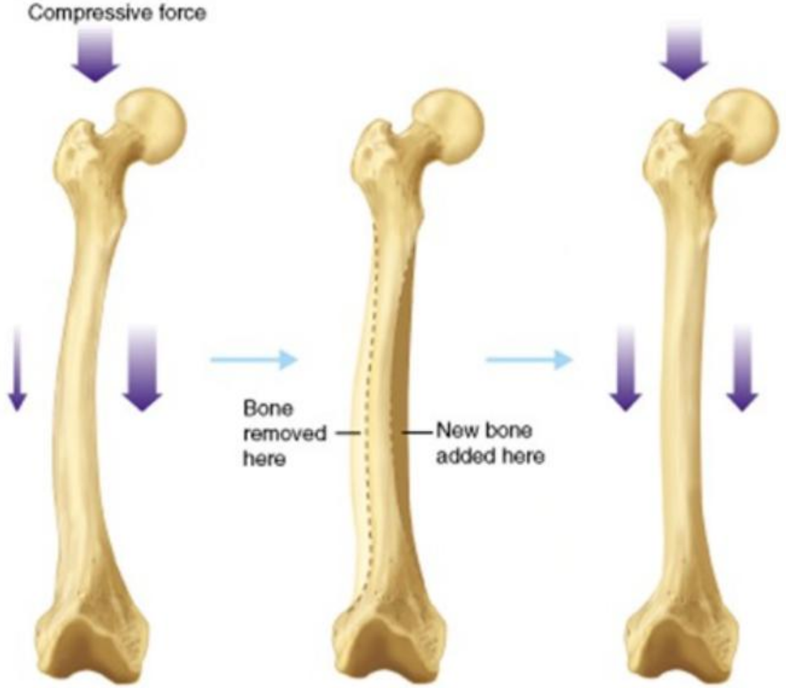
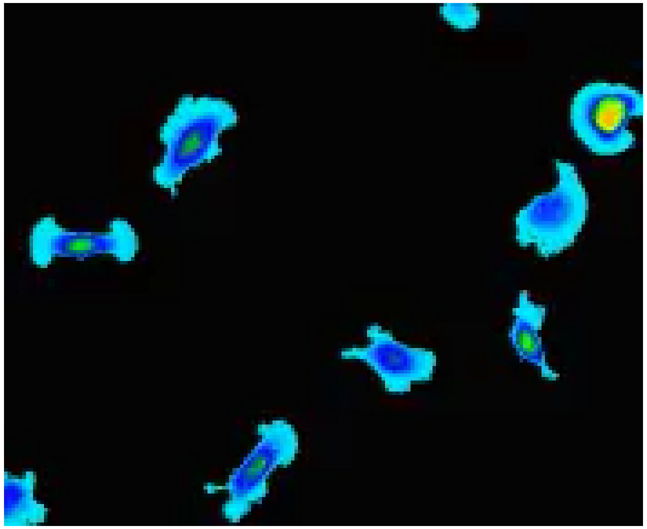
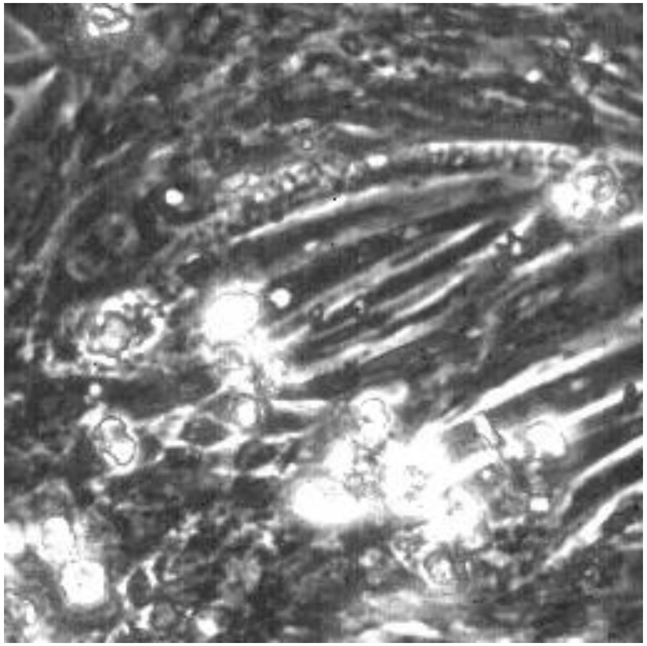

