Monday June 27th 2022 - Workshop

Mechanobiology and Physics of Life in Lyon

4th edition

Amphi Mérieux, Site Monod, ENS Lyon

9h-17h30

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5th workshop in *Mechanobiology and Physics of Life* in Lyon

Monday June 27th, 2022, Amphi Mérieux, ENS de Lyon

SCIENTIFIC PROGRAM

09h00-09h15	**Welcome, coffee**
09h15-9h25	**Opening**
	Session "Pathologies and cancer 1"
09h25-10h10	**Keynote: Gaëlle Recher** *Confined 3D cell culture used for both investigating cell self-organisation and engineering modular tissue units*, (LP2N, Institut d'Optique d'Aquitaine,)
10h10-10h25	**Pauline Bregigeon** *Microfluidic system for culture, monitoring and electroporation of spheroids based on a hydrogel scaffold* (Ampère, ECL, Lyon)
10h25-10h40	**Léa Chazot-Franguiadakis** *Nanofluidics for the study of viral particle transport* (LPENSL, Lyon)
10h40-10h55	**Poster teaser 1/2**
10h55-11h25	**Coffee break**
	Session "Pathologies et Cancer 2"
11h25-11h40	**Malèke Mouelhi** *Long-term nuclear regulation of cancer cells under confinement* (ILM, Lyon)
11h40-11h55	**Léa Barral** *Impairment in mechanotransduction pathways, a key for AML Chemoresistance* (CRCL, Lyon)
11h55-12h10	**Fabien Delebosse** *Characterization of the mechanical properties of lung adenocarcinoma* (LTM/Cell&Soft, Grenoble)
12h10-12h15	**Idylle-labs presentation**
12h15-12h30	**Poster teaser 2/2**
12h30 -14h15	**Lunch and Poster session**
	Session "Subcellular and Cellular mechanics"
14h15-14h30	**Sigolène Lecuyer** *Substrate stiffness impacts early biofilm formation by modulating bacterial surface motility* (LPENSL, Lyon)
14h30-14h45	**Delphine Débarre** *Mechanical regulation of cell adhesion to a soft wall under flow* (LiPhy, Grenoble)
14h45-15h00	**Sylvain Monnier** *Probing cell crowding in cells and tissues with Brillouin light scattering* (ILM, Lyon)
15h00-15h30	**Coffee break**
	Session "From cells to tissue and development"
15h30-15h45	**Benoit Landrein** *The mechanics of seed size control in plants* (RDP, Lyon)
15h45-16h00	**Vincent Mirouse** *WAVE regulatory complex facilitates cell rearrangements through the generation of a protrusive F-Actin subpopulation at tricellular junctions*. (iGReD, Clermont-Ferrand)
16h00-16h15	**Jocelyn Etienne** *Friction when changing neighbours: adhesion-regulated junction slippage controls cell intercalation dynamics in living tissue* (LiPhy, Grenoble)
16h15-17h00	**Keynote: Julien Derr** *Plant morphogenesis: motions, growth and mechanics* (RDP, ENS de Lyon)
17h00-17h15	**Poster prices and closing remarks**

Session « Pathologies and cancer »

Keynote speaker

Gaëlle Recher, Institut d'Optique d'Aquitaine

Confined 3D cell culture used for both investigating cell self-organisation and engineering modular tissue units

In both physiological and pathological context, 3D cell cultures are now the new standard for studying cell behaviours and how dependent they are on the 3D context, in comparison with 2D cultures. The diversity of 3D culture has also increased and ranges from arrays of isolated spheroids to bulk cultures of organoids in Matrigel domes. The group has been developing and upgrading a microfluidic-based encapsulation system to produce and grow cell aggregates, confined in hollow alginate capsules. We take advantage of this high-throughput production of spheroids and organoids to question cell-cell interactions in confined environment with custom imaging tools. These approaches lead us to study cell self-organisation depending on the formulation of the cell suspension: whether cells are mixed with solely medium or with extracellular matrix, or whether we add endothelial cells to promote micro-vascularisation. This presentation will end by illustrating the benefits of using pre-assembled building blocks made with both spherical and tubular capsules for manufacturing ‘vascularised’ tissues.

Microfluidic system for culture, monitoring and electroporation of spheroids based on a hydrogel scaffold

Pauline Bregigeon[a*], Théo Le Berre[a], Julien Marchalot[a], Laure Franqueville[a], Christian Vollaire[a], Marie Frénéa-Robin[a] and Charlotte Rivière[b,c,d]

[a]*Univ Lyon, Ecole Centrale de Lyon, INSA Lyon, Université Claude Bernard Lyon 1, CNRS, Ampère, UMR5005, 69130 Ecully, France*
[b]*Université de Lyon, Université Claude Bernard Lyon 1, CNRS, Institut Lumière Matière, F-69622, Villeurbanne, France*
[c]*Institut Universitaire de France (IUF), France*
[d]*Institut Convergence PLAsCAN, Centre de Cancérologie de Lyon, INSERM U1052-CNRS UMR5286, Université de Lyon, Université Claude Bernard Lyon 1, Centre Léon Bérard, Lyon, France*

*pauline.bregigeon@ec-lyon.fr

Keywords: spheroid, agarose hydrogel scaffold, microsystem, electrochemotherapy.

Abstract

In the search for a safer and more efficient treatment than classic chemotherapy, electro-chemotherapy (ECT) has emerged as a solid alternative[1]. This method is based on electroporation (EPN). This phenomenon occurs when a pulsed electric field is applied to cells, and results in an increase in cell permeability to drugs. As the interest in such treatment is growing, the need for reliable tumor models to study the effect of EPN on cells is rising. Spheroids have been identified as a relevant model, as they can better reproduce the tumor microenvironment, compared to cells grown in 2D on flat surfaces, thanks to the formation of junctions ensuring cellular cohesion and communication[2].

In the literature, the proposed approaches usually consist in first fabricating the spheroids using droplet-based microfluidics, hanging drop methods or ultra-low attachment plates[2], and then introducing them in an electroporation cuvette connected to a pulse generator or using hand-held electrodes. It involves several handling steps, which can potentially damage spheroids. Moreover, the location of the spheroids is not controlled, which may induce differences in the electric field perceived from one spheroid to the other. Thus, the parallel treatment of tens of spheroids of similar characteristics (size, shape) is required to produce relevant statistical data.

To address this challenge, we designed a microfluidic platform enabling culture and electroporation of a large number of spheroids sharing similar characteristics without requiring any manipulation (Figure 1a)[3]. Here, we demonstrate the application of the device to the delivery of an anti-cancer agent, bleomycin, in HT29 colorectal cancer cell spheroids using sine wave bursts.

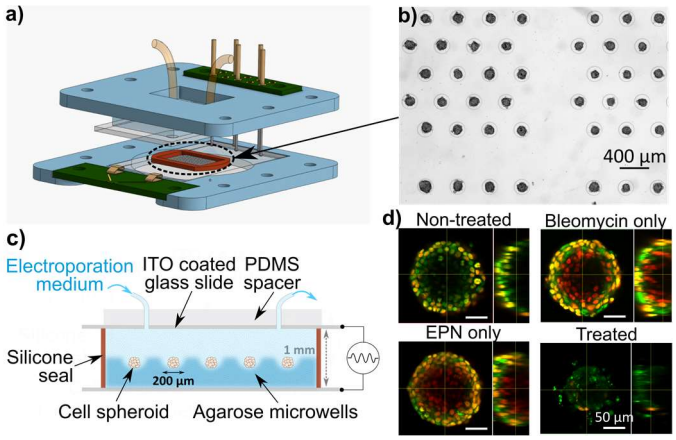

Figure 1: *(a) Computer Assisted Design (CAD) of the microsystem. (b) 2.5X bright field image of HT29 spheroids after 3 days of culture. (b) Schematic drawing of the microfluidic chamber (side view). (d) Orthogonal views of confocal images of spheroids 72h after the experiment. Green = cell nuclei, Red = proliferative cell, Yellow = both.*

To produce the spheroids, a 2% agarose hydrogel is molded and grafted on an amine-functionalized ITO-coated glass slide[4], used as an electrode for EPN. HT29 cells are seeded in the microwells to form spheroids of reproducible size and location (Figure 1b). A silicone seal is placed around the hydrogel to form a microfluidic chamber (Figure 1c), which is closed with another ITO coated glass slide, and mounted inside a sealing device enabling water tightness and electrical contact. Low conductive EPN Hepes buffer, supplemented with the anticancer drug (bleomycin, 20 µg/mL), is then injected in the microfluidic chamber. It enables to change the medium inside the chamber from culture to EPN buffer, with calibrated time and volume (data not shown). EPN is performed by applying 2 sine bursts[5] (300 V_{pp}, 10 kHz, 5 ms), which parameters were previously determined with fluorescence method (data not shown). Growth is monitored by microscope imaging and proliferation is characterized 3 days after the experiment thanks to a commercial kit (Click-iT EdU Alexa546, Invitrogen) and confocal microscope imaging.

Figure 1d shows that EPN parameters used here led to a reversible EPN as similar proliferation layers can be seen in yellow for non-treated and EPN only spheroids. Moreover, ECT (delivery of bleomycin in spheroids by EPN) is efficient as there are nearly no proliferative cells for treated spheroids. Figure 1d also shows that, as expected, for spheroids in contact with bleomycin without EPN, cell proliferation is not affected. Indeed, as bleomycin is only efficient on permeabilized cells, it is especially interesting for ECT, following the trend of safer therapy with fewer side effects. Spheroid growth was also quantified by bright field imaging and led to the same conclusion.

These results highlight the functionality of our device as an in vitro platform to test and monitor anti-cancer drug uptake in spheroids by EPN. After this first application, we intend to complexify the tumor model to analyze EPN efficiency in the presence of a dense extracellular matrix, produced by stromal cells. The microsystem also offers the possibility to perform bioimpedance measurements to study in real time the EPN of spheroids and to follow their growth.

