and disease, yet their translational relevance is often limited by the absence of key *in vivo*-like features [1], particularly functional neurovascular integration. While brain organoid studies have shown that exposure to a vascularized environment can profoundly enhance tissue maturation and functionality [2, 3], comparable humanized models for the spinal cord remain scarce, despite the critical role of neurovascular coupling in spinal cord physiology and injury.

Here, we establish humanized neurovascular models of the spinal cord by integrating neural and vascular organoids derived from human induced pluripotent stem cells (hiPSCs). Human spinal cord organoids (hSCOs) and vascular organoids (hVOs) were independently generated and fused at developmentally defined stages to promote coordinated neurovascular assembly, resulting in vascularized spinal cord organoids (hVSCOs) that serve as a physiologically relevant *in vitro* platform. *In vitro*, hVSCOs developed robust CD31⁺/VE-cadherin⁺ vascular networks, infiltration of Iba1⁺ microglia, and a complex neurovascular architecture resembling native human spinal cord tissue. When subjected to controlled contusive injury using an Infinite Horizon impactor, hVSCOs recapitulated hallmark features of spinal cord injury, including astrocyte-mediated glial scar formation, increased caspase-3-associated apoptosis, activation and proliferation of Ki67⁺ progenitor populations, disruption of vascular structures, and a pronounced reduction in electrophysiological activity measured by multielectrode array recordings.

In vivo, transplantation of GFP-labeled hSCOs into the spinal cord of immunodeficient juvenile NOD-SCID mice resulted in consistent graft survival and integration without macroscopic damage to host tissue or detectable motor impairment. Transplanted organoids exhibited vascular infiltration from the host and maintained neural identity within the spinal cord environment, supporting the physiological compatibility and stability of the humanized tissue *in vivo*.

This dual *in vitro*-*in vivo* platform enables the study of human-specific neurovascular interactions, injury mechanisms, and the preclinical evaluation of regenerative therapies with enhanced physiological fidelity.

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Agent-based modelling of 3D gastruloid symmetry breaking

Sotiris Samatas^{1,2}, Gabriel Torregrosa-Cortés^{1,2,3}, James Sharpe^{1,2,4}, Jordi Garcia-Ojalvo^{2,3}, Vikas Trivedi^{1,2,5} & David Oriola^{2,6}

¹EMBL Barcelona, Barcelona Biomedical Research Park, 08003, Barcelona, Spain

²Barcelona Collaboratorium for Modelling and Predictive Biology, 08005 Barcelona, Spain

³Department of Medicine and Life Sciences, Universitat Pompeu Fabra, Barcelona Biomedical Research Park, 08003 Barcelona, Spain

⁴Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain

⁵EMBL Heidelberg, Developmental Biology Unit, 69117 Heidelberg, Germany

⁶Polytechnic University of Catalonia - BarcelonaTech (UPC), Physics Department, 08034 Barcelona, Spain

Gastruloids are three-dimensional stem cell aggregates that spontaneously break symmetry, recapitulating key aspects of anterior–posterior axis formation. Symmetry breaking is commonly marked by the polarised expression of the mesodermal marker Brachyury/T at the posterior pole. Although experimental studies indicate that both cell fate transitions and collective cell rearrangements are essential for this process, how these mechanisms integrate to drive robust polarisation remains unclear. Here, we present a three-dimensional agent-based model that explicitly couples cell fate dynamics with tissue mechanics to investigate symmetry breaking in gastruloids. The model incorporates cell proliferation, differentiation, and mechanical interactions mediated by cell–cell adhesion and contractile protrusions. Guided by experimental observations, we implement differential adhesion between Brachyury/T-positive (T+) and Brachyury/T-negative (T–) cell populations. Simulations reproduce key experimental features, including an initial transient radial organisation with a T+ core and a T- outer layer, followed by robust polarisation. Moreover, the model captures experimentally observed dependencies of symmetry-breaking timing on both aggregate size and the initial fraction of T+ cells. We envision our agent-based model as a powerful tool to explore the self-organising potential of stem cell systems.

Self-organizing Murine Cardiac Organoids Towards Heart-on-chip and Modeling of Congenital Defects

Sébastien SART

Physical Microfluidics and Bioengineering Institut Pasteur

Objectives

Congenital heart diseases (CHDs) are among the most common birth defects. The limited ability to study cardiac organogenesis *in utero* or maintain embryos *ex vivo* underscores the need for advanced *in vitro* models that enable quantitative analysis of normal and pathological heart development.

Methods

We developed a morphogen-free protocol to differentiate murine embryonic stem cells (ESCs) into 3D, functional, and spatially organized cardiac organoids (cardioids). Cardiac development was tracked over time via transcriptional profiling. Cellular heterogeneity, tissue structure, and spontaneous beating were assessed using 3D quantitative imaging.

To model CHDs, *Greb1l*^{+/tm1a} ESCs were used for crisscross heart malformations, and Nodal inhibition was applied to simulate heterotaxy. A perfused droplet-based microfluidic platform was also developed for on-chip cardioid generation to enable scalable and reproducible production.

