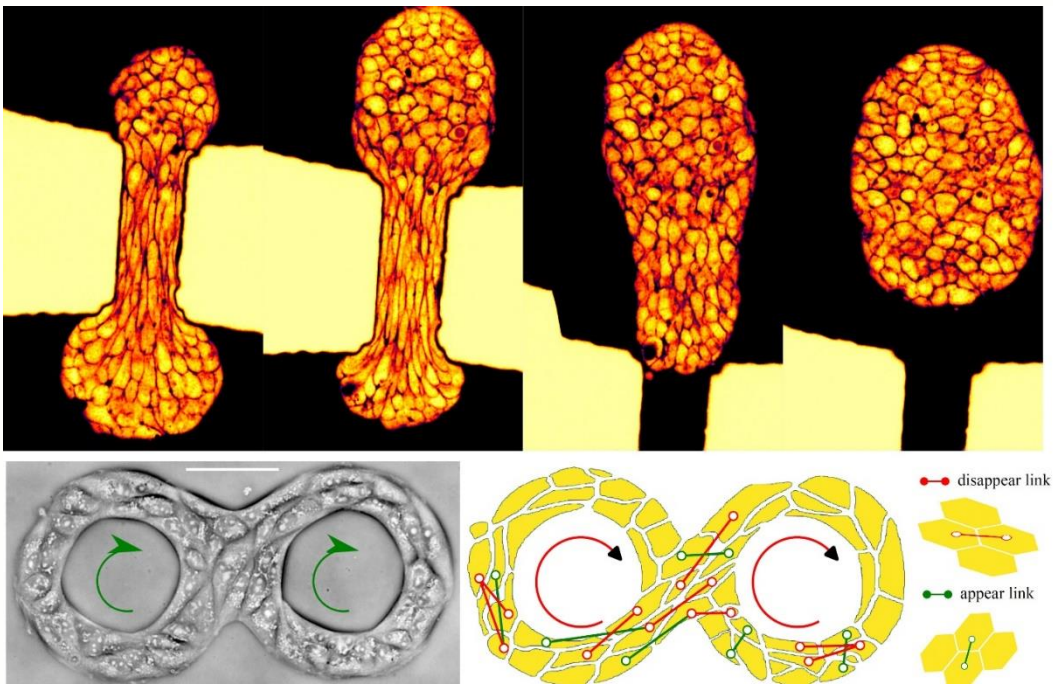
Invited speaker of GFR 2024

Probing biological tissues rheology through heterogeneous tissue flows in 2D cell monolayers and 3D organoids

Sham Tlili

(IBDM, CNRS - Aix Marseille Université)

Understanding how cells deform and exchange neighbors under mechanical constraints is crucial for deciphering dynamic changes in biological tissues. These mechanical interactions are pivotal in processes such as organ morphogenesis during embryonic development, the formation of organoids in vitro, and cancer progression. A significant challenge in studying spontaneously developing tissues lies in the limited understanding of both the active stresses generated by biological activity and the local rheological properties of tissues. In this talk, I will present experiments on two systems: 2D cell monolayers undergoing collective migration on adhesive substrates and 3D cell organoids subjected to microfluidic constriction. In both cases, tissues experience heterogeneous flows that drive cell deformation and neighbor exchange. I will discuss how combining microscopy with image analysis enables us to probe the mechanical behavior of these tissues.



Probing 3D tissue rheology with a high-throughput microfluidic aspiration pipette

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Preference : TALK at S1/Rheology of biological fluids and tissues

Keywords : Microfluidics, biological tissues, micropipette, rheology, poroelasticity

