4th workshop in *Mechanobiology and Physics of Life* in Lyon
Monday January 28th, 2019, Amphi Mérieux, ENS de Lyon

Abstract Booklet

1. Oral presentations

Olivier COCHET-ESCARTIN	iLM, Lyon
Catherine QUILLIET	LiPhy, Grenoble
Séverine URDY	IGFL, Lyon
Bruno LEGGIO	RDP, Lyon
Erwan TINTURIER	PIAF, Clermont-Ferrand
Karin JOHN	LiPhy, Grenoble
Marie DELATTRE	LBMC, Lyon
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Pol GRASLAND-MONGRAIN	Laboratoire Physique ENS de Lyon
Sylvain LEFORT	CRCL, Lyon
Magalie FAIVRE	INL, Lyon

2. Poster presentations

Olivier ALI	RDP, Lyon
Carine P. BEATRICI	MSC, paris
Sara BOUIZAKARNE	LiPhy, Grenoble
Fabiana CERQUEIRA-CAMPOS	GReD, UMR CNRS 6293 - INSERM U1103 - Clermont Ferrand
Antoine DELINIERE	Hôpital cardio, Lyon
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Sarah DINVAUT	INMG, Lyon
Antoine FRULEUX	RDP, Lyon
Thibault GARDETTE	INMG, Lyon
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Alain LOMBARD	LiPhy, Grenoble
Magali PHANER-GOUTORBE	Ecole Centrale, Lyon
Audrey PRUNET	iLM, Lyon
Gaël-RUNEL	CRCL, Lyon
Antoine FRULEUX	RDP, Lyon
Shogo TAKATANI	RDP, Lyon
Victor VIEILLE	G2ELab, Grenoble
Roland ZIMM	IGFL, Lyon
Nilankur DUTTA	LiPhy, Grenoble

Deciphering spatio-temporal signaling between BMP receptor and Integrin by optogenetics

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Understanding how cells integrate multiple signals from biochemical properties of extracellular matrix to achieve specific cell differentiation and acquire cell identity is a challenging question in cell biology. Cell integrate extracellular signals by forming anchorage sites through membrane receptor aggregation including adhesive receptors like integrins and growth factor receptor like BMPR. We have shown that matrix-bound BMP-2 is sufficient to induce b3 integrin-dependent cell spreading by overriding the soft signal of the biomaterial and by impacting actin organization and adhesion site dynamics. Whether cell differentiation and tissue specification depend on the differential spatio-temporal distribution between BMP receptor and integrins remains to be elucidated. Our study consists in evaluating how both receptors are able to communicate in space and time to interfere with adhesive behavior, to change cell identity or to favor cell processes like cell senescence, proliferation, migration or differentiation. The coordination between adhesive receptors and BMPR is investigated by integrating biomaterial development, molecular and biochemical approaches and cell imaging including live cell imaging, optogenetics and FRAP analysis.

Abstract — **Cell sorting, by which two cell populations spontaneously self-organize into distinct tissues is an important patterning process in development and regeneration. Here, we use *Hydra* regeneration from cellular aggregates as an *in vivo* model to quantitatively study this process. We compare our experimental data on sorting dynamics at different scales (single cells, separated tissues, aggregates) to existing theoretical models. We conclude that, in *Hydra*, cell sorting is driven by the physical properties of tissues, in particular their interfacial tensions.**

Keywords — **Cell sorting, *Hydra*, regeneration, Cellular Potts model.**

I. CONTEXT

How patterns emerge from an initially homogenous cluster of cells is a fundamental question in developmental biology. *Hydra* is a powerful model for studying mechanisms driving pattern formation because of its ability to regenerate after dissociation into individual cells. The first step of regeneration from cell aggregates involves segregation of the heterogeneous mixture of two epithelial cell types into their respective tissue layers, a process referred to as cell sorting. Two classes of physically motivated models have been put forward to explain this. One class explains sorting by intrinsic differences in motility between the two cell types [1]. The other treats each tissue as a complex fluid and attributes sorting to differences in interfacial and surface tensions [2], similar to liquid dynamics.

Experimentally, most studies of cell sorting have either relied on 2d cellular aggregates, including in *Hydra* [3], *in vitro* situations which do not have a direct counterpart *in vivo* [2], or have focused solely on single cell properties. We thus decided to revisit cell sorting in *Hydra*, taking advantage of current technology, such as transgenic animals and 2-photon microscopy. Using the versatility and experimental ease offered by *Hydra* as a model system, we developed an *in vivo* quantitative approach of cell sorting at multiple scales. By comparing our experimental results to existing models and our own numerical simulations, we clarify the physical mechanisms of cell sorting in this context.

II. EXPERIMENTAL APPROACH

We used fluorescent *Hydra* aggregates and measured key dynamical quantities (sorting time, typical cluster size) through time-lapse confocal imaging. Then, we used micro-aspiration, rounding up, and fusion assays to perform measurements of the rheological properties (elasticity, viscosity, surface tension) of both tissues separately. Finally, to examine the possibility of differences in cell motility, we achieved single cell tracking during regeneration using time-lapse two-photon microscopy. We compare these experimental measurements to simulations using a 3d Cellular Potts Model.

III. RESULTS AND CONCLUSION

We found that (1) differences in interfacial tensions between the tissues can drive sorting and (2) there are no intrinsic differences in cell motility between cell types [4]. Our results thus rule out differential motility as the driving force of *Hydra* cell sorting and point towards differences in interfacial tensions instead.
To further demonstrate this, we developed our own numerical simulations to mimic our experiments and compare their predictions to both experimentally measured quantities and previously published models. We found good agreement between experiments and theory showing that differential interfacial tensions are sufficient to explain cell sorting in aggregates.
Using a multi-scale, interdisciplinary approach, we answered a long lasting question in biology regarding the mechanisms driving cell sorting in *Hydra* regeneration.
As the importance of studying physical features in the context of development is increasingly recognized, our work demonstrates that a similar approach is also fruitful in the context of regeneration and we argue that this is an exciting research area waiting to be explored in more depth.

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Quantitative analysis of widefield ALEX-FRET in living cells

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Abstract

Based on the extreme sensitivity of FRET at the nanometer lengthscale, FRET-based biosensors were created to sense protein functions or molecular forces. The measurement of FRET can be extremely precise in single-molecule experiments where each photon can be properly assigned. In cells, the situation is more complex: ensemble measurement in each pixel reduces the dynamic range of the FRET probe and fluorescence signal can be affected by other environmental factors. Hence, measuring unbiased quantitative FRET efficiency remains challenging. FRET can be addressed by measuring the donor lifetime, method known to be fairly quantitative but generally limited to low temporal resolution. To study dynamical processes, the simplest and fastest method appears to be widefield imaging of sensitized-acceptor emission, but it generally fails at providing absolute FRET efficiencies.

We propose a theoretical framework for extracting quantitative FRET values based on a modified 3-image experimental scheme. From photophysical and experimental parameters, we define two specific factors to correct for excitation and emission independently, in addition to classical bleedthrough corrections. Inspired from the single-molecule approaches, we take advantage of the stoichiometry between donor and acceptor to determine in a robust way the two excitation/emission parameters simultaneously from the 3D-fitting of the 3-image data. This method was evaluated with FRET standards (Koushik et al. Biophys. J. 2006) showing unbiased and reliable FRET efficiencies. As an application, we show the response of Rac1, a small Rho GTPase (Dora biosensor, Y.I. Wu), to a local mechanical stimulation.

Evolution of mitotic spindle behaviour and mechanics in nematode embryos

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The cell is a level of biological organisation that has been poorly explored from an evolutionary perspective because basic cell functions (e.g. cell division) show remarkable conservation across phyla. Thus, an essential question remains: to what extent cellular mechanisms evolve without altering the basic function they sustain?

