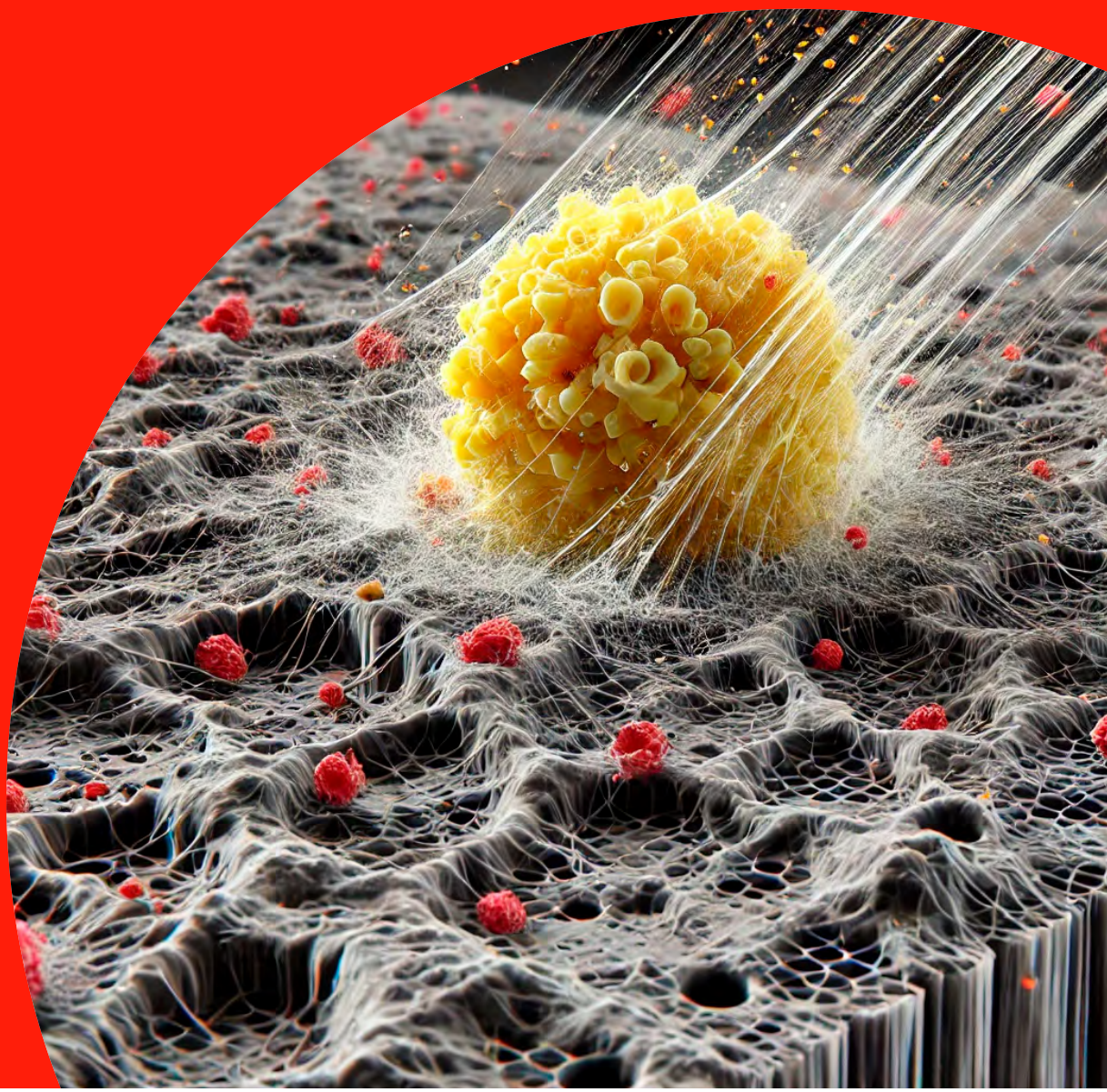
Mechanobiology Shaping Life: development, ageing, and disease

4–5 December 2024
Institute of Physics, London, UK



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Programme

Wednesday 4 December	
09:00	Registration and Coffee
10:00	Welcome and Introduction
	Session I
10:10	The role of the Piezo1 and TRP channel interactome in cellular Mechanotransduction **Boris Martinac,** Victor Chang Cardiac Research Centre
10:50	Oncogenic signaling and stiffness sensing **Mari Johanna Ivaska**, University of Turku
11:20	Shaping the Ear: Exploring the Physical and Mechanical Cues **Bowen Chen,** King's College London
11:23	Laminin-defined mechanics: key to retinal epithelium function and physiological plasticity **Aleksandra N. Kozyrina,** Interdisciplinary Centre for Clinical Research, RWTH Aachen University
11:26	Possible therapeutic effect through nanoparticle motion in magnetomotive ultrasound **Jules Reniaud**, Lund University
11:30	Signaling and mechanosensing at cell-cell junctions during intestinal homeostasis and regeneration **Andrew Clark,** University of Stuttgart
11:40	Forces in Motion: Mechanobiology of Gonad Development in C. elegans **Ronen Zaidel-Bar**, Tel Aviv University
11:50	Cell mechanics and mechanotransduction in cardiovascular morphogenesis **Julien Vermot**, Imperial College London
12:20	**Lunch**
	Session II
13:30	Mechanobiology of cellular senescence **Joe Swift,** University of Manchester
14:00	Application of high-frequency nanovibration to patient-derived glioblastoma cells **Kirsty Weighill**, University of Strathclyde
14:03	Interplay of Piezo1 and Ezrin in both inside-out and outside-in mechanotransduction **Marta Cubero Sarabia**, University of Glasgow
14:06	Modulation of Piezo1 channel kinetics by a naturally occurring fatty acid contributes to endothelial functions **Yurou Cai**, University of Leeds
14:10	Piezo1 is a mechanosensor of soft matrix viscoelasticity **Mariana Azevedo Gonzalez Oliva**, IBEC Parc Cientific Barcelona

14:20	PIEZO1 interaction with adhesion molecules mechano-regulates endothelial cell-cell junctions **Eulashini Chuntharpursat-Bon**, University of Leeds
14:30	Mechanical memory of morphology in confined migrating cells **Sylvain Gabriele**, University of Mons
15:00	Tea Break
	Session III
15:30	Engineered viscoelasticity in stem cell microenvironments **Manuel Salmeron-Sanchez**, University of Glagow / IBEC
16:00	Epithelial cell interactions in an overcrowded environment: exploring the phenomena of jamming and live cell extrusion **Ivana Pajic-lijakovic,** University of Belgrade
16:10	Mechanobiology Community Session
	Session III (continued)
17:00	Epithelial mechanics from the bottom up **Xavier Trepat**, Institute gor Bioengineering of Catalonia
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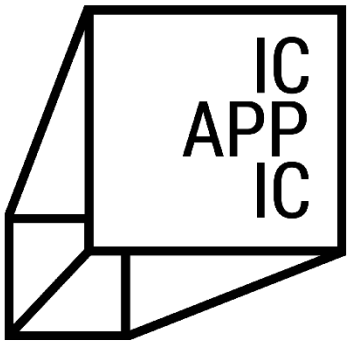
09:00	Integrating Mechanical Microenvironments; the Interplay of Shear Stress, Tissue Stiffness and Gene Expression **Elizabeth Jones**, KU Leuven
09:30	Towards a Perfusable Artery-On-Chip Model Replicating Human Atherosclerosis Development **Lorraine Couteau-Brisset**, Queen Mary University
09:33	Characterization of poroelastic diffusion in autosomal dominant leukodystrophy cells **Andrea Lagomarsino**, Università Degli Studi Di Genova, Italy
09:36	Intestinal Stem cell niche biomechanics in intestinal health and disease **Cai Johnson**, University of Glasgow
09:40	Decoding Piezo1-dependent Mechanotransduction Across Scales Using the GenEPi Biosensor **Konstantinos Kalyviotis**, The Francis Crick Institute/King's College London/Imperial College London
09:50	Spatial mechano-transcriptomics: mapping at single-cell resolution mechanical forces and gene expression in tissues **Adrien Hallou**, Kennedy Institute / University of Oxford
10:00	PIEZO1 force sensor in cardiovascular health, disease and physical exercise **David Beech,** University of Leeds
10:30	Coffee Break
	Session V
11:00	Tissue Fluidification in Pathophysiology **Scita Giorgio**, International Foundation of Medicine (IFOM)
11:30	Matrix viscoelasticity directs epithelial cell mechanobiology through substrate area confinement **Giuseppe Ciccone**, Institute for Bioengineering of Catalonia (IBEC)
11:33	Spatiotemporal regulation of nuclear deformation through modulation of cell cytoskeleton forces on photo-active interfaces **Francesca Mauro**, University of Naples Federico II
11:36	Extracellular matrix plasticity enables a pro-invasive mechanical cross-talk between cancer cells and cancer-associated fibroblasts **Hamid Mohammadi**, Crick Institute
11:40	Mechanical Homeostasis of Retinal Pigmented Epithelium across Space and Time **Jacopo Di Russo**, RWTH Aachen University
11:50	Mechanical regulation of metastasis by the brain vasculature **Marina Uroz,** Boston University

12:00	Mechanobiology and Bone Disease: Uncovering Novel Mechanisms in Osteoporosis and Cancer Bone Metastasis **Laoise McNamara**, University of Galway
12:30	Lunch
	Session VI
13:30	The Border Zone in Myocardial Infarction: a mechanobiological analysis at the cellular and supracellular scales **Vito Conte**, Eindhoven University of Technology
13:40	Computational modelling of mechano-mediated cardiovascular formation, growth, and remodeling **Tommaso Ristori**, Eindhoven University of Technology
13:50	Harnessing geometry and mechanics to engineer functional musculoskeletal microtissues **Sebastien Callens**, Eindhoven University of Technology
14:00	Hypertensive Pressure Mechanosensing Triggers Transdifferentiation of Vascular Smooth Muscle Cells to Foam Cells **Swiatlowska Pamela**, Imperial College London
14:03	Oncogenic molecular features triggered by the mechanoresponsive polycystin proteins in solid tumours **Angeliki-Ioanna Giannopoulou**, Biologist, Msc, Ph.d. Student
14:06	A Tour de Force through Cellular Nanoscale Mechanobiology in (Patho)Physiology **Carsten Schulte**, University of Strathclyde
14:10	3D Biomimetic piezoelectric scaffolds-bas **Oana Oana Dobre**, University of Glasgow
14:20	Controlled in-vitro ultrasound stimulation enhances actin and vinculin expression in osteoblast-like cells **Andrea Orthodoxou**, School of Engineering, University of Glasgow
14:30	Guiding Mechanotransduction in Blood Vessels **Ellie Tzima**, University of Oxford
15:00	Mechanobiology of Cancer Metastasis and Ageing: Insights from Microfluidic and Biophysical Models **Emad Moeendarbary**, University College London
15:30	Tea and Depart

Exhibitor



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The role of the Piezo1 and TRP channel interactome in cellular mechanotransduction

Boris Martinac[1]
[1]Victor Chang Cardiac Research Centre, Australia

TRP ion channels have been reported to serve as mechanoreceptors in cell mechanotransduction. However, in many instances it is unclear whether TRP channels are the primary transducers of mechanical force in these processes. The results of a recent study demonstrated that mammalian TRP channels were insensitive to tension caused by stretching cell membrane and prompted the idea of Piezo1-TRP channel interactome suggesting that Piezo1 and TRP channels act in tandem at the origin of cellular signaling processes. The example of Piezo1 and TRPM4 interaction in cardiomyocytes illustrates how the two channels act in tandem to activate a Ca2+ -calmodulin kinase II (CaMKII)-dependent hypertrophic signaling pathway leading to cardiac left ventricular hypertrophy upon pressure overload. This result suggests that TRP channels may act as amplifiers of cellular mechanosensory signaling cascades.

