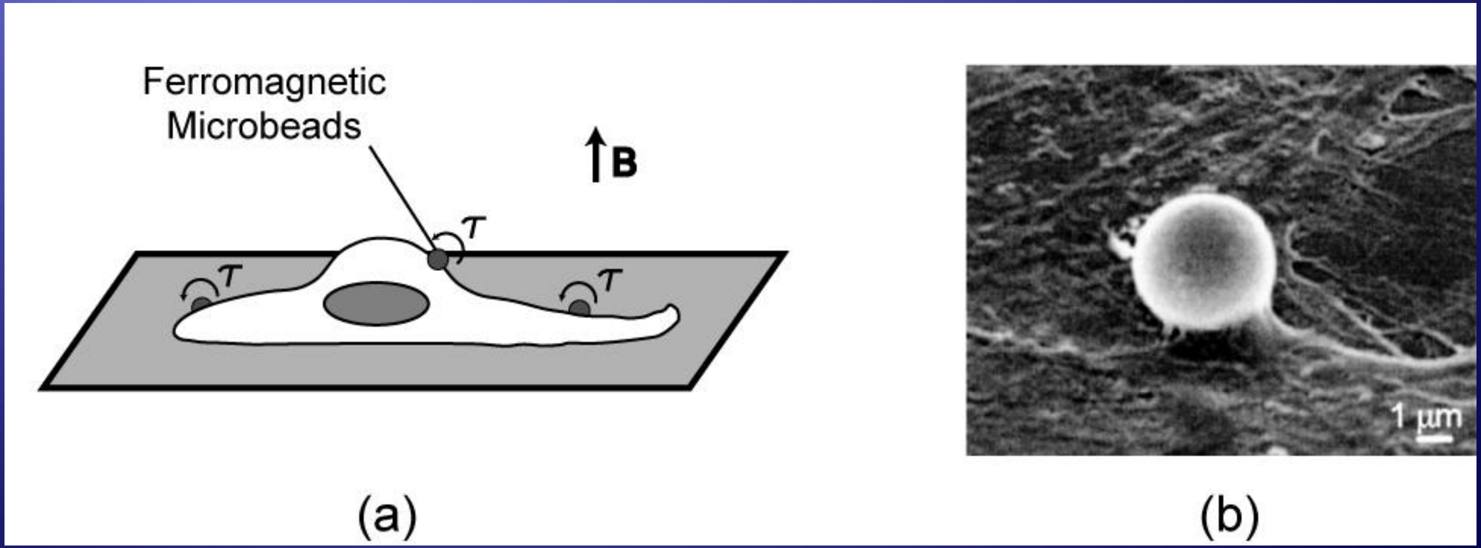
Session 10

MAGNETIC TWISTING CYTOMETRY

Approach

- Torque applied at surface of cells with magbeads
- Used to determine cell mechanics
  - Cellular viscoelasticity (Fabry, Fredberg)
  - Mechanotransduction (Wang, Ingber)

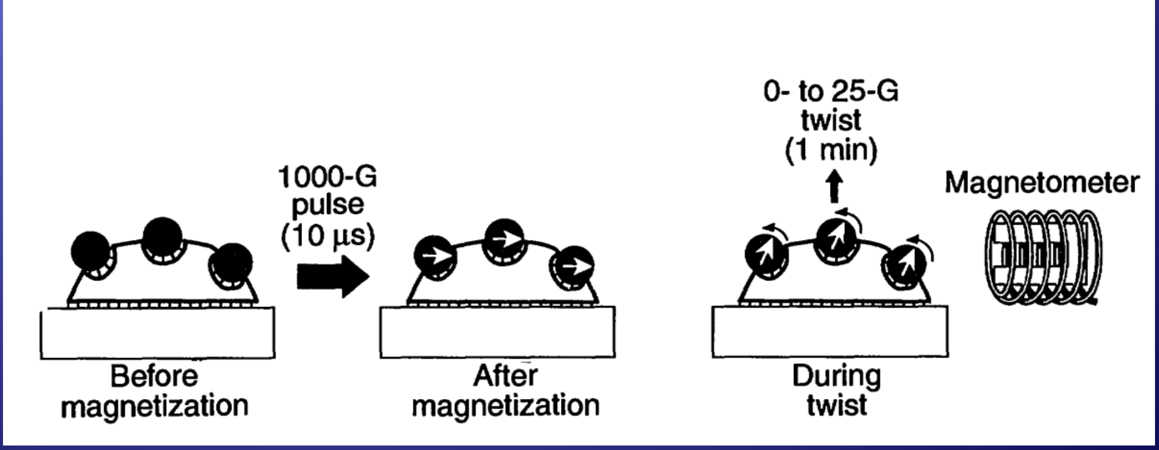


Technique

- Field applied briefly in one direction to magnetize
- Sinusoidal field applied in other direction to twist
- Torque applied: $\tau = \mu \times H$

