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Cell Biomechanics notes

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CELL BIOMECHANICS

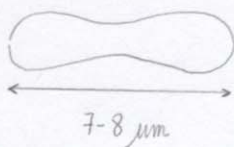
08 / 15 / 2006

Gubra Yuresh

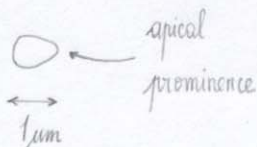
Case studies in the context of disease states

1. P. Falciparum malaria

red blood cell (RBC)



merozoite



Two critical effects of malaria on cell mechanics

- increased adhesion of RBC to endothelium
- reduced deformability of the RBC hence sequestration of RBCs.

Membrane & cytoskeleton mechanics coupled.

- optical tweezers : changes in RBC deformability large deformation stretching of healthy and infected cells



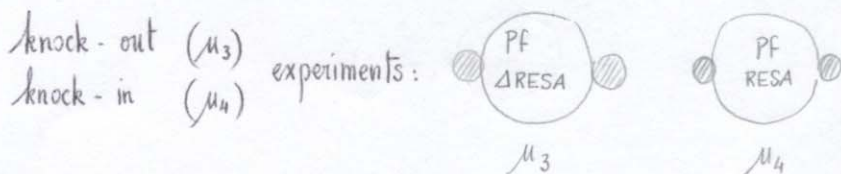
- biochemistry PFEMP, KAHRP, RESA } classes of proteins mediating mechanical properties

phospholipid bilayer anchored to spectrin network by some proteins.  
RESA - spectrin, KAHRP bound to ankyrin & actin : known interactions;  
how are biochemistry & mechanics related ?

- is the increase in stiffness due to change in membrane ?  
or presence of granular particles from infection ?



$\mu_1$                        $\mu_2 > \mu_1$



- from mechanics to biochemistry and gene inactivation  
RESA (green) parasites (blue / purple) band 3 = red blood cells (red)  
if  $\Delta$ RESA : purple, but no more green.

## Cell biomechanics - 2.

- the presence of RESA protein significantly stiffens the infected cell  
 $\mu_2 > \mu_1$ ,  $\mu_3 \approx \mu_1$  and  $\mu_4 > \mu_1$

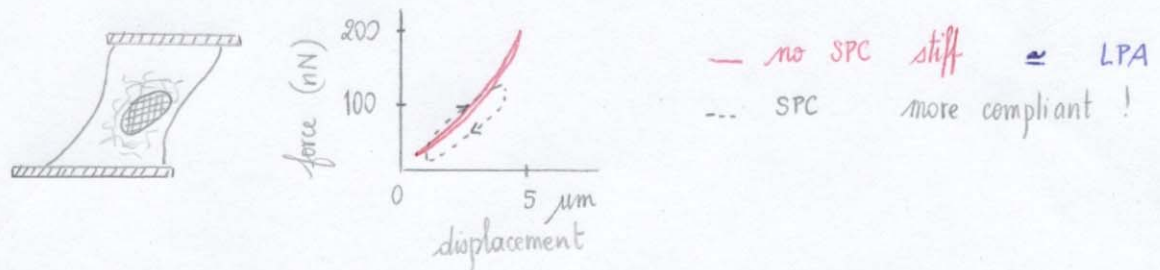
in-plane shear modulus : from  $\sim 8$  to  $\sim 15-20 \mu\text{N/m}$   
(J.P. Mills, M. Diez 2006)

- now known what specific site on spectrin RESA binds
- RESA (ring-stage erythrocyte surface antigen) affects stiffness in ring stage  
RESA's effects most dominant during febrile episodes ( $41^\circ\text{C}$ ) (not trophozoite)

## 2. Effect of SPC-induced reorganization of keratin network in human epithelial Panc-1 cells

- pancreatic cancer has high mortality, hard to detect.
- sphingosylphosphorylcholine (SPC) induced keratin rearrangement in cancer cells (fluorescence) collapse in perinuclear region within 1 hour

- how are single cell mechanical properties affected?  
increased propensity to metastatic invasions of cancer cells?  
microplate experiments:



- lysophosphatidic acid (LPA) promotes actin stress-fiber formation: no effect on cancer cell deformability (stiffness and lack of hysteresivity conserved)
- circumstantial evidence for role of single cell mechanical properties in cancer progression



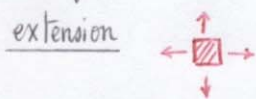
Membrane & cytoskeleton mechanics

Roger Kamm

- What are the primary structural elements in the cell?
- How do cells interact with their environment?
- How do cells generate force?
- How do cells migrate?

