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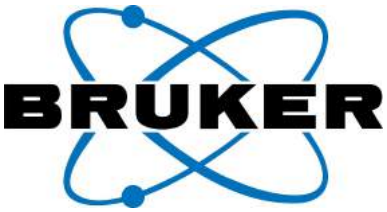
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1. Program

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14:45-15:00	**Bart Vos**: *Exploiting Onsager regression in passive measurements to reveal active mechanics of living systems*
15:00-15:30	**Léa-Laetitia Pontani**: *Emulsions as a tool to investigate tissue mechanics in vitro and in vivo*
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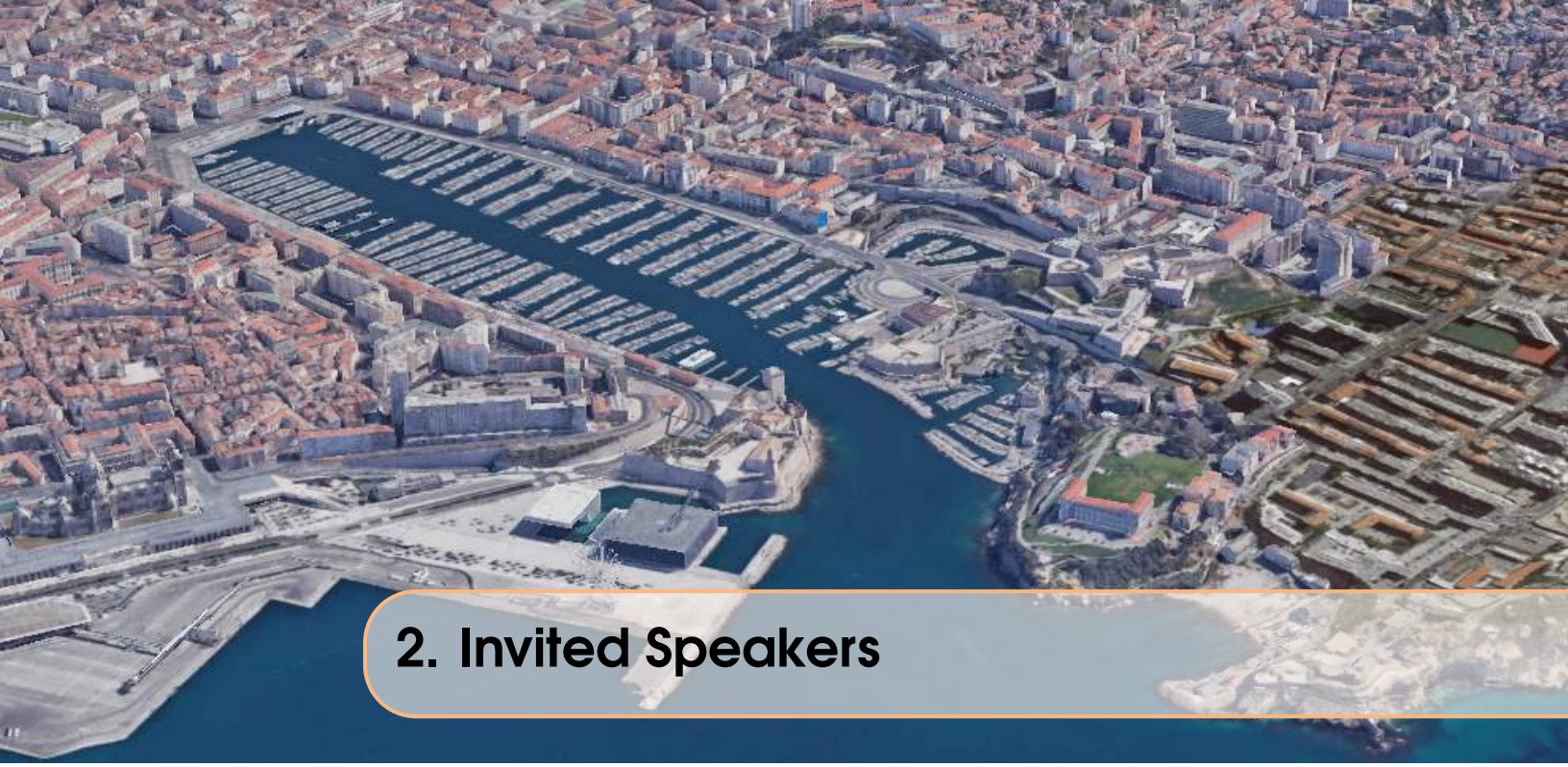
12:00-12:30	TALK ORGANIZERS



2. Invited Speakers

2.1 Pierre-François LENNE

Institut de Biologie du Développement de Marseille Luminy CNRS UMR 7288 Parc scientifique et technologique de Luminy - case 907 13288 Marseille cedex 09 - France

Shaping cell contacts during tissue morphogenesis

During morphogenesis, cell contacts are constantly remodeled, undergoing irreversible deformations. This stems from active contractile forces that work against adhesive forces at cell contacts. I will emphasize three aspects of cell contacts' mechanics during tissue morphogenesis: the time dependence of material properties, the importance of geometry, and feedback between adhesion and cytoskeletal mechanics.

2.2 Lea Laetitia PONTANI

Sorbonne Université, CNRS, Institut de Biologie Paris-Seine (IBPS), Laboratoire Jean Perrin (LJP), Paris, France

Emulsions as a tool to investigate tissue mechanics in vitro and in vivo

We develop biomimetic emulsions to understand the physical basis of collective remodeling in biological tissues. In particular, we focus on the interplay between adhesion and extrinsic mechanical forces to control the emergence of tissue architecture during morphogenesis. Therefore, we design our emulsions to reproduce the passive mechanical and adhesive properties of cells in biological tissues and we study their elasto-plastic response to an applied mechanical perturbation. In simple microfluidic constrictions we find that adhesion alone can polarize the droplets in the direction of the flow, indicating that adhesion alone could induce in-plane cell polarization in elongating tissues. We also showed that a percolation transition in the network of adhesive contacts was sufficient to control the collective deformation of droplets in static packings. In biological tissues, this indicates that tuning the adhesion properties in a limited number of cells around this transition could be sufficient to modify globally the mechanical properties of the tissue. We are now exploring how adhesion hierarchy inside such systems can lead to self-organization of the biomimetic tissue through repeated mechanical stimulations. In particular, we uncover a predominant role of adhesion differentials in the emergence of deformation and polarization of the biomimetic emulsions. Finally, in parallel to these in vitro approaches, we use oil droplets as force sensors in vivo, in developing zebrafish embryos. In particular, the injection of biocompatible oil droplets in their olfactory placode allowed us to measure the presence of anteroposterior compressive forces that can contribute to axon elongation in olfactory neurons. We are currently developing biocompatible self-functionalizing droplets in order to obtain the full force map in the placode and in surrounding tissues during development.

2.3 Marie-Hélène VERLHAC

Center for Interdisciplinary Research in Biology, Collège de France, CNRS, INSERM, Université PSL, Paris, France.

Cytoplasmic forces organize the oocyte nucleus across scales

Cells remodel their cytoplasm with force-generating cytoskeletal motors. Their activity generates random forces that stir the cytoplasm, agitating and displacing membrane-bound organelles like the nucleus in somatic and germ cells. These forces are transmitted inside the nucleus, yet their consequences on liquid-like biomolecular condensates residing in the nucleus remain unexplored. Here, we probe experimentally and computationally diverse nuclear condensates, that include nuclear speckles, Cajal bodies, and nucleoli, during cytoplasmic remodeling of female germ cells named oocytes. We discover that growing mammalian oocytes deploy cytoplasmic forces to timely impose multiscale reorganization of nuclear condensates for the success of meiotic divisions. These cytoplasmic forces accelerate nuclear condensate collision-coalescence and molecular kinetics within condensates. Inversely, disrupting the forces decelerates nuclear condensate reorganization on both scales, compromising condensate-associated mRNA processing and consequently hindering oocyte divisions that drive female fertility. We establish that cytoplasmic forces can reorganize nuclear condensates in an evolutionary conserved fashion in insects. Our work implies that cells evolved a mechanism, based on cytoplasmic force tuning, to functionally regulate a broad range of nuclear condensates across scales. This finding opens new perspectives when studying condensate-associated pathologies like cancer, neurodegeneration and viral infections.

2.4 Matteo RAUZI

University Côte d'Azur, France

Cell and tissue mechanics driving epithelial tube formation

The formation of epithelial tubes is essential to build organs responsible to direct vital factors outside-in or inside-out as well as within animals (e.g., food and water through the gut, air through the lungs, or blood in blood-vessels). Therefore, tube formation plays a critical role in multicellular life, organized in stratified layers and in which an inside and an outside are established. Unveiling the mechanisms and mechanics responsible for tube formation is key to understand the emergence of complex life forms and to decipher how tubulogenesis disorders that result from tube formation failure (e.g., spina bifida, polycystic kidney, tracheal atresia) may emerge. Tube formation often results from inpocketing: the bending of a tissue patch forming a pocket like shape. Nevertheless, the mechanisms and mechanics responsible for tissue inpocketing are not well understood. To dissect and study this process we use the sea urchin Paracentrotus lividus embryo: an ideal playground for biophysicists and a powerful model system to test and measure cell surface mechanics. More specifically, we will focus on better understanding the formation of the archenteron: a tubular epithelial structure that emerges from the inpocketing of the embryo vegetal plate eventually forming the digestive tube of the sea urchin larva. By implementing ultrashort infrared laser pulses coupled to 4D multi-view light sheet microscopy, μ-pipetting and μ-indentation, multidimensional image analysis and mathematical modelling, this work shines new light on the cell and tissue mechanics driving epithelial inpocketing for tube formation.

2.5 Anna LABERNADIE

Cell Behaviour and Tissue Bioengineering Lab CENTRO DE INVESTIGACIÓN PRINCIPE FELIPE, Calle de Eduardo Primo Yúfera, 3, 46012 VALENCIA (Spain)

Tumor-Stroma Interactions: Role in cancer spreading and immunotherapy outcomes

The tumor microenvironment (TME) is a complex array of cellular and non-cellular components including cancer-associated fibroblasts (CAFs), blood vessels, immune cells and the extracellular matrix (ECM) that tightly interact with cancer cells. Increasing evidence reveals that physical interactions as critical as chemical crosstalk can drive tumor dissemination and immunotherapy resistance. In the first part of the presentation, we will focus on the role of TME in tumor spreading. Using combined biophysical techniques and molecular tools we demonstrated the existence of force transmission between cancer-associated fibroblasts (CAFs) and cancer cells mediated by a heterophilic junction between E-cadherin expressed by cancer cells and N-cadherin expressed by CAFs. We discovered that this adhesion was mechanically active and enabled CAFs to guide collective cell migration and initiate cancer cell invasion. The second part of the talk will focus on the role of the TME in cancer immunotherapy outcomes. I will present our newly designed Micro Immune Response On-chip (MIRO) device that recapitulates a stromal barrier to assess immunotherapy efficacy. Using a HER2+ breast tumor model we show that the use of MIRO allows monitoring immune infiltration through the stromal barrier and quantifying Antibody-Dependent Cellular Cytotoxicity (ADCC) response upon drug treatment. Our study reveals that MIRO could offer a versatile tool to unveil fundamental features of the TME architecture driving immune response and a promising preclinical tool for precision medicine.

2.6 Ben FABRY

Department of Physics Friedrich-Alexander-Universität Erlangen-Nürnberg Erlangen, Germany

Mechanotransduction in immune cells

To reach targets outside the bloodstream, immune cells can extravasate and migrate through connective tissue. During tissue infiltration, immune cells migrate in an amoeboid fashion, characterized by weak matrix adhesions and low traction forces, that allows them to achieve high migration speeds of up to 10 μm/min. How immune cells reconcile amoeboid migration with the need to overcome steric hindrance in dense matrices is currently not understood. Here we show that when confronted with steric hindrance, immune cells can switch from their default amoeboid migration mode to a highly contractile, mesenchymal-like migration mode. We use time-lapse confocal reflection microscopy to obtain simultaneous measurements of migration speed, directional persistence, and cell contractility in 3D biopolymer networks. We find that NK92 (natural killer) cells are highly mechanoresponsive and exert substantial acto-myosin driven, integrin-mediated contractile forces of up to 100 nN on the extracellular matrix during short contractile phases. This burst-like contractile behavior is also found in primary B, T, NK cells, neutrophils, and monocytes, and is specifically used by the cells to avoid getting stuck in narrow pores of the surrounding matrix. Our results demonstrate that steric hindrance guides the rapid regulation of integrin-mediated adhesion to the ECM in a large number of immune cell subtypes.

2.7 Daniel COHEN

Princeton University Omenns-Darling Bioengineering Institute, and Department of Mechanical and Aerospace Engineering

Chiens de berger' cellulaires: understanding and controlling collective cell behaviors

Collective cell behaviors are fundamental to the coordinated migration, proliferation, and decision-making of cells in a multicellular organism. Imagine if we could program these behaviors, effectively 'herding' cells to produce patterns, grow organs, and accelerate healing. Our research focuses both on developing an understanding of how collective cell behaviors work and how we can better control them. This seminar will present three aspects of our work:

1. defining the 'rules' of large-scale collective cell migration;
2. new bioelectric tools to interactively control tissue, organoid, and stem cell colony physiology;
3. materials to manipulate collective cell behavior by targeting cell-cell adhesion.

2.8 Robert INSALL

Dept of Cell and Developmental Biology, University College London, Gower Street, London WC1E 6BT, UK

Self-generated chemotaxis - cells discovering their environments by steering themselves

Chemotaxis plays a crucial role in influencing cellular behavior during both developmental and adult phases. Additionally, it is implicated in various diseases and pathological conditions. While most chemotaxis studies focus on how cells passively interpret externally imposed gradients, we have directed our attention toward self-generated gradients. Here, cells establish gradients as they migrate, essentially creating their own signaling cues. This unique approach results in intricate scenarios that defy intuition and necessitate in-depth mathematical modeling for comprehension. These self-generated gradients yield remarkably satisfying outcomes. Notably, they enable cells to remotely detect environmental features, for example solving complex mazes. Intriguingly, certain self-generated gradients repel cells instead of attracting them. This phenomenon poses an unexpectedly challenging puzzle, prompting an exploration of interactions between multiple chemoattractants detected by the same receptors. Furthermore, cells can introduce fresh information by emitting secondary signals, leading to intricate and captivating outcomes. These intricate interactions empower cells to leverage simple guidelines to orchestrate complex and unforeseen migration patterns, akin to those observed during embryonic development.

2.9 Arjun NARAYANAN

New York University, Abu Dhabi

The physical chemistry of Actomyosin cortex self-assembly in C elegans oocytes

As the C. elegans oocyte begins to divide, it must first fill the region just under its plasma membrane with a contractile and dynamic meshwork of actomyosin filaments. But how is this first actomyosin cortex put together from constituent molecules? I will describe the discovery that in C. elegans oocytes, actomyosin cortical assembly relies on the emergence of thousands of short-lived protein condensates rich in actin filaments, and filament nucleators. We extract empirical growth laws governing the composition dependent chemical dynamics of these condensates. These growth laws show that - in contrast to condensate growth via diffusion - the growth dynamics of cortical condensates are chemically driven. Remarkably, the associated chemical reactions obey mass action kinetics despite governing both composition and size. This coupling of mass action kinetics and assembly kinetics allows the C.elegans oocyte to prevent runaway actomyosin filament nucleation as it rapidly assembles its first cortex. Importantly, the empirical growth laws reveal novel principles of intracellular physical chemistry likely to be applicable across cell biology.

2.10 Britta TRAPPMANN

Bioactive Materials Laboratory Max Planck Institute for Molecular Biomedicine Münster, Germany

Cellular mechanosensing within 3D extracellular matrices

Cell fate decisions are influenced by many cues, which together constitute the cell microenvironment. One critical regulator is the extracellular matrix (ECM), which varies not only in composition, but also in physical properties such as stiffness. The impact of matrix stiffness on cell spreading and differentiation has been studied intensively on 2D surfaces using synthetic hydrogels, but very little is known about stiffness sensing within more complex 3D matrices. Here, a major hurdle is to isolate the role of ECM stiffness from other matrix properties, in particular degradability. If cells are fully encapsulated, changes in bulk stiffness also influence the amount of matrix crosslinks that a cell has to cleave in order to spread and interact with its surroundings, impacting cell shape and function. Here, we have developed a sugar-based hydrogel system that offers independent control over mechanical properties, adhesive ligand density and matrix degradation rates. Using this system, we study the impact of matrix stiffness and degradability on cell spreading, mesenchymal stem cell differentiation and angiogenic sprouting. In particular, we demonstrate that matrix degradability, mechanics and adhesivity jointly control the multicellularity of 3D endothelial cell invasion.

2.11 Vincent STUDER

Institut interdisciplinaire de neurosceinces, CNRS, université de Bordeaux, France

Unveiling Neuronal Adhesion Complexes Through Subcellular Micropatterning

Understanding neuronal adhesion complexes establishment and stability is pivotal for unraveling the mechanisms underlying synaptic formation and plasticity. In this context, our research employs an innovative approach using subcellular micropatterning. This technique enables precise manipulation of cellular components, offering an unparalleled means to explore intricate interactions between neurons and their environment. By unveiling molecular structures and dynamic interplays within neuronal adhesion complexes, we aim to shed light on key processes involved in synaptic development. Additionally, we will highlight the broader relevance of our methodology by showcasing its applicability to various other cell types such as cancer or immune cells. This presentation provides a comprehensive overview of our methodology and preliminary findings, demonstrating its potential impact on advancing our understanding of fundamental brain mechanisms and its versatility in investigating diverse areas of biology.

2.12 Julie PLASTINO

Laboratoire de Physique de l'Ecole Normale Supérieure, ENS, Université PSL, CNRS, Sorbonne Université, Université Paris Cité

Mechanical roles for actin and the nucleus in cell invasion

Invasion of cells through basement membrane (BM) extracellular matrix barriers is an important process during organ development and cancer metastasis. The biochemical aspects of cell invasion have been well-studied, but the mechanical aspects, particularly as concerns the role of the nucleus, the largest and stiffest organelle of the invading cell, are less understood. Here we study an invasion event, anchor cell (AC) invasion, which occurs during the development of the nematode Caenorhabditis elegans. Due to the simplicity of genetically modifying and imaging the worm, this approach allows for a quantitative evaluation of in vivo cell invasion. We find that, during invasion, the AC deforms the BM barrier with its actin-rich protrusion. The protrusion exerts forces, estimated in the tens of nN range, which are key for breaking through the BM. Force production is driven by actin polymerization nucleated by the Arp2/3 complex and its activators, while formins, crosslinkers and myosin motor activity are dispensable. Furthermore we find that interfering with the protein components (the LINC complex) that link the cytoskeleton to the nucleus leads to reduced AC invasion efficiency. We note that AC nuclei with altered connection to the cytoskeleton are round, while wild type are deformed. We observe a similar round nucleus phenotype by reducing the actin network. Putting all our results together, we conclude that the actin protrusion is applying forces both on the BM and on the nucleus, and that this interplay is important for invasion efficiency. Overall this study demonstrates that the cytoskeleton-nuclear connection is important for BM disruption by invading cells.

