

Tissue rheology: a focus on short timescales

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Physique et
Mécanique des
Milieux
Hétérogènes
UMR 7636



Contents

- Background
- Rheology of magnetized multicellular aggregates
- Mapping cell cortex rheology to tissue rheology
- *Hydra* mechanics

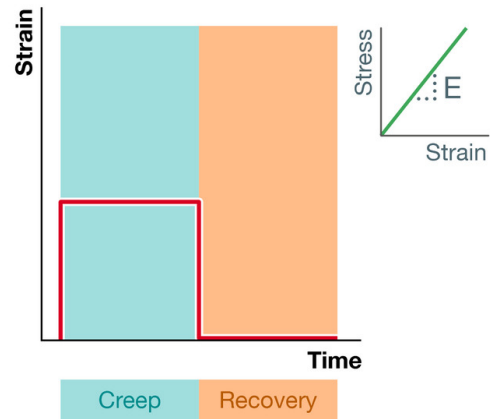
Acknowledgements

- All collaborators !
- G. Mary, F. Mazuel (MSC)
- A. Asnacios, M. Reffay (MSC)
- C. Wilhelm (PCC)
- V. Nier (PCC)
- C. Gay (MSC)
- E. Moisson (MSC)
- T. Perros, O. Cochet-Escartin (ILM)

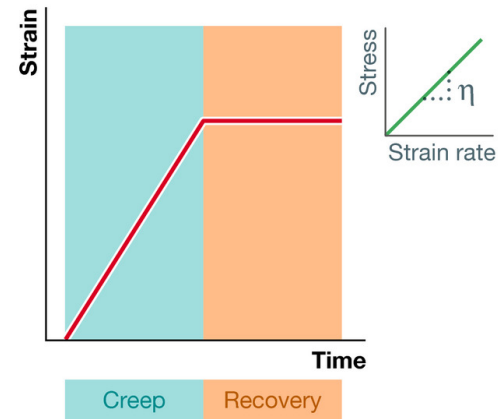
Background

Rheology: a primer

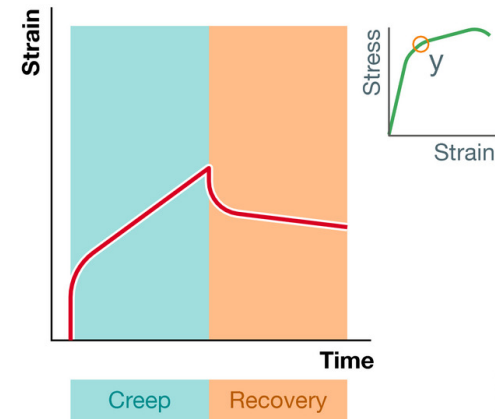
A Elastic solid



B Viscous fluid

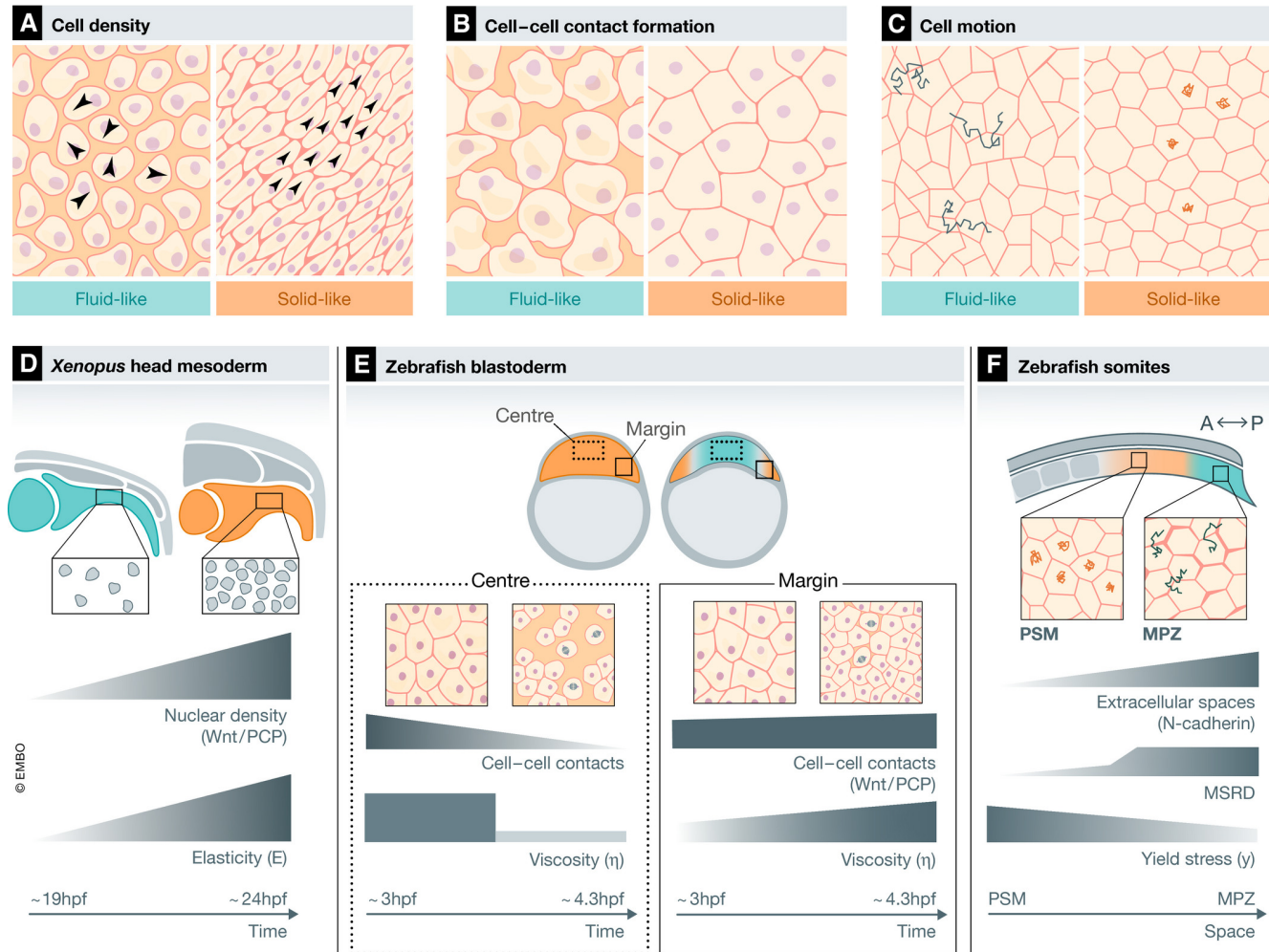


C Viscoelastic fluid



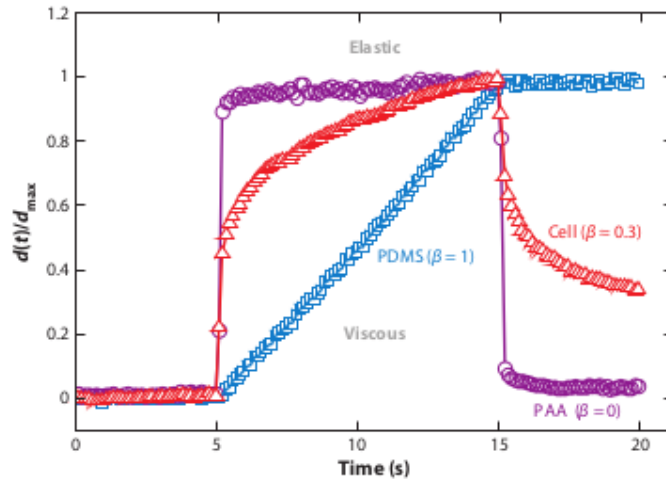
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Tissue rheology in embryonic organization



Single cell rheology

Power law rheology



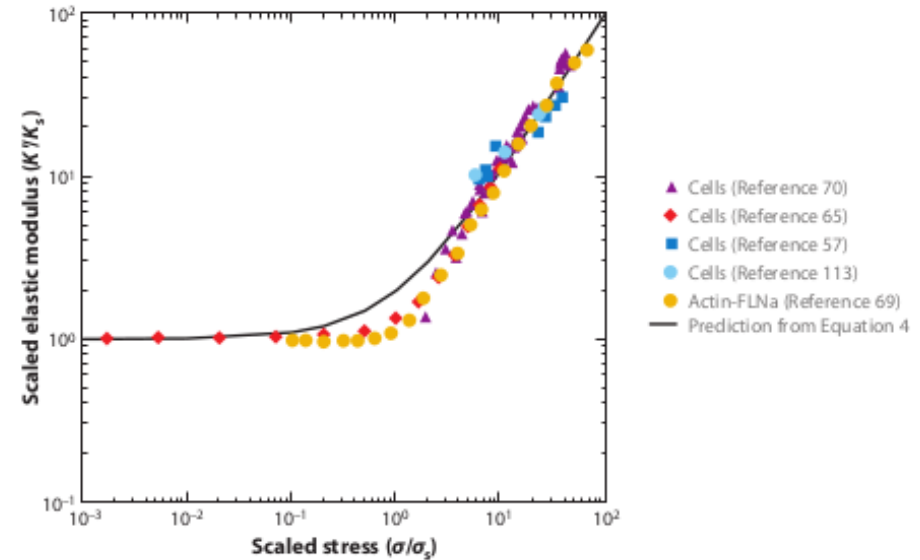
- Creep function: $J(t) = J_0 \left(\frac{t}{t_0} \right)^\beta$