We have developed the asymmetric cell division of nematode embryos as a study system. The first embryonic division of the nematode *C. elegans* gives rise to two daughter cells of asymmetric size and fate, due to the asymmetric positioning of the mitotic spindle. This initial event is crucial to embryogenesis and conserved in most nematode species. We characterized the intra and inter-species variation in spindle movements in embryos of 42 closely related species of nematodes. We found that significantly different combinations of spindle movements ultimately lead to an asymmetric displacement of the spindle and established that even between virtually identical phenotypes, mechanical optimization of the spindle differs.

We are now exploring which changes to spindle positioning mechanisms have occurred over the course of nematode evolution using comparative biophysics. In a subset of interesting species, we have performed spindle severing experiments to reveal the balance of forces. We have also measured cytoplasmic viscosity and centrosome size, in order to compare the forces in pN between species using the Stoke's law.

Mechanical signature of RBCs flowing out of a microfluidic constriction

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The Red Blood Cells (RBCs) membrane possesses a unique structure responsible of their remarkable ability to deform to go through the small capillaries of the microcirculation. RBCs have been extensively studied in the past fifty years in order to characterize mechanical phenotypes associated with both healthy and pathological states.

Indeed, various diseases such as malaria[1-3], Sickle Cell Anemia[4-6] (SCA) or Hereditary Spherositosis[7] (HS) are associated with variation of RBCs deformability. Although conventional techniques allowing the quantification of cellular mechanical properties, such as atomic force microscopy (AFM), micropipette aspiration and optical tweezers[8] are well-established, they present throughput too low (of the order of several tens of cells per day) to be envisaged as routine diagnostic tools. Currently only ektacytometry, which consists in shearing a blood sample into a Couette system, is used by hematologists to screen RBC membrane disorders.[9-11] However, the measurement is averaged over the whole cell population.

In the present study, we report the effect of several chemical treatments known to affect RBCs membrane surface area or membrane deformability, on the dynamical behaviour of RBCs flowing out a microfluidic constriction. We evaluated if the response of the cells at the exit of the constriction is sensitive enough to discriminate between the effect of excess surface area and membrane deformability. Finally, the mechanical response of malaria infected blood samples and blood samples from patients with HS and SCA are investigated.

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Lattice defects induce microtubule self-renewal

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ABSTRACT

Microtubules are dynamic polymers, which grow and shrink by addition and removal of tubulin dimers at their extremities. Within the microtubule shaft, dimers adopt a densely packed and highly ordered crystal-like lattice structure, which is generally not considered to be dynamic. Here we report a new dimension of microtubule dynamics, whereby thermal forces are sufficient to remodel the lattice, despite its apparent stability. Our combined experimental data and numerical simulations on lattice dynamics and structure demonstrate that dimers can spontaneously leave and be incorporated into the lattice at structural defects. We propose a model mechanism, where the lattice dynamics is initiated via a passive breathing mechanism at these dislocations, which are frequent in rapidly growing microtubules. These results show that we may need to extend the concept of dissipative dynamics, previously established for microtubule extremities, to the entire shaft, instead of considering it as a passive material.

BMPR1B and JAK2/STAT3 inhibition targets BMP4-niche mediated leukemic stem cells that persist during CML remission

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Keywords : BMP, Jak/Stat, niche, resistance, leukemic stem cells, targeted therapies.

Objectives: Despite improvements of Chronic Myeloid Leukemia (CML) patient care by Tyrosine Kinase Inhibitors (TKI) specific targeting of the BCR-ABL kinase, TKI are not yet curative since many CML patients still retain progenitors and leukemic stem cells (LSC) in bone marrow. The bone morphogenetic proteins (BMP) pathway, that co-regulates the fate and proliferation of normal hematopoietic stem cells (HSCs) and interactions with their niche, is deregulated early on during CML development *[1].* These alterations sustain a permanent pool of LSC and progenitors expressing high levels of BMPR1b receptor and evolve upon treatment to progressively implement a BMP4 autocrine loop, finally leading to TKI-resistant cells persistence and progression *[2]*. Recently, a major study revealed that a specific LSC sub-fraction survived in TKI-sensitive CML patients from which resistant cells emerge *[3]*. We investigated how BMP signaling could relate to those persisting LSC.

Methods: We use single cell RNA-Seq analysis of CML BCR-ABL+ persistent cells from patients. We developed a new model of $CD34^{+}CD38^{-}$ LSC that presents similar characteristics to persistent primary LSC. We compared presentation of soluble BMP4 to LSC as a free factor *versus* a matrix-bound BMP4 biomimetic films used to replicate an *in-vivo* microenvironment (biofilm system with a controlled rigidity).

Results: Q-PCR analysis of bulk CD34+ from CCyR or resistant patients, or single cell RNA-Seq analysis of BCR-ABL+ persistent cells show a co-enrichment of BMP signaling, quiescence and HSC signatures, without modulation of major canonical target genes. We identify a direct correlation between BMPR1b and Stat3 only in quiescent CML cells. We demonstrate that $CD34^{+}CD38^{-}$LSC model display also increased P-Smad1/5/8^{+} and P-$Stat3^{+}$ subset, and that a combination of Jak2 (AG490) and BMPR1b (E6201) inhibitors can efficiently prevent proliferation and increase differentiation of persistent CML cells. Importantly, we showed

that this presentation mode of BMP4 provided by the niche could promote resistance of immature cells. Dual targeting of BMPR1B and Jak2 pathways prevented the proliferation of IM-persistent cells in a biomimetic environment. In addition, AG490 alone displayed the unexpected ability to increase BMP4 production by mesenchymal bone marrow cells, thus promoting niche-protecting effects toward LSC. The addition of E6201 prevented this by abrogating BMP4-mediated LSC protection against TKIs.

Conclusions: Our data suggest that AG490-E6201 combination is optimal to impair the maintenance of CML persistent LSC and prevent BMP4-supporting mesenchymal stem cells protection.

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Reproducible epidermal morphogenesis in ascidians: an active-reactive mechanical model

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(Dated: December 14, 2018)

Cell division bears a great importance in developing multicellular organisms as it contributes to patterning, organs shaping, accommodating cells growth and organizing single cells in tissues. Overall, the tight regulation of oriented cell divisions is one fundamental strategy to achieve the control of developing biological forms. Recent years have seen a number of attempts to elucidate the mechanisms used to control the orientation of cell divisions in specific contexts: in cleaving embryos a physical model of dynamics and mechanical equilibrium of mitotic spindles accounts for the long-axis (or Hetwigs) rule for cell division; within animal tissues it has been shown that the coupling of externally induced strain and Hertwigs rule leads to the orientation of cell divisions with the main stress direction. What is still missing in this theoretical landscape is a view of oriented cell division as a many-body process in multicellular interacting systems. Given the importance of orchestrating several rounds of divisions of many cells with a tissue, intrinsic process protecting the coherence of several division orientations must play an important role. In our work, building on recent experimental and theoretical breakthroughs on the energetics of single-cell cytokinesis and on important chemical cues for oriented cell divisions in animal embryos, we develop a theoretical standpoint on the many-body energetic thermodynamics of cell divisions in passive and active tissues in developing embryos, which integrates the role of external anisotropic mechanical stress. We argue that cells would orient their division plane in such a way as to reduce the energetic cost of furrow ingression and cytokinesis. We show that Hertwig's rule emerges as a limiting-case behaviour, and demonstrate how anisotropic mechanical stress profiles in multicellular systems can provide important cues to guide cell divisions even in the absence of pronounced strains. This model provides a possible mechanism through which cells integrate external and internal information and correlate the orientation of their cleavage planes. Remarkably, our model can account for the robust division pattern observed in the epidermis of wilde-type and mutant embryos of the ascidian *Phallusia mammillata*, including those reproducible observed deviations from Hertwig's rule which have so far eluded explanation.