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Nanofluidics for the study of viral particle transport

Léa Chazot-Franguiadakis[a], Fabien Montel[a]

a. Laboratoire de Physique, UMR CNRS 5672, ENS de Lyon, Université de Lyon, Lyon, France
* lea.chazot-franguiadakis@ens-lyon.fr

The human cell is organized in compartments between which the transport of biomolecules is a key step. Among the cell's communication pathways, the nuclear pore complex (NPC), which regulates transport between the cell nucleus and the cell interior [1], is certainly the most complex. This pore has exceptional adaptability and selectivity properties, due to the presence of a network of dynamic polymers inside its central channel. Many viruses (adeno-associated virus, hepatitis B virus, HIV, ...) must transport their genetic cargo through the nuclear membrane, via the NPC to replicate inside the cell nucleus.

The strength of our project is to address the issue of virus transport through the NPC, in a biomimetic environment, i.e. simplified and controlled, to facilitate the study. To do so, we seek to mimic the nuclear pore by grafting nanoporous membranes with hydrophobic artificial polymers. We then use a highly sensitive optical system, developed within our team, which allows us to detect in real time and at the level of a single pore the transport of single viral particles [2]. More precisely, the device is based on the combination of fluorescence microscopy and the Zero Mode Waveguide effect which leads to the intensification of the electromagnetic field in the vicinity of a nanopore covered with a metallic film [3].

Using this device, we have measured the frequency of passage of viruses (labeled by a fluorophore) through the pores as a function of a control parameter (applied pressure), and we have highlighted a "jamming" phenomenon related to the confinement of viruses under flow. We studied the determinants (physical, chemical) of this effect and proposed a physical model of the phenomenon similar to a phase transition under flow. We were then able to extract parameters related to the interaction of the viruses with the pore.

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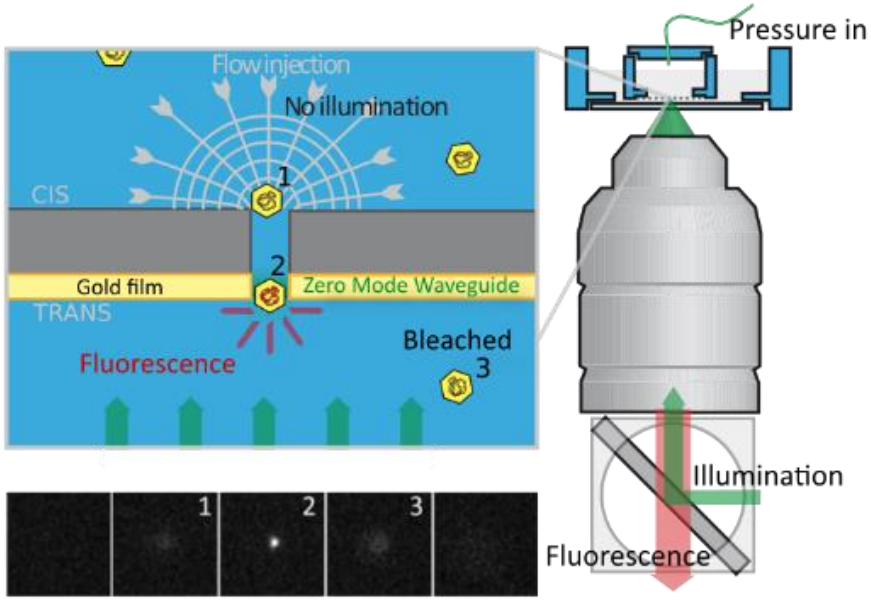

Figure 1 : Zero Mode Waveguide experimental setup for translocation of viral particles (fluorescently labeled) through a synthetic nanoporous membrane.

Long-term nuclear regulation of cancer cells under confinement

Malèke Mouelhi[1], Charlotte Rivière[1] and Sylvain Monnier[1]

[1] *Institute of Light and Matter (ILM), UMR5306 Univ. Lyon, Univ. Claude Bernard Lyon 1, France*

The physical properties of the tumor microenvironment are strongly modified during tumor growth and participate in the development and invasion of cancer cells [1], including not only stiffness, but also compression [2].

In particular, the nucleus is critically affected during compression [3] and is appearing as an important mechanosensor of deformations [4,5]. Nevertheless, most studies focus nowadays on short-term cell response (from minutes to few hours). New questions are open on the long-term adaptation to deformations and the mechano-sensing mechanism involved. We have recently developed a new agarose-based microsystem copping with media renewal impediment to investigate cell response to prolonged confinement [6].

We used this device to apply a tunable and controlled 1D confinement on the colorectal cancer cell line HT-29 up to several days. We evidenced a decrease of the nuclear volume after 24 hours under confinement. The overall nuclear shape is also dynamically regulated with the apparition of transient nuclear blebs. We are currently analyzing the mechanisms and consequences of such adaptation on cell division, transcription activity and protein expression. Such long-termed adaptation to mechanical constrains may be of importance for cancer cell plasticity and play a role in their resistance to treatments.

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Impairment In Mechanotransduction Pathways, A Key For AML Chemoresistance.

Léa BARRAL[1,2], Katharina RÖSEL[3], Charlotte RIVIERE[4], Magalie FAIVRE[5], Véronique MAGUER-SATTA[1,2], Sylvain LEFORT[1,2*].

[1] Inserm U1052, CNRS UMR5286, Cancer Research Center of Lyon, France; [2]University of Lyon; [3]University of Graz, Austria; [4]University of Lyon, Université Claude Bernard Lyon 1, CNRS, Institut Lumière Matière, F-69622, Villeurbanne, France; [5]Institut des Nanotechnologies de Lyon INL-UMR5270 CNRS, Université Lyon 1, Villeurbanne, France.

* : corresponding author.

Keywords: Acute Myeloid Leukemia; Bone Morphogenetic Proteins; Mechanotransduction; Treatment resistance.

Interaction with the hematopoietic niche is a key factor for the leukemic stem cells (LSCs) to survive. Cytokines and mechanical signals are important regulators for normal hematopoeisis and leukemogenesis. Among these cytokines, Bone Morphogenetic Proteins (BMP), that also display mechanosensing activity, have been shown to promote immature properties and cell survival in Acute Myeloid Leukemia (AML). On the other hand, adhesion to stromal cells as well as ExtraCellular Matrix remodelling ensure LSC protection. Thus, it was suggested that hyperproliferation of blasts confines hematopoietic cells and overall bone marrow microenvironment, as intracellular pressure increases. Since both mechanical and cytokinic contexts are impaired in AML, we investigated how mechanotransduction pathways and mechanical forces are involved in AML pathogenesis.

Using available AML transcriptomic datasets, we showed that two mechanoresponsive pathways, BMPs and YAP/TAZ, are upregulated in AML patients at relapse compared to diagnosis, suggesting an implication of both pathways in chemoresistance. Similarly, cytarabine-resistant AML cell lines (ML2 or OCI-AML3) display BMPR1b overexpression as well for TEADs and TAZ proteins. Interested by the mechanical properties, we first measured AML intrinsic rigidity: surprisingly, the AML resistant cell lines are less rigid than the sensitives. Thus, the cells adhere better to various substrate (fibronectin, stromal cells) as they seem more deformable and expressing more mechanoreceptors such as β1 integrins. Moreover, the activation of the β1 integrin of adherent cells is impacted by BMPR1B and YAP/TAZ inhibitors in an opposite manner: BMPR1B stimulates β1 integrin activation whereas YAP/TAZ prevents it. Wanting to mimic properties of the bone marrow niche, we used hydrogels of different stiffness to look at gene expression and localization of the different signalling effectors. Also, thanks to a confinement system, we showed for the first time, opposite expression of BMP2 and BMP4 in primary human Stromal Cells (from healthy donors vs AML patients). Finally, through a humanized 3D model of the bone marrow, we looked how combination of chemotherapeutic treatment and BMPR1B inhibitor would affect the number of leukemic cells.

Thus, resistant cells have different mechanical properties which help them to be maintained in the bone marrow microenvironment. These results help us understand the relationship between the miroenvironment and the leukemic cells and identify new therapeutic targets.

Characterization of the Mechanical Properties of Lung Adenocarcinoma

Fabien Delebosse[1], Camille Migdal[2], Lara Chalabreysse[3], Alice Nicolas[1]

[1]Université Grenoble Alpes, CNRS, LTM, 17 rue des martyrs 38054, Grenoble, France

[2]Cell&Soft, 17 rue des martyrs 38054, Grenoble, France

[3]Hospices Civils de Lyon, HCL, Hôpital Louis Pradel, 28 Avenue Doyen Lepine, 69677 Bron

Abstract:

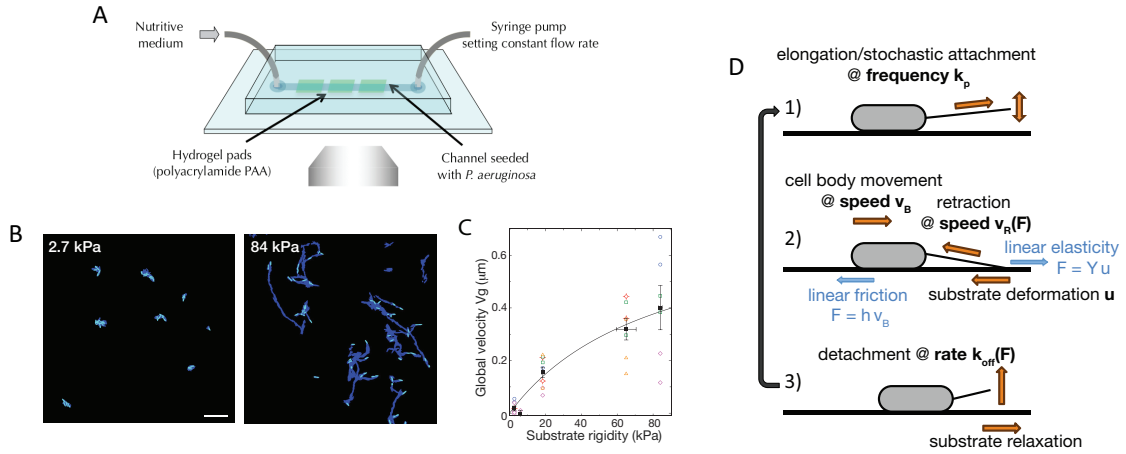
The adverse scenario of lung cancer worldwide encourages research of innovative methods to improve therapeutic arsenals. The advent of immune checkpoint blockade therapy and targeted-drugs to driver mutations has changed the prognosis of lung cancer patients in the past few years, but the majority of them will not benefit from those treatments. Thus, the development of creative tools and techniques is mandatory to facilitate research and discovery of new treatments. A primary step involved in cancer research is cell culture. To date, most cell culture has long been performed on surfaces that do not reflect the mechanical properties of most tissues in vivo. The use of soft culture environments that have a similar mechano-physiology of in vivo conditions is seen as a promising route for the discovery of new cancer drugs and tissue engineering. Based on this context, it is essential to understand how the mechanical properties are distributed in healthy and tumor tissues to design cell culture substrates that reproduce the in vivo mechanical microenvironment. Here, we present a methodology to characterize the mechanical properties of healthy and adenocarcinoma lung tissues using IT-AFM. Lung tissues are very sticky. In this context, data post-processing algorithms were implemented and optimized using contact mechanics models that consider adhesion effects. Preliminary results from 11 patients showed that the measured rigidities of both types of tissue obey a log-normal distribution with tumor tissues being slightly stiffer than healthy ones. A difference in rigidity texture was observed, showing more heterogeneity for tumor tissues compared to healthy ones. These findings give an indication of the initial parameters necessary to design a mechano-mimetic environment.

Session “Subcellular and Cellular mechanics »

Substrate stiffness impacts early biofilm formation by modulating bacterial surface motility

Gomez Sofia[a], Bureau Lionel[a], John Karin[a], Débarre Delphine[a], and Lecuyer Sigolène[b]
[a]Université Grenoble Alpes, CNRS, LIPhy, 38000 Grenoble, France; [b]Université Lyon, ENS de Lyon, Université Claude Bernard, CNRS, Laboratoire de Physique, F-69342, Lyon, France

Surface-associated lifestyles dominate in the bacterial world. Large muticellular bacterial assemblies, called biofilms, are essential to the survival of bacteria in harsh environments, and are closely linked to antibiotic resistance in pathogenic strains. Biofilms stem from the colonization of a substrate by bacteria: this process can take place on a wide range of surfaces, from living tissues to inert materials.



A. experimental approach B. surface coverage (cyan) and total explored area (blue) after 100 min (scale bar 20 μm) C. Population averaged bacterial surface velocity as a function of substrate rigidity, and model (solid line) based on the elements described in D.

Here, we have studied experimentally the impact of substrate rigidity on bacterial adhesion and early biofilm development. By imaging and tracking adhering bacteria *in situ*, on polyacrylamide hydrogels of defined stiffness (1-100 kPa), we demonstrate that the opportunistic promiscuous pathogen *Pseudomonas aeruginosa* explores substrates differently based on their rigidity (fig. A). This leads to striking variations in microcolony structure, strain mixing during co-colonization and phenotypic expression. Using simple kinetic models, we propose that these phenotypes arise through a purely mechanical interaction between the elasticity of the substrate and the type IV pilus (T4P) machinery, a contractile surface appendage that mediates surface-based motility ("twitching"). Our 1D model is based on a force balance between (i) a pilus that extends, attaches and retracts with a defined frequency; (ii) the deformation of the underlying substrate at the pilus tip upon retraction; and (iii) the friction force due to adhesion of the bacterial body when it is dragged across the surface at the other end of the pilus (fig. D). The efficiency of pili activity is thus modulated by the deformability of soft substrates [1].
Together, our findings reveal a new role for substrate softness in the spatial organization of bacteria in complex microenvironments, with far-reaching consequences on efficient biofilm formation.

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Mechanical regulation of cell adhesion to a soft wall under flow

Heather S. Davies[1], Natalia S. Baranova[2], Nouha El Amri[1], Liliane Coche-Guerente[3], Claude Verdier[1], Lionel Bureau[1], Ralf P. Richter[2,3], Delphine Débarre[1]

1. Université Grenoble Alpes, CNRS, Laboratoire Interdisciplinaire de Physique (LIPhy), 38000 Grenoble, France

2. Université Grenoble Alpes, CNRS, Département de Chimie Moléculaire (DCM), Grenoble, France

3. School of Biomedical Sciences, Faculty of Biological Sciences, School of Physics and Astronomy, Faculty of Mathematics and Physical Sciences, Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds LS2 9JT, United Kingdom

Monitoring interactions between objects and a nearby surface is a key aspect of soft matter studies ranging from wetting to complex fluid flow. In biophysics, they govern cell adhesion or the flow of microswimmers or blood cells. Blood cell - vessel wall interactions, in particular, are highly regulated as they are critical both for the flow of red blood cells, and for the control of white blood cell adhesion to the walls (e.g. at a site of inflammation). However, the biochemical and mechanical cues governing this regulation are still poorly understood, in particular because of the challenge of non invasive investigation of short-range interactions, in particular under flow where fast imaging is required.

To decipher the essential parameters controlling blood cell homing or repulsion to the vessel wall, we have developed an *in vitro* platform combining microfluidics, advanced biochemistry and reflection interference contrast (RIC) imaging at high speed [1]. RIC microscopy makes use of partially coherent light to probe optical distances between two objects based on their reflection interference pattern: typically a cell, bead or vesicle adhering or hovering over a substrate [2]. Our custom-built setup allows imaging at high frame rate (200 Hz) and tracking of cell mimetics with an accuracy of ~5 nm in all directions.

Using this platform, we have investigated experimentally the role of the softness of the vessel wall outer layer in the regulation of blood cell homing under flow. This brush, named glycocalyx and mainly composed of charged exopolysaccharides, is both thick (up to 1µm) and extremely soft (down to a few Pa in compression modulus). We have demonstrated that these peculiar mechanical properties induce a short-range repulsion of non-interacting cells, in good agreement with the theory of elastohydrodynamics (EDH) that accounts for the effect of substrate deformation under hydrodynamic forces, providing the first experimental evidence of this "soft biolubrication" effect at play at small scale [3]. On the other hand, we have shown that these same mechanical properties are a critical factor that stabilizes the homing of cells bearing specific receptors (CD44) for one of the main coumpound of the glycocalyx, hyaluronan (HA). Our results thus highlight the role of the glycocalyx as a gatekeeper for the adhesion to the blood vessel wall.

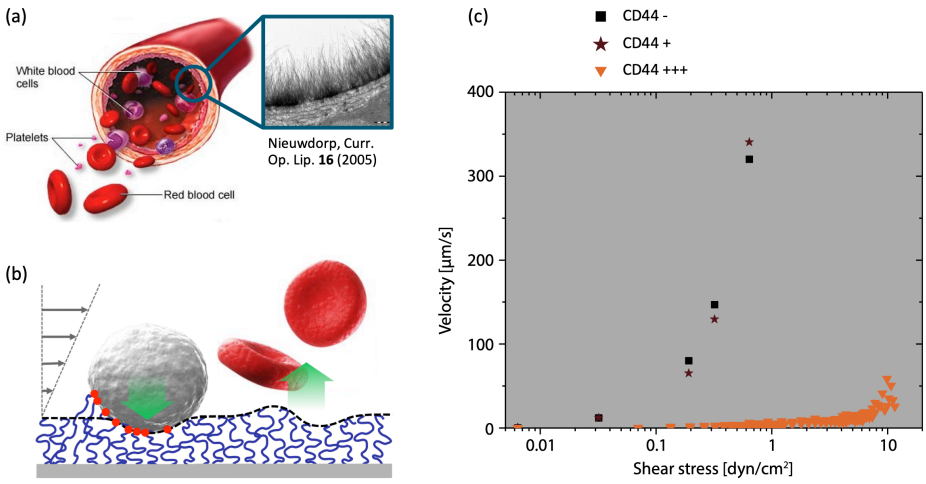

Fig. 1 (a), the glycocalyx lining the wall of blood vessels is the outermost structure encountered by circulating cells and hence regulates the first stages of their adhesion to the vessel wall. (b), schematic representation of the stabilized attractive interactions with the glycocalyx under a shear flow of an activated white blood cell bearing CD44 receptors, and of the repulsion of red blood cells with no specific biochemical interaction with the polymer brush. a synthetic vessel wall (polymer brush). (c), velocity under flow of cells bearing different densities of CD44 receptors, showing superlinear increase of speed for CD44- cells and stabilized rolling for CD44++ cells, up to physiological shear stresses.

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Probing cell crowding in cells and tissues with Brillouin light scattering

Guqi Yan, Alexis Viel, Gaëtan Jardiné, Malèke Mouelhi, Sylvain Monnier and Thomas Dehoux[1]

[1]*Institut Lumière Matière, UMR5306, Université Lyon 1-CNRS, Université de Lyon, 69622 Villeurbanne, France*

Volume regulation is key in maintaining important tissue functions, such as growth or healing [1]. This is achieved by modulation of active contractility, as well as water efflux that change molecular crowding within individual cells. Local sensors have been developed to monitor stresses or forces in model tissues, but these approaches do not capture the contribution of liquid flows to volume regulation. Here we use a new tool based on Brillouin light scattering (BLS) that uses the interaction of a laser light with inherent picosecond timescale density fluctuations in the sample [2]. To investigate volume and density variations, we induced osmotic perturbations to compress single cells and multicellular spheroids (MCS). During osmotic compressions we observe an increase in the BLS frequency shift that reflects local variations in the refractive index and compressibility. We also measure a non-linear increase of the linewidth of the BLS frequency for large compressions suggesting a phase transition. To elucidate these data, we propose a model based on a mixing law that describes the increase of molecular crowding upon reduction of the intracellular fluids. Comparison with the data suggests a non-linear increase of the compressibility due to the dense crowding that induces hydrodynamic interactions between the cellular polymers.