where $\varepsilon(t) = \int_0^t J(t-t') \dot{\sigma}(t') dt'$

- Complex modulus: $G(\omega) = \frac{\Gamma(1-\beta)}{J_0} (i\omega t_0)^\beta$

where $\sigma(t) = \int_0^t G(t-t') \dot{\varepsilon}(t') dt'$

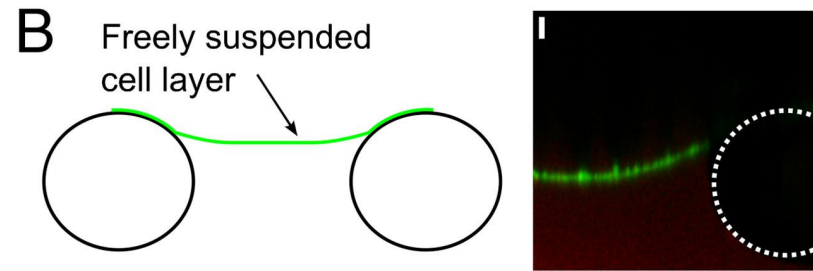
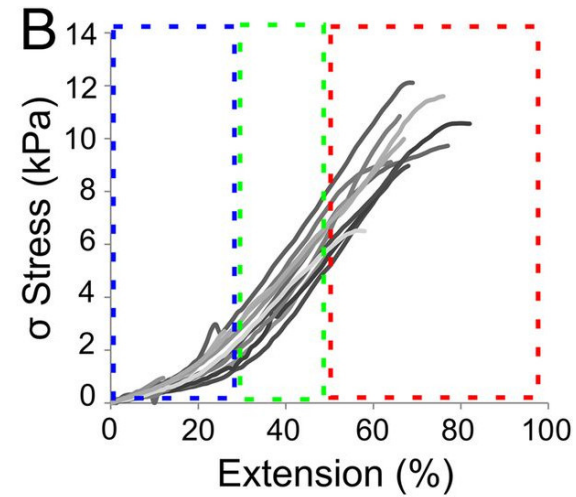
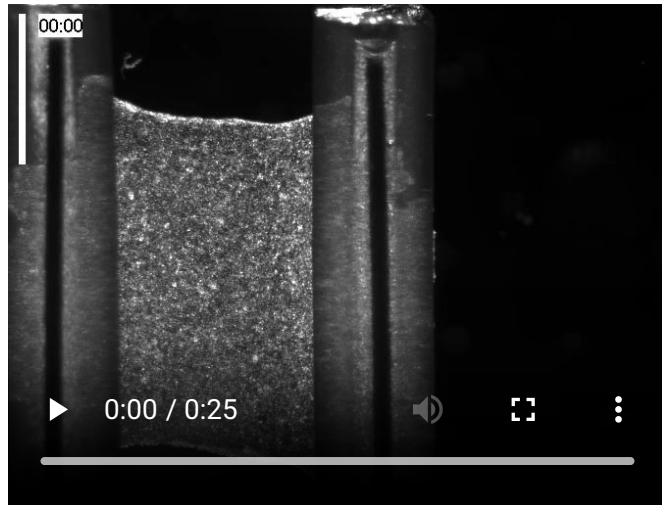
Stress stiffening



- Differential elastic modulus K'
- Black line: $K'(\sigma) = K'_0 + a\sigma$

Suspended cell monolayers: tensile testing

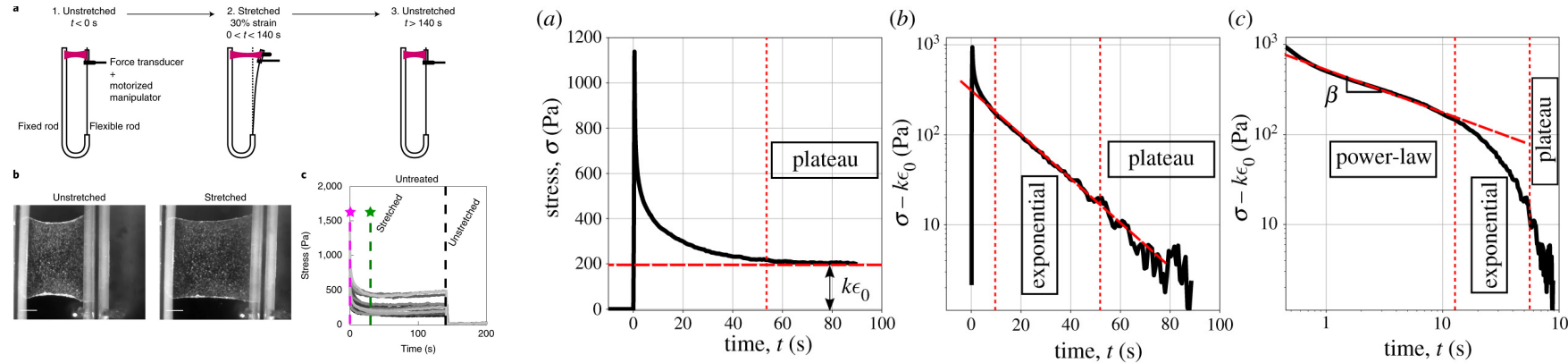
Madin Darby Canine Kidney (MDCK) cells



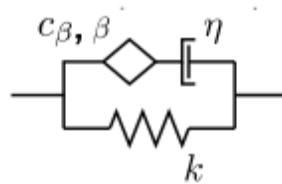
- Stress-extension curve
- $E \sim 10^4 \text{ Pa}$

Suspended cell monolayers: stress relaxation

Madin Darby Canine Kidney (MDCK) cells



Fractional viscoelastic model



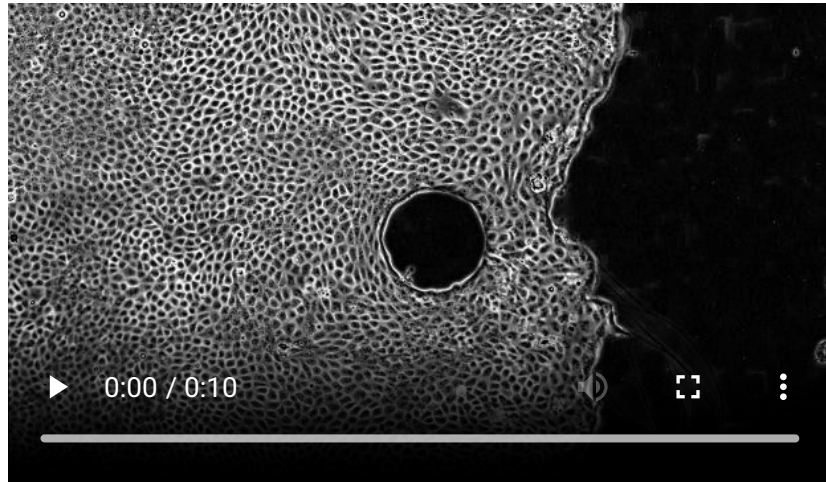
Fractional element \diamond :

$$\sigma(t) = c_\beta \frac{d^\beta \epsilon}{dt^\beta} = \frac{c_\beta}{\Gamma(1-\beta)} \int_0^t (t-t')^{-\beta} \frac{d\epsilon(t')}{dt'} dt'$$

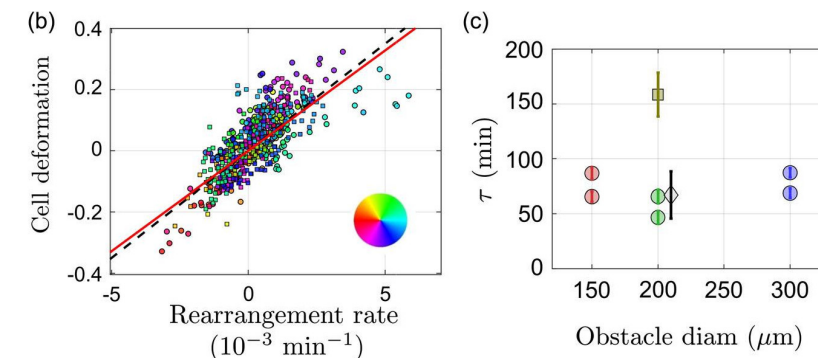
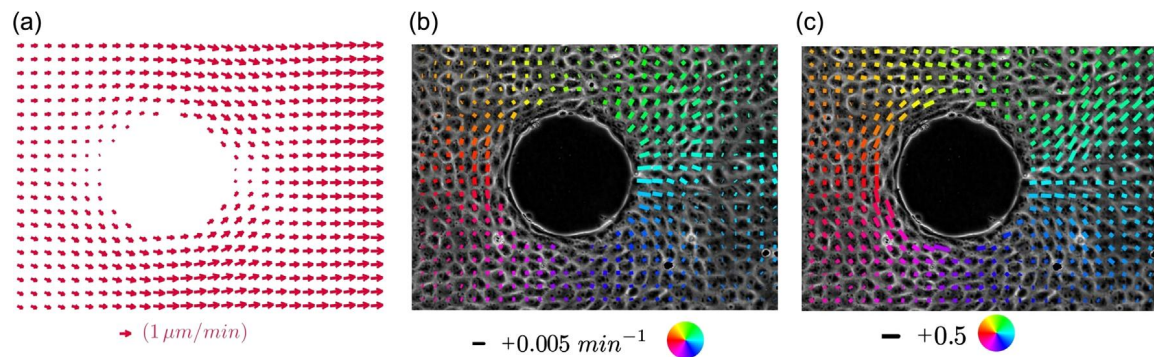
- $k \sim 10^3$ Pa
- $\eta \sim 10^4$ Pa s
- $\beta \sim 0.3$
- $c_\beta \sim 10^3$ Pa s $^\beta$
- $\tau = \left(\frac{\eta}{c_\beta}\right)^{\frac{1}{1-\beta}} \sim 10$ s

Cell monolayers: Stokes experiment

Madin Darby Canine Kidney (MDCK) cells



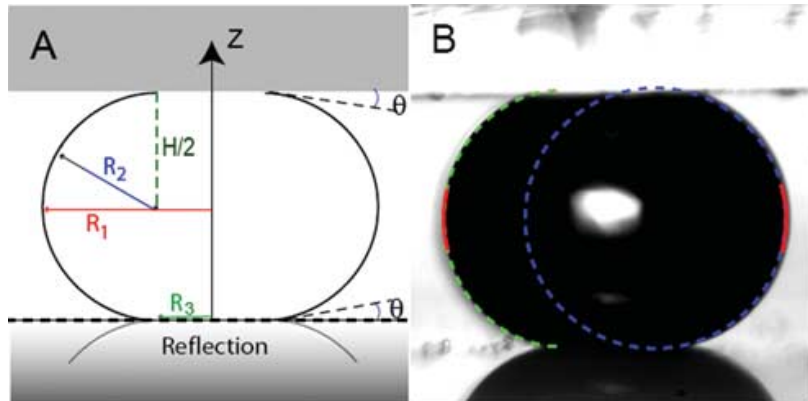
- Cell divisions blocked
- Strain rate $\vec{\nabla}\vec{v}$, cell deformation ϵ_e
- Rearrangement rate $\text{dev}\dot{\epsilon}_r = \text{dev}\vec{\nabla}\vec{v} - \text{dev}\dot{\epsilon}_e$
- Maxwell model: $E \text{dev}\epsilon_e = \eta \text{dev}\dot{\epsilon}_r$
- Viscoelastic time $\tau = \frac{\eta}{E} \sim 1 \text{ h}$