Results

Transcriptomic analysis revealed sequential activation of first and second heart field markers, followed by expression of cardiomyocyte, epicardial, and endothelial genes - mirroring stages of cardiac organogenesis. Key signaling pathways (Wnt, Nodal, BMP, p38-MAPK) were self-activated in a time-resolved manner, directing lineage commitment toward myocardium-, endocardium-, and epicardium-like tissues. Imaging showed organized tissue structure, cardiac myofibrils, heart-like cavities, and rhythmic beating. Functional heterogeneity across cardioids, reflected by variable beating frequencies, was linked with morphology, calcium transients, and cardiac marker expression. To assess capacity of cardioids to model CHDs, *Greb1l*-deficient ESCs exhibited disrupted organoid formation, absence of beating, and impaired cardiac progenitor development, consistent with *in vivo* crisscross phenotypes. Similarly, Nodal inhibition post-progenitor emergence mimicked heterotaxy traits such as reduced differentiation, slower beating, and increased proliferation. Cardioid generation was successfully implemented in a droplet microfluidic platform, supporting high-throughput applications.

Conclusion

Murine cardioids recapitulate key features of normal and pathological heart development. Their integration with microfluidic systems provides a robust, scalable platform for combinatorial drug screening and mechanistic studies of CHDs.

Mechanosensitive regulation and prediction of vascular dysfunction in disease

Shailaja Seetharaman

Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore
Mechanobiology Institute, National University of Singapore

Abnormalities in blood vessel and blood flow properties are key drivers of severe cardio- and cerebro-vascular pathologies. The remarkable ability of the vasculature to sense and respond to mechanical and biochemical signals across multiple scales presents both a challenge for understanding disease progression and an opportunity for therapeutic intervention. Here, we investigate the mechanisms by which the endothelial tissue lining of the vasculature responds to blood flow using *in vivo* and *in vitro* models of healthy and atherosclerotic tissues. Using bulk RNA sequencing, partial carotid artery ligation, and microfluidics, we find that the transcription as well as protein expression of Four-and-a-half LIM protein 2 (FHL2) are enriched in endothelial cells experiencing atherosclerosis-like flow profiles. We further demonstrate that the FHL2 perturbs actin-microtubule cytoskeletal crosstalk, resulting in aberrant cell junction morphology, heightened contractility and tissue permeability in a force-dependent manner. These results uncover a novel mechano-chemical feedback loop important for driving vascular dysfunction in disease. Next, building on these insights, we have developed machine learning (ML) models for the prediction of tissue-scale endothelial morphology and function in health and disease. In summary, our work highlights an integrated approach which enables the engineering of physiological vascular tissue function, with the ultimate goal of targeting the vasculature in therapies.

MorphoChip: A Minimalistic *In Vitro* Assay to Study Cell Intercalation in Morphogenesis

Daniel Selma Herrador[†], Vladimir Misiak, Giovanni Cappello, Thomas Boudou, and Martial Balland^{*}

LIPhy, Université Grenoble Alpes, CNRS (UMR5588), F-38000 Grenoble, France

ABSTRACT

Cell intercalation, or T1 transition, is a central morphogenetic process in which cells exchange neighbors to shape tissues while preserving overall integrity. It involves active mechanisms, such as cell crawling, and passive actomyosin pulsations. Traditional *in vivo* studies (e.g., *Drosophila*, zebrafish embryos) complicate analysis of isolated events, whereas *in silico* models require experimental validation.

We introduce an *in vitro* assay using four-cell assemblies (cell quadruplets) that replicate the minimal tissue architecture for T1 transitions. This setup allows real-time imaging and force measurement at single-cell resolution. Micropatterned glass or Polyacrylamide (PAA) gels coated with Extracellular matrix (ECM) proteins define cell boundaries, and Madin-Darby canine kidney (MDCK) cells self-organize into quadruplets, enabling statistical analysis of morphological and mechanical parameters. Cell intercalation rate can be tuned by changing the pattern aspect ratio, substrate stiffness, and imaging height, with each condition generating a distinct double-well energy landscape consistent with cell-vertex models. These results provide a direct link between geometrical constraints, mechanical stress, and intercalation dynamics.

Ongoing experiments may extend the assay to discriminate epithelial versus mesenchymal intercalation modes in the framework of cancer research. Targeted perturbations in human carcinoma, such as FAT1-KO to induce a mesenchymal state, are expected to yield higher transition rates and phenotype-specific energy landscapes. Finally, a recent collaboration with Eric Theveneau's lab with quadruplets derived from *Xenopus* explants will explore the assay as a potential platform to study the epithelial-to-mesenchymal transition (EMT) in a developmental context too.

This assay highlights the critical role of geometry and mechanics in epithelial organization, offering a quantitative framework for tissue development studies and cancer research *in vitro*. It provides novel insights into the mechanochemical principles governing topological rearrangements across molecular and tissue scales.