Compressive force
Bone removed here
New bone added here


us op


L
R

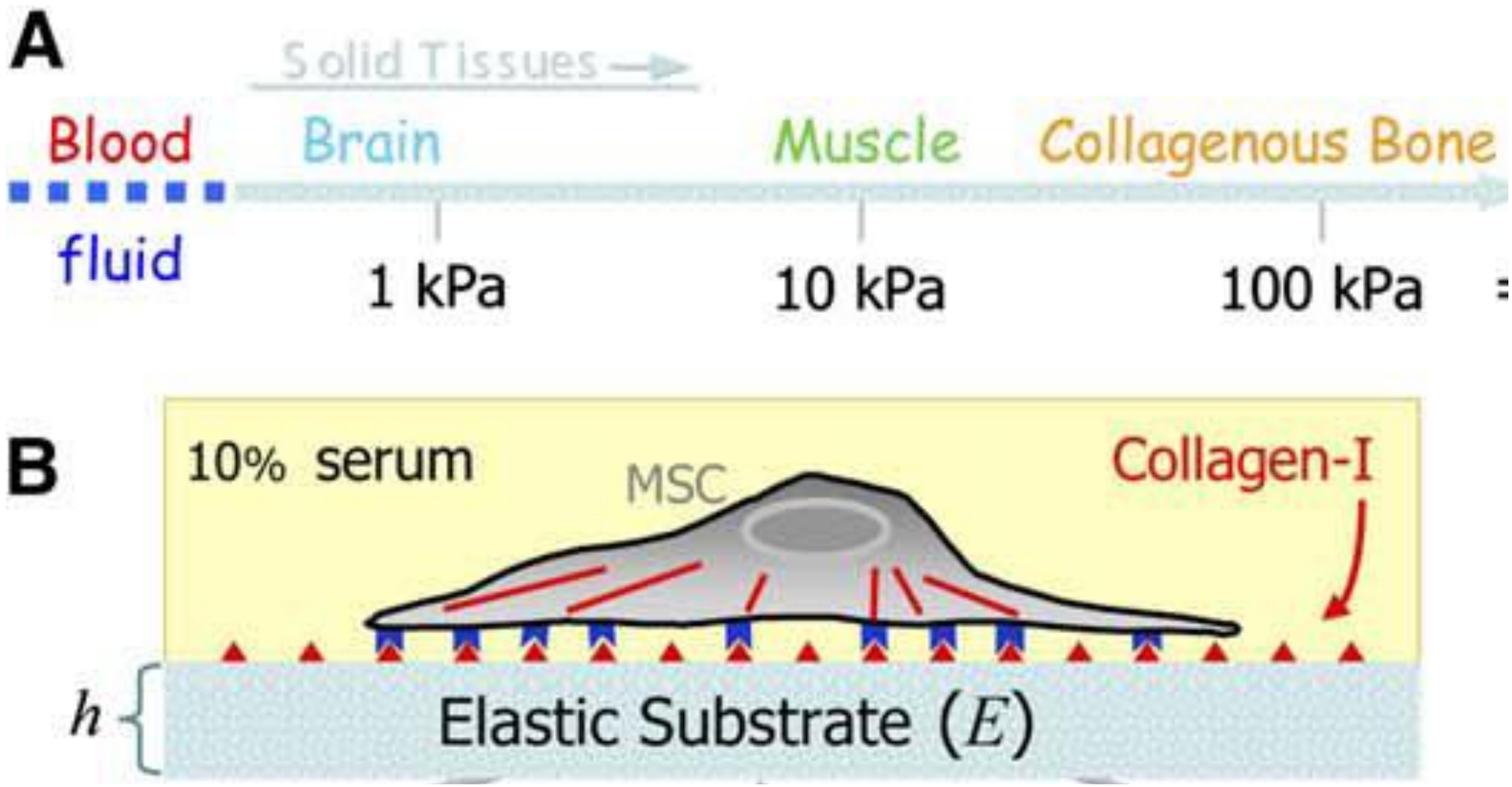
Cellular forces



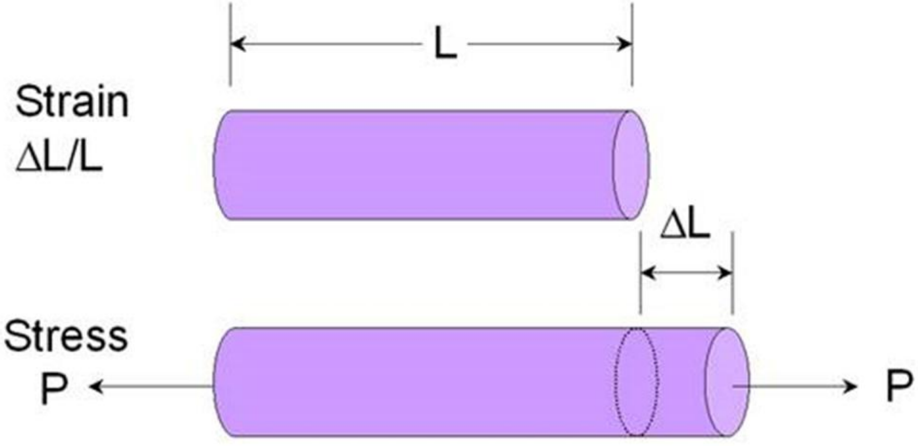


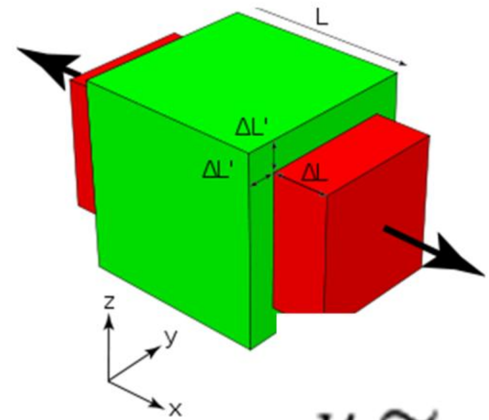
- Cells generate forces and use them to carry out numerous functions.
- Cells measure the mechanical response of the environment to forces

Engler, *et al.*, *Cell*, **126**:677-89 (2006).



Linear, isometric materials

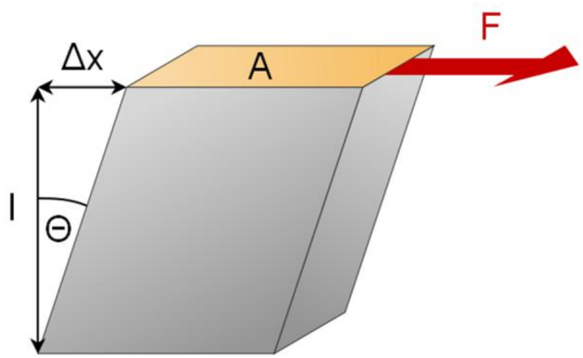




$$\nu \approx -\frac{\Delta L'}{\Delta L}$$

$$\text{Young's Modulus} = \frac{\text{Stress}}{\text{Strain}} = \frac{P}{\Delta L/L}$$