Cell spheroids

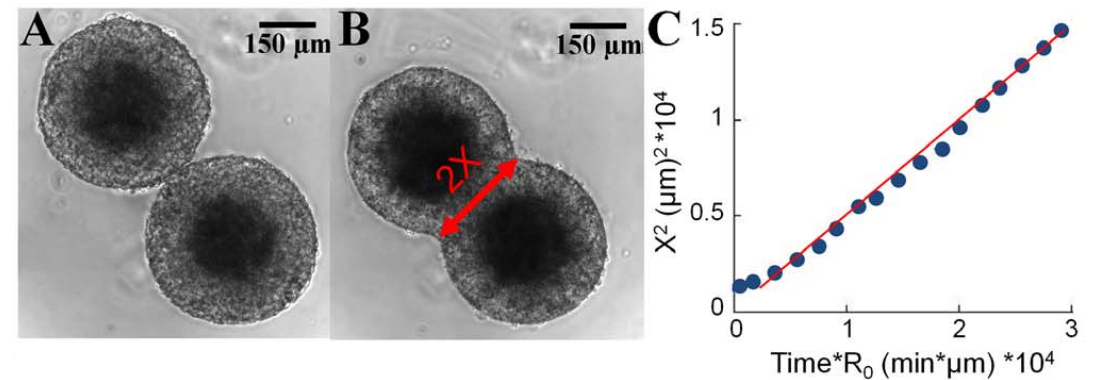
Mouse embryonic carcinoma F9 cells

Surface tension γ



- Parallel plate compression
- $F = L\gamma$, geometrical factor L
- $\gamma \sim 10^{-3} \text{ N/m}$

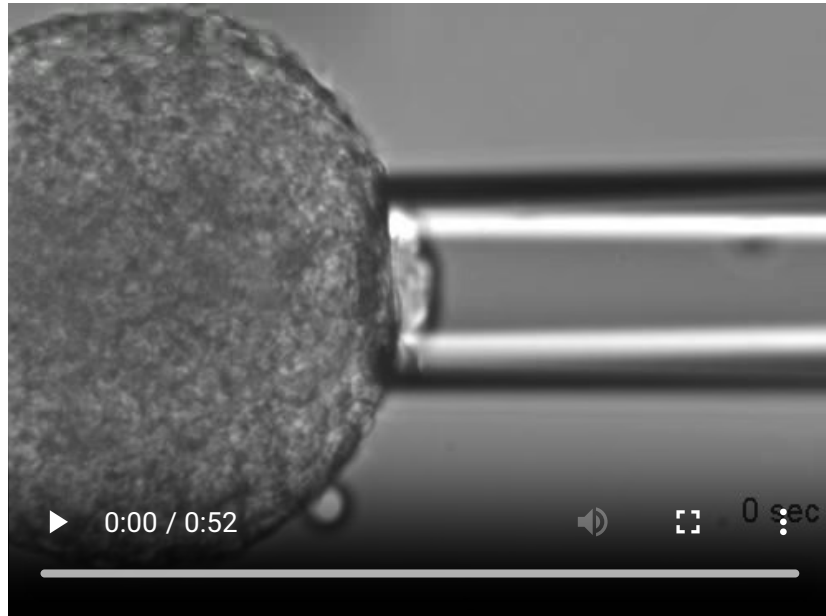
Viscosity η



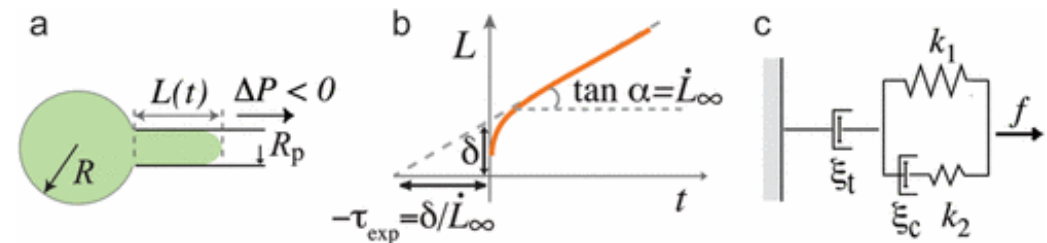
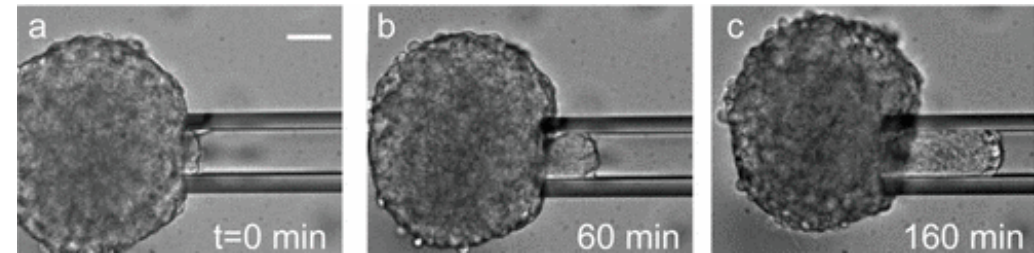
- Fusion experiments
- $X^2(t) = \frac{\gamma}{\eta} R_0 t$
- $\eta \sim 10^5 \text{ Pa s}$

Micropipette aspiration of multicellular aggregates

Murine sarcoma S180 cells



- $\Delta p = 1500 \text{ Pa}$
- $R_0 = 150 \mu\text{m}$
- $R_p = 25 \mu\text{m}$
- Aspiration then retraction

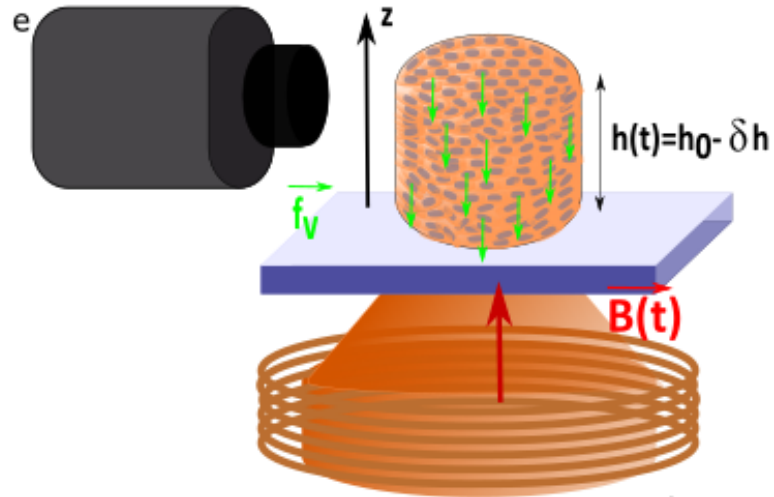


- Modified Maxwell model
- $E \sim 10^3 \text{ Pa}$
- $\eta \sim 10^5 \text{ Pa s}$

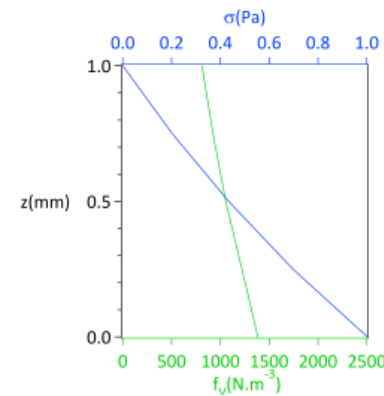
Rheology of multicellular aggregates

G. Mary, F. Mazuel, V. Nier, F. Fage, I. Nagle, L. Devaud, J.-C. Bacri, S. Asnacios, A. Asnacios, C. Gay, P. Marcq, C. Wilhelm, and M. Reffay, *All-in-one rheometry and non-linear rheology of multicellular aggregates*, Phys. Rev. E **105** 054407 2022

Magnetic rheometer

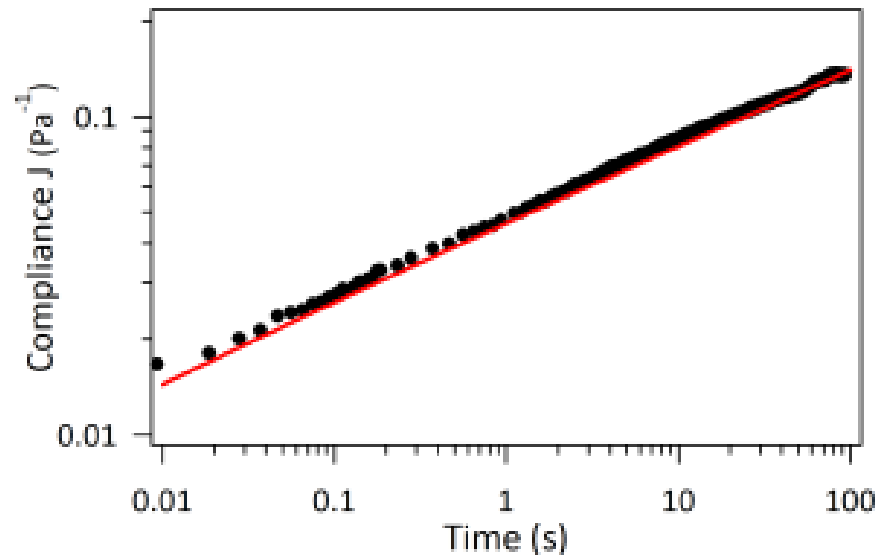


- Force f_V approximately constant
- Local stress $\sigma_{loc}(z) = \int_z^{h(t)} f_V(z') dz'$
- Average stress $\sigma \equiv \langle \sigma_{loc}(z, t) \rangle_z \approx \frac{1}{2} h_0 f_V$