CELLQUAKE ELASTOGRAPHY: ULTRAFAST IMAGING OF CELL ELASTICITY

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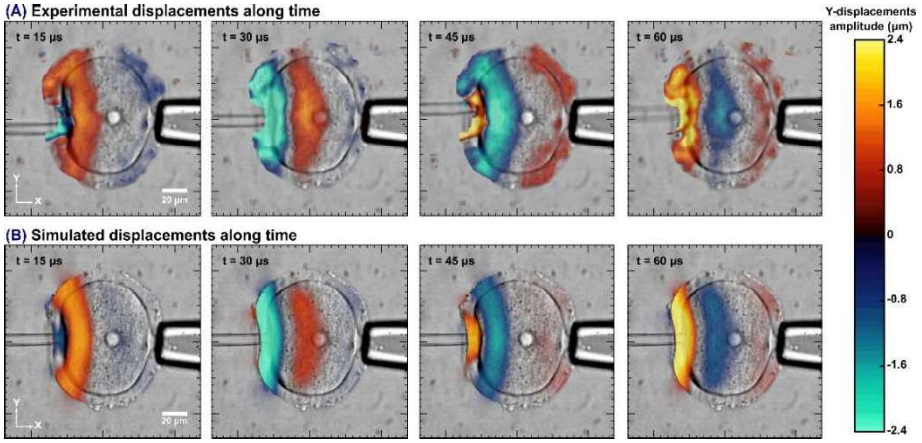
Background: Cell elasticity is mainly measured using static elastography method, i.e., by measuring the deformation following an applied force [1]. Measurements hardly make images of cell interior, and can take up to a few minutes. Shear wave elastography method, developed at the organ scale, could be adapted at the cellular level to get quick and robust measurements. It however requires high speed imaging, up to 100.000 images per second.

Aims: The cellquake elastography aims to apply the shear wave elastography technique on cells.

Methods: A 80 um diameter mouse oocyte was held by a pipette. A second pipette in contact with the oocyte was oscillating at 15 kHz with a piezoeletric actuator (piezo impact drive unit; Prime Tech Ltd, Japan). The cell was observed with a 100,000 frames/second camera (Phantom Research v2512, CA, USA) attached to a x100 microscope. Displacements were computed with a Lucas-Kanade optical flow method. Elasticity was computed using a passive elastography method [2].



Results: Shear wave have been successfully induced in the oocyte, with a good agreement between experimental and simulated results. Elasticity images of cells have been computed. Adding cytochalasin B, a softening toxin, to the cell lead to a significant ($p<0.02$) decrease of elasticity.

Conclusions: Feasibility of the shear wave elastography technique at the cellular level has been demonstrated [3].

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Mechanics in Drosophila during early gastrulation

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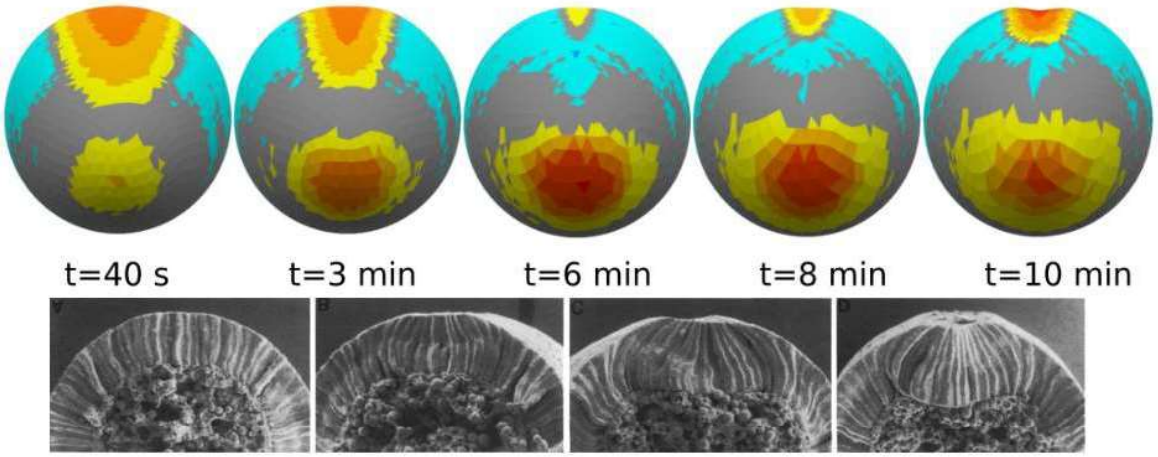

We propose new insights concerning the onset of gastrulation in the development of Drosophilia. Gastrulation takes place when the drosophilia embryo is mostly constituted by a closed monolayer of cells with elliptical shape. It consists in a morphogenetic movement in which a longitudinal furrow is formed. It is known to happen concomitantly with the increase of myosin activity in a ventral location ("mesoderm"). Previous models for this crucial developmental step mainly invoked local and sudden modification of the preferred ("spontaneous") curvature of the cell monolayer, despite the lack of biological evidence.

Here, we model the embyo as a closed surface in finite elements, which suits to the locally planar organization of the cytoskeleton. The isotropic myosin action is modelled as a gradual pre-stress (roughly : affine reduction of the equilibrium features of surface elements).

This mechanical model allows to retrieve the shape of the embryo at the beginning of the ventral furrow formation, as well as caracteristic features: a sudden change in the ventral curvature, which indicates a buckling, quantitative evolution of area changes in the mesodermal and peri-mesodermal zones, local evolution of the distance between the embryo and the vitelline membrane that surrounds it.
These results show the importance of the mesodermal location of the myosin, and the role of the three-dimensional geometry, in the folding of the epithelium.

Sollicitations mécaniques et transport de l'information à longue distance chez les plantes

Résumé

Les plantes sont constamment soumises à de multiples contraintes mécaniques générées par le vent ou le toucher. Elles répondent à ces stimuli en modifiant leur croissance. Cette réponse de croissance mécano-induite peut se produire rapidement et à longue distance du site initial de stimulation, impliquant l'existence d'un signal transportant l'information à travers la plante. La nature de ce signal est encore inconnue. Il a été mis en évidence la génération et la propagation d'un signal électrique chez le peuplier lorsque sa tige est fléchie. Pour cela, nous avons développé un dispositif de mesures électrophysiologiques extracellulaires couplé à un banc de flexion à amplitude contrôlée. De par sa forme et son déplacement bidirectionnel dans la tige, ce signal électrique ressemble à un potentiel d'action. Cependant, un point le distingue du potentiel d'action : un mode de propagation non autoentretenu ; démontré par la capacité du signal à se régénérer après avoir traversé une section de tige privé de cellules excitables. Cela amène à postuler que le signal électrique soit généré par une onde de pression hydraulique induite par la flexion de la tige selon une théorie en cours de développement dans la littérature.

Mots clés : Flexion, signal électrique, signal hydraulique, mécano-sensible, thigmomorphogenèse, peuplier.

Abstract

Plants daily undergo external mechanical solicitations such as wind or touch and respond to these stimuli by adjusting their growth processes. A fascinating feature of this mechanical-induced growth response is that it can occur rapidly and at long distance from the initial site of stimulation; suggesting the existence of a fast signal that propagates across the whole plant. The nature and the origin of the signal are still not understood. An experimental device was developed to record electrophysiological extracellular signal on poplar trees during controlled stem bending. Our results show that the bending of stem generates an electrical signal that propagates through the plant. Some characteristics of this electrical signal such as its shape and its bidirectional displacement in the stem are similar to the characteristics of an action potential. However, one point distinguishes this signal from the action potential: a non-self-propagating mode of propagation; demonstrated by the ability of the signal to propagate through a stem segment without bark. This leads us to suggest that the electrical signal is generated by a hydraulic pressure wave induced by the bending of the stem as described by a theory being developed in the literature.

