Tissue rheology:
a focus on short timescales

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Hétérogènes
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Contents

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

Acknowledgements

- All collaborators !
- G. Mary, F. Mazuel (MSC)
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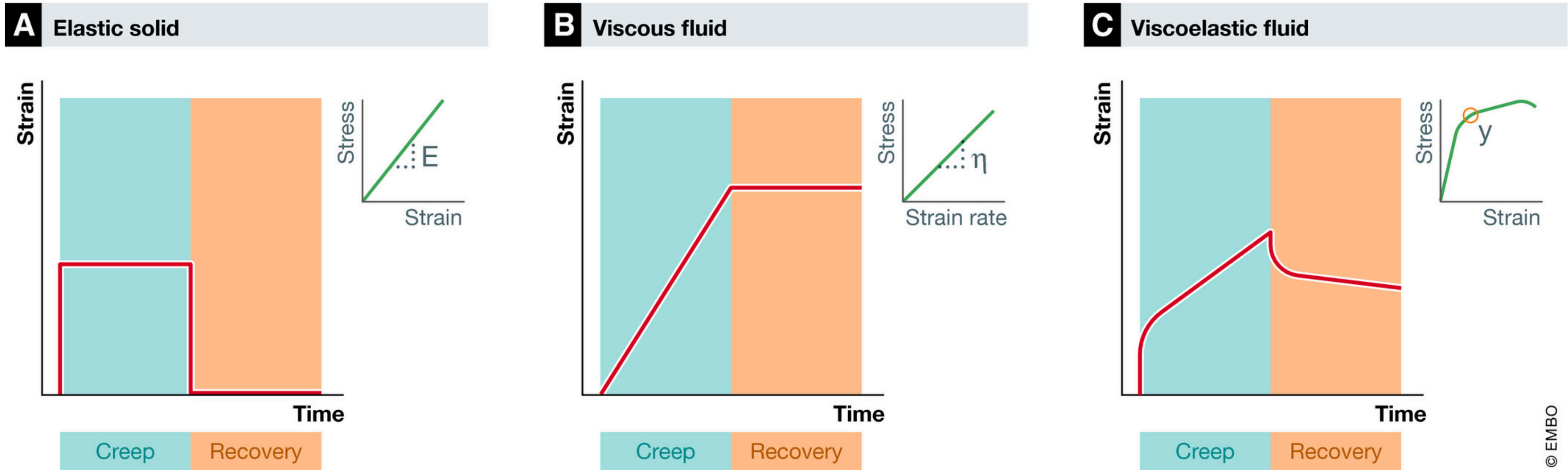
- V. Nier (PCC)
- C. Gay (MSC)
- E. Moisdon (MSC)
- T. Perros, O. Cochet-Escartin (ILM)

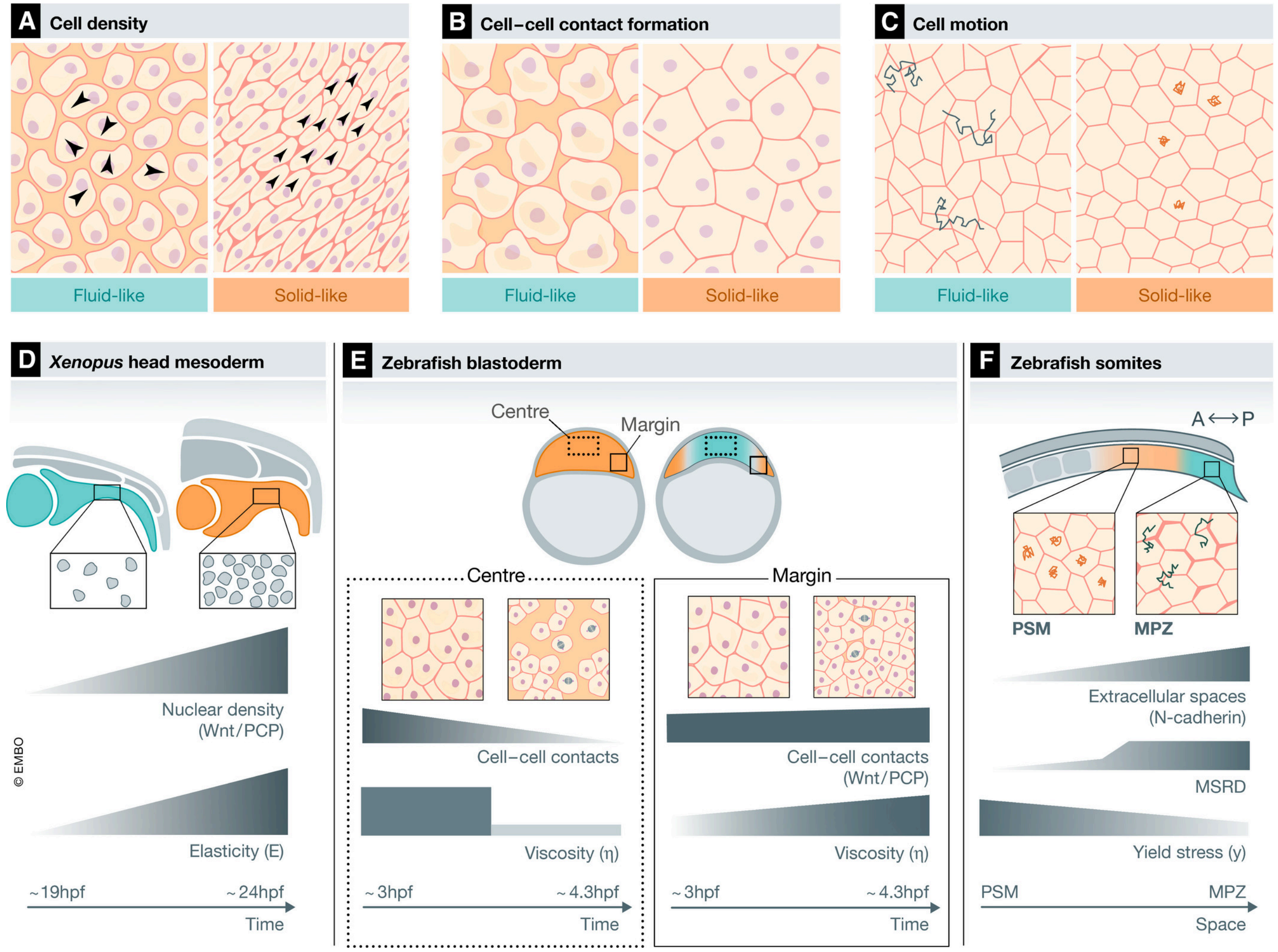
Background

Rheology: a primer



N. Petridou and C.P. Heisenberg, EMBO Journal **e102497** 2019

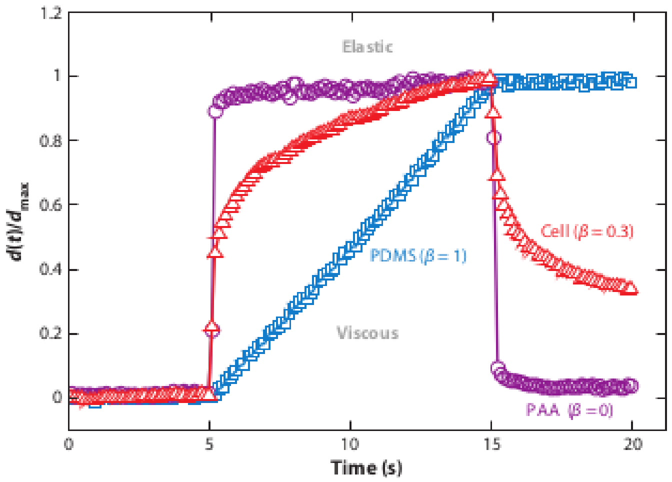
Tissue rheology in embryonic organization



N. Petridou and C.P. Heisenberg, EMBO Journal **e102497** 2019

Single cell rheology

Power law rheology



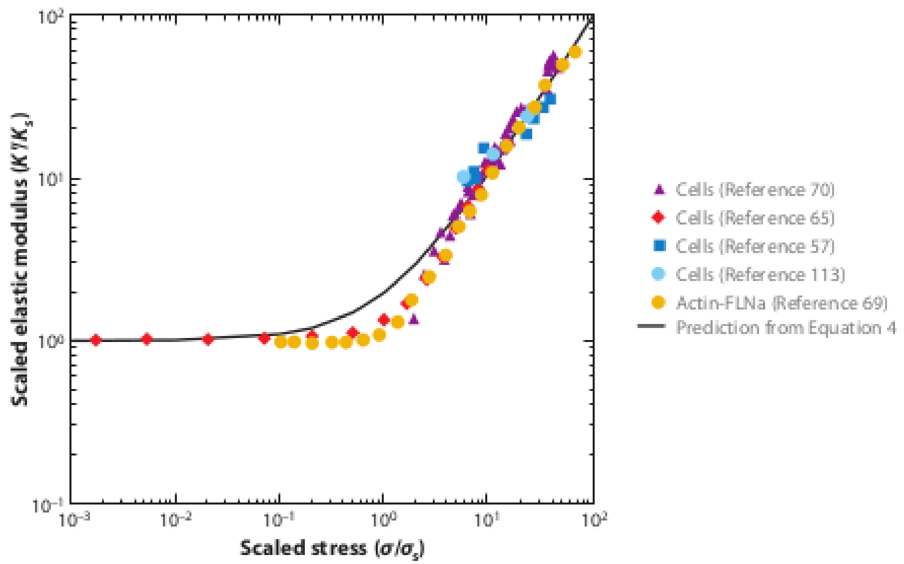
- Creep function: $J(t) = J_0 \left(\frac{t}{t_0}\right)^\beta$

