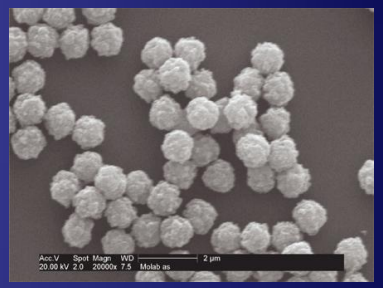
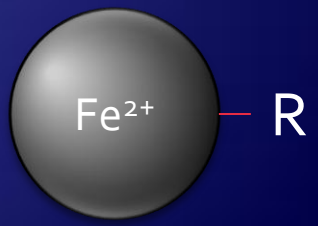
Session 9

MICRORHEOLOGY

Microbeads

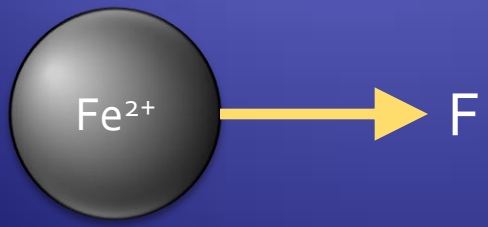
- In 1977, J. Ugelstad made batches of "identical" polystyrene beads
- Two step swelling process:
  - Polymer seed particle in water
  - Grows by incorporating slightly water soluble organic compounds
- Magnetic beads:
  - Porous beads with oxidative groups
  - Mixed with Fe^{2+} solutions
  - Forms magnetic oxides
- Surface chemistry tailored

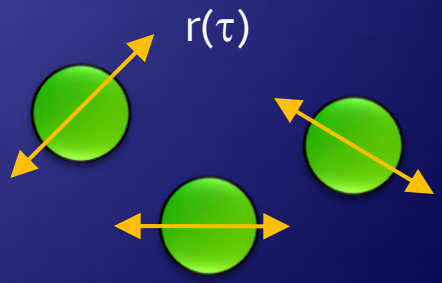




Microbeads for Forcing and Sensing

- Force system: Magnetic Tweezers
  - Coat the bead with ECM
  - Apply magnetic field to pull on bead
- Probe system: Microrheology
  - Inject fluorescent beads inside cell
  - Particle tracking of thermal fluctuations inside cell