Oncogenic signaling and stiffness sensing

Mari Johanna Ivaska[1]
[1]University of Turku, Finland

Tissue homeostasis is dependent on the spatially controlled localization of specific cell types and the correct composition of the extracellular stroma. Integrin-mediated adhesions, in conjunction with the actin cytoskeleton and signaling by receptor tyrosine kinases, regulate cell fate and identity and allow cells to migrate and invade the surrounding extra-cellular matrix (ECM). We have previously uncovered key differences between normal and cancer-associated stroma, whereby the mechanical and architectural features of normal stroma inhibit tumour growth and may epigenetically reprogram aggressive breast cancer cells towards a more benign phenotype. Recently, we turned our attention to other putative crosstalk mechanisms between cancer cells and the tumor microenvironment as well as tumor cell interactions with distinct tissue borders during systemic dissemination in the body. I will describe different control mechanisms guiding cancer cell invasion across physiological borders and their relevance to cancer progression and metastasis.

Shaping the Ear: Exploring the Physical and Mechanical Cues

Bowen Chen[1], Eileen Gentleman[1], and Andrea Streit[1]
[1]King's College London, UK

The intricate three-dimensional architecture of the inner ear is essential for auditory function. However, the interaction between mechanical forces and molecular signals that shapes this organ during development remains poorly understood. This project aims to elucidate the role of mechanical forces in the early stages of inner ear development in chicks, focusing specifically on the regional specification and outgrowth of the cochlear duct.

The inner ear originates from an otic vesicle (OV), a single-layered epithelium that undergoes significant morphogenesis. Despite its importance, the molecular mechanisms of mechanosensing and mechanotransduction that guide OV development are not well explored. To address this gap, we are currently conducting single-cell RNA sequencing on the OV cells harvested at various developmental stages. This comprehensive profiling will enable us to analyse the expression patterns and distribution of putative mechanosensitive genes, thus highlighting the OV-specific key candidates in the response to mechanical cues.

To further investigate the role of mechanical forces in OV development, we have engineered a hydrogel-based ex vivo culture system for chick OVs. By fine-tuning the mechanical properties of the hydrogel and incorporating specific molecular components, our system precisely manipulates the in vivo conditions to promote OV development. This provides a cutting-edge platform for studying tissue morphogenesis under synthetic modulation, offering unique insights into the mechanical regulation.

Our research will shed light on the critical interplay between mechanical forces and molecular signalling in shaping the intricate architecture of the inner ear. By elucidating these fundamental processes, this study not only expands our understanding of tissue morphogenesis and organ development but also has the potential to influence broader fields of developmental biology and regenerative medicine.

Laminin-defined mechanics: key to retinal epithelium function and physiological plasticity

Aleksandra N. Kozyrina[1,2], Teodora Piskova[1,2], Francesca Semeraro[1,2], Iris C. Doolaar[3,4], Taspia Prapty[1,2], Tamás Haraszti[3,4], Maxime Hubert[5,6], Reinhard Windoffer[2], Rudolf E. Leube[2], Ana-Sunčana Smith[5,6], and Jacopo Di Russo[1,2,3]
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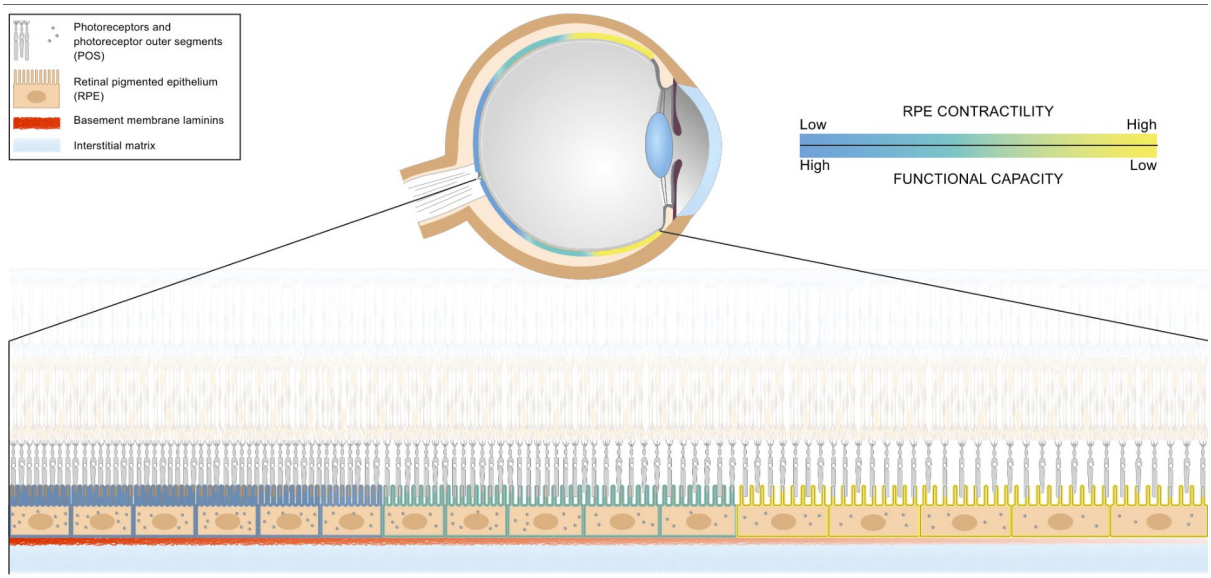
Epithelial cells are interconnected and rely on intricate mechanical properties to sustain essential functions. In homeostasis, these properties depend on a balance between intercellular tension and adhesion to the underlying extracellular matrix (ECM). This balance is crucial for tissue function, particularly in postmitotic epithelium like retinal pigment epithelium (RPE), where the lack of cell division must be dynamically compensated to maintain tissue integrity. While ECM regulates this force balance, how changes in ECM composition influence RPE function remains poorly understood.

For the first time, we show the existence of a laminin isoform density gradient in outer retinal ECM in vivo, where laminin 332 and α5-containing isoforms are less abundant at the retinal periphery. This gradient correlates with changes in RPE cellular morphology, with lower laminin density associated with more elongated cells. These variations in cellular shape factors directly relate to the mechanical properties of the epithelial monolayer, including contractility and cell-to-neighbourhood relationships.
To explore this connection, we developed a reductionist model using human stem cell-derived RPE monolayers on soft hydrogels coated with varying laminin concentrations to simulate the in vivo

environment. Mechanical properties were characterized using traction force microscopy, monolayer stress microscopy, and nanoindentation, correlating cellular contractility with key functional outcomes such as adhesion properties and phagocytic efficiency—a vital function for retinal health. Our results reveal that laminin density modulates RPE mechanical homeostasis and function via β1 and β4 integrins, aligning with in vivo observations of altered cytoskeletal organisation at the retinal periphery.

More broadly, this research expands our understanding of ECM's role in mechanical homeostasis and physiological plasticity, not only in RPE but also in other epithelial tissues like skin and lungs. The discovery of a laminin-defined mechanical gradient modulating cellular function highlights the importance of mechanical balance in tissue physiology and epithelial mechanobiology.



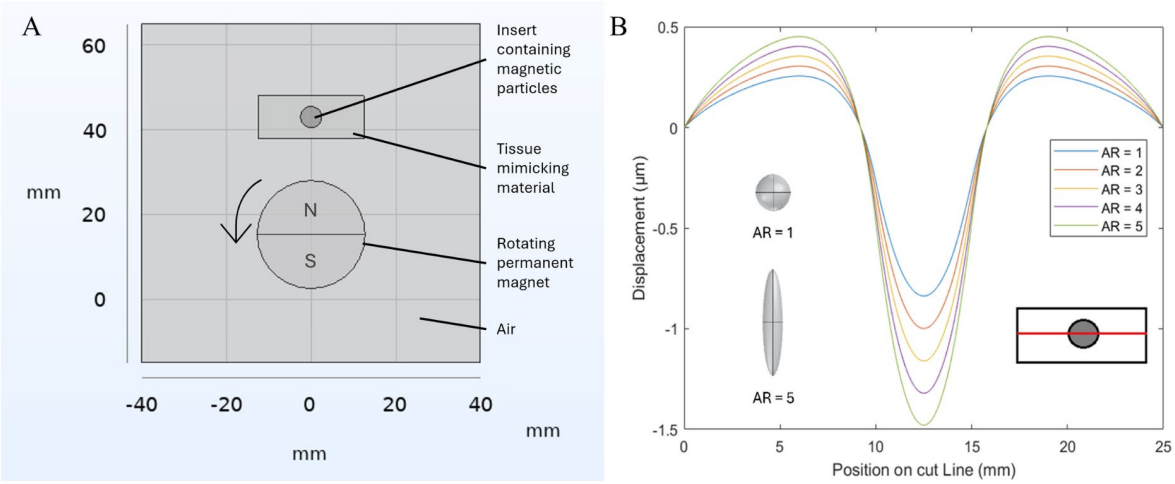
Possible therapeutic effect through nanoparticle motion in magnetomotive ultrasound

Jules Reniaud[1], Maria Evertsson[1], Magnus Cinthio[2], and Tomas Jansson[1,3]
[1]Department of Clinical Sciences Lund, Lund University, Sweden, [2]Department of Biomedical Engineering, Lund University, Sweden, [3]Clinical Engineering Skåne, Sweden

Magnetomotive Ultrasound (MMUS) is an imaging technique used to detect the presence of a magnetic contrast agent. An external magnetic field causes oscillation of the agent, producing tissue motion, detectable with ultrasound. MMUS has applications in cancer diagnosis, and possibly therapy, but low particle concentration and distance pose limitations. We propose modifying the particle shape to increase sensitivity, suggesting ellipsoids. Indeed, altering particle geometry affects the magnetic field inside the particle and thus the force pulling the particles and the induced motion. This also impacts the particle's magnetic moment, thereby affecting the torque exerted on surrounding tissue. We hypothesize that these forces may be strong enough to cause a therapeutic effect. We report on a finite element analysis investigating the effect of particle shape on magnetomotion.

A magnetic object in the proximity of a magnet sees a total magnetic field that varies with the object's shape. The total magnetic field within a particle was expressed as the sum of the external magnetic field and a geometry dependent perturbation. This was computed with a finite element analysis software (COMSOL, Version 6.1, COMSOL AB, Stockholm, Sweden) for a single particle to obtain a so-called demagnetizing function. A geometry dependent expression of the force was obtained from the demagnetizing function and used as input in a second model (panel A). Displacement induced by particles with different aspect ratio (AR) was compared. The single particle model was also used to compute the magnetic torque for different AR.

For the same iron content, particles with a higher AR showed a larger displacement in a gel-based tissue-mimicking phantom material (panel B). The force originating from the magnetic moment of a single nanoparticle and pressing on the surrounding cell was estimated to be in the same order of magnitude as that necessary for disrupting cell membrane.