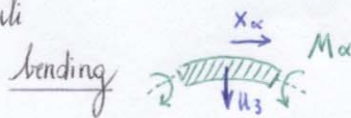
- cell simplified by membrane + cytoskeleton + nucleus but more complex, heterogeneous, crowded in reality!
- neutrophils present microvilli, and a dense cortex of actin, with fluid inside.
- the cytoskeleton is made of actin microfilaments:  $7-9 \mu\text{m } \phi$ ,  $15 \mu\text{m } l_p$   
 microtubules:  $25 \mu\text{m } \phi$ ,  $6000 \mu\text{m } l_p$   
 intermediate filaments:  $10 \mu\text{m } \phi$ , (less dynamic family)  
 persistence length, bending stiffness and Young's modulus } characterize stiffness
- the membrane is a lipid bilayer (2 leaflets of hydrophobic tails / hydrophilic heads)  
 lipids can organize into micelles, bilayers or liposomes.  
 glycolipids, phospholipids, and proteins are anchored in membrane  
 fluid mosaic model: molecule freely diffuse within membrane, and membrane shears easily
- forces are transmitted through membrane by adhesion receptors (anchored in both ECM and cytoskeleton)  
 cells are not passive, they can generate force (muscles).  
 cells are dynamic: can change their modulus in seconds and become activated / migrate (example of neutrophils deforming in capillaries / microchannels)

- membrane deformations and moduli



$$N = \frac{Eh}{2(1-\nu)} \cdot \frac{\Delta A}{A_0} \equiv K_e \frac{\Delta A}{A_0}$$

{ N: surface tension  
 t or h: thickness of membrane



$$M_\alpha = - \frac{Et^3}{12(1-\nu^2)} \cdot \frac{\partial^2 u_3}{\partial x_\alpha^2}$$

$$= - K_B \frac{\partial^2 u_3}{\partial x_\alpha^2}$$

shear

$$N_{12} = \sigma_{12} h = 2G E_{12} = K_s E_{12}$$

derive governing equations for linear deformations and the reduced forms for bending or tension dominance.

$$\frac{\kappa_B}{N\lambda^2} \gg 1 \quad \kappa_B \left( \frac{\partial^4 u_3}{\partial x_1^4} + 2 \frac{\partial^4 u_3}{\partial x_1^2 \partial x_2^2} \dots \text{etc!} \right)$$

- cell peeling experiments to measure  $\kappa_B \sim 10^{-18} \text{ N}\cdot\text{m}$
- micropipet aspiration experiments to characterize viscoelastic responses.

but membranes are more complicated than a mere sheet: liquid vesicles exhibit fluctuations - dominated (entropic) regime and an elastic (enthalpic) regime when inflated.

$$\frac{\Delta A}{A_0} \approx \frac{k_B T}{8\pi \kappa_B} \ln \left( \frac{NA}{\pi^2 \kappa_B} \right) + \frac{N}{\kappa_e}$$

- cell adhesion and membrane receptors:  $\left\{ \begin{array}{l} \text{integrins} \\ \text{cadherins} \\ \text{N-CAM} \\ \text{selectins} \end{array} \right.$  to form  $\left\{ \begin{array}{l} \text{tight junctions} \\ \text{adherens junctions} \\ \text{desmosomes} \\ \text{gap junctions (sign)} \\ \text{hemidesmosomes} \end{array} \right.$

measure { adhesion: patterned deformable substrates  
force generation: on pillars substrates

on beaded polyacrylamide gels (fluorescent markers = displ)  
observe size of focal adhesions,  $\sigma_{FA} \sim 5 \text{ kPa} \gg \sigma_{\text{shear flow}}$  !  
regardless of size of FA.