  μ is magnetic moment

  H is sinusoidal external field

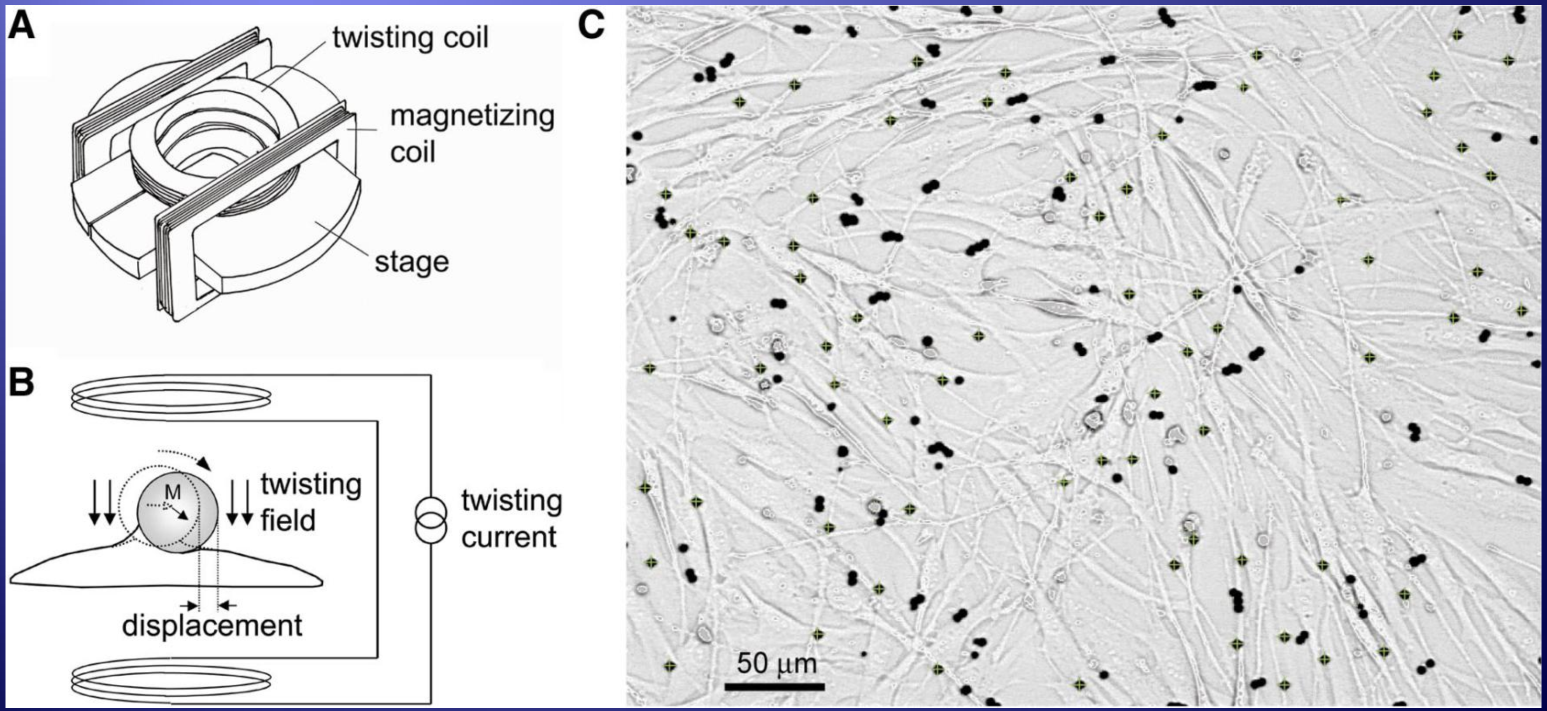


Apparatus

A) Microscope stage with electromagnetic coils

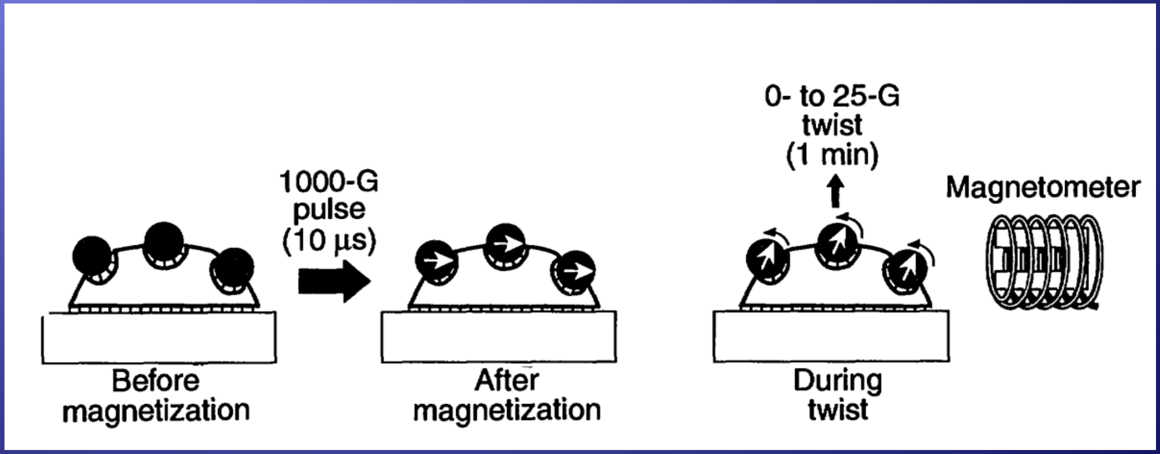
B) Twist causes magbead rotation and translation

C) Human airway smooth muscle cells with magbeads



Magnetic Measurement

- Magnetometer detects field produced by dipoles
- Twisting field rotates beads (and dipoles)
- Decrease in B field at magnetometer ≈ twist