Key words: Bending, electrical signal, hydraulic signal, mechanosensing, thigmomorphogenesis, poplar.

Quantification of the cell behaviors involved in the development and regeneration of the limb epithelium of the crustacean *Parhyale hawaiensis*

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Abstract

The development and regeneration of epithelial tissues involves dynamic cell behaviors that most often occur in highly curved tissues. Quantifying cell size and shape changes, cell proliferation, apoptosis and cell-cell rearrangements in such tissues necessitates: 1. stacks of live images taken at single-cell resolution for days; 2. reliable image analysis methods to segment and track the cells in 4D and; 3. statistical mechanics methods to decompose whole tissue deformation into distinct cellular contributions. Over the last decade, the crustacean *Parhyale hawaiensis* has been established as a model for both development and regeneration, taking advantage of its small size, its transparency and its propensity to heal amputated limbs. During development and regeneration, the limb epithelium progressively elongates and folds, forming the leg segments. While the elongation of the limb during development is mostly driven by oriented cell divisions [1], it appears - during regeneration - to be driven by other cell behaviors, such as cell-cell rearrangements [2]. To compare further the similarities and differences involved in development and regeneration, we first segment and track epithelial cells in 4D. After generating a cartographic atlas of the curved epithelial surface [3], we decompose whole tissue deformation into the aforementioned distinct cell behaviors using tracked cell neighbors links [4]. This quantitative approach will allow us to shed light on the old questions pertaining to the comparison of the processes involved in development and regeneration, as well as to generate spatio-temporal data that could be used to define and test cell-based mechanical morphogenetic models.

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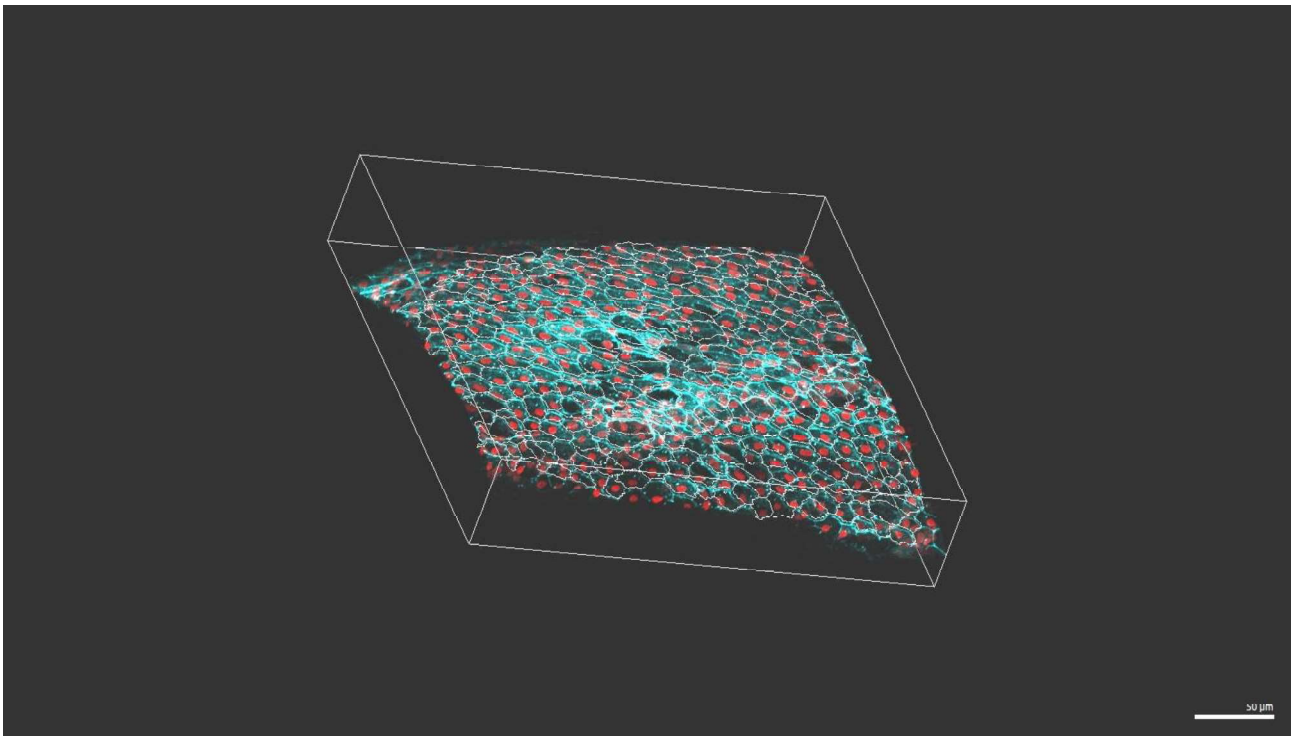
Figure



Example of 2.5D cell segmentation of the adult leg epithelium of *Parhyale*.

Simulations & analysis of turgor-induced stress patterns in multi-layered plant tissues.

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December 11, 2018

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Abstract

The intertwining between Mechanics and Developmental Biology is extensively studied at the Shoot Apical Meristem (SAM) of land plants. Indeed, plants morphogenesis heavily relies on Mechanics: Tissue deformations are fueled by turgor-induced forces and cell mechano-sensitivity plays a major regulatory role in this dynamics. Since measurements of forces in growing meristems are still out of reach, our current knowledge relies mainly on theoretical and numerical models. So far, these modeling efforts mostly focused on the epidermis, where aerial organs are initiated. From this perspective, the epidermis is usually assimilated to its outermost cell walls and described as a thin continuous *shell*, resisting to the pressure exerted by the turgidity of inner cells. In recent years, however, a growing number of experimental evidence has been suggesting a more complex mechanical role of inner walls. The aim of this work is to investigate the influence of these inner walls on the mechanical homeostasis of meristematic tissues. For this, we performed numerical simulations of the loading by turgor-induced forces of both realistic young flower buds as well as purely virtual structures. These simulations were performed on high-resolution meshes. Our analysis sheds light on the mechanics of growing plants by demonstrating the strong influence of inner walls on the epidermis mechanical stress pattern especially in negatively curved regions. Our simulations also revealed some strong and unsuspected features, such as correlations between stress intensity and cells size as well as differential responses to loading between epidermal and inner cells. Finally, we were able to monitor the time evolution of the mechanical stresses felt by each cell and its descendants during the early steps of flower morphogenesis.

Tissue Mechanics: Stokes Flow in Confluent Cell Simulations

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Cell migration plays an important role in embryogenesis, wound healing and tumor metastasis. Cell monolayer migration experiments help to understand what determines the movement of cells. However the usual monolayer set up can not discriminate the differences in migration created by each cell ingredient. That is why we find useful to observe cell monolayers dynamics in a discriminant benchmark: a heterogenous flow, i. e. a cells flow around a circular obstacle (known as Stokes geometry)[1,2]. We work with experiments *in vivo* (cell monolayer migration in culture) and *in vitro* (in drosophila thorax during metamorphosis). We simulate cell monolayer evolution using different computation models of active agents. We compare several computation cell models among themselves and with *in vitro* experiments. To compare the data from different sources we take the same measurements in each one: velocity field, density field, deformation field and others. The aim is to list the minimum cellular ingredients necessary to correctly simulate real cell migration. We plan to compare the measurements in the several active matter models[3,4,5] (including the so-called Vicsek and Potts ones) which all have their own specific advantages. Altogether, the dialog between *in vivo*, *in vitro* and *in silico* assays, and analytical calculations will enable to evidence specific cell-level contributions to tissue mechanics and collective active migration.