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

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

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

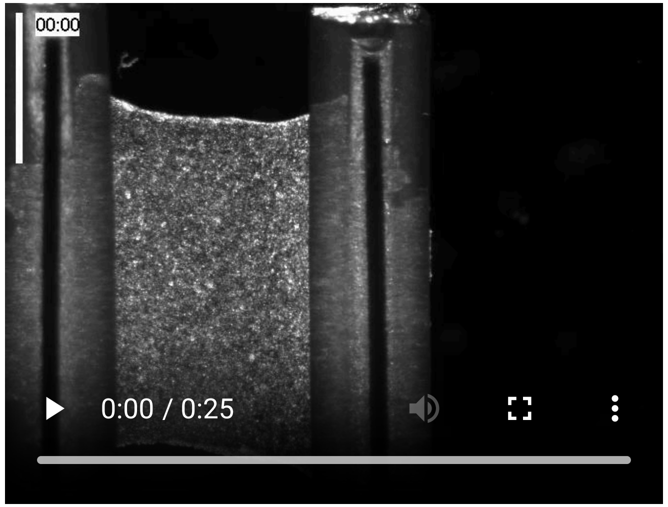
Stress stiffening

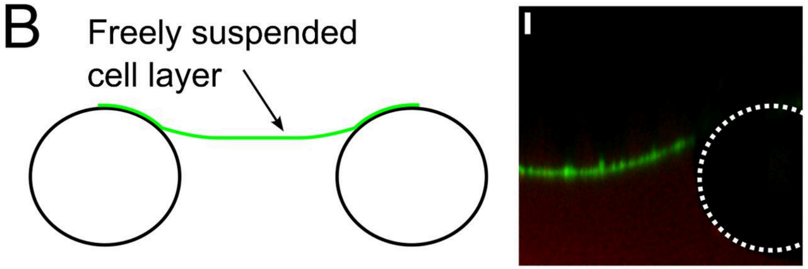


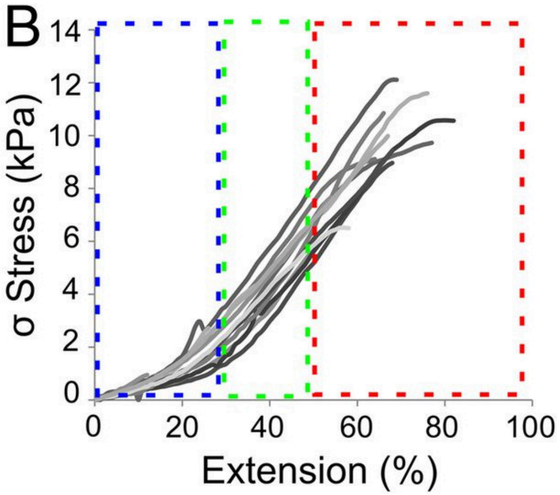
- Differential elastic modulus K'
- Black line: $K'(\sigma) = K'_0 + a\sigma$

Suspended cell monolayers: tensile testing

Madin Darby Canine Kidney (MDCK) cells



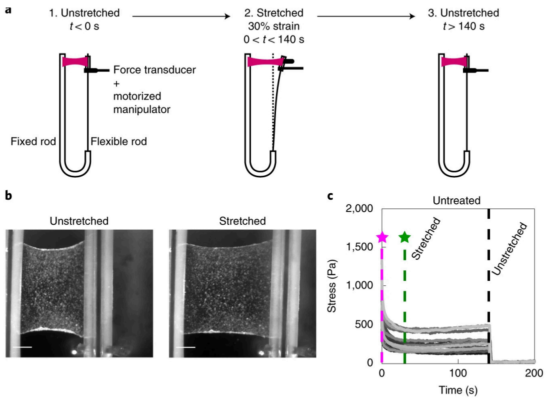


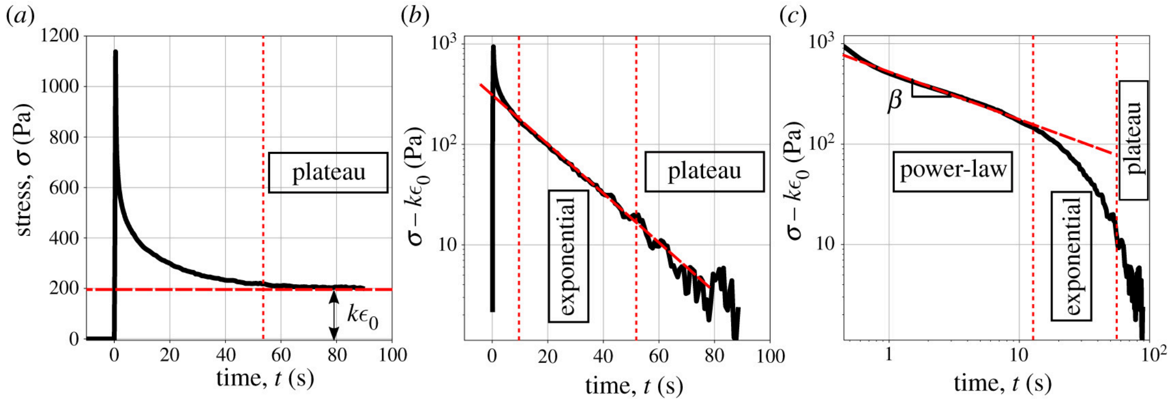


- Stress-extension curve
- $E \sim 10^4\,\mathrm{Pa}$

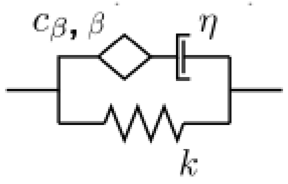
Suspended cell monolayers: stress relaxation

Madin Darby Canine Kidney (MDCK) cells





Fractional viscoelastic model



Fractional element $\Diamond$:

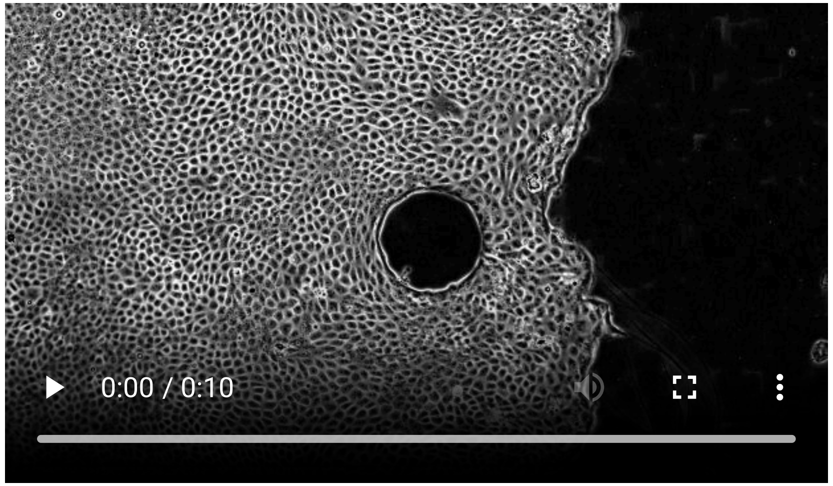
$$\sigma(t) = c_\beta \frac{d^\beta \epsilon}{dt^\beta} = \frac{c_\beta}{\Gamma(1-\beta)} \int_0^t (t-t')^{-\beta} \frac{d\varepsilon(t')}{dt'} dt'$$

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

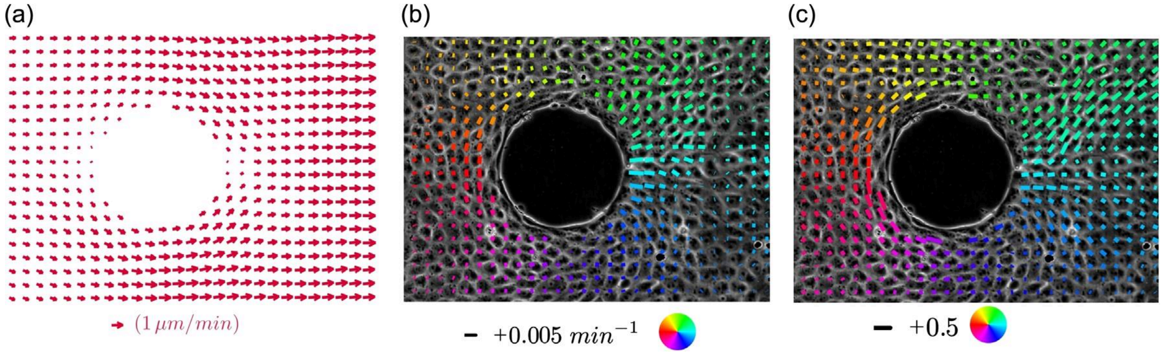
N. Khalilgharibi *et al.*, Nat Phys **15** 839 2019; A. Bonfanti *et al.*, Roy Soc Open Sci **7** 190920 2019