Power-law rheology

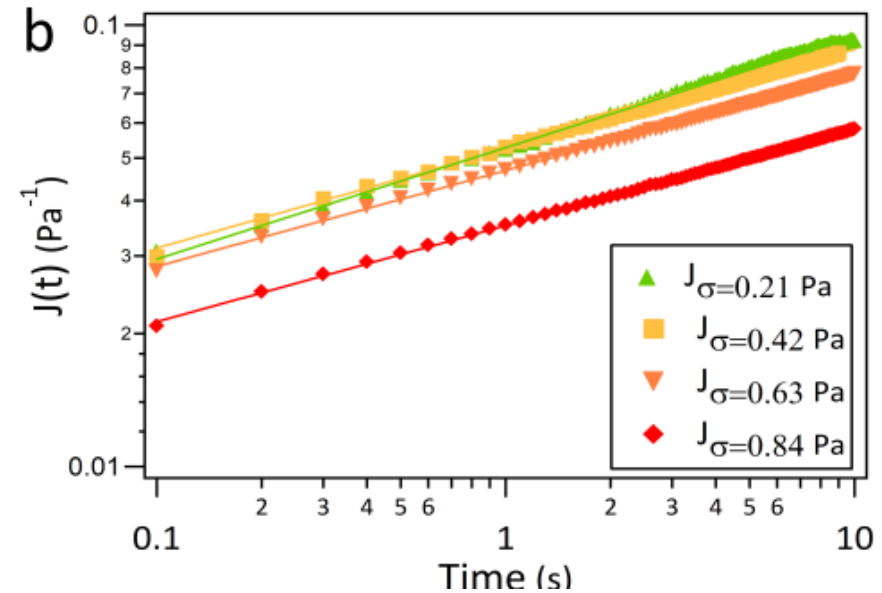
Creep function



- Force step signals

- Creep function $J(t) = J_0 \left(\frac{t}{t_0}\right)^\beta$

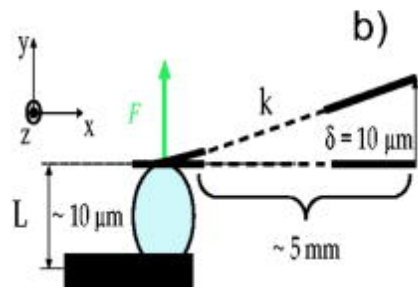
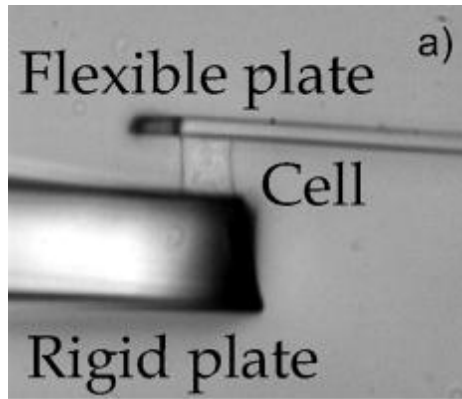
Stress-stiffening



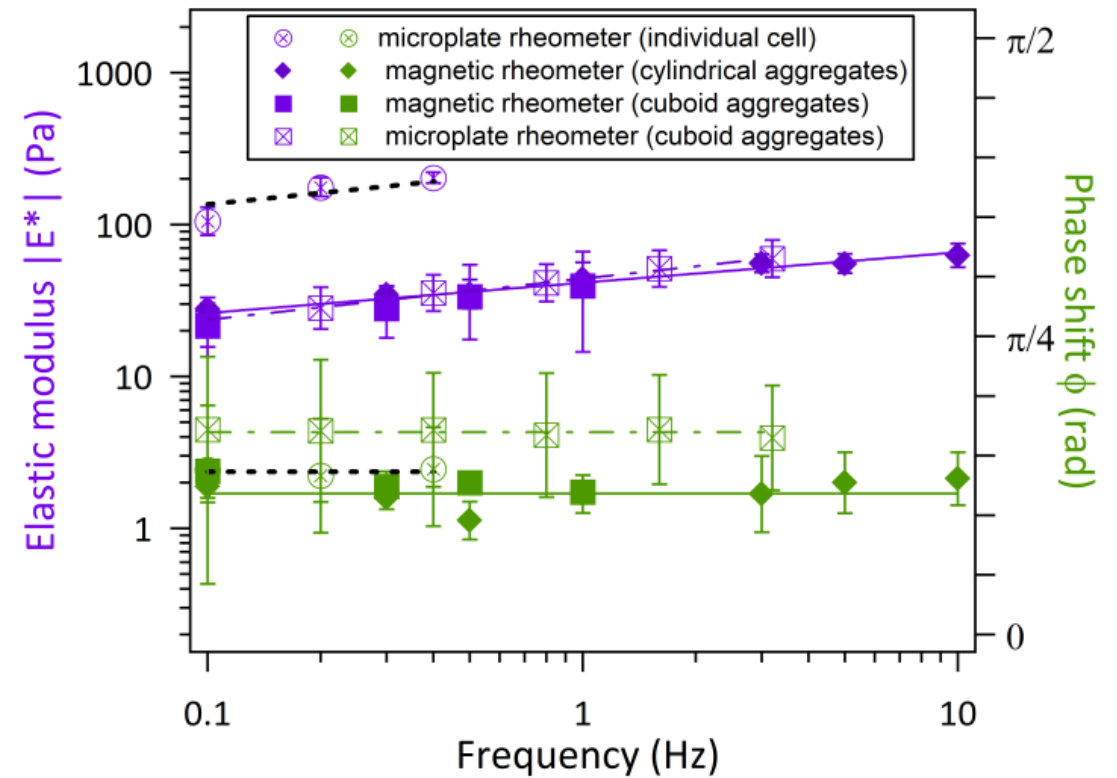
- $\beta \sim 0.25$
- J_0 decreases with the applied stress

Comparison with parallel plate rheology and single cell rheology

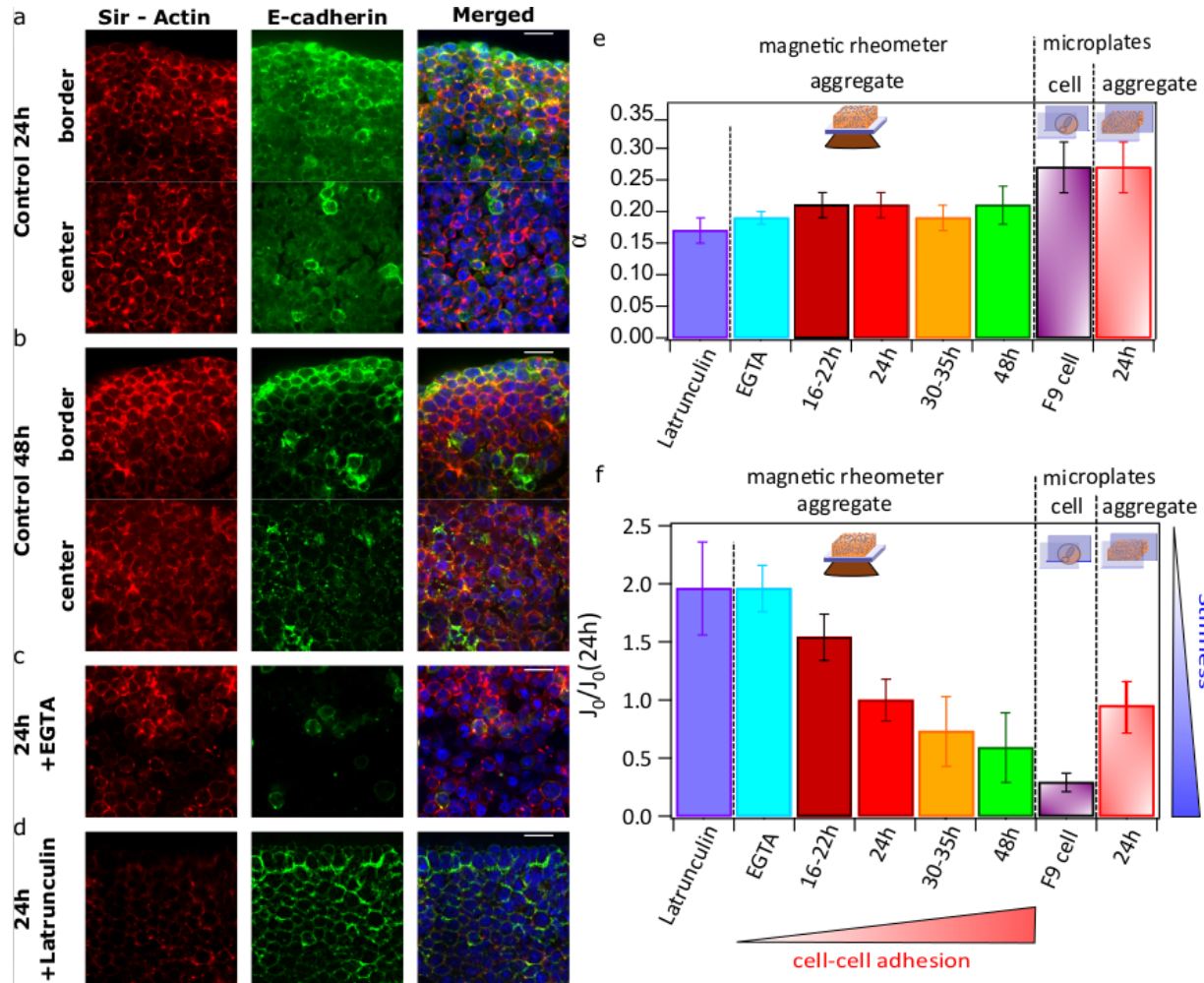
Parallel plate rheometer



Harmonic regime



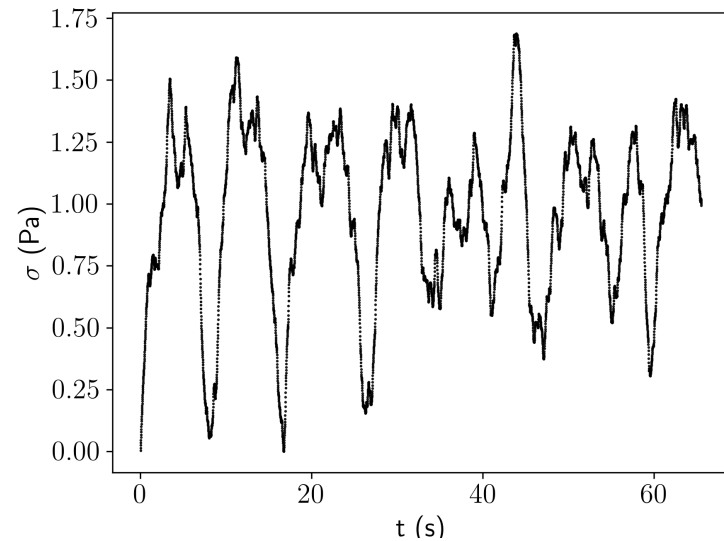
Contribution of cell-cell contacts and cytoskeleton to the rheology