2.13 Yekaterina MIROSHNIKOVA

NIDDK/NIH, USA

Nuclear mechanotransduction and stem cell fate regulation

During embryonic development and organogenesis, large-scale changes in tissue elongation, stretching, compression, folding/buckling, and budding impact the shape, position, packing, and contractility of cells. Conversely, changes in single cell contractility, shape and position locally alter tissue organization and mechanics. Thus, forces function as important ques that are transmitted to the nucleus to coordinate gene expression programs to control cell states. In my presentation I will discuss our recent work that implicatesmechanical force, by activating biochemical signaling in the cytoplasm as well as triggering mechanotransduction through nuclear deformation, in remodeling nuclear architecture, chromatin state, and global gene expression patterns in both somatic and embryonic stem cells. We show that cell deformation leads to rapid changes in gene activity and transcription, that are strictly dependent on the biochemical context and that have long term impact on cell states. Collectively this work reveals how mechanosignals are integrated with biochemical inputs to alter cell states and to generate and maintain tissue architecture.

2.14 Ana-Suncana SMITH

Institute of Theoretical Physics, Erlangen

Mechano-sensitivity of epithelium across scales

Despite the primary role of cell proliferation in tissue development and homeostatic maintenance, the interplay between cell density, cell mechanoresponse, and cell growth and division is not yet understood. In this talk, I address this issue by reporting on an experimental investigation of cell proliferation on all time- and length-scales of the development of a model tissue, grown on collagen-coated glass or deformable substrates until homeostasis. Through extensive data analysis, we demonstrate the relation between mechanoresponse and homeostasis. We also evaluate the probability for cell division, as a function of the local cell density. Motivated by these results, we construct a minimal model of cell proliferation. By parametrizing the growth and the dividing phases of the cell cycle, and introducing such a proliferation model in dissipative particle dynamics simulations, we recover the mechanoresponsive, time-dependent density profiles in 2D tissues growing to macroscopic scales, as well as the organisation of the homeostatic state. The results show that the mechanoresponse on the level of a constitutive cell and its proliferation results in a matrix-sensitive active pressure, as evidenced by adapted Fisher-Kolmogorov equations. Together with local regulation of cell shape, the active pressure evokes massive cooperative displacement of cells in the invading tissue and is a key factor for developing large-scale structures in the steady state.

2.15 Marino ARROYO

Universitat Politècnica de Catalunya Institute for Bioengineering of Catalonia (IBEC) International Centre for Numerical Methods in Engineering (CIMNE)

Emerging spatiotemporal patterns of actomyosin gels across scales

The actomyosin cytoskeleton is remarkably polymorphic and multifunctional, enabling movement, force generation and morphogenesis from sub-cellular scales to organs. To adopt different architectures and perform different functions, it relies on emergence and self-organization. In this talk, I will describe theoretical and computational models of actomyosin gels that allow us to understand the emergence of spatiotemporal patterns from sub-cellular scales (patterns of dense nematic bundles) to tissue scales (pulsations and folds).

2.16 Anne-Cécile REYMANN

CERBM IGBMC Development and stem cells Department Strasbourg, France

Actin variants affecting cell mechanical properties scales up to a range of symptoms at the organismal level.

Non-muscle actinopathies (NMA) are a set of ultra-rare human diseases caused by heterozygous single point mutations or gene deletion in cytoplasmic β or γ actin. Patients presenting NMA develop a wide range of symptoms with different severities, notably in terms of intellectual disability and facial or organ malformations. Together with a team of collaborators, the Reymann team aims to understand the molecular to functional consequences of some cytoplasmic actin variants using the model organism C. elegans. Nine human substitutions, were successfully recapitulated in C. elegans actin-coding gene act-2 using CRISPR/Cas9 mediated genome engineering. Interestingly, our preliminary results highlight that the general healthiness of mutant worms is correlated with patients' disease severity. Using a variety of techniques, we assess the consequences of individual actin variants at different scales; from general worm fitness to embryo development up to in vivo molecular dynamics. Overall, we observed the presence of defects with different penetrance, such as increased embryonic lethality and reduced brood sizes. Accordingly, we observed mutation-specific gonad malformations and early embryogenesis defects regarding actin cortex structure and mechanical properties such as cell blebs or cytokinesis failure. We also observe variant-specific embryo arrest during, later, key events of development such as gastrulation or ventral enclosure. Nonetheless, we did not detect major changes in motility, touch response, or neuron positioning and identity in surviving worms. In conclusion, we show that C. elegans is a suitable model for the study of NMAs and we currently are integrating our in vivo results with those in vitro from our collaborators to propose some mechanism explaining the observed perturbations.

3. Abstracts

3.1 Françoise ARGOUL *Poster*

Laboratoire Ondes et Matière d'Aquitaine

Yeast wall viscoelasticity mirrors its metabolism and growth stage: an AFM investigation

Among natural kingdoms, plants, fungi and unicellular microorganisms (yeasts, bacteria, algae, ...) differ from multicellular animals by their intracellular structure and by the strengthening of their extracellular membrane by rigid walls that can sustain quite high turgor pressure (from 0.5 MPa in exponential phase to 1.5 MPa in the stationary phase for yeast). In this work, we combine AFM experimental compression of single yeast cells with flat cantilevers (tipless) and non-linear analysis of force-indentation curves, based on a multi-scale methodology. The force versus displacement curves are corrected by cantilever stiffness, filtered, and derivated to extract different characteristics such a scaling law exponents, effective maximal tension, dissipative loss. This study required a very large number of force curves to reach a statistical relevance for the estimated mechanical parameters in each situation. Comparing different compression velocities (fixed velocity scans) we observe that in the limit of small deformations the yeast wall behaves as quasi-elastic material. Strinkingly, the force vs displacement curves follow unpredicted scaling exponents, as compared to theoretical predictions for pressurized shells, that suggests a multi-layered structuration of this wall, each layer having different mechanical strengths. Experiments were performed simultaneously to the proliferation of yeast (S. cerevisiae) in different culture media, with different sources of carbon, and at different stages of the proliferation. For a given carbon source, we discuss here how the yeast mechanical properties depend on the cell growth stage, by focusing on initial (exponential), intermediary and final (stationary) stages. On another side, we compare different carbon sources (glucose, lactate, galactose), and we discuss how the cell energetic metabolism influences the yeast growth dynamics and the wall mechanics.

3.2 María Isabel ARJONA HIDALGO *Poster*

IJM-CNRS

Reconstituting Cytoplasm Mechanics from individual cell extracts

The cell cytoplasm is a crowded heterogeneous medium of macromolecules, active cytoskeleton networks and endomembranes. Cytoplasm rheology needs to coordinate for the regulation of fundamental cellular processes such as molecular diffusion or cell division. However, the contribution of different cellular constituents to the material properties of the cytoplasm and their role in cell division and early embryogenesis is poorly understood. To tackle this problem, we implemented in-vitro encapsulation of individual cell extract fractions within synthetic cell-like droplets. By using a bottom-up approach, we fabricated $\approx$100 μm water-in-oil droplets filled with magnetic probes of diverse sizes. These allow to actively examine the rheology of different viscoelastic fluids with magnetic tweezers. To map synthetic-cell flows, we also encapsulated fluorescence non-magnetic beads and analyzed the viscoelastic response by particle imaging velocimetry methods under calibrated forces. Following this, we are working on the encapsulation of sea urchin embryo extract fractions obtained by eggs centrifugation on sucrose gradients. By examining these fractions with biochemistry and electron microscopy, and by monitoring with magnetic tweezers their rheological response, we aim to unravel fundamental aspects of spatiotemporal changes of cytoplasm mechanics, and how they correlate with the location, density, size or activity of the different macromolecules, cytoskeleton polymers or endomembranes inhabiting the cytoplasm.

This multidisciplinary project promises to define the spatiotemporal connections between cytoplasm structure and rheological behavior by examining the role of diverse cellular components and their influence on cell division and early embryo development.

3.3 Lama AWADA *Poster*

Aix-Marseille University/ LAI/ CIML

Imprint of mechanical forces on antibody affinity maturation in B cell immune responses

Antibodies play a crucial role in vaccine development and cancer treatment. The selection of antibody-producing B cells during affinity maturation requires the uptake of membrane-bound antigen from follicular dendritic cells. This antigen-BCR bond takes place between membrane-tethered molecules (in two dimensions 2D), and its formation and rupture dynamics are governed by disruptive forces and many other factors. Hence, the need for appropriate physical methods to measure this interaction instead of classical three-dimensional (3D) solution measurements. We aim to decipher the impact of in vivo B cell differentiation-induced amino acid substitutions on the mechanical characteristics of antigen-antibody bonds, and how the latter factor into the maturation process. For this purpose, we sorted antigen-specific B cells undergoing affinity maturation in ovalbumin-injected mice using single-cell RNA sequencing. B cell lineages derived from B cells at various stages of the maturation process are used to produce recombinant antibodies of different affinities. Our collection includes 15 antibodies of 3 lineages. Subsequently, we use an automated laminar flow chamber to perform 2D measurements of the association/dissociation kinetics of our antigen-antibody bonds under a physiologically relevant force range. We've also been systematically comparing our results to 3D measurements using biolayer interferometry. Interestingly, the affinities of antibodies in

solution were found to be within the same range for the mutated antibodies of the same lineage. Our measurements show that although mutations did not significantly impact affinity values, sensitivity to force among antibodies of the same lineage varied, with the germline antibody being the most sensitive to force. To assess the cellular relevance of the findings, we are currently quantifying NK cells' activation by the different mutated antibodies. We accumulate data combining antibody sequence, 2D and 3D binding properties of antibodies varying by one or several amino-acid allowing refined theoretical work to link the sequence and function of antibodies.

3.4 Avin BABATAHERI *Poster*

Ecole Polytechnique

1D confinement mimicking microvessel geometry controls pericyte shape and motility

Pericytes are mural cells of the microvasculature, they wrap around small vessels, support the vessels mechanically and regulate blood flow. Although pericytes participate in a wealth of biological processes, little is known about pericyte mechanobiology. In this work we build a custom in vitro tool that replicates key features of the microvessels to unravel underlying laws driving pericyte behavior, focusing on cell shape and motility in 1D.Lanes of fibronectin are produced with controlled width, length and lane spacing. Lateral confinement induces in vivo-like shape, namely long thin processes of several hundreds of micrometers and a protruding soma. Vessel coverage defined as cell to lane area ratio shows values similar to those observed in vivo. Morphological parameters for endothelial cells cultured on lanes of same dimensions demonstrate the uniqueness of pericyte behavior.
Lanes of varying length are then used to study the effect of constraint on kinetics of pericytes. The velocity probability distributions follow an exponentially decaying curve. A simple theoretical rationalization, models the pericytes as particles undergoing 1D Brownian motion, as conceptualized by De Gennes. Each cell is hypothesized to have a fixed energy, from internal biological processes such as ATP consumption, akin to the thermal agitation of traditional Brownian particles. The internal agitation generates a propulsion force which is then balanced by friction with the substrate. The friction is shown to be dry friction. In the last part of this work, we study migration across gaps between 1D lanes. In vivo, pericytes migrate along small vessels by creating anchors to disconnected spots of fibronectin and are even able to bridge separated capillaries, here discontinuous lanes with gaps of different sizes are produced.The model predicts the size of the gaps that the cells could systematically cross, regardless of the pattern length.

3.5 Pradeep Kumar BAL *Poster*

Universitat Politècnica de Catalunya

Theoretical and Computational Modelling of Cell-Cell Adhesion

Cell-cell adhesion and decohesion are important in biology, to keep cellular tissues together and, in addition, to allow disengagement of cells during remodeling. Due to the fluid nature of the surface of the animal cells, the molecular bonds that keep cells together are laterally mobile. It limits the application of classical theories of adhesion and interfacial fracture in this context. These molecular bonds form clusters, attach to the cytoskeleton through mechanosensitive adapter protein

molecules, and undergo turnover by endocytosis. Cells can tune various properties of these molecular bonds including diffusivity, stiffness, and force sensitivity. We lack a fundamental understanding of how mechanics, chemistry, and biological regulation integrate to support the adaptable function of cell-cell adhesion, and how effective mechanical properties of adhesions such as strength and toughness depend on the molecular properties of bonds.

We develop a mathematical and computational model for cell-cell adhesion, based upon Onsager's principle, coupling active gel models of actomyosin cortex to the adhesion dynamics of mobile binders, that would allow us to understand how the actin cortex and the adhesion complexes work together to give rise to adaptable junctions. For computational analysis in full three-dimensional generality, we consider an ALE parametrization based on an offset to represent the fluid deformable cell surface and use the Local Monge Parametrizations (LMP) method to approximate tensor fields on general surfaces given by a collection of local parametrizations using a FEM setup based on subdivision surfaces. Our work provides a conceptual background to guide new experiments that probe cell-cell adhesion and decohesion. We show how the interplay of mechanics and chemistry at adhesion patches leads to a wide range of behaviors that cells can use to stabilize cell-cell junctions during physiological stretch or to selectively detach during morphogenesis.

3.6 Amlan BARAI *Talk*

IBDM - Aix-Marseille Université

The emergence of "actin stars" for epithelium coordination and tissue patterning

Epithelia constitute cellular groups on the surface of organs acting as a physical barrier against external aggressions and ensure organ functionality. Faithful epithelial morphogenesis is crucial during development and tissue homeostasis. The dynamic intestinal epithelium is an appropriate model to study this field of research. Constant regeneration of epithelium is one of the main characteristics of intestinal homeostasis mediated by a finely controlled balance between intestinal stem cell proliferation and differentiation. Therefore, it is instrumental to study mechanisms controlling intestinal organization to understand tissue homeostasis and function. Recently, by using intestinal organoids as a working model, we have discovered a unique multicellular actin assembly specifically located in the basal domain of the columnar differentiated epithelium, and absent from the crypt domain. This network is characterized by 6-branched star-shaped actin clusters, thus termed "actin stars" (AcSs), with each AcS located at the centroid of the basal side of each epithelial cell. Each branch of the AcS orthoradially connects plasma membrane at cell-cell contacts and mirrors a corresponding branch from a neighbouring cell. Importantly, we could confirm the presence of such actin structures in vivo, along villi in the mouse small intestine. Thus, the AcS network represents a large-scale inter-connecting meshwork in the differentiated epithelial domain. Collectively, the AcS network, because of its regular multicellular architecture, "crystallize" or freeze cell packing and lock epithelial order in the differentiated domain during intestinal morphogenesis. As a consequence, it likely limits changes in cell shape, intercalation, and migratory events. In addition, the existence of such a cytoskeletal scaffold in the differentiated domain could establish a physical barrier to prevent expansion of the proliferative compartment and ultimately control cryptogenesis. We anticipate that our results will have a significant impact on understanding the role of AcSs in epithelial differentiation, tissue homeostasis in physiological and pathological conditions.

3.7 Zoé BARBIER *Poster*

UMons

How matrix curvature changes can direct collective cell migration through modulation of Erk waves

Collective migration is a key function of many epithelial tissues, both in physiological (wound healing) and pathological (cancerous metastases) processes. Recent findings suggest that propagation waves of extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase activation determine the direction of the collective cell migration. In the mean time, accumulative evidence shows that the migration of single cells is affected by cell-scale curvature variations (curvotaxis). However, it remains elusive how local changes of curvature can modulate the propagation of ERK and be integrated to coordinate collective movements. Here we use a photopolymerization method to form well-defined corrugation patterns in soft hydrogels of different wavelengths to mimick the natural folding of epithelial tissues. Our results show that corrugations induce a uniaxial collective flow of MDCK cells in the direction of the corrugation axis, demonstrating a curvotaxis effect on collective migration. By combining Förster resonance energy transfer(FRET)-based biosensors in MDCK cells with long time-lapse experiments, our findings show that Erk protein activation spreads from cell to cell in a defined dynamic pattern (waves) during collective cell migration on flat hydrogels. We then investigate how the modulation of the local curvature can lead to a mechanical stretch at the single cell level, which can activate ERK through epidermal growth factor receptor (EGFR) activation, and ERK activation triggers cell contraction. The contraction of the activated cell pulls neighboring cells, evoking another round of ERK activation and contraction in the neighbors. Our study raises the question of the critical role of cellular response to external stimuli such as matrix curvature in intercellular signal transduction.

3.8 Jorge BARRASA FANO *Talk*

KU Leuven

User-friendly platforms and novel methods for cell-ECM force calculation in 3D in vitro systems

Cell-matrix mechanical interactions play a critical role in disease progression. Traction Force Microscopy (TFM) is a widely used technique for quantifying these interactions. However, there is a lack of TFM methods compatible with 3D in vitro systems, and their codes are either inaccessible or not shared with non-technical users. In this study, we introduce TFMLAB, an accessible MATLAB-based platform that enables biologists to employ complex 3D computational methods. TFMLAB covers all computational steps necessary for 3D TFM, including microscopy file reading, image denoising, cell segmentation, matrix displacement measurement, and cell traction recovery, without requiring programming expertise.

While TFMLAB is valuable for quantifying cell forces in 3D linear elastic hydrogels, we also present a novel approach for quantifying these forces in fibrillar materials at the individual fiber level, closer to mechanotransduction scales. Traditional 3D TFM considers the extracellular matrix (ECM) as a continuous, homogenized medium, limiting the recovery of cell forces and neglecting the effects of local remodeling of the fibrillar network by cells. We propose a novel 3D data-driven TFM

approach using a discrete fiber model that explicitly captures the ECM's fibrillar network as well as single fiber dimensions and mechanics. The model is tuned based on two sources of data. First, fiber network is obtained from second harmonic imaging of collagen hydrogels and segmentation of individual fibers. Second, the single fiber diameter and mechanics are obtained by simulating network mechanical behavior and fitting model simulations to real stress-strain curves obtained from bulk shear rheology measurements of collagen hydrogels. Moreover, we adapted and validated our physics-based inverse method (PBIM) in this novel discrete fiber framework using in silico simulations.

We conclude that our data-driven TFM approach opens possibilities for future applications in real experimental setups, enabling comprehensive investigations of cell force information at length scales relevant to mechanotransduction processes.