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Oriented basement membrane fibrils provide a memory for F-actin planar polarization via the Dystrophin-Dystroglycan complex during tissue elongation

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Summary

The elongation of the fly ovarian follicle is as model of tissue morphogenesis involving the extracellular matrix. It is proposed that, following a Fat2-dependent planar polarization of the basal domain of the follicle cells, oriented basement membrane (BM) fibrils and F-actin stress fibers constrain follicle growth, promoting its axial elongation. However, the relationship between BM fibrils and stress fibers and their respective mechanical impact on elongation are unclear. We found that Dystroglycan (Dg) and Dystrophin (Dys) are involved in BM fibril deposition. Moreover, they also orient stress fibers, acting locally and in parallel to Fat2. However, this cell autonomous control of F-Actin orientation by the Dg complex also relies on the previous BM fibril deposition, indicating two distinct but interdependent functions. Thus, this complex works as a critical organizer of the epithelial basal domain, acting on both F-actin and BM. Moreover, BM fibrils act as a persistent cue for the orientation of stress fibers, which are the main mechanical effector of elongation.

Mechanically triggered atrial arrhythmia and catecholaminergic polymorphic ventricular tachycardia: a misleading association

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Introduction

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inheritable cardiac channelopathy associated by occurrence of ventricular arrhythmia triggered by exertion or stress. Atrial tachycardia (AT) has been suggested to be a potential initial presentation in patients diagnosed with CPVT.

Method - Result

A 23-year-old Caucasian female was admitted for palpitations occurring during exercise or strong emotions. A stress test revealed ventricular bigeminy that appeared soon after the start of the test. A heterozygous RyR2 mutation was found. Despite beta blockers, she remained symptomatic. A second look at the stress test identified a slightly irregular tachycardia described as atrial fibrillation and arising at a very early stage of the test that was missed as the culprit arrhythmia. Numerous episodes of atrial tachycardia occuring exclusively during minimal physical activity completely disappeared after the last ablation procedure (Fig 1). On the third ablation attempt, a non-sustained atrial tachycardia originating in the inferior part of the Crista Terminalis was mechanically induced (cycle length of 270ms) and successfully ablated. After 6 months of follow-up, the patient was asymptomatic with nadolol alone (80 mg/day).
In the present patient, the diagnosis of AT was missed for a period of nearly 6 years. Diagnosis was delayed because it is usually thought that ventricular arrhythmias are the main cause of symptoms in CPVT patients.

Conclusion

The present case illustrates that in patients with CPVT: 1) failure of stellectomy and beta blockers to eradicate the arrhythmia may indicate a mechanism that is different from a single hypersensitivity to sympathetic stimulation; 2) the source of the atrial arrhythmic focus can be identified by intra-cardiac mechanical stimulation.

Figure 1 A **Figure 1B**

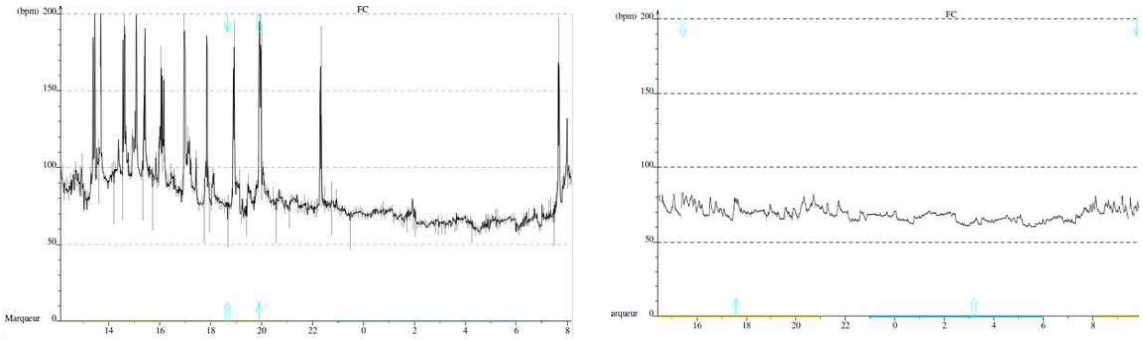

Figure 1: 24 hours Holter recordings A) before and after (B) catheter ablation of the right atrial tachycardia.

Geometry can provide long-range mechanical guidance for embryogenesis

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Morphogenesis is a three-dimensional process during which an organism undergoes complex deformations to acquire a given shape and organisation. The genetic patterning of Drosophila embryos and the way this regulates key molecules and complexes, such as actomyosin, is well described. How the motor Myosin II generates local mechanical action is understood, however, the way this is integrated at the scale of the embryo to drive morphogenetic movements is still to be characterised. Axis extension in Drosophila is a good model system for this, since it involves the deformation of the whole of the embryonic epithelium. It is dependent on a well-characterised anisotropic myosin recruitement pattern in the germband tissue, where actomyosin organises in oriented supracellular cables through a planar-polarisation mechanism.

In order to resolve the stresses and deformations produced at the scale of the whole embryo, we develop a novel finite element technique which allows us to solve the three-dimensional mechanical balance resulting from a given global distribution of myosin-generated prestress. Our prediction of local mechanical behaviour is based on a rheological law recently validated for cortical actomyosin [1,2] and extend to the case when myosin generates an anisotropic prestress [3].

Numerical simulations confirm that the planar-polarised arrangement of myosin in the germband can trigger embryo-scale flows similar to those observed experimentally. Interestingly, this mechanical behaviour is shown not to rely necessarily on cell intercalation, but rather on the anisotropy of myosin action, which can entail cell elongation as well as intercalation. We also show that the mechanical balance that leads to axis extension towards the posterior of the embryo is crucially dependent on the embryo's geometry, including the presence anteriorly of the cephalic furrow, which can act as a guide for morphogenetic movements.

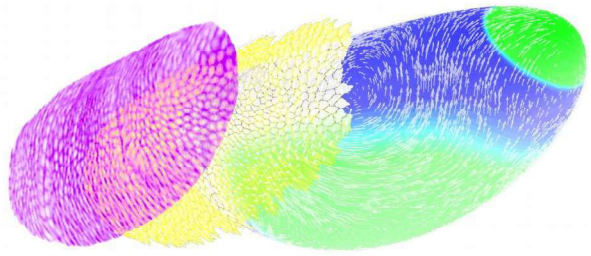

Image from laser sheet imaging (left), tracked cells and velocity vectors (center) and assumed Myosin localisation and resulting velocity in numerical simulation (right)

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ELECTRIC FIELDS: A POTENTIAL NOVEL AXON GUIDANCE SYSTEM TO CODE POSITIONAL INFORMATION

Long distance navigation of axons is marked by choice points, instructing highly stereotyped directional changes of axon trajectories. In this stepwise model of axon guidance, each step is thought essential for the next one, nevertheless intriguingly examples suggest that such sequential pathway experience can be dispensable for axons to reach their final destination. We further investigated this pathway-independent ability of axons to locate their target, using as model systems, two populations of spinal cord neurons having drastically different target location in the organism: the dorsal interneurons, which target the central nervous system and ventral motoneurons, which target the periphery. Dorsal and ventral explants were grafted at ectopic positions in the chicken embryo. Both classes of axons succeeded to orientate towards central or peripheral direction and to reach their physiological target territories. This suggests that the growing axons might perceive an overall guidance information that enable them to locate over large scale tissue position in the embryo.