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Session “From cells to tissue and development”

The mechanics of seed size control in plants

Audrey Creff, Olivier Ali, Vincent Bayle, Gwyneth Ingram and Benoit Landrein
Laboratory of Plant Development and Reproduction (RDP), ENS de Lyon, France

How cells stop their growth once an organ has reached a certain size is a key question in developmental Biology. In animals, it has been proposed that mechanical signals could act, together with biochemical factors, in the control of organ growth and size. In plants, mechanical signals regulate a variety of cellular processes such as growth, division or gene expression; but their contribution to the regulation of organ size remains to be demonstrated. Using the seed of *Arabidopsis thaliana* as a model system, we are studying the mechanical regulation of organ growth in plants. By combining experimental approaches with modelling, we propose that seed size is regulated through an incoherent mechanosensitive feedforward loop where the turgor pressure of the inner tissues (endosperm) induces growth directly but represses it indirectly by promoting the stiffening of the cell walls of the outer tissues (seed coat) in a tension-dependent manner. Our work sheds new light on the mechanisms of organ size control in plants and allows us to redefine the contribution of turgor pressure to plant organ growth.

Creff A., Ali O., Bayle V., Ingram G. and Landrein B. *Endosperm turgor pressure both promotes and restricts seed growth and size*. **BioRxiv**
https://www.biorxiv.org/content/10.1101/2021.03.22.436392v1

WAVE regulatory complex facilitates cell rearrangements through the generation of a protrusive F-Actin subpopulation at tricellular junctions.

Lisa Calvary, Pierre Pouchin, Graziella Richard, Hervé Alégot, Olivier Bardot, **Vincent Mirouse**

Institute of Genetics, Reproduction and Development (iGReD), University Clermont Auvergne, CNRS, INSERM, Clermont-Ferrand, France.

How cells change their respective position to allow a tissue to acquire a specific shape is a major question. Drosophila ovarian follicle elongates along its anteroposterior axis and this elongation is driven by the follicular epithelium that covers each follicle. We have previously described that the first step of this elongation involved rearrangements such as cell intercalation (Alégot et al., eLife, 2018). However, in these cells there is no junctional planar polarization of Myosin II as it is the case in some other epithelia experiencing cell intercalations. In this context, we became interested in F-actin role in cell rearrangements. By performing a reverse genetic screen of F-Actin regulators, we identified that the WAVE regulatory complex (WRC), involved in the generation of branched F-Actin, is required for follicle early elongation. In follicular cells, WRC is localized at tricellular junctions where it generates an intense F-Actin population. Tricellular junctions are important hotspots for tissue dynamics thought their role is not yet fully understood. High-resolution analysis reveals that F-actin at tricellular junction corresponds to very dynamic protrusions emanating from one cell and extending between the bicellular junction of the two others. In addition, our work show that WRC protrusive activity is required for new junction extension in the cells at the extremities of this junction. Thus, WRC promotes oriented cell intercalations, thereby permitting follicle elongation. Data of our current analyze on living samples of the relationship between F-Actin protrusions induced by WRC and cell junction dynamics will be shown. Finally, we will present genetic and biochemical evidence on how WRC is recruited at tricellular junction, deciphering a critical mechanism for epithelium dynamics at the molecular, cellular and tissular scales.

Friction when changing neighbours

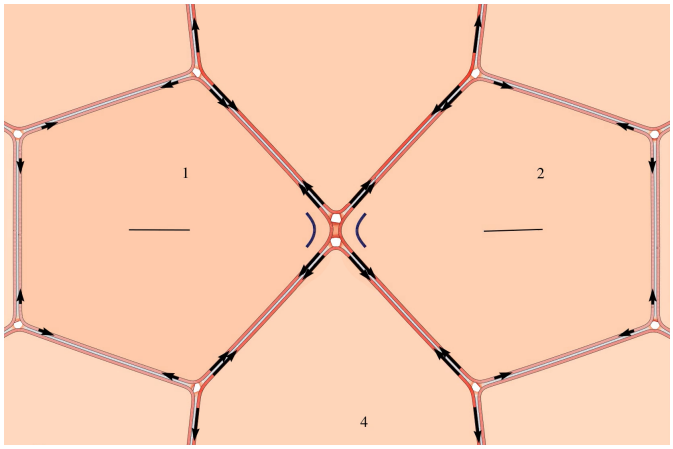
adhesion-regulated junction slippage controls cell intercalation dynamics in living tissue

Alexander Nestor-Bergmann○, Guy B. Blanchard○, Nathan Hervieux○,
Alexander G. Fletcher⊙, Jocelyn Étienne⊗ and Bénédicte Sanson○

○ Univ. Cambridge, ⊙ Univ. Sheffield, ⊗ Univ. Grenoble Alpes – CNRS

During development tissues undergo dramatic shape changes to build and reshape organs. In many instances, these tissue-level deformations are driven by the active reorganisation of the constituent cells. This intercalation process involves multiple cell neighbour exchanges, where an interface shared between two cells is removed and a new interface is grown. The key molecular players involved in neighbour exchanges, such as contractile motors proteins and adhesion complexes, are now well-known. However, how their physical properties facilitate the process remains poorly understood. For example, how do cells maintain sufficient adhesive contact while actively uncoupling from one another? Then, how does a new interface grow in a contractile environment? Many existing biophysical models cannot answer such questions, due to representing shared cell interfaces as discrete elements that cannot uncouple.

Here, we develop a model where the junctional actomyosin cortex of every cell is modelled as a continuous viscoelastic rope-loop, explicitly representing cortices facing each other at bicellular junctions and the adhesion molecules that couple them [2]. The model parameters relate directly to the properties of the key subcellular players that drive dynamics, providing a multi-scale understanding of cell behaviours. The code is distributed as an open-source free software [1].



Mechanical balance during neighbour exchange in large cell–cell friction conditions. The junction outlined in dark blue is actively contracting. Black arrows, tension in the cortices of each cell. Cortices interact via spring-like adhesions. Observe that tension propagates across tissue, this is due to adhesion resistance to shear.

We show that active cell neighbour exchanges can be driven by purely junctional mechanisms. Active contractility and cortical turnover in a single bicellular junction are sufficient to shrink and remove a junction. Next, a new, orthogonal junction extends passively. Our Apposed-Cortex Adhesion Model (ACAM) reveals how the turnover of adhesion molecules regulates tension transmission and junction deformation rates by controlling slippage between apposed cell cortices. The model additionally predicts that rosettes, which form when a vertex becomes common to many cells, are more likely to occur in actively intercalating tissues with strong friction from adhesion molecules.

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Session “From cells to tissue and development”

Keynote speaker

Julien Derr, Ecole Normale Supérieure de Lyon

Plant morphogenesis: motions, growth and mechanics

Although they were already observed as early as 400 years before common era by Androsthenos of Thasos, an admiral of Alexander the Great who reported about the ``sleep movements'' of the tamarind's leaflets and later popularized by Darwin, the active motions of plants were never quantitatively exploited. In this talk, I will try to convince you that plant motions are not only beautiful. They're also important phenomena to observe because they carry information about the very nature of plant growth. The first part of the talk will be a general introduction to plant growth and morphogenetic motions, as well as some observations of the phenomena. In a second part, we will investigate in more detail the case of leaves development for simple or compound leaves. We will show how the rich morphogenetic motions are strongly related to posture regulation mechanisms and mechanosensitivity. Finally, we'll focus on the mechanics of developing plant by looking at force generation associated to motion in tendrils of climbing plants.

POSTERS

Session "Pathologies and Cancer"

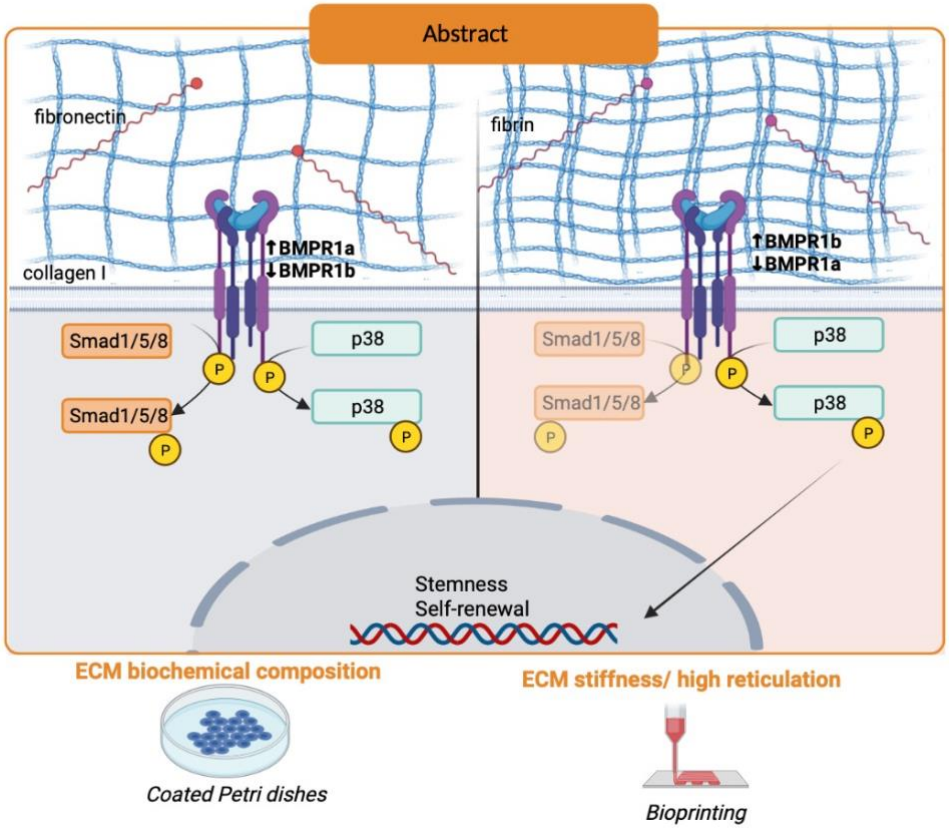
Hydrogel-based microsystem to study coupling between diffusion and cellular volume in living tissues