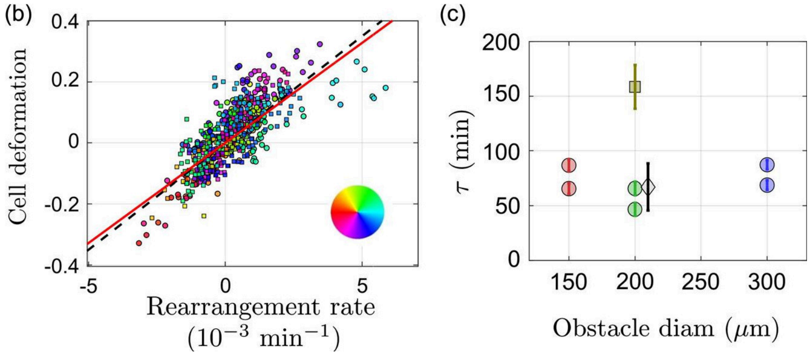
Cell monolayers: Stokes experiment

Madin Darby Canine Kidney (MDCK) cells



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

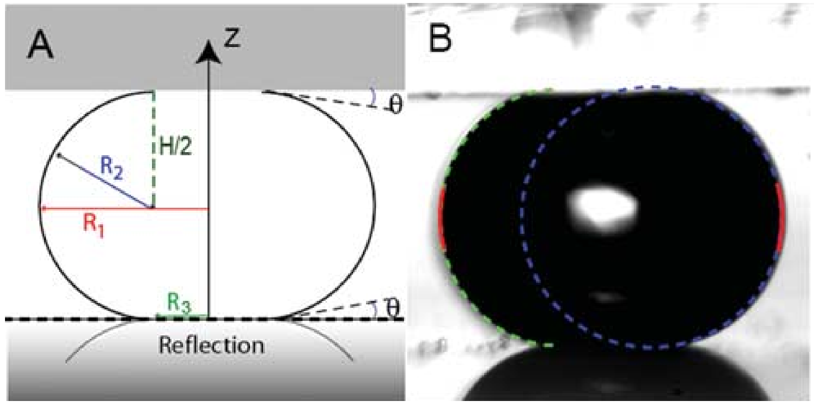




Cell spheroids

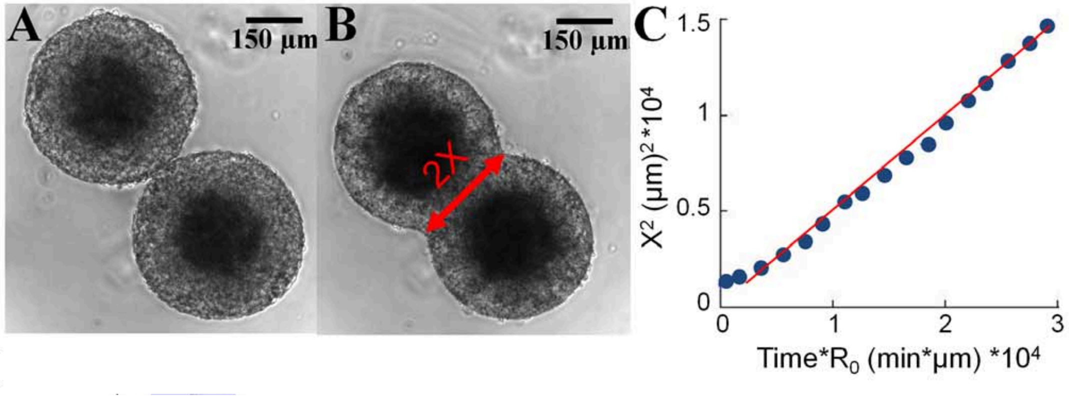
Mouse embryonic carcinoma F9 cells

Surface tension γ



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

Viscosity η

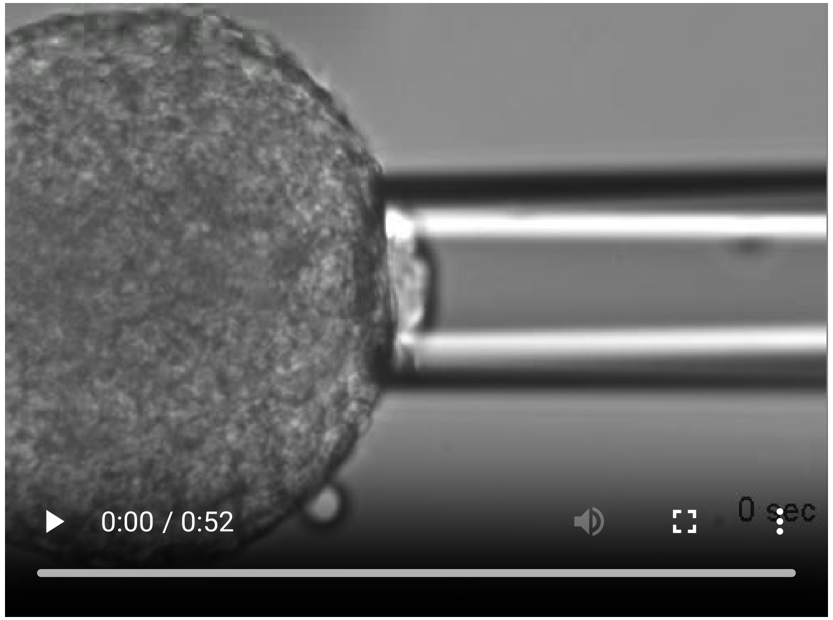


- Fusion experiments
- $X^2(t) = \frac{\gamma}{\eta} R_0 t$
- $\eta \sim 10^5\,\mathrm{Pa\,s}$

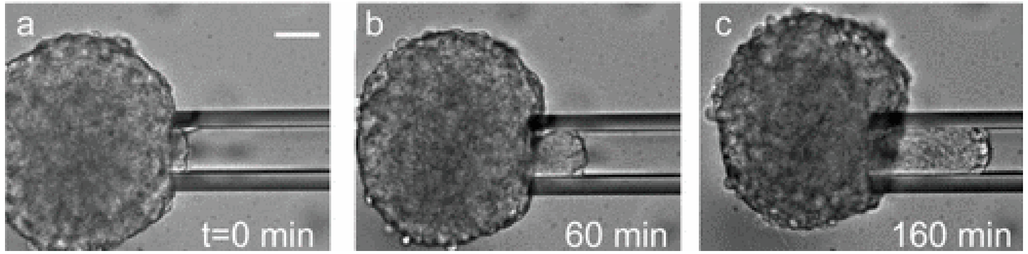
A. Mgharbel *et al.*, HFSP Journal **3** 213 2009; T.V. Stirbat *et al.*, PLoS One **8** e52554 2013

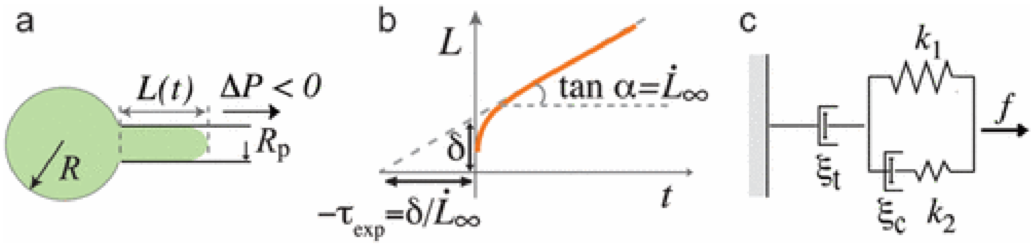
Micropipette aspiration of multicellular aggregates

Murine sarcoma S180 cells



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





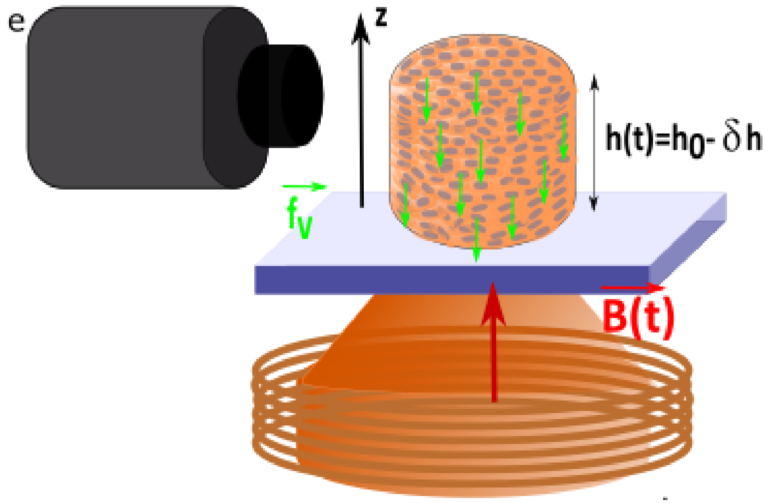
- Modified Maxwell model
- $E \sim 10^3\,\mathrm{Pa}$
- $\eta \sim 10^5\,\mathrm{Pa\,s}$

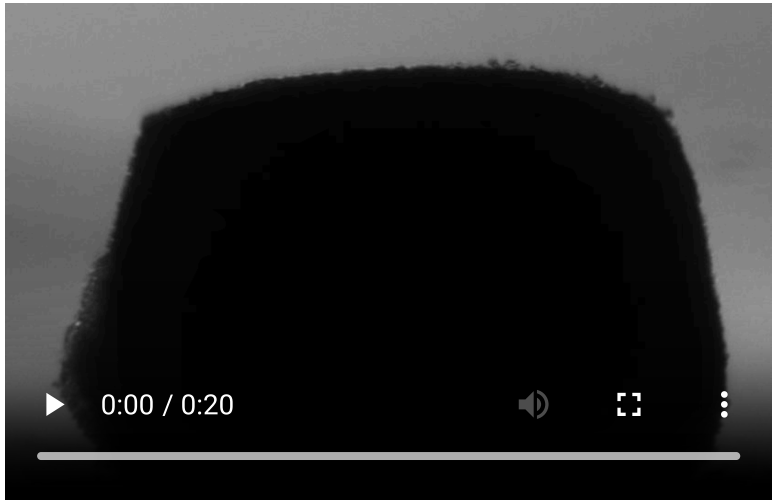
K. Guevorkian *et al.*, Phys Rev Lett **104** 218101 2010

Rheology of multicellular aggregates

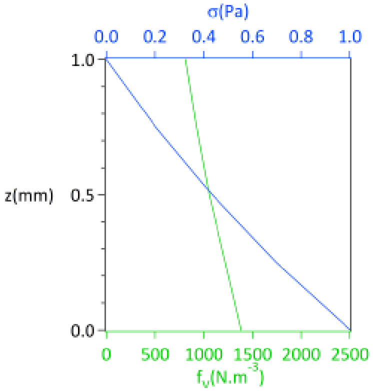
G. Mary, F. Mazuel, V. Nier, F. Fage, I. Nagle, L. Devaud, J.-C. Bacri, S. Asnacios, A. Asnacios, C. Gay, P. Marcq, C. Wilhelm, and M. Reffay,
All-in-one rheometry and non-linear rheology of multicellular aggregates, Phys. Rev. E **105** 054407 2022