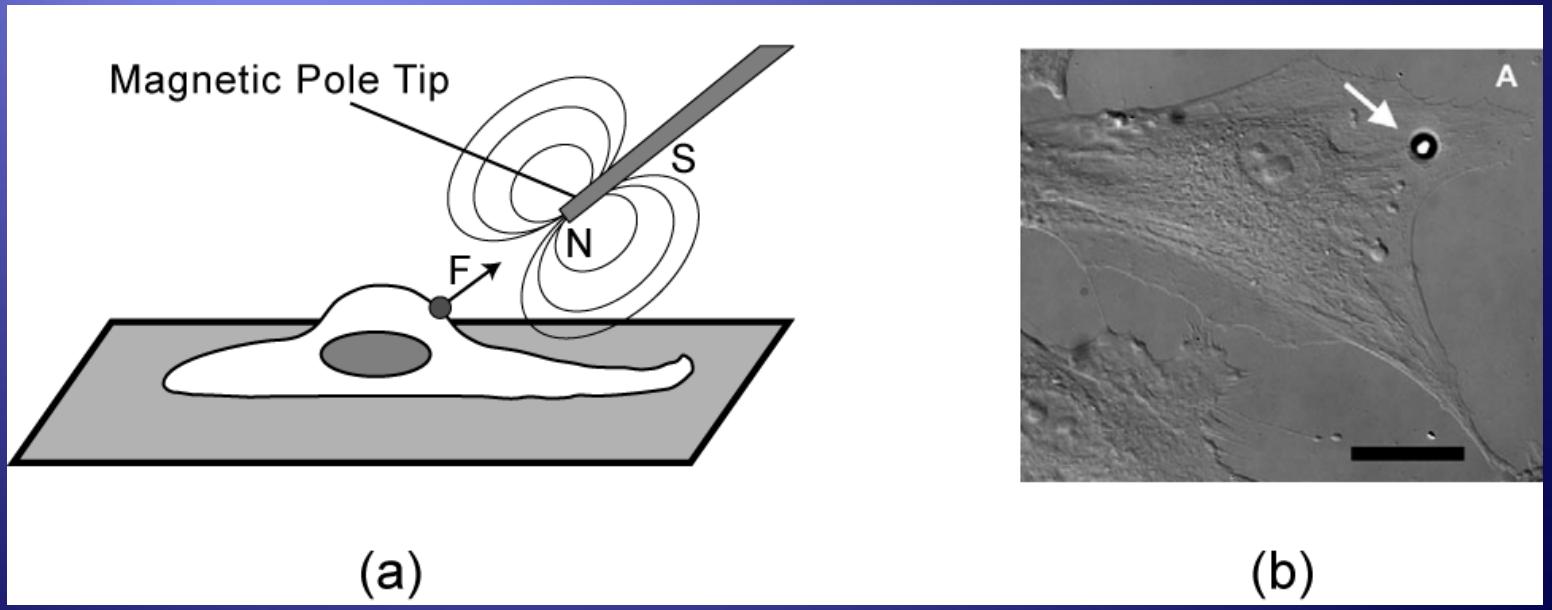


The Approach – Magnetic Tweezers

- Magnetic force from magnetic field gradient

$$F = \mu \bullet \nabla B$$

μ = magnetic moment B = magnetic field



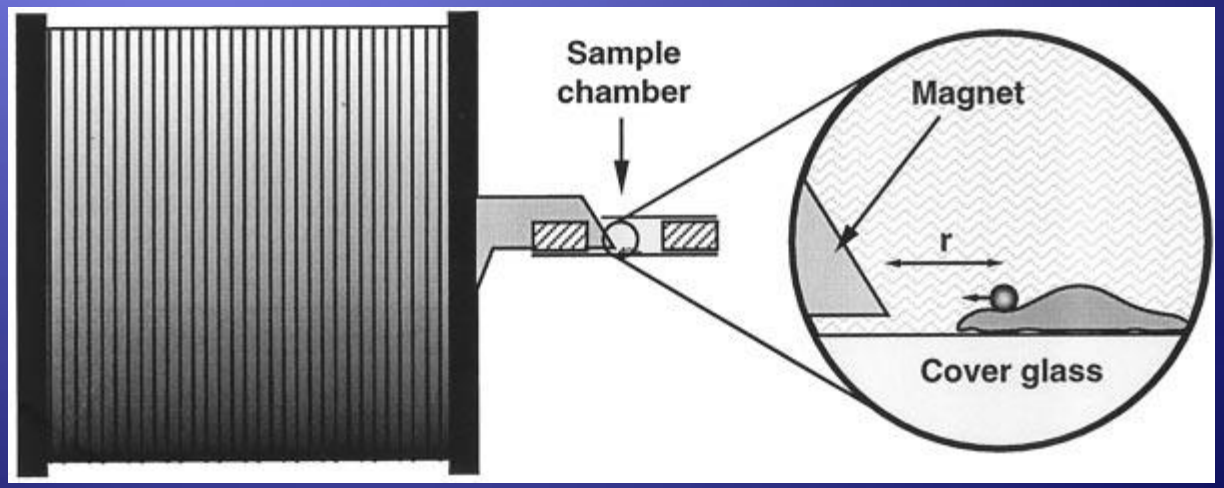
The System

- Electromagnetic Coil (Biot-Savart's Law)