3.9 Sara BARRASA-RAMOS *Talk*

LadHyX, CNRS, Ecole Polytechnique

Role of Endothelial Cell Shape and Orientation in Angiogenic Sprouting

Angiogenesis is the emergence of new microvessels from pre-existing ones. Favoring or thwarting this process is essential in fields as diverse as oncology, cardiovascular disease, and tissue engineering. The very early stages of angiogenesis are characterized by endothelial cells (ECs) that sprout from the parent vessel and extend into the surrounding extracellular matrix. Both biochemical and biophysical cues have been shown to play a critical role in angiogenic sprouting; however, the underlying mechanisms remain incompletely understood. A major biochemical pro-angiogenic factor is vascular endothelial growth factor (VEGF). Interestingly, this protein has also been shown to induce elongation in a portion of the ECs in a monolayer, leading to the appearance of clusters of alternately highly ordered (i.e. elongated and aligned) cells and less ordered (more isotropic) cells. In the present work, we assess the relative positions of EC clusters and sprouts in vitro in order to establish if stresses resulting from local changes in cellular nematic order play a role in the initiation of angiogenic sprouting. To this end, human umbilical vein ECs (HUVECs) are cultured to confluence on the surface of a fibronectin-coated Type I collagen hydrogel. Addition of VEGF elicits sprouting into the hydrogel as well as EC cluster formation. Early results suggest that sprouts are more likely to initiate in zones of local order singularity. Interestingly, the changes in cellular nematic order appear to be accompanied by a localized wrinkling of the underlying collagen hydrogel, which may provide a link between cell order and sprouting. Patterning the free surface of the hydrogel using a PRIMO (Alveole) system allows physical confinement of the EC monolayer and hence control of cell morphology. We are currently trying to tune the occurrence of angiogenesis by manipulating cell alignment through surface patterning.

3.10 Hiba BELKADI *Talk*

Institut Pasteur - Ecole Polytechnique

Characterizing the rheology of cell spheroids using a new microfluidic platform

A wide range of methods is available to measure single cell mechanics, identifying key mechano-biological features of disease. However, there is a lack of high-throughput methods to measure the mechanics of multicellular aggregates, even though these provide much better models of health and disease. Indeed, understanding the mechanical properties of a tissue must account for cell-cell contacts, the extracellular matrix, geometric factors, etc, in addition to the mechanics of individual cells. Here we present a method to actuate and observe many three-dimensional cellular aggregates, such as spheroids or organoids, within one deformable micro-device. The device is a single piece of PDMS bonded to a coverslip. It compresses cellular aggregates of 100um in diameter up to an amplitude of 80um. This compression can be static, cyclic with a chosen amplitude and frequency - up to 2 Hz -, or generally dynamic. The device is compatible with various microscopy techniques. From brightfield images, we measure the two-dimensional deformation field of an aggregate under compression. This allows us to detect heterogeneities in stiffness within it, namely in the co-culture of different cell types. Alternatively, confocal microscopy allows us to link the mechanical response of the whole aggregate to local deformations and displacements of its constitutive elements, such as the nuclei or the cell membranes. This platform therefore allows a wide range of actuation and measurement approaches, in order to probe the mechanical properties of 3D tissues. Current work is focused on using this device to investigate the rheological properties of spheroids, as a function of forcing frequency, and their adaptation to hour-long cyclic stimulation.

3.11 Chandini BHASKAR NAIDU *Poster*

Institut Curie- PSL University

Mechanotransduction at the Golgi apparatus

Cells can sense and respond to external forces and mechanotransduction events appear to be critical for most cellular functions. While mechanotransduction has been extensively studied at the plasma membrane and at the nucleus, the impact of forces on other organelles is poorly known. Our project focuses on the study of mechanotransduction at the Golgi apparatus (GA), a central organelle for intracellular transport pathways. We aim to answer several questions:

1. Can external and internal forces propagate to the GA and impact its tension?
2. Is the tension of the GA regulated by actin dynamics and/or the composition of Golgi membranes and the Golgi matrix?
3. Do post-Golgi trafficking and polarized secretion depend on the tension of the GA?

To achieve these aims, we have first applied internal forces directly on the GA by manipulating a bead with optical tweezers and will monitor the effects on tension at the GA using fluorescent HaloFlipper probes. Alongside, we have applied external forces through altering the substrate stiffness and followed post-Golgi trafficking of synchronously secreted cargoes. Our preliminary

results indicate that substrate stiffness has a strong impact on trafficking kinetics of the selected cargoes.

Our results should provide new fundamental insights in the role played by mechanical tension in force transduction at the level of the GA as well as a better understanding of the physical mechanisms underlying polarized secretion.

3.12 Francoise BROCHARD-WYART *Talk*

Institut Curie-Sorbonne université

Gas vesicles: role of compressibity and porosity

Gas vesicles GV are produced in bacteria as a mean to achieve cellular buoyancy. These air-filled proteins nanostructures have been genetically encoded in cells to serve as contrast agent for ultrasound imaging and cell killing agent through inertial induced cavitation. In order to study their mechanical properties, they have to be formed at a larger scale. Here, we report the formation and properties of giant gas vesicles, microbubbles encapsulating perfluoro-n-butane and coated by surface-active proteins,produced by filamentous fungi (hydrophobin HFBI) adsorbed on their surface. We use the micropipette aspiration technique to study their surface tension and viscoelastic properties. Depending upon the concentration of proteins, the gas vesicles are in a liquid or a glassy state and flow only above a yield stress. We develop a model to analyze the suction of these compressible coated bubbles and compare it with incompressible liquid-filled capsules. The sucked bubble does not reach a stationary state, and the length of the tongue increases at constant velocity. This is interpreted by a leak-out of the gas through the porous membrane. In the case of a porous capsule, we discuss the validity of the classical picture of E.Evans, based on the Laplace law relating the membrane tension to P and defined a "sealing" parameter as the ratio of two characteristic times Q, where is the viscous membrane flow relaxation time, and is the leak-out flow relaxation time. If Q is large, the membrane tension is given by the Laplace relationship as in the classic picture, but if Q is small, the classical picture does not hold anymore. It will be interesting to test our model to ultra-porous cell membranes. GV studied here are sprerical because the proteins HFBI look like janus particles. It will be interesting to use the bacterial proteins forming elongated GV.

3.13 Shuai BU *Poster*

The University of Glasgow

Dynamically modulated core-shell droplets study stem cell fate based on mechanical properties

This study aims to investigate the effect of mechanical properties on stem cell fate using dynamically modulated core-shell droplets. Inspired by depth sensing by cells, the droplets were formed using microfluidic chips, with the human mesenchymal stem cells (hMSCs) encapsulated in a collagen core and surrounded by a shell made of alginate, a synthetic biomaterial that can be modulated to vary in stiffness. By adding strontium Sr^{2+} to the media, the alginate shell can be made stiffer, while the collagen core remains soft. This difference in stiffness promotes osteogenesis differentiation of hMSCs. The study demonstrates that despite the low stiffness of the collagen core,

the effective modulus of the matrix "felt" by the cells is substantially higher due to the stiffening of the alginate shell. These results provide insight into the role of mechanical properties in stem cell fate determination and offer a potential approach for controlling stem cell differentiation by dynamically modulating the mechanical properties of the microenvironment.

3.14 Alessandra CAMBI *Poster*

Radboud university medical center

Mech(n)anoimmunology: podosome architecture and dynamics in 2D and 3D environments.

Tissue stiffness alteration, resulting in disrupted homeostasis of mechanical forces, is involved in many pathologies including pulmonary fibrosis, atherosclerosis and cancer. Dendritic cells (DCs) are specialized leukocytes involved in these pathologies. In tissues, DCs patrol for pathogens or aberrant cells. Upon danger recognition, DCs migrate to lymph nodes to initiate immune responses. DCs experience multiple, elastically diverse extracellular environments both in healthy and in diseased tissues. Yet, little is known about how mechanical forces in these microenvironments contribute to the regulation of DC immunobiology. DCs use podosomes, mechanosensitive actin-rich protrusions, to generate forces, migrate, and ingest large foreign antigens. Individual podosomes probe their microenvironment through periodic protrusion and retraction cycles (vertical oscillations), while oscillations of multiple podosomes in a cluster are coordinated in a wave-like fashion. To understand the molecular mechanisms orchestrating this complex dynamic behavior, we integrated advanced quantitative bioimaging, biomimetic substrates of variable stiffness, immunological assays and a chemo-mechanical model. We demonstrated that collective wave dynamics arise from the coupling between chemo-mechanical signaling and actin diffusion, proving the role of podosome clusters in DC mechanosensing. By perturbing the septin network, we altered podosome architecture and cluster dynamics, identifying a novel interplay among septins, myosin IIa and actin. Finally, preliminary observations by superresolution microscopy of DCs migrating on collagen bundles in decellularized dermis reveal for the first time podosome nanoscale architecture and dynamics in a semi-physiological 3D environment. Tissue-resident DCs are the orchestrators of immune responses in pathologies associated with changes in extracellular matrix mechanical properties and are the first cells to respond to implanted biomaterials, playing a role in persistent implant infection and subsequent biofilm growth. A better understanding of the mechanisms regulating DC mechanoimmunology will foster the development of new immunotherapeutic approaches aimed at targeting aberrant extracellular matrix and for medical interventions to reduce implant rejection.

3.15 Giuseppe CICCONE *Poster*

Centre for the Cellular Microenvironment, University of Glasgow

Matrix viscoelasticity regulates epithelial cell spreading, morphology and migration

There is growing evidence that viscoelastic properties of the extracellular matrix (ECM) are a key determinant of cell fate. Indeed, recent studies have shown that matrix viscoelasticity mediates important processes, such as cell spreading and migration. However, our current understanding of these complex processes is mainly based on purely elastic matrices, limiting our knowledge of how

cells interpret changes of ECM viscoelasticity. To address this question, we developed soft (0.3 kPa) and stiff (3 kPa) polyacrylamide (PAAm) hydrogels with independently tuenable Young's Modulus (E) and viscous dissipation, mimicking the complex mechanical microenvironment of normal and tumoral breast tissues, respectively. We show that viscous properties alone have a strong effect on epithelial (MCF-10A) cell spreading, morphology and migration. Supporting this, we show that viscous dissipation affects focal adhesion formation and actin retrograde flow. In addition, important cellular processes involve a physical restriction imposed by neighbouring cells and the surrounding ECM. For instance, cancer cell migration is directly related to the spatial confinement imposed by the native collagen matrix. To better recapitulate the complexity of the cellular microenvironment, we therefore combined viscoelastic cues with physical confinement by functionalising PAAm hydrogels with fibronectin micropatterns of various geometries to study cell mechanotransduction in terms of YAP nuclear translocation, and cell migration on thin ECM adhesive lines. All together, our platform allows to decouple the effects on elasticity, viscoelasticity and confinement on cell behaviour.

3.16 Natasha COWLEY *Poster*

University of Manchester

Effects of environmental rigidity and geometry on confined cell migration

Cell migration is central to many important biological processes such as wound healing and immune response. It is also prominent in many pathological processes for example cancer metastasis. Migrating cells experience a wide variety of external environments in vivo. We have modelled a cell migrating in deformable channels to investigate how the rigidity and confining geometry of the external environment affect cell motility and shape. We model a confined cell migrating using adhesion-independent strategies as a viscous droplet with an active boundary, analogous to the acto-myosin cortex. Using the immersed boundary method to implement the deformable active boundary, we numerically solve the model equations. We establish behaviour in rigid channels and contrast to deformable channels. By varying the stiffness of the channel, we explore how the deformation of the channel under cell generated forces affects migratory behaviour. We find that decreasing the stiffness of the channel increases translational velocity of the droplet and gives a more persistent trajectory. Channel deformation is inversely proportional to the deformation of the droplet. Cells in vivo often encounter constricting geometries in their environment, for example pores in the extracellular matrix or narrowing vessels. We introduce constrictions into the deformable channels to understand which parameters are important in determining whether cells can pass through constrictions. Cell speed, persistent motion and deformation are all important factors in cell migration. Using this general model we show how the environment in which a cell moves can affect all of these factors. This has important implications for targeting medical interventions for diseases where migration is a key process, and in better understanding immune response.

3.17 Joseph D'ALESSANDRO *Poster*

Institut Jacques Monod, CNRS / Université Paris Cité

Mechanical plasticity revealed by traction forces of migrating epithelial cell trains

In order to move, adherent cells generate active forces, which are transmitted to the substrate through cell-substrate adhesions. In cell groups, such as epithelial tissues, those internally generated forces can also be transmitted to some extent at cell-cell junctions. How internal forces distribute between cell-substrate and cell-cell adhesions is not understood. The concept of tug-of-war conceptualised the fact that traction forces - exerted on the substrate - are on average directed centripetally. Consequently, on average, the stress within the cell colony is tensile and increases towards its centre. Yet, large fluctuations of both stress fields accompany this overall trend. We showed recently that in closed rings, which experimentally approximates a 1d periodic boundary condition, single cells behave as almost independent mechanical entities with dipolar traction force patterns.

How do those forces distribute within a migrating group of epithelial cells, and how do they collectively contribute to cell migration? To tackle this, we took a reductionist approach, by confining MDCK cells to linear fibronectin tracks.

We found that traction forces cluster into patches along the line, spanning subcellular to multicellular sizes. Those patches evolve during the lifetime of cell "trains". Thus, cell groups dynamically explore all the configurations between a fully coupled "megacell" to fully independent, single-cell force dipoles. The distribution of those mechanical states is modified upon perturbing cell-cell and cell-substrate adhesions. We also studied the correlations between the traction forces distribution and the motion of the cells. We found that, consistently with what was found on single cells, those correlations lie rather in the spatial symmetries of traction forces than in their magnitude.

Overall, our work sheds a new light on the dynamic regulation of force-transmitting modules in epithelial clusters. The mechanical plasticity that ensues allows those cell groups to deform and move while maintaining their cohesiveness.

3.18 Louise DAGHER *Talk*

Institut Curie

Using Microfluidics for Characterizing Fluid Transport Dynamics of the First Mammalian lumen

Abstract: Understanding how fluid-filled lumens form and grow is key to a broad variety of biological processes in physiological and pathological contexts. During preimplantation development, the position of the first mammalian lumen breaks the radial symmetry of the embryo. This establishes the first axis of symmetry of the mammalian embryo, which determines the implantation site in the uterus.

To grow the lumen, epithelial cells at the surface of the embryo build up an osmotic gradient across the epithelium. This draws water from the outside medium that accumulates into microlumens trapped between cell-cell contacts, which fracture under the hydrostatic pressure of the fluid. Then, guided by the cell contractile activity and the cell adhesion, microlumens pour into a common lumen

at the embryo periphery. This lumen will continue to fill, the embryo swelling to a final size where it will shed its shell and potentially be able to implant.

However, how fluid dynamically accumulates into the embryo could influence the lumen growth and positioning. This could not be investigated due to the lack of tools to measure the fluid transport properties of the embryo. In my talk, I would like to present a microfluidic device that I have developed to control the environment of preimplantation embryos in space and time, and how we can use it to characterize in vivo the biophysical parameters of blastocyst fluid transport. Preliminary results suggest that both inward and outward transport rates scale equally with the osmotic gradient built by the embryo. However, preliminary analysis suggests that elastic constraints provided by the egg shell and the epithelium itself could influence embryo final size. This work also focuses on distinguishing the relative contributions between the different molecular regulators involved in this fluid transport, and how this might relate to concomitant cell differentiation.

3.19 Simon DE BECO *Poster*

Université Paris Cité

Role of RhoA-induced mechanical forces in cell extrusions and cell fate

Cell extrusion is one mechanism allowing abnormal or supernumerary cells to be eliminated from epithelia in order to control the integrity of the tissue. Mechanical stresses in epithelial tissue, such as induced by cell compaction or topological defects, have been shown to trigger the initiation of extrusion events. However, the details of decision-making during mechanically-induced extrusions remain poorly known. In particular, why a specific cell is extruded out of a globally crowded epithelium is still not well understood. Neither is the fate of extruded cells: they can activate apoptotic pathways and die before being removed from the tissue, or can be extruded alive. What are the mechanisms that regulate these different outputs? Optogenetic approaches are used in this project as local mechanical force tuners: by controlling RhoA activation and subsequent myosin contraction, they allow us to trigger cell contractility in a local and semi-quantitative manner to study extrusion events and associated fate. Using this tool, we show that enhancing cell contractility increases tensile stress and cell extrusion rate. We also show that these RhoA-induced cell extrusions are increasingly oriented at the basal side of the tissue, and that they have an increased probability to be independent of caspase-3 activation as compared to unstimulated cells. Altogether, this points to a relationship between RhoA-induced cell contractility, the apico-basal orientation of cell extrusions and the fate of extruded cells.

3.20 Alexandra DEGTYAREVA *Poster*

Max Planck Institute of Molecular Cell Biology and Genetics

Mechanical Control of Mesenchymal Stem Cell Niche Emergence

Cell differentiation lies at the heart of embryonic development, tissue repair, and regeneration. While research has advanced our understanding of the genetic aspects of cell differentiation, mechanical control of fate choice during development in vivo is poorly understood. Cells constantly "sense" their mechanical environment, through the engagement of membrane receptors with the

extracellular matrix (ECM) to transmit forces to the nucleus via the cytoskeleton, and respectively adapt to the change. In vitro, soft and simple matrices support mesenchymal stem cell (MSC) maintenance while cells seeded in stiff environments (i.e., high collagen) differentiate. Intriguingly, one in vivo stem cell niche resists differentiation in a collagen-rich, mechanically loaded environment. These are the suture stem cell (SuSC) niches, which lie between the flat bones of the skull. To understand how SuSCs resist differentiation to maintain a progenitor identity in such a complex mechanical environment we tested the role of emergent collagen structure in developing sutures. We found that collagen organization, not simply abundance, controls differentiation vs progenitor fate in this mesenchymal stem cell niche as inhibition of collagen crosslinking drove osteogenic gene expression during suture emergence. However, we also found that these collagen perturbations disrupted the tight associations between cell clusters in the prospective suture. Here, I show that loss of both collagen organization and cellular organization through tissue dissociation drives the immediate expression of the osteogenic marker Osx1 (or Sp7). This suggests that the complex physical interactions within the SuSC microenvironment protect progenitor gene expression. To understand the mechanical link between collagen organization, cellular organization, and gene expression I quantitatively explore the dynamics of actin microfilaments shown to mediate mechanical regulation of osteogenic gene expression in vitro. In doing so, I aim to interrogate context-dependent mechanical control of fate choice in mesenchymal tissues.

3.21 Janne DE JONG *Poster*

KU Leuven

Quantifying cellular tractions upon CCM2 loss and modulation through ROCKs using in-vitro models.