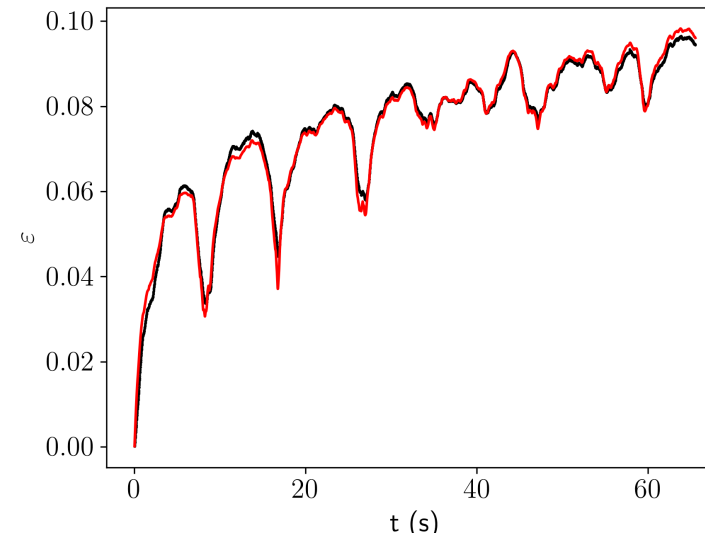
Broad-spectrum rheology

$$\sigma(t) = \sigma_0 \tanh(\lambda t) \sum_{i=1}^n \rho_i \cos(2\pi f_i t + \phi_i)$$

Applied stress signal



Measured deformation and fit

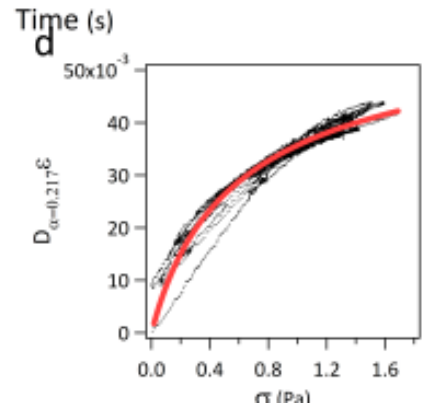
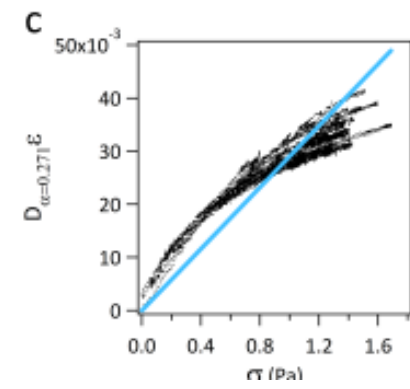
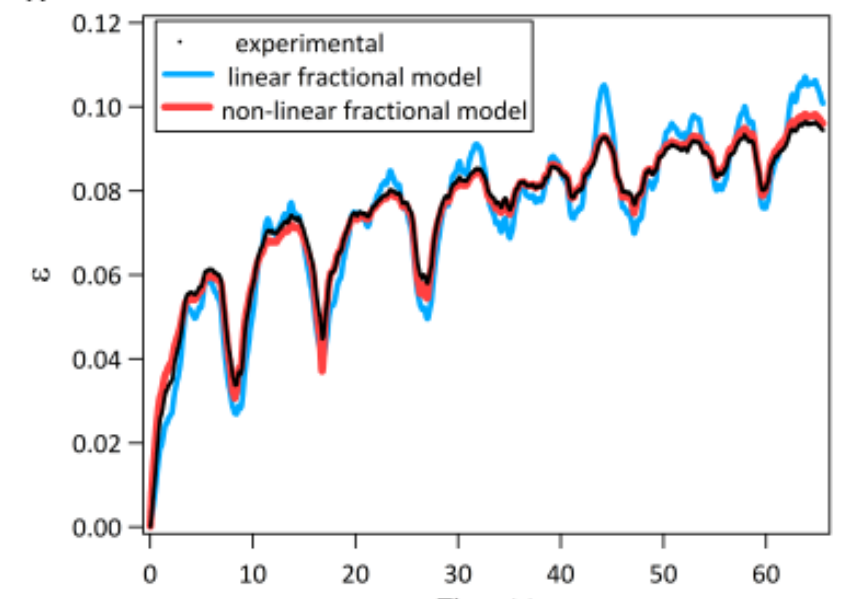


- Black: Data
- Red: Fit (maximum likelihood)

Nonlinear fractional rheology

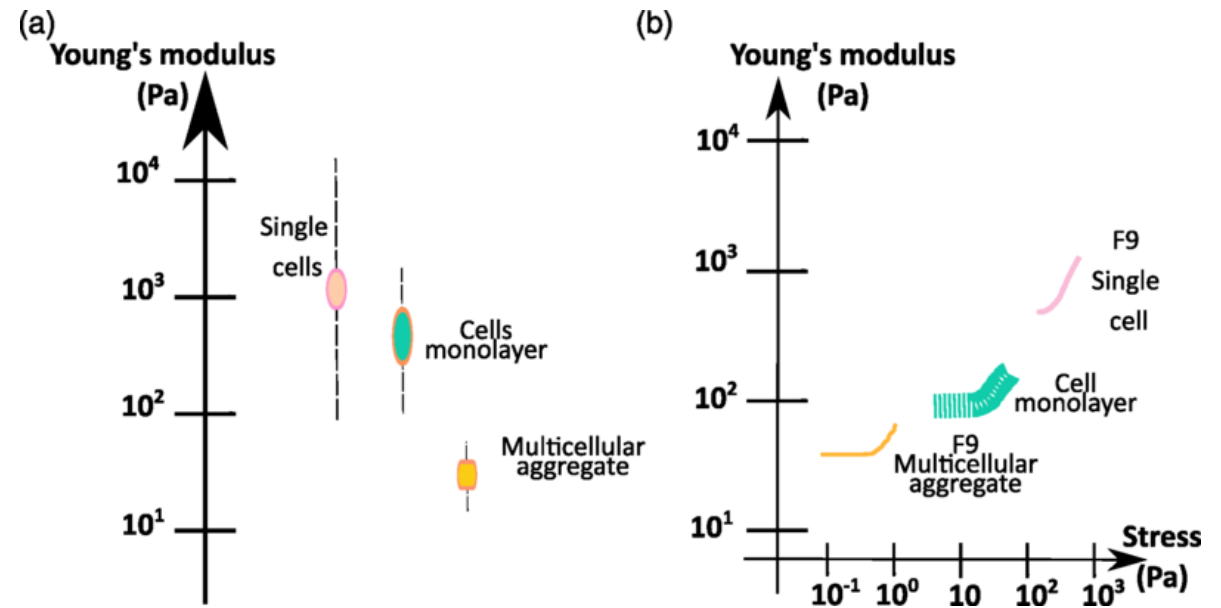
Best fit:
$$\sigma = c_\beta \left(1 + \frac{\sigma}{\sigma_0} \right) \frac{d^\beta \epsilon}{dt^\beta}$$

- $\beta \sim 0.2$
- $\sigma_0 \sim 0.5 \text{ Pa}$
- $c_\beta \sim 10 \text{ Pas}^\beta$



Summary

- Nonlinear fractional rheology
- Stress-stiffening
- Similarities across scales
- How to bridge the gap between cell scale and tissue scale?



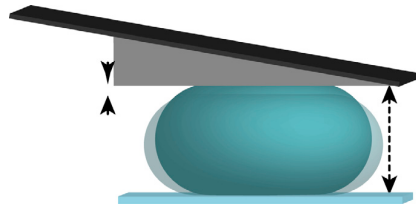
Mapping cell cortex rheology to tissue rheology

E. Moision, P. Seez, F. Molino, P. Marcq and C. Gay,

Mapping cell cortex rheology to tissue rheology, and vice-versa, Phys. Rev. E **106** 034403 2022

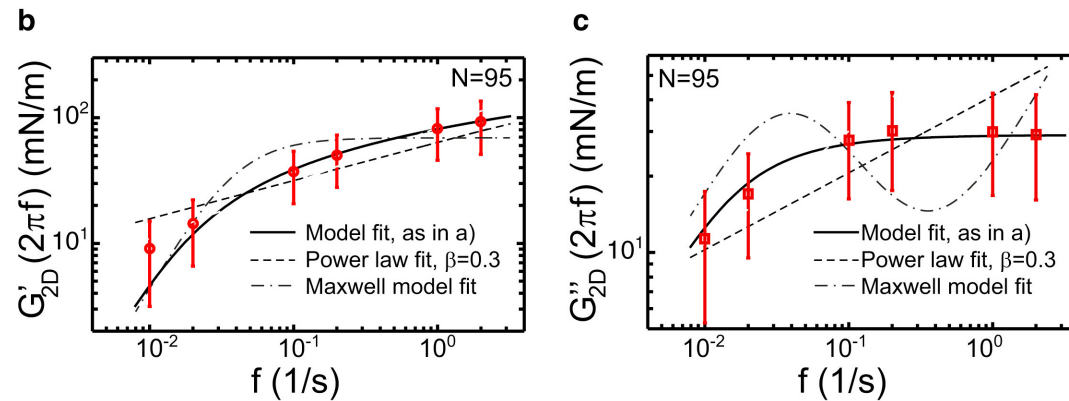
Cell cortex rheology

Viscoelastic cell stiffness is dominated by the cortical layer



Compression plate experiment

HeLa cells



Broad relaxation spectrum model

- Rest tension σ_0
- $\sigma_{2D}(t) = \sigma_0 + \int_{-\infty}^t G_{2D}(t-t') \dot{\epsilon}_{2D}(t') dt'$
- $G_{2D}(t) = \int_0^{\infty} h(t') e^{-t/t'} d \ln t'$
- $h(t') = K_h, t \leq t_{\max}$
- $h(t') = 0, t > t_{\max}$
- Cut-off t_{\max}
- Amplitude K_h