$$|B| = N\mu_0 I/D$$

N = no. of loops μ_0 = permittivity

I = current D = coil diameter

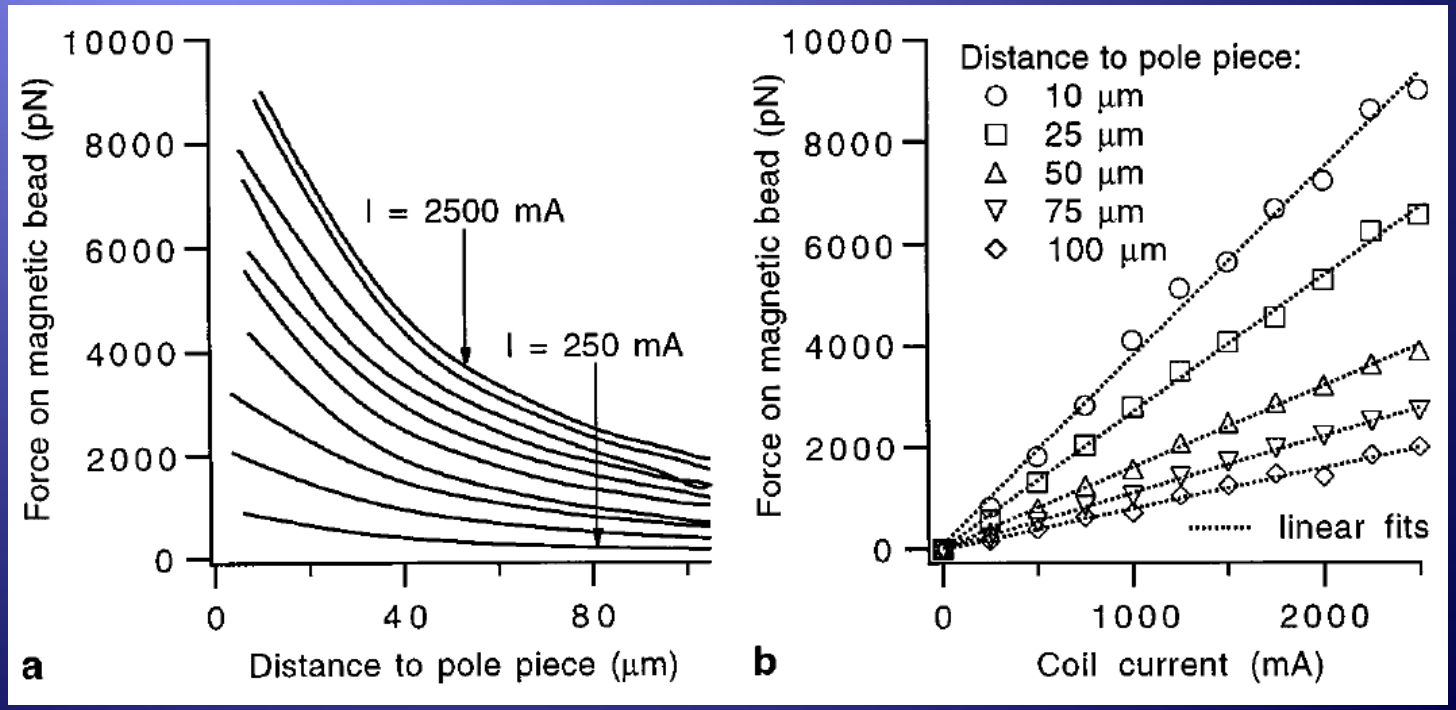


Calibration

- Stokes' Law for low Reynolds numbers

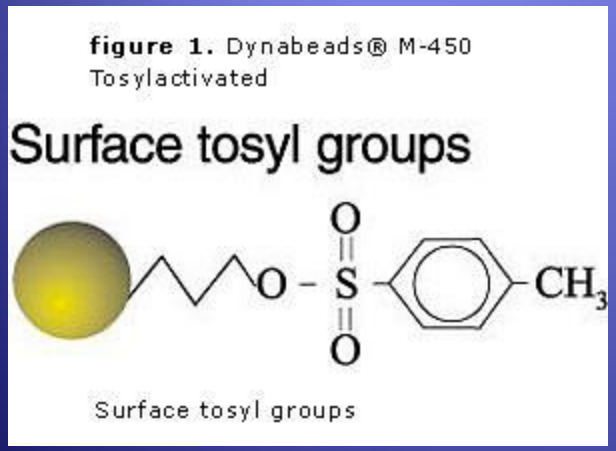
$$F = 6\pi\eta Rv$$

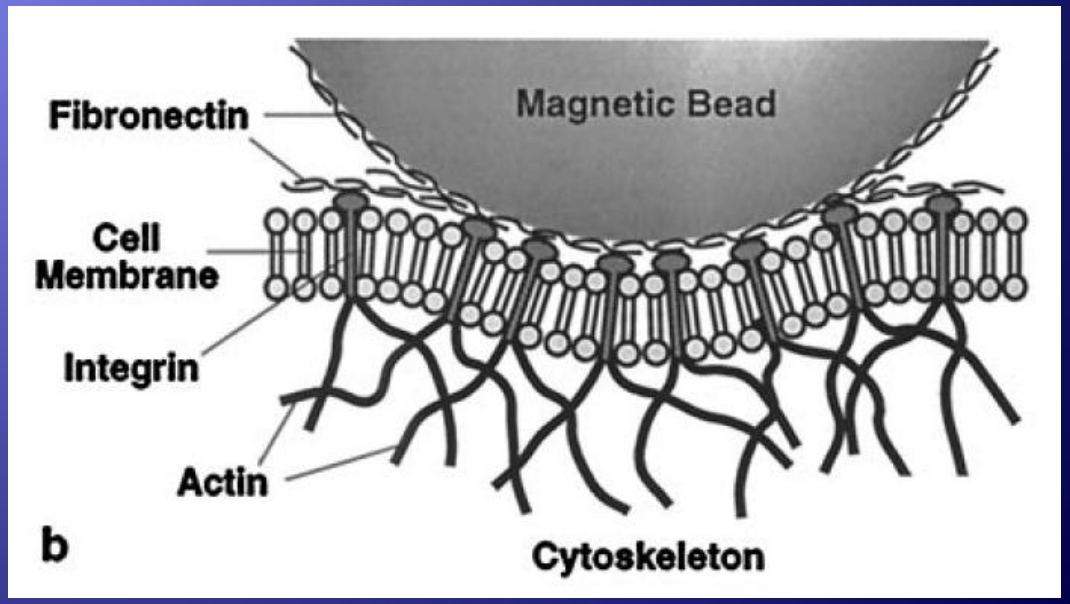
η = viscosity R = particle radius v = velocity



Adhesion-CSK Connection

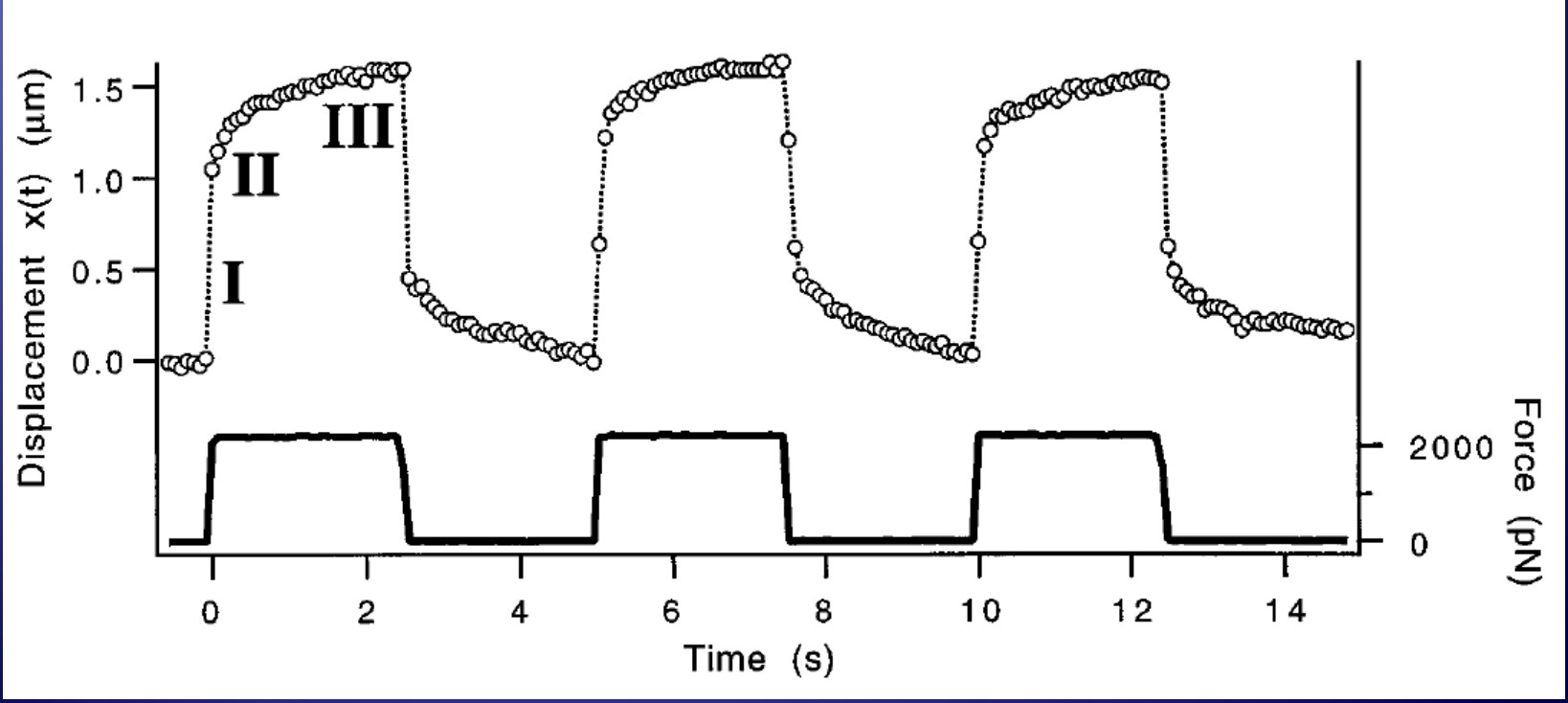
- Bead treated with *p*-toluene-sulfonyl (tosyl)
- Sulfonyl esters react covalently with fibronectin
- Direct coupling to CSK via integrins