Beyond chemical gradients of extracellular cues, bioelectric signals are attractive candidates to this function as they were shown to encode spatial information essential for regeneration and to be present in the developing embryo. Thus, we investigated whether electric field could influence the navigation of chick motor and commissural axons, using *in vitro* set-ups. We found that electric fields, in the ranges of the measures already reported in chick embryos, could orient the trajectories of both types of axons. Furthermore, different sub-populations seem to have specific sensitivities, which could result in different trajectory choices *in vivo*. Pharmacological inhibition of different ion channels and pumps *in vitro* led to identify a Na^+/K^+ ATPases as potential mediators of electric field-induced axon orientation. We are currently testing siRNAs against different Na^+/K^+ ATPases subunits to confirm this role *in vitro*. In parallel, we are investigating the effect of siRNA mediated Na^+/K^+ ATPases knockdown on axon navigation, notably after grafting. If siRNAs decrease axon sensitivity to electric field, changes in axon behavior *in vivo* would support a contribution of electric field to axon guidance.

Collectively, our findings could provide novel insights into the mechanisms ensuring axon guidance fidelity and resilience and uncover novel contribution of bioelectric signals and Na^+/K^+ ATPases during neuron development.

Mechanical regulation of morphogenesis

Ecole Normale Supérieure de Lyon
RDP, *Biophysics and Development* team
Antoine FRULEUX & Arezki BOUDAOUD

Biophysics — Modeling & Data analysis — Morphogenesis — Plant development

Figure 1: Cell growth is heterogeneous in space and time. Example of a sepal (green organ that protects a flower before it opens) from the model plant Arabidopsis thaliana. The colour scale corresponds to growth rates (high in red, low in blue).

The two hands of most humans almost superimpose. Similarly, flowers of an individual plant have similar shapes and sizes. This is in striking contrast with growth and deformation of cells during organ morphogenesis, which feature considerable variations in space and in time, raising the question of how organs and organisms reach well-defined size and shape. In order to link cell and organ scales, we built a theoretical model of growing tissue with fibre-like structural elements that may account for for the plant cell wall or animal cytoskeleton or extracellular matrix [1]. We made two important predictions. First, fluctuations occurring at cellular scale exhibit long-range correlations. Second, the response of fibres to growth-induced mechanical stress may enhance or buffer cellular variability of growth, making it possible to modulate the robustness of morphogenesis.
I will present in more details the results of the model we did to reveal how tissue response to mechanical signals controls robustness in development. I'll also present preliminary results of multi-scale growth analysis during plant development.

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Spatio-temporal cartography of repulsive guidance forces during commissural axon navigation

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During embryonic development, commissural axons cross the spinal cord midline in the floor plate (FP) and gain responsiveness to local repellents, which they did not perceive before reaching the midline. This switch towards sensitivity prevents axons from crossing back and expels them away from the FP. We set-up a paradigm for live imaging and super resolution STED analysis of guidance receptor dynamics during commissural growth cone navigation in chick and mouse embryos. This analysis revealed a remarkable program of delivery and allocation of receptors at the growth cone surface, generating receptor-specific spatial and temporal profiles. This provides a mechanism whereby commissural growth cones can discriminate coincident repulsive signals that they functionalize at different time points of their navigation.

Our next aims are to characterize the spatial and temporal distributions of the repulsive ligands and also to map ligand-receptor interactions during FP navigation. We will concentrate on midline Slit proteins, processed into SlitN and SlitC and acting via Robo and PlexinA1 receptors. PlexinA1 is also recruited by Neuropilin2, forming a receptor complex mediating the repulsive effect of an additional midline repellent, the semaphorin3B. We generated a knock-in mouse model, baring PlexinA1 mutation of a tyrosine that specifically abrogates SlitC but not Sema3B signaling.

We generated molecular tools (i) to track Slit processing with a fluorescent cleavage reporter, (ii) to map the subsequent distribution of SlitN and Slit C fragments, (iii) and to cartography SlitC/PlexinA1 and SlitN/Robo interaction by Bimolecular Fluorescent Complementation in space and time during commissural axons navigation. In parallel, we investigate the mode of action of SlitC-PlexinA1 signaling. Analysis of our knock-in mouse model indicates that abrogation of this signaling induces some growth cones to break their forward trajectory, making a U-turn within the FP. Using ex vivo set-ups, we are testing whether this reflects alterations of axon-growth cone stiffness, morphology, and/or exploratory dynamics as well.

Mechanobiology and Physics of Life in Lyon - Jan 28th 2019

Coupling magneto-active substrates with FRET biosensors to decode mechanotransduction

Alain Lombard

Abstract

Living cells process mechanical signals into intracellular biochemical ones that regulate essential cellular functions ; this mechanism is called mechanotransduction. We think of cells as signal processing machines that convert mechanical inputs into biochemical outputs. Our goal is to experimentally study the coupling between the two types of signals to infer the mechanotransduction "transfer function" in space and time. To apply local and dynamic mechanical constraints at the single cell scale through a continuous surface, we have developed and modelled magneto-active substrates made of magnetic micro-pillars embedded in an elastomer. Constrained and unconstrained substrates are analysed to map surface stress resulting from the magnetic actuation of the micro-pillars and the adherent cells. These substrates have a rigidity in the range of cell matrices, and the magnetic micro-pillars generate local forces in the range of cellular forces, both in traction and compression. As an application, we followed the protrusive activity of cells subjected to dynamic stimulations. The biochemical response to the spatio-temporally controlled forces exerted by our substrates is read with FRET-based biosensors. They report in live the biochemical activity of Rho-GTPases in fibroblasts given a quantitative computation of the FRET efficiency. Our goal is to compute the spatial maps of FRET efficiency over time and correlate them with the spatio-temporal maps of the mechanical stimulation or the membrane activity. Our magneto-active substrates combined with FRET-based biosensors thus represent a new tool to study mechanotransduction in single cells.

References

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In vivo actin dynamics during C. elegans embryonic morphogenesis

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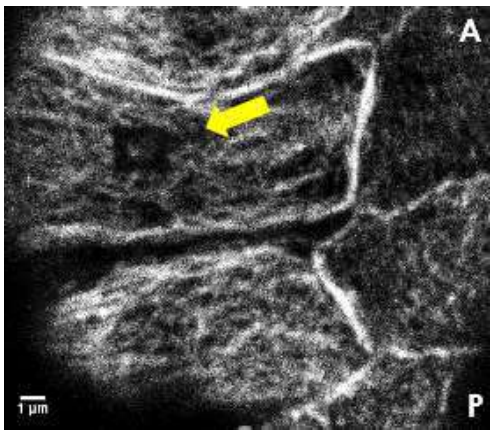

Morphogenesis is a developmental process by which shape is acquired. During morphogenesis, tissues change their shape, a process often mediated by myosin-driven cell contractility. The *C.elegans* embryo has been established as a model to investigate the relationship between cell contractility and shape changes. One important morphogenetic step in *C. elegans* is elongation, that converts the embryo from bean-shaped to the characteristic elongated worm shape. Elongation occurs via changes in cells' shapes that depend on the forces created by the embryonic cells and the resistance of the biological tissue.

Actin organization plays an essential role during this elongation. Elongation requires that actin organizes in thick parallel bundles in the dorsal and ventral epidermis, while it initially consists of randomly oriented, thinner filaments in the lateral one. These distinct organizations of actin are suspected to be at the origin of an anisotropic tension that correlates with a planar polarization of the cortex.

Using laser ablations in the actin cortex, we probed actin tension during the morphogenetic step of elongation of *C.elegans* embryos. The ablation is performed in a rectangular-shaped torus, to mechanically separate a small patch of actin from the rest of the cortex and allow the analysis of the tissue relaxation with well-defined boundary conditions. By tracking the dynamical changes of size of these patches of actin, we measure strain rates and can assess a potential anisotropy of the stresses in the tissue: an enhanced rate of strain in a specific

direction indicates an anisotropy of the stress prior to ablation, which we correlate with the organization of actin. Exploitation of this technique is expected to provide a better characterization of actin dynamics during the course of *C. elegans* embryonic elongation.

