CELL BIOMECHANICS

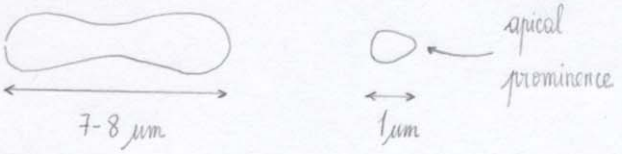
08 / 15 / 2006

Subra Suresh

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

healthy cells undergo 80% stretch

infected cells cannot deform

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

phospholipid bilayer anchored to spectrin network by some proteins.

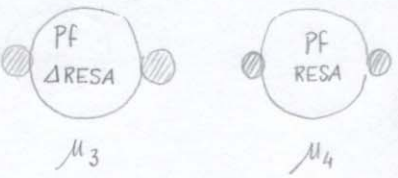
RESA - spectrin , KAHRP bound to ankyrin & actin : known interactions ;
how are biochemistry & mechanics related ?

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

μ_1 $\mu_2 > \mu_1$

knock - out (μ_3)
knock - in (μ_4)

experiments :



μ_3 μ_4

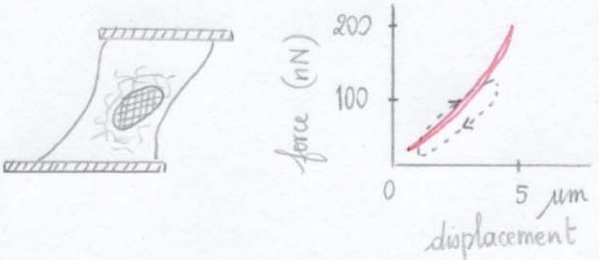
- from mechanics to biochemistry and gene inactivation
  RESA (green) parasites (blue / purple) band 3 = red blood cells (red)
  if ΔRESA : purple, but no more green.

- the presence of RESA protein significantly stiffens the infected cell
  $\mu_2 > \mu_1$, $\mu_3 \simeq \mu_1$ and $\mu_4 > \mu_1$
  $\mu_4 \simeq \mu_2$
  in-plane shear modulus : from ~ 8 to ~ 15-20 $\mu N/m$
  (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 °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 :



— no SPC stiff ≃ LPA
--- SPC more compliant !

- 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 progressi

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 μm ϕ, 15 μm l_p
  microtubules : 25 μm ϕ, 6000 μm l_p
  intermediate filaments : 10 μm ϕ, (less dynamic family)

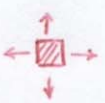
  persistence length, bending stiffness and Young's modulus } characterize stiffne

$$l_p = \frac{K_B}{k_B T} \qquad K_B = EI = E \frac{\pi}{4} a^4 \quad , \; E \sim \text{GPa} !$$

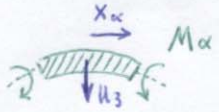
- the membrane is a lipid bilayer (2 leaflets of hydrophobic tails / hydrophilic heads)
  lipids can organize into micelles, bilayers or liposomes.
  glycolipids, phospholipids, and proteins are anchored in membrane
  fluid mosaic model: molecule freely diffuse within membrane, and membrane shears easi

- forces are transmitted through membrane by adhesion receptors (anchored in both ECM an
  cells are not passive, they can generate force (muscles). cytoskeleton)
  cells are dynamic : can change their modulus in seconds and become activated / migra
  (example of neutrophils deforming in capillaries / microchannels)

- membrane deformations and moduli

extension 

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

bending 

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

shear

$$N_{12} = \sigma_{12} h = 2G\varepsilon_{12} = K_S \varepsilon_{12}$$

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

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

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

- cell peeling experiments to measure $K_B \sim 10^{-18}$ N 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) regi when inflated.

$$\frac{\Delta A}{A_0} \cong \frac{k_B T}{8\pi K_B} \ln\left(\frac{NA}{\pi^2 K_B}\right) + \frac{N}{K_e}$$

- <u>cell adhesion</u> and membrane receptors : { integrins, cadherins, N-CAM, sélectins } to form { tight junctions, adherens junctions, desmosomes, gap junctions (sign, hemidesmosomes }

measure { adhesion : patterned deformable substrates
force generation : on pillars substrates
on beaded polyacrylamide gels (fluorescent markers ≡ displ

observe size of focal adhesions, $\sigma_{FA} \sim 5$ kPa $\gg \sigma_{\text{shear flow}}$! regardless of size of FA.

- cell adhesion and the rolling leukocyte , Bell equation = rate for unbinding if f

$$k_r = k_r^\circ \exp(\gamma f / k_B T)$$

- different measurement techniques cover different orders of magnitude in force / displac
  - magnetocytometry
  - 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. McMahan
- 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 interaction maps insufficient to predict integrative function ?

{ CSK = ?
{ mechanism = ?
{ protein - protein interactions ?

can we establish laws ?