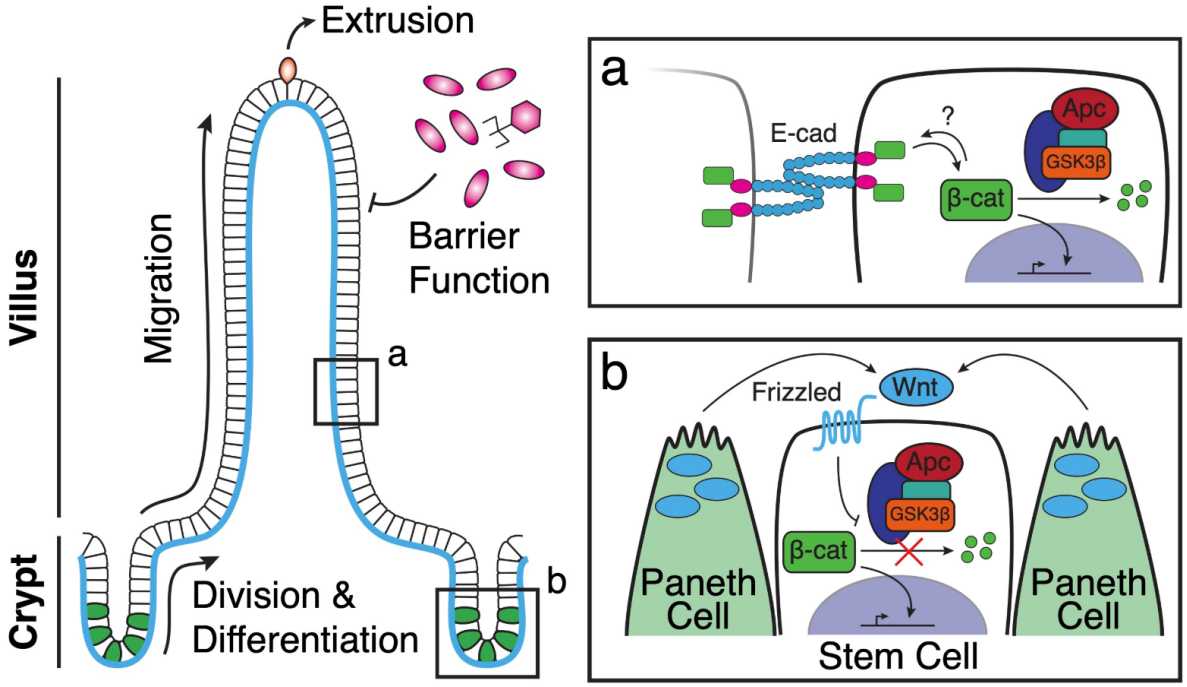


Signaling and mechanosensing at cell-cell junctions during intestinal homeostasis and regeneration

Thao Nguyen[1,2], Sarbari Saha[1,2], and **Andrew Clark**[1,2]
[1]University of Stuttgart, Germany, [2]University of Tübingen, Germany

The mammalian intestine is a highly dynamic adult tissue that requires constant regeneration due to rapid cell turnover. Epithelial stem- and progenitor cells in intestinal crypts divide and differentiate into various specialized cell types before migrating collectively toward the tips of intestinal villi, where cells are extruded into the intestinal lumen. Intestinal stem cell activity is regulated by various pathways, most prominently canonical Wnt/β-catenin signaling. Using primary intestinal organoid monolayer culture combined with quantitative high-resolution microscopy and live imaging, we find that up- or down-regulation of Wnt signaling by chemical perturbation leads to disruption of the organization of the stem cell compartment and changes in stem cell differentiation. We also observe changes in intracellular β-catenin dynamics, cell shape, tissue fluidity and barrier function. These findings suggest that Wnt signaling not only controls stem cell activity, but also cell-cell junctions and tissue integrity, likely via β-catenin, which, in addition to its signaling functions in the Wnt pathway,

is an essential component of adherens junctions. Interestingly, modulation of cell-cell junctions by enhancing or perturbing E-cadherin leads to changes in intracellular β-catenin localization, tissue organization and downstream Wnt signaling. Furthermore, tissue-scale β-catenin localization patterns depend on substrate stiffness, indicating that these processes are mechanosensitive. These mechanosensitive localization patterns can be further modified by tuning E-cadherin activity. These data further suggest an intricate balance between cell-substrate and cell-cell adhesions, which is supported by the observation that changes in substrate stiffness can regulate mechanical tension at cell-cell junctions, as measured using two-photon laser ablation. Through this work, we aim to address crosstalk mechanisms between mechanosensing and morphogen signaling at cell-cell junctions and to better understand intestinal homeostasis as well as intestinal tumorigenesis, where changes in canonical Wnt signaling and loss of overall tissue organization play a major role.



Forces in Motion: Mechanobiology of Gonad Development in C. elegans

Priti Agarwal[1], Tom Shemesh[2], and **Ronen Zaidel-Bar**[1]
[1]Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel, [2]Technion - Israel Institute of Technology, Israel

Gonad development in C. elegans serves as a model for understanding organogenesis and cell migration. While many of the genes involved have been discovered, the cellular and mechanobiological aspects underlying gonad elongation and turning remained poorly understood. Traditionally, it has been assumed that the somatic distal tip cell (DTC) actively leads gonad elongation, with germ cells following.
Here, we used live-imaging, laser ablations, DTC-specific genetic manipulations, and a qualitative physical model, to show that the gonad does not elongates by a pulling force from the leader cell, but rather due to a pushing force generated by the proliferating germ cells, which are confined by a

basement membrane behind the DTC. Local release of matrix-degrading metalloproteases by the DTC determines the direction of gonad elongation. Moreover, we identified the mechanism of gonad turning: the DTC concentrates integrin-mediated cell-matrix adhesions on one side and that this temporally controlled asymmetric adhesion generates a bending moment. Genetic perturbations that interfere with adhesion polarity lead to turning defects.

A key feature of DTC migration is the forward positioning of its nucleus. We identified that the KASH domain protein UNC-83 links the nucleus to kinesin-1, moving it along a polarized, acentrosomal microtubule network to counter frictional forces that would otherwise push the nucleus backward. Remarkably, disrupting nuclear positioning alone does not impair morphogenesis, but when combined with reduced actomyosin contractility, the DTC splits, causing gonad bifurcation. Long-term imaging shows that the lagging nucleus stretches the DTC, eventually leading it to fragment into a nucleated cell and an enucleated cytoplast, each forming an independent gonadal arm.

Our findings offer a novel framework to understand normal tissue morphogenesis, as well as cancer metastasis, in cases where cells are confined by a basement membrane and perform directed invasion.

Cell mechanics and mechanotransduction in cardiovascular morphogenesis

Julien Vermot[1]
[1]Imperial College London, UK

How physical forces influence morphogenesis remains unclear. We study the formation of the zebrafish atrioventricular canal (AVC) where cardiac valves develop to assess the role of mechanical and osmotic forces using zebrafish as a model organism. Using live imaging, we found that the AVC forms within a zone of tissue convergence associated with the increased activation of the actomyosin meshwork and cell-orientation changes. Our recent work aims at providing a cellular understanding of the process. We will discuss the cellular and molecular mechanisms explaining tissue convergence and the role of physical forces in process. I will also introduce our recent methods to visualize, analyse and infer mechanical forces when tissue organises in the embryo.

Mechanobiology of cellular senescence

Joe Swift[1]
[1]University of Manchester, UK

Tissues are maintained by homeostatic feedback mechanisms in which cells can respond to, but also modify, the chemical and mechanical properties of the surrounding extracellular matrix (ECM). Mechano-sensitive mesenchymal stromal/stem cells (MSCs) resident in the marrow niche experience a diverse mechanical environment, but ageing can affect the composition and quality of bone and marrow tissues. Senescence is a protection mechanism against uncontrolled proliferation, but senescent cells accumulate in ageing tissues as they are no longer effectively removed by the immune system. As well as reducing the capacity of tissues for regeneration, the accumulation of senescent cells has negative effects on tissue function, for example by secreting inflammatory

factors. Previous work from my laboratory has shown that proliferating (i.e., non-senescent) MSCs are able to rapidly and reversibly remodel their proteomes when challenged with mechanical stress, for example to break protein linkages between the nucleus and cytoskeleton. However, new results show that replicative senescence in MSCs leads to significant loss of chaperone proteins responsible for maintaining the cytoskeleton. Furthermore, senescent MSCs lack the translational capacity to rapidly remodel their proteomes in response to stress, even when transcriptional responses are functional. These mechanisms are compounded to give senescent MSCs a blunted response to their environments, evidenced by changes to morphology and their ability to remodel the surrounding ECM. As the potential of MSCs to be used in regenerative medicine continues to be explored, this work shows how our ability to direct cell behaviour is affected by cell replication, senescence and ageing.

Application of high-frequency nanovibration to patient-derived glioblastoma cells.

Kirsty Weighill[1], Melanie Jiminez[2], Peter G. Childs[2], and Natividad Gomez-Roman[1]
[1]Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Dennistoun, UK, [2]Biomedical Engineering, University of Strathclyde, UK

Glioblastoma is an aggressive malignant primary brain tumour [1] with an average survival time of 12-18 months [2]. Recurrence following treatment is inevitable due to resistant glioblastoma stem cells that persist and repopulate a tumour [3]. To improve therapeutic efficacy of current treatment, glioblastoma stem cells need to be targeted. Nano-amplitude vibration is a novel, potential, therapy that mechanically stimulates cells and alters the gene expression and differentiation of mesenchymal stem cells [4]. Here, we explore the effect of nanovibrational stimulation on glioblastoma cells, with a focus on proliferation and differentiation.

Laser Doppler Velocimeter (LDV) analysis was carried out on cultureware during nanovibration at frequencies of 1 and 20 kHz. The variability between 1 kHz and 20 kHz is presented in Figure 1A. Patient-derived glioblastoma cells (G7) were cultured in stem cell enriched conditions (STEM), STEM conditions plus nanovibration (NANO), and media promoting differentiation as a positive control (DIFF). The impact of 30 nm nanovibration at 1 kHz on proliferation, viability, stemness, radiation effect and differentiation was investigated by cell counting, trypan blue staining, neurosphere assay (including 3 Gy radiation), and western blotting, respectively. The effect of 40 nm, 20 kHz, nanovibration on proliferation, differentiation and YAP translocation is being investigated by microscopy and western blotting.

Nanovibration at 1 kHz had no effect on proliferation, viability or differentiation of G7 glioblastoma cells ($p>0.05$). Ionising radiation (3 Gy) significantly reduced the ability to produce neurospheres across all conditions. However, a lower response was observed in NANO ($p<0.05$) compared to STEM and DIFF ($p<0.001$). Changing the frequency to 20 kHz is expected to apply a greater accelerative force on the cells, with initial results indicating altered morphology (Figure 1B).

Our findings suggest that glioblastoma cells respond in a frequency-dependent manner. Further studies are underway to evaluate the effects of 20 kHz.

[1] Gilard, V., Tebani, A., Dabaji, I., Laquerriere, A., Fontanilles, M., Derrey, S., Marret, S. & Bekri, S. 2021. Diagnosis and Management of Glioblastoma: A Comprehensive Perspective. Journey of Personalised Medicine, 11(4); pp258

[2] Rios, S.A., Oyervides, S., Uribe, D., Reyes, A.M., Fanniel, V., Vazquez, J. & Keniry, M. 2024. Emerging Therapies for Glioblastoma. Cancers, 16(8); pp1485
[3] Mattei, V., Santilli, F., Martellucci, S., Monache, S.D., Fabrizi, J., Colapietro, A., Angelucci, A. & Festuccia, C. 2021. The Importance of Tumour Stem Cells in Glioblastoma Resistance to Therapy. International Journal of Molecular Sciences, 22(8); 3863
[4] Kennedy, J.W., Tsimbouri, P.M., Campsie, P., Sood, S., Childs, P.G., Reid, S., Young, P.S., Meek, D.R.M., Goodyear, C.S. & Dalby, M.J. Nanovibrational stimulation inhibits osteoclastogenesis and enhances osteogenesis in co-cultures. Scientific Reports. 11;22741

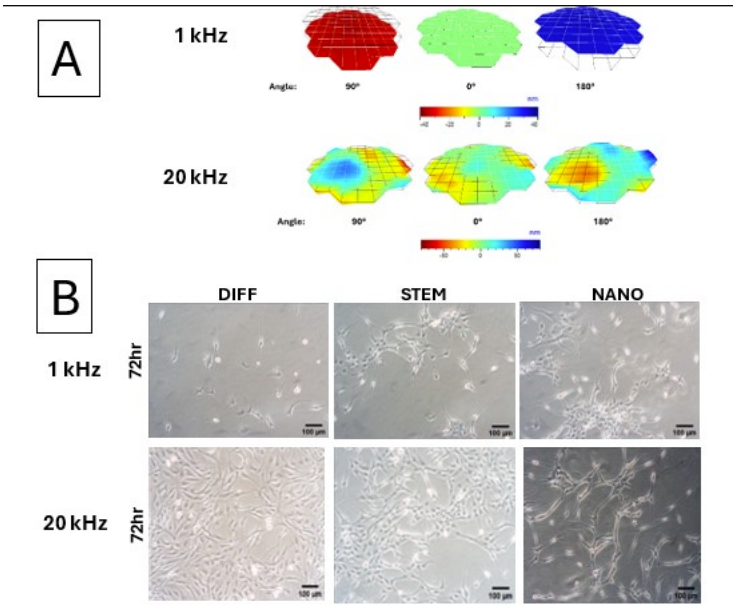


Figure 1 Nanovibration of glioblastoma cells. **A** LDV analysis comparing the vibration applied to cells at 1 kHz and 20 kHz. Image highlights the uniform vibration at 1 kHz applied to cells compared to the variability of vibration applied at 20 kHz, with ranging amplitudes across the cultureware. Thus, nanovibration at 20 kHz exerts a different force on the cells compared to 1 kHz. Scale bars represent amplitudes of wave. **B** Phase contrast images of G7 Glioblastoma cells taken at 72-hour timepoint for both 1 kHz and 20 kHz. From left to right: DIFF (cells in media promoting differentiation), STEM (cells in stem cell enriched conditions), NANO (cells in stem cell enriched conditions and nanovibrated at 20 kHz). 1 kHz images from 6-well plates and 20 kHz images from 10cm petri dish. Images captured using VisiCam5 camera and Waveimage software at 10X magnification.