Poisson ratio
0.5 for incompressible material

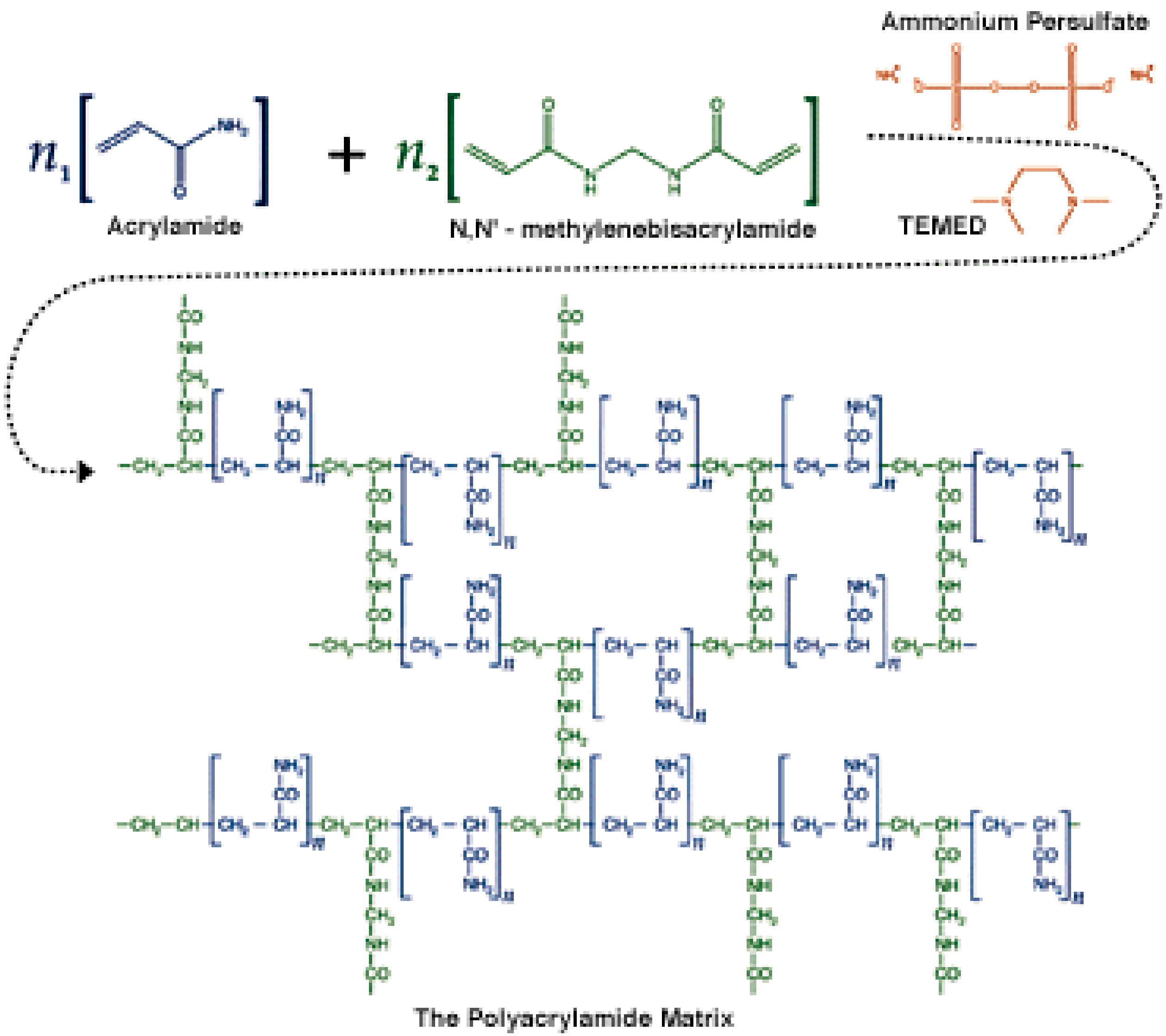


$$E = 2G(1+\nu) \qquad G = \frac{E}{2(1+\nu)}$$

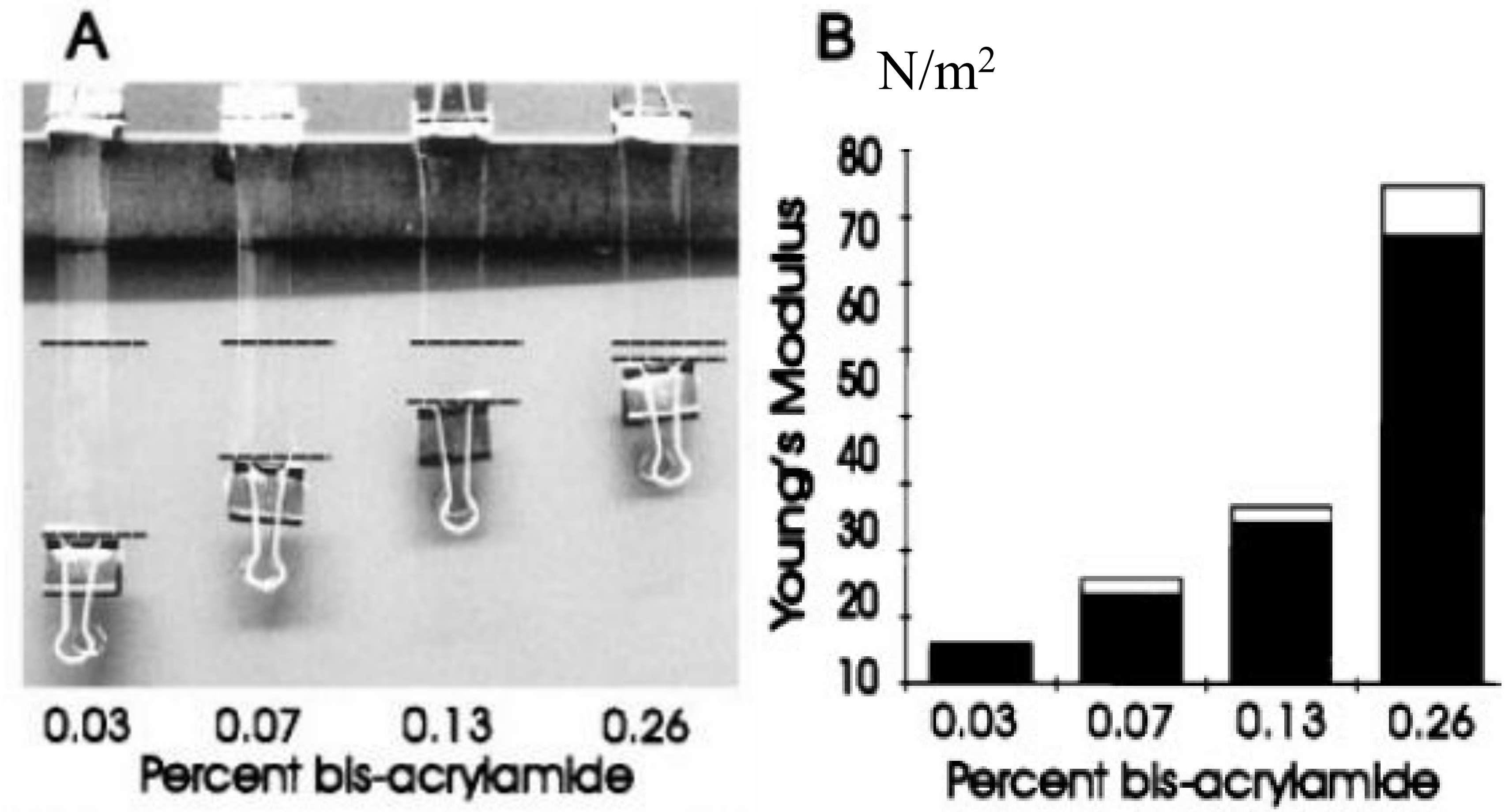
$$\nu = \frac{E}{2G} - 1$$

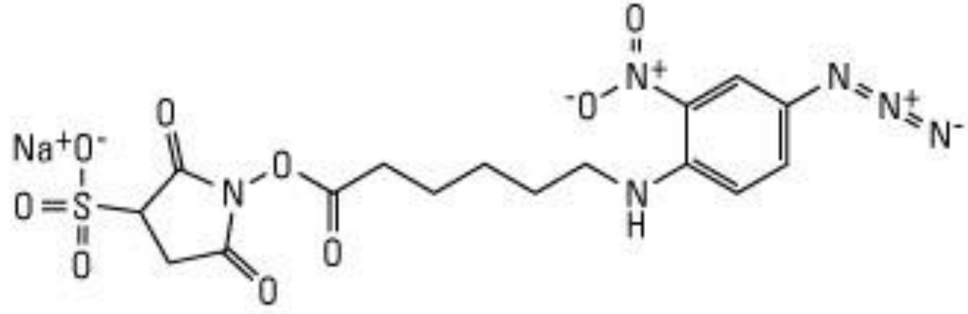
Shear modulus $G = \frac{F/A}{\Delta x/I} = \frac{FI}{A\Delta x}$

Pelham, RJ, and Wang, Y-L, *PNAS*, **94**: 13661 (1997)



Pelham, RJ, and Wang, Y-L, *PNAS*, **94**: 13661 (1997)