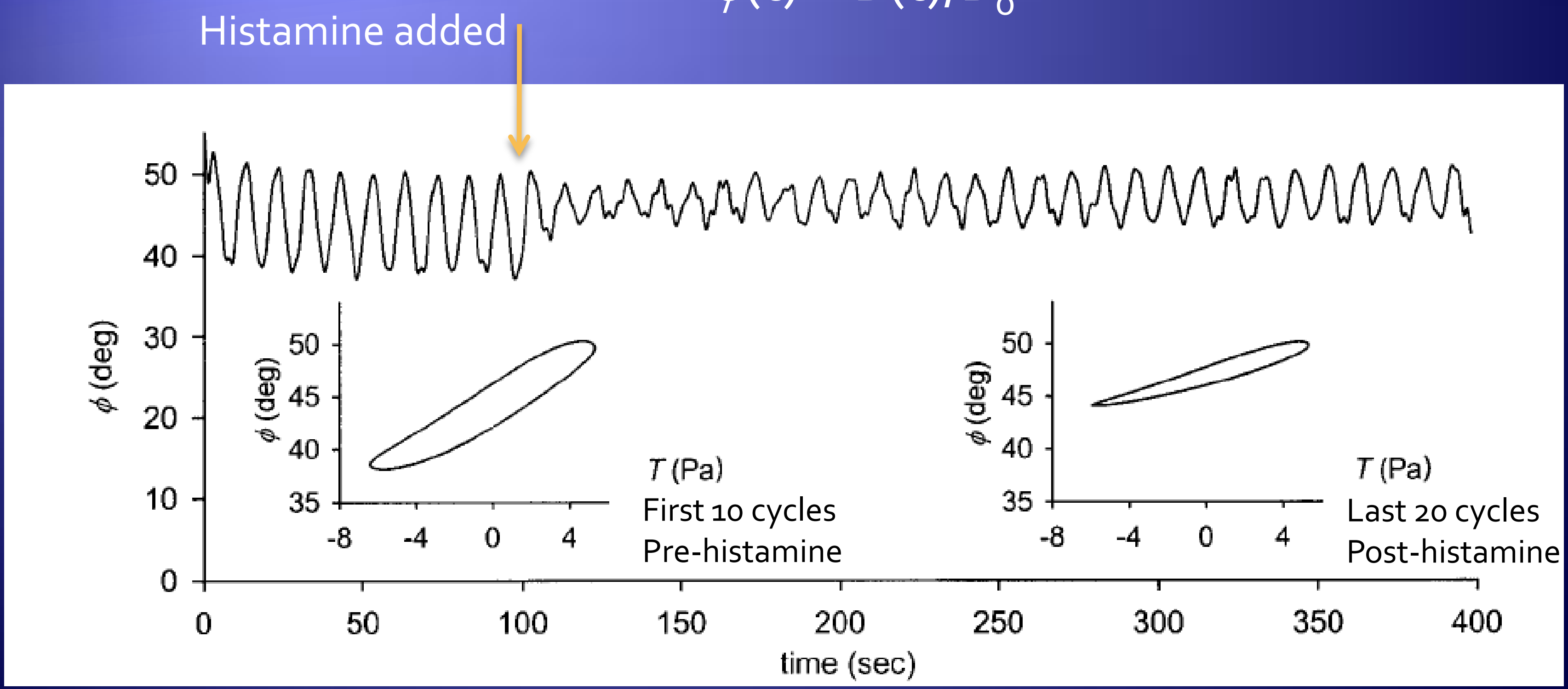
Torque Response

- For magbeads: $T(t) = c\,H(t)\cos\phi(t)$

$c = 0.41$ Pa/gauss

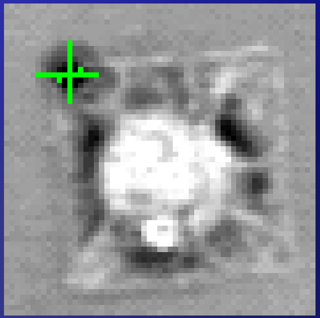
$H(t) = H \sin(t)$

$\phi(t) = B(t)/B_o$



Particle Tracking Measurement

- Fast, real-time image analysis
- Individual particle tracking better than aggregated magnetometry
- Pixel-to-displacement, intensity weighted algorithm of centroid position per frame



					9	7	9				
				9	7	2	7	9			
			9	7	2	0	2	7	9		
			7	2	0	0	0	2	7		
			9	7	2	0	2	7	9		
				9	7	2	7	9			
					9	7	9				

→

		9	7	9							
	9	7	2	7	9						
9	7	2	0	2	7	9					
7	2	0	0	0	2	7					
9	7	2	0	2	7	9					
	9	7	2	7	9						
		9	7	9							