Interplay of Piezo1 and Ezrin in both inside-out and outside-in mechanotransduction

Marta Cubero Sarabia[1], Massimo Vassalli[1], Medha Pathak[2], Manuel Salmeron-Sanchez[1], Matthew Dalby[1], Gabriella Bertaccini[2], Ignasi Casanellas[2], and Matthew Walker[1]
[1]University of Glasgow, Glasgow, UK, [2]University of California, Irvine , USA

The membrane-to-cortex attachment (MCA) is highly dynamic and constantly in reconstruction, playing a fundamental role in many physiological processes and being fundamental in mechanotransduction (1). Ezrin, a scaffolding protein that physically links the cytoskeleton and the cellular membrane, is part of the machinery involved in the MCA(2). In the last decade, studies have

suggested its involvement in cellular mechanics, being involved in membrane tension maintenance and embryonic stem cell pluripotency exit by modulating this attachment (3,4). Moreover, two recent publications have suggested that Ezrin and Piezo1 act in the same signalling pathway in breast cancer cells and neurons (5,6).

Here, Ezrin inactivation on endothelial cells (ECs) have shown to affect Piezo1 activity and diffusion across the membrane in "inside-out" mechanotransduction (i.e. with no external stimulation). Moreover, protein inhibition affects morphological changes differently depending on Piezo1 expression, suggesting that these proteins could be acting on a common signalling pathway in ECs.

Additional experiments were performed on Mesenchymal Stem Cells (MSCs) under nanovibrational stimulation (1 kHz, 30 nm of frequency and amplitude, respectively), previously proved to be osteoinductive (7–9). Results suggest that Piezo1 is directly activated as a result of this stimulation and that Ezrin inhibition enhances the osteogenic potential of this nanovibrational stimulation, decreasing cellular stiffness during the first 8 hours of stimulation and significantly increasing ERK activation. Moreover, activation of Ezrin during long-term nanovibrational stimulation was not observed until day 7 of stimulation, suggesting that the protein needs to be inactive during early differentiation stages.

These results suggest that Ezrin modulates the MCA and ultimately affects mechanotransduction, specifically Piezo1 activity, in physiological conditions.

[1] DOI: 10.1016/j.ceb.2020.04.001
[2] DOI: 10.1186/s12929-016-0246-3
[3] DOI: 10.1038/srep14700
[4] DOI: 10.1016/j.stem.2020.10.017
[5] DOI: 10.1101/2023.01.17.524464
[6] DOI: 10.1242/jcs.258809
[7] DOI: 10.1098/rsta.2017.0290
[8] DOI: 10.1021/nn400202j
[9] DOI: 10.1126/sciadv.abb7921.

Modulation of Piezo1 channel kinetics by a naturally occurring fatty acid contributes to endothelial functions

Yurou Cai[1], Claudia Bauer[1], Antreas Kalli[1], David Beech[1], and Jian Shi[1]
[1]Leeds Institute of Cardiovascular and Metabolic Medicine, School of Medicine, University of Leeds, UK

PIEZO1, a mechanically-activated Ca2+ permeable channel, is expressed across various cells and tissues. It plays a pivotal role in mechanoelectrical transduction and physiological processes. Understanding the factors that influence Piezo1 function is essential for elucidating its role in physiology and pathophysiology. Recent studies have shed light on the regulatory effects of lipids, especially endogenous membrane lipids, on Piezo1 channels. Furthermore, it has been suggested that fatty acids have a conditional role in cardiovascular diseases. However, our knowledge of their effects on Piezo1 is incomplete and we do not know if Piezo1-mediated fatty acid effects are relevant to endothelial cell functions.

To investigate, intracellular calcium was measured in HEK-293 cells overexpressing Piezo1 and human umbilical vein endothelial cells (HUVECs) natively expressing Piezo1. Endothelial cell functions including cell viability, cell migration and cell alignment to fluid flow were investigated. The underlying mechanism was interrogated by the patch-clamp technique.
Intracellular Ca2+ measurements showed that dodecanoic acid (DDA) induced a concentration-dependent increase in Ca2+ influx evoked by the Piezo1 agonist Yoda1, while patch-clamp recordings showed that DDA enhanced the peak current response to mechanical pressure and delayed Piezo1 channel inactivation. The combined application of DDA and Yoda1 showed no effect on HUVEC viability but a combination of Yoda1 (0.3 µM) and DDA (10 µM) increased HUVEC migration depending on Piezo1 expression. DDA treatment enhanced cell alignment to fluid shear stress.

To conclude, DDA enhances activity of overexpressed and native Piezo1. Its effects on Piezo1 are relevant to endothelial cell migration and response to shear stress.

Piezo1 is a mechanosensor of soft matrix viscoelasticity

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In the last 30 years, it has been shown that cells exert and perceive mechanical forces as part of physiological (e.g., tissue formation) and pathological (e.g., cancer progression) processes; leading to what is now known as the field of mechanobiology. The most common approach in the field has been to study cellular processes in response to substrates of defined elasticity (commonly referred to as stiffness); however, tissues behave as viscoelastic solids, and energy dissipation (e.g., stress relaxation) has recently shown to strongly influence cell behaviour. Mechanosensitive ion channels have emerged as fundamental proteins in sensing extracellular matrix (ECM) mechanics. Among those, Piezo1 has been proposed as a key mechanosensor in cells. However, whether and how Piezo1 senses time-dependent ECM mechanical properties (i.e., viscoelasticity) remains unknown. To address this question, we combined an immortalised mesenchymal stem cell (MSC) line with adjustable Piezo1 expression with soft (400 Pa) and stiff (25 kPa) viscoelastic hydrogels with independently tuneable elastic and viscous moduli. We demonstrate that Piezo1 is a mechanosensor of viscoelasticity in soft ECMs, consistent with the actin-talin-integrin-fibronectin molecular clutch model. The model was extended in this work to account both for the cell's interaction with viscoelastic substrates as well as for the overall effect of Piezo1 knock-down in clutch engagement. Finally, by performing RNA sequencing (RNA-seq), we identified the transcriptomic phenotype of MSCs response to matrix viscoelasticity and Piezo1 activity, highlighting gene signatures that drive MSCs mechanobiology in soft and stiff viscoelastic hydrogels.

PIEZO1 interaction with adhesion molecules mechano-regulates endothelial cell-cell junctions

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The endothelium is a monolayer of cells forming the inner lining of all arteries, veins, capillaries and lymphatics. These cells form a crucial interface between blood and tissues to regulate the exchange of substances and cells, partly via specialised cell-cell junctions. Key junctional proteins involved in mechanoregulation are the adhesion molecules CDH5 and PECAM1. Subsequently, came the discovery of PIEZO1 mechanosensitive ion channel that was found to be a blood flow sensor in endothelial cells. We have recently shown that PIEZO1 is an integral part of this biochemical zipper between cells, merging two prominent ideas for force sensing (1).

In this study, we have used super-resolution (STED) and Förster resonance energy transfer measured by fluorescence lifetime imaging microscopy (FRET/FLIM) to identify interactions of PIEZO1 with PECAM1 and CDH5. Endogenous proteins were examined in tissue using our CRISPR-modified HA-tagged PIEZO1 mouse. We used patch clamp and fluorescent calcium recordings to determine ion channel activity and calcium switch assays to recapitulate junction formation on endothelial monolayers.

We identified a pool of PIEZO1 located at cell junctions where PECAM1 and CDH5 are in complex with PIEZO1. PECAM1 formed a stable complex that leads to dampening of PIEZO1 activity. Shear activation of PIEZO1 showed dynamic recruitment of junctional CDH5 and PIEZO1 knockdown impaired CDH5 junction formation. PIEZO1 is required in the Ca2+-dependent formation of adherens junctions and associated cytoskeletal organisation, consistent with conferring force-dependent Ca2+ entry for junctional remodelling.

[1] E Chuntharpursat-Bon et al., Commun. Biol. (2023) https://doi.org/10.1038/s42003-023-04706-4.

Mechanical memory of morphology in confined migrating cells

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Cell migration in narrow microenvironments is a hallmark of numerous physiological processes, involving successive cycles of confinement and release that drive significant morphological changes. However, it remains unclear whether migrating cells can retain a memory of their past morphological states, which could potentially enhance their navigation through confined spaces. By combining cell migration assays on standardized microsystems with biophysical modeling and biochemical perturbations, we demonstrate that local geometry governs these morphological switches, thereby facilitating cell passage through long and narrow gaps. We uncovered a long-term memory of past confinement events in migrating cells, with morphological states correlated across transitions through actin cortex remodeling. These findings suggest that mechanical memory in migrating cells plays an active role in their migratory potential in confined environments.

Engineered viscoelasticity in stem cell microenvironments

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The physical properties of the extracellular matrix (ECM) and the use of growth factors are powerful tools to control cell behaviour, including (stem) cell differentiation. Integrins are mechanotransductors that feel and respond towards the mechanical properties of the ECM. We have developed material systems that allow simultaneous stimulation of integrins and growth factors receptors. We have engineered polymers and 3D hydrogels that unfold and assemble proteins to allow exposure of the integrin and growth factor binding regions. For example, we show the use of BMP-2 in synergy with α5β1 integrins to promote osteogenesis and regeneration of critical-sized defects. Further, we have developed interfaces that bind latent proteins that induce integrin-mediated mechanical activation of growth factors. We will demonstrate the use of TGF-β1 that is released and activated by using engineered surfaces that organise fibrinectin to promote binding of LTBP1 and enable integrin B1 to pull on active TGFB1.

In the second part of the talk, we will use surfaces of controlled viscosity in our pathway to engineer and understand the viscoelastic properties of the ECM. We use supported lipid bilayers that are functionalised with either RGD (integrin binding) or HAVDI (cadherin binding) to demonstrate the molecular clutch is engaged on surfaces of high enough viscosity and, importantly, that it is weaken upon N-cadherin binding, controlled by the competition between vinculin and α-catenin for actin filaments. We then introduced substrates of controlled elasticity and viscosity, first in 2D using polyacrylamide hydrogels that were further patterned using fibronecin and then in 3D using PEG-hydrogels functionalised with fibronectin. We will discuss the unexpected interplay between viscoelasticity, cell adhesion and molecular clutch engagement. We introduce Brillouin microscopy as a way to follow the evolution of the viscoelastic properties of cells and the engineered hydrogels in 3D in a non-invasive way and in real time.