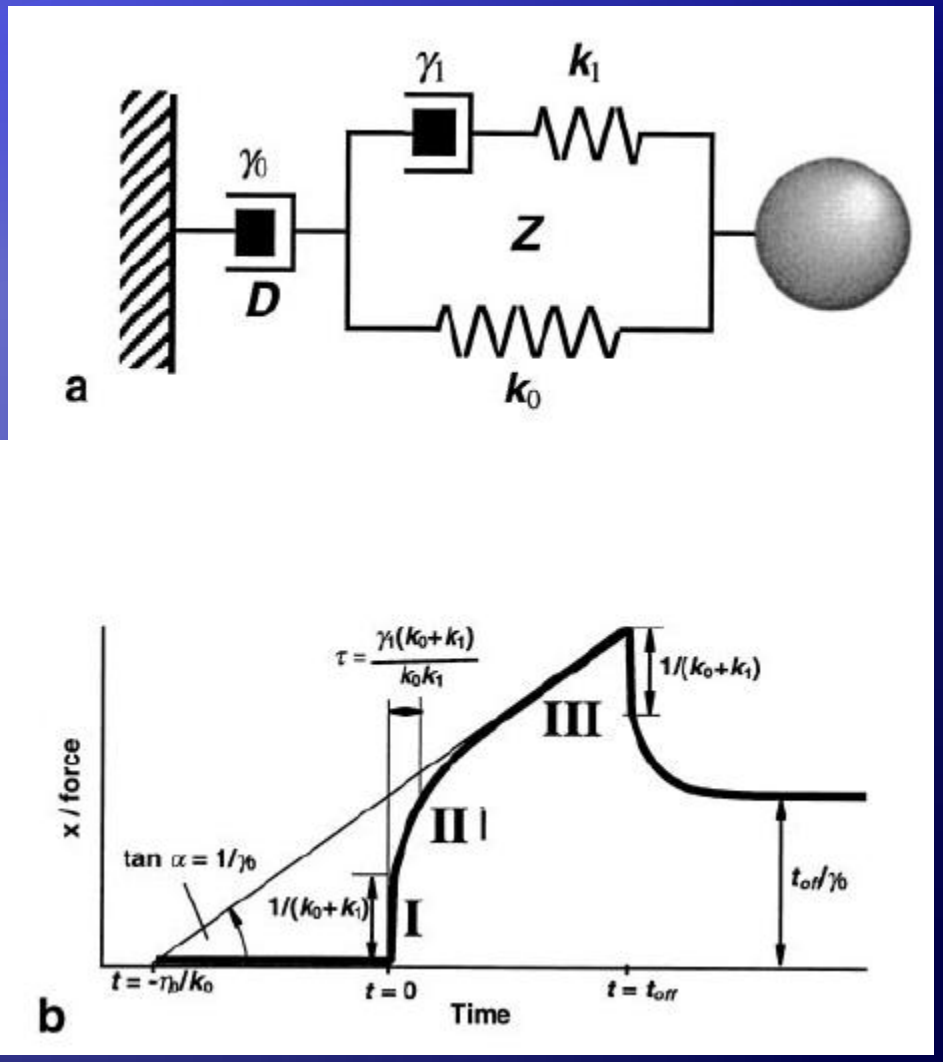
The Measurement

- "Classic" Creep Response
  - Elastic displacement (Regime I)
  - Relaxation (Regime II)
  - Steady-state Flow (Regime III)