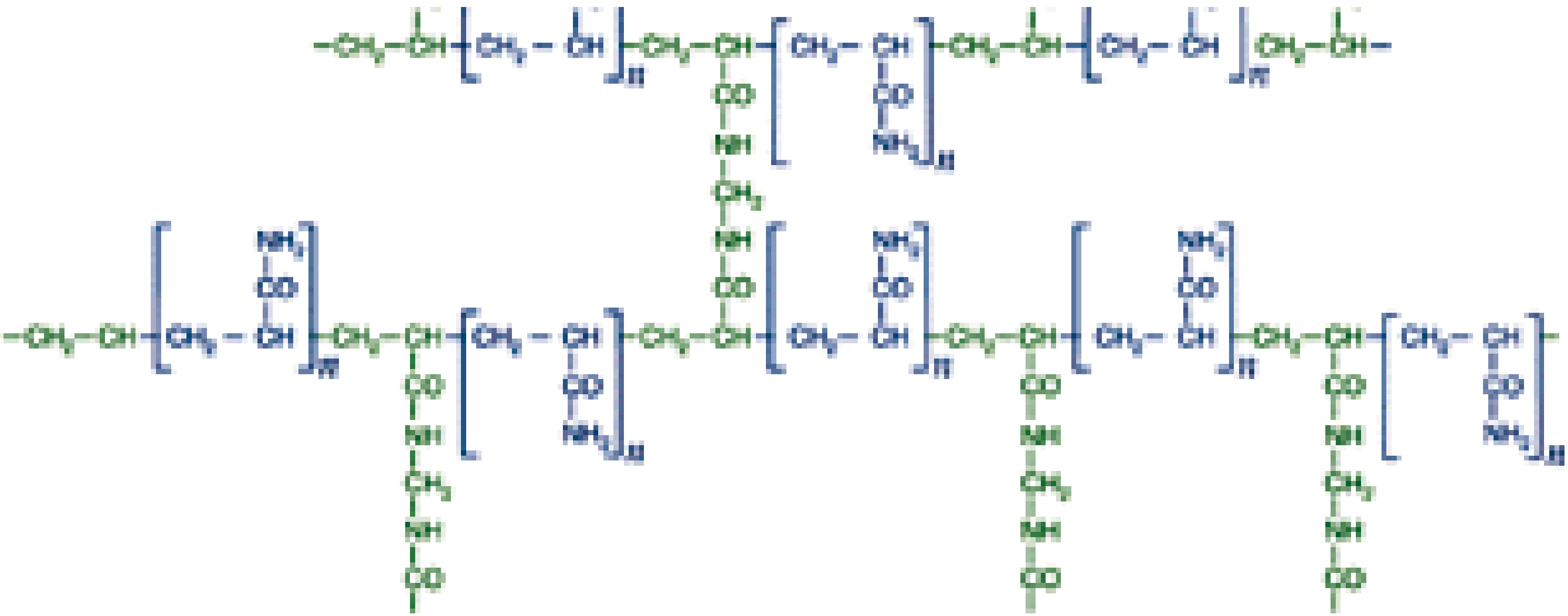


Sulfo-SANPAH

Sulfosuccinimidyl 6-(4'-azido-2'-nitrophenylamino)hexanoate

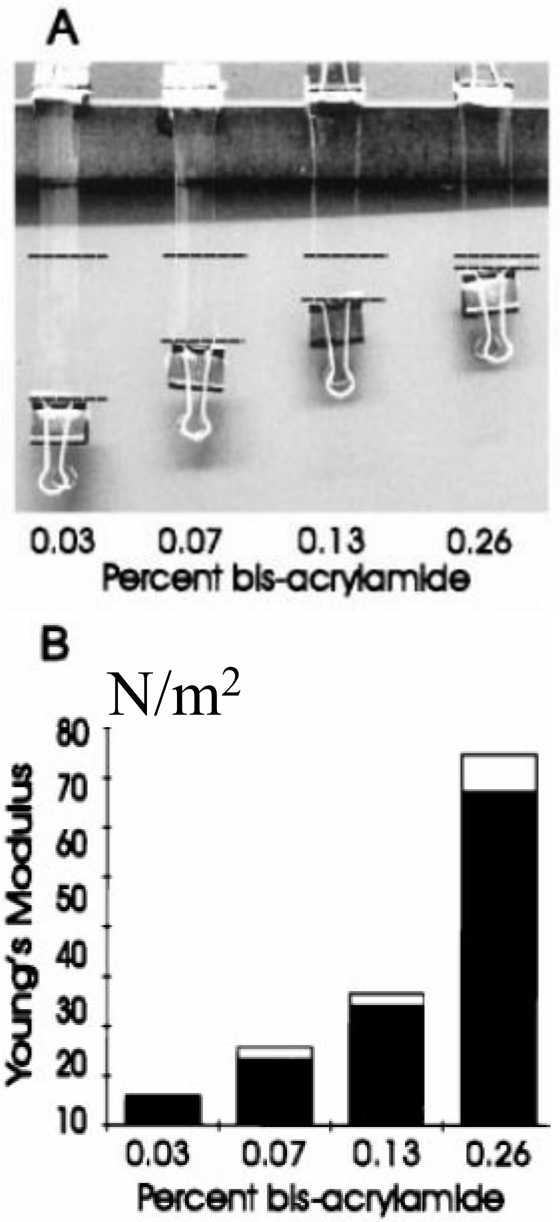
MW 492.40

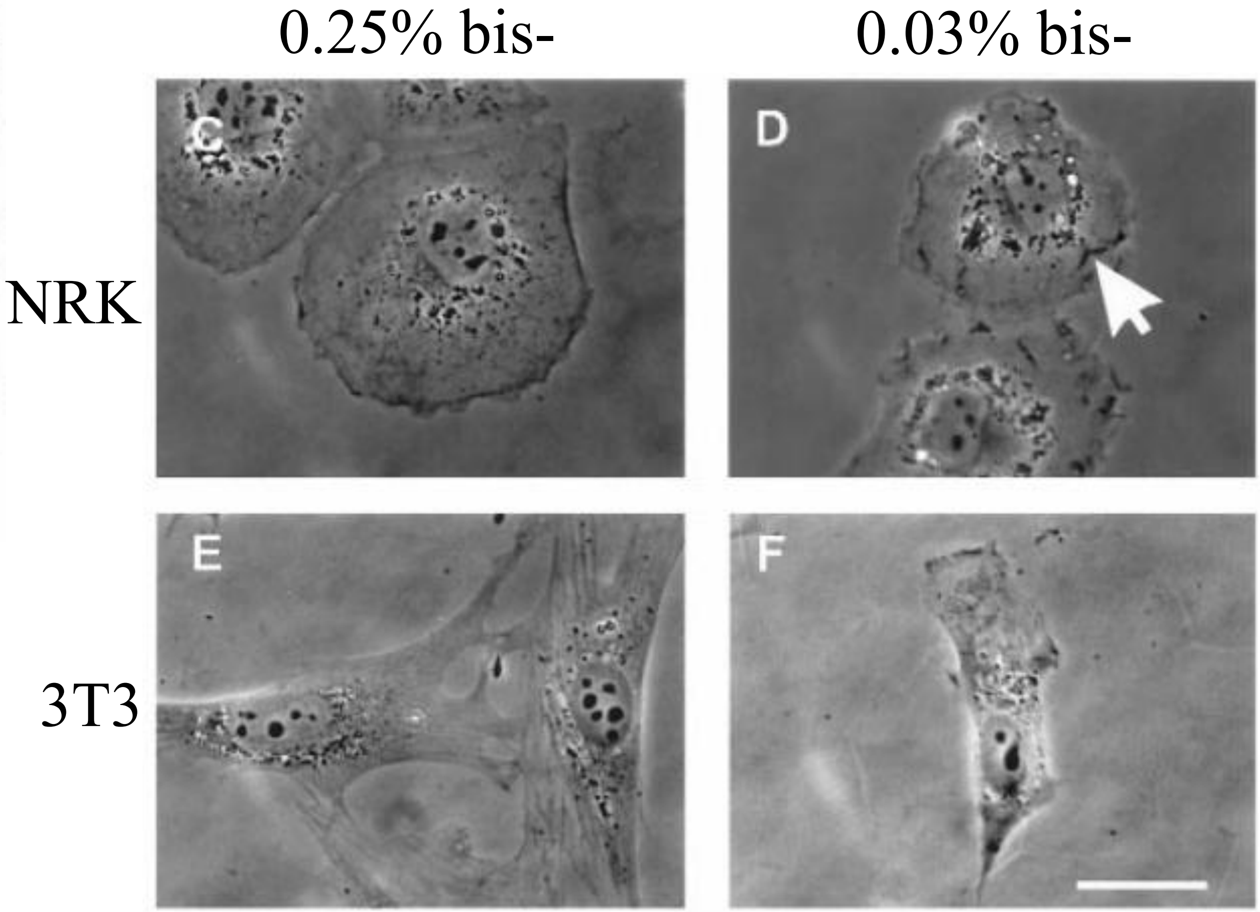
Spacer Arm 18.2 Å



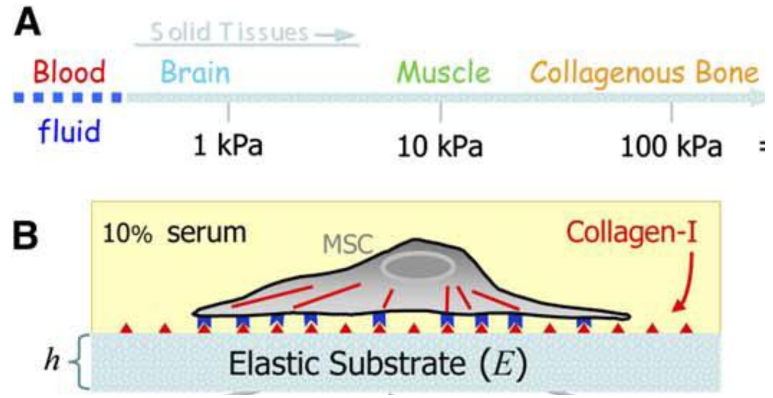
The Polyacrylamide Matrix

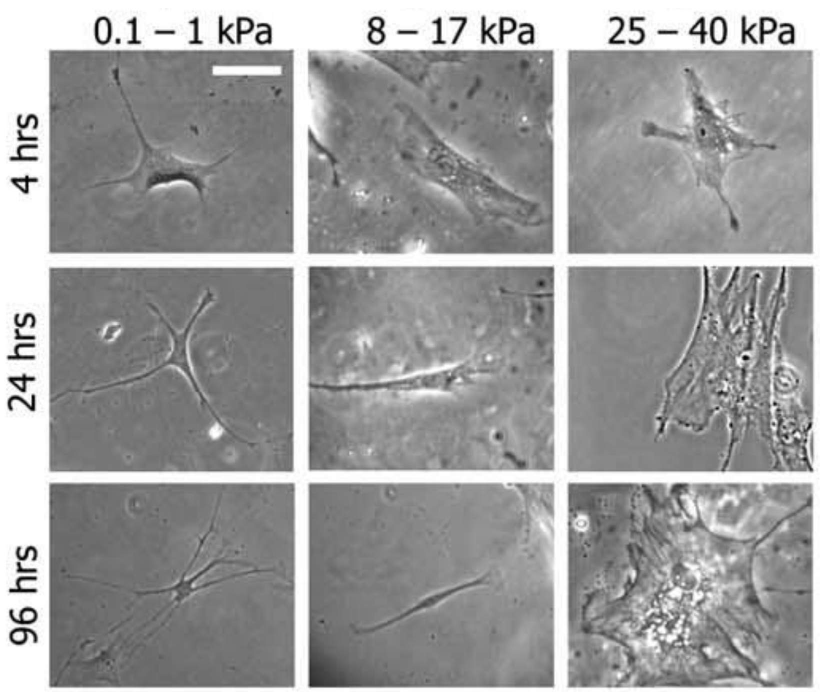
Pelham, RJ, and Wang, Y-L, *PNAS*, **94**: 13661 (1997)