Firm Integrin and CSK connection

- Ligand-bound integrins *actively* resist the twist

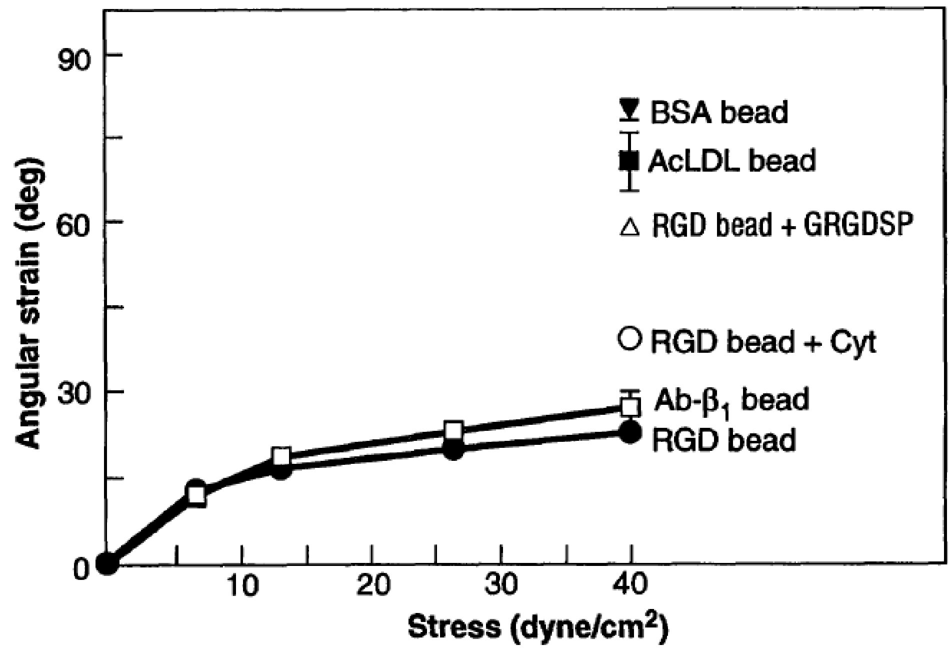
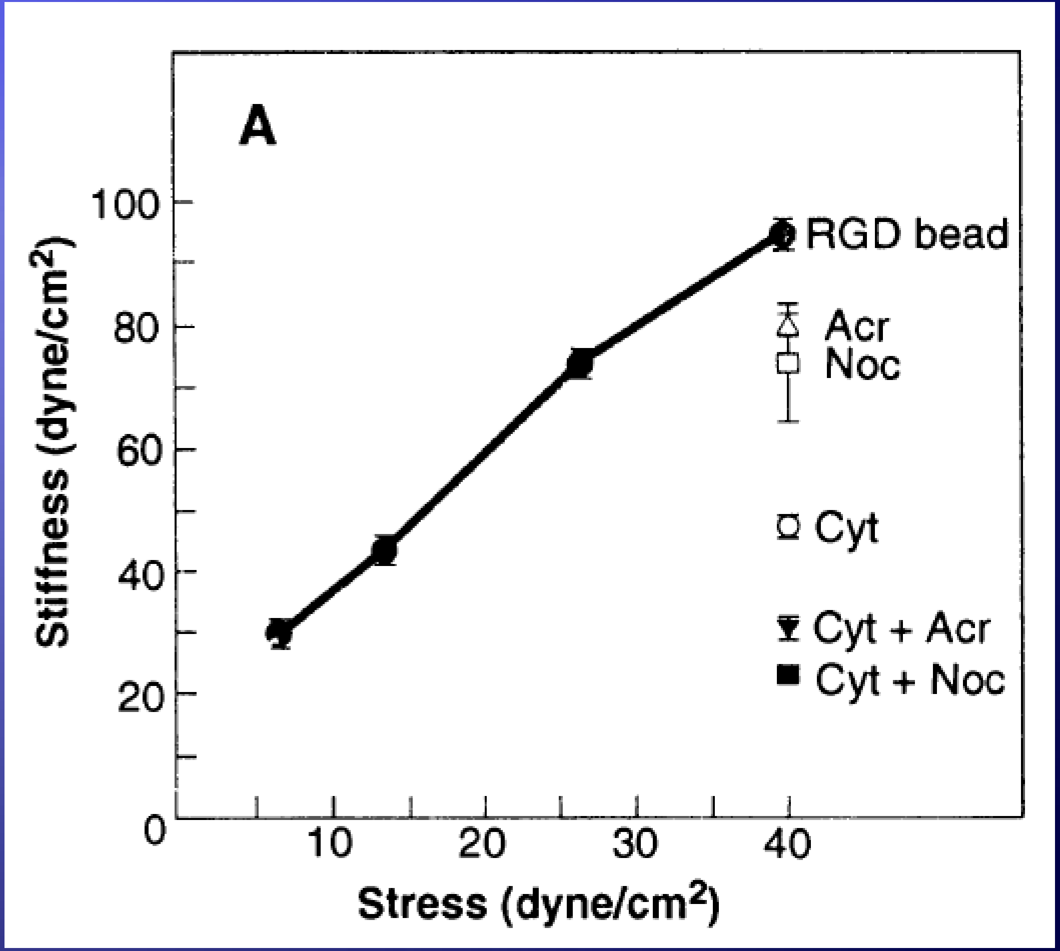


Fig. 2. Stress-strain relation measured with magnetic microbeads attached to the surfaces of living cells. Applied stress was determined by a calibration technique in which the same beads were twisted in a standard solution of known viscosity (*22*). Angular strain (bead rotation) was calculated as the arc cosine of the ratio of remanent field after 1 min of twist to the field at time 0. Angular strain is plotted here as degrees. Bead coatings were as follows: RGD, Arg-Gly-Asp–containing synthetic peptide; Ab-β_1, antibodies against integrin β_1; AcLDL, acetylated–low density lipoprotein; BSA, bovine serum albumin; GRGDSP, soluble fibronectin peptide (1 mg/ml added for 10 min); Cyt, cytochalasin D (0.1 μg/ml). Measurements analyzing the effects of different bead coatings with or without GRGDSP were made at stresses from 0 to 40 dyne/cm^2; for clarity, intermediate data points are shown only for Ab-β_1 and RGD beads that exhibit integrin-dependent stiffening. The effects of cytochalasin D were measured only at the highest stress. Error bars = SEM.