Epithelial cell interactions in an overcrowded environment: exploring the phenomena of jamming and live cell extrusion

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Epithelial tissues are highly responsive to the mechanical stress caused by collective cell migration, possessing the ability to modulate this stress effectively by remodelling cell-cell and cell-matrix adhesion contacts. This modulation is vital for several biological functions, including morphogenesis, wound healing, and the suppression of cancer spread. When epithelial monolayers are either actively or passively wetted or de-wetted on substrate matrices, they experience different stress components, including compressive, tensional, and shear stress. An increase in compressive stress among the cells enhances cell-cell interactions by raising the frequency of variations in cell-cell distances, subsequently triggering a range of signalling pathways within the cells. The phenomenon may result in either cell jamming or the extrusion of viable cells. Although considerable research has been conducted in this area, the mechanisms by which cells determine whether to undergo jamming or extrusion, as well as their strategies for mitigating compressive mechanical stress, remain poorly understood. The extrusion of live cells from densely populated regions within monolayers is linked to

the existence of topological defects in cell alignment, which arise from the interaction between compressive and shear stress components acting on the cells.

These topological defects stimulate cell re-alignment, as a part of the cells' tendency to re-establish an ordered trend of cell migration, by intensifying the glancing interactions in overcrowded regions. In addition to individual cell extrusion, collective cell extrusion has also been documented during monolayer active de-wetting, depending on the cell type, matrix stiffness, and boundary conditions. Cell jamming has been discussed in the context of the cells' contact inhibition of locomotion caused by cell head-on interactions.
Since cell-cell interactions play a crucial role in cell rearrangement in an overcrowded environment, this presentation is focused on physical aspects of these interactions in order to stimulate further biological research in the field.

Epithelial mechanics from the bottom up

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Epithelial sheets form specialized 3D structures suited to their physiological roles, such as branched alveoli in the lungs, tubes in the kidney, and villi in the intestine. To generate and maintain these structures, epithelia must undergo complex 3D deformations across length and time scales. How epithelial shape arises from active stresses, viscoelasticity and luminal pressure remains poorly understood. I will present different approaches to study the mechanobiology of epithelial shape from the bottom up. I will discuss new technologies to design epithelia of arbitrary size and geometry and to subject them to controlled mechanical deformations in 3D. I will show that monolayers exhibit superelastic behavior when stretch is applied and that they readily buckle when tension is released. We use this phenomenology and a 3D vertex model to rationally direct spontaneous pattern formation, and hence engineer tissue folding. I will also present our recent advances to understand the mechanobiology of intestinal organoids. We show that these organoids exhibit a non-monotonic stress distribution that defines mechanical and functional compartments. From these experiments we conclude that the stem cell compartment folds through apical constriction and that cells are pulled out of the crypt along a gradient of increasing tension, rather than pushed by a compressive stress downstream of mitotic pressure as previously assumed. This experimental and theoretical work unveils how patterned forces enable folding and collective migration in the intestinal crypt.

Integrating Mechanical Microenvironments; the Interplay of Shear Stress, Tissue Stiffness and Gene Expression

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Endothelial cells (ECs) are sensitive to their mechanical environment. Though endothelial cells can sense shear stress, they are also sensitive to the stiffness of the matrix on which they reside. Whether different mechanical forces interact is poorly studied, and we were specifically interested whether tissue stiffness altered mechanotransduction. To approach this in a large-scale unbiased

manner, we performed a factorial experiment across 14 combinations of shear stress and stiffness and studied gene expression in all these conditions by bulk RNA-Seq. We ran likelihood ratio tests on bulk RNA-Sequencing data across all conditions to determine the impact of the interaction between shear stress and stiffness on the endothelial gene expression. We identified which genes were modulated uniquely by shear stress, by stiffness, and –especially – by their interaction (i.e. genes whose flow-induced mechanotransduction is altered by tissue stiffnesses). Notably, the “interaction genes" were predicted to be involved in several vascular remodeling processes including angiogenesis and EC migration. The expression patterns of these genes were, moreover, comparable with in vitro flow-induced EC migration rates on different stiffnesses. We further identified 21 interaction genes whose expression correlated with the nuclear translocation of Yes-associated protein 1 (YAP1) across conditions. From these, upon YAP1 gene silencing, MGLL and KRT15 revealed as novel putative YAP1 targets modulated in a shear stress-by-stiffness interaction-dependent manner. Our unique mechanobiology dataset and approach proves as a new reference to identify endothelial genes and processes modulated by shear stress-by-stiffness interactions.

Towards a Perfusable Artery-On-Chip Model Replicating Human Atherosclerosis Development

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Introduction
Atherosclerosis is the most ubiquitous cardiovascular disorder worldwide [1]. Progression of the disease is attributed to a dysregulation of arterial cells behaviour. Endothelial cells (EC), vascular smooth muscle cells (vSMC) and monocytes participate jointly to the accumulation of lipids, cells and cell debris within the intima layer. The principal triggers of atherosclerosis were identified as disrupted mechanical stimuli which motivates cells maladaptive response [2]. Mechanotransduction studies of atherosclerosis are usually done in 2D models and rarely consider the impact of vSMC mechanosensing over EC behaviour. We propose a new perfusable Artery-On-Chip model replicating the architecture of the native artery through a two-layer 3D construct, where vSMC and EC are co-cultured together to study their interactions under mechanical stimulation.

Methods
Our Artery-On-Chip device is made through a double needle templating technique. A tubular construct made of vSMC-loaded dECM hydrogel is cast within a low-density matrix. Endothelial cells are subsequently seeded on the walls of the tubular lumen. Our design allows for the modulation of mechanical parameters associated with atherosclerosis through the modulation of hydrogel stiffness and the passage of flow through its lumen.

Results & Discussion
We characterised the physicochemical parameters of dECM hydrogel derived from bovine arteries and optimised its use within the artery-On-Chip model. Vascular smooth muscles cells and endothelial cells are maintained in co-culture multiple days successfully and show signs of crosstalk. Finally, stimulation of PIEZO1 pressure sensing channel modulated both arterial cells behaviour towards a pro-atherosclerotic phenotype.

Conclusion
To our knowledge, our current microfluidic model is the only perfusable device permitting the co-culture of arterial cells in an architecture similar to the native artery. This Artery-On-Chip versatility

allows for precise mechanical stimulation towards the study of mechanosensing in early atherosclerosis.

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Characterization of poroelastic diffusion in autosomal dominant leukodystrophy cells

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The mechanical characteristics of the cell nucleus, often compromised in disease, influence essential processes such as chromatin accessibility. The architecture and structural mechanics of the nucleus are determined by the nuclear lamina, which is composed of A- and B-type lamins. Laminopathies are a group of genetic disorders resulting from mutations in nuclear lamins.

Recently, gene duplication and overexpression of lamin B1 (LB1) have been identified in families affected by autosomal dominant leukodystrophy (ADLD), with increased nuclear stiffness observed in correlation with this overexpression [1].

The primary method for characterizing the mechanics of biological materials at the sub-micrometer scale is atomic force microscopy (AFM). This technique allows us to determine the Young's modulus of biomaterials, which could potentially serve as a biomarker for certain pathologies. However, this method requires significant time investment due to the need for a large statistical sample.

To address this challenge, we explored an alternative approach using a poroelastic model of the cell combined with the stress-relaxation technique, allowing us to calculate the poroelastic diffusion coefficient (Dp) in living cells [2]. This investigation revealed a significant difference in poroelastic behavior in ADLD cells, which was more pronounced than changes in stiffness alone. This newly identified diagnostic biomarker could offer an innovative approach to studying pathologies related to dysregulated nuclear mechanics.

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Intestinal Stem cell niche biomechanics in intestinal health and disease

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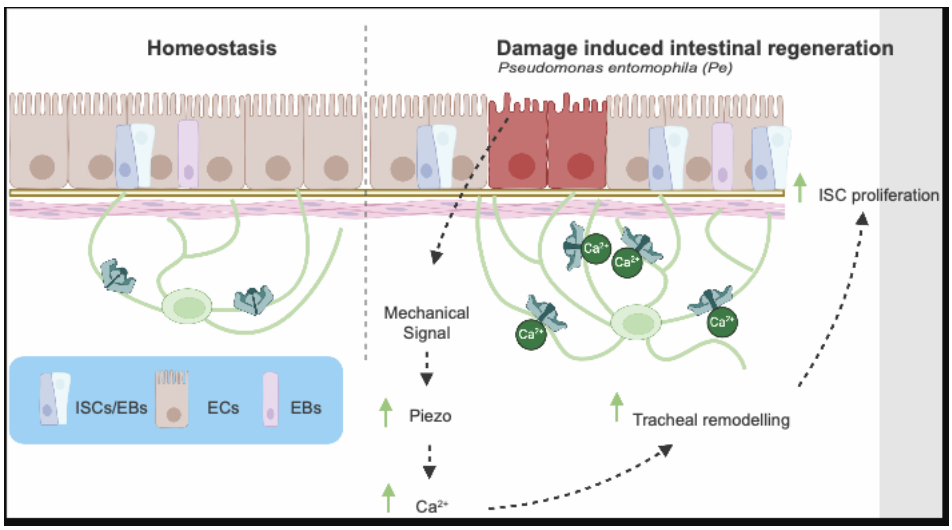
The intestinal epithelium is subject to sustained mechanical and biological insult, requiring dynamic intestinal stem cell (ISC) activity to replenish the loss of damaged or senescent epithelial cells. The ISC microenvironment plays a critical role in regulating proliferative dynamics in health and disease, with much of our understanding in the intestine stemming from model organisms: Drosophila melanogaster and Mus musculus. The Drosophila midgut, a functionally and anatomically analogous organ to the mammalian intestine, has provided invaluable insight into regulatory mechanisms that govern ISC behaviour, both physiologically and in response to disease. Upon prolonged and concentrated enteric insult, such as Pseudomonas entomophila (Pe), the Drosophila midgut undergoes notable cell death and tissue-wide morphological changes. Subsequently, compensatory ISC proliferation is induced, with recent studies highlighting the importance of reciprocal crosstalk between the midgut epithelium and the vascular-like tracheal system, for ISC activity (Perochon et al. 2021; Tamamouna et al. 2021). Notably, tracheal remodelling precedes and drives ISC division during intestinal regeneration. While biochemical trachea-gut crosstalk has been characterised in considerable detail, the biomechanical consequences of gut resizing and its implications in tracheal remodelling remain poorly understood. My research, alongside others in the lab, has highlighted upregulation of Piezo-mediated mechano-signalling in the gut trachea in various damage models. Implementing multifaceted techniques, spanning biochemistry, biophysics, and computational mathematics, I aim to characterise and model the local and global biomechanical properties of the regenerating intestine. I have begun by characterising the viscoelastic properties of the fly midgut which will facilitate predictions of mechanical attributes required to recapitulate ISC/vascular dynamics. My objective is to identify force dependent mechanisms in the intestine required for vascular remodelling, and to develop a comprehensive understanding of how forces are generated and sensed in intestinal health and disease.