Cerebral Cavernous Malformation (CCM) is a disease affecting endothelial cells of the micro-capillaries in the central nervous system resulting in lesions. The disease has a strong ROCK-dependent mechanical component. Loss of the CCM1-CCM2 complex is known to weaken intercellular junctions by preventing ROCK2 interaction with Adherens Junctions components, while also overactivating ROCK1 to thereby increase stress fiber and focal adhesion formation [1]. In this work, we explore the change in cellular tractions upon CCM2 loss in different in-vitro models and investigate the role of ROCK1 and ROCK2 in the aberrant behavior of CCM silenced cells. First, we performed 2D Traction Force Microscopy (TFM) on siRNA transfected HUVECs seeded on collagen-coated polyacrylamide (PAA) gels to determine tractions of control, CCM2-depleted, CCM2/R1-depleted and CCM2/R2-depleted cells in a 2D single cell model. Next, we used the same cell types in a biomimetic polyethylene glycol (PEG) invasion assay to investigate tractions in a 3D angiogenic context. Finally, we have optimized micropatterning techniques on PAA gels to explore cell-matrix as well as intracellular stresses in 2D monolayers by combining 2D TFM and Monolayer Stress Microscopy (MSM). Our data show that CCM2-depleted single cells exert higher tractions than control single cells in 2D. Additional depletion of either ROCK1 or ROCK2 restores the modified force exertion of CCM2-depleted cells to the control level. 3D TFM on angiogenic sprouts showed similar trends with higher tractions upon CCM2-depletion and a restoration upon additional ROCK1 or ROCK2 depletion. Preliminary dynamic data on micropatterned islands show, however, a decreased ability of the monolayer of cells to collectively move beads embedded in the gel upon CCM2-depletion. We are currently optimizing our MSM workflows to explore the

cell-matrix and cell-cell force exertion of CCM2-depleted cell monolayers in comparison with the 2D single cell and 3D angiogenic invasion model.

3.22 Claire DESSALLES *Talk*

Université de Genève

Actin dynamics drives active tissue mechanics under anisotropic tension

During development and throughout adult life, tissues have to acquire and maintain a proper shape, continuously generating and responding to mechanical forces. Although tissue mechanics have been investigated at different scales, most effort focused on short term responses, leaving the dynamics of living tissue and the role of the underlying active cellular remodeling poorly understood. To tackle this question, we developed a collagen-based microvessel-on-chip that uses hydraulic actuation whereby normal forces induced by the luminal pressure compress the surrounding soft hydrogel, dilate the channel, and create circumferential tension in the tissue. Longitudinal and circumferential laser ablations of the monolayer confirmed the tension to be anisotropic. Monolayer stiffness of control and drug-treated tissues was measured by recording the stress-strain relationship in response to a rapid pressure ramp. We then sought out to investigate the dynamics of tissue mechanics. Over the course of three days, the monolayer underwent a viscoelastic extension in response to a constant pressure step. Anisotropic tissue elongation was driven by cell elongation and alignment in the direction of the stress. The magnitude-dependent cell reorientation was accompanied by cytoskeletal reorganization from cortical actin to ventral stress fibers. The adherens junctions remodeled towards comb-like configurations that enabled transendothelial actin cables. These collective structural responses were highly dynamic and depended on the presence of adherens junctions. Finally, we propose a mechanical model based on active matter theory linking the actin dynamics to the tissue mechanics and recapitulating the various experimental observations. In summary, we show that cytoskeletal and junctional remodeling enable viscoelastic extension in response to anisotropic tissue tension. This organ-on-chip was also shown to be compatible with epithelial cell culture, which opens up the possibility of extending our study of active tissue mechanics to numerous organ-specific tissues.

3.23 Claire DESSALLES *Poster*

Université de Genève

Topology and geometry organize the morphogenesis of active nematic surfaces

Morphogenesis, the process by which tissues acquire their shape, hinges on a finely orchestrated collective motion of cells. Accumulating evidence shows that many biological tissues behave as active nematics, both in vitro and in vivo. The collective motion of cells is controlled by the nematic order, and topological defects have been proposed as morphogenic organizers via active stresses. However, the generation and control of tissue-scale forces involved in morphogenesis remain poorly understood, in particular within 3D surfaces. The goal of my project is to understand how geometry and topology controls the spontaneous organization of cells that drives morphogenesis, i.e. the growth from a 3D surface to tissues with complex shapes. To investigate this phenomenon, I grow

cells on the surface of deformable capsules and monitor the nematic field, cellular flows, and tissue growth. Capsule shape can be altered, to control the local gaussian curvature and its anisotropy. Shell rigidity can be tuned, and forces inferred from the elastic deformations of the shell. The nematic field is shown to depend on confinement and curvature. Confinement on a surface of finite area constrains the number of defects, while the topology of a surface dictates the total nematic charge, +2 in the case of our spherical capsules. For instance, four equidistant +1/2 defects are observed in the actin network of a monolayer of C2C12 on a spherical capsule. Subsequent growth of the monolayer shows the formation of multilayers with orthogonal orientation. The high long-range contractile stresses due to the nematic ordering leads to anisotropic folding of the capsule. In future work, by quantifying cell motion, collective alignment, and stress fields as a response to topology and geometry, I aim uncover the coupling terms between nematic order and active stresses that shape tissues.

3.24 Lucie ERGOT *Poster*

University of Mons

The extracellular matrix stiffness promotes the invasiveness of breast cancer epithelial cell.

Tumor progression alters the composition and physical properties of the extracellular matrix (ECM). Particularly, increased matrix stiffness has profound effects on tumor growth and metastasis in breast tissues. While one of the major contributing factors is increased density of collagen fibers in the ECM, the influence of the ECM stiffness on the epithelial-mesenchymal transition (EMT) and dissemination of breast cancer epithelial cells remain unclear. Here we used Gelatin hydrogels (GelMA) derived from native type I collagen through partial hydrolysis and functionalized with methacrylate groups to reproduce in vitro the main physico-chemical properties of breast tissues. We used the Irgacure 2959 photoinitiator to control the polymerization of GelMA hydrogels through UV illumination. Our findings show that the rigidity of the hydrogels increases from 2 kPa (soft) to 15 kPa (stiff) by doubling the gelatin concentration, which allows to mimic the rigidity of healthy and tumoral breast tissues. Normal (MCF-10A) and tumoral (MDA-MB-231) epithelial cells were cultured on soft and stiff GelMA to investigate the influence of the ECM stiffness on the epithelial-mesenchymal transition (EMT) and dissemination of breast cancer epithelial cells.

3.25 Dalia El Arawi *Poster*

Aix-Marseille University/ LAI-IBDM

Leukocyte migration in a 3D confined environment

Leukocyte migration is a pivotal process in the immune system's defense against infection and inflammation. During migration, leukocytes encounter various microenvironments that require them to use different mechanisms to reach their destination, including 2D and 3D migration. In 2D migration, leukocytes migrate along the vessel walls, where they spread and develop large lamellipods. Conversely, 3D migration allows leukocytes to squeeze and migrate between endothelial cells and through the extracellular matrix. In addition, leukocytes can move through tissues by either relying on adhesion to the substrate or by swimming in an adhesion-independent manner using

their own motility machinery. However, the underlying mechanisms that govern these processes are still not fully understood, particularly concerning adhesion and confinement impacts. This project aims to explore how leukocytes respond to various microenvironments using microfluidic devices, offering a controlled and reproducible setting for studying their migration mechanisms. By creating microfabricated fluidic channels of different geometrical parameters, we can study leukocyte migration in real time and under hydrodynamic shear stress. Channels of adherent and non-adherent surfaces were used to characterize cells' mobility and relate the effect of geometrical parameters and external mechanical cues such as confinement and applied pressure, on their interactions with surrounding surfaces. Our results show that leukocyte can migrate independently of adhesion in a confined environment and are able to resist hydrodynamic stress in serrated channels. When adhering, cells move faster in confined environments, and withstand higher pressure. Furthermore, we used TIRF imaging to investigate actin and integrins dynamics during leukocyte migration. Advanced image analysis revealed a retrograde flow of both integrins and actin after pressure application and this molecular paddling is responsible for leukocyte propulsion and their ability to resist to hydrostatic pressure. Overall, our study provides valuable insights towards comprehending the mechanisms involved in leukocyte migration.

3.26 Sara FAOUR *Poster*

university de technologie de troyes

Study of fibroblast contractility on 2D and 3D soft gels

Fibroblast activation is a multi-step process defined by increased contractile properties and associated processes (increased ECM production, tissue remodeling, proliferation...). In the presence of persistent stimuli from cancer lesions, these fibroblasts become CAFs (Cancer-Associated Fibroblasts) that are pro-tumorigenic cells that can chemically and mechanically remodel the tumor micro-environment, promoting the proliferation and invasion of cancer cells [1]. A growth factor (TGF-β- a key mediator in activation) was used to activate two different subsets - normal fibroblasts (WPMY-1) and activated fibroblasts (exp-CAF1 [2]). This activation was verified by the implementation of a functional assay using 3D gel composed of type-1 collagen. As the involvement of matrix stiffness has become more apparent in the differentiation of fibroblasts, hydrogels of different stiffness (1 kPa - 100 kPa) were prepared to mimic physiological and pathological conditions [3]. The stiffness of these hydrogels were characterized with active microrheology using optical tweezers to define the frequency-dependent viscoelastic modulus G*(ω).

1] R.Kalluri,Nat. Rev. Cancer,16 (2016582-598. [2] Y.Kojima et al. PNAS, 107 (2010) 20009-20014. [3] M. Carrancá et al. Journal of Biomedical Materials Research Part A, 109 (2021) 926-937.

3.27 Laura FAURE *Talk*

Institute for Bioengineering of Catalonia

A new system revels that single epithelial cells can exert pushing forces on their environment

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

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

3.28 Sandrine FRABOULET *Poster*

UGA

Nuclear deformation and cell fate

Cells in a tissue are subjected to different mechanical forces and it is now known that the mechanical properties of cellular microenvironment greatly influence many fundamental aspects of their physiology including their differentiation, growth, and migration. For a long time, cell surface adhesions were considered to be the main transducers of mechanical stress. Recent studies indicate that the nuclear envelope itself may also play a role in mechanotransduction regulating chromatin organization and signalling pathways that will have a profound impact on cell behaviour and fate.

In this context, we have studied the long-term consequences of a lack of contractility in fibroblasts and characterized the induced phenotype. The relaxed cells enter a senescent state associated with DNA damage and telomere dysfunction. We have shown that DNA damage is a late component and does not seem to initiate the senescent phenotype. Surprisingly, we also show that the molecular complex LINC connecting the nuclear envelope and the cytoskeleton is not necessary to induce the senescent phenotype. We have dissected the molecular changes preceding the induction of the senescent phenotype. Lamin A/C changes, pre-lamin A processing and the consequences on nuclear deformation using confocal imaging is dissected. Understanding these mechanisms may potentially lead to the identification of new pharmacological targets influencing cell cycle and cell fate and is therefore useful for anti-cancer therapy.

3.29 Emile GASSER *Poster*

Institut Curie - LIED - MSC

Biomechanics of different breast cancer cell subtypes differ in response to fast induced deformation

As part of the metastatic cascade, cancer cells enter the blood circulation, where they encounter high shear stress and strong deformations due to the topology of blood capillaries. This step is probably the less well understood one within the dissemination process, due to difficult experimental access. We investigate here how cancer cells respond to the harsh bloodstream conditions, and how this relates to their metastatic potential. We developed a microfluidic device to decipher short-term dynamics of deformation under flow and, most importantly, morphological recovery at the single cell level in flow-free conditions. We report different mechanical behaviors in cancer cell lines from breast cancer subtypes of different aggressiveness.

In our dedicated microfluidic device, cancer cells are deformed under pressure in a capillary-like constriction, then allowed to recover in a pressure-free trap chamber. We show that the arrest time of circulating breast cancer cells in 6x15µm2 constrictions depends firstly on their size, and then on their aggressiveness: for the same size, the more aggressive MDA-MB-231 cells cross the constriction more quickly than the less aggressive SK-BR-3 and MCF-7 cells. At the sub-cellular scale, the arrest time is under the control of the nucleus positioning into the constriction. The recovery of the whole cell consists in a quasi-instantaneous elastic regime, followed by a longer-term viscoelastic regime in the order of ten seconds. This elastic regime requires an intact acto-myosin cortex, and thus disappears for deformations in narrower constrictions inducing cell blebbing (typically 6x6µm2 constrictions). The recovery time constant is larger for more metastatic cells. Interestingly, we observed that the nuclei of more aggressive cells recover almost instantly in an elastic way. The extent of this dominant elastic recovery regime might be linked to the presence of a Vimentin cage-like structure around the nucleus, missing in the less aggressive cancers.

3.30 Sophie GEIGER *Poster*

Heidelberg University

Single Cell Force Spectroscopy: The Impact of Cell Contact Area

Single cell force spectroscopy is a powerful method to characterize cell-substrate interactions. It has already been applied in several studies to investigate the effect of photomechanical stimulation as well as the influence of structuring molecules on cell detachment forces. One important aspect is the contact area between cell and substrate, since an increase contact area usually leads to higher cell detachment forces. The contact area between cell and substrate varies with the deformation of the cell pressed onto the surface and also with cell size. By limiting the adhesion area, we here aim to avoid the distortion that these variations exert on the cell detachment forces. This is achieved using micropatterned substrates. We use light-induced molecular adsorption of proteins (LIMAP) to generate circular and hexagonal fibronectin micropatterns on a passivated background. In a systematic study, we investigate the dependence of cell detachment forces on the cell-substrate interaction surface.

3.31 Caroline GIUGLARIS *Talk*

Laboratoire PhysicoChimie Curie, Institut Curie, PSL Research University

Hydrodynamics of active cells migrating under mesoscopic confinement

When interacting in large ensembles, cells can undergo collective cell migration, reminiscent of the motions observed in fish schools or sheep herds. The importance of collective migration in various biological processes such as morphogenesis or cancer progression has been pointed out in recent years. In vivo, cells migrate in effective "channels" defined by the local environment. Such a situation can be recapitulated in well-defined synthetic mesoscale structures or patterns. Physical models of interacting particles can then be proposed to explain the experimental observations. These interpretations allow measuring physical properties of the system (diffusion, viscosity...), leading to a better understanding of its dynamics. Here, we plate Human Bronchial Epithelial Cells (HBECs) on tracks and disks, of various supracellular sizes. We observe that all cells migrate in the same direction, which we interpret as the signature of a polar flocking. Analysing the velocity field and the density field showed striking transverse oscillations that can be interpreted in the framework of the hydrodynamics of active polar fluids. Theory and experiments are in very good agreement, which allows quantitatively measuring rheological parameters of the tissue. Our results outline the impact of geometrical confinement on collective cell behaviour, and show how such experiments allow to quantitatively characterize the rheology of cell assemblies.

3.32 Cyril GRANDJEAN *Poster*

Univeristé de paris cité

Mechanical properties of plant cells with newly formed walls

Unlike animal cells, plant cells are surrounded by a wall composed of various interlocking polymers that provide mechanical support, flexibility and cell protection. Since this stiff wall prevents cell migration, cell shape changes during development depend on controlling how cell walls yield to wall stresses generated by turgor pressure (Trinh et al., 2021). We currently lack a quantitative understanding of the interplay between wall mechanics, turgor regulation and cell growth even at the single-cell scale. Because a cell wall is built up as a series of layers, its properties depend on its history of formation and deformation, which is typically unknown. One way of addressing these issues is to study the formation of a cell wall de novo, starting from wall-less cells, or protoplasts. With a combination of live imaging and mechanical measurements with a parallel plate technique (Desprat et al, 2006), we followed the formation of a new wall around isolated protoplasts and the evolution of the cell's mechanical properties along the regeneration process. While protoplasts exhibited the same power-law rheology as animal cells, with comparable values of elastic and loss moduli (Durand-Smet et al, 2014), we show that both the elastic and loss moduli increase by two orders of magnitude within seven days of wall regeneration. We now aim at disentangling the contributions of the wall and turgor pressure to the increase of the cell's moduli during regeneration. We are also evaluating how different biopolymers from the cell wall contribute to cell mechanics via targeted exogenous enzymatic cell wall digestion or synthesis inhibition.

3.33 Mathis GRELIER *Poster*

Friedrich-Alexander-Universität Erlangen-Nürnberg

A Cell Mimetic Multiscale Model of Cadherin Adhesion Dynamics Corresponding Author Presentation type

Cadherin mediated adhesion is a fundamental process in multicellular organisms, enabling cells to sense, signal, and respond to physical changes in their environment. Many biophysical determinants of adhesion can be reconstituted using receptor-decorated Giant Unilamellar Vesicles (GUVs) as cell-mimetic systems. These vesicles are then decorated with cadherins and allowed to adhere to an underlying substrate, also functionalized with cadherins. In the past, this system has been used to understand membrane-mediated forces between cadherin bonds (Fenz et al. Nature Physics 2019). In the extension of this study, we are now focusing on the effect of the mobility of cadherins on the dynamics of adhesion in a joint experimental and modeling study. We observe a significantly faster growth of clusters containing trans-cadherin bonds, when proteins can diffuse on both GUV and SLB membranes, compared to the system where cadherins retain mobility only on the GUV. To explain this result, we construct a multiscale Monte Carlo simulations of the GUV spreading dynamics, due to cadherin trans-binding using an adaptive Euler method. We reveal that membrane-mediated trans interactions between cadherins lead to the recruitment of freely diffusing receptors into the adhesion zone. This is a result of the equilibration of the chemical potential difference that emerges when cadherins become part of large agglomerates of trans-bonds. Furthermore, like in the experiments, we demonstrate a significant enhancement in adhesion when cadherins diffuse on the SLB compared to fixed receptors. This comprehensive model sheds light on the complex interplay between cadherin interactions and membrane fluctuations during spreading of GUVs.

In conclusion, our research enhances the understanding of cell behavior and adhesion mechanisms at a multiscale level, encompassing binding dynamics and adhesion zone growth. This knowledge can be extended to investigate more complex cellular systems, involving a broader range of underlying mechanisms.

3.34 Simon GSELL *Poster*

Aix-Marseille University, IRPHE

Marangoni-like tissue flows enhance symmetry breaking of embryonic organoids

During development, embryonic tissues self-organize to achieve spatial organization and shape. While this process has been widely described through bio-chemical approaches, many aspects of the role of active tissue mechanics during embryo morphogenesis remains to be explored. In particular, we do not yet understand the possible role of mechanics in the emergence of the body plan, when the main axes and symmetries are set in the early embryo. In this work, we address this question using 3D aggregates of embryonnic stem cells recapitulating embryo-like development in-vitro. Once cell differentiation is activated, these embryonic organoids exhibit spontaneous elongation and symmetry breaking of key proteins involved in axis formation of embryos (e.g. T/Bra). We show that this symmetry breaking is accompanied by coherent tissue flows that contribute to the aggregate polarization. We develop a mode decomposition analysis showing that these flows exhibit

a dominant Marangoni-like recirculation pattern. In order to understand how such flows can robustly emerge, we build a minimal continuum model assuming that large-scale tissue stresses are controlled by key protein concentration patterns within the tissue. Our simulations are able to reproduce the dominant flow pattern based on experimentally measured protein concentration fields. Our work thus suggests that a positive feedback loop between protein concentration patterns and tissue flows may enhance axis formation of embryo-like bodies.