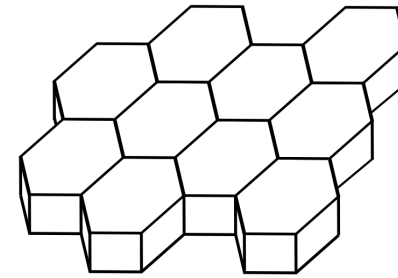
Planar cell monolayer

Cell cortex rheology (2D)

$$\begin{aligned}\text{dev}(\sigma) &= 2g \text{dev}(\varepsilon_{\text{face}}) \\ \frac{1}{2} \text{trace}(\sigma) &= \sigma_0 + k \text{trace}(\varepsilon_{\text{face}})\end{aligned}$$

- Rest tension σ_0
- $k(\omega)$ measured experimentally
- Assume $\nu_c = \text{constant} \rightarrow$ deduce $g(\omega)$

Hexagonal tiling of the plane



- Ordered lattice
- Incompressible material
- Homogeneous and affine deformations
 - Planar faces
- Distinguish horizontal (H) and lateral (L) faces
- Tension aspect ratio $\Psi = \frac{\sigma_{0H}}{\sigma_{0L}}$

Analytical procedure

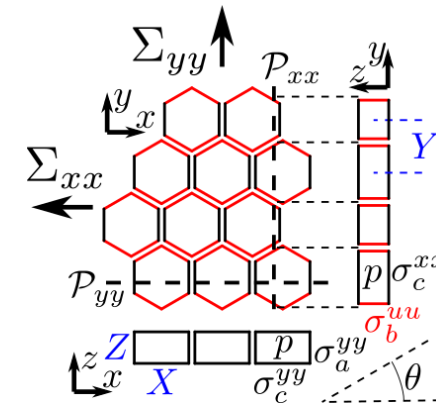
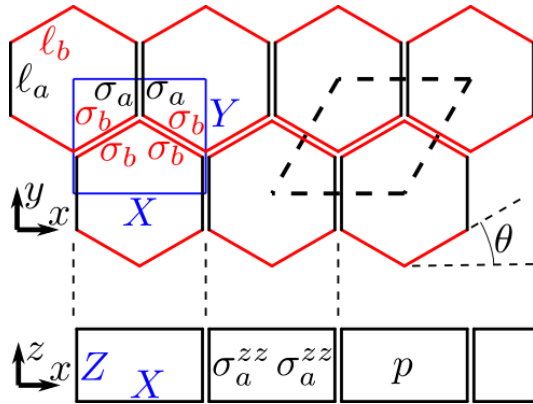
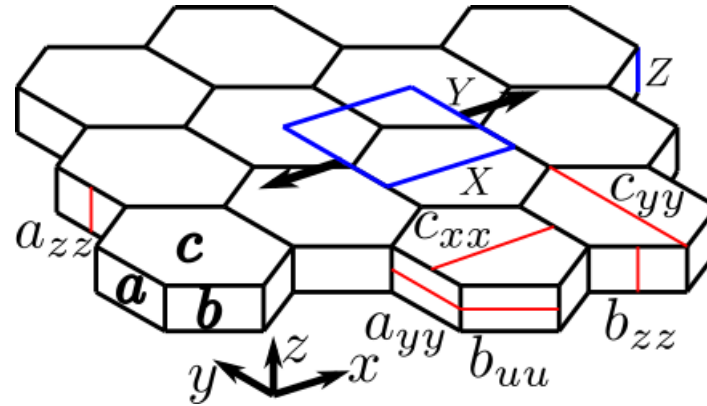
- Define microscopic stress components σ_{ij}
- Define macroscopic stress components Σ_{ij} (2D)
- Express force balance at vertices
- Compute the monolayer rest state (e.g. $\Psi = \frac{Z_0}{X_0}$)
- Expand variables to first order close to the rest state
- Imposing a stretch on the x axis, solve the resulting system of equations to linear order
- Compute the Young's modulus E and Poisson ratio ν :

$$E = \frac{\Sigma_{xx}}{\epsilon_x} \quad \nu = -\frac{\epsilon_y}{\epsilon_x}$$

- Deduce the shear and compression moduli (G, K):

$$G = \frac{E}{2(1 + \nu)} \quad K = \frac{E}{2(1 - \nu)}$$

Schematics



Micro-rheology \rightarrow macro-rheology

Moduli

$$K = 3\sigma_{0H} + 2k_H + \Psi k_L + 9\Psi g_L$$

$$G = \sigma_{0H} + 2g_H + \frac{1}{\frac{1}{2\sigma_{0H}} + \frac{1}{2\Psi k_L + 2\Psi g_L}}$$

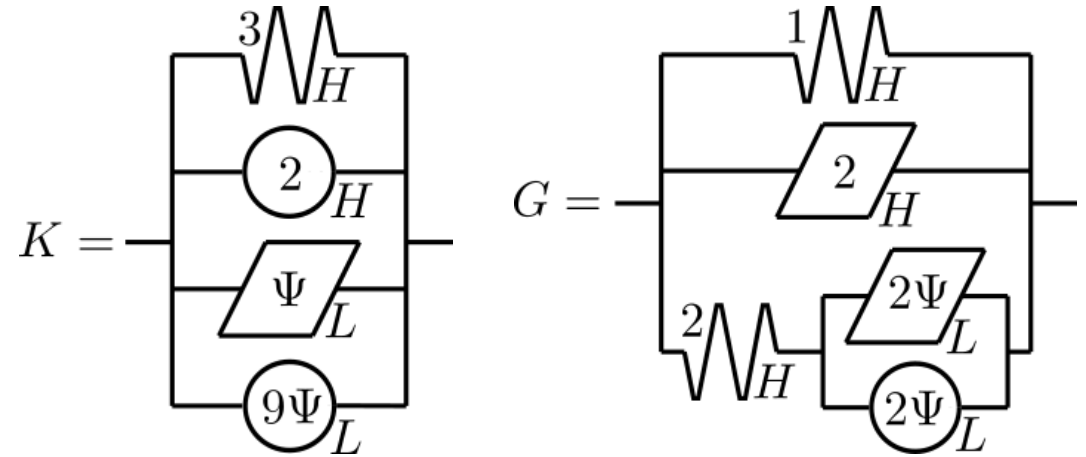
Low frequency limit

$$K_0 = 3\sigma_{0H}$$

$$G_0 = \sigma_{0H}$$

Low frequency moduli:

- are real \Rightarrow elastic behaviour
- are determined by the cortex rest tension of horizontal faces



Micro-rheology → macro-rheology

Low frequency limit: elastic material

$$K_0 = 3\sigma_{0H}$$

$$G_0 = \sigma_{0H}$$

High frequency limit

$$K_\infty \sim 2k_H + \Psi k_L + 9\Psi g_L$$

$$G_\infty \sim 2g_H$$

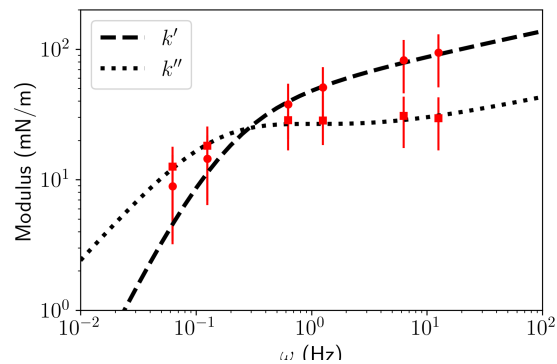
Moduli

$$K = 3\sigma_{0H} + 2k_H + \Psi k_L + 9\Psi g_L$$

$$G = \sigma_{0H} + 2g_H + \frac{1}{\frac{1}{2\sigma_{0H}} + \frac{1}{2\Psi k_L + 2\Psi g_L}}$$

The high-frequency tissue rheological behaviour is analogous to the high-frequency cortex rheological behaviour !

Cell cortex rheology



Alternative visco-fractional model

$$G_{2D}(\omega) = \frac{1}{\frac{1}{i\omega\eta} + \frac{1}{c_\beta(i\omega)^\beta}}$$

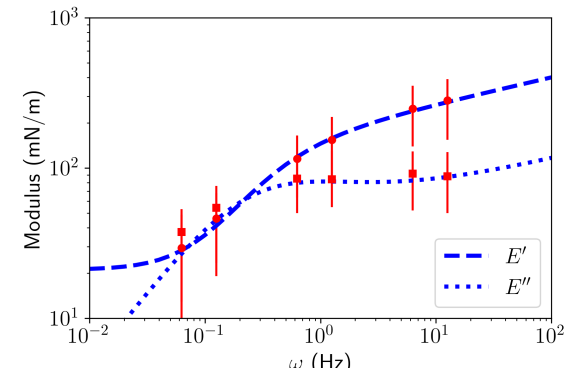
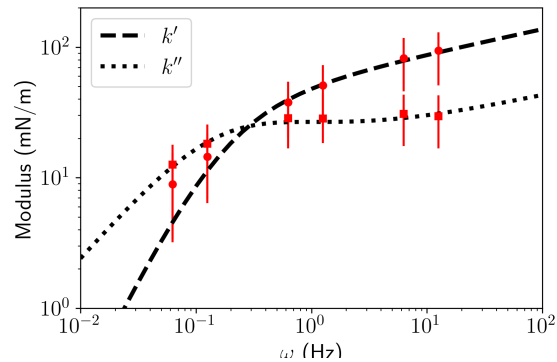
- $\beta = 0.19$
- $c_\beta \sim 10^{-2} \text{Ns}^{-\beta} \text{m}^{-1}$
- $\eta \sim 10^{-1} \text{Ns m}^{-1}$

Visco-fractional rheology is invariant under this mapping

Cortex rheology



Tissue rheology



Cortex parameters (HeLa cells)

- $\beta^c = 0.19$
- $c_\beta^c = 60 \text{ mN s}^{-\beta} \text{ m}^{-1}$
- $\eta^c = 250 \text{ mN s m}^{-1}$

Not measured for MDCK cell cortex

Tissue parameters

- $\beta^T = 0.19$
- $c_\beta^T = 170 \text{ mN s}^{-\beta} \text{ m}^{-1}$
- $\eta^T = 500 \text{ mN s m}^{-1}$

Not measured for HeLa monolayer

Perspectives

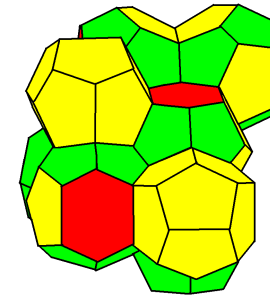
We hope that this work will foster more experimental work, measuring both cortex and tissue rheology.