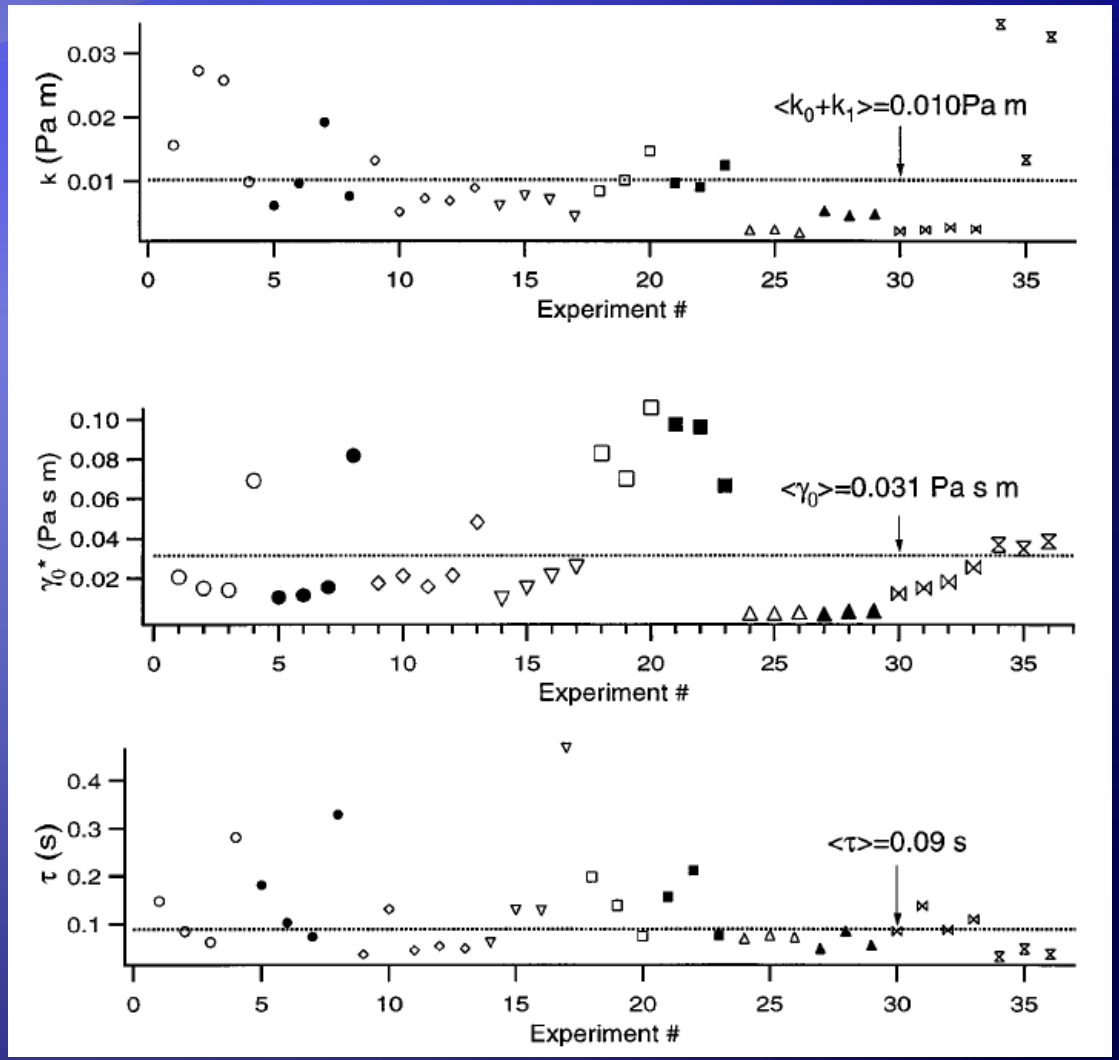
The Model

- Effective elastic modulus $k = k_1 + k_2$
- Viscosity γ_0
- Relaxation time τ



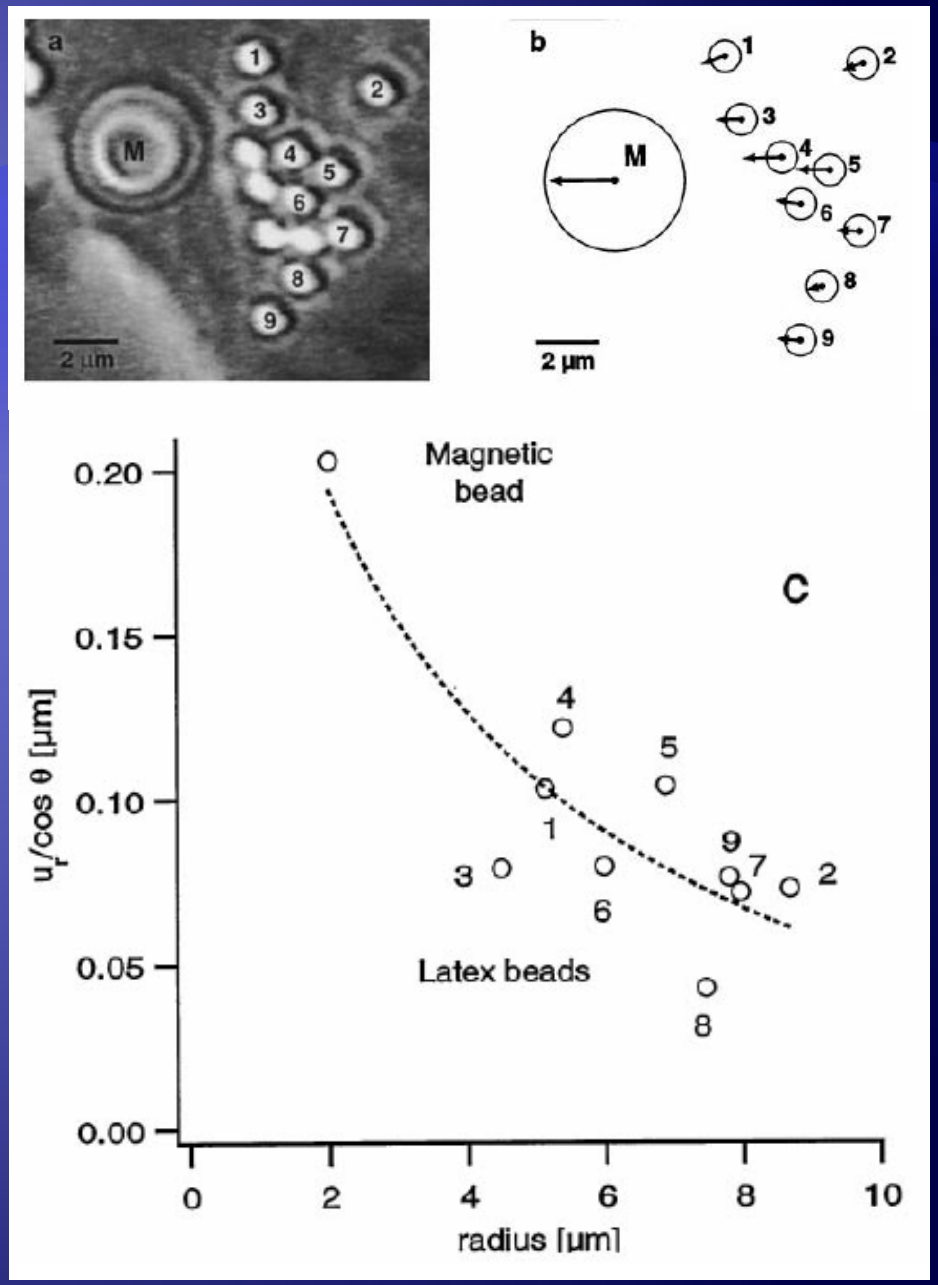
Viscoelastic Properties

- Same symbol:
  - Multiple measurements, one location
- Open/closed symbols:
  - Same cell, different locations
- Parameters vary cell-to-cell



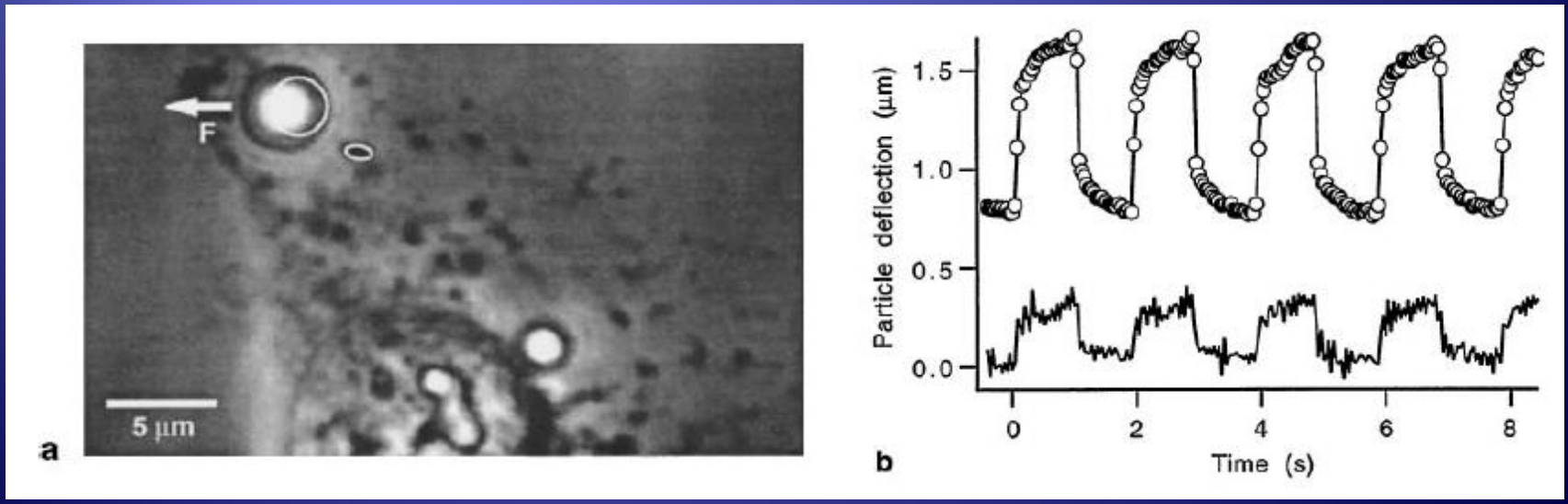
Strain Field

- Magnetic bead (M)
- Latex beads (#1-9)
  - Nonmagnetic, 1 μm
  - Fibronectin coated
  - 3 beads untrackable
- F = 3.7 nN, 1 sec