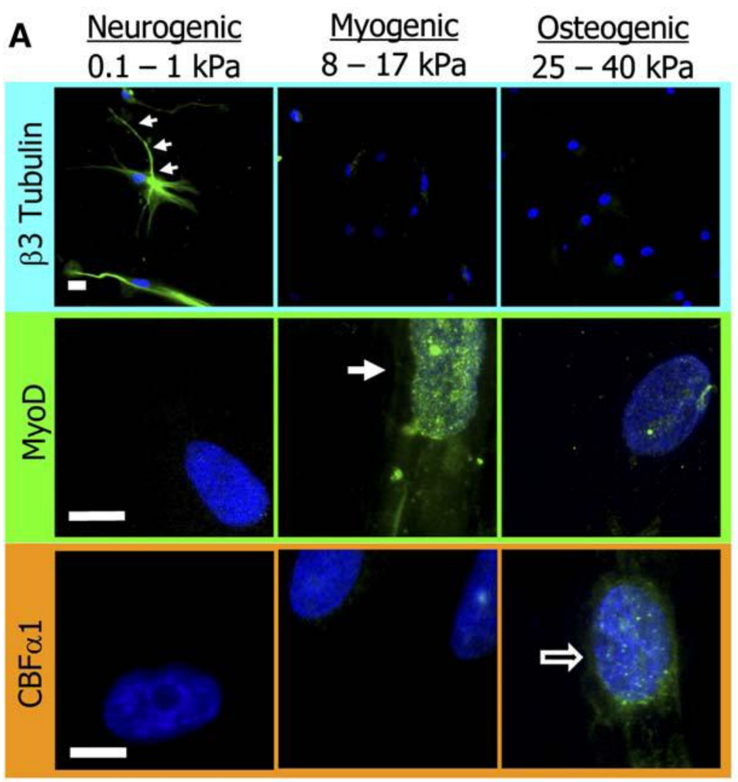




Engler, *et al.* (2006) - Matrix Elasticity Directs Stem Cell Lineage

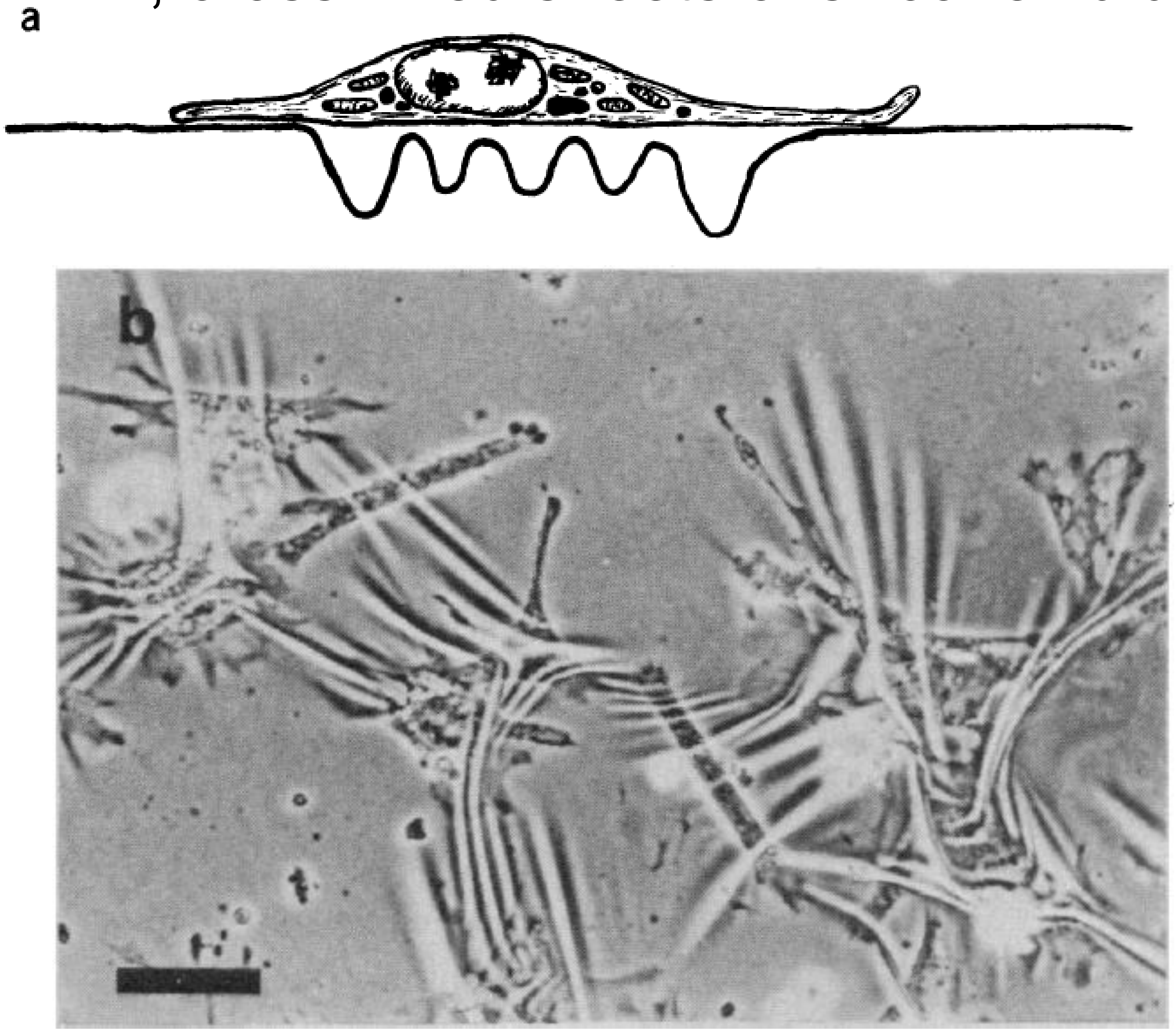






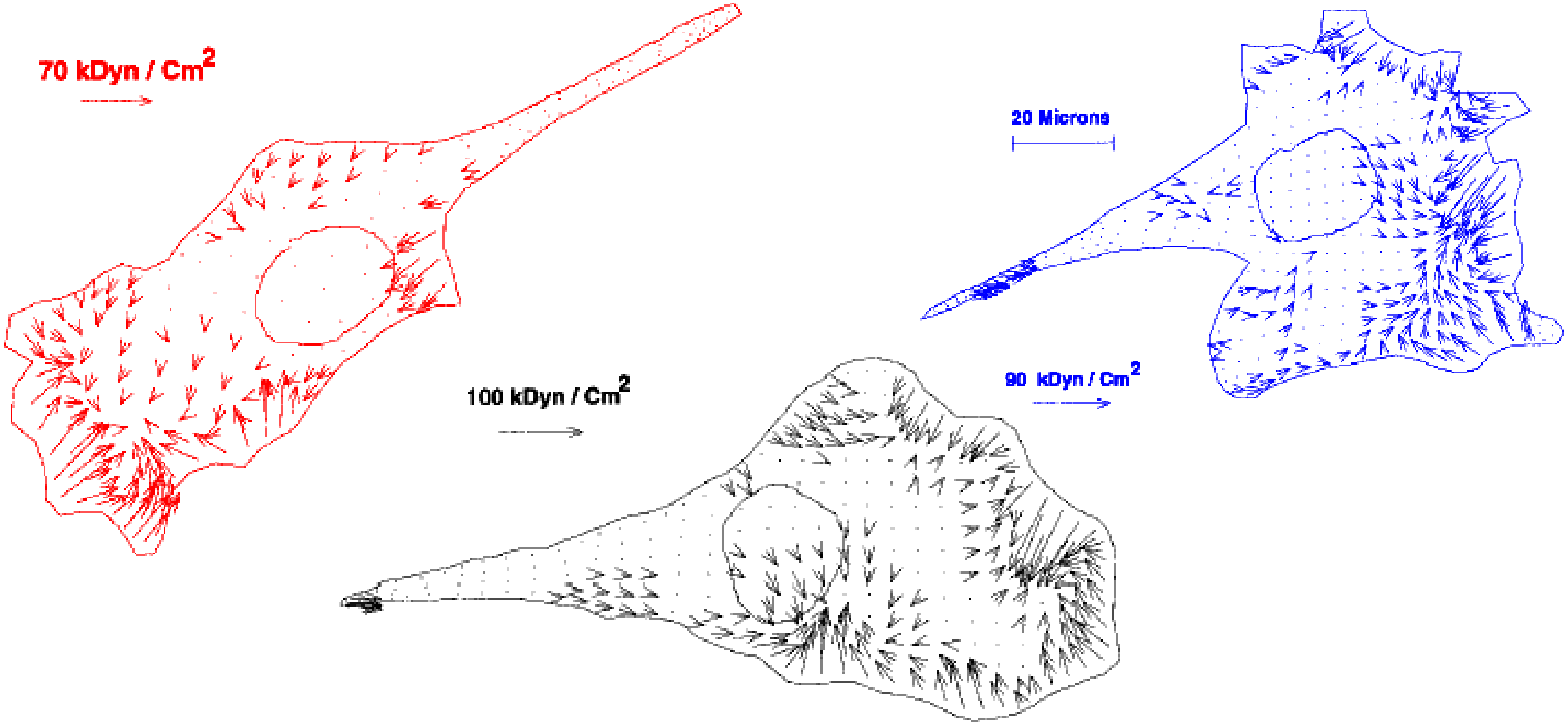
Harris AK, Wild P, Stopak D. *Science*, **208**:177-9 (1980).

- Thin, crosslinked sheets of silicone fluid



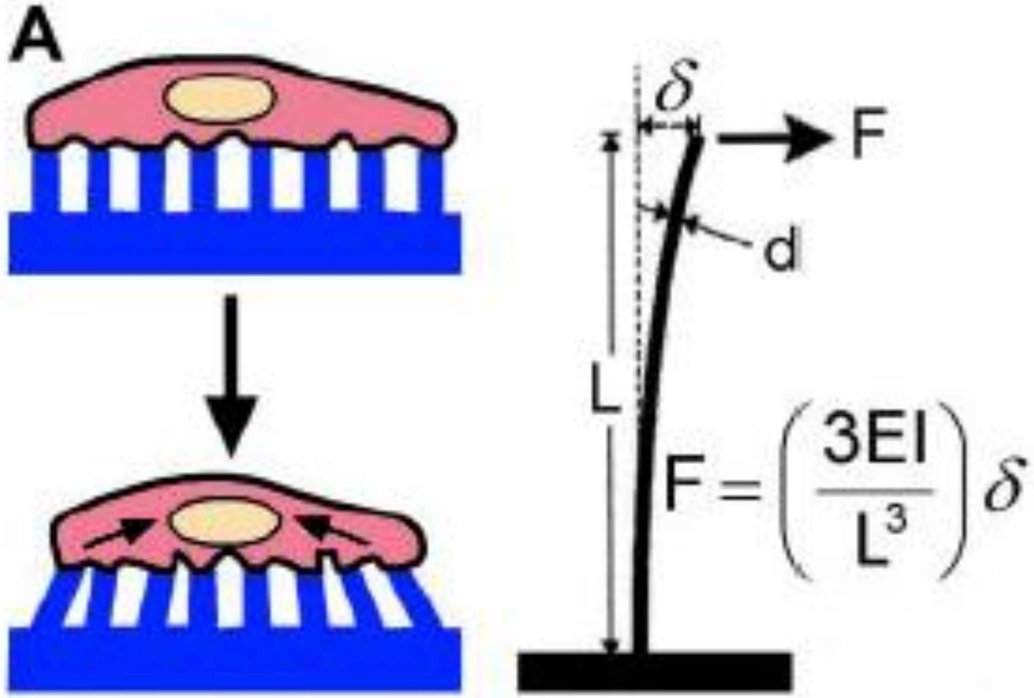
Estimating Cell-generated Traction Forces
Dembo, M., and Wang, Y-L., *Biophysical Journal*, **76**:2307-16 (1999).