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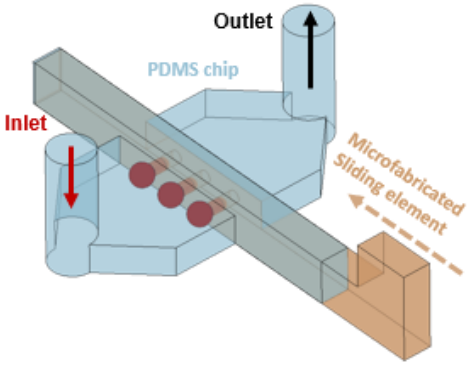
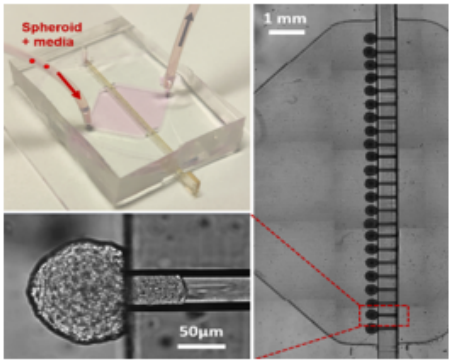
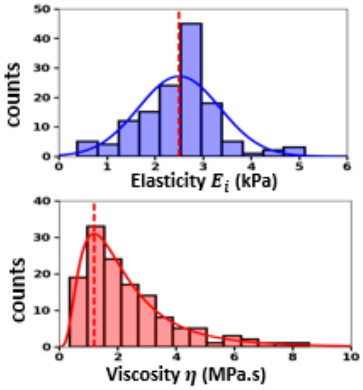
Abstract: The rheological properties of biological tissues play a key role in wound healing and morphogenesis. Most tissues are described as viscoelastic fluids, but their behavior can be complex, with non-linearity that could originate from biological activity and/or from tissue permeability.

We have developed an innovative microfluidic device made up of two elements, in order to study in depth the rheology of model tissues by realizing micropipette aspiration of ~20 spheroids (3D cell aggregates) in parallel. The device also permits dynamic stimulation: by applying forced sinusoidal regime, we observe that the high frequency response (time scale <1 min) deviates from the predicted viscoelastic behavior. We hypothesize that the coupling of fluid flow inside the aggregate with its deformation (poroelasticity) needs to been taken in account to properly describe the tissue response: permeability contributes to the transmission of stress, followed by cell deformation and finally cell reorganization in the tissue. We are currently realizing permeability measurements in steady state, as well as finite element simulation, in order to confirm this hypothesis. Cell scale imaging and immunostaining are also used to assess the relationship between microstructure and rheological properties. Finally, the approach can be extended to other tissues.







Mechanics of the cellular microenvironment as probed by cells in vivo

Marie Pochitaloff (1, 2), Alessandro Mongera (2), Hannah Gustafson (2, 3), Georgina Stooke-Vaughan (2), Payam Rowghanian (2), Sangwoo Kim (2), Otger Campas (1, 2, 4), Marie Pochitaloff

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Preference : TALK & rhéologie des tissus cellulaires et fluides biologiques
Keywords (5 max): Rheology, Morphogenesis, Microdroplet

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

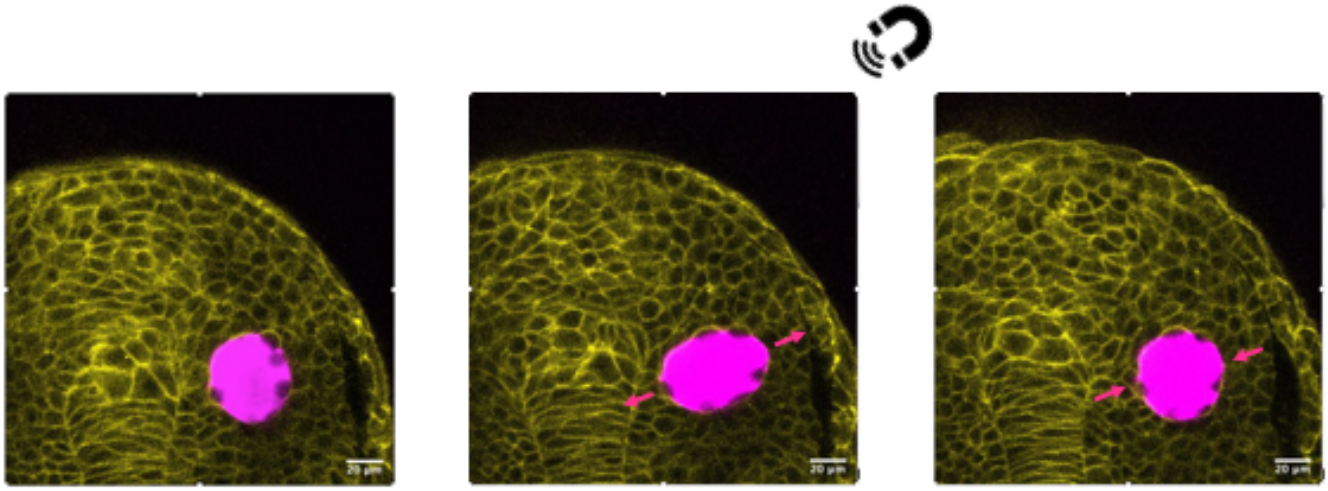

Figure: Magnetic microdroplet enable to apply controlled stresses in the tissue and measure the rheological response of the tissue.