Magneto-mechanical approaches to modulate Cellular and Multicellular Dynamics

Mechanical forces play essential roles across all levels of cellular organization: from single cells generating traction on their substrates to groups of cells exerting coordinated forces that shape developing tissues and organs. Yet, despite their importance, our understanding of how cells sense, interpret, generate, and exploit these forces remains incomplete. To address this gap, we have developed magnetic nanoparticle and microparticle-based approaches that enable the application of controlled forces on the cells in 2D and 3D context, with simultaneous long-term, super resolution time-lapse imaging. Using these tools, we are investigating the role of mechanical forces in single cell behavior, collective cell dynamics, and tissue-scale morphogenesis. This bottom-up approach will advance our understanding of mechanical force-driven biological processes and support the development of improved methods for engineering complex tissues and organs for regenerative medicine and disease modelling.

A Multi-Throughput Microfluidic Platform for Flow-Regulated Microvessels-on-Chip

Farid Seral, Joana Silva, Marta Cherubini, Roberto Paoli, Kristina Haase

EMBL Barcelona, Barcelona Spain

Shear stress is a key regulator of microvascular physiology, influencing vessel morphology, perfusion and barrier function. However, current microfluidic vascular models often lack precise and independent control over the applied pressure, limiting systematic investigation of flow-dependent vascular responses. Here, we present a multi-throughput microfluidic platform that enables accurate control of the differential pressure across multiple devices in parallel. The system integrates a valve layer actuated by pins of a braille display combined with pressure-controlled pumps, allowing fine tuning of flow regimes across 8 separate chips. To validate the system, FRAP was performed in hydrogels under pressure-driven flow – revealing an even diffusivity quantified along the gel length.

As a proof of concept for vessel formation, GFP-labeled HUVECs and lung fibroblasts were co-cultured under flow and static conditions for seven days. Interstitial flow was applied from day 4 to 7 via a differential pressure of 6 mbar, resulting in perfusable vascular networks, whereas static cultures formed non-perfusable networks. Barrier function was quantified through perfusion of FITC dextran, yielding permeability values on the order of 10^{-7} cm/s, consistent with our previous reports. Additionally, distinct morphological phenotypes were quantified using a deep learning analysis pipeline, revealing significant changes in key vascular features. The platform further supports alternative configurations, including media recirculation and inter-device crosstalk.

Overall, this system provides a robust and scalable tool for investigating how biomechanical cues regulate microvascular structure and function, with applications in vascular biology, hydrogel-embedded multicellular tissues, disease modeling, and drug screening.

Tissue crowding activates Cdx2 to maintain trophoblast stem cells during mouse blastocyst implantation

Giovanni Sestini^{1,2}, Barbara Diaz-Rohrer³, Takaki Yamamoto⁴, Paula Llanos³, Alexandr A. Kalinin³, Jinwoo Seong¹, Ronan Quenec'hdu¹, Jana Slováková¹, David Rozema⁵, Diana Pinheiro⁶, Jean-Leon Maître⁵, Edouard Hannezo⁷, Anne E. Carpenter³, Beth Cimini³, Kyogo Kawaguchi⁴, Shantanu Singh³, Nicolas Rivron¹.

Affiliations:

¹Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna BioCenter, 1030 Vienna, Austria.

²Vienna BioCenter PhD Program, Doctoral School of the University of Vienna, Medical University of Vienna, 1030 Vienna, Austria.

³Imaging Platform, Broad Institute of MIT and Harvard, Cambridge MA, USA

⁴Nonequilibrium Physics of Living Matter RIKEN Hakubi Research Team, RIKEN Center for Biosystems Dynamics Research, 2-2-3 Minatojima-minamimachi, Chuo-ku, Kobe 650-0047, Japan.

⁵Institut Curie, Université PSL, CNRS UMR3215, INSERM U934, Paris, France.

⁶Research Institute of Molecular Pathology (IMP), Vienna BioCenter (VBC), Vienna, 1030, Austria, ⁷Institute of Science and Technology Austria 3400 Klosterneuburg, Austria.

Abstract:

Stem cells are often maintained by niches that locally secrete signals to control potency. In mouse blastocysts, trophoblast stem cells (TSCs) are maintained by epiblast-derived inducers, particularly FGF4.

Here we show that the cells of the polar trophoblast, expressing the progenitor compartment markers Cdx2, Eomes and Esrrb, crowd and stiffen following the implantation of the blastocyst in the uterus, transitioning from a squamous to a columnar epithelium.

By recreating this crowding *in vitro*, we show that this process establishes a mechanical niche that temporarily prevails over FGF4 signaling and it is sufficient for the nuclear translocation of the protein Yap, which induces the progenitor compartment markers.

Thus, we propose that the blastocyst exploits the crowding of the polar trophoblast to generate a mechanical niche that supports the maintenance of the stem-cell gene regulatory network.