- Polyacrylamide gels
  - covalently immobilize collagen into this substrate
  - Young's modulus of 62 kdyn/cm^2 (6.2 x 10^{10} kPa)
- Randomly embed beads into this matrix, track their displacements over time

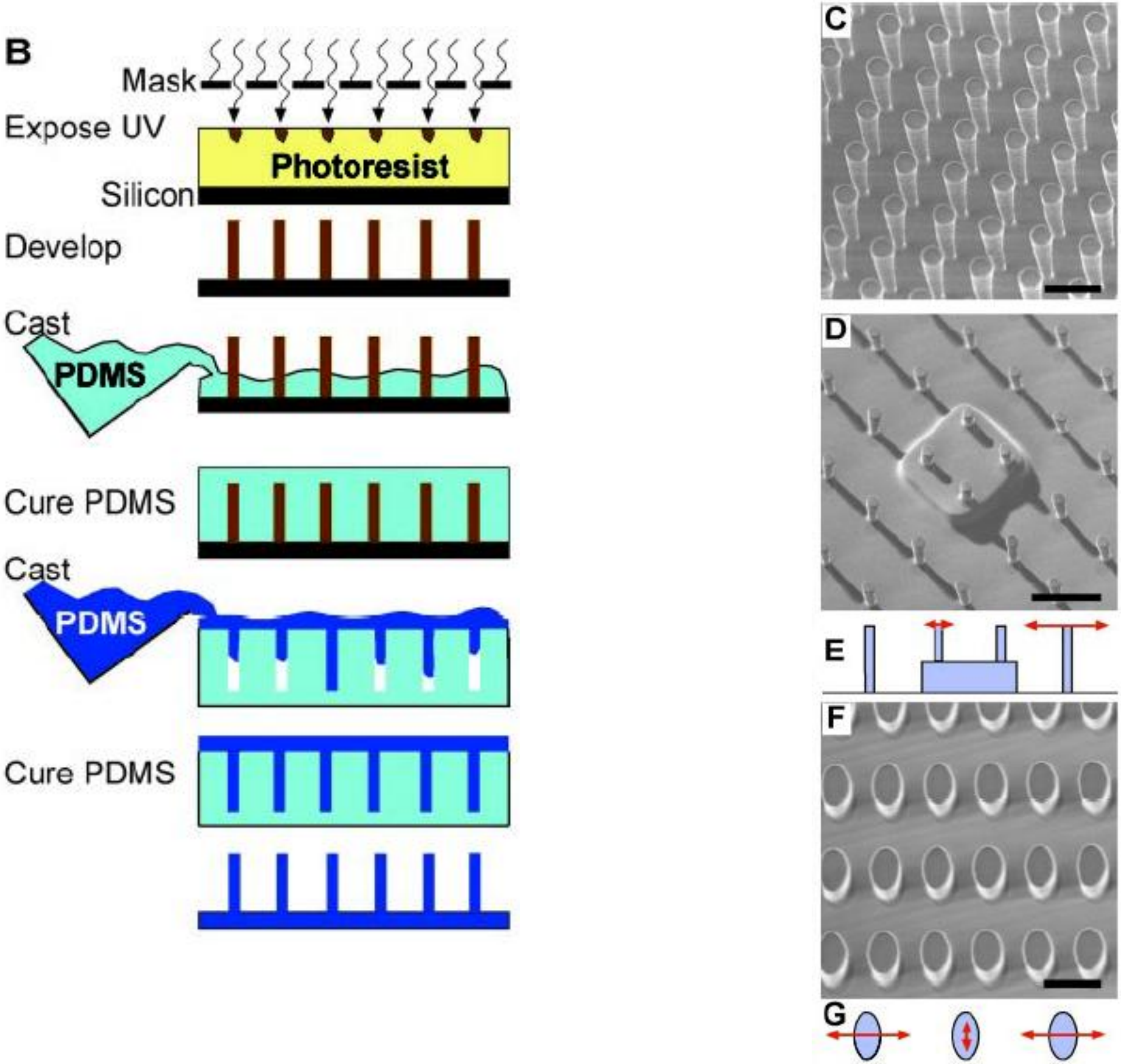


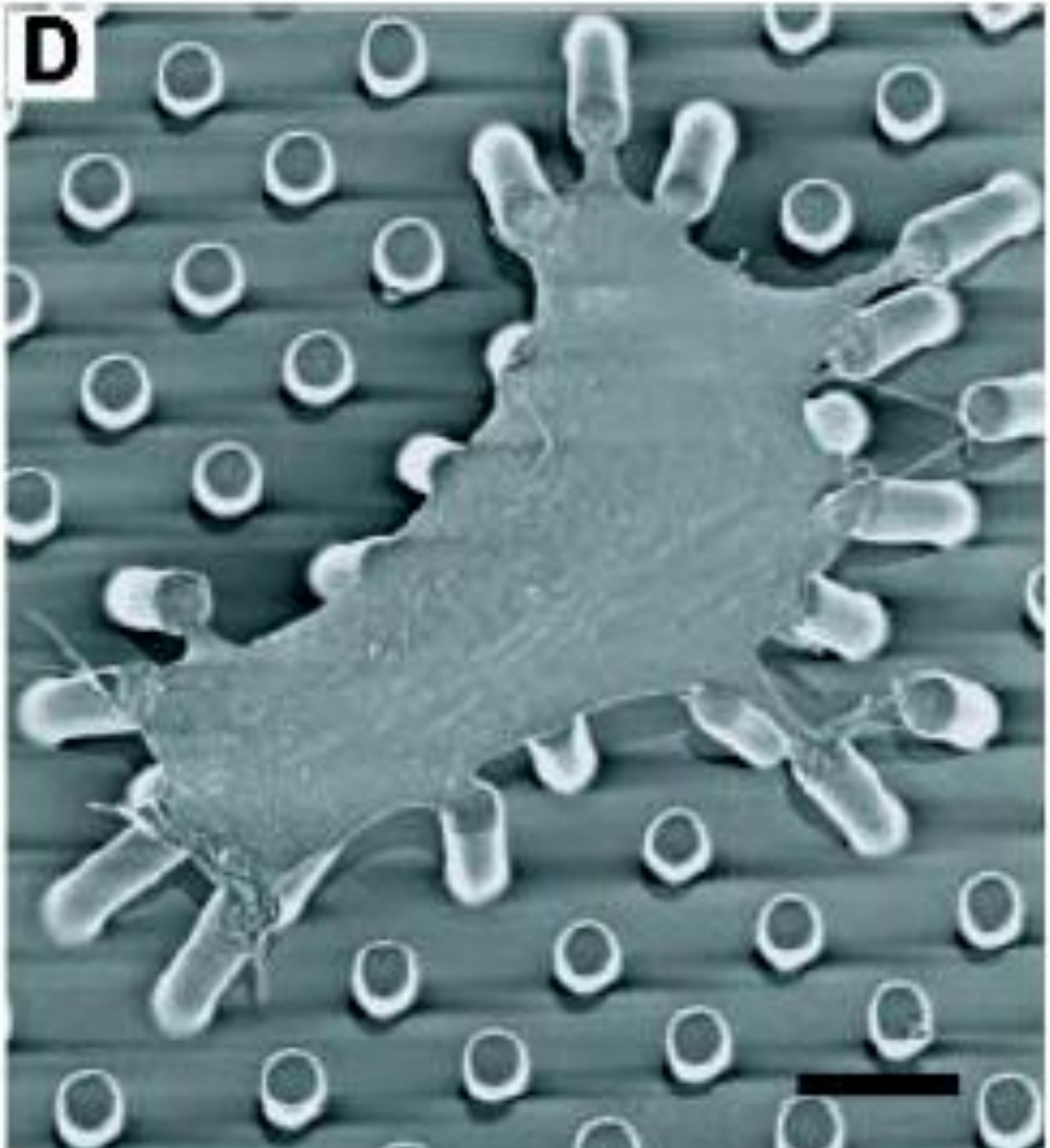
- Most intense forces along the leading edge of the cell
- Little forces under cell nucleus and along tail
- Considerations of this method:
  - Measurements of force per area
  - Spatial resolution of this force/area of 5 μm
  - Details dependent on random spacing of beads
  - Force-induced deformation at one place produces deflection at nearby locations; hard to decouple