2D cell aggregates

- Relax model hypotheses?
 - Curved faces
 - Compressible material

3D cell aggregates

- Use regular Weaire-Phelan tiling of 3D space?



Numerical simulations

- Large deformations/nonlinear rheology
- Disordered lattice
- Boundary effects

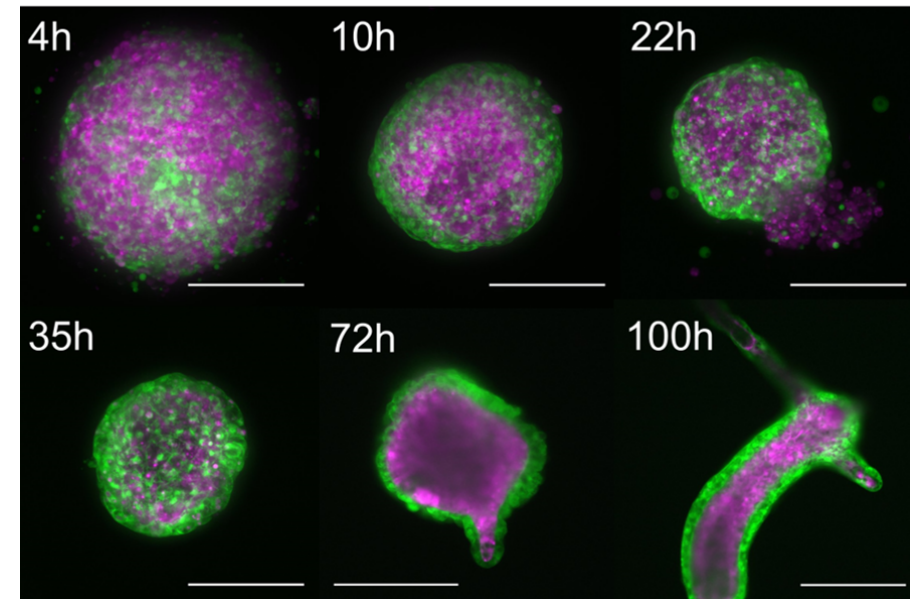
Hydra mechanics

Hydra regeneration

From a piece of adult tissue

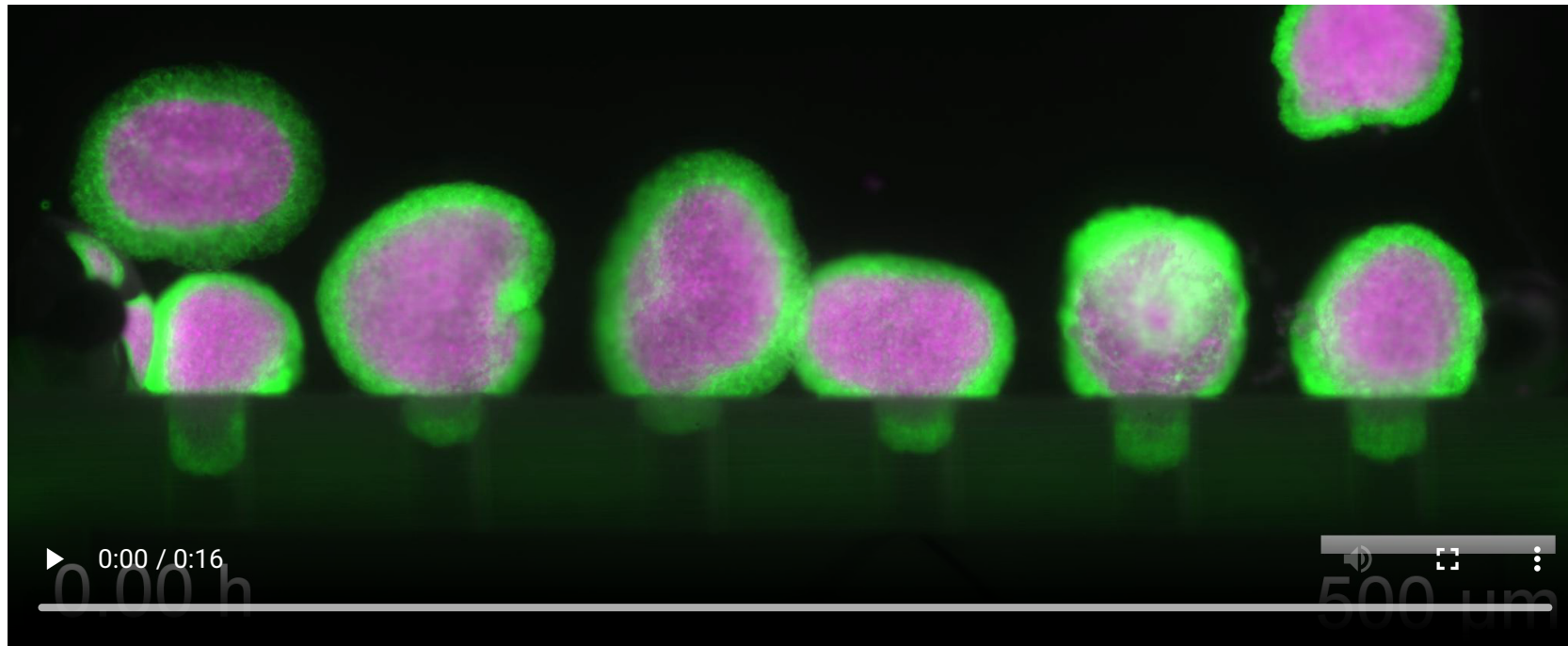


From a mixed cell aggregate



- **Green:** Ectoderm
- **Magenta:** Endoderm

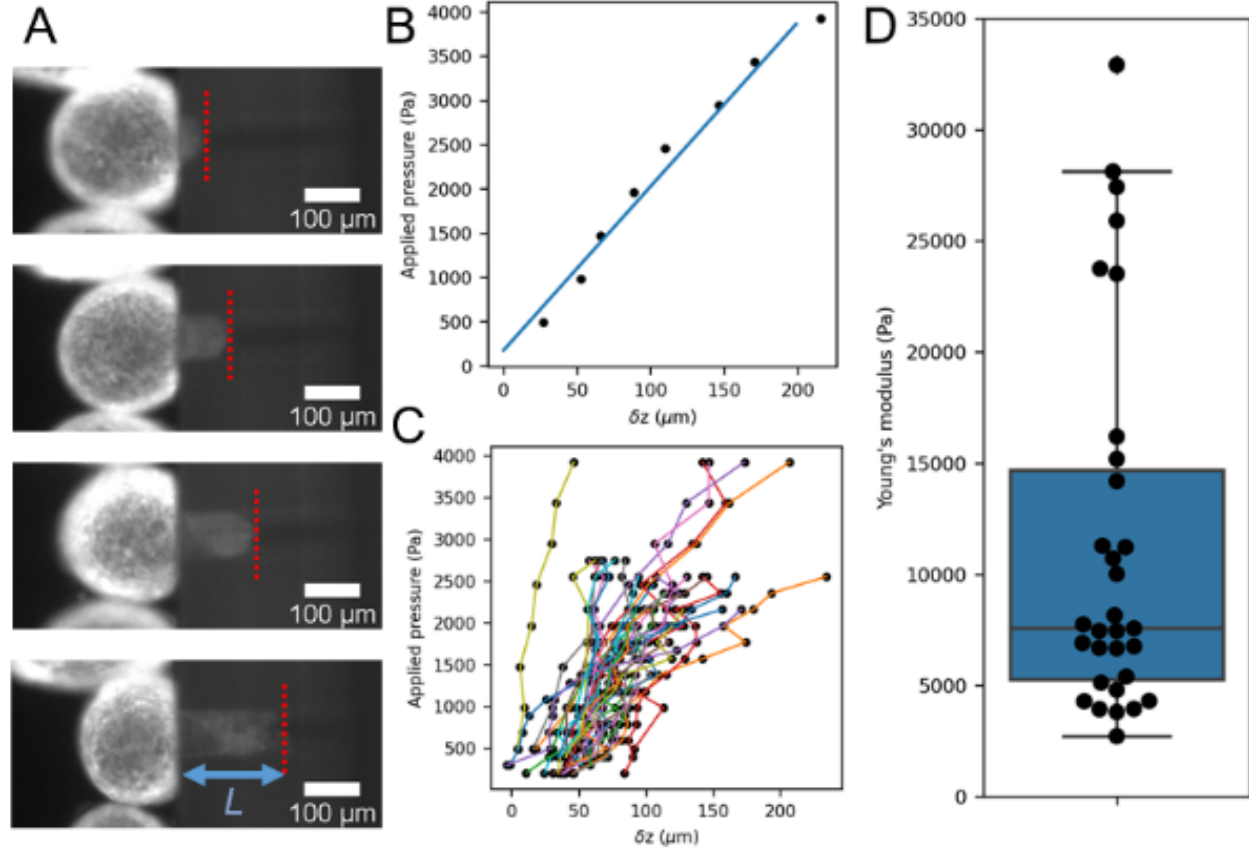
Parallelized micropipette aspiration of *Hydra* spheres