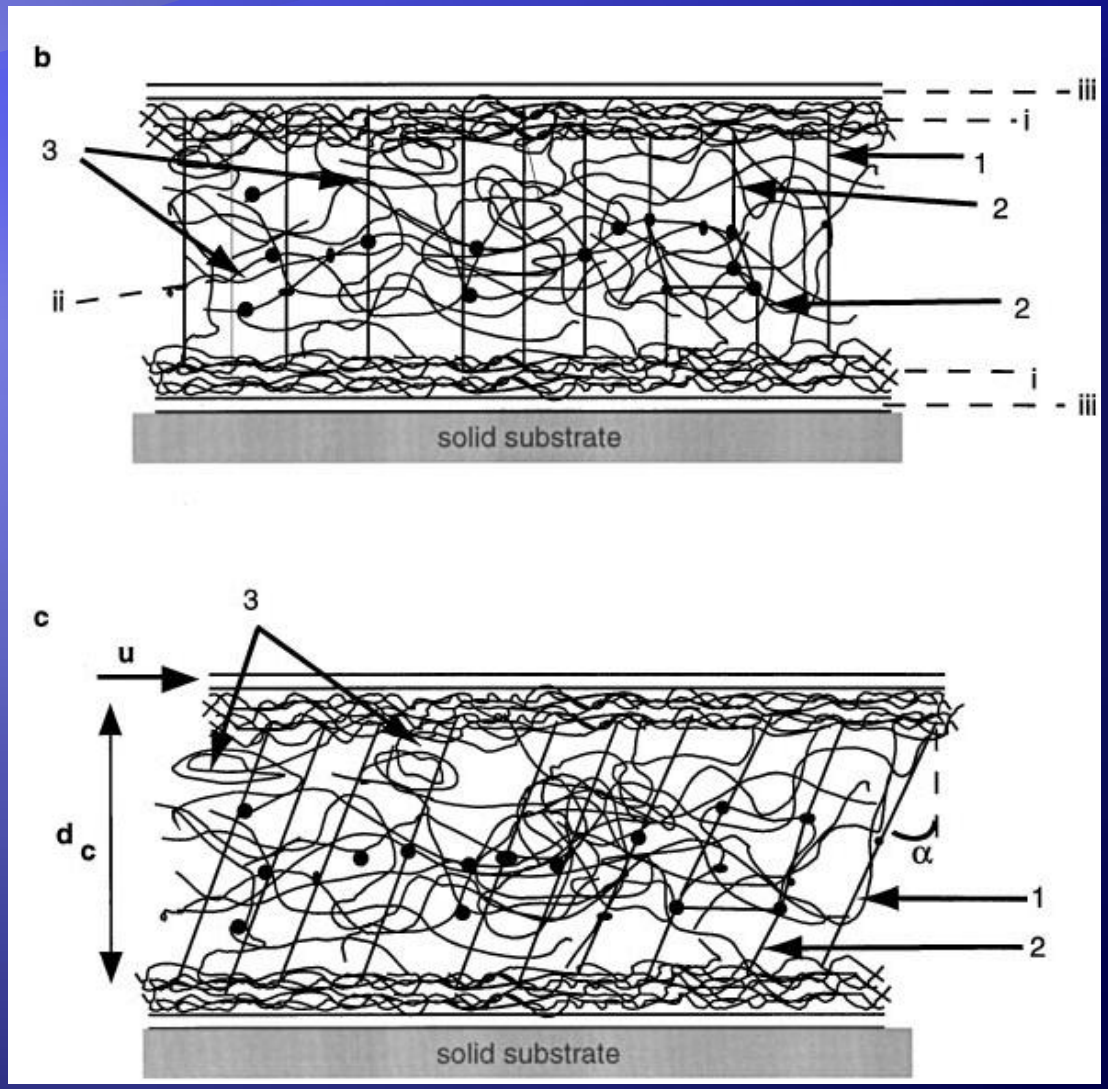
Deep strain fields

- Cell vacuoles show displacement under magnetic bead force.
- Shear on the cell surface penetrates partially into the cytoplasm



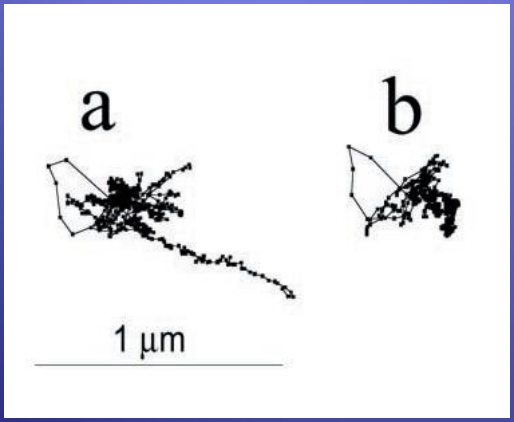
Cell Mechanical Coupling Model

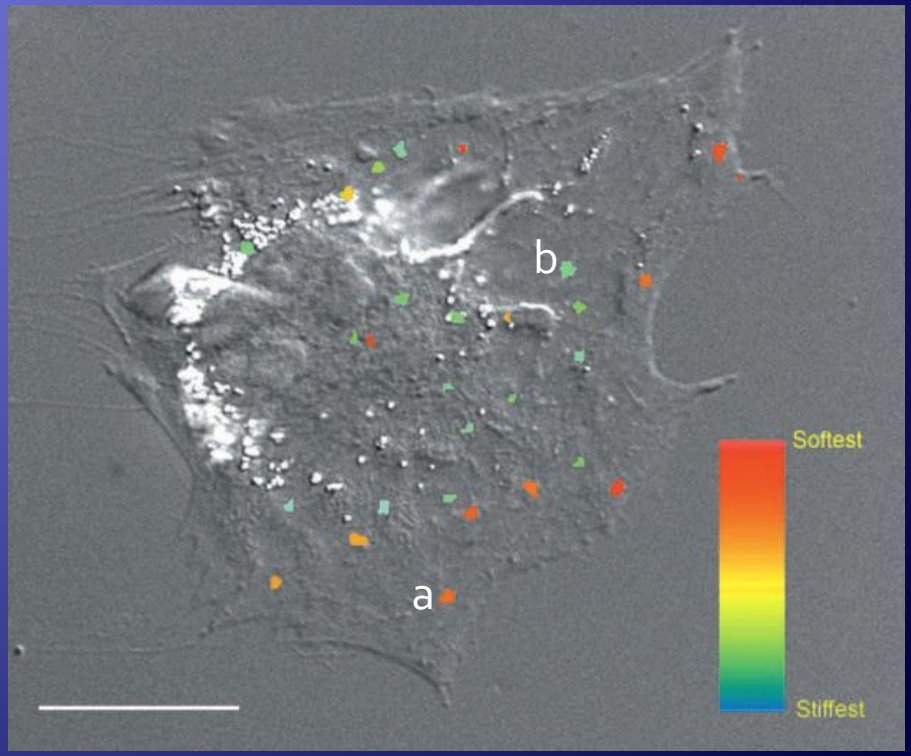
- Composite shell
  - Lipid-protein bilayer
  - Cortical actin