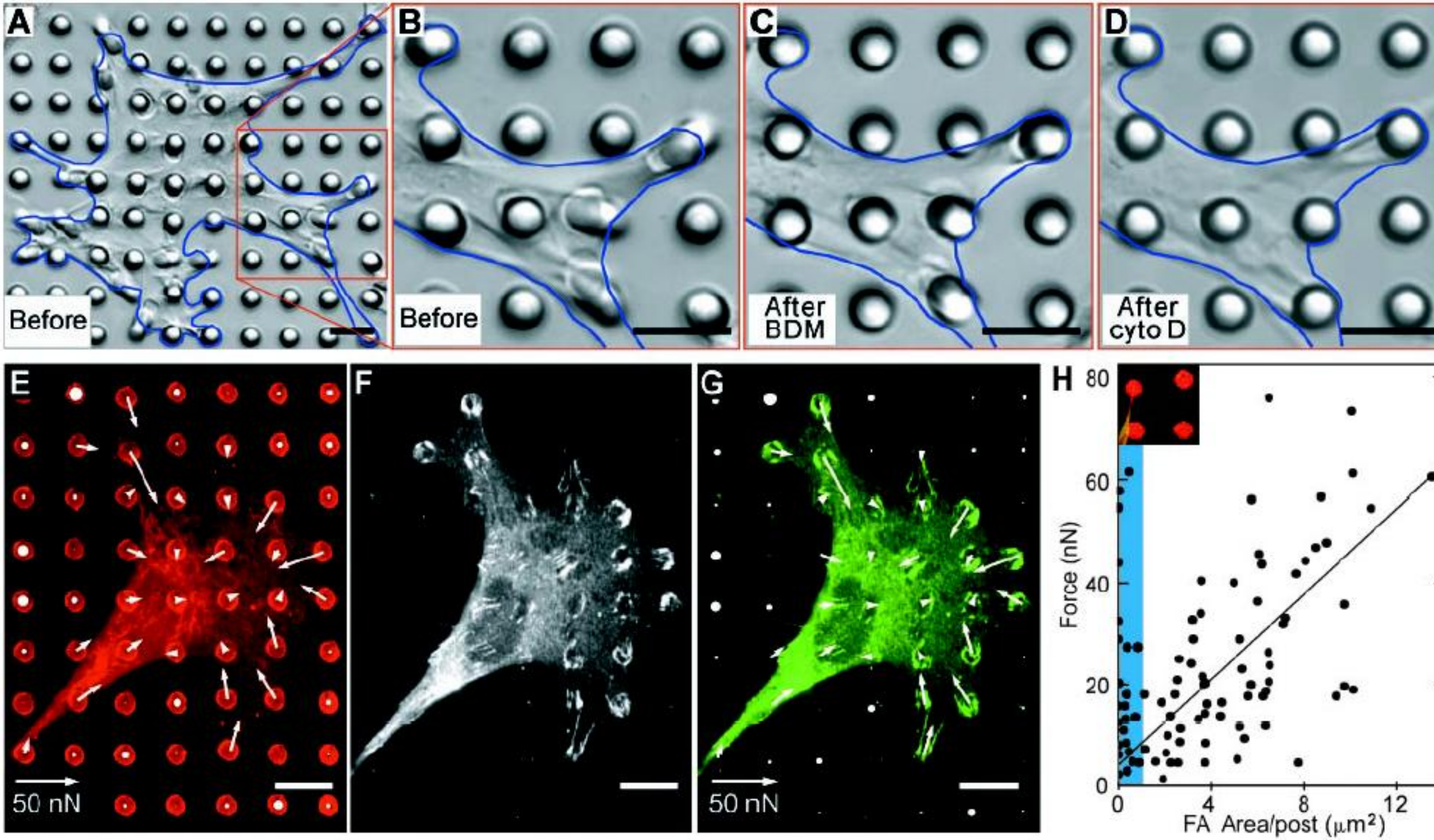
Traction force microscopy using elastomer pillar arrays Tan, *et al.* (2003) – Cells lying on a bed of microneedles:



- One of the key, widespread methods in use today.
- Simple measurement of force
- Many considerations

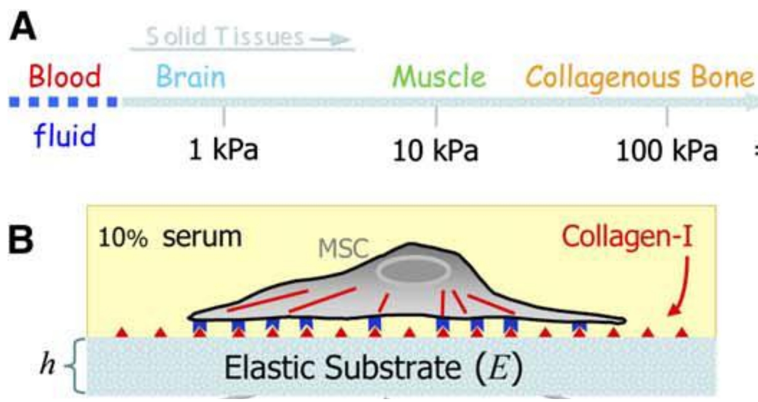

B
Mask
Expose UV
Photoresist
Silicon
Develop
Cast
PDMS
Cure PDMS
Cast
PDMS
Cure PDMS
C
D
E
F
G


D

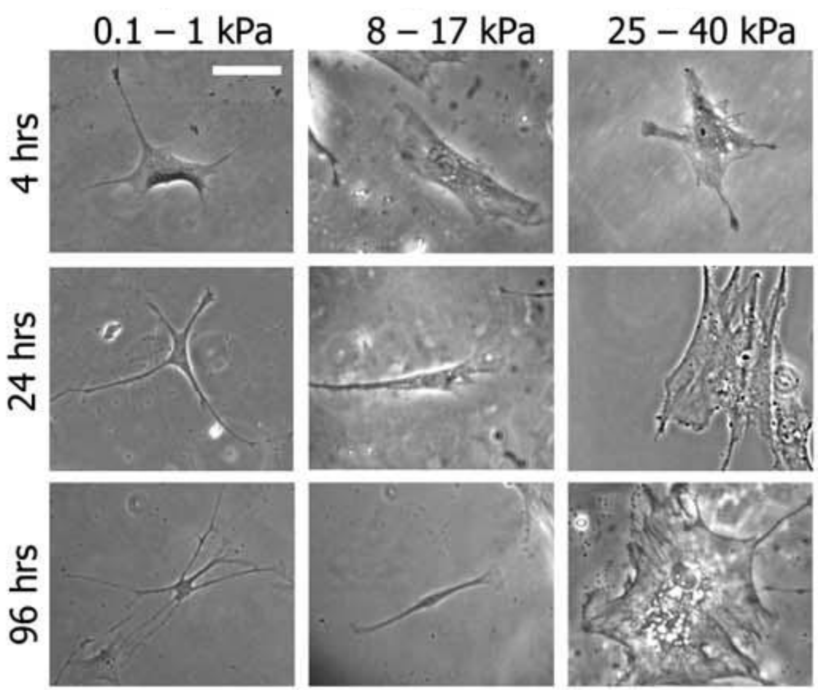


A
Before
B
Before
C
After
BDM
D
After
cyto D
E
50 nN
F
G
50 nN
H
Force (nN)
FA Area/post (μm²)
0
20
40
60
80
0
4
8
12

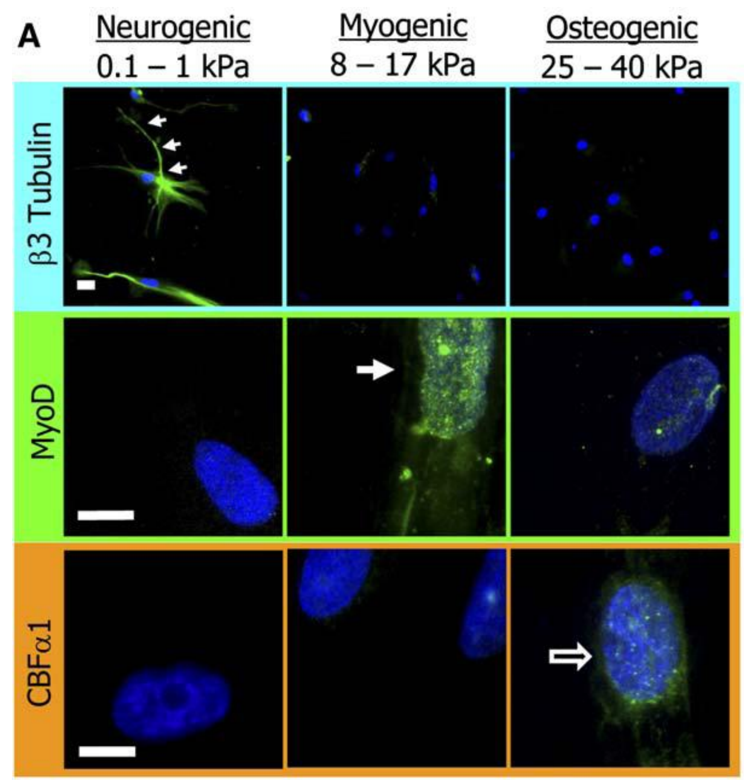
Is this real?