We conclude that spontaneous, intrinsic tissue compaction and stiffening serves as a self-organising principle to preserve stem cell identity.

Engineered morphogen gradients applied basally to human embryonic stem cells to control and dissect tissue patterning

Tom Wyatt, Mingfeng Qiu, Julie Stoufflet, Hassan Omais, Gabriel Thon, Sara Bonavia, Pascal Hersen, Vincent Hakim & Benoit Sorre

Morphogen gradients are used repeatedly during development to pattern embryonic tissues. Absolute concentration, duration or even temporal derivative of morphogen concentration have all been proposed to carry positional information depending on the context. However, establishing the causal relationship between the spatio-temporal profile of the gradient and the resulting cellular diversity and tissue patterning is difficult to address in live embryo because of lack of tools to take control of those variables. This is especially true during mammalian gastrulation, where the primitive streak is patterned by a complex, time evolving signalling landscape of the BMP, WNT and NODAL pathways

Here, using microfluidics devices able to apply well defined morphogen landscapes on the basal side of human embryonic stem cells colonies, thus mimicking how BMP4 is delivered to the pluripotent epiblast during mouse gastrulation, we show that in this configuration absolute concentration of BMP4 provides positional information and the cell identities emerging during differentiation can vary according to the shape of the gradient¹.

As morphogen gradients and signaling centers are ubiquitous during development, our toolbox provides powerful mean to dissect the logic of patterning at all developmental stages and to engineer tissue precisely.

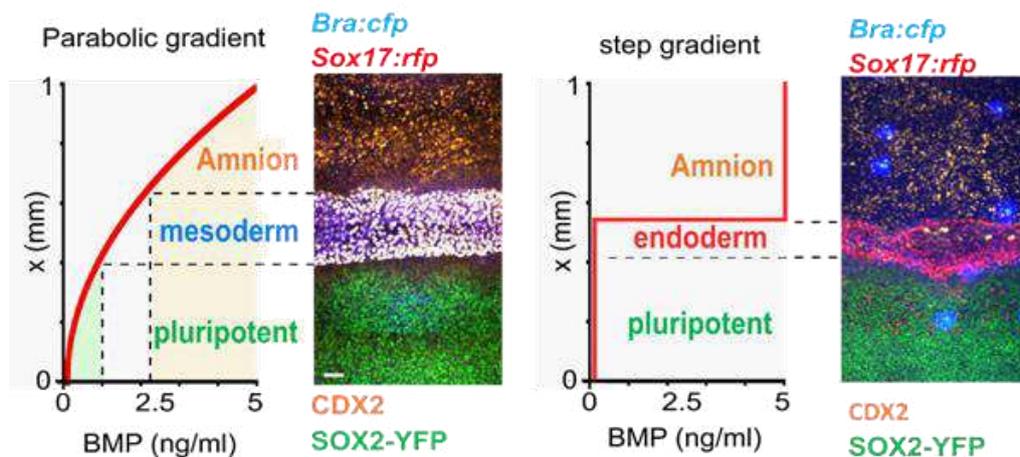


Figure 1: Different cell types are generated in human embryonic Stem Cells colonies depending on the steepness of the applied BMP gradient.

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Abstract

In non-equilibrium (active) systems, increased driving is commonly assumed to amplify energy dissipation. This frames the efficiency of protein-based machines as a fixed or monotonically decreasing function with driving. Using picowatt-sensitive calorimetry and advanced entropy production metrics in reconstituted actomyosin networks, we show that energy dissipation depends non-monotonically on myosin-generated stress (driving). At low driving, dissipation increases proportionally with stress, consistent with near-equilibrium linear response. At high driving, however, dissipation decreases, revealing a far-from-equilibrium regime in which excessive load suppresses motor ATPase activity. This non-monotonicity reflects a transition from spatially localized stress at low driving to delocalized stress at high driving, where force per motor, and thus ATPase suppression, is maximized. Crosslinker mechanics tune this transition as fascin (slip bonds) amplifies stress localization and shifts the dissipation peak to higher driving, whereas α -actinin (catch bonds) stabilizes under load, delocalizes stress, and shifts the peak to lower driving. Thus, enhanced mechanochemical coupling causes additional driving to restructure rather than amplify dissipation, revealing how material system organization (bonding), and not driving alone, governs energy flow far from equilibrium.