Baush & 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, $E \sim 1$ GPa for actin, $E \sim 10$ Pa for actin gels !
$E \sim 1$ 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)

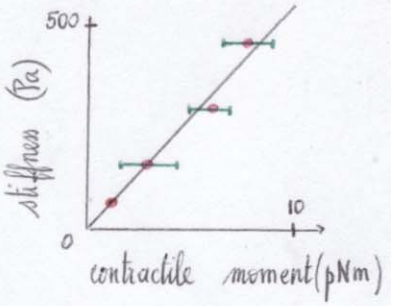
Phillips & Quake, Physics Today 2006 : in biology, a confluence of energy scales
covalent, ATP hydrolysis, hydrogen bonds, thermal energies $\sim 10^{-18} - 10^{-20}$ J
all terms come together in active biology ($10^{-8} - 10^{-10}$ m

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

what is the stiffness of biology ? Young's modulus $E = \frac{F}{A} = \frac{\text{ATP hydrolysis energy}}{\text{volume}}$

$E \sim 10^3$ Pa

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



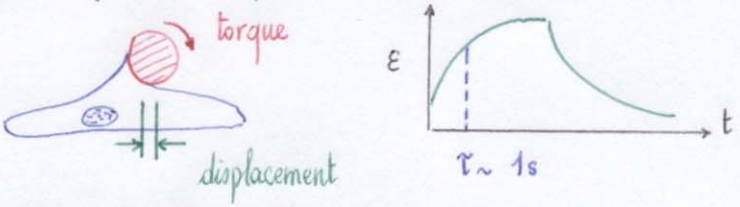
Butler AJP '02, Stamenovic JAP '04, Gardel PRL'06

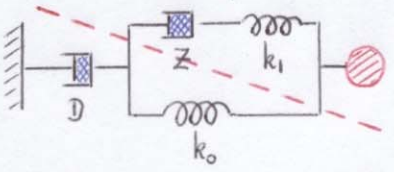
speculation of tensegrity

Kumar BJ '06 laser nanoscissors cut stress fibers

#2 - Magnetic twisting cytometry : creep compliance

Bausch BJ 1998
Fabry PRL 2001



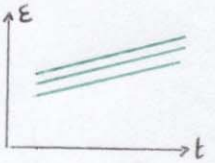


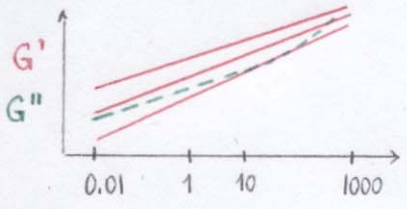
to explain experimental data, fit parameters
NO !!

but if you cover 5 orders of magnitude in frequency

slow dynamics : $t^{x-1} \sim t^{0.2}$

scale-free
- creep
- approach to equilibrium slower than exp.
- no instantaneous elasticity
- no distinct relaxation times



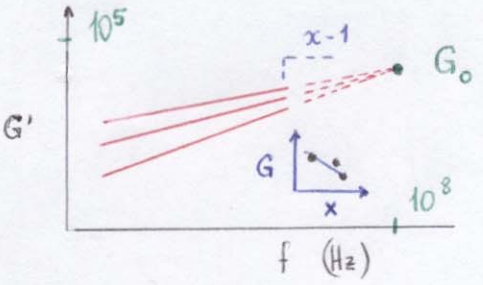


independent of cell type and scale of probe,
weak power law prevails

structural damping law

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

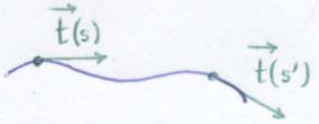
- Alcaraz BJ '03, Smith BJ '05 : AFM, Desprat BJ '05 : microplates, Fabry : MTC
- also conformation of nucleus
- 2-point microrheology Hoffmann PNAS'0



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

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

- <u>semi-flexible polymers</u> :
what accounts for the behavior of gels ? actin gels K_B bending stiffness
$l_p = \frac{K_B}{k_B T}$ persistence lengt



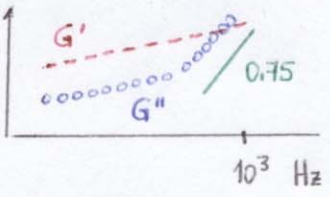
$\langle \vec{t}(s) . \vec{t}(s') \rangle = \exp\left(-\frac{s-s'}{l_p}\right)$

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

elasticity : "mechanical" spring constant at zero temperature $K_M \sim E a^2 l^{-1}$
<u>"thermal" spring</u> 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 : ρ density of filaments, a radius, ζ drag coefficient

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



observed in cells as well.
at high f frequency.

#3 - <u>aging and rejuvenation</u>
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 ⟹ aging
no steady state, trapping in deeper wells
- but ATP hydrolysis in biology ~ 25 $k_B T$
shear stress can give energy
⟹ rejuvenati
reset clock