Decoding Piezo1-dependent Mechanotransduction Across Scales Using the GenEPi Biosensor

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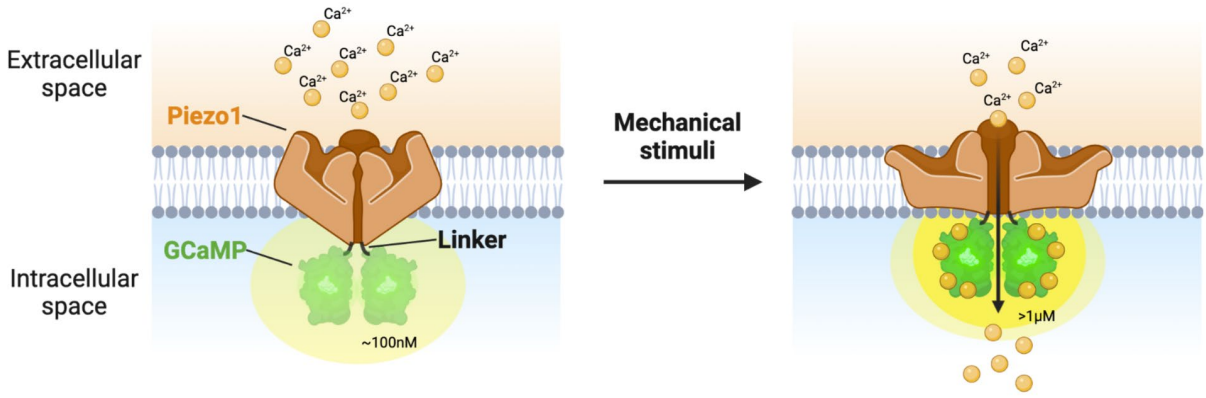
Mechanosensing translates mechanical stimuli into essential biological responses, with Piezo1 ion channels acting as key mediators of this process. Traditionally, assessing Piezo1 activity has relied on invasive or indirect approaches like electrophysiology and cytosolic calcium imaging. To overcome these limitations, we developed GenEPi, a genetically encoded fluorescent biosensor that offers a novel solution for non-invasive, high-resolution imaging of Piezo1-dependent activity. GenEPi captures Piezo1-dependent activity with high spatiotemporal resolution across scales from single cells to whole organisms. Specifically, it reveals transient, localised mechanical stimuli at the plasma membrane of individual cells, detects repetitive contraction-triggered stimulation of beating cardiomyocytes within microtissues, and enables robust, reliable monitoring of Piezo1-dependent activity in vivo. Using GenEPi, we now capture synchronised Piezo1 activity in cell monolayers under shear stress and pioneer the first knock-in GenEPi mice, allowing for imaging of endogenous Piezo1 dynamics across multiple organs. Additionally, in a recent collaboration we explore the immunomodulatory function of Piezo1 using GenEPi. Overall, GenEPi and similar biosensors open new windows into non-invasive, multiscale imaging of Piezo1 activity, deepening our understanding of how mechanochemical feedback loops regulate development, homeostasis, and disease.



Spatial mechano-transcriptomics: mapping at single-cell resolution mechanical forces and gene expression in tissues

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Advances in spatial profiling technologies are providing insights into how molecular programs are influenced by local signaling and environmental cues. However, cell fate specification and tissue patterning involve the interplay of biochemical and mechanical feedback. Here, we propose a new computational framework that enables the joint statistical analysis of transcriptional and mechanical signals in the context of spatial transcriptomics. To illustrate the application and utility of the approach, we use spatial transcriptomics data from the developing mouse embryo to infer the forces

acting on individual cells, and use these results to identify mechanical, morphometric, and gene expression signatures that are predictive of tissue compartment boundaries. In addition, we use geoadditive structural equation modeling to identify gene modules that predict the mechanical behavior of cells in an unbiased manner. This computational framework is easily generalized to other spatial profiling contexts, providing a generic scheme for exploring the interplay of biomolecular and mechanical cues in tissues.

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PIEZO1 force sensor in cardiovascular health, disease and physical exercise

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PIEZO1 forms a trimeric calcium permeable non-selective cation channel that activates in response to mechanical force. We found significance of it in cardiovascular biology, initially showing its sensing of physiological fluid shear stress and role in embryonic vascular maturation, and therefore development [1]. Through conditional deletion of endothelial PIEZO1 in adult mice, we identified its roles in blood pressure regulation during physical activity, the determination of capillary density in skeletal muscle, physical exercise performance, and lipid homeostasis via signalling to parenchymal cells of the hepatobiliary and intestinal systems [2-4]. We found that PIEZO1 confers force sensing on other physiologically important mechanisms that include the calcium-regulated proteases CAPN2 [1] and ADAM10 [5], nitric oxide synthesis via NOS3 [1, 3], gene expression via NOTCH1 intracellular domain [5], and cell apoptosis via NOS3 and thrombospondin-2 [3]. We found a pool of PIEZO1 located to endothelial cell-cell junctions where it interacts with cell adhesion molecules such as PECAM1 [6]. With genetics collaborators, we found natural missense variants that disrupt the channel's force sensing ability and associate with the unsolved disease problems of non-immune fetal hydrops and generalized lymphatic dysplasia [7]. With computational biology collaborators, we generated molecular dynamics simulations of the full-length human channel in endothelial membrane to understand its dynamic states, revealing lipid rearrangements in the channel that regulate its ion pore opening. With chemistry collaborators, we developed pharmacology that activates or inhibits the channels [8]. In summary, we found that PIEZO1 forms an exceptional mechanical force sensor of the cardiovascular system with important downstream consequences and the possibility for therapeutic intervention. We expect that PIEZO1's roles will become more important in ageing when there is often blood pressure elevation, lipid dysregulation, tissue stiffness and physical exercise intolerance.

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Tissue Fluidification in Pathophysiology

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The process in which locally confined epithelial malignancies progressively evolve to become invasive cancer cells is associated with the acquisition of cell motility, fostered by a tissue-level phase transition (PT) from a solid-like to a liquid-like state, known as unjamming. The biomolecular machinery behind unjamming and its pathophysiological relevance have only begun to be unraveled. Using a combination of physical approaches, ex vivo and in vivo model systems, we will address these issues and discuss whether an endocytic-driven PT between “solid” and “liquid” states of cell collectives is a complementary gateway to cell migration in pathology, focusing specifically on the progression of early breast cancer lesions that become locally invasive. We will show how the dynamic changes associated with PT feature the coexistence of long-range coordinated motion and local cell re-arrangement and are sufficient to promote matrix remodeling, and local invasion and exert mechanical stress on individual cell nuclei. This is accompanied by profound transcriptional rewiring, with the unexpected activation of an inflammatory response, change in cell state, and the emergence of malignant traits. Noticeably, carcinoma is composed of a heterogeneous set of cells that differ not only in their genetic landscape but also in their mechano-phenotypes. The impact of mechano-heterogeneity on tissue-level jamming transition is poorly understood. Here, we will also discuss unpublished findings that suggest that contact percolation, a purely geometrical feature, can impact the collective migratory behavior of tissues and, strikingly, promote the activation of an inflammatory gene transcription program in normal and breast carcinoma models.

Matrix viscoelasticity directs epithelial cell mechanobiology through substrate area confinement

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Extracellular matrix (ECM) viscoelasticity has emerged as potent regulator of physiological and pathological processes. Spatial confinement in the ECM is also considered as a regulator of cell behaviour. However, although the ECM is viscoelastic, the relationship between matrix mechanics and spatial confinement in driving epithelial cell mechanotransduction is not well understood and relies on experiments done using purely elastic hydrogels. For the first time, we micropattern viscoelastic hydrogels with independently tuneable Young’s modulus and stress relaxation, specifically designed to mimic the mechanical properties observed during breast tumour progression, transiting from a soft dissipative tissue to a stiff elastic one. Using this system, we demonstrate that viscoelasticity modulates breast epithelial cell spreading, adhesions, YAP nuclear import and cell migration differently on soft and stiff matrices. By restricting the cell’s adhesive area,

we show that spatial confinement modulates viscoelasticity sensing in terms of cell spreading, mechanotransduction and migration. Our findings establish ECM viscoelasticity as a key regulator of epithelial cell mechanobiology and unravel the role of spatial confinement in this process.

Spatiotemporal regulation of nuclear deformation through modulation of cell cytoskeleton forces on photo-active interfaces

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Numerous investigations have provided evidence that the cellular cytoskeleton is capable of exerting external forces upon the nucleus, thereby influencing its morphological characteristics and the organization of DNA [1]. Diverse methodologies aim to regulate the nuclear shape and genome organization by adjusting cytoskeletal forces on the nucleus. For example, biointerfaces with specific topographies have proven effective in modulating gene expression, through nuclear deformation caused by cytoskeleton reorganization [2]. In the present study, a photo-switchable azopolymer-based cell culture platform was employed to control the behaviour of MCF10A cells. Specifically, the in-situ optical inscription/erasure of nanotopography in parallel lines allowed the dynamic modulation of nuclear shape and the architecture of genetic material. This modulation was achieved by spatiotemporally tailoring the intensity and distribution of the forces generated by the cell cytoskeleton acting on the nucleus. Analyses of the cell tensional state revealed cell stiffening following the physical signal presentation, which manifested consequential effects on chromosome organization, chromatin compaction levels and the arrangement of heterochromatin domains within the nucleus. Intriguingly, the disruption of these forces after the removal of nanotopography induced a recovery of the configuration of subcellular and subnuclear structures that cells assumed without the physical signal (Figure 1). Furthermore, investigations of cancerous cells' behaviour revealed that cells with diverse invasiveness respond differently to the dynamic stimulation. Consequently, this study offers a strategic approach to control “on-demand” cellular behaviour and unravel many biological events involved in dynamic cell-material interaction, also within the tumour context.

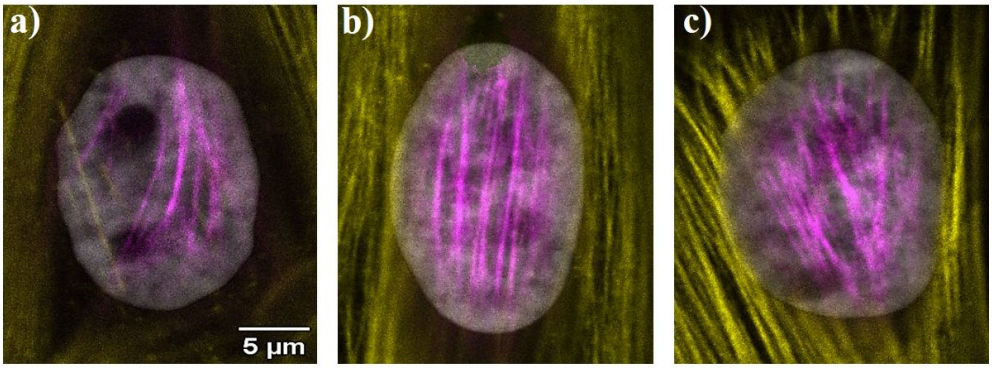

Figure 1. Nucleus (grey) and F-actin (yellow, lateral-associated fibres and magenta, actin cap) of MCF10A cells on flat (a), photo-patterned (b) and cyclically stimulated (c) azopolymer surfaces.

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Extracellular matrix plasticity enables a pro-invasive mechanical cross-talk between cancer cells and cancer-associated fibroblasts.

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The extracellular matrix of tumours undergoes substantial biomechanical alterations during cancer development, including cross-linking events. Crosslinking of the matrix has a pro-invasive role in cancer progression by increasing cancer cell aggressiveness, yet it remains unclear whether these changes affect stromal cells in a manner that promotes or impedes metastasis. To investigate this, we employed intact and lysyl oxidase-induced cross-linked collagen matrices, molecular dynamics simulations, and xenograft mouse models. Our findings demonstrated that the transition from a plastic to an elastic matrix due to crosslinking impaired a pro-invasive mechanical interaction between cancer-associated fibroblasts (CAFs) and cancer cells. Cancer cells migrated in a directed manner in response to the millimetre-scale remodelling caused by CAFs in plastic collagen matrices, which was restricted following cross-linking. Notably, laser ablation of the collagen network disrupted matrix remodelling by CAFs and inhibited the migration of cancer cells, indicating a biomechanical signal. The signal polarised the force between cancer cells and the matrix, favouring the migration of cancer cells towards the origin of the signal. Weakening or strengthening cancer cells contractility inhibited or enhanced their directed migration, respectively. Further, our results showed that linear and nonlinear elastic properties of the matrix were inadequate to account for force transmission at the millimetre scale. Instead, the plastic deformation enabled long-range transmission of the biomechanical signal by permanently reconfiguring matrix fibres through fibre slippage. Our findings identified a new pro-invasive role for CAFs in the tumour microenvironment that was strictly regulated by extracellular matrix plasticity.