Tissue-engineered outer retina model to study the influence of extracellular matrix on retinal cell interactions

Vasudha Turuvekere Krishnamurthy^{1,2*}, Laura Klasen^{2,3}, Ramin Nasehi^{2,3}, Lisa Jungbluth¹, Eva Miriam Buhl⁵, Alice Podesta⁶, Si-Ting Wu¹, Björn Kampa⁶, Laura De Laporte^{2,3,4}, Jacopo Di Russo^{1,2}

1. Institute of Molecular and Cellular Anatomy, RWTH Aachen, Germany
2. DWI - Leibniz Institute for Interactive Materials, Aachen, Germany
3. Institute for Technical and Macromolecular Chemistry, RWTH Aachen, Germany
4. Department of Advanced Materials for Biomedicine (AMB), CBMS—Center for Biohybrid Medical Systems, AME—Institute of Applied Medical Engineering, RWTH Aachen, Germany
5. Institut für Pathologie & Medizinische Klinik II, Uniklinik RWTH Aachen, Germany
6. Department for Molecular and Systemic Neurophysiology, Institute for Biology II, RWTH Aachen University

Keywords: Retina, 3D model, extracellular matrix, stem cells

More than 20% of the human cortex is dedicated to vision, making vision arguably the most dominant sensory modality compared with touch (~10%) and hearing (~3%). Vision is mediated by the retina, a specialised neural tissue that converts light into neural signals. Despite its importance, most knowledge of retinal biology derives from animal models that differ substantially from humans in anatomy and ageing. While retinal organoids have opened new horizons for studying human retinal development and disease, current models lack a fully mature outer retina and fail to recapitulate the essential interactions between the extracellular matrix (ECM), the retinal pigment epithelium (RPE), and photoreceptor cells (PRs).

To address this gap, we developed a human-relevant co-culture system that recapitulates ECM-RPE-PR interactions and enables controlled perturbation of ECM biochemical and mechanical cues. Using a recently optimised platform (1), human induced pluripotent stem cells (hiPSCs) were differentiated into mature PRs within three months, significantly faster than conventional retinal organoid protocols, by assembling cells into a 3D microporous annealed particle (MAP) scaffold composed of spherical poly (ethylene glycol) microgels. The PR MAP scaffolds were co-cultured with stem cell-derived RPE on ECM-mimicking hydrogels building on previous work (2).

PRs formed light-responsive outer segments displaying the “eat-me” signal, phosphatidylserine, confirmed by pSIVA staining. When co-cultured with hiPSC-derived RPE on tunable hydrogels, PR outer segment fragments were efficiently phagocytosed and localised to endosomes. Phagocytosis was reduced by MERTK receptor inhibition, confirming specificity. Following dark-to-light transition, phagocytosis increased, consistent with *in vivo* behaviour. Finally, increasing substrate stiffness reduced phagocytic efficiency, confirming earlier evidence obtained with porcine outer segment fragments (2).

Collectively, this human-based *in vitro* model enables mechanistic studies of how ECM changes associated with ageing and disease regulate RPE-photoreceptor interactions, providing a new platform for investigating outer retinal mechanobiology.

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Macrophage-mesenchymal stem cell interactions in viscoelastic microenvironments

Mireia Valero Puigdomenech¹, Mariana A. G. Oliva¹, Manuel Salmeron-Sanchez^{1,2,3}

¹Institute for Bioengineering Catalonia (IBEC), Barcelona Institute of Technology (BIST), Spain

²University of Glasgow, United Kingdom

³Institució Catalana de Recerca i Estudis Avançats (ICREA), Spain
mvalero@ibeccbarcelona.eu

The extracellular matrix (ECM) is a dynamic 3D environment that displays not only elastic, but viscoelastic behaviour to mechanical loads, such as creep and stress relaxation [1], which are critical to tissue repair and regeneration. Macrophages play a central role in these processes through phagocytosis, cytokine secretion and coordination of tissue remodelling [2]. Their phenotype shifts across a continuum of activation states in response to microenvironmental cues, from pro-inflammatory (M1-like) to pro-regenerative (M2-like) profiles [3]. Human mesenchymal stem cells (hMSCs), which have immunomodulatory effects, are also regulated by macrophage phenotype [4]. While substrate elasticity has been shown to regulate macrophages behaviour, how matrix viscoelasticity affects macrophage polarization and immune-stem-cell interactions remains poorly understood [5]. Our aim is to investigate how local matrix mechanics, especially viscoelasticity, influences macrophage polarization and their crosstalk with MSCs spheroids in engineered multicellular microenvironments.

We engineer polyethylene glycol-maleimide hydrogels via Michael reaction and functionalize them with full-length fibronectin to enhance cell-matrix interactions [6]. By tuning crosslinking density and macromer-crosslinker molecular weight, we generate families of soft and stiff hydrogels with either elastic or viscoelastic behaviour. Bulk mechanical properties are quantified via nanoindentation. Human macrophages and hMSC spheroids are encapsulated within these matrices, and their morphology and organization are assessed using confocal microscopy. Brillouin spectroscopy enables non-invasive, real-time characterization of microscale mechanical properties within the cell-laden hydrogels.

This strategy allows a mechanistic understanding of how ECM viscoelasticity influences multicellular immune-stem cell interactions, offering insights into regenerative processes and guiding the design of physiologically relevant biomaterials.