Microrheology

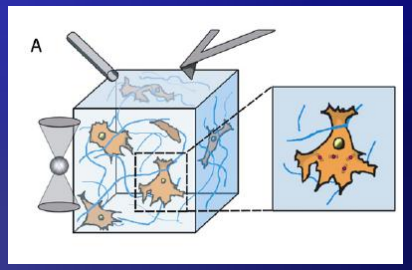
- Noninvasive probe of local viscoelasticity
- Monitor the motion of particles (100 nm diameter)

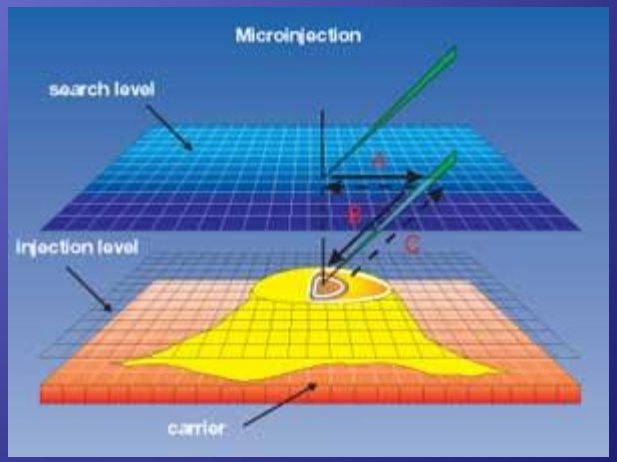


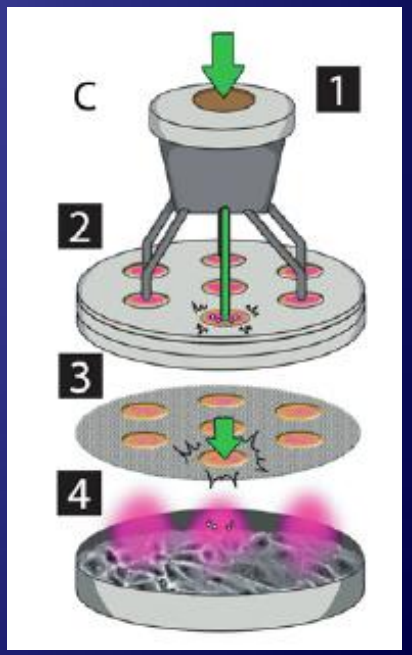


The Method

- Injecting probes into cytoplasm
  - Microinjector for 2D loading
  - Ballistic injection for 3D loading

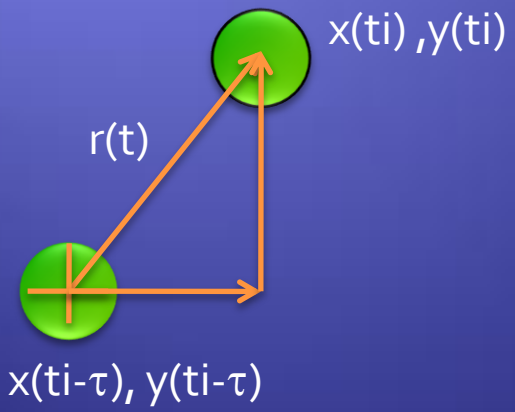






Particle Tracking

- Mean squared displacement (MSD)… $\langle \Delta r^2(\tau) \rangle$
  - τ is the time lag (a/k/a frame rate)
  - t is the elapsed time (a/k/a video length)
  - $\langle \Delta r^2(\tau) \rangle = MSD_x(\tau) + MSD_y(\tau)$



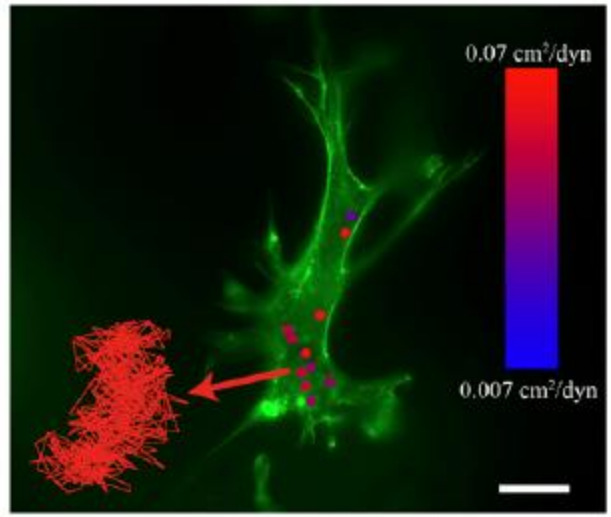
$$MSD_x(\tau) = \frac{\sum_{i=1}^{N} (x(t_i - \tau) - x(t_i))^2}{N+1}$$

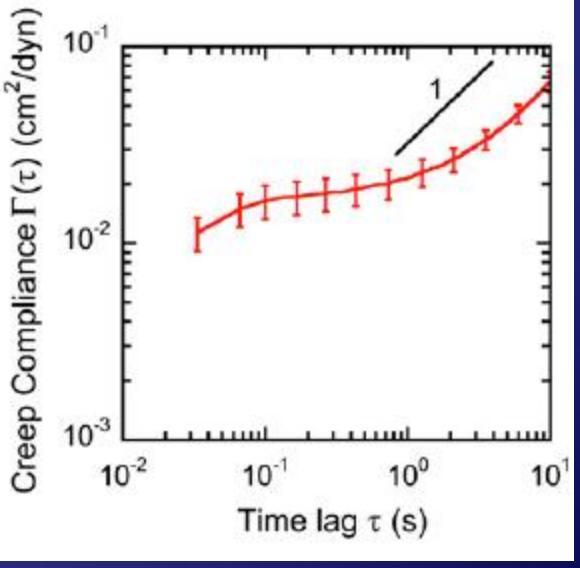
$$MSD_y(\tau) = \frac{\sum_{i=1}^{N} (y(t_i - \tau) - y(t_i))^2}{N+1}$$