Rôle des forces mécaniques au cours de la progression du mélanome cutané

Le mélanome est le cancer de la peau le plus meurtrier en raison de son fort potentiel métastatique et de sa résistance aux thérapies actuelles. Comprendre les mécanismes qui sous-tendent l'acquisition du potentiel invasif du mélanome cutané est donc crucial. Depuis plusieurs années, nous travaillons à décortiquer les mécanismes moléculaires qui conduisent à l'initiation tumorale et au franchissement de la jonction dermo-épidermique indispensable à la dissémination des cellules de mélanome, et nous avons identifié de nouveaux acteurs importants (EMT-TFs et Tspan8).A ce jour, l'identification de mutations et moléculaires et de résistances aux traitements ne cessent de s'enrichir et permettent une meilleure prise en charge des patients, en développant de nouvelles thérapies ciblées. Malgré de nombreuses avancées dans la compréhension de ces mécanismes, peu d'études s'intéressent à l'organisation des cellules tumorales dans leur ensemble et au entre la tumeur et son microenvironnement.

Durant la mélanomagenèse et l'acquisition du caractère invasif, des changements structuraux au sein de la cellule et du tissu sont observés. Cependant, peu d'études se sont intéressées aux pressions pouvant s'exercer au sein d'un épiderme et sur la tumeur mélanocytaire pour limiter ses capacités d'invasion. C'est pourquoi, nous proposons d'étudier les propriétés nano-mécaniques de pression qui s'exercent mutuellement entre épiderme et mélanome en croissance, dans deux modèles *in situ* : un modèle de peau reconstruite en 3D et chez le poisson Medaka qui développe spontanément des mélanomes cutanés. Pour appréhender cette mécanique complexe, la microscopie à force atomique (AFM) se présente comme une technique de choix.

Bone is a living tissue able to adapt itself to its mechanical environment through bone remodeling process. It has been discovered that ultrasound waves can influence bone remodeling. A lot of studies have reported the beneficial effect of low intensity pulsed ultrasound to treat pathological fracture healing and a commercial device based on this principle exists. Nevertheless, biomechanical underlying mechanisms of ultrasound mechanotransduction are poorly understood. Our hypothesis is that the ultrasound (US) stimulation at the meso-scale level can induce fluid shear stress acting on osteocytes at micro-scale level, inducing a biological answer. To investigate this question, we developed a numerical model combining acoustics, fluid and structure. The goal is to better understand the interaction of ultrasound with bone, from the tissue scale to the cell scale in order to investigate the possibility of using ultrasound to help treat another dysfunction of bone remodelling, that induced by bone metastases.

Single Cell Force Spectroscopy study of Bacteria/Cell adhesion : Effect of the introduction of glycoclusters for cystic fibrosis pathology

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Abstract

Single Cell Force Spectroscopy (SCFS) has been shown be a relevant mode of Atomic Force Microscopy to study the processes involved in the adhesion between cells and bacteria at the cellular scale [1-3].The force-distance curves measured by SCFS are representative of the detachment process. In this work, we used SCFS to characterize the adhesion between an opportunistic human pathogen *Pseudomonas aeruginosa* (PA) and epithelial cells. In particular, PA is responsible for the infection of the human epithelium of patients with cystic fibrosis, causing breathing difficulties and, in the worst case, the death of the patient. It has developed antibiotic resistance because of his ability to create a biofilm on the surface of the epithelial cells. To do that, PA takes advantage of an arsenal of virulence factors, among them one lectin LecA which specifically recognizes the galactose molecules present on the surface of the host cell. A therapeutic approach against PA relies on the inhibition of lectins by additional carbohydrates, resulting in decreased cytotoxicity and hence increased cell survival [4]. Good candidates for lectins inhibition are the galactoclusters, synthesized molecules which present higher affinity to LecA than the natural carbohydrates. We showed that the introduction of the glycoclusters induced a reduction of the adhesion of PA on the cell that manifests by a decrease of the detachment work [5].

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CELLQUAKE ELASTOGRAPHY AT LOW FRAME RATE

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Ali Zorgani[2], Stefan Catheline[2]

Background: Shear wave elastography has recently been introduced to measure cell elasticity, under the name of microelastography. In this technique, the sample is put into vibration by a micropipette. The measurement of the vibration by an ultrafast camera allows to map the mechanical wave speed, related to the sample elasticity.
However, depending on the reconstruction algorithm, a lower frame rate camera could also be used

Aims: The objective was to evaluate the feasibility of using a low frame rate camera to make cellquake elastography measurements.

Methods: We used a 1200 frames sequence where a mouse oocyte was put into vibration and pictured with a 200.000 fps camera. Elasticity was reconstructed using 200 frames with variable subsampling, i.e., taking 1 frame over 2 (100.000 fps), over 3 (66.667 fps), over 4 (50.000 fps)… Then, elasticity maps were reconstructed for each subsampling. Elasticity maps were compared qualitatively and quantitatively using a relative error measurement from the 200.000 fps image.

Results: Resulting elasticity maps are very close qualitatively. Quantitatively, we get a relative error smaller than 2%. These results show that low frame rate camera could be theoretically be used without much decrease of quality. However, it comes with some limitations: the exposure time of the camera still has to be low (smaller than 10 microsecond) to avoid blurring due to displacement; quantitative information cannot be extracted if the frequency is lower than Nyquist frequency (half sampling frequency); and the total experience acquisition time naturally increase when frame rate decreases.

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Control of the micro-environment to decipher cancer cell response

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Many drug candidates for cancer treatment show potential when examined *in-vitro* but fail in clinical trials. This failure may stem at least in part from the use of conventional *in-vitro* systems that fail to replicate the physiological microenvironment in humans as well as the lack of cell-phenotypic measurements. In addition, there are now number of evidences that mechanics is playing an important role in the malignant transformation of cells during tumor progression and dissemination. We try to tackle these important issues (micro-environment and mechanics) by developing original techniques enabling to precisely control cell micro-environment, including the applied mechanical stress. The aim is to design *in vitro* assays reproducing as much as possible the environment encountered by tumor cells *in vivo* and to provide quantitative parameter of cell dynamic behavior that can be used as novel read-outs of cancer stage. Using time-lapse microscopy and cell tracking, as well as Traction Force Microscopy, such phenotyping cell characterization is performed at the multicellular cell level using colorectal cancer cells spheroids (HCT116) as model of tumor growth, and at the single cell level using the Chronic Myeloid Leukemia model to investigate the interplay between mechanics and Bone Morphogenic Protein Signaling.

Straight organ growth requires NEK6-dependent dampening of microtubule response to mechanical stress

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Many plant organs, like stem, petiole or hypocotyl exhibit straight growth. This phenotype requires intercellular growth coordination. Mechanical stress may play an important role in this process : tissue shape and growth prescribes a supracellular pattern of mechanical stress, which may channel consistent microtubule alignment between adjacent cells. This would in turn control growth direction *via* the guidance of cellulose deposition in the wall. To investigate that scenario, we focus on Arabidopsis tubulin kinase NEK6, which promotes microtubule depolymerization. The *nek6* mutant exhibits wavy hypocotyl, with stop and go growth patterns. To our surprise, through microtubule and growth quantifications, combined with micromechanical tests, we found that the *nek6* mutant is hyper-sensitive to mechanical stress. Interestingly, we found that NEK6 preferentially localizes on the microtubules that are aligned with tensile stress. Thus, we propose that NEK6 depolymerizes the microtubules along tensile stress, to avoid an over-response of microtubules to the variation in organ growth, and associated stress. NEK6-dependent stress dampening thus appears as a prerequisite for straight growth.