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Experimental model of the mechanobiology of the immunocompetent tumor ecosystem

Abstract

The progression of a tumor and its response to therapy depend on the evolution of a complex tumor microenvironment (TME) that includes cancer cells, cancer-associated fibroblasts (CAFs), endothelial cells and immune cells, among others. In many types of solid tumors, the TME prevents immune cells from eliminating the disease by inhibiting their capacity to migrate into the tumor, often leading to immunotherapy failure. Growing evidence indicates that CAFs are major contributors to immune exclusion, as they envelop the tumor and exert active compression on cancer cells. This physical barrier, together with the secreted signaling proteins and ECM components create a barrier to immune infiltration. To understand how mechanochemical interactions drive tumor progression and influence treatment efficacy, there is a need to develop experimental models of the tumor ecosystem. Here we present TEOC (Tumor Ecosystem On Chip), a microphysiological system that enables control and measurement of the mechanobiology of the TME, focusing on immune exclusion. Our TEOC platform consists of two microchannels separated by a porous membrane that enables controlled compartmentalization of tumor, stromal, and immune components. CAFs and tumor organoids can be cultured either within the same channel to directly examine CAF-tumor interactions such as encapsulation, compression, and ECM remodeling; or on opposite sides to model a stromal barrier. Immune cells introduced into the upper channel can then be tracked as they migrate through the fibroblast layer and across the membrane toward the tumor, enabling quantitative analysis of immune infiltration. Additionally, this platform is compatible with surface micropatterning to control cell alignment. This will allow us to investigate by which mechanisms the spatial alignment of CAFs and the mechanobiological properties of the TME determine immune infiltration.

Janet van der Graaf-Mas 1, 2, Alice Perucca 1, 2, Yarden Spialter 1, Pau Guillamat 1, Eleni Dalaka 1, Eduard Batlle 3, 4, 5, Alexandre Calon6, Xavier Trepas 1, 4, 7, 8

1. Institute for Bioengineering of Catalonia (IBEC), 08028 Barcelona, Catalonia. 2. Facultat de Medicina, Universitat de Barcelona, 08028 Barcelona, Catalonia. 3. Institute for Research in Biomedicine (IRB), Barcelona,

Catalonia. 4. Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain 5. Centro de Investigación Biomédica en Red en Cáncer (CIBERONC), Barcelona, Catalonia. 6. Cancer Research Program,

Hospital del Mar Research Institute (IMIM), 08003 Barcelona, Catalonia. 7. Unitat de Biofísica i Bioenginyeria, Facultat de Medicina, Universitat de Barcelona, 08036 Barcelona, Catalonia 8. Center for Networked

Biomedical Research on Bioengineering, Biomaterials and Nanomedicine (CIBER20BBN), 08028 Barcelona, Catalonia

Title: Force transmission and mechano-transduction from cell-cell adhesions to the nucleus

Valeria Venturini, Laura Faure, Guillermo Martinez-Ara, Ona Baguer Colomer, Gotthold Fläschner, Xavier Trepap, Pere Roca-Cusachs

Abstract:

Cells sense and respond to mechanical signals from their environment via a process known as mechanotransduction. Among other mechanisms, single cells can sense the stiffness of their extracellular environment (ECM) via direct mechanical coupling of the nucleus through the LINC complex to the actin cortex, coupled via integrin-based adhesions, to the ECM. In stiff environments, the traction force induces nucleus flattening and nuclear envelope stretch that leads to nuclear pore opening. This further regulates nucleo-cytoplasmic transport of mechanosensitive molecules and transcription factors, thereby regulating transcription.

However, how these processes are controlled in cells that are connected to neighbors via cell-cell junctions is not known. To dissect this question, we design in vitro minimal systems to specifically address the role of eCadherin-based adhesions in nuclear force transmission and mechanotransduction, using human breast cancer epithelial cells as a model. Preliminary results show that eCadherin binding prevents cell spreading and consequent nucleus flattening by re-organizing the microtubules network, further controlling nucleo-cytoplasmic transport and downstream mechanosensitive pathways.

Spherical Skin Model: Stratified Co-Culture of Fibroblasts and Keratinocytes on Spherical Beads Toward Compound Screening

Elisa Lenzi¹, Nadia Vertti-Quintero^{2*#}, Julien Husson³, Anne-Laure Bulteau², Carine Nizard², Karl Pays², Sébastien Sart¹, Charles N. Baroud^{1,3*}

¹ Institut Pasteur, Université Paris Cité, Physical Microfluidics and Bioengineering Unit, 25-28 Rue du Dr. Roux, 75015 Paris, France

² LVMH Recherche, 185 avenue de Verdun, 45804 Saint Jean de Braye, France

³ Laboratoire d'Hydrodynamique (LadHyX), CNRS, Ecole Polytechnique, Institut Polytechnique de Paris, 91128 Palaiseau, France