3.35 Daniel HAMMER *Talk*

University of Pennsylvania

Upstream migration: a common feature of amoeboid cell motility

Upstream migration is a fascinating mechanochemical phenomenon in which amoeboid cells of the immune system crawl upstream against the directon of flow on surfaces coated with intracellular adhesion molecule-1 (ICAM-1). Upstream migration is mediated by leukocyte function antigen-1 (LFA-1), which is known to form catch bonds. Although this phenomenon was first elucidated by the Theodoly and coworkers on T-cells, we have found that other cells of the immune system, including neutrophils and macrophages, can exhibit upstream migration. In particular, in neutrophils it was necessary to block a competing integrin for ICAM-1, macrophage-1 (MAC-1) antigen, and have a sufficiently low density of ICAM-1 to observe upstream migration. We present here our initial findings on macrophage upstream migration. We also found that hematopoeitic stem cells, as well as the KG1a human hematopoeitc cell line, will migrate upstream. will migrate upstream on ICAM-1 surfaces. We show that molecules downstream of LFA-1, notably the adaptor protein CRK and the the ubiquitin kinase cCBL, are necessary for upstream migration. Experiments in upstream migration on activated HUVEC monolayers indicate that upstream migration facilitates transendothelial migration. Furthermore, the strong flows that give rise to upstream migration suggest that traction forces between upstream migrating cells and substrates might be quite strong. We show our early attempts at meauring the upstream migration of amoeboid cells on compliant polyacrylamide hydrogels with embedded beads, which will enable measurements of traction forces.

3.36 Benedikt HARTMANN *Poster*

Max Planck Institute for the Science of Light

Linking cell mechanics and circulation

During their round trip through the body, blood cells flow through capillaries and encounter constrictions of similar or even smaller size as themselves. To better understand this microcirculation, it is crucial to understand how exactly a cell's mechanical properties contribute to the transit through multiple constrictions. However, previous studies have focused either on transit through a single constriction or on comparing transit times in multiple constrictions without connecting them to the viscoelastic properties of the cells. Here we measure the viscoelastic properties of cells using a novel hyperbolic microfluidic chip in which the timescale of cell deformation is similar to that at which cells transit through constrictions in vivo. Additionally, using custom channel designs with multiple constrictions and relaxation regions of various sizes, we test how the viscoelastic properties and

other parameters, such as cell size, are linked to the transit time through several constrictions. Our preliminary data show that the initial deformation facilitates transit through subsequent constrictions, yet it is unclear if this is due to a plastic deformation of the cell or a reversible shape change which needs time depending on the viscosity of the cell. These investigations into the role of mechanical properties in the context of microcirculation will improve our understanding of blood-related diseases or circulating tumor cells.

3.37 Leon HERMANS *Talk*

Eindhoven University of Technology

Matrix stiffness-TGF-β Synergy Regulates Collective Cardiac Fibroblast Force Generation

The generation of contractile forces by cardiac fibroblasts plays a pivotal role in the progression of cardiac fibrosis after myocardial infarction (MI). Harnessing cardiac fibroblast contractility to engineer the infarcted heart could therefore be a promising approach prevent fibrosis expansion and heart failure following MI. Various cues within the fibroblast microenvironment, including matrix stiffness, TGF-β1, and cell-cell interactions, are known to regulate fibroblast contractility. However, the collective impact of these cues on cardiac fibroblast-generated forces remains unclear. To address this, we systematically investigated the relationship between pluripotent stem cell-derived cardiac fibroblast contractility, matrix stiffness, TGF-β1 signaling, and cell-cell interactions. Our findings demonstrate that matrix stiffness monotonously increases cardiac fibroblast contractility, regardless of TGF-β signaling or cell-cell interactions. However, we discovered that in specifically in collectives, matrix stiffness synergizes with TGF-β1 to amplify contractility at severely fibrotic matrix stiffness. This discovery emphasizes the significance of targeting matrix stiffness and cell-cell communication to effectively control cardiac fibroblast contractility and mitigate fibrosis expansion following myocardial infarction (MI).

3.38 Andrea IGLESIAS RAMAS *Poster*

Institut Curie

The role of resting membrane potential in cell behaviour and cancer

All mammalian cells present an electric potential across the plasma membrane called resting membrane potential (RMP). The role of RMP in non excitable cells - cells that do not generate action potentials - remains largely unexplored. Interestingly, a striking correlation between the RMP and cancer has been observed in previous studies. Healthy cells tend to be hyperpolarized (i.e. have strongly negative RMP) while cancer cells tend to be depolarized (i.e. RMP close to 0 mV). It is still not known if the increased RMP in cancer cells is a simple consequence of the enhanced metabolism in these highly proliferative cells, or if the RMP also has a causal role on the cancerous phenotype. Recent studies have shown that the RMP directly affects intracellular signaling pathways, thereby potentially steering cell behavior. In this work, we investigate quantitatively the link between RMP and cell behaviour. First, we want to see if the RMP has a causal role on cell migration and proliferation (two hallmarks of cancer cells) using chemical and optogenetic perturbations that affect the RMP. Second, we explore the role of the RMP in cancer by monitoring the RMP over long

timescales (hours and days) in healthy and cancerous cell lines to detect systematic differences between the two cell types, apart from the changes due to cell density and cell cycle. Third, using mixed cell populations we address the role of cell-cell electrical connections and supra-cellular spatial patterning of the RMP in the outcome of cancer cells surrounded by healthy cells. Altogether, our work proposes to advance our understanding of the role of the RMP in cell behaviour and in particular in cancer, and to advance imaging methods aimed at measuring the RMP over long timescales and in many cells.

3.39 Munoz JOSE *Poster*

Universitat Politecnica de Catalunya

Inference of cytoskeletal structure from TFM cellular tractions

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

We here propose a novel technique for inferring a plausible cytoskeletal structure and contractility distribution mechanically compatible with the tractions extracted from TFM results. The technique is based on an iterative minimisation process, where a set of contractile dipoles are generated. From the mismatch of the TFM traction field and the traction generated by the mesh of dipoles, we design a filtering and reconstruction process for different dipoles sizes and connectivities in an efficient manner. The process is also complemented from the contractility profile obtained assuming a continuous elastic media adhered to the substrate.

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

3.40 Yohalie KALUKULA *Talk*

Mechanobiology Biomaterials group, Interfaces and Complex Fluids Laboratory, Research Institute for Biosciences, CIRMAP, University of Mons, Place du Parc, 20 B-7000 Mons, Belgium

The transient migration dynamics of confined epithelial cells is controlled by a morphological switch

The migration of epithelial cells through dense tissues and tight spaces is a crucial process in tissue development, homeostasis, and diseases such as cancer. However, how spatial confinement affects cell migration dynamics is still not well understood. We investigated the transient migration events of epithelial cells on adhesive dumbbell-shaped micropatterns that lead to repeated back and forth migration events. By tuning the dimensions of the central narrow bridge that connect two squared-shape adhesive sites, we show that the spatial confinement imposed by the bridge geometry

influences the migration velocity. Our findings show that imposing narrower bridges increases the cell migration speed through large cellular extensions. Interestingly, extending the length of the narrower bridges increases significantly the success rate of crossing up to 95%. We show that the crossing rate and the dynamics of transient migration are both controlled by a morphological switch imposed by the bridge aspect ratio. Indeed, epithelial cells on longer bridges switch from an extended and slow morphology to a fast and compacted phenotype with a steady polarization state, raising the question of the existence of a polarization memory in confined cells. To address this question, we investigated the role of the actin cytoskeleton and characterized the expression of epithelial to mesenchymal markers in these two opposite phenotypes.

3.41 Leda LACARIA *Poster*

CNRS/IBDM/Aix-Marseille Univeristy

Oncogenic Ras induces differences in cell mechanics and cytoskeleton organization in HMLE cells

Cellular mechanical properties are crucial for biological functions in health and disease, including cell migration and adhesion. Malignant cells generating distant metastasis in cancer are reported to be softer than benign cells. The cytoskeleton, critical for cell mechanical stability, is affected during malignancy and tumorigenesis. However, the correlation between cytoskeletal structure, composition, and mechanical properties in cancer cells is poorly understood. This work employs atomic force, traction force, and advanced fluorescence microscopy, breast epithelial cells (HMLE) and Ras-induced variant (HMLER) as model of tumorigenicity and micropatterning for a robust averaging of cell images to answer this question. Results confirm that Ras-expressing cells are softer, particularly in peri-nuclear regions. These cells are thinner and more spread, with less variation among cells of elasticity. Traction force microscopy shows similar forces, but distinct distribution in the two cell lines. Immunofluorescence reveals in Ras cells vimentin overexpression around the nucleus, cytokeratin 5 upregulation at the cell front, and f-actin more organized in stress fibers and transverse arcs. Advanced polarization resolved fluorescence microscopy indicates less ordered F-actin in Ras cells, supported by nematic order parameter calculations. These results took all together suggest that the denser cytoskeleton of malignant cells allows the formation of locally oriented stress fibers, supporting the transmission of forces at short length scales, and a more crowded but less interconnected cytoskeleton structure that finally leads to a more compliant cell.

3.42 Zoë LANGE *Talk*

Frankfurt Institute for Advanced Studies, Goethe-Universität Frankfurt am Main

Hatching on a budget: The economized energy expenditure of beetle embryos

Embryogenesis of egg-laying animals is a biological example of a thermodynamically quasi-isolated system. Additional to the genetic code, most energy and matter necessary for embryo development is stored inside the egg from the time point of fertilization up to the time point of hatching. It remains controversial how energy is stored and transferred, especially in quiescent periods between large-scale deformations. Here, we study extra-embryonic tissue dynamics in two key processes

during embryo development, gastrulation and dorsal closure, in the serosa membrane of the red flour beetle Tribolium castaneum. Is serosa membrane retraction a passive process enabled by prestress from serosa window closure. We argue that energy in the serosa tissue is close-to-fully conserved between serosa window closure during gastrulation and dorsal closure. We identify serosa migration during gastrulation as a passive process driven by the compacting embryo using 3D non-invasive stress estimation techniques. We localize the point of origin of forces driving serosa tissue rupture and retraction. We believe that this study of extra-embryonic tissue dynamics signifies the importance of studying non-equilibrium mechanics in embryogenesis.

3.43 Claire LECLECH *Poster*

Ecole Polytechnique

Contact guidance of vascular endothelial cells on microgrooved substrates

Adherent cells in vivo often reside on extracellular matrices (ECMs) that possess a topographical organization at different scales. Various types of engineered microstructured substrates have been developed to study the impact of basal topographical cues on cell behavior in vitro. Amongst them, microgrooves mimicking the anisotropic organization of the ECM have been shown to align and elongate different cell types. However, how cells detect and respond to microgrooves remains unclear, particularly for cellular monolayers. We are investigating these questions using vascular endothelial cells (ECs), which form a monolayer lining the inner surfaces of blood vessels. Relative to ECs on control flat surfaces, single ECs cultured on parallel arrays of microgrooves are highly elongated, and they align and migrate in the groove direction. The extent of elongation and alignment increases with groove depth, and this dependence relies on focal adhesion (FAs) and protrusion guidance. Interestingly, confluent EC monolayers on microgrooves, develop a specific type of collective movement in the form of periodic antiparallel cell streams that require intact cell-cell junctions and whose dimensions are principally driven by groove depth. Modeling the EC sheet as an active fluid with the microgrooves acting as constraints on cell orientation accurately predicts the occurrence of the antiparallel streams. Further increasing cell density on the microgrooves leads to progressive loss of cell alignment and elongation, remodeling of FAs and actin, and markedly reduced cell migration. We propose that the secreted basement membrane and the increased strength of cell-cell junctions in very dense monolayers counteract the effect of substrate topography. The present results provide evidence for different cell density-dependent regimes of response to substrate topography. Beyond highlighting fundamental mechanisms of contact guidance, these results may prove useful in the field of implantable endovascular devices where surface topographic motifs constitute a potentially promising strategy for improving device efficacy.

3.44 Shaozhen LIN *Poster*

Aix-Marseille University

Tilt-induced clustering of substrate-binding proteins

Cell adhesion proteins are transmembrane proteins that play a crucial role in the binding of cells to one another, as well as to the extracellular matrix that surrounds them. Cell adhesion proteins can typically organize into clusters that can take various forms, from circular patches to long linear structures. Here, we propose that a membrane height gradient leads to the emergence of an average tilt conformation of adhered proteins associated with a relaxation of their conformational energy. We show that such coupling between the membrane undulation and the symmetry-breaking effect of the protein tilt yields an effective line tension that is negative, allowing for stable clusters to form. Our model predicts transitions from circular spots to long linear structures (as in Swift-Hohenberg's theory) and then to Turing-like patterns for increasing membrane-protein tilt strength or protein-substrate binding chemical potential. Our findings suggest a potentially critical role of the tilt effect of cell adhesion proteins in regulating the cluster formation of cell adhesion proteins.

3.45 Marine LUCIANO *Poster*

University of Geneva

Mechanical response of epithelial monolayers to curvature sensing over various length scales.

In a wide range of epithelial tissues, cells organize into bidimensional monolayers whose physiological functions are strongly linked to their microenvironment, which is mostly curved rather than flat. This topological feature is required to ensure the regulation of functions in many organs, such as the exchange area of the intestine. However, the complex curvature patterns of many organs span different length scales and are difficult to reproduce in vitro, preventing a better understanding of the link between curvature and functions. To address this question, we developed a new microfabrication method that couples a corrugated polyacrylamide hydrogel onto an inducible rolling PDMS multilayer to create tunable culture substrates with two scales of curvature. The rolling method is based on an elastic instability originally observed in bilayer membranes that comes from a contrast of elastic moduli and thicknesses. We ensured a strong adhesive cohesion between hydrogel and PDMS rolling bilayer by using a benzophenone-based strategy to bind covalently polyacrylamide hydrogels to PDMS membranes. After rolling, we obtained the spontaneous formation of a tube with a corrugated inner surface layer that allows to study under standardized conditions how epithelial monolayers adapt to a rapid anisotropic change of curvature over two length scales. Our findings show that the alternance of concave and convex structures inside a tubular structure affects the cytoskeletal organization, as well as nuclear morphology and positioning, suggesting a collective response of epithelial cells to changes of curvature over different length scales. Altogether, our results provide insights into the emerging architectures of epithelial tissues grown on folded surroundings, which are reminiscent of the in vivo scenario.

3.46 Qiyan MAO *Poster*

Institut de Biologie du Développement de Marseille

Tension-driven multi-scale self-organisation in human iPSC-derived skeletal muscle fibers

Human skeletal muscle is a hierarchically organised tissue with hundreds of contractile myofibres packed into large bundles connecting two skeletal elements. This allows chains of contractile sarcomeres within each myofibre to generate an optimal level of mechanical tension. Contrary to the paradigm that human muscle bundles only form under the guidance of connective tissue, our recent study showed that human muscles spontaneously bundle and fuse in vitro without any external cues. The stable attachment of these self-organised bundles coincides with an increase in mechanical tension and the formation of long chains of periodic sarcomeres within individual myofibres. Together, these results suggest a key role for mechanical tension in providing feedback to coordinate muscle self-organisation from the tissue to the molecular level. Here, we set out to investigate how tension is generated and sensed during human muscle self-organisation. By manipulating substrate stiffness, we show that the characteristic scale of alignment in human muscle bundles is greatly extended on ultra-compliant substrates. This coincides with a delay in sarcomere assembly, which in turn suggests a delay in the build-up of tension in these muscle bundles. This strongly supports our model that human muscle bundles are mechanoresponsive. As a potential tension-sensing mechanism, we found that YAP-1 is transiently enriched in the nuclei of myofibers prior to their stable attachment. Taken together, these results provide initial insights into the molecular and cellular mechanisms of tension-driven human muscle self-organisation.

3.47 Genesis MARQUEZ-VIVAS *Poster*

Université Grenoble Alpes

Self-sustained velocity waves and pattern emergence in tissues

Supra-cellular organization is crucial to establish and maintain the structure, function and homeostasis of biological tissues. Several recent works reported that wave-like patterns of the velocity spontaneously appear in colonies of epithelial cells. Strikingly, supra-cellular waves are characterized by precise wavelength and period. Our objective is to investigate whether supra-cellular waves induce a transcriptomic divergence between the cells situated in the wave nodes and those in the antinodes.

3.48 Rudolf MERKEL *Poster*

Forschungszentrum Jülich, IBI-2

An in vitro Study of the Effects of Mechanical Strain on the Epidermis

Our body is protected against external forces by several structures, the most important of which is the epidermis, the outermost layer of the skin. In this multilayered structure, cells differentiate while they progress from the basal to the apical layer. Adaptation of epidermal cells and the overall tissue to mechanical loads is well known and central for mechanical integrity. However, mostly for technical reasons the underlying mechanisms are still poorly understood. Recently we introduced simplified epidermal equivalents (SEE) as cell culture-based model systems for the epidermis [1]. They enable the application of well-defined mechanical loads and show skin-like behavior under stretch.

In further work, we find that SEEs display hallmarks of the epidermis like layer-specific proliferation or formation of tight junctions. SEEs grown on stretchable elastomer deform with uniform mechanical strain throughout all layers. We use cyclic stretch as mechanical cue and observe reorientation of cells, nuclei, and cytoskeleton as response. In the basal cell layer, actin bundles connected via adhesions to the underlying substrate rotate away from strain direction and align. Surprisingly, in response to cyclic strain the seemingly uniform apical actin cortices display a fine-grained structure that can be quantified by Fourier transform methods. Inhibition of myosin 2 or the upstream rho associated protein kinase (ROCK) strongly reduces order formation while inhibition of myosin light chain kinase has no effect.

[1] Püllen, R.; Konrad, J.; Merkel, R.; Hoffmann, B. Skin under Strain: From Epithelial Model Tissues to Adult Epithelia. Cells 2021, 10, 1834. https://doi.org/10.3390/cells10071834

3.49 Waleed Ahmad MIRZA *Poster*

European Molecular Biology Laboratory (EMBL)

Theory of active self-organization of dense nematic structures in actin gels

The actin cytoskeleton is remarkably adaptable and multifunctional. It often organizes into nematic bundles such as contractile rings or stress fibers. However, how a uniform and isotropic actin gel self-organizes into dense nematic bundles is not understood. Here, using an active gel model accounting for nematic order and density variations, we identify a novel active patterning mechanism leading to dense nematic structures. Linear stability analysis and two-dimensional nonlinear finite element simulations establish the conditions for nematic bundle self-assembly and how active gel parameters control the architecture, orientation, connectivity and dynamics of self-organized patterns. Moreover, we substantiate with discrete network simulations the main requirements for nematic bundle formation according to our theory, namely increased active tension perpendicular to the nematic direction and generalized active forces conjugate to nematic order. Lastly, we show that on a three-dimensional curved cytoskeleton surface, the self-organization of dense and highly aligned bundles enhances the efficiency of cellular processes such as cell division and motility. Our work portrays actin gels as reconfigurable active materials with a spontaneous tendency to develop patterns of dense nematic bundles.