Micro-rheology of soluble and adherent small intestinal mucus

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Preference: TALK & S1 Rheology of biological fluids and tissues

Keywords (5 max): Micro-rheology, Intestinal mucus, Duodenum

Abstract (250 Words Max): Mucus is secreted by different organs to protect underlying tissues from external bacteria and pollutants. In the small intestine, the mucus is spatially organized into a bi-layered structure: the adherent mucus protects the mucosa, whereas the soluble mucus mixes with the luminal content [1]. Previous research on intestinal mucus rheology often involved scraping it from the intestinal or colonic mucosa, which overlooks the properties and the role of soluble mucus. For this purpose, we isolated living portions of the small intestine of the rat in an organ bath and infused it with a physiological buffer to collect soluble mucus. The rheological properties of the collected sample were characterized by particle tracking of Brownian tracers using a confocal microscope. Then, the segment was directly mounted on the confocal microscope to study the micro-rheology of the adherent mucus. Confocal imaging of the soluble mucus revealed heterogeneous regions, with a predominance of viscous areas where the tracers diffuse, and a few gel-like areas where the tracers were trapped. Conversely, the adherent mucus showed gel-like properties, with characteristics changing to the distance from the mucosa.

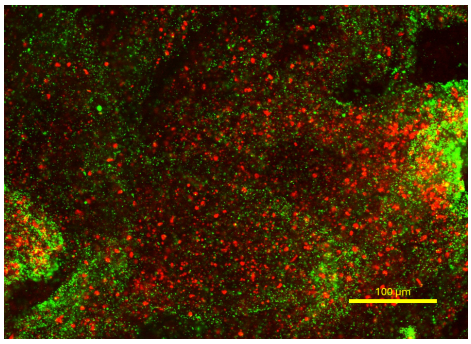

Figure 1: Microscopic image of intestinal mucosa (red) and tracers (green) trapped in the adherent mucus.

[1] A Allen and NJH Carroll. "Adherent and soluble mucus in the stomach and duodenum". In: *Digestive diseases and sciences* 30 (1985), 55S–62S.

Micro-rheology along motile intestinal villi

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Preference: TALK & S1 — Rheology of biological fluids and tissues

Keywords: bio-rheology, intestinal flow, lattice Boltzmann method, CFD

Abstract: Understanding the emergent micro-rheology and particulate transport near the intestinal walls is vital for building models for digestion and absorption of food/drugs in the gut. The small intestines have a lining of finger-like or leaflet-like projections called the 'villi'. Length-wise muscle motion leads to oscillatory motion of the villi – termed 'pendular motility' [1]. We examine role of pendular motility at the villi scale on flow and emergent suspension rheology near the villi, using 2D simulations. We choose the geometric dimensions of the duodenal section of the rat intestine as our model. In the fluid, we simulate a dilute suspension of coupled point-particles of volume fraction, ϕ. The oscillatory fluid inertia imposed by this motion is captured by the non-dimensional Womersley number, Wo. Rich, non-Newtonain flow complexity emerges even at low ϕ, with increasing Wo. We find that a mixing layer forms above the villi, whose height decays with increasing Wo at low ϕ. Above the mixing layer, an unidirectional 'pumping' flow layer manifests. Our results are consequential for understanding nutrients/drugs transport towards the villi surface, and their subsequent absorption.

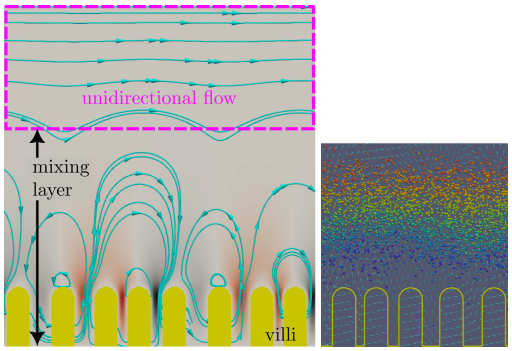

Figure 1: Left panel plots instantaneous flow streamlines, showing the mixing layer and the axial flow layer. The right panel shows the transport of particle suspension within the mixing layer.

[1] R. G. Lentle et al. In: *Neurogastroenterology & Motility* 24.7 (2012), 686–e298.