* Corresponding authors

Presenting author

Advanced skin models are critical for pursuing non-animal approaches in drug and cosmetic testing. However, existing 3D models remain complex and time-consuming, which limits their adoption. Spherical skin model (SSM) is presented, a platform that balances biological fidelity with experimental robustness. The SSM is based on a core-shell structure where the dermal core is modeled by embedding human fibroblasts into collagen microcarriers (150 μm), while the epidermal shell is formed by outer layers of immortalized keratinocytes. The collagen beads are generated using droplet microfluidics to enable rapid and reproducible production. The biological relevance of SSM is revealed through elevated expression of epidermal differentiation markers (loricrin, involucrin, keratin 1, keratin 10) and the dermal-epidermal junction marker collagen VII. The barrier function is validated by permeability assays that show strong exclusion of fluorescent dextran above 4 kDa. Moreover, their usefulness for screening is shown by identifying a dose-dependent effect of vitamins in reducing oxidative stress and apoptosis against tert-butyl hydroperoxide. As such, this 3D microphysiological model recapitulates key structural, molecular, and functional features of human skin while offering rapid generation, scalability, and compatibility with high-throughput applications in dermatological and cosmetic research.

Engineering the auxin-inducible degron system for tunable in vivo control of organismal physiology

Jeremy Vicencio¹, Daisuke Chihara¹, Matthias Eder¹, Lucia Sedlackova¹, Julie Ahringer², Nicholas Stroustrup^{1,3}

¹ Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Dr. Aiguader 88, Barcelona 08003, Spain

² The Gurdon Institute and Department of Genetics, University of Cambridge, Cambridge, UK

³ Universitat Pompeu Fabra (UPF), Barcelona, Spain

ABSTRACT

The auxin-inducible degron (AID) is designed for the rapid and near-complete degradation of a specific target protein in vivo. However, to understand the dynamics of complex physiological networks, researchers often need methods that produce graded, quantitative changes in degradation rates for multiple proteins simultaneously. Here, we develop the AID system for in vivo, quantitative control over the abundance of multiple proteins simultaneously. First, by measuring and modeling the on- and off-target activities of different AID system variants in *Caenorhabditis elegans*, we characterize a variant of the TIR1 E3-ubiquitin ligase enzyme with improved degradation activity compared to the original AID and AID2 systems. Then, we develop a TIR1 expression construct that enables simultaneous pan-somatic and germline protein degradation. Finally, we expand the AID toolkit to allow independent, simultaneous degradation of two distinct tissue-specific proteins. Together, these technologies enable new in vivo approaches for studying quantitative cellular biology and organismal dynamics.

A versatile 3D bioprinting platform for engineering physiologically relevant and high throughput human blood-brain barrier models

Gal-la Vinyes-Bassols, Anna Vilche, Oscar Castaño, Anna Lagunas, Josep Samitier

The blood–brain barrier (BBB) is a highly selective interface preserving central nervous system (CNS) homeostasis by regulating molecular exchange and blocking toxins, pathogens, and inflammatory mediators. While essential for protection, the BBB’s restrictive permeability hampers therapeutic delivery for neurodegenerative diseases, highlighting the need for physiologically relevant in vitro models that replicate human neurovascular architecture and cellular dynamics.

We present a versatile three-dimensional (3D) bioprinting platform to generate physiologically relevant, high-throughput human BBB models. This platform employs a novel bioink optimized for rheological properties and biocompatibility, compatible with microvalve-based embedded 3D bioprinting—a technique not previously applied to BBB modeling. The bioink allows precise, low-shear deposition while maintaining excellent cell viability. Using this approach, 48 uniform 3D ring-shaped hydrogel scaffolds are fabricated in nine minutes, demonstrating scalability and reproducibility.

Human brain microvascular endothelial cells (BMECs) bioprinted within fibrin-rich constructs remain viable and proliferative over seven days, organizing into microvascular-like structures. The scaffolds sustain structural integrity and biological functionality, validating the material and printing parameters.

While this study focuses on the endothelial component, the platform’s modular and adaptable design offers future potential for integration with co-cultures of astrocytes and pericytes, as well as incorporation into microfluidic systems and brain organoids. These advancements could further enhance physiological relevance and translational capacity by combining the strengths of multiple contemporary BBB models.

This work represents the first successful application of microvalve-based embedded 3D bioprinting for BBB modeling, establishing a rapid, reproducible, and adaptable platform with broad utility for drug screening, disease modeling, and neuroengineering applications.

Reducing the scaffold cleft angle boosts microtissue growth and broadens the width of the unjammed growth front

Tassilo von Trotha^{1,*}, Konstantin M. P. Wolf^{1,*}, Roman Vetter^{2,3}, Dagmar Iber^{2,3}, Mario C. Benn^{1,#,*}, Viola Vogel^{1,#}

¹Laboratory of Applied Mechanobiology, Institute of Translational Medicine, Department of Health Sciences and Technology, ETH Zurich, Gloriastrasse 37/39, 8092 Zürich, Switzerland

²Department of Biosystems Science and Engineering, ETH Zürich, Schanzenstrasse 44, 4056 Basel, Switzerland