3.50 Vladimir MISIAK *Poster*

Université Grenoble Alpes

Study of an elementary paving of tissue morphogenesis

Morphogenesis is the unfolding of a biochemical and mechanical patterns in a biological tissue. A common phenomenon of morphogenesis in the convergence-extension process where the tissue elongates in one axis by cell shaping, proliferation and rearrangement of the cells. Convergence-Extension is mostly due to cell medio-lateral intercalations which is also called T1 transitions. This transition can be described in a simple way with a system of only four cells in which two neighboring cells are separated by an intercellular junction that shrinks until forming a four-cell vertex which is then remodeled in a new cell-cell junction that lengthens between the other two cells. Nowadays, the models that describe and use this process still need improvement. To this end, we adopt a bottom-up approach by first studying a minimalistic system of cell intercalation: a cell quadruplet. The architecture of the system is shaped by micropatterning of extracellular matrix (ECM) proteins which allows for high throughput experiments. Cell contractility and cell-cell adhesions are disrupted with the help of optogenetic tools. The mechanical readout of the system is reported by coupling these techniques with Traction Force Microscopy (TFM).

3.51 Sylvain MONNIER *Poster*

University of Lyon

The key role of mitosis in nuclei adaptation to prolonged squeezing of cancer cells

During their life, mammalian cells are subjected to numerous mechanical constraints, especially in pathological contexts such as cancer. Most studies on cell confinement focus on short periods, and little is still known about cell adaptation to prolonged squeezing, over several cell divisions. Using a hydrogel-based confinement system, we reveal the unsuspected role of mitosis in long-term adaptation to prolonged uniaxial confinement, in a contractility-dependent manner. To adapt to the level of confinement and to alleviate the imposed mechanical stress, nuclei are reaching a new homeostatic state following the first confined cell division: cells down-regulate their nuclear volume, together with a reset of their nuclear envelope folding. A simple geometric model suggests that this new nuclear volume is triggered by the apparent surface of the nuclear envelope. Our findings have important implications for the fundamental understanding of nuclear regulation under mechanical constraints and are critical to better comprehend cancer cells plasticity.

3.52 Adrien MÉRY *Poster*

EPFL

Harnessing Mechanobiology for Synthetic Tissue Morphogenesis

The study of tissue morphogenesis is instrumental to understand the scientific principles of embryonic development, and these principles could drive innovations in engineering tissues for regenerative medicine. We aim to replicate key morphogenetic events such as invagination, branching, evagination, and villi formation in vitro using microengineered tissue constructs. By identifying the key mechanical loading conditions to synthetically initiate these native events, our goal is to propose a generic design framework capable of generating arbitrary shapes. Robotic micromanipulation tools allow spatiotemporally controlled interventions to cut, inject or aspire specific areas of the tissues to create mechanical stresses and boundary conditions that are expected to drive programmed tissue deformation. Finally, contractility and mobility of cells can be influenced directly through optogenetic control of pathways such as RhoA, providing a non-invasive technique to manipulate the mechanical state of the tissues. In collaboration with computational mechanicians, we aim to develop a digital twin of our microengineered tissues, thus yielding the basis for an anatomical compiler resorting only to mechanical stimuli.

3.53 Eric NEIVA *Talk*

Centre Interdisciplinaire de Recherche en Biologie, Collège de France, CNRS UMR7241

An unfitted finite element model for myosin-mediated coupled surface-bulk active flows

Cytoplasmic reorganization intervenes in several cell patterning and polarization processes, such as the establishment of PAR-polarity in the C. Elegans zygote or the even distribution of syncytial nuclei in Drosophila embryos. Since both the cytoplasm and cortex are viscous, their flows are hydrodynamically coupled; cortical actomyosin flows can trigger cytoplasmic flows and vice versa. However, the precise nature and relative contribution of active forces generated at the cell cortex and bulk of the cytoplasm are yet to be elucidated.

In this talk, we will describe our efforts to shed light onto this matter with theoretical and numerical modelling. Together with biophysical measurements, they can test the plausibility of certain proposed mechanisms of cytoplasmic reorganization. In particular, we will describe a new model for myosin-mediated coupled surface-bulk active flows, along with its numerical implementation with unfitted finite elements methods. The model considers the dynamics of surface-bulk viscous flows on general and smooth closed surfaces. They are coupled to surface-bulk myosin transport equations via an active term, representing active tensions generated by the activity of myosin motors. We will briefly detail the numerical implementation of the model and, to conclude, we will discuss the resulting system, in terms of its characteristic time-scales and dimensionless numbers.

3.54 Aude NOMMICK *Talk*

Université Paris Cité - Institut Jacques Monod - CNRS

Actin-microtubule interactions promote the emergence of mitotic spindle planarity in early embryos

Early embryos are shaped by the geometries of their reductive cell divisions. In many species, these divisions transit from a radial pattern to a more planar pattern as mitotic spindles progressively orient parallel to the embryo surface. These planar divisions are key for the formation of the blastula, the first monolayered epithelium of an organism, that prepares the embryo for subsequent morphogenetic movements. To address mechanisms that promote the emergence of spindle planarity during embryo development, we tracked nuclei, centrosomes and spindle positions with 3D live and fixed imaging of sea urchin embryos from the 1 to 500 cells stage, when the blastula forms. We found that nuclei and centrosomes are initially well centred but undergo an apical shift towards the embryo surface from the 16-32 cell stages onwards. This apical migration pre-positions and orients spindle poles parallel and close to the embryo apical plane during mitosis, to promote planar divisions. Remarkably, this shift in centrosome positioning and division patterns is associated with an interaction between astral microtubules and intracellular "actin fingers" that extend from the apical cortex. These apical actin fingers prolong actin-rich microvilli at the outer surface of the embryo, and appear to pull astral microtubules to decenter centrosomes towards the apex. We propose a self-organized model, in which cortical actin fingers promote the emergence of planar polarity, by catching and pulling astral microtubules as blastomere cells pass below a size threshold.

3.55 Guillaume PERNOLLET *Talk*

UNIGE

Identifying the impact of non-biochemical cues on epithelial monolayers organisation

Correlating tissue shape and function has been one of the main interest of biophysicists in the past decade with for example studies on cells organisation during drosophila wing development or cell position within the intestinal niche. Although it is well known that physical constrains such as curvature or substrate rigidity impact cellular behaviour, quantifying the impact of these different factors on tissue organisation and function remains challenging due to the difficulty of isolating these different factors in vivo. To overcome these obstacles, ex-vivo models are used to allow the analysis of the impact of a single factor. In those studies, the emphasis is often put on a single observable that acts as the sole reporter of the effect of the impacting factor, with work on the impact of tissue stress on cell elongation, or the impact of curvature on monolayers height. Here we present a more holistic approach with at its core the development of a segmentation pipeline to quantify multiple relevant observables like cell tilt, cell volume, nuclei orientation etc. and their correlations. Combination of this tool with state-of-the-arts biomaterials technic such as ultrasoft hydrogels, hollow alginate capsules and patterning to grow our tissues under various conditions like curvature and confinement, allowed us to observe interesting phenomenon such as cell volume shrinking due to confinement, cell height tight regulation, curvature impact on cell volume and height, as well as the induction of tilt, cell skew and apical-basal cell junction transition. Overall, our approach allows us to better

understand the mechanisms at play controlling tissue organisation, how the various external physical constrains impact those mechanisms, and what exactly tissue organisation means.

3.56 Marie POCHITALOFF *Poster*

TU Dresden, Physics of Life

Mechanics of the cellular microenvironment as probed by cells in vivo

Tissue morphogenesis, homeostasis and repair require cells to constantly monitor their three-dimensional microenvironment and adapt their behaviours in response to local biochemical and mechanical cues. Yet the mechanical parameters of the cellular microenvironment probed by cells in vivo remain unclear. Here, we report the mechanics of the cellular microenvironment that cells probe in vivo and in situ during zebrafish presomitic mesoderm differentiation. By quantifying both endogenous cell-generated strains and tissue mechanics, we show that individual cells probe the stiffness associated with deformations of the supracellular, foam-like tissue architecture. Stress relaxation leads to a perceived microenvironment stiffness that decreases over time, with cells probing the softest regime. We found that most mechanical parameters, including those probed by cells, vary along the anteroposterior axis as mesodermal progenitors differentiate. These findings expand our understanding of in vivo mechanosensation and might aid the design of advanced scaffolds for tissue engineering applications.

3.57 Giulia POZZI *Talk*

DISMA - Politecnico di Torino

Mechano-biological model of glioblastoma cells in response to osmotic stress

This work investigates the mechano-biological features of cells cultured in monolayers in response to different osmotic conditions. In-vitro experiments have been performed to quantify the long-term effects of prolonged osmotic stresses on the morphology and proliferation capacity of glioblastoma cells. The experimental results highlight that both hypotonic and hypertonic conditions affect the proliferative rate of glioblastoma cells on different cell cycle phases. Moreover, glioblastoma cells in hypertonic conditions display a flattened and elongated shape. The latter effect is explained using a nonlinear elastic model for the single cell. Due to a crossover between the free energy contributions related to the cytosol and the cytoskeletal fibers, a critical osmotic stress determines a morphological transition from a uniformly compressed to an elongated shape.

3.58 Jyotsana PRIYADARSHANI *Poster*

KU Leuven

Integrating mechanics with proangiogenic cues of CCM-mutated endothelial cells on a microfluidic chip

The advent of microengineering technology in the area of cell biology for developing dynamic in vitro models has gained significant attention in the past few decades. Vascularization has been an integral component of such platforms for precise fluidic delivery. The cerebral cavernous malformed (CCM-mutated) capillaries have been shown to possess proangiogenic invading traits while forming blood-filled dilated lesions in the area of low-shear venous capillaries of the central nervous system. Hence, developing a 3D perfusable capillary mimicking platform for reconstructing the key features of CCM disease will be of great interest. The present work aims to combine such a CCM-on-a-chip platform with the mechanical quantification of cell-generated forces within the extracellular matrix using 3D Traction Force Microscopy (TFM) to develop a next-generation biomimetic model, which can be used to explore the mechanotransduction events and therapeutic designs in vitro.

3.59 Felix REICHEL *Talk*

Max Planck Institute for the Science of Light and Max-Planck-Zentrum für Physik und Medizin, Erlangen

High-throughput viscoelastic characterization of cells in hyperbolic microfluidic channels

Research over the last decades revealed that single-cell mechanical properties can serve as label-free markers of cell state and function and that mechanical changes are a sign of alterations in the cell's molecular composition. This led to the development of a number of microfluidics tools to rapidly measure the deformability and also the viscoelastic properties of cells. However, accurately quantifying stresses within these systems is often challenging and with that the derivation of a stress-strain relation becomes a complex task. Here, we used hyperbolic channels to create an extensional flow field, where the acting stresses can be measured using calibration particles and yield a simple relationship between acting stress and resulting cell strain. We then used the setup to measure the Young's modulus, bulk viscosity and complex modulus of different cultured cell types and blood over a wide range of time scales. Drug induced changes to the cell state could be measured by a change in cell mechanical properties. Our simple setup provides a straightforward and time-efficient approach for evaluating the viscoelastic properties of large cell samples and microscale soft particles.

3.60 Alexandre REMSON *Poster*

University of Mons

Chirotaxis: matrix chirality modulates the cell migration speed

Chirality is ubiquitous in Nature, from living organisms to biomolecules, and influences fundamental processes that involve intermolecular interactions. Important biological processes are based on cell proliferation and migration, that both take place in interaction with the components of the extracellular matrix (ECM). Among them, collagen is the most abundant protein in ECM and connective tissues. Collagen consists of left-handed helical chains supercoiled into a right-handed triple helix. While various physico-chemical properties (e.g. stiffness, topography, confinement, etc.) of the cell microenvironment have been studied extensively, the influence of the ECM chirality on cellular migration has been overlooked. To address this issue, we used a microcontact printing technique to fabricate well-controlled culture surfaces coated with either collagen I as natural matrix or biomimetic matrices made of collagen-mimetic-peptides (CMPs) presenting opposite chirality (L vs. D peptides). The surfaces were characterized by circular dichroism, showing a specific polyproline type II helix (PPII) conformation of the chains. We show that D-surfaces prevent the total spreading of epithelial keratocytes which are less spread and more rounded, demonstrating that keratocytes are sensitive to the ECM chirality. Interestingly, our findings show that migrating cells on D-surfaces exhibit a lower migration speed than those on collagen I and L substrates but are significantly more persistent, suggesting that the molecular chirality of the ECM regulates key aspects of cell migration referred to as "chirotaxis". To better understand the role of the molecular chirality on cellular mechanotransduction pathways, we characterized focal adhesions and used specific inhibitors of collagen-binding integrin receptors during migration assays

3.61 Jean-Paul RIEU *Poster*

Université Claude Bernard Lyon 1/ Institut Lumière Matière

Collective migration by self-generated aerotactic gradients

At the origin of multicellularity, cells may have evolved aggregation in response to predation, for functional specialization or to allow large-scale integration of environmental cues. Multicellular Dictyostelium (Dicty) slugs possess emergent sensing abilities toward light or heat that do not possess single cells [1]. Malignant B and T lymphocytes upon exposure to chemokine may assemble into small aggregates that migrate directionally into chemotaxis gradients much more efficiently than single cells [2]. Small clusters of neural crest (NC) cells chemotax to the chemoattractant Sdf1 while single NC cells cannot [3]. We recently demonstrated that dense Dicty populations are able to consume most available oxygen (O2) and create self-generated gradients to which they respond aerotactically. This creates various self-organized patterns depending on the initial boundary conditions: spreading of confined colonies with propagating wave fronts (rings of cells) [4] or microphase separation between aggregates and a cellular gas phase [5]. We will here present novels results concerning the motile properties of Dicty aggregates depending on their size. Mean squared displacements resulting from the tracking of aggregates of various sizes exhibit three regimes: diffusive, ballistic and diffusive again from short to long time scales. Surprisingly, larger aggregates are migrating at larger distances than smaller ones even if the smaller exhibit a higher instantaneous

speed. We try to understand whether the self-generated O2 landscape which is mandatory to explain microphase separation is also involved in this migrative properties scaling, or alternately, if an ad hoc Vicsek type model with cooperativity between cells is sufficient. Numerical simulations to test these models are performed with the CompuCell3D extended Potts model.

[1] Miura and Siegert,PNAS (2000). [2] G. Malet-Engra et al., Curr. Op. Bio (2015). [3] Theveneau et al., Dev. Cell (2010). [4] Cochet-Escartin et al. eLife (2021). [5] Carrère et al. Nature Communications (2023).

3.62 Romain ROLLIN *Poster*

Institut Curie

Physical origin of the nuclear folds and their role in nuclear volume homeostasis

Despite their usual textbook representations, nuclei are often wrinkled and folded. These irregularities of the nuclear envelope (NE) have attracted attention in the recent years because they were shown to have functional significance, for example in mechanosensing. While their function has been established, their origin is still unclear. In this talk, I will provide both experimental and theoretical evidence showing that these folds originate from a well-known mechanical instability. Stiff elastic sheets bound to compliant substrates buckle upon compressive stresses. Using quantitative order of magnitude estimates, I will show that this instability is indeed favorable for the system lamina, chromatin under study. We propose that the compression in the system is induced by the competition between the NE formation just after mitosis and the geometrical transition induced during chromosome decondensation. One salient prediction of the mechanical model is the non-linearity in the tension response of the NE. For small extensions, the folds effectively act as membrane reservoirs and the tension remains small. At larger extensions, the folds disappear, and the tension increases. We confirm these predictions with AFM experiments. By further coupling this mechanical model of the NE to an osmotic model, I will show that this effective non-linear elasticity of the envelope defines two distinct regimes in the volume response of nuclei to perturbations. First a "safe" regime, where the osmotic pressure is balanced at the NE, implying that the nuclei deform at constant volume upon uniaxial confinement and scale with the cell volume during a hypo-osmotic shock. Followed by a regime of nuclear volume loss and nuclear scaling breakdown upon folds disappearance. Together, our osmo-mechanical model of the nucleus demonstrates new important roles of the nuclear folds, both for the structural integrity of the NE, for the nuclear scaling, and for the nuclear volume response to external stresses.

3.63 Guillaume ROMET-LEMONNE *Talk*

Institut Jacques Monod

Self-repair of branched actin filaments exposed to mechanical load

In cells, several key actin filament networks are nucleated by the Arp2/3 complex. In most cases, the Arp2/3 complex is activated to generate actin filaments as branches that grow off the sides of pre-existing 'mother' filaments. While this nucleation mechanism is now well understood, much less is known about the stability and turnover of actin filament branches. I will present

unpublished work from our lab, where we use microfluidics and optical microscopy to manipulate and monitor individual Arp2/3-nucleated branches in vitro, under controlled biochemical and mechanical conditions. Unexpectedly, we found physiological conditions where, upon mechanically-induced branch dissociation, the Arp2/3 complex remains bound to the mother filament and is reactivated to nucleate a new branch. We have quantified the different reactions responsible for this regeneration mechanism, under different mechanical conditions. I will discuss how these observations shed new light on the active conformation of the Arp2/3 complex, and on the means by which the turnover and stability of the resulting filaments may be controlled in cells. In particular, the branch regeneration mechanism we uncover here provides a means for actin filament networks to adapt to mechanical stress.

3.64 Artur RUPPEL *Poster*

CRBM - CNRS

Minimalistic tissue models to study the role of cell-generated forces in tissue morphogenesis

Forces play a critical role in biological processes, driving cell migration, division, and cell and tissue morphogenesis in development and disease. They also have signalling character, participating in the coordination of cellular behavior. To investigate the role of cell-generated forces in tissue morphogenesis, we used minimalistic tissue models and a combination of techniques, including traction force microscopy, micropatterning, non-neuronal optogenetics, and mathematical modeling.

Our results demonstrate that force signals travel fastest perpendicular to the orientation of cells in epithelial cell doublets and small monolayers, providing valuable insight into how forces are transmitted and distributed within tissues. The minimalistic nature of our assay allowed us to quantify force signal transmission, without the confounding effects of more complex tissue models.

Furthermore we recently developed a minimal 4-cell in vitro assay to study force distribution during cell intercalation, a major mechanism driving and accommodating tissue morphogenesis. We validated this assay using embryonic stem cells from the Xenopus laevis embryo and adult epithelial MDCK cells.

By combining our minimal in vitro assay with mathematical modeling, we aim to gain new insights into the fundamental cellular and physical principles of cell intercalation.

3.65 Gautham SANKARA-NARAYANA *Talk*

Pathogenesis of Vascular Infections Unit, Inserm U1225, Institut Pasteur, Paris, France.