µRobots magnétiques pour la manipulation de cellules

Un éventail de nouvelles possibilités pour la manipulation à distance de cellules uniques

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Thibaut Devillers, Orphée Cugat, Olivier Stephan, Guillaume Dellatre

Nous présentons une nouvelle technique pour fabriquer des **µ-robots magnétiques 3D hybrides et contrôlables à distance**. Cette technique permet de fixer des blocs magnétiques puissants à des endroits précis d'une **structure 3D de géométrie quelconque et de résolution submicrométrique faite en polymère biocompatible, transparent** et imprimée par polymérisation à deux photons. Ces blocs magnétiques permettent de **déplacer et déformer les structures à distance** par l'action d'un champ magnétique appliqué depuis l'extérieur. Ainsi, de nouvelles fonctionnalités d'actionnement et de manipulation deviennent possibles à l'échelle de la cellule biologique, notamment grâce à la diversité des géométries possibles. En guise d'exemple, nous avons réalisé une micro-pince pouvant se déplacer et s'ouvrir à distance: cette dernière peut donc **saisir, déplacer et relâcher** des billes de dimension semblable à des cellules biologiques et placées dans une chambre microfluidique **confinée**. Dans l'avenir, un tel outil pourrait permettre de réaliser des **mesures de forces par palpation de cellules** ou encore de faire de la **microchirurgie sur cellules**.

Matériau massif, opaque et dur: **aimant permanent** sphérique de NdFeB avec revêtement

+

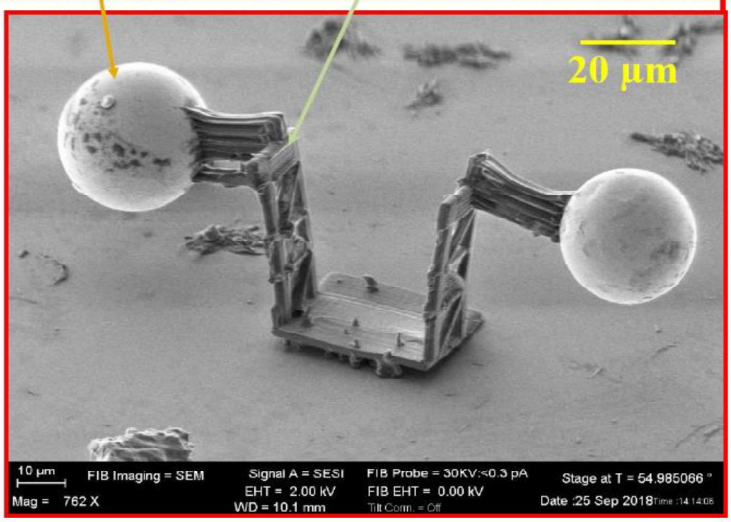
Structure polymère 3D souple, biocompatible, transparente, fabriqué en stéréolithographie à deux photons (SolGel, Collagène…)

=

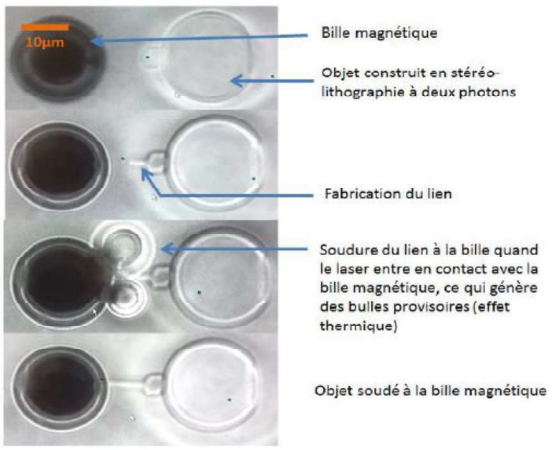
Micro-robot 3D hybride, déformable, magnétique et transparent permettant un actionnement efficace et complexe

→

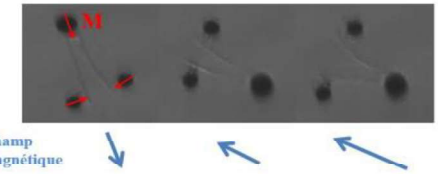
Micro-actionnement à distance de **déplacement et déformation** pour la réalisation **de fonctions complexes** sur des petits objets (billes, cellules biologiques…)



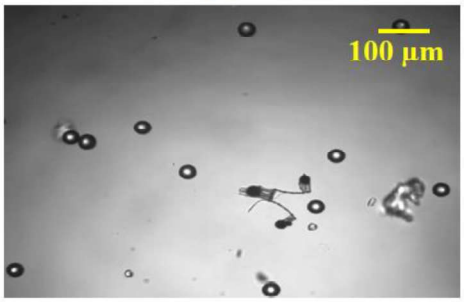
Principe de fixation des blocs magnétiques à la structure polymère 3D

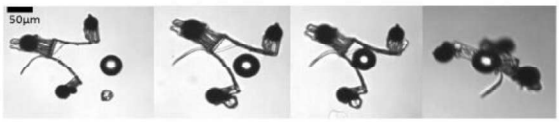


Principe de fonctionnement de la pince



La pince permet de saisir, déplacer et relâcher une bille de polystyrène de 40µm





Prospectives

-Déplacement/tri de cellules
-Stimulation mécanique de cellules
-Mesure de force par palpation de cellules
-Microchirurgie: ponction et énucléation d'une cellule par ajout d'outils en bout de pince

Modelling biomechanics during shark odontode morphogenesis.

Shark dentition shows an astonishing diversity of shapes, between species, between different individuals of the same species and between different places along a given individual's jaw. In addition, the shark body is covered by a regular array of diversely shaped dermal denticles, which are serially homologous to oral teeth. Odontode (a generic term for teeth and denticles) morphogenesis involves the interaction of a highly conserved gene regulatory network and coordinated changes in cell and tissue biomechanics. In order to understand the contribution of each component within this developmental network, we use, complementing experiments in catshark embryos, a mathematical model that combines gene regulatory dynamics and biomechanics in tissues by explicitly implementing different cell types and layers and their interactions via cell-cell signalling and mechanical forces. In particular, we are interested in elucidating the role of the mechanical microenvironment of the developing odontode in controlling its size and shape. For instance, we try to understand how the local curvature of the underlying jaw cartilage as well as growth rates in the surrounding tissues impact the size and orientation of the teeth. We suggest shark odontogenesis can be considered a paradigm of the dynamical role of mechanics in animal development and introduce an in silico approach to help testing developmental hypotheses.

Investigating the dynamics of apico-medial Myosin-II foci

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Abstract

During the development of embryos,, tissue morphogenesis is often driven by pulsatile cell shape changes. These changes are mediated by Myosin-II which localises at the apical cortex to forms, highly concentrated, dynamic aggregations called 'foci'. While it is well known that there is a strong correlation between Myosin pulses and cell shape changes, the apparent motion of these foci has been less studied. We investigate whether the dynamics of Myosin foci can be explained by biochemical or mechanical factors within the tissue, since these may have implications on the function of foci themselves. Using automated image analysis of amnioserosa cells with tagged Myosin-II during Drosophila dorsal closure, we isolate the foci and track the movements of thousands of them within hundreds of cells from multiple embryos. We observe that it is common that several foci are present simultaneously within the same cell apex, and that events of merging and splitting are equally frequent. Asking whether the motion of foci behaves as a random walk, we found that though the motion is in the diffusive regime, the distribution of angles between two consecutive steps is not random but clusters around zero and that the distance they travel grows nearly linearly in time, showing that the motion is not random. It is therefore possible to define a direction of movement for each focus. We find that this direction is correlated with the orientation of the cell when it has an elongated shape. We hypothesise and test three possible contributions that may explain the dynamics of foci: the role of the underlying tissue motion, a self-avoiding behaviour due to cortex properties, and the directed motion of foci due to a biochemical or mechanical cause.