Microstructure of red blood cell suspensions: effect of the cell deformability

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Preference: TALK or Poster & S1- Rheology of biological fluids and tissues

Keywords (5 max):

Abstract: (250 Words Max)

The reduced red blood cell (RBC) deformability in patients with sickle cell disease (SCD) induces a modification of blood rheology and leads to painful vaso-occlusive crises. Probing RBC's change deformability has great potential for the development of new monitoring plans for SCD. The aim of this work is to evaluate if spectral-based quantitative ultrasound can detect variation in RBC deformability by conducting measurements and numerical simulations.

Experiments are conducted in a Couette cell, combined with an ultrasonic transducer to probe the anisotropic microstructure of suspensions at different insonification angles. Suspensions consist of healthy human (deformable) or artificially rigidified (pathological) RBCs in a fluid of equivalent density, and with different volume fractions. By comparing the backscatter coefficients of one concentrated and one diluted suspension, we measure the angular-dependent structure factors, that are related to the spatial arrangement of the RBCs inside the flow. Numerical simulations of the RBC microstructure are also performed using the YALES2BIO software, where the deformability is modulated using the ratio of internal-to-external viscosity of RBCs.

Significant changes in the structure factor amplitude are found between deformable and artificially rigidified RBCs in the experiments (Fig. a) and in the simulations (Fig. b). At the same hematocrit, larger structure factor amplitude for rigidified RBCs means more random cell positioning than for healthy RBCs. These results demonstrate that the microstructure is impacted by the change in RBC deformability. This paves the way to the monitoring of the pathological state of RBCs using quantitative ultrasound approach.

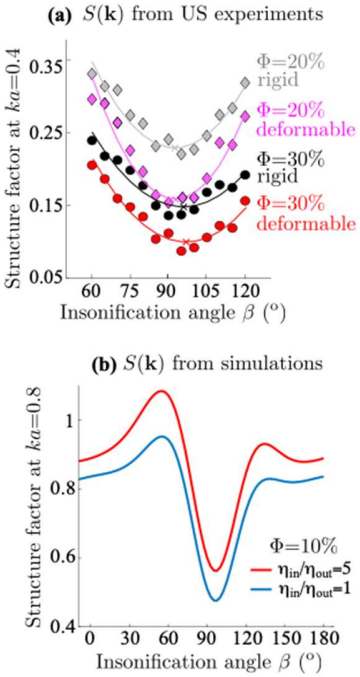

Figure: a) Structure factors measured at different insonification angles for deformable and rigid RBCs and hematocrits of 20% and 30%. b) Simulated structure factors for RBCs with ratio of internal-to-external viscosity ηin/ηout=1 (deformable) and 5 (less deformable).

Modelling the rheology of an *in vitro* blood vessel under pressure

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Preference : Talk in “Rheology of biological fluids and tissues” session

Keywords (5 max): organ-on-chip, blood vessel rheology, actin, active surface model

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Abstract : (250 Words Max)

Modelling the rheology of biological tissues made of living cells is of prime importance in many domains like developmental biology, medicine or bioengineering. To do so, it is notably important to address the coupling between tissue biology and mechanics.

In this talk, I will briefly present an experimental setup allowing to both probe the rheological behavior of an *in vitro* blood vessel made of a cylindrical monolayer of endothelial cells and submitted to a step of pressure and to capture its biological constituents behavior during the mechanical response. With this setup, we observe that the radius dynamics after a step of pressure exhibits an instantaneous elastic response and a long-time viscous behavior. At the same time the actin fibers forming the cells cytoskeleton undergo a strong reorientation from a mainly longitudinal orientation at low pressure to a circumferential orientation after a significant step of pressure (Fig. 1).

I will then show how we modeled this *in vitro* blood vessel by an anisotropic viscoelastic tube which anisotropy is controlled by the actin fibers. Those last are described as nematic rods trying to align with the tension anisotropy in the tissue and generating both active and elastic stresses. With this model we were able to reproduce both the radius and the actin fibers dynamics and to explain this behavior by the coupling between the overall tissue mechanics and the cytoskeleton dynamics.

Dissociation of Red Blood Cell aggregates under Extension

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Preference: TALK

Keywords: Micro-haemodynamics, RBC aggregation, Extensional stress, Cell dynamics

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Abstract : Blood rheology and microcirculation are strongly influenced by red blood cell aggregation. We investigated and quantified the dissociation rates of red cell aggregates in extensional flow, using hyperbolic microfluidic constrictions and image analysis in coordination with a convolutional neural network (CNN). Our findings reveal that the aggregate dissociation increases sharply when a critical extension rate is reached. These critical hydrodynamic conditions, which falls within the range of microcirculatory blood flow, suggest that a large variations of aggregate sizes should be expected in-vivo microcirculation. Our project contributes to a deeper understanding of the behavior of red blood cell aggregates in response to extensional stress in microcirculatory networks. This provides crucial experimental data to validate theoretical and numerical models, and constitutes the basis for improved evaluation of blood aggregability in clinical contexts.