Mechanical Homeostasis of Retinal Pigmented Epithelium across Space and Time

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The mechanical properties of tissues are critical to their function and state. Tissue mechanical homeostasis ensures these properties remain within an optimal range, but this balance is continually challenged by extracellular matrix (ECM) remodeling and cellular senescence. These challenges are particularly significant in postmitotic tissues, where the inability of cells to divide necessitates unique temporal adaptations to change.
The retinal pigment epithelium (RPE), a postmitotic tissue crucial for photoreceptor health and vision, exemplifies these dynamics. Using in vivo and in vitro models, we show that ECM-regulated actomyosin contractility in the RPE governs the phagocytosis of photoreceptor outer segments (POS), thereby sustaining mechanical homeostasis. In vivo, we identified a gradient of basement membrane laminins that supports a corresponding gradient of actomyosin contractility, locally influencing tissue functionality [1].
These processes become increasingly relevant with aging, as ECM remodeling coincides with RPE changes. Aging RPE undergoes significant alterations, including cell loss from uncompensated apoptosis, leading to hypertrophic cell accumulation and disrupted monolayer organization, which suggests impaired mechanical homeostasis.
To investigate this further, we developed a novel in vitro model to study the mechanobiology of postmitotic epithelial aging. By inducing apoptosis via controlled caspase-8 activation in mature, low-proliferative hiPSC-derived RPE monolayers, we mimic cell loss observed in aged native RPE. Our model revealed significant biomechanical shifts, transcriptional changes, and functional impairments resembling those in elderly individuals.
Overall, our findings highlight the critical role of RPE mechanical properties in maintaining visual function and emphasize the need to understand mechanical phase transitions over time and space. This work opens new avenues for the development of innovative “mechano-diagnostic” strategies to detect and address pathological aging.

[1] https://doi.org/10.1101/2023.02.24.529913

Mechanical regulation of metastasis by the brain vasculature

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In brain metastasis, cancer cells often remain in close contact to the existing vasculature and travel using blood vessels as migratory paths – a process known as vessel co-option. Although it promotes the dissemination of metastatic cells within the brain, the mechanisms regulating this form of migration are poorly understood. Combining the use of ex vivo brain slices and an organotypic in vitro model for vessel co-option, we show that adherent cells invade brain tissue most rapidly when moving along the vasculature. To migrate, cells adhere to the vascular basement membrane extracellular matrix (ECM) through focal adhesions. However, providing this layer of ECM alone fails to replicate the cell migration patterns observed during vessel co-option. By selectively modulating the stiffness of the vascular network using synthetic hydrogels, we show that cancer cells also sense and respond to vascular stiffness, where a stiffer network promotes a faster invasion through the brain. We found that vessel co-option is enhanced by both the stiffness of brain vasculature which reinforces focal adhesions through a talin-dependent mechanism, and the softness of the surrounding environment that permits cellular movement. Our work reveals a mechanosensing mechanism that guides cell migration in response to the tissue's intrinsic mechanical heterogeneity, with implications in cancer invasion and metastasis.

Mechanobiology and Bone Disease: Uncovering Novel Mechanisms in Osteoporosis and Cancer Bone Metastasis

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Biophysical stimuli are crucial for bone development and function, from the earliest embryo and throughout life. Osteoporosis is a devastating disease, and many drug treatments fall short. Our research has shown that osteoporosis is not simply a disease of bone loss. We revealed that bone tissue composition and the mechanical environment of bone cells are altered osteoporosis. We uncovered changes in the mechanosensation mechanisms of bone cells in estrogen deficiency, which may play an important role in disease progression. Recently, we developed ex-vivo mechanobiological models of bone tissue, which incorporated multicellular niches within 3D matrices and a bioreactor. We applied these models to study bone cell activity governing bone loss and osteogenesis in estrogen deficiency and to investigate treatment approaches.

Our research has also advanced understanding of breast cancer-bone metastasis. We conducted temporal and spatial analysis of bone composition and mechanical properties. In combination with computational mechanoregulation theory, we predicted that early changes in bone tissue properties may drive extensive osteolysis in late-stage metastasis. Applying our ex-vivo bone models to study breast cancer cell invasion into bone tissue, we revealed the synergistic influence of osteoclasts, osteoblasts, and the mechanical environment on tumor growth and osteolysis.

The Border Zone in Myocardial Infarction: a mechanobiological analysis at the cellular and supracellular scales

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Myocardial infarction, commonly known as a heart attack, occurs when a local region of the heart muscle (myocardium) suffers from insufficient blood supply. The resulting lack of oxygen leads to the local death of vast numbers of cardiomyocytes, the cells responsible for the myocardium's cyclic contraction that ensures heart beating. Lost cardiomyocytes are replaced by colonies of cardiac fibroblasts activated by the pathological pro-fibrotic signalling that follows infarction. Cardiac fibroblasts eventually stiffen the injured myocardial tissue through increased mechanical stress and collagen deposition, preserving myocardial wall integrity in the immediate aftermath of infarction but ultimately leading to scar formation and adverse ventricular remodelling over time. Between the infarcted myocardial tissue and the healthy non-infarcted tissue lies the border zone, a transitional region with a hybrid physiological/pathological phenotype. This area shows impaired contractility, as the cardiomyocytes are still physiologically viable but pathologically hypocontractile. Importantly, we are learning that the border zone can act as a hub for fibrosis, gradually spreading from infarcted regions to healthy ones and potentially leading to a worsening of the patient's prognosis. I will show how we create in vitro mimics of the post-infarct myocardium by using cardiomyocytes and cardiac fibroblasts derived from human pluripotent stem cells. Upon systematically quantifying the mechanobiological behaviour of the post-infarct cardiac microtissues at both cellular and supracellular scales, we identify a physical mechanism that can sustain the expansion of the border zone in the infarcted heart.

Computational modelling of mechano-mediated cardiovascular formation, growth, and remodeling

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Cells in the cardiovascular system are constantly exposed to various mechanical cues. It is thus not surprising that mechanobiological mechanisms play a crucial role in cardiovascular processes, from blood vessel formation to growth and remodeling of vessels and heart valves. Fully understanding and controlling these mechanisms can lead to developing successful strategies to restore diseased tissues or create functional tissue-engineered replacements. Mechanistic computational models addressing cell mechanobiology can have a groundbreaking impact in this context; they can unravel and inspire new experiments and optimize regenerative medicine strategies.

Via computational modelling, we showed that cytoskeleton-regulated YAP/TAZ nuclearization mediates the effects of stiffness on endothelial cell-cell (Notch) signaling and blood vessel formation [1]. Once formed, the vessel wall thickness grows up to a homeostatic thickness determined by mechanoresponsive Notch [2]. Notch mechanoregulation also mediates the increase in wall thickness in response to hypertension [3,4]. Our simulations indicate that Notch manipulations can indeed steer growth and remodeling of hypertensive [3,4] and tissue-engineered blood vessels [5]. Mechanical cues play a crucial role also for tissue engineering of heart valves. Via computational models accounting for cell and collagen mechanoregulation, we could predict the remodeling of native and tissue-engineered heart valves [6,7]. The simulations led to a breakthrough in the design of tissue-engineered valves, ensuring their functionality with a follow-up of one year [8]. Overall, our studies demonstrate that computational modelling of cell and tissue mechanobiology can accelerate discoveries and have a groundbreaking role in cardiovascular regenerative medicine.

[1] Passier et al. NPJSBA, 2024;
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[4] van Asten et al. BMMB 2023;
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[6] Ristori et al. Acta Biomaterialia 2018;
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[8] Emmert et al. Sci Adv Trans Med 2018.

Harnessing geometry and mechanics to engineer functional musculoskeletal microtissues

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Musculoskeletal tissues are structurally optimized to sustain the complex biomechanical loading patterns they are subjected to throughout daily life activities. The cells in these tissues are highly mechanosensitive, and balance tissue growth, maintenance, and remodeling in response to various biophysical cues. However, musculoskeletal injuries and degenerative diseases that disturb this balance are becoming more prevalent in the aging population, resulting in pain, disability, and a large socio-economic burden. Our group integrates engineering and biology to develop novel in vitro, in silico and ex vivo (micro-)tissue platforms. We use these to enhance our understanding of musculoskeletal tissue mechanobiology and organization, and to develop regenerative strategies that help restore biomechanical functionality.

By engineering bone-like tissues on rationally structured biomaterial surfaces in vitro, we have shown that local geometry (curvature), mediated by cell contractility, plays an important role in the spatial organization of cells and their extracellular matrix [1,2]. This has implications for the design of biomaterial scaffolds used for tissue regeneration. Using organoid models [3], we are exploring how directional growth-induced stresses control collagen organization in articular cartilage, which is indispensable for its mechanical properties [4]. We take inspiration from processes during postnatal development, and try to harness those to engineer larger tissue constructs in a bottom-up fashion. Furthermore, we design and employ a variety of dynamic bioreactors to mechanically stimulate engineered and ex vivo tissues during culture, including osteochondral tissue, tendons, and intervertebral discs [5]. Combined, our efforts help us understand how (patho-)physiological loading

contributes to tissue growth, remodeling, and degeneration, and guides our development of novel tissue engineering strategies that can restore musculoskeletal tissue functionality.

[1] Callens et al., Nat. Comm. (2023); [2] Callens et al., in preparation; [3] Crispim & Ito, Acta Biomater. (2021); [4] Peters et al., in revision; [5] Salzer et al., JOR Spine (2023)

Hypertensive Pressure Mechanosensing Triggers Transdifferentiation of Vascular Smooth Muscle Cells to Foam Cells

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Arterial vascular smooth muscle cells(VSMCs) play central role in the onset and progression of atherosclerosis, where they undergo phenotypic switching by downregulating vSMC-specific genes and transition to a different cell type [1]. Our previous work indicated that vSMC respond to hypertensive pressure and differential extracellular matrix compliance, exhibiting an atherosclerotic phenotype [2]. Our aim here was to study pressure sensing in further detail.

A7R5 rat vSMC were plated on glass or 1kPa-polydimethylsiloxane and subjected to hypertensive pressure(HT; 200/120mmHg). Hyperspectral Stimulated Raman Spectroscopy(hsSRS) was applied for lipid imaging. Scratch, proliferation, TUNEL assays and RT-PCR were used to verify the foam cell phenotype. Electroporation, Western-Blot, immunocytochemistry were used to visualize Piezo1 channel. CUT&Tag analysis were applied for Piezo1-driven gene regulation changes. Lamin-associated heterochromatin was visualized with Transmission Electron Microscope. Nanoindentation was used to examine nuclear stiffness. Mouse tissue, primary rat and human vSMC were used to confirm the A7R5 findings.

Following hypertensive pressure stimulation, we observed lipid droplet accumulation in vSMC as indicated by hsSRS($p>0.05$; Fig.1). Similar result was obtained upon acute vSMC Piezo1, mechanosensitive channel, stimulation($p>0.01$). Further results demonstrate upregulation of CD68, KLF4, LDLR and downregulation of ABCA1 genes($p>0.05$), higher proliferation rate($p>0.001$) and lower cell motility($p>0.01$).; characteristics specific to foam cells, indicating that Piezo1 $Ca2+$ transients can trigger vSMC-to-foam cell transition. We identified that nuclear Piezo1 activation leads to changes in nuclear $Ca2+$ uptake, reactive oxygen signalling, downregulation of histone-3-lysine-9-trimethylation($p>0.001$) and softer nucleus($p>0.05$). CUT&Tag experiment confirmed Piezo1-epigenetic regulation of VSMC phenotypic switching.

These results show that vSMC-to-foam cell transition can be triggered by mechanical stimulation.