- $\Delta p \sim 10^3 \text{ Pa}$
- $R_0 \sim 200 \mu\text{m}$
- $R_p = 50 \mu\text{m}$

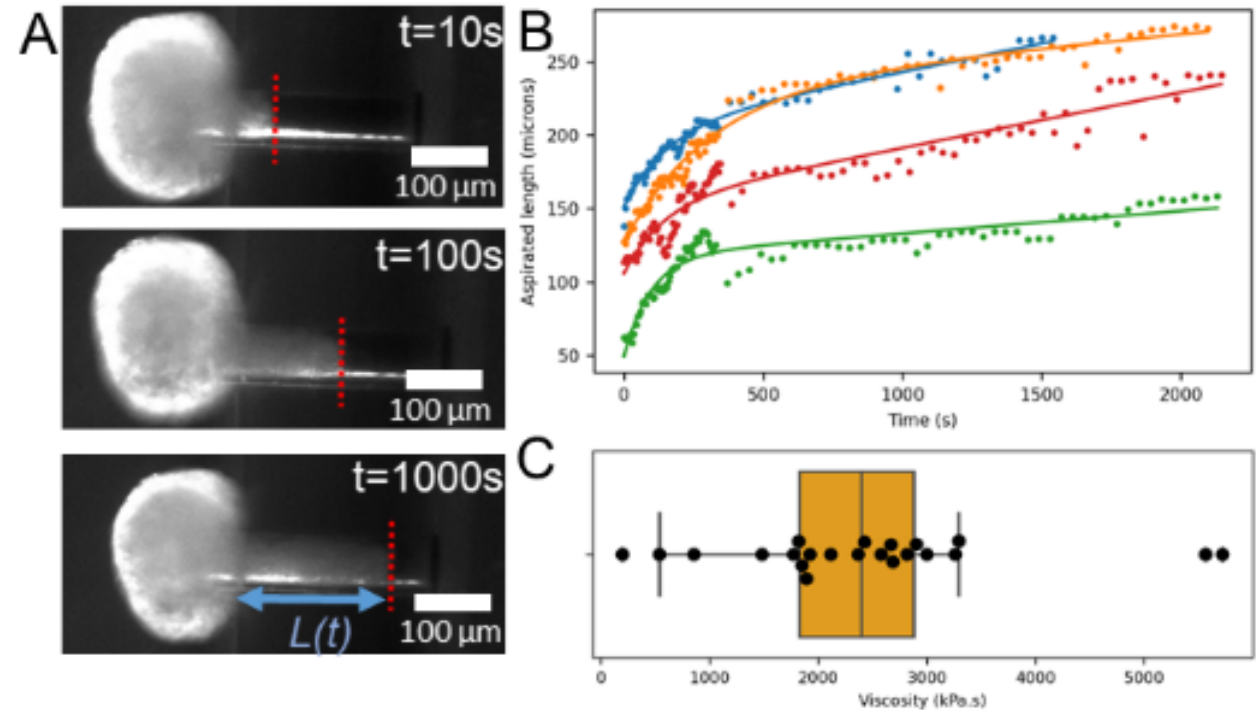
Hydra elasticity

- "Low" applied pressure
- Elastic shell of thickness h
- Initial radius R_0
- $\Delta p = C_E \frac{2Eh}{(1-\nu)R_0^2} \delta z$
- Young's modulus $E \sim 10^4 \text{ Pa}$

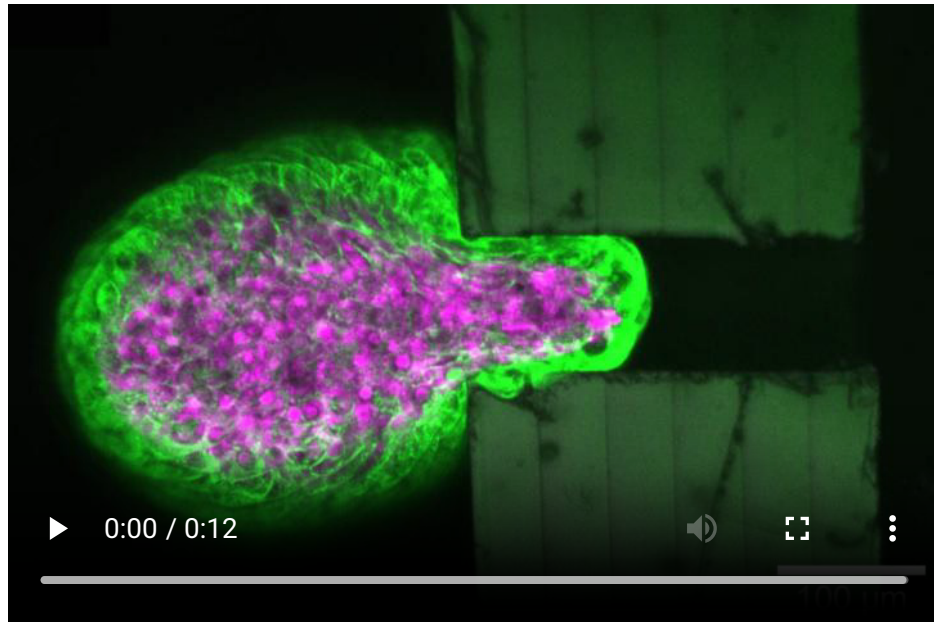


Hydra viscosity

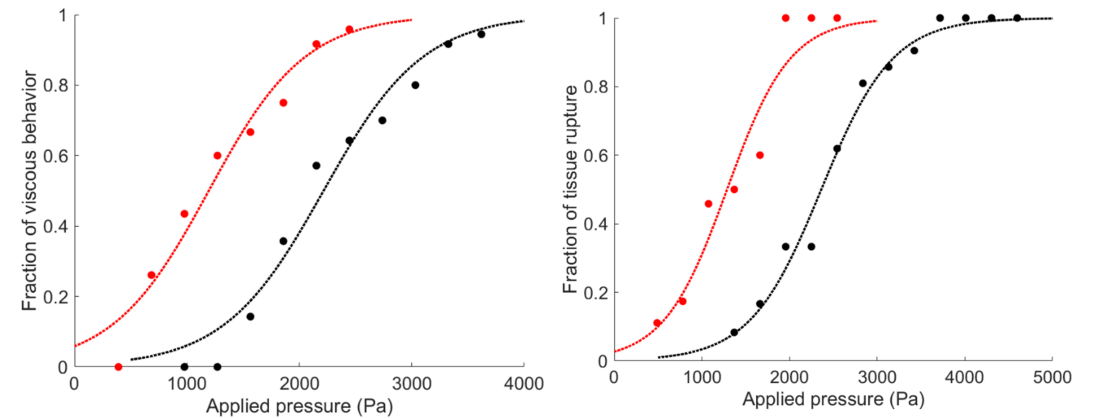
- "High" applied pressure
- Modified Maxwell model
- Flow at constant velocity U when $t \gg \tau$
- Micropipette radius R_p
- $\Delta p = C_\eta \eta \frac{U}{R_p}$
- Shear viscosity $\eta \sim 10^6 \text{ Pa}\cdot\text{s}$



Elastic \rightarrow viscoelastic \rightarrow rupture



- $\Delta p = 3 \text{ kPa}$
- Green: Ectoderm
- Magenta: Endoderm



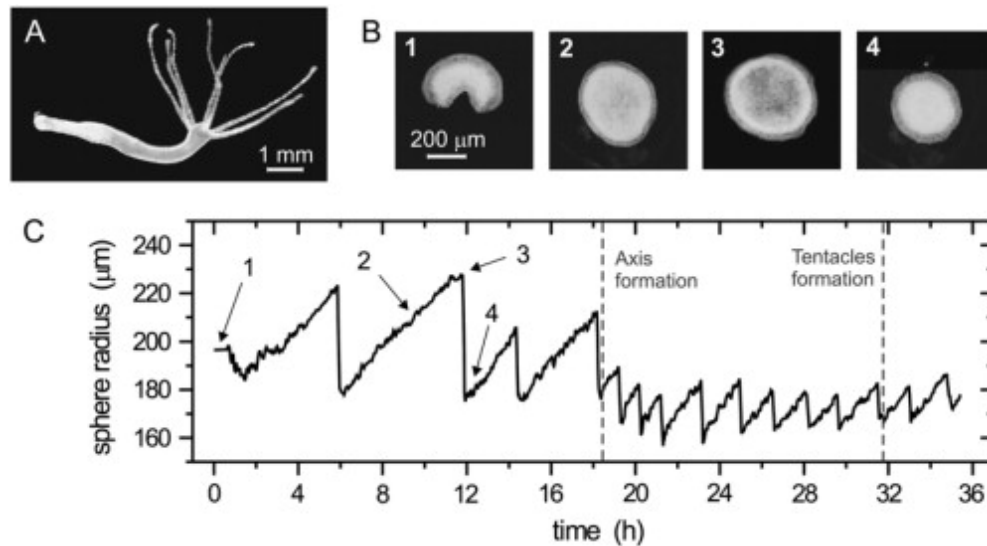
- Black: WT
- Red: EDTA, weaker cell-cell adhesion

Thresholds:

- to viscoelastic behavior: $\Delta p_c = 2.22 \pm 0.14 \text{ kPa}$
- to rupture: $\Delta p_c = 2.37 \pm 0.08 \text{ kPa}$

Early regeneration

Osmotic oscillations



- $\frac{\delta R}{R_0} \sim 0.3$
- $\Delta p = \frac{2Eh}{(1-\nu)R_0} \frac{\delta R}{R_0} \simeq 10^3 \text{ Pa}$
- Elastic regime !

Perspective

Hydra Mechanics

- How to measure the Poisson ratio ν ?
- How to go beyond scaling relationships when evaluating E and η ?
- Investigate the biomechanics of rupture?

Establishment of head-foot axis

- First symmetry-breaking during regeneration
- Molecular players?
- Role of osmotic oscillations?
- Relevance of mechano-chemical coupling?

Merci / Thank you