CSK Resists the Twist

- CSK Inhibitors
  - Acrylamide (Acr) inhibits IF
  - Nocodozal (Noc) inhibits MT
  - Cytochalasin D (Cyt) inhibits actin
- Full inhibition of CSK needed for "free" twisting



Rheological Measurements

- Complex Modulus: (s, Fourier transform)
  - Magnetometry: $G^*(s) = T(s) / \phi(s)$
  - Optical Tracking: $G^*(s) = T(s) / x(s)$

- Storage and Loss Moduli:

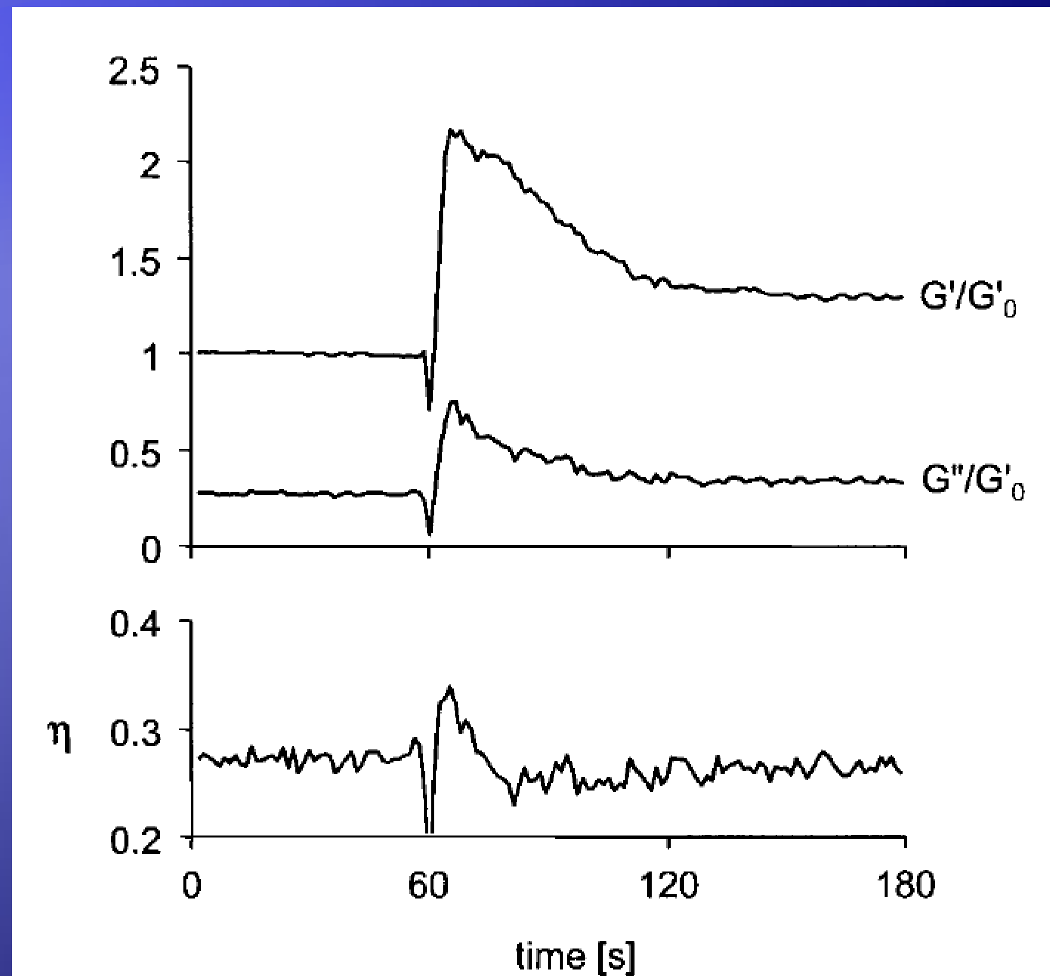
$$G^*(s) = G'(S) + iG''(s)$$

- Hysteresivity (lag time):