Gaëtan JARDINÉ, 1; Hélène DELANOË-AYARI, 1; Gilles SIMON, 1; Charlotte RIVIÈRE, 1; Sylvain MONNIER, 1;

1 - Institut Lumière Matière, UMR5306, Université Lyon 1-CNRS, Université de Lyon, 69622 Villeurbanne, France

Abstract

Tissues are spatially heterogeneous structures which give rise to their specific biological functions. This heterogeneity is essential in both physiological, e.g. patterning in development, and pathological processes, e.g. the organization of multiple cell subpopulations within a tumour. The emergence of such spatial distribution can be due to gradients of biomolecules, reaction-diffusion systems and mechanical cues. In confined environment, tissues will exert forces on their surroundings which will in turn reacts by generating stresses. Tissues are composite systems including cells, ExtraCellular Matrix (ECM) and interstitial fluids. ECM is at least 2 orders of magnitudes more deformable than cells, so its porosity can easily be modified under stress. This can then impede diffusion and lead to the establishment of chemical gradients of morphogens within the interstitial fluid. This phenomenon could therefore influence the emergence of a biological heterogeneity in growing tissues. Our aim is to assess fluxes within the intercellular space in growing tissues under compression. As cellular volume is highly coupled to water fluxes and linked to biological functions, we want to measure its distribution and dynamics. We hypothesize that fluxes and volume are influenced by mechanical stresses which will be assessed using soft probes. All these features are dynamics and need to be characterized in dense living tissues which makes them challenging to image. To this end, we developed a hydrogel-based microsystem to limit the thickness of model tissues to assess cell volume, diffusion in the intercellular space and stress in confined growing tissues. Moreover, we have setup images analysis tools and extracted cellular volumes in these densely packed 3D systems. Ultimately, we will spatially correlate these physical parameters to cancer stem-like cell fate



The mammary gland is a dynamic organ, where transformation leads to breast cancer (BC), the most widespread and deadly cancer in women. Previous data supported that tumors are not only characterized by neoplastic cells, but also by a shaped microenvironment composed of stromal cells and the extra-cellular matrix (ECM). The mammary gland is indeed in tight communication with its niche that generates mechanical and biochemical cues, the disruption of which leading to cancer. Indeed, the team demonstrated a strong deregulation of the *Bone Morphogenetic Proteins* (BMPs) pathway in response to biochemical variations of the niche. Other studies also highlighted BMP dysfunctions in response to biomechanical fluctuations like stiffness variations. However, in the mammary context, the impact of both microenvironment composition and stiffness is far from clear, due to the lack of relevant models. Consequently, we aimed to develop models mimicking both features, to investigate their impact on cell behavior and the BMP pathway. We addressed these features by developing a 3D-bioprinted breast model. Higher rigidity led to a switch from BMPR1a to BMPR1b cellular expression, mainly followed by the activation of the MAPK non-canonical cascade *via* p38. This also led to the amplification of the immature cells compartment. Our results therefore highlight that biochemical and biomechanical cues from the cell microenvironment could combine their influence on cellular plasticity and molecular machinery to sense them, and pinpoint as crucial the design of relevant models recapitulating both types of signals to address mammary cell transformation.

Exploring BMP2 impact on breast epithelial integrity through integrin dynamics Breast

Line Chaar, Fabrice Senger, Amaris Guevara-Garcia, Anne-Pascale Bouin
and Corinne Albiges-Rizo
Institute For Advanced Biosciences
Site Santé, Allée des Alpes, 38700 La Tronche

cancer is the most common cancer affecting women worldwide, accounting for about 30% of new cancer cases diagnosed each year and for 15% of cancer deaths in women. Breast cancer is not a single disease but rather represents a collection of tumor subtypes with diverse pathological features, molecular signatures and clinical outcomes. Intra-tumoral heterogeneity reflects distinct breast epithelial cells that serve as the cells of origin for malignant transformation. Understand how an aberrant microenvironment impinges on the phenotypic diversity of breast cells or affects tumor heterogeneity is not well understood. Bone Morphogenic Protein 2 (BMP2), which controls extracellular matrix composition and tissue biomechanics is abnormally elevated in luminal breast tumors. Moreover, abnormal BMP-Receptor signaling has been reported in breast cancers. Our lab has previously shown the cooperation between BMPR and integrin to drive cell adhesion processes and Smad signaling as effector downstream BMP receptor in 2D. We are repositioning the contribution of the cooperation between integrin and BMPR in spatially organized multicellular contexts by considering the geometry (2D versus 3D), the polarity and the biomechanics of the breast epithelium. Our hypothesis is that signaling resulting from BMPR/ integrin cross-talk may impact on the organization of epithelial cells by affecting cell sorting, cell competition and the direction of cell extrusion within epithelial tissue. We are investigating whether and how BMP2 targets a subpopulation of MCF10A breast cells to promote intra-tumoral heterogeneity. Our results show that BMP2 induces a partial EMT which is associated with a change in the expression pattern of integrin and extracellular matrix in 2D environment. MCF-10A cells adopt an elongated shape when treated with BMP2. PIV analysis of time-lapse data obtained on MCF-10A monolayers indicates that BMP2 stimulation induces a fluidization of the monolayer by driving collective cell migration in 2D monolayer. In 3D environment, BMP2 treatment reveals a transition from acini to spheroid which correlate with a redistribution of integrin. Based on organoids, micropatterns and adaptative microscopy, we anticipate an integration of integrin dynamics as a molecular basis for tissue fluidization within the context of intratumoral heterogeneity.

Molecular Bases of Osteogenesis and Therapeutic Perspectives against Osteoporosis

Nawal HAJJ SLEIMAN[1], **Samir MERABET**[1,#].

[1] *Institut de Génomique Fonctionnelle de Lyon, Ontogenesis and Molecular Interactions, Ecole Normale Supérieure de Lyon, 46, allée d'Italie 69364 Lyon Cedex 07 France.*

[#] *To whom correspondence should be addressed.*
Tel: +33(0)6.22.82.11.88; E-mail: samir.merabet@ens-lyon.fr

Many pathologies grouped under the term "osteoporosis" correspond to a set of disorders affecting the balance between osteoclasts and osteoblasts and leading to a loss of bone tissue with a higher risk of fracture. Osteoporosis represents a major public health problem: 39% of women over 60 years old suffer from this pathology. Despite this, osteoporosis remains an underdiagnosed and undertreated disease. In addition, current treatments target the osteoclast and cause side effects that limit their long-term usage. A promising alternative approach is to target osteoblasts to stimulate bone formation, thereby promoting osteogenesis. My PhD project focuses on the transcriptional co-activator TAZ (also known as WWTR1) that acts as a downstream regulatory target in the Hippo signaling pathway. TAZ plays a pivotal role in regulating fate determination including osteogenesis and homeostasis. In the Hippo pathway, which is activated upon mechanical stress, upstream kinases phosphorylate TAZ, inducing its interaction with 14-3-3 protein and sequestration in the cytoplasm. Dephosphorylation of TAZ promotes its shuttling to the nucleus, where TAZ interacts with key transcription factors such as TEAD or RUNX2 to regulate the expression of specific target genes. Based on TAZ shuttling activation, we aim to capture the TAZ interactomes in a compartment specific manner to understand the mode of action of the major regulators of osteogenesis. To this end, we have developed innovative tools that allow to specifically capture the full interaction network of nuclear TAZ/TEAD or cytoplasmic TAZ/14-3-3 complexes in live cells. These pioneer tools provide a unique opportunity to capture cofactors implicated in TAZ activation, opening new therapeutic perspectives against osteoporosis.

POSTERS

Session "Subcellular and Cellular Mechanics"

Investigation of lengthscales in cell rigidity sensing

Abbas Mgharbel, Camille Migdal, Nicolas Bouchonville, Paul Dupenloup, David Fuard, Eline Lopez-Soler, Caterina Tomba, Marie Courçon, Danielle Gulino-Debrac, Hélène Delanoë-Ayari, Alice NICOLAS

Laboratoire des technologies de la microélectronique (LTM)
Centre National de la Recherche Scientifique : UMR5129
LETI/DTS 17 rue des martyrs 38054 GRENOBLE CEDEX 9 - France

alice.nicolas@cea.fr

Determining the scales at which cells sense the mechanical properties of their environment has become central since it was evidenced that tissues bear micron-scaled rigidity textures with sharp gradients. Here we show that rigidity sensing takes place at the adhesion and at the cell scales. By quantifying cellular forces and contractility together with immunostaining analysis, we show that both types of forces perform a mostly decoupled probing of the rigidity: the pulling forces that the adhesions transmit to the extracellular matrix locally adapt to rigidity while cell intracellular stresses adapt marginally to the adhesion-scaled rigidity and predominantly to the cell-averaged rigidity. These findings evidence parallel rigidity-sensing machinery in cell adhesion and question signaling pathways that may control this parallel adaptation.

Title:

Magnetomechanical stimulation of 3T3 fibroblast by low-frequency-vibrating magnetic particles: effect on the contractility of the actin cytoskeleton

Authors

Andrea Visona1,2, Bernard Dieny2, Robert Morel2, Alice Nicolas1

1 Univ. Grenoble Alpes, CNRS, LTM, 38000 Grenoble, France

2 Univ. Grenoble Alpes, CEA, CNRS, Spintec, 38000 Grenoble, France.

Abstract

Cell mechanical stimulation is an active research topic which arouse increasing interest among the scientific community in the recent years. In this field magnetic nanoparticles have proven to be valuable assets thanks to their peculiar properties of being actuated remotely through magnetic field and being able to exert mechanical stimuli very locally even inside the cell, upon internalization. Among the different mechanosensitive molecules of a cell, an interesting target is the actin cytoskeleton and therefore the forces organization which determine the contractile state of the cell. This can be achieved thanks to traction force microscopy (TFM), which allows to measure the forces the cells transmit to an elastic substrate.