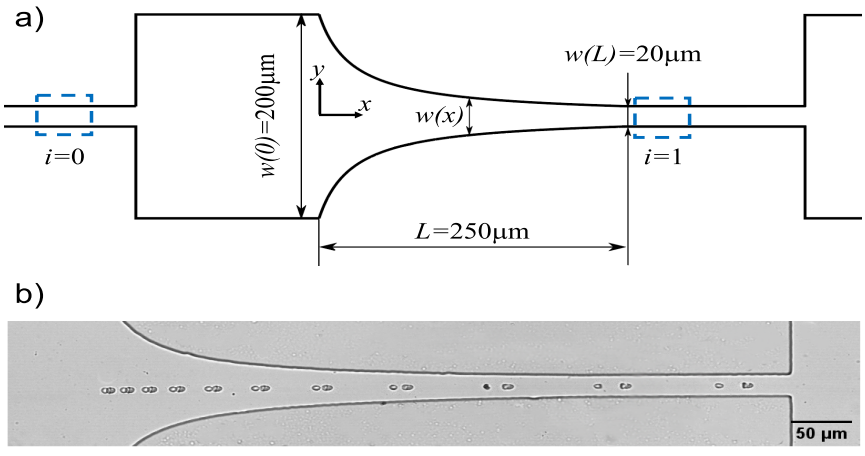

Figure 1: Hyperbolic flow geometry producing extensional flow: (a) Design of the central part of the microfluidic setup. (b) Example temporal sequence of a 3-cell aggregate breaking into $2+1$ cells in the extensional flow with $\dot{\varepsilon} = 204\ \mathrm{s}^{-1}$

Mechanical behavior of a confined yeast clog

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Preference: TALK in S1/ Rheology of biological fluids and tissues

Keywords (5 max): friction, filtration, living particles, confinement, microscale

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Abstract : (250 Words Max) Studying the filtration of biological particles involves multiple processes taking place at various spatiotemporal scales. Due to this complexity, reaching a consensus based on membrane scale studies is challenging. In this presentation, we studied the mechanical behavior of a clog of living particles based on observations at the microscale in a model configuration: we used baker's yeast *Saccharomyces cerevisiae*, with well-known mechanical and biological properties, to form clogs that were observed in microfluidic devices with well-controlled dimensions. Compression and decompression cycles were applied to the clogs, demonstrating their porous and deformable nature. The results show that the observations diverge from the poroelastic theory's predictions and conventional interpretations in the literature. A continuous model is proposed, considering the coupling between the fluid flow, the deformation of the clog (assumed to be a linear elastic material) and the friction against the device's walls. This model reproduces all observations remarkably well. Collectively these results reveal the non-trivial role of the friction between a soft porous medium and its confining environment, provide a first theoretical framework for the study of bioclogging on small scales and provide information that could contribute to improving filtration methods.

A new acoustic technique to study suspended cells viscoelasticity at long timescales

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Preference : Talk & **Rheology of biological fluids and tissues**

Keywords (5 max): Acoustic force spectroscopy (AFS), Mechanics, Cells, Active microrheology

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Abstract : (250 Words Max) There is an emerging realization that cells mechanical properties could reflect the cell state, in health and disease, and consequently many techniques have been developed to measure cell viscoelasticity, which is inherently dependent on the timescale of measurement. While several works report data at medium to short timescales (from seconds to microseconds), only very few techniques allow viscoelastic measurement at long timescales (minutes to hours). We propose to use Acoustic Force Spectroscopy (AFS) coupled to Reflection Interference Contrast Microscopy (RICM) to perform measurements on several suspended cells at the same time, in physiological conditions, without any labeling or medium modification. The AFS setup consists of a microfluidic channel with a transparent piezo on top to generate standing acoustic waves inside the channel. These acoustic waves are used to push suspended cells towards the bottom of the channel while RICM allows to determine the resulting deformation (Figure a and b). The applied acoustic forces are modulated in order to achieve active-microrheology measurements (Figure c) and the analysis of the resulting deformation amplitude allows the determination of the complex shear modulus G*(ω) of leukocytes. This method opens the way to low-frequency (10^{-3} Hz) active microrheology experiments, a regime still unexplored at the single-cell level.