- cell adhesion and the rolling leukocyte, Bell equation = rate for unbinding if  $k_r = k_r^0 \exp(\gamma f / k_B T)$

- different measurement techniques cover different orders of magnitude in force / displacement

- magneto cytometry
- optical tweezers
- magnetic trap
- atomic force microscopy
- substrate deformation
- embedded particle tracking
- micropipette aspiration
- ...



Mechanical properties of the cytoskeleton

Jeff Fredberg

Books: Muscle reflexes and locomotion T. McMahon  
 Biological physics P. Nelson  
 Molecular driving forces Dill & Bromberg  
 Mechanisms of motor proteins and cytoskeleton J. Howard

- What are the physical laws governing elasticity, contraction, remodeling?  
 that play roles in adhesion, spreading, crawling, invading

22,000 human genes, 100,000 proteins, intricate connectivity maps

How do we understand such complexity? splitters: normative reductionist biology  
 lumpers: seek integrative unifying networks

\* systems biology: complete parts list, detailed interactive maps insufficient to predict integrative function?

{ CSK = ?  
 mechanism = ?  
 protein-protein interactions? can we establish laws?

Bausch & Kroy, Nature 2006: A bottom-up approach to cell mechanics

- Portrait of CSK elasticity, contraction and remodeling:  
4 hallmarks soft & stressed  
 scale-free dynamics  
 aging & rejuvenation  
 hopping, intermittency

# 1 - in the stiffness universe,  $E \sim 1 \text{ GPa}$  for actin,  $E \sim 10 \text{ Pa}$  for actin gels!  
 $E \sim 1 \text{ kPa}$  for cells and soft matter (foam, colloids)

explanations: volumic fraction (dilution), bending (not only stretching), non-affine relations, prestress (exist in cells, not in actin gels)

Phillips & Quake, Physics Today 2006: in biology, a confluence of energy scales  
 covalent, ATP hydrolysis, hydrogen bonds, thermal energies  $\sim 10^{-18} - 10^{-20} \text{ J}$   
 all terms come together in active biology  $(10^{-8} - 10^{-10} \text{ m})$

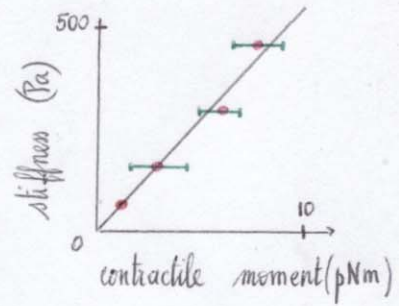
biology is very crowded! densely packed (not dilute) space.

what is the stiffness of biology? Young's modulus

$$E = \frac{F}{A} = \frac{\text{ATP hydrolysis energy}}{\text{volume}}$$

$$E \sim 10^3 \text{ Pa}$$

- stress in biology? traction microscopy  
measure deformation of substrate to infer forces exerted  
the cell is in a state of tension everywhere  
stiffness controlled by prestress (= tension) in cells



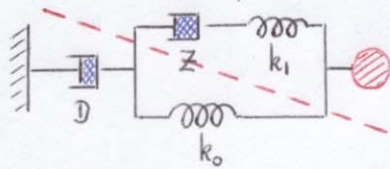
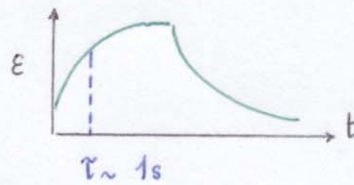
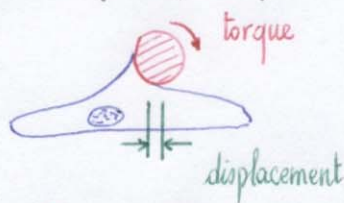
Butler AJP '02, Stamenovic JAP '04, Gardel PRL '06