$$\eta(s) = G''(S) / G'(s)$$

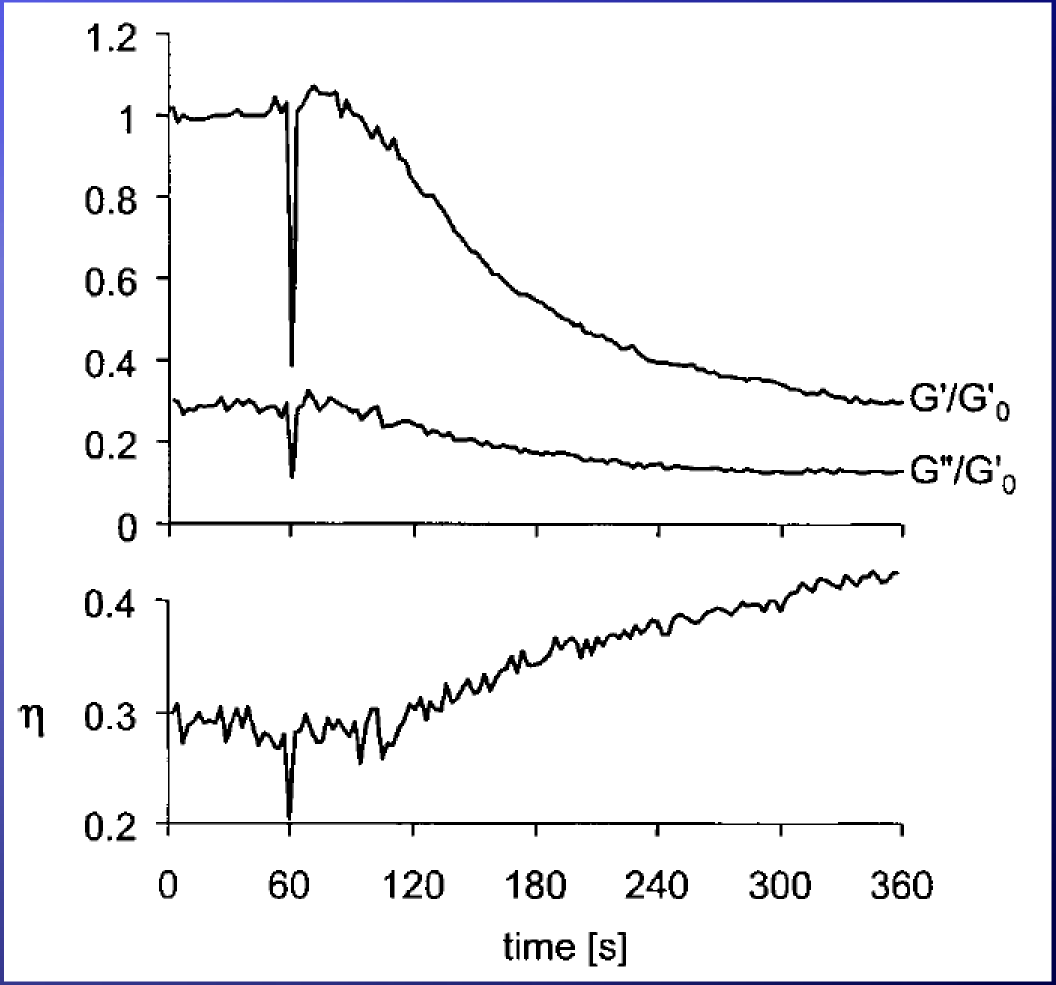
Histamine Stiffens hASMCs

- Histamine activates myosin
- Baseline steady before histamine
- Storage modulus increased 2.2X
- Loss modulus increased 3X
- Transient hysteresistivity response