Some preliminary results have shown that low-frequency-vibrating particles, inside the cell or in contact with the cell membrane, increase the contractility of NIH 3T3 single cells few hours after the magnetomechanical stimulation. Moreover, it was highlighted a correlation between magnetic stimulation and NIH 3T3 filopodia development, hinting to a possible response of the cell to its increased contractile state.

Magnetic actuation of polymer membranes mimicking the intestinal barrier

Valentin Chalut[1], Anne-Laure Deman[1], Caterina Tomba[1]

[1] Univ Lyon, Université Claude Bernard Lyon 1, CNRS, INSA Lyon, Ecole Centrale de Lyon, CPE Lyon, INL, UMR5270, 69622 Villeurbanne, France

As part of the digestive system, the intestine is a folded organ that is responsible for the absorption of nutrients in particular thanks to its specific micro-structure, formed by villi and crypts. This organ also allows the movement of the food bolus to facilitate digestion. Its dysfunction is also at the origin of serious pathologies, such as the Hirschsprung's disease.

To understand intestinal embryonic development and the origin of these pathologies, and to develop treatments, it is necessary to propose new approaches. Indeed, the classical 2D in-vitro models does not allow to replicate the microstructure of the intestine and its movements, and the animal model is relatively too different from human beings, or not viable for very serious pathologies.

The organ-on-chip approach is very promising because it consists in replicating all or part of an organ on a small scale, with in addition the possibility of using human cells. Various works have been recently conducted to mimic the microstructure of the intestine (refs). However, these intestine-on-chip are microstructured but static and do not accurately reproduce the movements of the intestine, although these movements may influence the tissue structure and organization in the intestine and are essential to the digestion function. The aim of this project is therefore to develop an intestine-on-chip with villi and crypts that can be set in motion in a controlled and reproducible manner.

Inspired by soft robots, we have created deformable magnetic polymer membranes with shape memory. This wavy membrane can simulate intestinal movements by producing a deformation amplitude of about 1.4 to 1.7mm at 140 mT and a radius of curvature of about 1 to 2mm (figure 1). It can also support replicas of villi (made of PDMS or hydrogel), which are 500µm high and 200µm wide. This technology has the advantage of being remotely operable using an external magnetic field, ranging from 5 to 140 mT. It is therefore possible to tune the motion of the structures non-invasively to study cell growth under dynamic constraints.

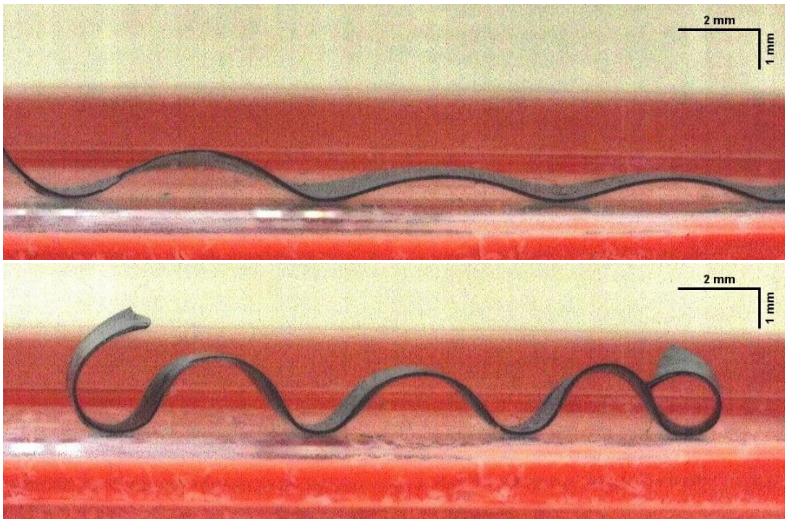


Figure 1: Magnetic membrane under a magnetic field of 5 (top) and 140 mT (bottom).

How molecular motors alter microtubule turnover

William Lecompte[1], Sarah Triclin[2], Laurent Blanchoin[2,3], Manuel Théry[2,3] and Karin John[1]

[1]Univ. Grenoble-Alpes, CNRS, Laboratoire Interdisciplinaire de Physique, 38000 Grenoble, France.
[2]Univ. Grenoble-Alpes, CEA, CNRS, INRA, Institute de Recherche Interdisciplinaire de Grenoble, Laboratoire de Physiologie Cellulaire & Végétale, CytoMorpho Lab, 38054 Grenoble, France.
[3]Univ. Paris Diderot, INSERM, CEA, Hôpital Saint Louis, Institut Universitaire d'Hematologie, UMRS1160, CytoMorpho Lab, 75010 Paris, France.

ABSTRACT

Microtubules (MTs) and molecular motors are ubiquitous in eukaryotic cells and are vital for many key cellular functions (eg. chromosome segregation, intracellular protein transport). Recent experiments have shown that processive molecular motors may modify the under- lying microtubule lattice yet a mechanistic model has remained elusive. Here we investigate theoretically how molecular motors could potentially participate in remodeling the shaft lattice. Using a kinetic Monte Carlo method we model the MT lattice structure at the tubulin dimer scale. Molecular motors can attach, detach and walk along the microtubule lattice.

Our leading concept is, that the walk of molecular motors locally destabilizes the lattice and may facilitate the exchange of tubulin dimers with the surrounding medium. This view is implemented via two constitutional mechanisms: (1) a transient conformational change in the underlying lattice induced by the continuous flow of motors, and (2) the stabilization of a single lattice vacancy due to a steric hindrance for GTP-tubulins to integrate a GDP-lattice environment. The model reveals that a small transient perturbation (< 3kT) induced by the motor's walk is sufficient to modify significantly the lattice dynamics along the shaft on length and time scales observed experimentally, even at low motor density.

Mechanical balance of dividing cells in an epithelial tissue

Ali Alhadi Wahhod, Jocelyn Eteinne

The shape taken by cells is intrinsically linked to the mechanics of their cytoskeleton, and importantly the actomyosin cortex that localizes just beneath the cell membrane. Because this membrane is impermeable in epithelial tissue, the cell shape is constrained by its interaction with neighboring cells. It is known that the optimal shape of the cells at their apical surface is typically hexagonal. Because they adhere on a basement membrane, single-layer epithelial cells are generally prismatic. However, when a cell within the epithelial tissue undergoes cell division, cell rounding is observed, that is, the cell detaches from the basement membrane and adopts a shape closer to spherical.. This alters the position of the neighbor cells as well. Our main purpose is to understand whether we can understand this shape evolution using a surface tension approach. Indeed, this shape evolution is simultaneous to an increase of myosin presence in part of the cell cortex, which is known to increase mechanical tension in the cortex.Thus, we modeled mechanical tensions as surface tensions and we tested whether these surface tensions and consequently the intracellular pressure, would explain the shape observed. Analytically, we used the Lagrange multiplier method to set the whole Lagrangian of the system, knowing that the cells are constrained by geometry and volume that alter the cell arrangement, and then establish the equilibrium equations, dynamically as well. The results allow a better understanding of the 3D mechanical balance of cells.

Molecular recognition between biotin and streptavidin adsorbed on different silane monolayers studied by Single Molecule Force Spectroscopy (SFMS) and Steered Dynamics simulations

Solène LECOT[1], Antonin LAVIGNE[1], Frédéric DUBREUIL [2], Thomas GEHIN[3], Yann CHEVOLOT[3], Christelle YEROMONAHOS[1*], **Magali PHANER-GOUTORBE[1*]**

[1] *Univ Lyon, Ecole Centrale de Lyon, CNRS, INSA Lyon, Université Claude Bernard Lyon 1, CPE Lyon, INL, UMR5270, 69134 Ecully Cedex, France*

[2] *Université Lyon, CNRS, ECL, LTDS, UMR5513, 69130 Ecully, France;*

[3] *Univ Lyon, CNRS, Ecole Centrale de Lyon, INSA Lyon, Université Claude Bernard Lyon 1, CPE Lyon, INL, UMR5270, 69134 Ecully Cedex, France*

In a certain number of clinical diagnostic tools, the capture of the analyte to be detected, target-molecule, by an adsorbed probe molecule is carried out thanks to molecular recognition. This recognition process between the two target/probe molecules can be limited by the conformation adopted by the probe molecule when it is adsorbed on the solid substrate. Silane monolayers are widely used to functionalize SiO_2 or Glass surfaces and are well adapted as anchoring layer for the adsorption of probe-molecules.

In this study, we consider the Streptavidin/biotin (Probe/Target) classical model and we focus on two different silane monolayers, with two different head-groups: one terminated with an amine group, hydrophilic and positively charged (NH_3+) in PBS solution at pH=7.4, and one terminated with a CH_3 group, hydrophobic and neutral in the same solution.

Single Molecule Force Spectroscopy (SFMS) has proven to be a relevant mode of Atomic Force Microscopy to study the processes involved in the molecular recognition between a ligand and a receptor [1,2]. The force-distance curves measured by SMFS are representative of the detachment process between the 2 molecules. In our study, the AFM tip was functionalized with a PEG molecule terminated with a biotin and put in contact with streptavidin molecules adsorbed on the two silane monolayers. Force map curves were registred and analysed indicating the unbinding force representative of the bioactivity. Our results showed that under the same experimental conditions, the detachement force is approximately twice as high for the CH_3 silane layer as for the NH_3+ silane layer.

The same results were also obtained by Steered Molecular Dynamics (SMD), that mimic Atomic Force Spectroscopy. They were attributed to a difference in the conformation of the streptavidin on the two silane layers. In particuler, the streptavidin deforms to fix on the NH_3+ silane layer, limiting access to the biotin molecule [3,4].