Magnetic rheometer



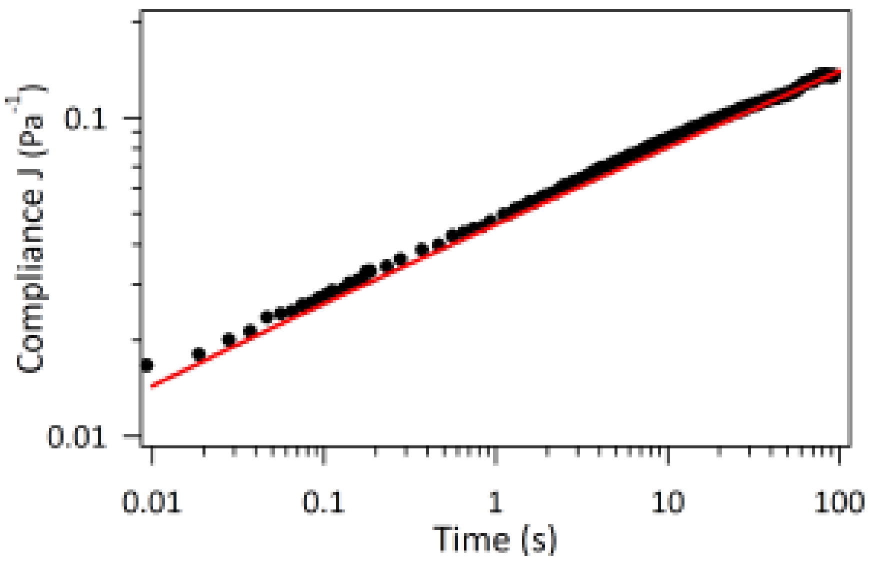


- Force f_V approximately constant
- Local stress $\sigma_{\rm loc}(z) = \int_z^{h(t)} f_V(z')\,{\rm d}z'$
- Average stress $\sigma \equiv \langle \sigma_{\rm loc}(z,t) \rangle_z \approx \frac{1}{2}\, h_0\, f_V$



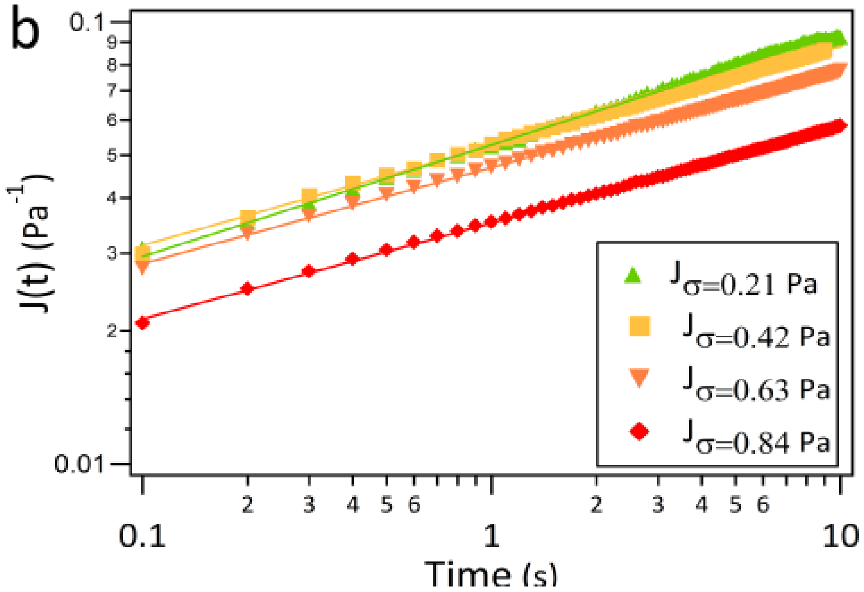
Power-law rheology

Creep function



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

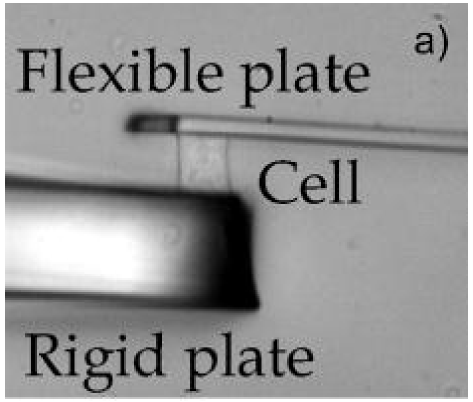
Stress-stiffening

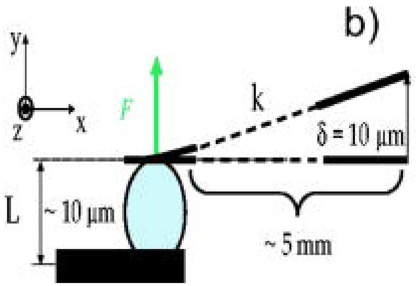


- $\beta \sim 0.25$
- J_0 decreases with the applied stress

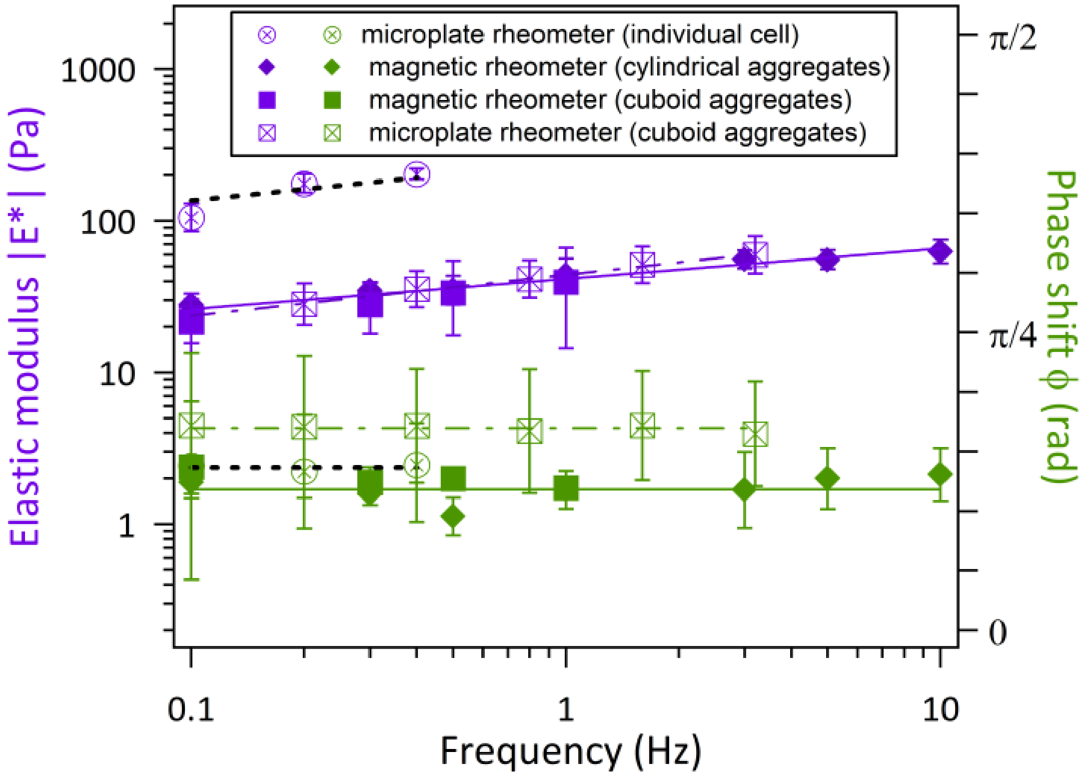
Comparison with parallel plate rheology and single cell rheology