speculation of tenegrity

Kumar BJ '06 laser nanoscissors cut stress fibers

#2 - Magnetic twisting cytometry : creep compliance

Bausch BJ 1998  
Fabry PRL 2001

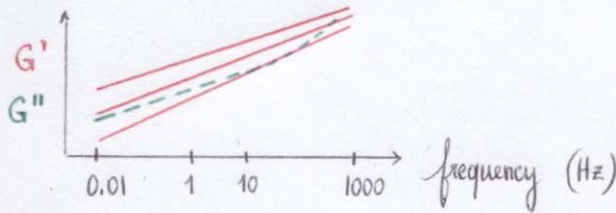
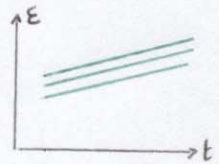


to explain experimental data, fit parameters  
NO !!

but if you cover 5 orders of magnitude in frequency  
slow dynamics : creep  $t^{x-1} \sim t^{0.2}$

scale-free

approach to equilibrium slower than exp.  
no instantaneous elasticity  
no distinct relaxation times



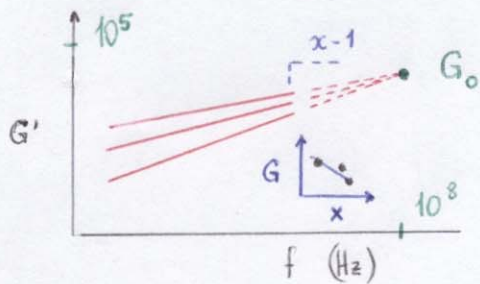
independent of cell type and scale of probe,  
weak power law prevails

structural damping law

$$G^*(\omega) \sim G_0 \left( j \frac{\omega}{\omega_0} \right)^{x-1} + j\omega\mu$$

Alcaraz BJ '03, Smith BJ '05 : AFM,  
Desprat BJ '05 : microplates, Fabry: MTC  
also conformation of nucleus  
2-point microrheology Hoffmann PNAS '0





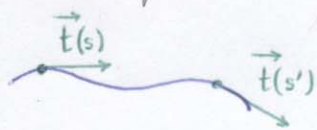
$G^* \sim j\omega^{x-1}$   
 slope  $x$   
 $x$  plays a key role in dynamics  
 fluid / solid transition  
 $x=1$  Hookean solid  
 $x=2$  Newtonian fluid

$x$ , non-universal exponent, has "temperature-like" properties glass transition theory.

- semi-flexible polymers:

what accounts for the behavior of gels? actin gels

$K_B$  bending stiffness  
 $l_p = \frac{K_B}{k_B T}$  persistence length



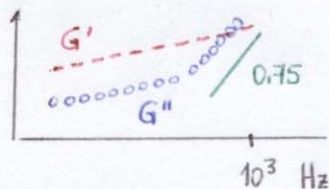
$\langle \vec{t}(s) \cdot \vec{t}(s') \rangle = \exp\left(-\frac{s-s'}{l_p}\right)$

$l_p \sim 17 \mu\text{m}$  actin not very flexible! ( $l_p \sim 50 \text{ nm}$  DNA)

elasticity: "mechanical" spring constant at zero temperature  $K_M \sim E a^2 l^{-1}$   
 "thermal" spring constant at finite temperature  $K_T \sim a^2 l_p l^{-3}$   
 which dominates? softer spring will  
 thermal effects can dominate even if  $l < l_p$

dynamics:  $\rho$  density of filaments,  $a$  radius,  $\zeta$  drag coefficient

$G^*(f) \sim (\rho K_B l_p \div 15) * (4 i \zeta \pi f \div K_B)^{3/4}$



observed in cells as well.  
at high frequency.

slope in gels 3/4

#3 - aging and rejuvenation

molecule in an energy landscape (that describes all possible molecular configurations) to remodel, the system must overcome energy barriers (Boltzmann, Eyring processes)



- if  $E \gg k_B T$ , rearrangements cannot be driven by  $k_B T$  only  
kinetics would progressively slow down  $\Rightarrow$  aging  
no steady state, trapping in deeper wells
- but ATP hydrolysis in biology  $\sim 25 k_B T$   
shear stress can give energy  $\Rightarrow$  rejuvenation reset clock