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**Correspondance : magali.phaner@ec-lyon.fr for AFM experiments*

**Correspondance : christelle.yeromonahos@ec-lyon.fr for Molecular Dynamic simulations*

POSTERS

Session "From cells to tissue and development"

Collective movements in complex environments: Fish School evacuation through a bottleneck

Renaud Larrieu[a], Philippe Moreau[a], Christian Graff[b], Philippe Peyla[a] et Aurélie Dupont[a]

[a] Univ. Grenoble Alpes, CNRS, LIPhy, F-38000 Grenoble, France
[b] Univ. Grenoble Alpes, Univ. Savoie Mont Blanc, CNRS, LPNC, 38000 Grenoble, France

The collective behavior of animals is a fascinating example of coordinated movements that spontaneously emerge on a large scale despite the local and limited communication capacities between individuals. One striking observation is that pedestrians, sheep or granulars behave similarly when urged to pass through a bottleneck [1] with the apparition of clogging events. On the contrary, ants have been shown to avoid jamming and the "faster-is-slower" effect[2]. Here, we propose to test the collective behavior of non-hierarchical groups of small fish (*Paracheirodon Innesi*) in a typical evacuation experiment. We observe that, when urged to pass through a small circular aperture in shallow water, schools of fish show a collective behavior closer to ants than to humans. No clogging events were detected even when the opening was close to their body size; the statistics of passage follow an exponential law of queuing. The fish tend to maintain a defined surface density before the opening, hence avoiding contacts and solid friction. The constant fish-to-fish distance seems to be controlled by social interactions and cognitive rules.

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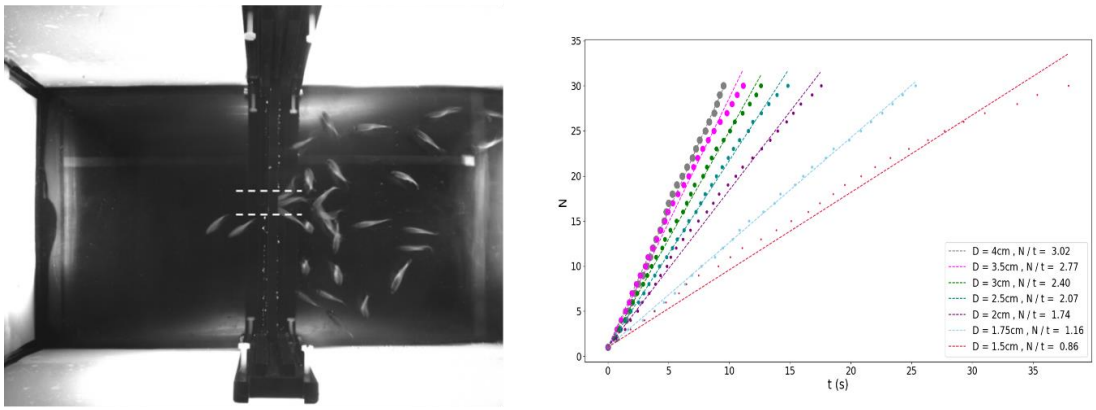


Figure 1 : Fish evacuation through a circular aperture image from the top (left). Averaged cumulative passage of fish as a function of time for different aperture diameters (right). The smaller the opening, the slower the fish evacuate. For visualization, marker sizes are proportional to the real aperture.

Linear correlation between active and resistive stresses informs on force generation and stress transmission in adherent cells

Hélène Delanoë-Ayari,[1,*] Nicolas Bouchonville,[2] Marie Courçon,[3] and Alice Nicolas[2,†]

[1] *Univ. Claude Bernard Lyon1, CNRS, Institut Lumière Matière, 69622 Villeurbanne, France*

[2] *Univ. Grenoble Alps, CNRS, LTM, 38000 Grenoble, France*

[3] *Univ. Grenoble Alps, CEA, Inserm, BIG-BGE, 38000 Grenoble, France*

Animal cells are active, contractile objects. While bioassays address the molecular characterization of cell contractility, the mechanical characterization of the active forces in cells remains challenging. Here by confronting theoretical analysis and experiments, we calculated both the resistive and the active components of the intracellular stresses that build up following cell adhesion. We obtained a linear relationship between the divergence of the passive stress and the traction forces, which we show is the consequence of the cell adhering and applying forces on the surface only through very localized adhesion points (whose size is inferior to our best resolution, of 400 nm). This entails that there is no measurable forces outside of these active point sources, and also that the passive stresses and active stresses inside cells are proportional.

* helene.delanoe-ayari@univ-lyon1.fr

† alice.nicolas@cea.fr

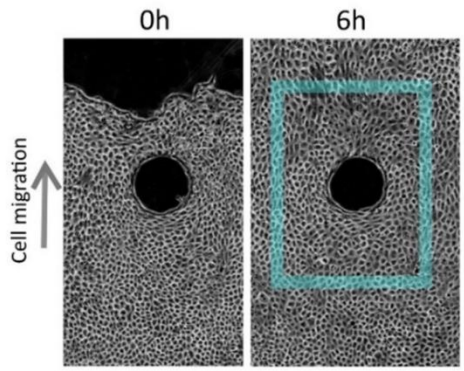
Boundary conditions in cell monolayer and collective migration

Melina DURANDE[1], Guillaume DUPREZ[2], Sham Tlili[3], François GRANER[1], Hélène DELANOE-AYARI[2]

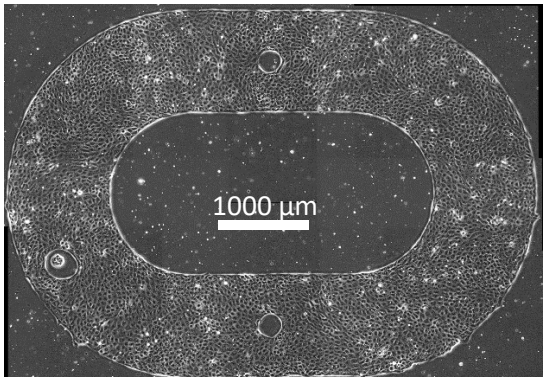
[1]Université Paris Cité, CNRS, UMR 7057, Matière et Systèmes Complexes, F-75205 Paris Cedex 13, France
[2] University of Lyon, Université Claude Bernard Lyon 1, UMR 5306 CNRS, Institut Lumière Matière, F-69622, VILLEURBANNE, France
[3] Aix-Marseille Université, UMR 7288 CNRS, Institut de Biologie du développement de Marseille, 13288 Marseille, France

Résumé :

Beside being an archetype of active matter collective migration, epithelial cell monolayer migration is involved in tissue development, wound healing or metastases. Different controlled experimental set-ups have already been designed to quantitatively investigate the mechanisms underlying the observed migration. We use two different set-ups, a classical one where the cell monolayer has a free front, and an original one where a monolayer of migrating cells uniformly covers a circular racetrack. We let MDCK cells migrate on either set-up, on large scale (over millimeters) and long time scales (over a day). In each of these spontaneous flows, we introduce an obstacle to induce a localised spatial heterogeneity which stimulates cell-cell neighbour changes (rearrangements). By characterising sub-cellular organisation, and supra-cellular long-range coherences of velocity and deformation fields, we can quantitatively compare in details both geometries. We discuss the existence of two distinct migration mechanisms. The first one does not require a free front and exists in both geometries; while when there is a free front, a second mechanism coexists with the first one.



Cell migration with a free front around a circular obstacle (diameter : 150 µm)



Circular racetrack with several obstacles

Mots-clés : Microrheology, Active Matter, Epithelial Cells, Cell Migration

Bacterial motility and early colony formation on a stiffness gradient.

Chagua Encarnacion Kennedy Nexon & Lecuyer Sigolene
Laboratoire de physique de l'ENS de Lyon (LPENSL UMR 5672)

Many bacteria adhere to surfaces, forming macrocolonies called biofilms in response to various environmental signals, which are still being studied. This transition, from planktonic to sessile state induces phenotypic changes in the bacteria. One cue that has gained attention in recent years as an active participant in the phenotypic modification of living organisms is mechanical signals. How does a mechanical interaction activate the genes that control a collective lifestyle? Recent work has shown that the twitching motility (powered by contractile filaments called type-4 pili) of the pathogen *Pseudomonas Aeruginosa* on a polyacrylamide substrate is affected by the stiffness of the substrate: single bacteria move faster on stiffer substrates, and are immobile on very soft ones (< 10 kPa) [1]. This directly influences the shape of the microcolonies that form on these substrates. On stiff substrates, bacteria tend to move over the surface, and form flat, spread-out microcolonies, whether on soft substrates, bacteria do not move and microcolonies are compact and hemispherical.

Within this framework, our objective is to explore how a polyacrylamide stiffness gradient influences the individual (mainly the twitching motility) and collective (microcolony formation) behavior of *Pseudomonas Aeruginosa* bacteria. For this purpose we perform a multidisciplinary approach using microfluidics (adhesion and growth take place in flow cells), soft matter (photopolymerization method is used for the elaboration of the stiffness gradient), microscopy (bacteria are imaged *in situ* by phase contrast) and machine learning (Weka plugins of ImageJ for bacterial segmentation). We elaborate substrates of controlled stiffness (170-340 Pa/μm) and mm-sized spatial (500-250 μm) gradients, as well as carry out studies at an individual and collective level. These first results show that bacteria have a preference for moving in the direction of the gradient and that microcolonies form with an elongated pattern, which differentiates them from those forming on homogeneous substrates. Overall, these results show a correlation between the mechanical feedback sensed by individual bacteria when they adhere on a stiffness gradient, bacterial displacement and microcolony formation in *Pseudomonas Aeruginosa*. Our future work will be to investigate at the single bacteria level whether the bacteria have a preference towards stiff or soft substrate (directional displacement) and whether displacement is affected by the polar disposition of the pili filaments at the time of bacterial division.

These results shed new light on the way promiscuous pathogens such as *Pseudomonas Aeruginosa* might have developed strategies to adapt and thrive in many different environmental niches.