Parallel plate rheometer



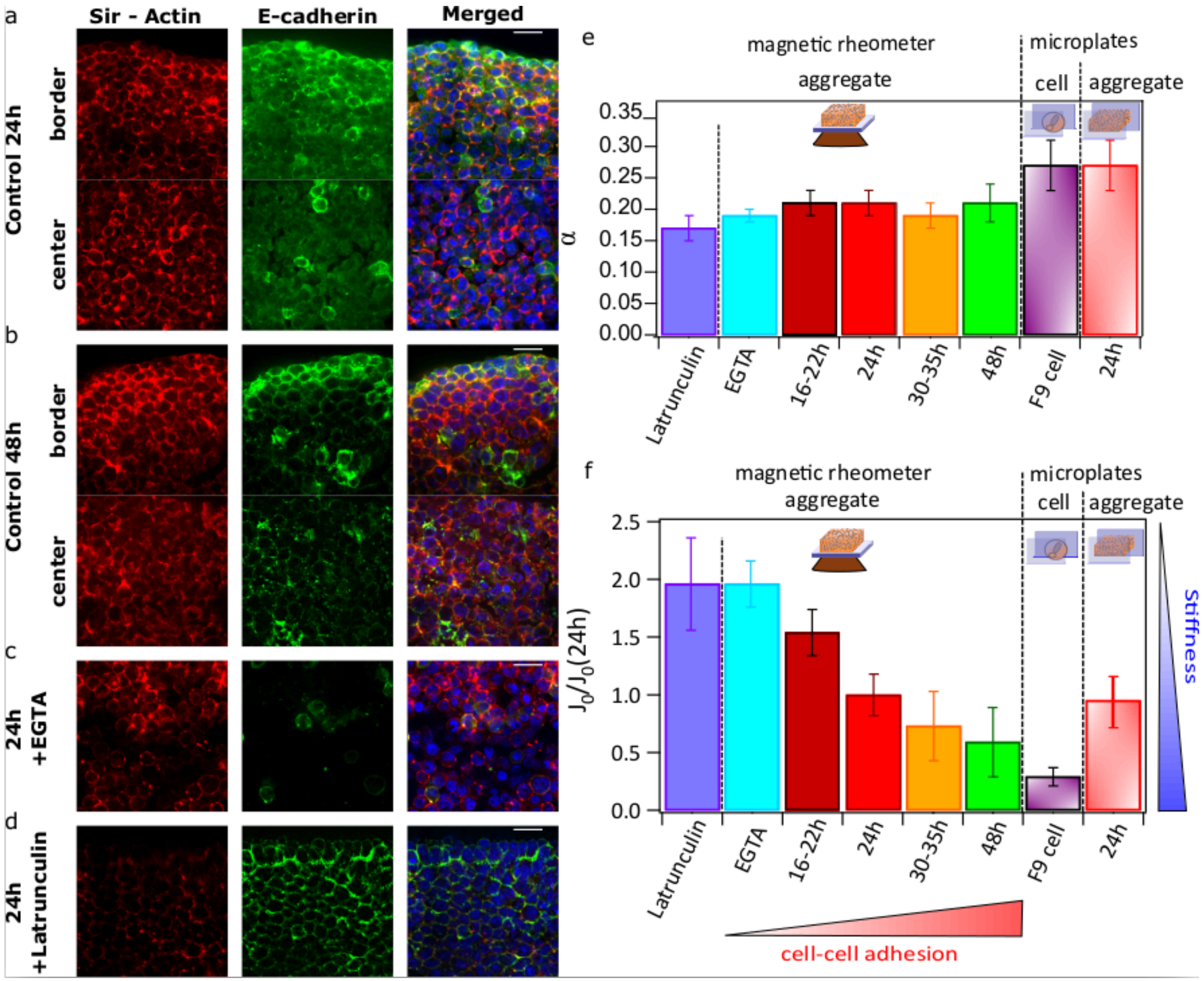


Harmonic regime



N. Desprat *et al.*, Rev Sci Instr **77** 2006

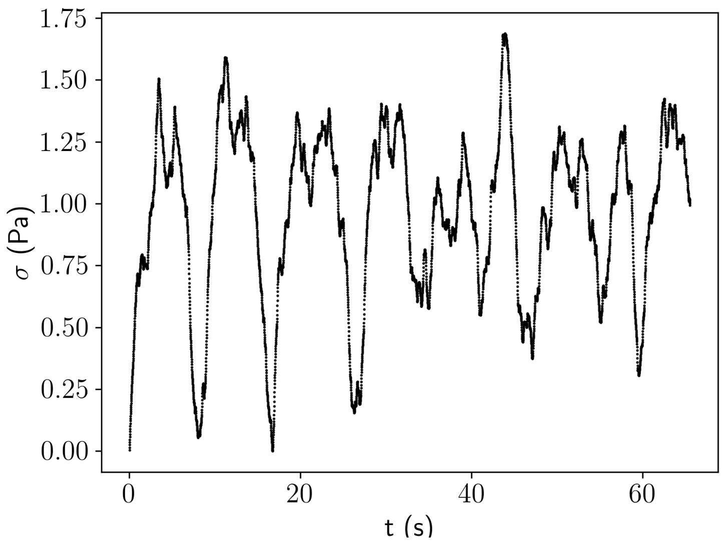
Contribution of cell-cell contacts and cytoskeleton to the rheology



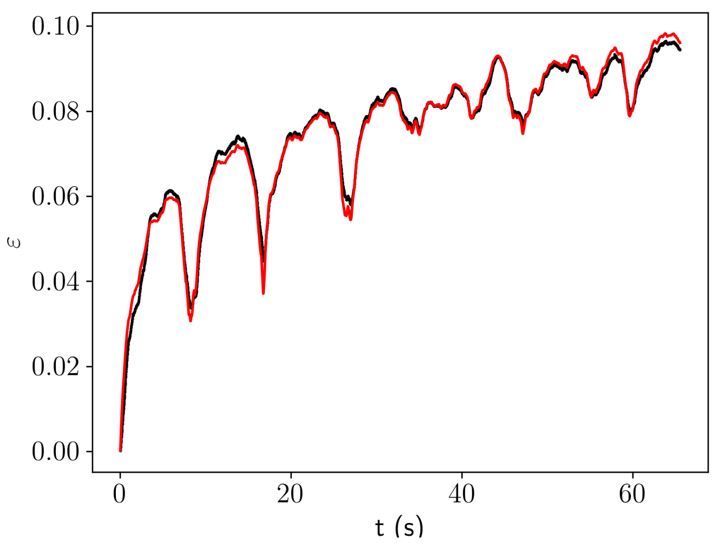
Broad-spectrum rheology

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

Applied stress signal



Measured deformation and fit

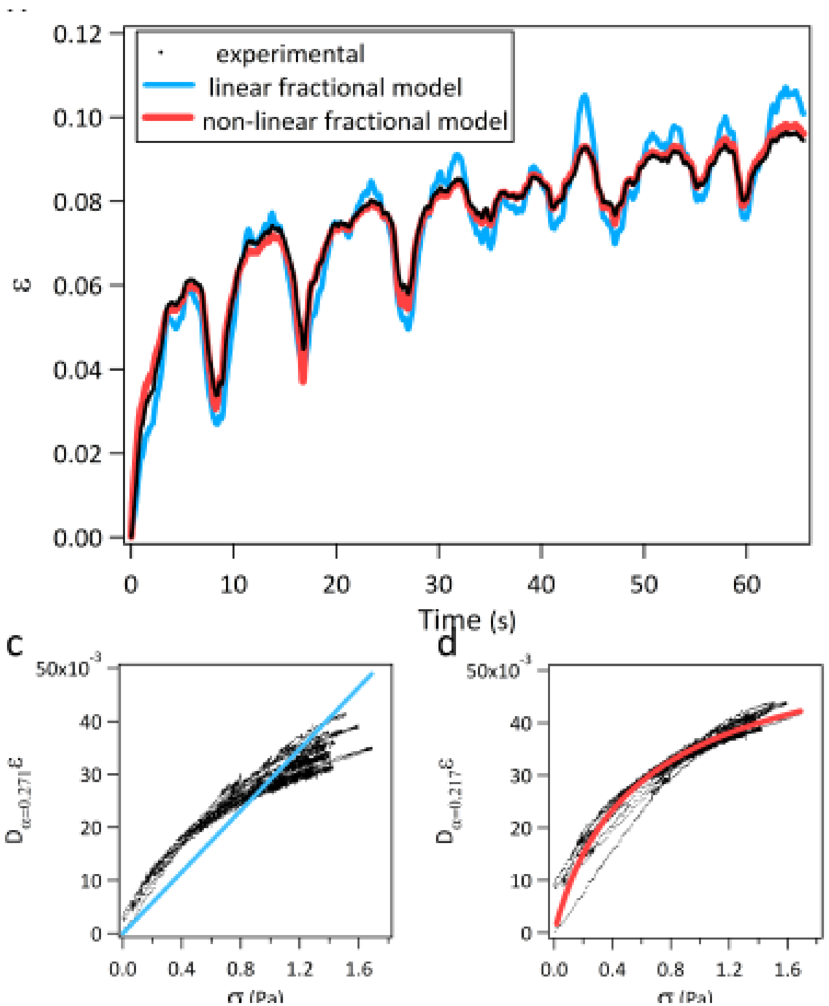


- Black: Data
- Red: Fit (maximum likelihood)

Nonlinear fractional rheology

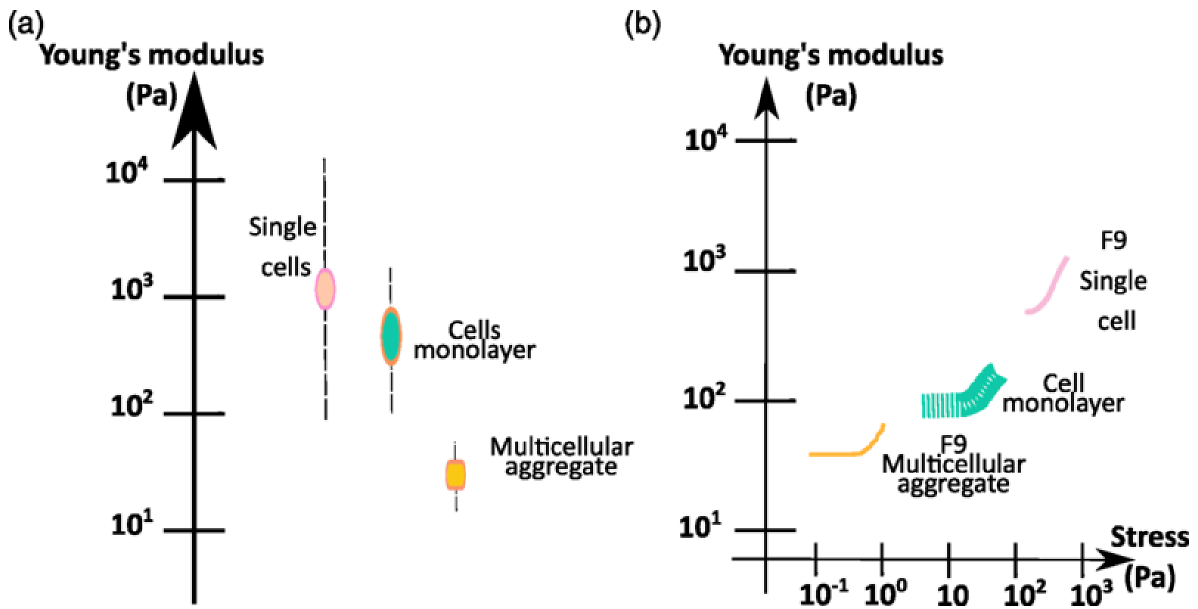
Best fit: $$\sigma = c_\beta \left(1 + \frac{\sigma}{\sigma_0}\right) \frac{d^\beta \epsilon}{dt^\beta}$$

- $\beta \sim 0.2$
- $\sigma_0 \sim 0.5\,\mathrm{Pa}$
- $c_\beta \sim 10\,\mathrm{Pa\,s}^\beta$