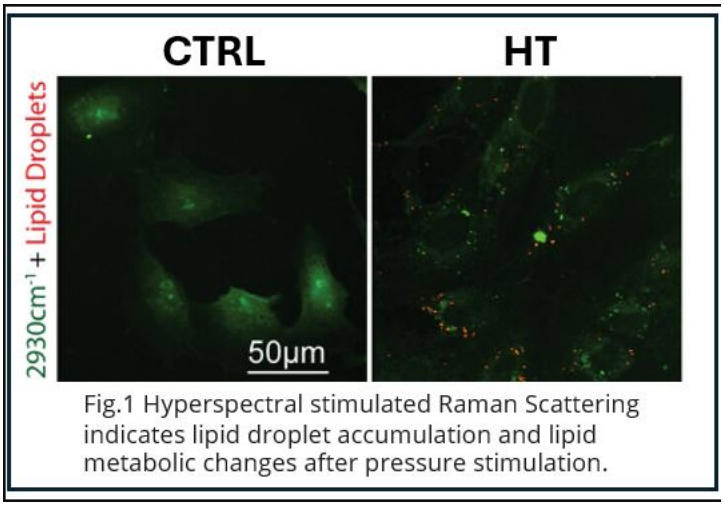

Fig.1 Hyperspectral stimulated Raman Scattering indicates lipid droplet accumulation and lipid metabolic changes after pressure stimulation.

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[2] Swiatlowska P, et al., "Pressure and stiffness sensing together regulate vascular smooth muscle cell phenotype switching". Science Advances 2022, doi:10.1126/sciadv.abm3471

Oncogenic molecular features triggered by the mechanoresponsive polycystin proteins in solid tumours

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Mounting evidence indicates that mechanical signals play a critical role in regulating cancer cell behaviour. Polycystins (mainly polycystin-1, PC1 and polycystin-2, PC2) are emerging as key proteins that orchestrate the overall mechanosensitivity of cells. We investigated the expression of these two mechano-induced proteins in human tissues from breast, glioma, and prostate solid tumours via immunohistochemistry. The results revealed that the expressions of PC1 and PC2 are mutually positively correlated. Increased expression of both proteins was identified in the peri-necrotic and peri-vascular areas of glioma tissues. PC2 also displays increased expression in grade 3 and 4 compared to grade 2 tumours. Both proteins were correlated with extraprostatic extension, PC2 was associated with infiltration of seminal vesicles and advanced stage in prostate cancer. In breast cancer, PC1 presents increased expression in Her2- patients. PC2 expression was correlated with the expression of progesterone receptor and PC2 lower expression was associated with prolonged survival in Her2+ patients. To evaluate the impact of polycystins' expression on cancer cell properties and behaviour, we conducted functional assays on respective cancer cell lines. PC1 gene (PKD1) silencing decreased the proliferation rate and migration potential of cancer cells. To further corroborate our results, we developed tumours in NSG mice and treated them with IgPC1, which serves as a functional inhibitor of PC1 protein. The treated mice exhibited a decrease in tumour volume compared to the untreated ones. Additionally, lower cell proliferation and higher tissue

necrosis was revealed in the treated tumours. In conclusion, our data suggest that polycystins play a role in tumour development and influence progression of the disease across various cancers, potentially acting as oncogenic factors.

A Tour de Force through Cellular Nanoscale Mechanobiology in (Patho)Physiology

Matteo Chighizola[2,3], Hatice Holuigue[2], Tania Dini[2,3], Mirko D'Urso[2,4], Stefano Marchesi[3], Stefania Marcotti[5], Francesca Borghi[2], Claudio Piazzoni[2], Nicholas Kurniawan[4], Brian Stramer[5], Giorgio Scita[3], Cristina Lenardi[2], Paolo Milani[2], Giuseppe Diaferia[6], Alessandro Podesta[2], and **Carsten Schulte**[1,2]
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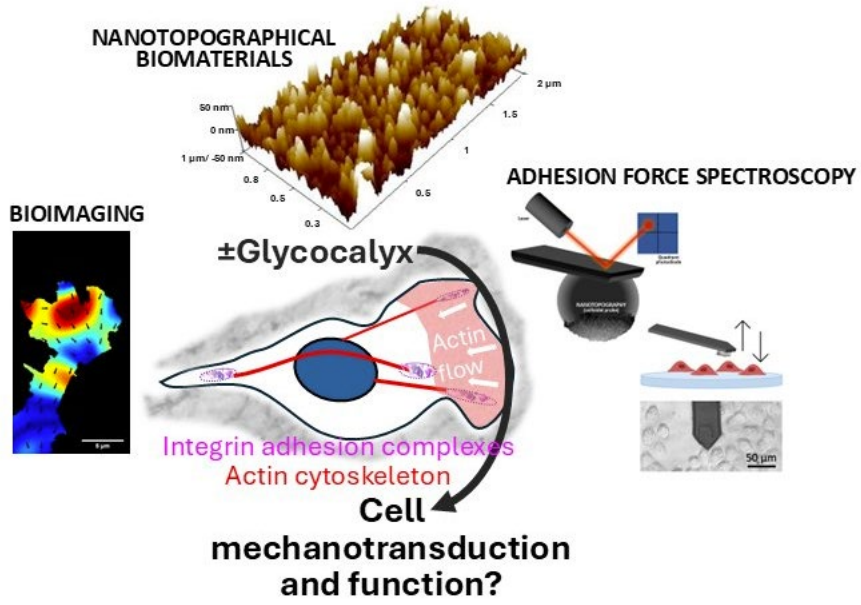
Albeit it is an acknowledged concept nowadays that mechanosensing/transduction strongly affect cellular behaviour in health and disease, there is an urgent need to better understand the intricate underlying force-based cell/microenvironment dialogue.

By applying versatile interdisciplinary approaches (integrating techniques, such as nanoengineering, advanced bioimaging, adhesion force spectroscopy, and omics), we dissected 1) integrin-mediated and nanotopography-sensitive events at the cell/microenvironment interface, and 2) their effect on cellular shape, differentiation and migration, studying e.g. different cell types from a neuronal context and murine embryonic stem cells.

We found a strong impact of the nanotopography and the glycocalyx on force loading-related processes that affect mechanotransductive processes and cellular responses. The glycocalyx configuration, e.g., influences the way the cell perceives its biophysical microenvironment with effects on migratory behaviour [1, and unpublished]. We furthermore established a proof-of-principle approach that enables the measurement of cellular interaction with native extracellular matrix at the nanoscale and piconewton range by atomic force microscopy [2].

We aspire to leverage such approaches to further our comprehension of how these force-related mechanotransductive processes are involved in pathophysiological situations, such as brain cancer and inflammatory responses.



[1] Chighizola et al. 2022 Journal of Nanobiotechnology, doi: 10.1186/s12951-022- 01585-5
[2] Holuigue et al. 2023 Nanoscale, doi: 10.1039/D3NR01568H

3D Biomimetic piezoelectric scaffolds-based therapeutic approach for volumetric muscle loss repair

Oana Oana Dobre[1], Xenofon Karagiorgis[2], Gerasimos Balatsoukas[1], Merna Maung[2], and Aleixandre Rodrigo-Navarro[3]
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Volumetric muscle loss (VML), a debilitating disease that poses a significant challenge to regenerative medicine. Current treatments, often relying on acellular scaffolds, frequently lead to fibrotic tissue formation, limiting functional recovery. We investigate potential of bio-piezoelectric scaffolds, a novel class of biomaterials that mimic the natural electrical and mechanical properties of muscle tissue, to revolutionize VML repair. Muscle tissue is not a passive structure but a dynamic environment constantly responding to electrical and mechanical cues. These signals are crucial for regulating muscle growth, repair, and function, are often absent in conventional scaffolds.

Our research focuses on the development of 3D biomimetic piezoelectric scaffolds, composed of a biodegradable and biocompatible polymer from the Polyhydroxyalkanoates (PHA) family. Electrospun fibers, incorporated into an extracellular matrix (ECM) hydrogel, provide a robust and biocompatible scaffold that mimics the mechanical properties of muscle tissue. Importantly, PHAs exhibit a piezoelectric effect, generating an electrical charge upon mechanical deformation. This intrinsic property allows the scaffold to respond dynamically to muscle contraction and relaxation, developing a bio-electric environment that closely resembles the native tissue.

In vitro studies using myoblast cells demonstrate enhanced cell adhesion, proliferation, and differentiation within the 3D bio-piezoelectric scaffolds. These cells exhibited increased expression of muscle-specific markers, such as MyoD, indicative of robust muscle regeneration. Preliminary in

vivo studies using a mouse model of VML showcase the potential of bio-piezoelectric scaffolds for inducing functional muscle regeneration. The scaffolds promoted the formation of new muscle tissue, improving muscle function and reducing fibrosis.

Controlled in-vitro ultrasound stimulation enhances actin and vinculin expression in osteoblast-like cells

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The first step of mechanotransduction, termed mechanosensing, involves cells detecting external mechanical signals, such as physical forces from exercise or gravity, ultrasonic waves, and electromagnetic waves, aiding bone cell adaptation to environmental changes at a genetic level [1]. The expression of integrins upon ultrasound exposure has been studied in the past with results suggesting that integrins are involved in sensing the ultrasonic signals [2].

Low intensity pulsed ultrasound (LIPUS) is approved by the FDA and NICE as an adjunctive treatment for accelerating healing in fresh bone, delayed union and non-union fractures however effectiveness is uncertain [3]. Attempts to optimise LIPUS parameters for fracture healing have yielded inconsistent results, due to standing waves that vary based on the cell culture platforms employed [4]. To overcome this, our group developed an ultrasound-compatible cell culture platform that provides a controlled ultrasound field, enabling the controlled and measured investigation of ultrasound effects on MG63 osteoblast-like cells.
This study examined the effects of LIPUS on the expression of vinculin, a mechanosensitive protein, and actin filament expression via immunofluorescence staining. A significant increase in the expression of vinculin as well as actin was observed when cells were subjected to ultrasound at higher intensities than previously seen. Ultrasound pressure, pulse repetition and duty cycle may be adjusted to optimise upregulated expression. LIPUS promotes cytoskeletal organisation and adhesion in MG63s, essential components in promoting functions related to mechanically stimulated bone repair. By applying a controlled and measured dosage of ultrasound to cells, this research establishes a reproducible in-vitro model for exploring the mechanobiological responses of cells to LIPUS as well as providing guidance for optimising therapeutic ultrasound to better understand the mechanisms involved in ultrasound-mediated mechanotransduction and developing an effective treatment.

[1] https://doi.org/10.3390/ijms241814326
[2] https://doi.org/10.1242/jcs.192781.
[3] https://doi.org/10.1136/bmj.j656.
[4] https://doi.org/10.1016/j.ultrasmedbio.2022.05.001

Guiding Mechanotransduction in Blood Vessels

Ellie Tzima[1]
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The Tzima lab investigates the role of mechanotransduction in regulating cardiovascular function in health and disease. This talk will focus on the recent discovery of a new class of mechanosensors which determine which determine vascular function in development, homeostasis and pathology.

Mechanobiology of Cancer Metastasis and Ageing: Insights from Microfluidic and Biophysical Models

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I discuss the mechanobiological principles governing cancer metastasis, focusing on cell interactions within the tumour microenvironment, intravasation, and extravasation. I present how microfluidic and in vitro models reveal the role of extracellular matrix mechanics in regulating cancer cell behaviour. Key findings include the modulation of cancer cell invasion by the biomechanical properties of endothelial and subendothelial matrices, which influence transendothelial migration. By exploring the forces that drive cancer metastasis, the presentation highlights new therapeutic targets and methodologies for understanding tumour progression and metastasis. Finally, I discuss how 3D vascular microfluidic models can be utilised to explore the mechanics of vascular morphogenesis in development and ageing.

Mechanobiology Shaping Life:
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