Bacterial adhesion on the apical cell surface modifies the traction force field on the basal side through a new actomyosin structure, called ancreopodia

Pathogenic bacteria have various strategies to invade the host, which generally involve the hijacking of host cell components such as cell membrane, cytoskeleton, cell-cell junction, and/or cell-ECM adhesion molecules to mediate bacterial adhesion, proliferation, and dissemination. Recent studies have proposed that these processes also involve major changes in host cell mechanics; however, the forces bacteria generate or induce in the host and their functional impact on tissue physiology and tissue integrity remain largely unexplored, especially in the case of extracellular

pathogens. To address this question, we investigate how Neisseria meningitidis (Nm) modulates endothelial cell mechanics upon adhesion, and its potential relationship with key pathological signatures of this infection, e.g. vascular damage. Previous work has shown that Nm binding on the host cell membrane (apical surface) induces major remodeling of the plasma membrane and actin cortex. This is mediated by Type-IV pili and leads to the formation of a honeycomb-like structure at the infection site termed cortical plaque, with thin protrusions intercalating between bacteria. This has been shown to be important for bacterial resistance to blood flow-generated shear stress, however, its impact on global host cell mechanics is unknown. For the first time, we identify that portion of cortical plaque from then apical cell-membrane extends perpendicularly to the basal cell membrane. These columnar extensions are actin-rich, and we term them "Ancreopodia". We bring to light that Ancreopodia assists the apical Nm to sense the basal extracellular matrix (ECM), and establish strong anchoring, and transmit forces. Furthermore, two consequences that we observe are 1) Ancreopodia are dynamic and induce cell organelle deformation; 2) Ancreopodia's base at the cell-ECM interface, additionally induces a topological defect (+1) that might lead to misalignment on the basement membrane. Both consequences could facilitate Nm-mediated cell death, tissue disintegration, and eventually vascular damage.

3.66 Pierre SARAMITO *Poster*

CNRS, lab LJK, Grenoble

Numerical resolution with a viscoelastic fluid model for describing the tissue reorganization

Tlili et al (Dev, 2022) have proposed a microfluidic platform to investigate the role of mechanical constraints on tissue reorganization. A cell aggregate is aspired in a rectangular channel, and this geometry provides a benchmark to discriminate between rheological models of tissue mechanics. Using an Oldroyd-B viscoelastic fluid model and numerical computations based on the finite element method, we are able to compare both the velocity vector field and the deformation and elastic stress tensors, and found that this continuous mathematical model explains correctly the experiments. Moreover, we estimate the dimensionless Weissenberg number which quantifies the ratio of two characteristic times.

3.67 Sebastien SART *Poster*

Institut Pasteur

Microfluidic Droplets for Mapping and Regulating Self-Organization of Organoids

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

3.68 Andreas SCHOENIT *Talk*

Institut Jacques Monod, CNRS and Universite Paris Cite

Cell-cell adhesion strength regulates mechanical cell competition in epitheli

Cell competition is a tissue surveillance mechanism important in development, infection pathology and tumorigenesis. In these processes, cells with reduced fitness compared to their surrounding are eliminated and outcompeted. Cells can compete by exerting mechanical forces on one another. However, what determines the competition outcome remains unclear. In this completely unpublished work, combining modelling and experiments, we show that the competition outcome is governed by relative cell-cell adhesion strength. By mixing various cellular populations expressing different levels of E-cadherin, we demonstrate that the weakening of cadherin-based junctions favored cell elimination. The elimination occurred at the interface between mechanically different tissues and was caused by the emergence of large mechanical stress fluctuations. These fluctuations can be more efficiently dissipated by cells with stronger cell-cell adhesion, preventing their elimination. This competition mechanism could have important consequences in the formation of tissue boundaries, tissue shaping and cell invasion initiation.

3.69 Valentine SEVEAU DE NORAY *Poster*

Aix Marseille Université

Keratocytes migrate against flow with a roly-poly like mechanism

Guided migration is a complex phenomenon that rely on several mechanisms, such as biochemical and/or mechanical cues. In the case of flowtaxis (mechanical guidance by flow), it has been shown that cells, such as leukocytes, can migrate against the flow. This counterintuitive phenomenon has been interpreted in two different ways: an active mechanism of mechanotransduction mediated by integrin molecules, or a so-called "passive" mechanism relying on a mechanical bias without mechanotransduction. Here, we performed micro-fluidic experiments on fish epithelial cells (keratocytes) and reveals phenotypes of upstream or downstream migration with respect to the flow. We show that each cell has an intrinsic orientation that results from the mechanical interaction of the flow depending on the cell morphology. For upstream keratocytes, we show that the torque exerted by flow interacting with cell's tailing edge can orient the cells against the stream. This mechanism reminds a « roly-poly » body that tends to right itself when pushed over its equilibrium position. Conversely, for downstream keratocytes, the flow interacting on the cell leading edge, the lamellipodium, generates a supplementary torque that causes destabilization of upstream migration. A simple mechanistic model, taking into account the geometry of the cell and the hydrodynamic drag on keratocytes, recapitulates the phase diagram of cells orientations according to their morphology without adjusting parameters. Our observations suggest that upstream cell phenotypes can be interpreted as a passive mechanism, thus implying a potential absence of an active physiological function.

3.70 Apeksha SHAPETI *Talk*

KU Leuven

Forces and degradation underlie angiogenesis driven mosaicism in cerebral cavernous malformations

Cerebral Cavernous Malformation (CCM) is a cerebrovascular disease characterized by tumour-like lesions that co-localize with sites of strong postnatal angiogenesis1. ROCK1-dependent intracellular tension and enhanced angiogenesis are known factors that modulate CCMs2,3. Recruitment of wild-type (WT) endothelial cells (ECs) is also a prerequisite for lesion growth4. We combined traction force microscopy (TFM), dynamic matrix degradation visualization, immunostaining and single cell RNA sequencing (scRNAseq) to explore mechanisms underlying WT-EC recruitment and its modulation by ROCKs during 3D in-vitro mosaic (WT+siRNA-silenced ECs) angiogenic invasion. Silenced CCM-ECs (further denoted CCM-ECs) show increased invasion, matrix degradation and cell tractions during both mosaic (WT+silenced-ECs) and non-mosaic (silenced-ECs) sprouting. Interestingly, WT-ECs invade further and faster in CCM mosaics but are restricted to stalk positions by hyper-angiogenic leading CCM-ECs. This is altered through inhibition of contractility or ECM degradation. Dynamic co-visualization of ECM degradation and invasion shows that WT-ECs can follow locally degraded paths created by CCM-ECs. Meanwhile, 3D-TFM on mosaic cell pairs illustrates that highly contractile tip CCM-ECs generate cell-cell pulling forces on stalk WT-ECs to also encourage migration. Additional silencing of ROCK1 or ROCK2 in CCM-ECs showed

that degradation is mediated only by ROCK1 while force exertion requires but ROCKs. Strikingly, follower WT-ECs reveal higher 1-integrin activation in CCM mosaics. Single-cell transcriptomic signature of invading CCM-ECs matched upregulated pathways identified in-vivo. Comparing highly expressed genes between WT-ECs in control versus CCM mosaics showed activation of matrisome and DNA replication programs in WTs from CCM mosaics. We demonstrate here that WT-ECs in CCMs show modified transcriptomic signatures and enhanced angiogenic invasion mediated by dysregulated mechanics and ECM degradation. Our novel 3D TFM workflows combined with multicellular in-vitro systems provide new tools for identifying disease mechanisms.

3.71 Nathan SHOURICK *Poster*

Université Grenoble Alpes - CNRS - LJK - TIMC

Mathematical modelling of collective cell movement

The migration of an epithelial cell monolayer on a solid substrate plays a role for instance during embryogenesis, tumor growth or wound healing. Each cell has a polarity, that is a preferred direction to actively exert forces and move. Its alignment with polarity of neighbouring cells contributes to the emergence of collective migration. In close interaction with experiments in Lyon and Paris, we developped a thermodynamically consistent mathematical model at tissue scale based on continuum mechanics to better understand the appearance of collective movement. Based on results of numerical resolutions, we will discuss the feedbacks between polarity and activity.

3.72 Matis SOLEILHAC *Poster*

CNRS

Role of EpCAM in intestinal crypt morphogenesis

Intestinal tissue's proper functioning relies on a balanced cell composition and organization, facilitated by stem cell niches. Organoids offer a valuable model to study biomechanical cues' impact on intestinal morphogenesis. Disrupted homeostasis in the crypt compartment contributes to diseases, including cancer. EpCAM (Epithelial Cell Adhesion Molecule) is a protein exclusively expressed in epithelial cells in physiological conditions and is described as a key player in epithelial morphogenesis. Interestingly, EpCAM's loss leads to the development of a rare human disease so-called Congenital Tufting Enteropathy (CTE). The impact of CTE development on the differentiated intestinal domain has been well characterized (Salomon et al., 2017; Gaston et al., 2021); the latter displays the formation of distinctive tissue lesions called "tufts". However, the consequences of EpCAM's loss on the proliferative compartment of the intestine have been very poorly studied. We tackle this question by using 2D and 3D intestinal organoids as models. Constitutive EpCAM's silencing in intestinal organoids using CRISPR-Cas9 system leads to degeneration and death of KO organoid clones. These data suggested that EpCAM is required for organoid self-renewal and crypt integrity. Thus, we have developed inducible lentiviral Cas9 system targeting EPCAM to generate conditional inducible EpCAM-KO organoids. We show that the silencing of EpCAM leads to a disruption of morphogenesis in 3D and 2D organoids. EpCAM-KO in organoids leads to a dramatic expansion of crypt-like domains in which the niche cell composition and arrangement are altered.

Analyses of EpCAM-mutated CTE patient biopsies validate these observations in vivo. Moreover, patient intestinal tissue exhibits frequent crypt fission events and numerous signs of asymmetric crypt fission events which testify to a defective cryptogenesis in the absence of EpCAM. We conclude that EpCAM is required for the morphogenetic developmental events that ensure proper cryptogenesis in the intestinal tissue.

3.73 Raimon SUNYER *Talk*

University of Barcelona

Stiffness-dependent active wetting enables optimal collective cell durotaxis

The directed migration of cellular clusters enables morphogenesis, wound healing and collective cancer invasion. Gradients of substrate stiffness direct the migration of cellular clusters in a process called collective durotaxis, but the underlying mechanisms remain unclear. Here we unveil a connection between collective durotaxis and the wetting properties of cellular clusters. We show that clusters of cancer cells dewet soft substrates and wet stiff ones. At intermediate stiffness—at the crossover from low to high wettability—clusters on uniform-stiffness substrates become maximally motile, and clusters on stiffness gradients exhibit optimal durotaxis. Durotactic velocity increases with cluster size, stiffness gradient and actomyosin activity. We demonstrate this behaviour on substrates coated with the cell-cell adhesion protein E-cadherin and then establish its generality on substrates coated with extracellular matrix. We develop an active wetting model that explains collective durotaxis in terms of a balance between in-plane active traction and tissue contractility and out-of-plane surface tension. Finally, we show that the distribution of cluster displacements has a heavy tail, with infrequent but large cellular hops that contribute to durotactic migration. Our study demonstrates a physical mechanism of collective durotaxis, through both cell-cell and cell-substrate adhesion ligands, based on the wetting properties of active droplets.

3.74 Singha TAPAS *Talk*

Institut Curie - Paris

Contraction-based cell motility against viscoelastic resistance

A migrating cell in a complex environment such as tissue is typically surrounded by extracellular matrices and neighboring cells. In such an environment, the motility of a single cell is regulated by the contraction of the actomyosin cortical cytoskeleton, which can be reproduced when cells are confined in microchannels. We study a model of Contraction-based cell motility inside a microchannel to investigate the regulation of cell polarization and motion by the mechanical resistance of the environment. In a viscous environment, we find that spontaneous symmetry breaking of the acto-myosin cortex and motility occurs beyond a contractility threshold that depends on the extracellular viscous resistance. If cell motion is opposed by a viscoelastic resistance, we find an oscillatory regime where the cell polarization and motion exhibit periodic oscillations. In the latter case a crucial parameter is the ratio of the viscoelastic relaxation time scale of the environment compared to the typical turnover time of the cell cytoskeleton.

3.75 Isabelle TARDIEUX *Talk*

Grenoble Alpes Universtiy (UGA) - IAB

Decoding the minimal requirements behind Toxoplasma high speed gliding motility

The tachyzoite of Toxoplasma gondii is a polarized single-cell eukaryote which uses a high-speed unidirectional helical gliding motility to navigate between cells, cross cellular barriers or move in and out of permissive host cells. While the prevalent mechanistic gliding model is centered around a sub-membranous linear actomyosin motor to generate continuous forces, compelling evidence argue for a more complex model of force production. We previously uncovered a periodic apical traction force that couples with the disengagement of the posterior pole from the substrate along with dragging forces. We demonstrated that the tachyzoite no longer glides if the apical contact is prevented whereas forbidding the basal disengagement leads to parasite over-contraction and implosion. We have now investigated the minimal requirements for productive force during helical gliding. First, designing a set of micropatterns with alternating permissive and not permissive contact area, we found that the tachyzoite performs equally well on discontinuous substrates when compared to continuous ones. Expansion microscopy and 3D reconstruction images of tachyzoites gliding on the micropattern provide unique details on how the parasite undergoes gliding while only adhering through the apical and basal poles. We next built biochemical tunable surfaces with quantitatively controlled exposed moieties using a bio-conjugated layer-by-layer assembly strategy. We characterized these surfaces biophysically using the sensitive Quartz Crystal Microbalance with Dissipation technique. This combinatorial screening approach coupled to micropattern and video-microscopy points for glycosaminoglycan species being sufficient substrates for engaging into productive interaction and helical gliding. We will discuss our current progress to decode tachyzoite motile force production in a minimalist work-frame.

3.76 Hervé TURLIER *Talk*

CNRS, Collège de France

From microscopy images to mechanical models of tissues and back

Fluorescence microscopy has become the most common technique for quantifying biological systems, from the subcellular scale to the tissue scale. Yet, extracting meaningful physical information from fluorescent images, especially in 3D, remains a challenging task. At the same time, physical and computer models of tissues are becoming more and more realistic, but their direct comparison, calibration or initialization from biological images remains generally out of reach. Here I will present our recent efforts to bridge the gap between images and mechanical models. I will start with the presentation of a novel segmentation and 3D tension inference method that can generate 3D atlases of the mechanics of embryos or tissues comprising up to a thousand cells from microscopy images [1,2]. Then I will present a novel cell-resolved computational model of 3D tissues based on tension, which explicitly accounts for viscous dissipation at cell interfaces, can handle cell divisions or other topological events (T1, T2) and can coupled to a discrete reaction-diffusion scheme to model multicellular mechanochemical feedbacks. Finally, I will show how we can close the loop with a generic pipeline to create realistic fluorescence microscopy images from such simulations - and more

generally from other frameworks such as Cytosim - for devising, training or benchmarking novel image analysis methods.

[1] Ichbiah, S., Delbary, F., McDougall, A., Dumollard, R., Turlier, H. (2023). Embryo mechanics cartography: inference of 3D force atlases from fluorescence microscopy. bioRxiv, 2023-04. [2] Ichbiah, S., Delbary, F., Turlier, H. (2023). Differentiable rendering for 3d fluorescence microscopy. arXiv preprint arXiv:2303.10440.

3.77 Pierre UCLA *Talk*

Institut Curie

Photopolymerization of 3D fiber networks to study the dynamics of cell-matrix interactions

During the tumor process, the physical properties of the extracellular matrix are greatly altered by the action of chemical processes, but also mechanical ones, cells exerting traction forces. The tumor matrix typically exhibits increased stiffness, alignment of collagen fibers, and changes in porosity, playing a determining role in the initiation and progression of cancer, by controlling the behavior of cancerous and surrounding cells. The characterization of forces exerted by cells within the tumor microenvironment is the subject of intensive efforts and methods such as Traction Force Microscopy are commonly used to study the mechanobiological processes at work. However, these methods have limitations resulting in particular from the non-linearities of the material considered, and the measurement of 3D cellular traction forces in contact with individual fibers requires the development of innovative approaches. While conventional approaches allow to adjust the macroscopic mechanical properties of gels, the control of local physical properties (stiffness of individual fibers, local density, geometry) calls for the development of new nano- and micro-fabrication techniques.

We propose an innovative technology to study interactions with the matrix of cancer cells and neighboring cells, with the development of fiber networks with fully controlled physical and chemical properties. The produced fibers span a wide range of sizes and mechanical properties are characterized by means of force spectroscopy using Atomic Force Microscopy. We combine this approach with the development of an original method for measuring cellular traction forces in 3D, relying on automated 3D segmentation of the deformed fibers coupled with a Finite Element Modeling framework. We demonstrate that this technique is suitable to study traction forces in mesenchymal cells such as endothelial cells (HUVEC) and fibroblasts (NIH-3T3) but also in amoeboid-like cells such as dendritic cells, using fast volumetric imaging by Lattice Light-Sheet Microscopy to capture low intensity and short-lived traction forces.

3.78 Kotryna VAIDZIULYTE *Talk*

Institut Curie

Atypical nuclear phenotypes in confined circulating tumor cells

The physically diverse environment that a metastasizing cancer cell undergoes has been shown to induce functional changes through signaling pathways and selection pressure. However, their consequences on the specific stage of circulating tumor cells (CTCs) has, as yet, not been investigated. Using a unique model - CTC permanent cell lines derived from a colon cancer patient - we identified a novel nuclear phenotype that CTC cells demonstrate under strong mechanical confinement.

Cancer cells from a primary tumor undergo several functional changes up to the point where they colonize distant tissues and form secondary tumors - metastases. During this process, cancer cells experience physical deformation caused by growth and crowding in the primary tumor, passage through small capillaries in the blood circulation, and extravasation and migration during invasion of secondary tissues. We found that the confined nucleus displays nuclear envelope ruptures, exposing nuclear DNA to the cytoplasm. However, instead of repeated blebbing – rupture and repair events – the nucleus of confined CTCs folds into a unique "sickle-shape", which has not been observed before. The formation of this "sickle-shape" nuclei seems to depend on the contractility level of the cell and actomyosin generated cortical flows: it can be completely blocked by the drug blebbistatin, which inhibits myosin II. To explain this nuclear deformability and possible fragility, we investigated the changes in the nuclear envelope composition and adjacent cytoskeleton proteins, focusing on nuclear lamins and vimentin intermediate filaments. Our new insights provide more information on the least researched step of the metastatic cascade - circulation in the blood. They might suggest which functional changes in cancer cells help them gain the capacity to survive circulation in the blood, and invade secondary tissues.

3.79 Pim VAN DEN BERSSELAAR *Talk*

TU Eindhoven

The interplay between hRAS activation and environmental cues on epithelial monolayer mechanics.