³Swiss Institute of Bioinformatics, Schanzenstrasse 44, 4056 Basel, Switzerland

*These authors contributed equally

#corresponding authors

Biochemical cues are widely used to accelerate tissue growth *in vitro*, but how geometric parameters of engineered scaffolds can be tuned to physically boost tissue growth remains poorly defined. We investigated how scaffold cleft angles, by modulating tissue tension, regulate 3D tissue morphogenesis. Microtissues grown from human dermal fibroblasts or pancreatic stellate cells were cultured in V-shaped clefts of 22.5°, 45°, and 90°. Smaller angles produced enhanced growth and increased curvature of the tissue-liquid interface (growth front). Computational modeling integrating cleft angles and relative surface tensions recapitulated both the curvature of the growth front and the enhanced growth rates in smaller angles. Immunostaining revealed a thin layer of α SMA-positive, proliferative myofibroblasts with nuclear YAP1 at the growth front, while smaller angles also induced nuclear YAP1 and α SMA deeper in the core, indicating tension across a broadened growth front. Live-cell tracking showed rapid, tangential migration in an unjammed growth front zone that widened with decreasing angles, in contrast to the largely immobile core cells. Occasionally, core cells escaped toward the open cleft which correlated with collagen fiber alignment along scaffold walls. These findings are physiologically relevant and offer practical applications in both regenerative medicine and the emerging field of lab-grown food.

Mechanical Contributions of Intestinal Cell Populations to Organoid Regeneration.

Meng Wang^a, Marija Matejčić^a, Xavier Trepata*

^a *Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology 8 (BIST), Barcelona, Spain.*

Regeneration of the intestinal epithelium following injury requires rapid resealing of the luminal barrier and subsequent restoration of tissue homeostasis. Despite its physiological importance, the mechanobiology underlying this process remains incompletely understood, and different cell populations—such as villus cells^[1] and transit-amplifying cells^[2]—have been proposed as key drivers of regeneration.

Here, we introduce a hydrogel-based 2D intestinal organoid model that enables direct, spatiotemporal monitoring of cell dynamics and mechanical forces in both crypt and villus regions following the induction of a localized gap within the villus domain. Using this system, we identify differentiated enterocytes as the primary cell population responsible for covering and resealing the injured area through active migration. Gap closure emerges as a strongly mechanically regulated process, characterized by elevated traction forces at the wound edge.

Following gap closure, migratory enterocytes upregulate regenerative markers and re-enter the cell cycle, thereby repopulating the wounded region and preventing mechanical failure of the epithelial monolayer under sustained contractile stress. Together, these results reveal a central mechanical role for differentiated enterocytes in intestinal wound healing and regeneration. More broadly, this experimental platform provides a powerful framework to dissect how cell-type-specific mechanical behaviors govern regeneration and homeostasis in highly dynamic epithelial tissues.

References

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Engineering epithelial cell shape and mechanics to create a new generation of biohybrid devices

Thomas Wilson ^{1,2}, Nimesh Chahare ^{1,3}, Özge Özguç ¹, Tom Golde ¹, and Xavier Trepat ^{1,2,4}

1. *Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute for Science and Technology (BIST), Barcelona, Spain.*
2. *Facultat de Medicina, Universitat de Barcelona, Barcelona, Spain.*
3. *LaCàN, Universitat Politècnica de Catalunya-BarcelonaTech, Barcelona, Spain.*
4. *Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.*
5. *Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina, Barcelona, Spain.*

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 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 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.

Building a Culture of Sustainable Science: Insights from IBEC and EMBL Barcelona

IBEC Sustainability Committee and EMBL Sustainability Committee

Integrating sustainable practices into biomedical research is essential to reduce the environmental impact of laboratories and to advance toward a more responsible scientific model. This poster presents the main initiatives implemented at the Institute for Bioengineering of Catalonia (IBEC) and at EMBL Barcelona, highlighting strategic, operational, and awareness-raising actions that foster an institutional culture of sustainability.

Since 2019, IBEC has developed a progressive roadmap that includes the creation of a Sustainability Committee, the publication of its *Action Plan for Sustainability*, the incorporation of sustainability into the institutional Strategic Plan, and the achievement of *My Green Lab* certifications, positioning the institute as a pioneer in Spain. Among the actions implemented are waste audits, programs for the reuse and donation of scientific material, carbon footprint analysis, and outreach activities for both the research and educational communities. These efforts have received international recognition, including a feature in *Nature Physics*.

In parallel, EMBL Barcelona has strengthened its commitment to sustainability through the publication of its own *Sustainability Action Plan*, the achievement of the *Silver LEAF* award, the organization of the “Sustainable Research – Fit for the Future” symposium, a 23% reduction in its carbon footprint since 2022, and the launch of the *Green Heroes Challenge*, an initiative designed to promote sustainable actions within laboratories.

Together, these actions illustrate a cross-cutting approach that combines institutional management, community engagement, and the transformation of laboratory practices. The shared goal is to advance toward a more efficient and environmentally conscious research model aligned with global sustainability challenges, demonstrating that sustainability is not only compatible with scientific excellence but actively strengthens it.

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