Summary

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

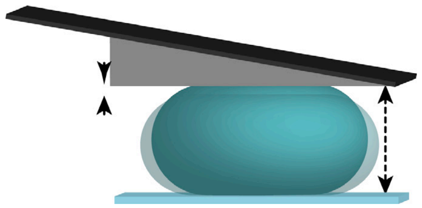


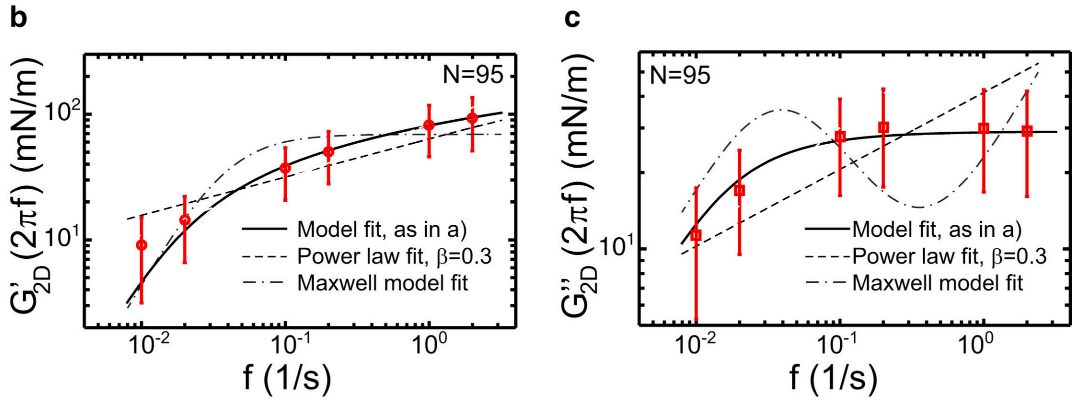
Mapping cell cortex rheology to tissue rheology

E. Moisdon, 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
- $\sigma_{2D}(t) = \sigma_0 + \int_{-\infty}^{t} G_{2D}(t-t')\,\dot{\varepsilon}_{2D}(t')\,\mathrm{d}t'$
- $G_{2D}(t) = \int_0^{\infty} h(t') e^{-t/t'} \mathrm{d}\ln t'$
- $h(t') = K_h, t \leq t_{\max}$
- $h(t') = 0, t > t_{\max}$
- Cut-off $t_{\max}$
- Amplitude K_h

E. Fischer-Friedrich *et al.*, Biophys J **111** 589 2016

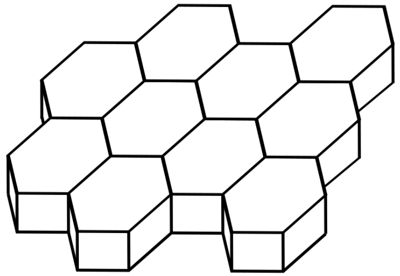
Planar cell monolayer

Cell cortex rheology (2D)

$$\mathrm{dev}(\sigma) = 2g\,\mathrm{dev}(\varepsilon_{\mathrm{face}})$$
$$\frac{1}{2}\,\mathrm{trace}(\sigma) = \sigma_0 + k\,\mathrm{trace}(\varepsilon_{\mathrm{face}})$$

- 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

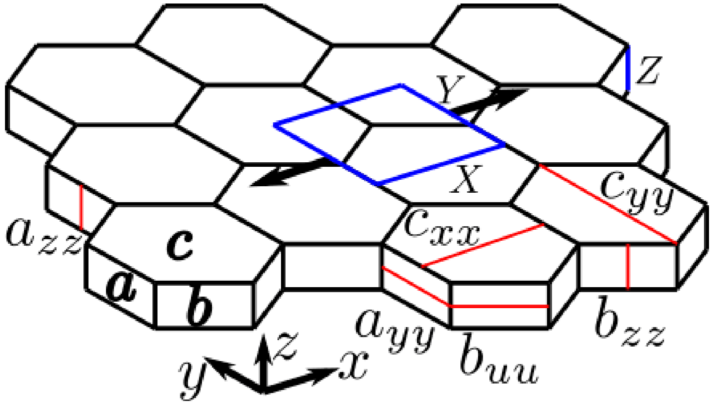
- Define microscopic stress components σ_{ij}
- Define macroscopic stress components Σ_{ij} (2D)
- Express force balance at vertices
- Compute the monolayer rest state (*e.g.* $\Psi = \frac{Z_0}{X_0}$)
- Expand variables to first order close to the rest state
- Imposing a stretch on the x axis, solve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

$$E = \frac{\Sigma_{xx}}{\varepsilon_x} \qquad \nu = -\frac{\varepsilon_y}{\varepsilon_x}$$

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

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

Schematics


Z
Y
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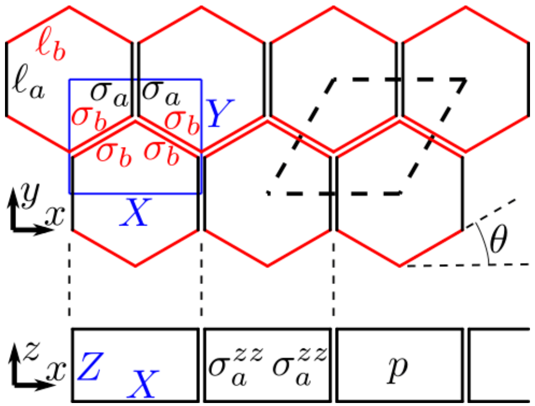
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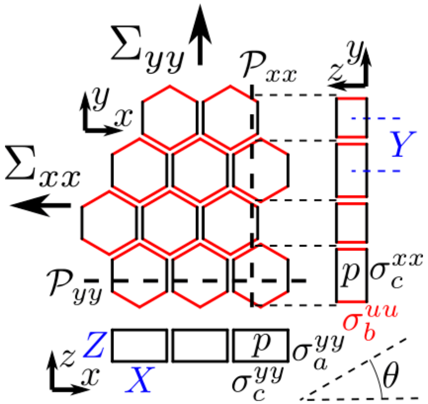
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p
σ_a^{yy}
z
x
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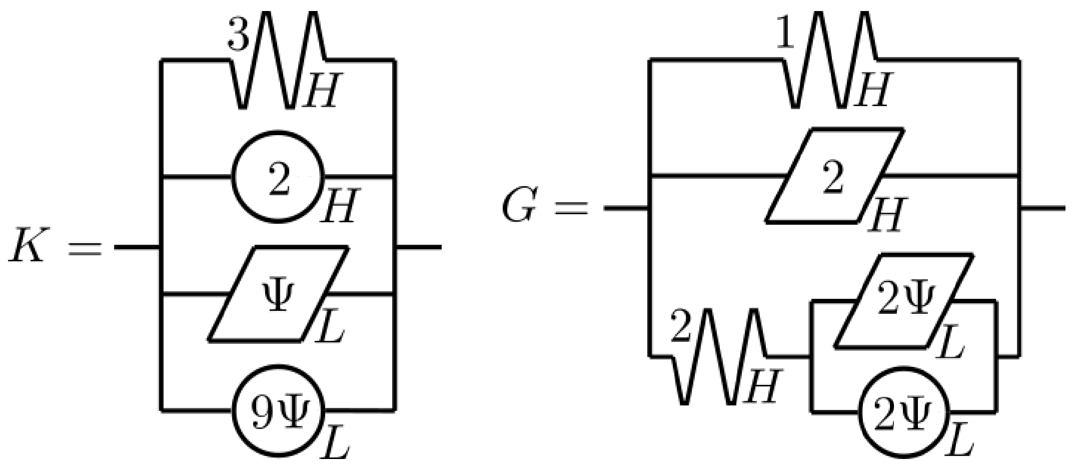
σ_c^{yy}
θ

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 $\to$ macro-rheology

Low frequency limit: elastic material

$$K_0 = 3\sigma_{0H}$$
$$G_0 = \sigma_{0H}$$

High frequency limit

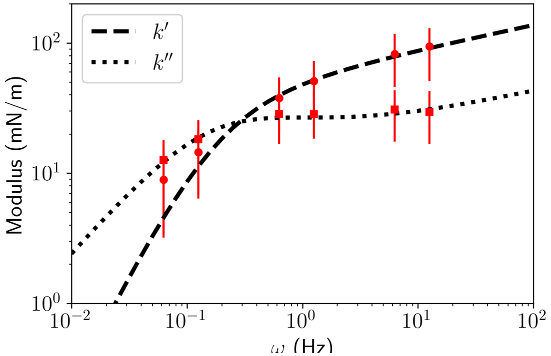
$$K_\infty \sim 2k_H + \Psi k_L + 9\Psi g_L$$
$$G_\infty \sim 2g_H$$

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



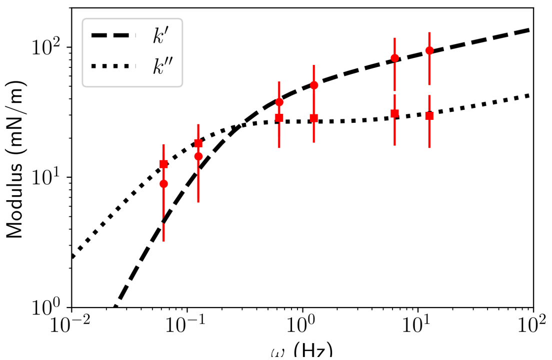
Alternative visco-fractional model

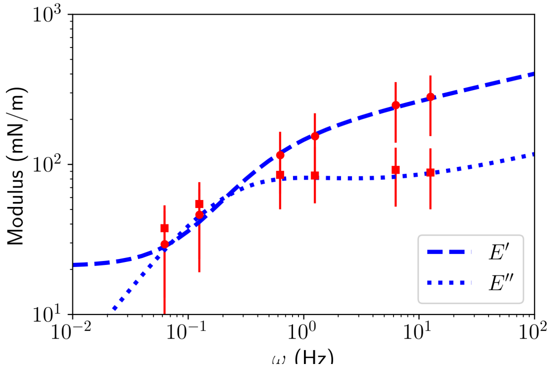
$$G_{2D}(\omega) = \frac{1}{\frac{1}{i\omega\eta} + \frac{1}{c_\beta (i\omega)^\beta}}$$