Heightened cell mechanics is a hallmark of carcinogenesis. Hence, unraveling the interplay between oncogene activation and environmental physical cues on cell monolayer mechanics can elucidate various aspects of cancer cell progression, especially in the context of cancers of epithelial origin: carcinomas. Here, we systematically investigated the interplay between oncogene activation and other physical cues from the cell's microenvironment on epithelial monolayer mechanics. By using MCF10a microtissues which can be made progressively malignant upon activation of the hRAS oncogene, we could methodically study the effect of several mechanical cues of the tissue's microenvironment on monolayer mechanics. Quantification of cellular kinematics (motion, velocity, correlations, shape, deformations) and dynamics (forces and stresses) showed that mechanical alterations induced by oncogene activation can be systematically modulated by the mechanical cues present in the microenvironment through crucial signaling pathways within the cell. These findings provide valuable insights into the mechanical regulation of kinematics and dynamics in

transformed epithelial monolayers and offer potential avenues for further investigations into the underlying molecular mechanisms and therapeutic interventions for disease progression.

3.80 Lorijn VAN DER SPEK *Poster*

Université Paris Cité

Does geometric constraint of individual muscle cells promote differentiation?

Understanding the interplay between electrical coupling, mechanical stimuli and transcription factors in the context of muscle cell differentiation is a challenge for mechanobiology and muscle tissue engineering. We aim to determine the correlation between mechanical and geometrical constraints and spatially resolved differentiation, focusing on single cell scale. I will present my research done on myoblast cells (C2C12) on square and rectangular adhesive micropatterns.

Myoblast cells are specifically interesting as their shape changes from round to elongated in vivo during differentiation. While it has been shown that transcription factor expression of C2C12 depends on their shape, it remains unknown whether this results in a different differentiation fate. Therefore, the aim of our research is to correlate geometrical constraints with differentiation in 2D. The degree of differentiation can be measured along different types of markers, like expression of various transcription factors, proliferation rate, and myoblast membrane potential to shed light both on proteomics and on function.

We focus on early differentiation (timespan of 3 hours). Even at such short time scales, our results point towards an important impact of geometric constraints on membrane potential and proliferation. I will also present our first results on protein expression and transcription.

3.81 Maurizio VENTRE *Poster*

Interdisciplinary Research Centre on Biomaterials - University of Naples Federico II

Decellularized Dermal Matrix as a Biomimetic Scaffold for Dendritic Cell Mechanobiological Studies

The importance of the extracellular matrix (ECM) - immune cell interactions has been largely acknowledged. However, systematic studies aimed at addressing the influence of specific ECM biophysical features on immune cell functions are lacking. Myeloid cells, especially Dendritic Cells (DCs) and Macrophages, migrate through tissues and are subjected to spatiotemporal changes of ECM mechanics, composition and microarchitecture both in health and disease. Current experimental models are mostly based on 2D substrates of 3D reconstituted gels that cannot replicate the mechanical and structural complexity of the native ECM. We hypothesize that decellularized skin might represent an accessible, viable and versatile alternative to bio-based scaffolds for investigating DCs functions in a 3D in vivo-like environment. To test our hypothesis, we fabricated decellularized dermal matrices starting from partially processed ovine leather samples. The matrices are constituted by a network of collagen bundles, which can be further processed with mechanical stretching and enzymatic treatments to modulate fibre alignment and stiffness. Advanced confocal imaging revealed that immature DCs (iDcs)? remain viable for at least 48 hrs in the matrices where they associate with collagen bundles. iDCs exhibit their characteristic shape with clear dorsal membrane ruffles and

the typical actin-rich podosome-like protrusions at the ventral-collagen fibre interface. Furthermore, iDCs are able to internalize both soluble and particulate antigens, as shown by colocalization of fluorescent ovalbumin with the lysosomes and fluorescent zymosan particles with the C-type lectin CD206. Additionally, iDCs positively responded to prostaglandin E2, by acquiring a mature phenotype. Finally, we provide evidence that DC behaviour and functions is affected by the mechanical and strucutral features of the dermal matrices. Additionally, decellularized dermal matrices are suitable for heterotypic multicellular cultures such as DCs, fibroblasts and melanoma cancer cells, possibly recreating a homogeneous or stratified 3D tumor microenvironment.

3.82 Maurizio VENTRE *Poster*

Interdisciplinary Research Centre on Biomaterials - University of Naples Federico II

Production and characterization of decellularized dermis matrices for dendritic cell cultures

Our understanding of mechanobiology is mostly based on two-dimensional culturing platforms. Current three-dimensional (3D) systems are constituted by hydrogels or synthetic scaffolds. However, the added dimensionality is not sufficient per se to endow the system of a thorough biomimicry. The in vivo extra cellular matrix (ECM) is composed of a complex network of fibres. Also, the spatial assembly and orientation of the fibres is position dependent, giving rise to graded 3D structures. Reconstituted collagen gels, as well as 3D bioprinted systems do not capture neither such a structural complexity nor the mechanical features of natural ECMs. Tissue decellularization has gained popularity as it enables to obtain ECMs whose structure and composition resemble those found in vivo. However, tissue decellularization is very time consuming and the presence of cellular/nuclear remnants, may affect the cell culturing process. Here we report on the fabrication, characterization and manipulation of ovine decellularized dermis specimen for dendritic cell (DCs) mechanobiological studies. Matrices were obtained from partially processed leather samples and are almost totally constituted by collagen fibres, which are arranged in the form of tens of micron wide bundles. Decellularised samples display a compact papillary layer that cover a more open reticular stratum. Stiffness falls in the 1 - 10 kPa range. This can be altered through enzyme treatments (i.e. collagenase), whereas fibril structure can be oriented through macroscopic stretching. Through rheological, mechanical (AFM) and morphological (SHG and microCT) analyses we provide evidence that the biophysical features of matrices can be manipulated. DCs firmly adhere and envelope collagen bundles in a manner reminiscent of convoluted 2D surfaces. Also, soluble and particulate compounds diffuse through the matrix. Decellularized matrices can be used as a low-cost, readily available and versatile systems for mechanobiological studies, thus helping to identify the biophysical effectors of DC functions in a biomimetic environment.

3.83 Antoine VIAN *Poster*

TU Dresden, Physics of Life

Double emulsion droplets as osmotic pressure sensors in biological systems

Mechanics is known to play a fundamental role in many cellular and developmental processes. Beyond active forces and material properties, osmotic pressure is believed to control essential cell and tissue characteristics. However, it remains very challenging to perform in situ and in vivo measurements of osmotic pressure. Here we introduce double emulsion droplet sensors that enable local measurements of osmotic pressure intra- and extra-cellularly within 3D multicellular systems, including living tissues. Double emulsion droplets volume can change when submitted to an osmotic shock because the surfactant dissolved in the oil membrane surrounding their aqueous core act as a transport vehicle for water molecules. In this work, we produced droplets with a microfluidic device to obtain great control over their sizes and loading. We then calibrated the droplet to relate the size measured when the osmotic pressure applied and determined their shrinking kinetics behavior. Finally, we measured the osmotic pressure in blastomeres of early zebrafish embryos as well as in the interstitial fluid between the cells of the blastula by monitoring the size of droplets previously inserted in the embryo.

Our results show a balance between intracellular and interstitial osmotic pressures, with values of approximately 0.7 MPa, but a large pressure imbalance between the inside and outside of the embryo. The ability to measure osmotic pressure in 3D multicellular systems (developing embryos, organoids, etc.) will help understand its role in fundamental biological processes.

3.84 Catherine VILLARD *Poster*

CNRS Université Paris Cité

Helical Fungi under Physical Constraints

Helical filaments are represented in living organisms from the molecular to the multi-cellular scale. Described by Darwin in plants as early as the 19th century, they still raise fundamental questions about their roles and associated mechanisms. We here focus on the oscillatory shapes adopted by the filaments (hyphae) of the fungus Candida albicans in different physical confinement situations. Microfluidic channels successively presenting portions of different heights, more precisely just below (1.5 μm) and above (6.5 μm) the hyphal diameter (2 μm), allow the observation of a reversible geometrical transition between wavy shapes and helices. Unexpectedly, curvatures in wavy shapes are not built from a regular forward oscillatory movement of the tip alone, but by successive sliding events of a significant portion of the hypha at the rear of the apex. Moreover, these events follow relatively straight hyphal trajectories, suggesting a phenomenon of periodic release of elastic stress. Overall, our observations are in line with the squeelix ("squeezed helix") concept developed by Kulic et al., based on an interplay between bending and twisting energies of filaments [1]. We have also evaluated the possible advantages of these oscillatory growth patterns in the colonization of complex spaces by implementing micro-mazes in microfluidic devices. The hyphae navigate through networks of obstacles by making successive coordinated choices among the 3 possibilities offered at the corner of each individual obstacle. Strikingly, hyphae preferentially change their curvature at each step, drawing regular oscillations along a constant growth direction.

This phenomenon of directional memory, which highlights an elementary sense of proprioception in these filamentous organisms, might result from the same mechanisms than the ones governing the spontaneous oscillations in confined environments.

3.85 Bart VOS *Talk*

University of Göttingen

Exploiting Onsager regression in passive measurements to reveal active mechanics of living systems

Understanding life is arguably among the most complex scientific problems faced in modern research. From a physics perspective, living systems are complex dynamic entities that operate far from thermodynamic equilibrium. This active, non-equilibrium behaviour, with its constant hunger for energy, allows life to overcome the dispersing forces of entropy, and hence drives cellular organisation and dynamics at the micrometer scale. Unfortunately, most analysis methods provided by the powerful toolbox of statistical mechanics cannot be used in such non-equilibrium situations, forcing researchers to use sophisticated and often invasive approaches to study the mechanistic processes inside living organisms. Inspired by Onsager's regression hypothesis, we introduce here a Mean Back Relaxation (MBR) observable, which detects active motion in purely passive measurements of particle fluctuations. The MBR, which is based on three-point probabilities, is theoretically and experimentally shown to exhibit markers of non-equilibrium, i.e., of detailed balance breaking dynamics. We furthermore observe an astonishing relation between the MBR and the effective non-equilibrium energy in living cellular systems. This is used to successfully predict the viscoelastic response function and the complex shear modulus from a purely passive approach, hence opening the door for rapid and simple passive mechanics measurements even in active systems.

3.86 Fanny WODRASCKA *Talk*

Institut Jacques Monod

Study of cell extrusion mediated by RhoA protein activation: optogenetic approach

Cell extrusion is one of the mechanisms allowing abnormal or supernumerary cells to be eliminated from epithelia in order to control the integrity of the tissue. Mechanical stresses in epithelial tissue, such as induced by cell compaction or topological defects, have been shown to trigger the initiation of extrusion events. However, the details of decision-making during mechanically induced extrusions remain poorly known. In particular, why a specific cell is extruded out of a crowded epithelium is still not well understood, neither is the fate of extruded cells. They can activate apoptotic pathways before being removed from the tissue or can be extruded alive: what are the mechanisms that regulate these different outputs? We address this question through a combination optogenetic and microscopy techniques. Optogenetics approaches are combined with Traction Force Microscopy experiments to take the quantitative aspect of the tool to a higher level. Optogenetics are used in this project as local mechanical force tuners: by controlling RhoA activation and subsequent myosin contraction, they allow us to trigger cell contractility in a local and

quantitative manner. The measurement of physical parameters such as traction forces associated with our optogenetics system allow us to study precisely the role of mechanical forces in cell extrusion. Combining optogenetics and TFM enabled us to show that enhancing cell contractility by stimulating cells increases rate of extruding cells. It also show that mechanically induced cell extrusions have an increased probability to be independent of caspase-3 activation as compared to unstimulated cells. Moreover, these extruded cells are increasingly oriented at the basal side of the tissue and show a delayed onset of apoptosis as compared to apically extruded ones. Altogether, these results show a strong relationship between cell contractility, the apico-basal orientation of cell extrusions and the fate of extruded cells.

3.87 Valentin WÖSSNER *Poster*

Universität Heidelberg

Minimal Active Gel Model for Single-Cell Motility with Adhesion

Explaining cell polarization in the absence of external cues is necessary in order to understand single-cell motility. Active gel theory has demonstrated that actomyosin contractility is sufficient for self-sustained cell migration. However, minimal models developed within this theoretical framework cannot explain the stick-slip motion often observed for migrating cells. Simple models have been suggested for the required adhesion dynamics, but these do not include intracellular flows. Here we show that in a one-dimensional setting active gel theory can be extended by such adhesion dynamics and that load sharing is the cooperative effect that is required to obtain symmetry breaking. For intermediate adhesiveness, symmetric polymerization leads to self-polarization and robust motility exists in a bistable regime. Our model predicts adhesion density profiles in qualitative agreement with experimental results and may serve as a starting point to describe the different migration patterns observed in adhesion-based motility.

3.88 Wang XI *Talk*

Institut Jacques Monod

The emergence of spontaneous coordinated epithelial rotation on cylindrical curved surfaces

Collective epithelial rotation (CeR) in three-dimensional tissues represents a coordinated cellular movement driving tissue morphogenesis and transformation. Questions regarding such behaviors and their relationship with tissue geometry are intimately linked to spontaneous active matter processes and to vital morphogenetic and embryonic processes. Here, using in vivo and in vitro biophysical experiments and theoretical modeling we study the emergence of CeR in response to different tissue curvatures. We observe large-scale, persistent and circumferential rotation in both concavely and convexly curved cylindrical tissues. While epithelia of inverse curvature show an orthogonal switch in actomyosin network orientation and opposite apicobasal polarities, their rotational movements emerge and vary similarly within a common curvature window and require cell-cell adhesion and cell polarity. Using an active-polar-gel model, we reveal the different relationships of collective cell

polarity and actin alignment with curvature, which lead to coordinated rotational behavior despite the inverted polarity and cytoskeleton order.

3.89 Wang XI *Poster*

Institut Jacques Monod

Ex vivo biomimetic intestinal model for developmental studies

Intestinal epithelium (IE) constitutes the second largest epithelium in the human body and exposes to a highly dynamic and complex microenvironment. Healthy intestine withstands various mechanical stimuli, extreme pH variations, contains different biochemical/physical gradients and hosts trillions of bacteria. The single-layer epithelium organizes into highly regular tridimensional (3D) crypt and villus structures, where cells of varied functions are found in distinct areas and undergo rapid proliferation, differentiation, coordinated cell migration, and extrusion. These spatiotemporal organization and dynamics preserve the intestinal stem cell niche and enable constant tissue replenishment, making IE a unique dual functional barrier for both uptakes of nutrients and protection against pathogens. Recent research has revealed that the villus-crypt architecture and environmental factors play critical roles in IE homeostasis and mechanisms in physiological and pathological conditions. However, current planar petri-dish-based and organoid culture systems lack the control of different environmental parameters. Combining micro-engineering techniques and cell biology, we address such a disadvantage by developing hydrogel scaffolds that represent the similar 3D crypt-villus architecture of intestinal epithelia. These scaffolds are further functionalized with proteins and integrated into microfluidic or Transwell set-ups for mimicking in vivo biochemical gradients. We present that primary intestinal stem cells from organoid culture can grow on our 2D and 3D scaffolds and replicate critical features of the small intestine. Our novel approach to an ex vivo intestinal model can serve as a versatile platform for broad bio-mimicking applications of IE and is adaptable to model other tissues. For example, it would be applicable to decipher mechanisms for developing IE layer and its homeostasis and to study the pathogenesis of rare congenital intestinal diseases, which are technically challenging to investigate in vivo.

3.90 Spela ZEMLJIC JOKHADAR *Poster*

Institut of biophysics, Faculty of medicine Ljubljana, University of Ljubljana

The influence of intravenous lipid emulsions on metabolism and mechanical properties of HUVEC cells

Critically ill patients with impaired digestive system are essentially dependent on parenteral nutrition, i.e., intravenously administered nutrition. An important component of parenteral nutrition are lipid emulsions, which can be administered in combination with other nutrients, such as amino acids and sugars, or as an individual infusions. Intravenously supplied fatty acids are taken up by immune cells circulating in the bloodstream and by the endothelium. They are metabolized via various cellular processes such as cellular energy metabolism to generate ATP and cell membrane synthesis, which is also critical for cell proliferation. Importantly, fatty acids are involved in various cellular signaling pathways and may, for example, influence T lymphocyte differentiation and

survival. Because cellular metabolism is involved in virtually every cellular process, its changes can be also reflected in the mechanical properties of cells. The aim of our work is to better understand the influence of lipid emulsions on the metabolism and mechanics of endothelial cells. Specifically, we investigated the influence of two standard clinical lipid emulsions SMOFlipid (a compound lipid emulsion), and Omegaven (a fish oil lipid emulsion) on the metabolism and mechanical properties of human umbilical vein endothelial cells (HUVEC). The viability of HUVECs was measured with the MTS assay, and the rate of ATP production from glycolysis and mitochondrial respiration was analyzed using the Seahorse analyzer. The mechanical properties of the cells were assessed with deformation cytometry using a custom open-source stroboscopic imaging system. Preliminary results indicate that Omegaven significantly reduced cell viability after 48 hours of exposure, whereas exposure to SMOFlipid did not affect viability or even resulted in a slight increase. We also observed an altered distribution of the actin cytoskeleton and the presence of intracellular vesicles in treated cells. Differences in the deformability of the cells, as measured by deformation cytometry, were also observed.

3.91 Jack ZHANG ZHOU *Poster*

Universidad de Zaragoza

Quantification of T-cell migration in confined and 3D conditions

The immune system plays a crucial role in the defense against pathogens and aberrant cells, such as tumoral cells. In order to carry out its function of immune surveillance, migration is one of the fundamental processes required. Therefore, it is essential to characterize this mechanism in physiologically and pathologically relevant scenarios to comprehend the immune response. In this context, we have adopted a novel microfluidic-based approach that recreates the biomechanical aspects of solid tumors (Juste-Lanas et al., 2022; Movilla et al., 2022). Two different microfluidic geometries were employed: one of them based on a central chamber which allowed hydrogel polymerization (Shin et al., 2012), while the other one on microstructures of confined channels with varying widths (Paul et al., 2016). The microfluidic devices were fabricated with polydimethylsiloxane (PDMS) owing to its many advantages, including biocompatibility, transparency, flexibility and gas permeability. Then, T cells were seeded on the microchips and were visualized via time-lapse microscopy under controlled conditions of temperature, humidity and CO2 concentration. The resulting images were processed with ImageJ and Matlab to quantify cell migration. We found that T lymphocytes display higher velocity under confinement compared to 3D migration. This is consistent because in 3D hydrogel matrices cells must squeeze through different pores in three possible dimensions, leading to an irregular track and slower migratory speed. These results demonstrate that confinement is a key factor in immune migration and its characterization can provide a better understanding of the infiltrating capacity of immune cells in solid tumors, as well as in wounds or other pathological conditions.

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