Dibutryrl cAMP softens hASMCs

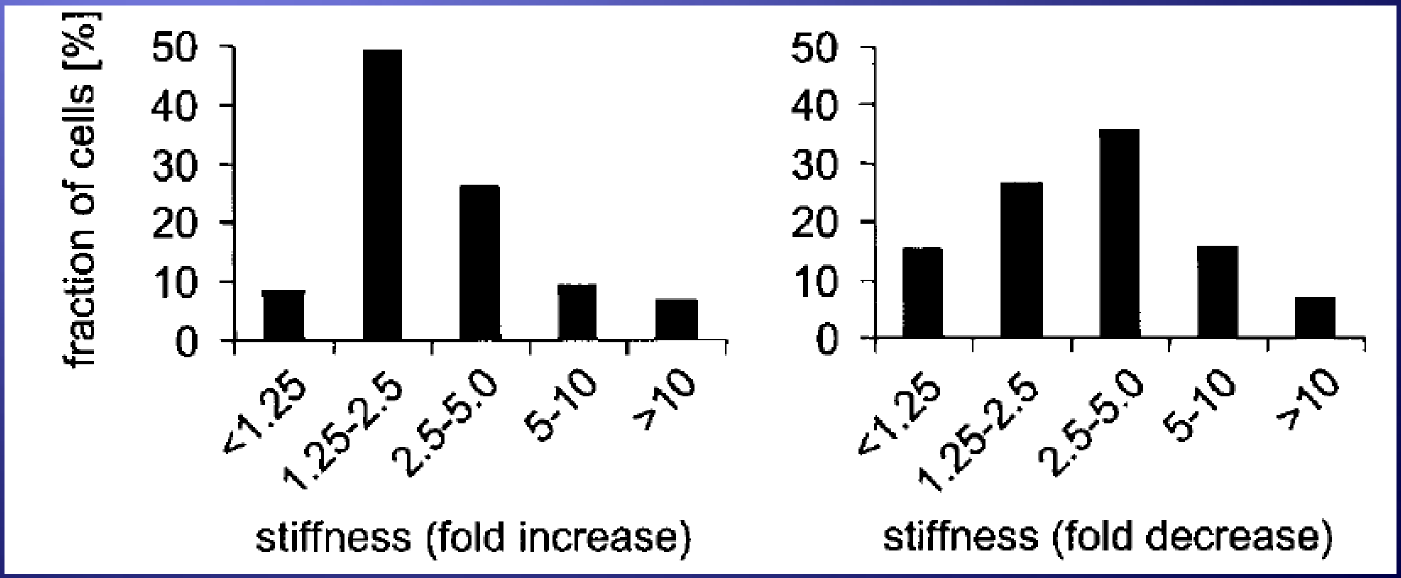
- DBcAMP inhibits myosin, leads to SM relaxation
- G′ falls to 30%
- G″ decreased to 45%
- η increased 1.5X



Wide Variability in Cells

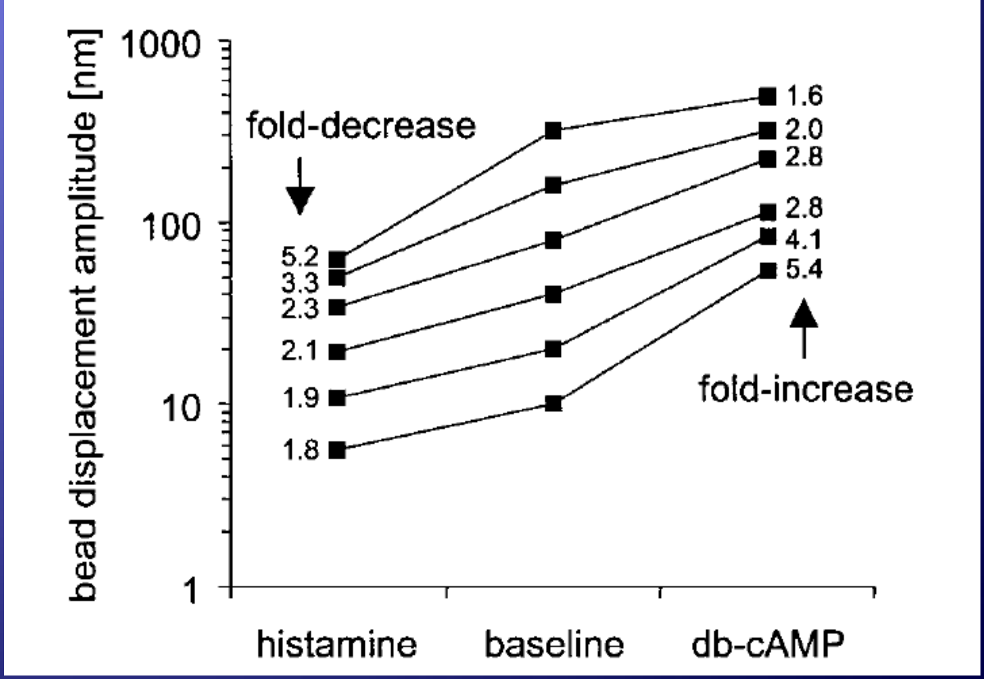
- Not donor-to-donor, day-to-day, or culture-to-culture, but cell-to-cell variability
- Wide distribution requires large sample populations

Histamine-stiffening DBcAMP-softening



Baseline Affects Stiffening/Softening

- "Soft" baseline cells: More histamine-stiffening, less DBcAMP-softening
- "Hard" baseline cells: More DBcAMP-softening, less histamine-stiffening



Questions?