- $\beta = 0.19$
- $c_\beta \sim 10^{-2}\mathrm{N\,s^{-\beta}\,m^{-1}}$
- $\eta \sim 10^{-1}\,\mathrm{N\,s\,m^{-1}}$

Visco-fractional rheology is invariant under this mapping

Cortex rheology $\Rightarrow$ $\Rightarrow$ $\Rightarrow$ $\Rightarrow$ **Tissue rheology**





Cortex parameters (HeLa cells)

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

Not measured for MDCK cell cortex

Tissue parameters

- $\beta^T = 0.19$
- $c^T_\beta = 170\,\mathrm{mN\,s^{-\beta}\,m^{-1}}$
- $\eta^T = 500\,\mathrm{mN\,s\,m^{-1}}$

Not measured for HeLa monolayer

Perspectives

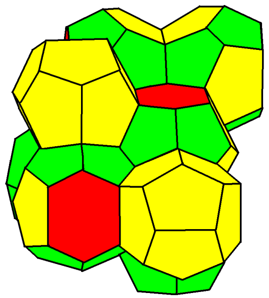
We hope that this work will foster more experimental work, measuring both cortex and tissue rheology.

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

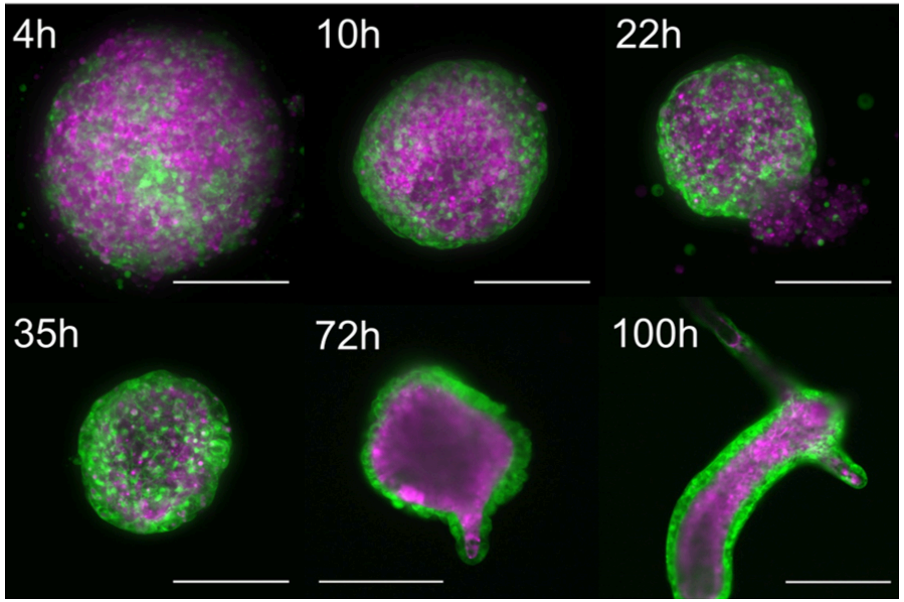
Hydra mechanics

T. Perros, A. Biquet-Bisquert, Z. Ben Meriem, M. Delarue, P. Joseph, P. Marcq and O. Cochet-Escartin,
Mechanical characterization of regenerating Hydra tissue spheres, BioRXiV 2023

Hydra regeneration

From a piece of adult tissue

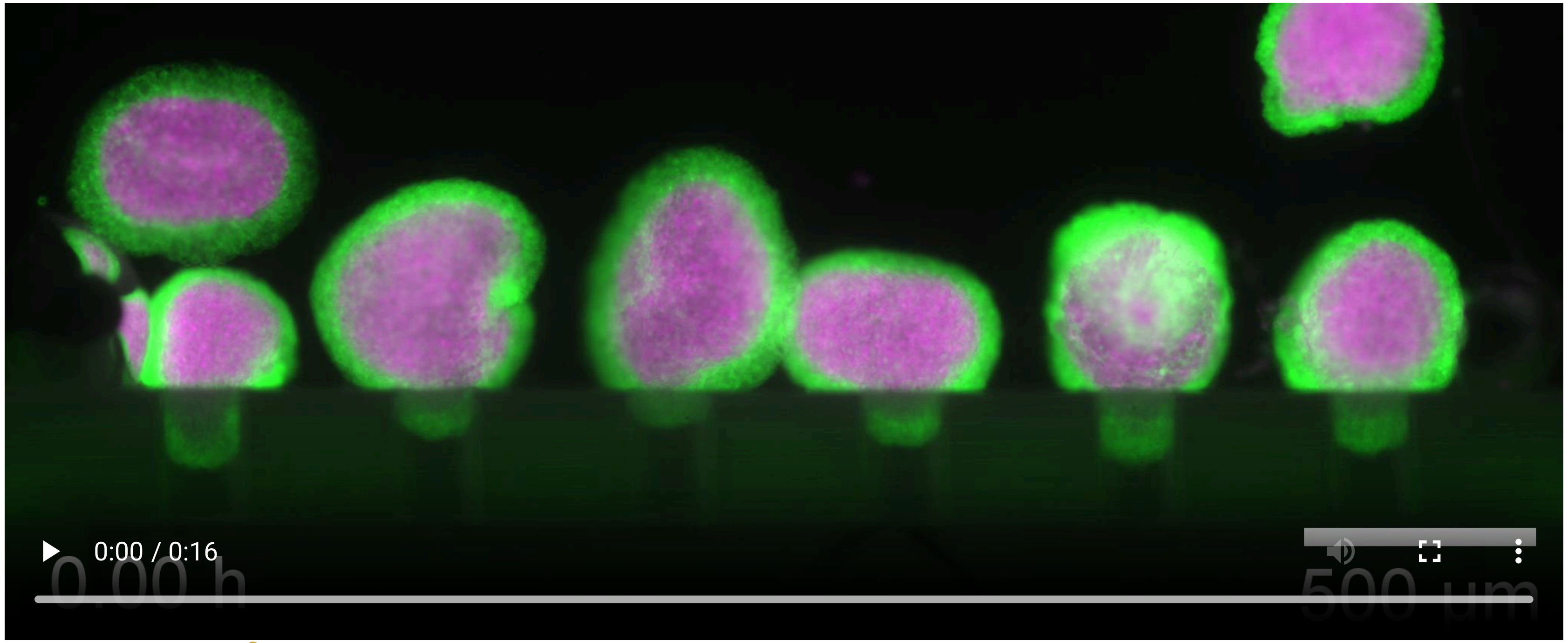
From a mixed cell aggregate



- Green: Ectoderm
- Magenta: Endoderm

Courtesy O. Cochet-Escartin

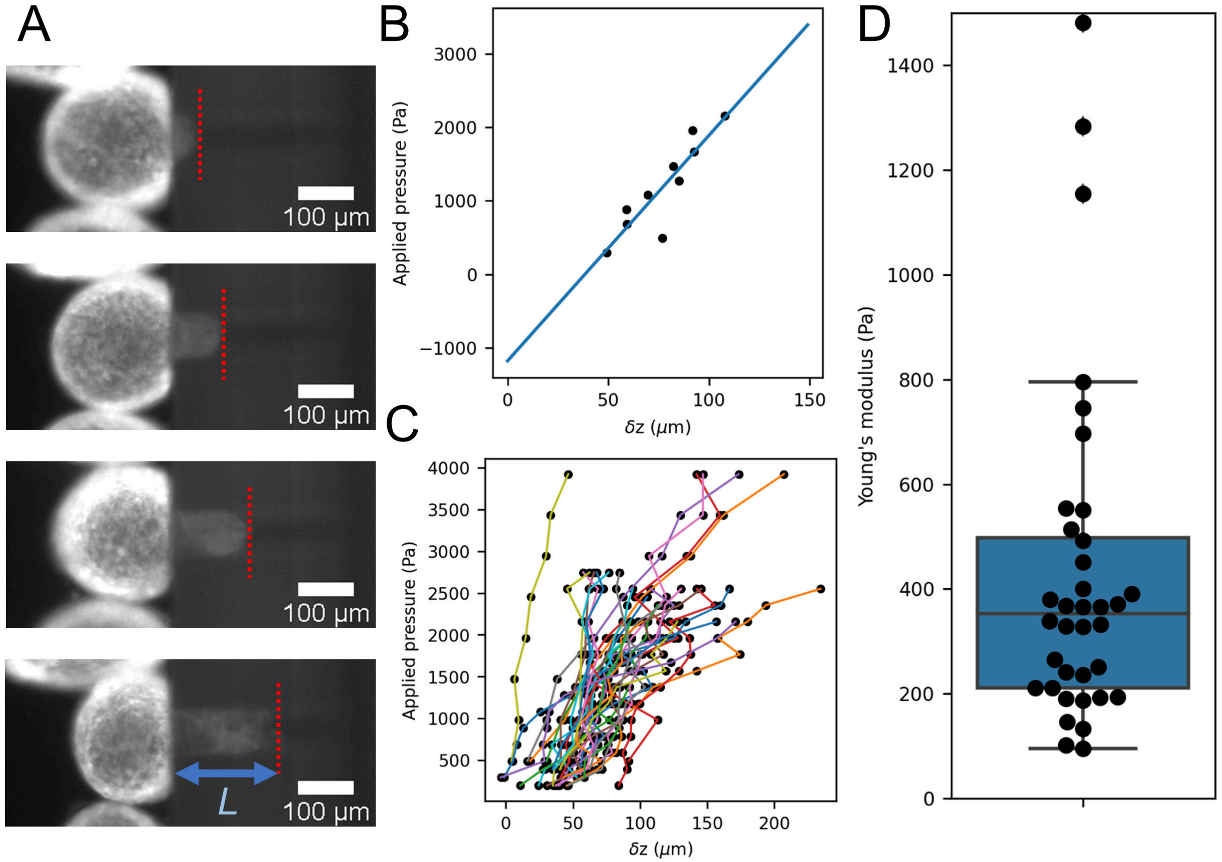
Parallelized micropipette aspiration of *Hydra* spheres