Local Creep Compliance, $\Gamma(\tau)$

- Describes local deformations of cytoplasm by thermal motion of particles

$$\Gamma(\tau) = \frac{3\pi D}{4k_B T} < \Delta r^2(\tau) > \quad [\text{cm}^2/\text{dyne}]$$





Viscoelastic Properties

- Complex viscoelastic shear modulus, G*(ω)

$$|G^*(\omega)| = \frac{2k_BT}{3\pi\langle\Delta r^2(1/\omega)\rangle\Gamma(1+\alpha(\omega))}$$

where $\omega = 1/\tau$ and $\alpha(\omega) = \partial(\ln\langle\Delta r^2(\tau)\rangle)/\partial(\ln t)$

- Shear storage modulus

$$G'(\omega) = |G^*(\omega)|\cos(\pi\alpha(\omega)/2)$$

- Shear loss modulus

$$G''(\omega) = |G^*(\omega)|\sin(\pi\alpha(\omega)/2)$$

Viscous Liquids vs. Elastic Solids

- For water or glycerol

$$\left\langle \Delta r^2(\tau) \right\rangle = \frac{2k_B T D_0 \tau}{\pi \eta D} \qquad G^*(\omega) = i\eta\omega$$

  - Storage Modulus: $G'(\omega) = \mathrm{Re}(G^*) = 0$
  - Loss Modulus: $G''(\omega) = \mathrm{Im}(G^*) = \eta$

- For a solid (Laplace transformed)

$$< \Delta r^2(s) >= \frac{2k_B T}{\pi G D} \frac{1}{s} \qquad G^*(\omega) = G$$

  - Storage Modulus: $G'(\omega) = \mathrm{Re}(G^*) = G$
  - Loss Modulus: $G''(\omega) = \mathrm{Im}(G^*) = 0$

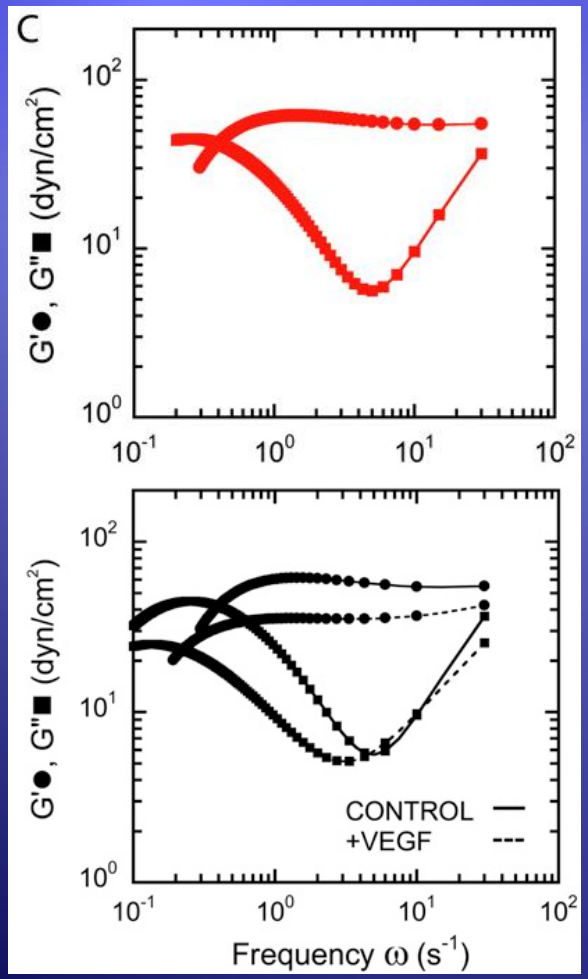
Shear

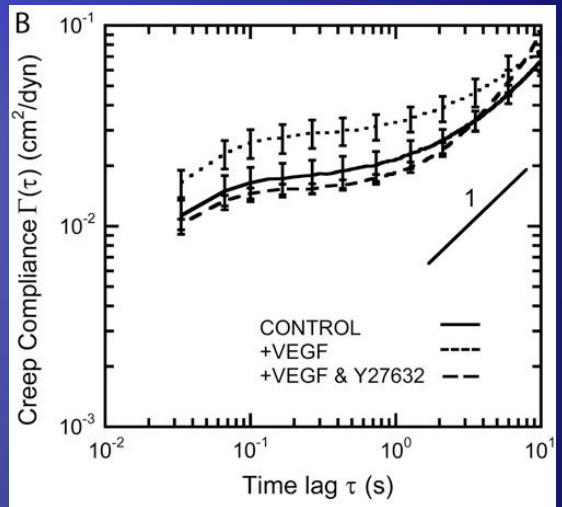
- Shear Stress $\tau = F/A$
- Shear Strain $\gamma = \delta/H$
- Shear Rate $d\gamma/dt = (d\delta/dt)/H$

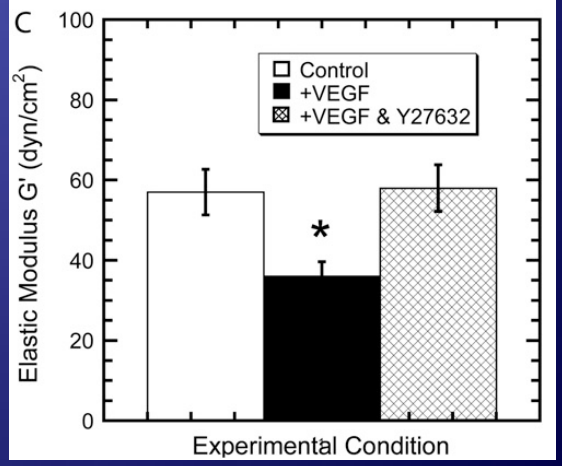
- $\tau = G\,\gamma$ (Solid) storage of strain
- $\tau = \eta\, d\gamma/dt$ (Fluid) dissipation of strain

Viscous Liquid to Viscous Solid

- VEGF softens a cell, but ROCK inhibition restores stiffness







QUESTIONS?