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Oxygen regulated microphase separation of living *Dictyostelium* cells

J.P. Rieu[a*], A. Carrère[a], O. Cochet-Escartin[a], C. Anjard[a] et F. Detcheverry[a]

a. Institut Lumière Matière, Université Claude Bernard Lyon 1, 43 boul. du 11 Novembre, Villeurbanne

* jean-paul.rieu@univ-lyon1.fr

Self-organization occurs in a variety of biological systems and the concept of active and condensed matter have proven very useful in explaining some of the mechanisms. Microphase separation is a classic example of self-organization, long studied in polymeric material and inert system [1] or more recently in biomolecular condensates, which are micron-scale compartment in eukaryotic cells [2]. To the best of our knowledge such microphase separation have never been reported in assemblies of living cells.

When place in a nutritive medium below a millimetric culture medium film, cells of the social amoeba *Dictyostelium Discoideum* (*Dicty*), grow until some critical density. Then they stop to grow, and assemble in compact domain with a characteristic size of typically 100µm surrounded by a dense cellular gas phase. These domains stay mobile and stable in size during several days. Moreover, if one changes the O_2 atmospheric level or the height of the medium, aggregate will quickly equilibrate with a new characteristic size and spacing. These observations as well as our previous experience about aerotatic behaviours of *Dicty* single cells [3] show that this cellular microphase separation of *Dicty* cells is oxygen regulated. It results from a balance between competing interaction; a long range repulsion, trough self-generated O_2 gradients due to O_2 consumption, and a short-range attraction due to cell-cell adhesion. A simple analytical model and lattice based Monte Carlo simulations support this simple mechanism which highlights the importance of oxygen regulation and self-generated gradients as an emergent organizing principle for biological matter.

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[2] S.F. Banani et al, Nat Rev Mol Cell Bio, 2017 May; 18(5) 285-298
[3] O. Cochet-Escartin et al, elife 2021 10:e64731

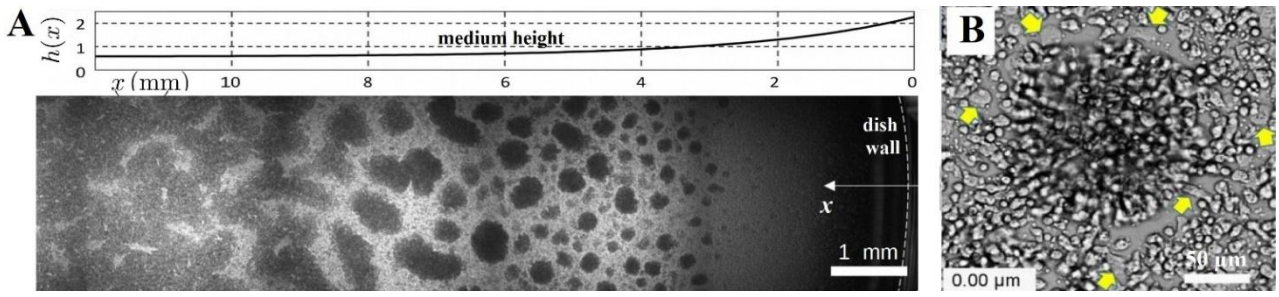


Figure 1: (A) *Dicty* aggregates under a varying milimetric culture medium layer with the height profile from the dish wall above. (B) Zoom on one aggregate surrounded by a cellular gas phase (arrows point to the first layer of cell flattened on the surface)

Hypoxia triggers collective aerotactic spreading of eukaryotic cells

J.-P. Rieu[1,*], N. Ghazi, A. Chauviat[4], S. Fabre[4], O. Cochet-Escartin[1], M. Demircigil[2], S. Hirose[3], V. Calvez[2], K. Funamoto[3], C. Anjard[1].

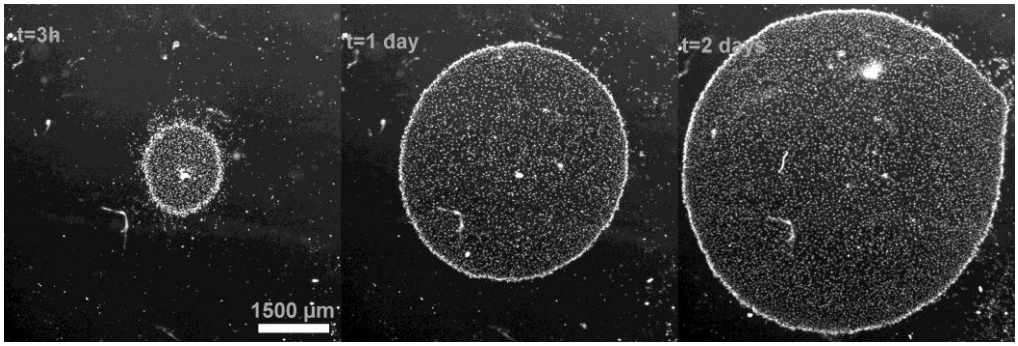
Affiliations :
1-Institut Lumière Matière, Université Claude Bernard Lyon 1, Villeurbanne, France
2- Institut Camille Jordan, Université Claude Bernard Lyon 1, - Villeurbanne, France
3- Institute of Fluid Science, Tohoku University, Sendai, Japan
4- UMR5557, INRA-Université Claude Bernard Lyon 1, Villeurbanne, France

* jean-paul.rieu@univ-lyon1.fr

A state of low oxygen occurs frequently in soil, water and multicellular tissues and has played a pivotal evolutionary role in shaping multicellularity. While both the social amoeba Dictyostelium discoideum (*Dd*) and the asocial amoeba Acanthamoeba castellani (*Ac*) are obligatory aerobic organisms, their ecological niche in the soil exposes them to reduced oxygen availability in particular due to competition with bacteria.

We have recently observed that vertically confining a micro-colony of *Dd* cells in a growth medium triggers cells to move quickly outward of the self-generated central hypoxia area and thus form an expanding ring propagating for days (Figure, [1]). The analysis of the cells' behavior within the micro-colony reveals a complex response to hypoxia depending upon their position:
- Cells closer to the ring present a clear outward directionality and are capable of dividing.
- Inner cells are not dividing, very motile but with limited outward directionality.
Using both oxygen sensors and microfluidic devices, we showed that *Dicty* cells are aerotactic within 0-2% O_2 and that the ring of cells occurs at that level where the O_2 gradient exists.

The variety of cell behaviors within the micro-colony were mimicked using a mean field PDE model that includes the cell division rate r, the motility D (diffusion constant) and a positive aerotactic bias a_0 where each quantity may depend on the oxygen level C. This model predicts that if the bias a_0 is not too steeply decreasing or if D and/or r are too large, then the ring may disappear [1].
We are testing this model with *Ac*. Interestingly, upon spreading, no ring formation occurs even if the cells respond aerotactically as demonstrated by microfluidic experiments. A detailed quantification of r, D and a_0 will be presented to explain the differences between the social and asocial amoebae.



[1] Cochet-Escartin O. et al. “Hypoxia triggers collective aerotactic migration in Dictyostelium discoideum.” eLife [Online]. 2021. Vol. 10, p. e64731. Available at : < https://doi.org/10.7554/eLife.64731 >

Integrating aspiration-assisted bioprinting and near-field electrospinning for tissue engineering

Alexandre Dufour [1], Javier Arduengo Garcia [2], Jean-Yves Hascoët [2], Luciano Vidal [2], Victor Vuillet-a-Ciles [3], Vincent Salles [3], Christophe Marquette [1]

[1] 3d.FAB, Univ Lyon, Université Lyon1, CNRS, INSA, CPE-Lyon, ICBMS, Villeurbanne, France ; [2] Rapid Manufacturing Platform, Centrale Nantes, UMR CNRS 6183, Nantes, France ; [3] Laboratoire des Multimatériaux et Interfaces, UMR CNRS 5615, Univ Lyon, Université Claude Bernard Lyon 1, F-69622 Villeurbanne, France.

Keywords. Spheroids, Solution electrospinning writing, Aspiration-assisted bioprinting

Background. Tissue engineering strategies often rely on the homogenous distribution of cells throughout a biomaterial scaffold, resulting in tissues of homogeneous composition and lacking the complexity of their native counterparts. Besides, cell spheroids can be used as building blocks, generating tissues with composition and architecture mimicking native tissues. For example, spheroids can be assembled into an organized array of polymeric chambers whose function is to support the growth of cellular aggregates and provide mechanical reinforcement to the resulting tissues.
Materials & Methods. Here, we propose to use Solution Electrospinning Writing (SEW) to generate a supporting framework for the assembly of mesenchymal stem cell (MSC) spheroids deposited by aspiration-assisted bioprinting with the aim to generate cartilage tissues with biomimetic mechanical properties and content [1].
Results. We will show–that SEW can be used to produce up to 2 mm thick organized array of polymeric chambers by stacking 4 µm fibers, in which MSC spheroids can be successfully seeded and cultured for several weeks. In parallel, we will present a specially developed aspiration-assisted bioprinter able to pick and place MSC spheroids into a defined location safely.
Conclusions. Future investigations will look into the automated placement of MSC spheroids into the SEW scaffold to generate large-scale tissues based on spheroid fusion.

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Mechanics of Epithelial cell flattening in Drosophila

Muriel Grammont, ENS Lyon, ***muriel.grammont@ens-lyon.fr***

It is unknown how external forces and constraints impact morphogenesis in a neighboring tissue. To address this, we used the Drosophila ovarian follicle, where a cluster of 15 nurse cells and a posteriorly located oocyte are surrounded by a layer of epithelial cells, which are themselves resting on a basement membrane. As the nurse cells grow, the overlying epithelial cells flatten in a wave that begins in the anterior. We previously showed that ***muriel.grammont@ens-lyon.fr***this flattening depends on the TGFß signaling. We now demonstrate that an anterior to posterior gradient of decreasing cytoplasmic pressure is present across the nurse cells and that this gradient acts through TGFß to control both the triggering and the progression of the wave of cell flattening. We also prove that BM softens around the flattening cells and that this softening depends on TGF pathway. Finally, we reveal that nurse cell pressure and BM softening combine to increase follicle elongation in the anterior, which is crucial for allowing nurse cell growth and pressure control. Altogether, these results show that BM mechanical properties and the inner cytoplasmic pressure in the nurse cells have an important role in shaping cells and tissues