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

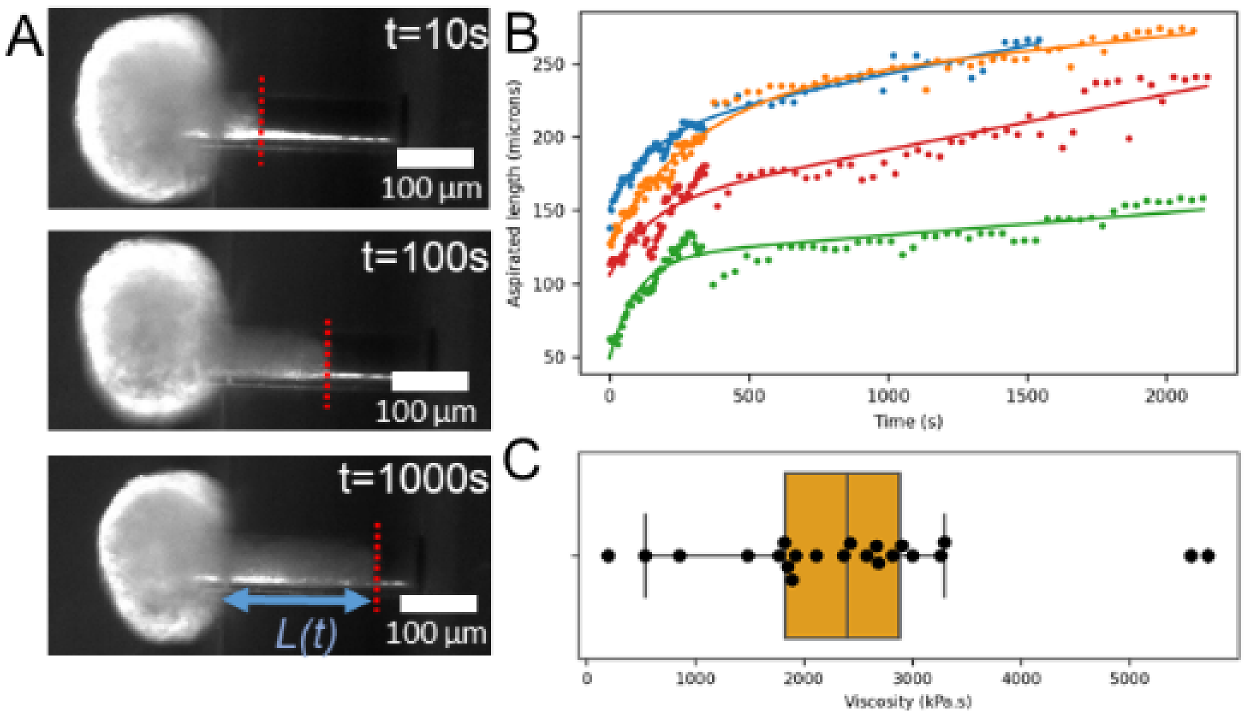
Hydra elasticity

- "Low" applied pressure
- Hyperelastic shell of thickness h
- Saint-Venant-Kirchhoff model
- Young's modulus $E \sim 10^2\,\mathrm{Pa}$

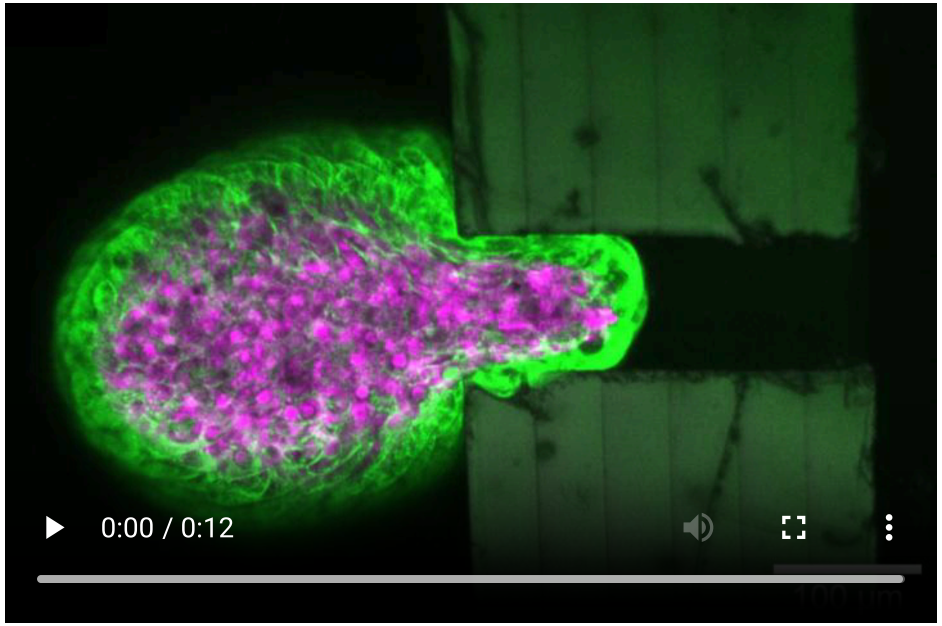


Hydra viscosity

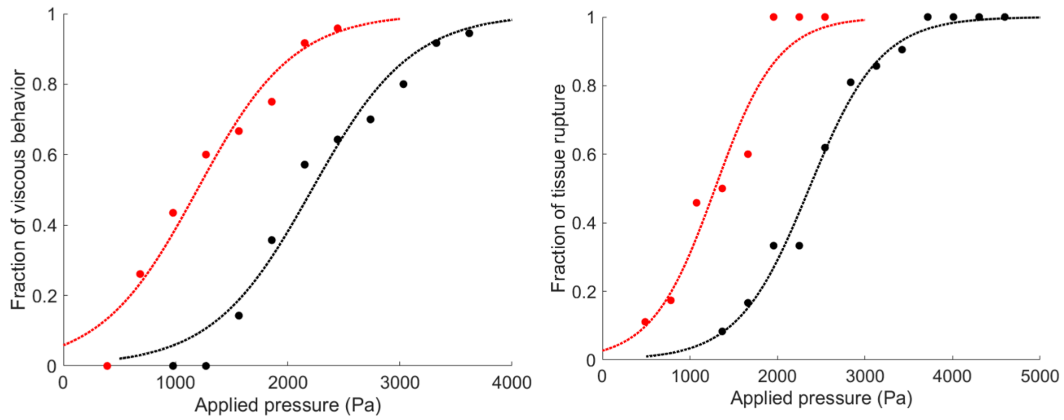
- "High" applied pressure
- Modified Maxwell model
- Flow at constant velocity U when $t \gg \tau$
- Micropipette radius R_p
- $\Delta p = C_\eta \eta \frac{U}{R_p}$
- Shear viscosity $\eta \sim 10^6\,\mathrm{Pa\,s}$



Elastic $\rightarrow$ viscoelastic $\rightarrow$ rupture



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



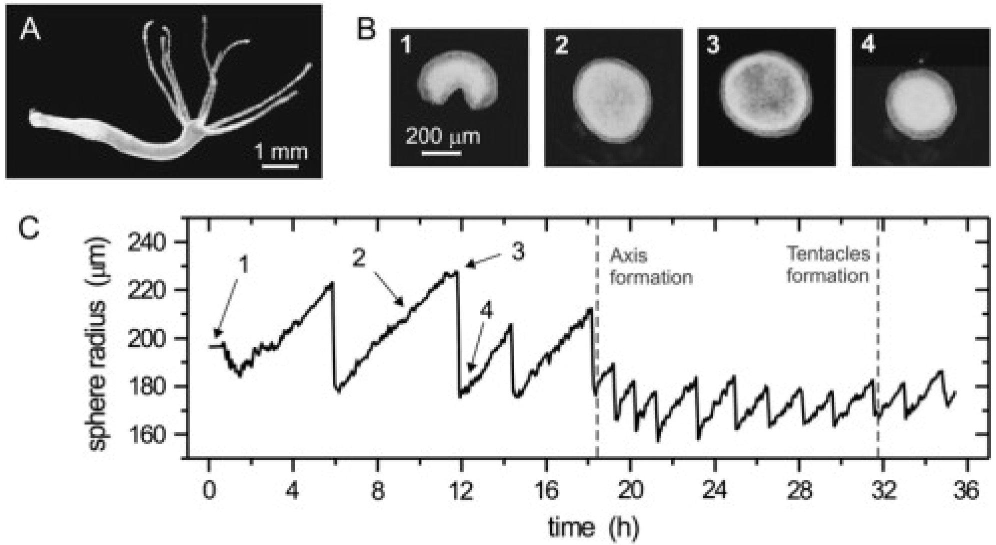
- Black: WT
- Red: EDTA, weaker cell-cell adhesion

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



- $\alpha(t) = \frac{R(t)}{R_0}$
- $\sigma = \left(\alpha^4 - \frac{1}{\alpha^2}\right) \left(\left(\frac{\lambda}{2} + \mu\right)\left(2 + \frac{1}{\alpha^6}\right) - \left(\frac{3\lambda}{2} + \mu\right)\frac{1}{\alpha^2} - \mu\right)$
- $\Delta p = \frac{2h}{R}\sigma$
- $\alpha_{\max} \sim 1.3, \Delta p_{\max} \sim 10^3\,\mathrm{Pa}$
- 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?

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Merci / Thank you