A
Solid Tissues
Blood
fluid
Brain
Muscle
Collagenous Bone
1 kPa
10 kPa
100 kPa
B
10% serum
MSC
Collagen-I
h
Elastic Substrate (E)

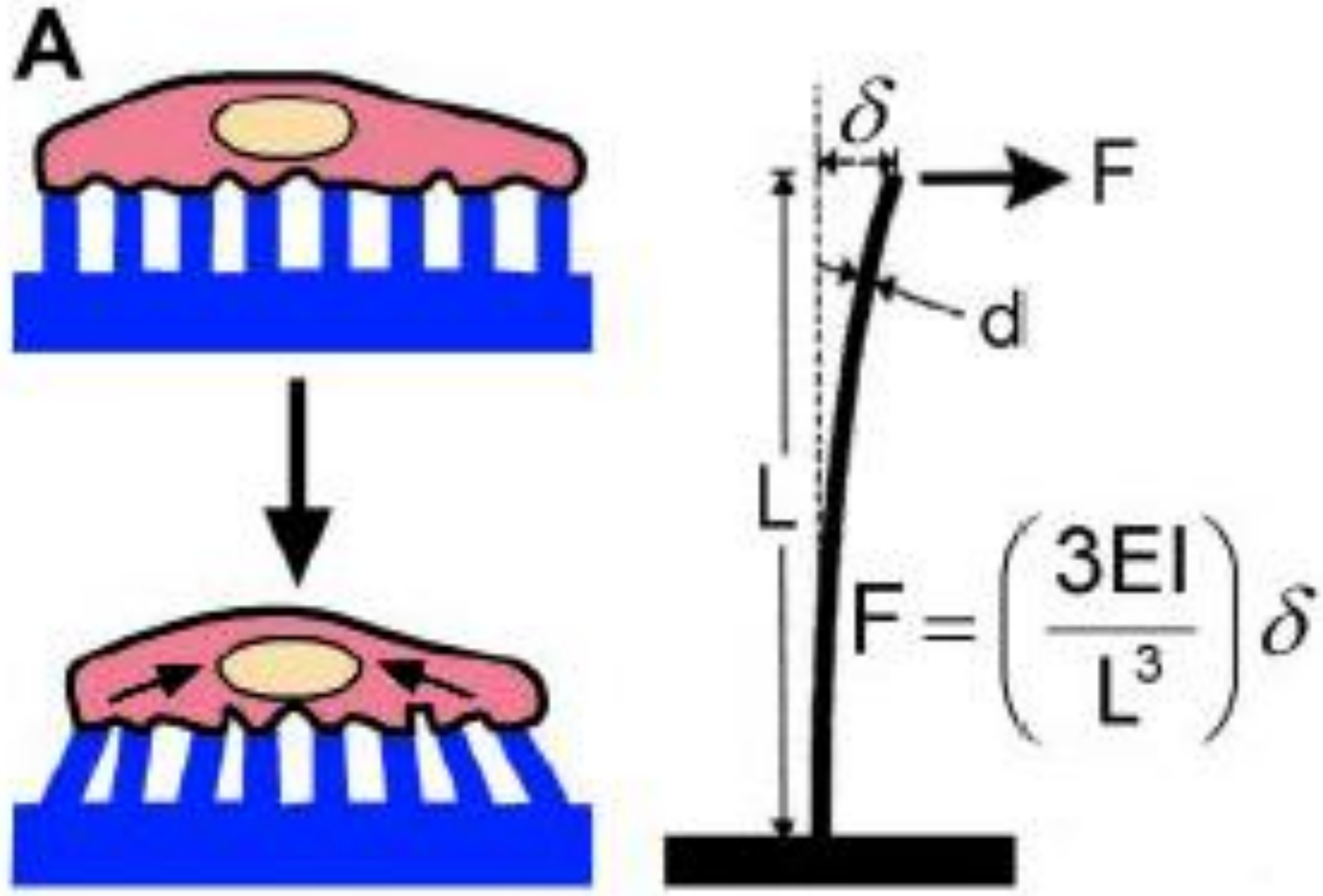


0.1 – 1 kPa
8 – 17 kPa
25 – 40 kPa
4 hrs
24 hrs
96 hrs



A
Neurogenic
0.1 – 1 kPa
Myogenic
8 – 17 kPa
Osteogenic
25 – 40 kPa
β3 Tubulin
MyoD
CBFα1

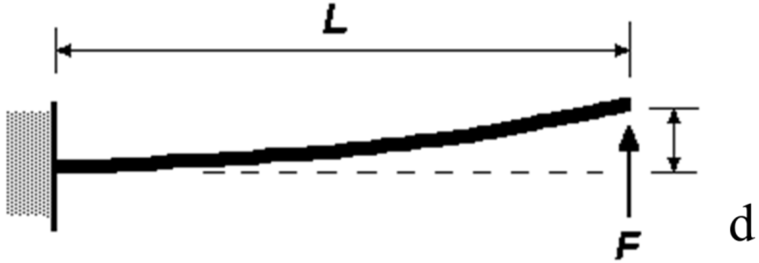
Cantilevers to simulate bulk rigidity



Saez, *et al.* (2005), Biophysical Letters:

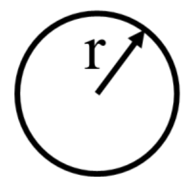
- Is the mechanical activity of epithelial cells controlled by deformations or forces?
- Relate pillars to slabs

pillars



$$d = \left(\frac{L^3}{3*E*I}\right)F = \left(\frac{4}{3\pi E}\frac{L^3}{r^4}\right)F$$

slabs, ala Landau & Lifschitz



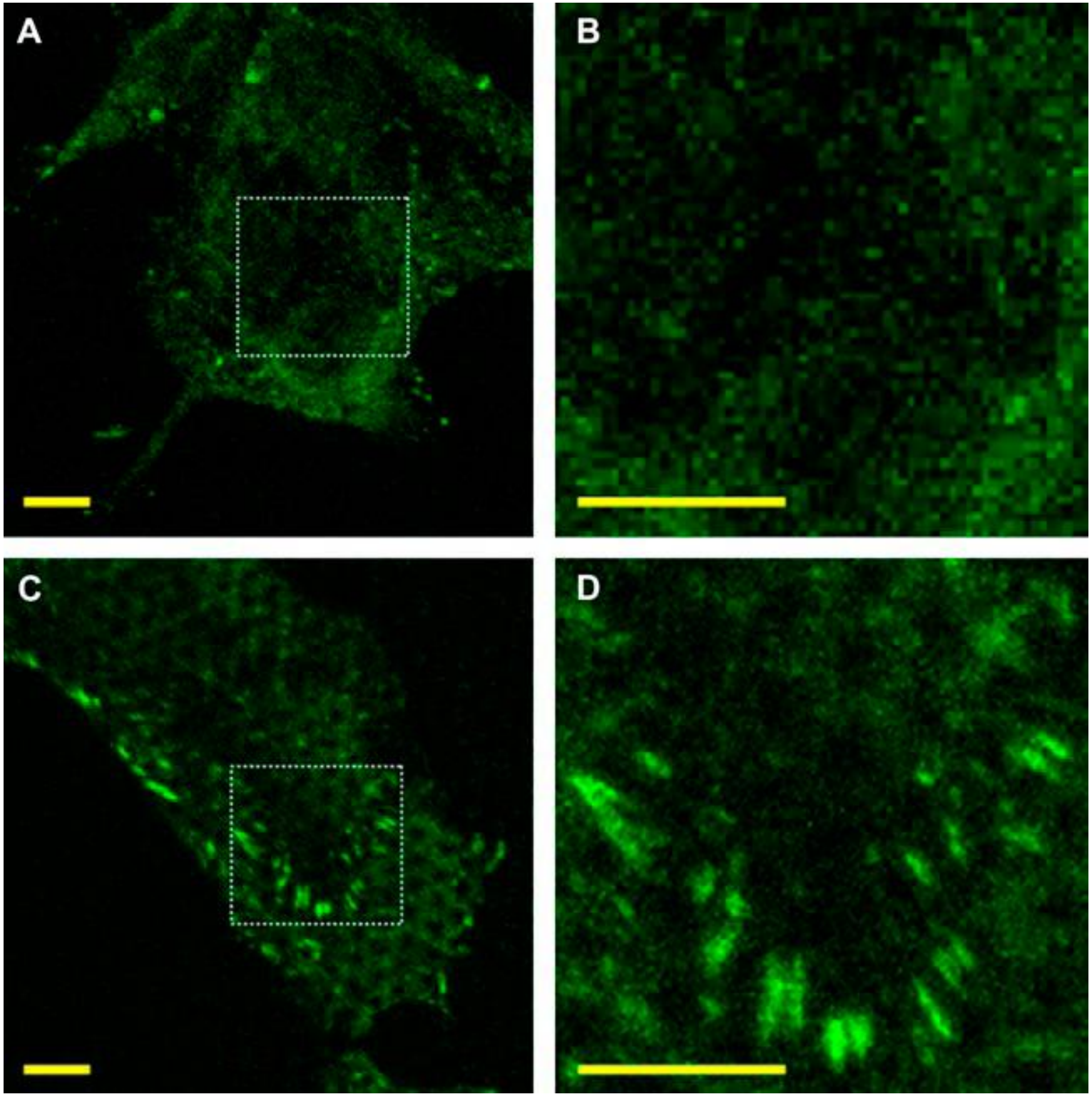
$$d = \left(\frac{9}{4}\frac{1}{\pi r E_{eq}}\right)F$$

(assume area of force application is equal to pillar diameters)

$$E_{eq} = \left(\frac{27}{16}\frac{r^3}{L^3}\right)E$$

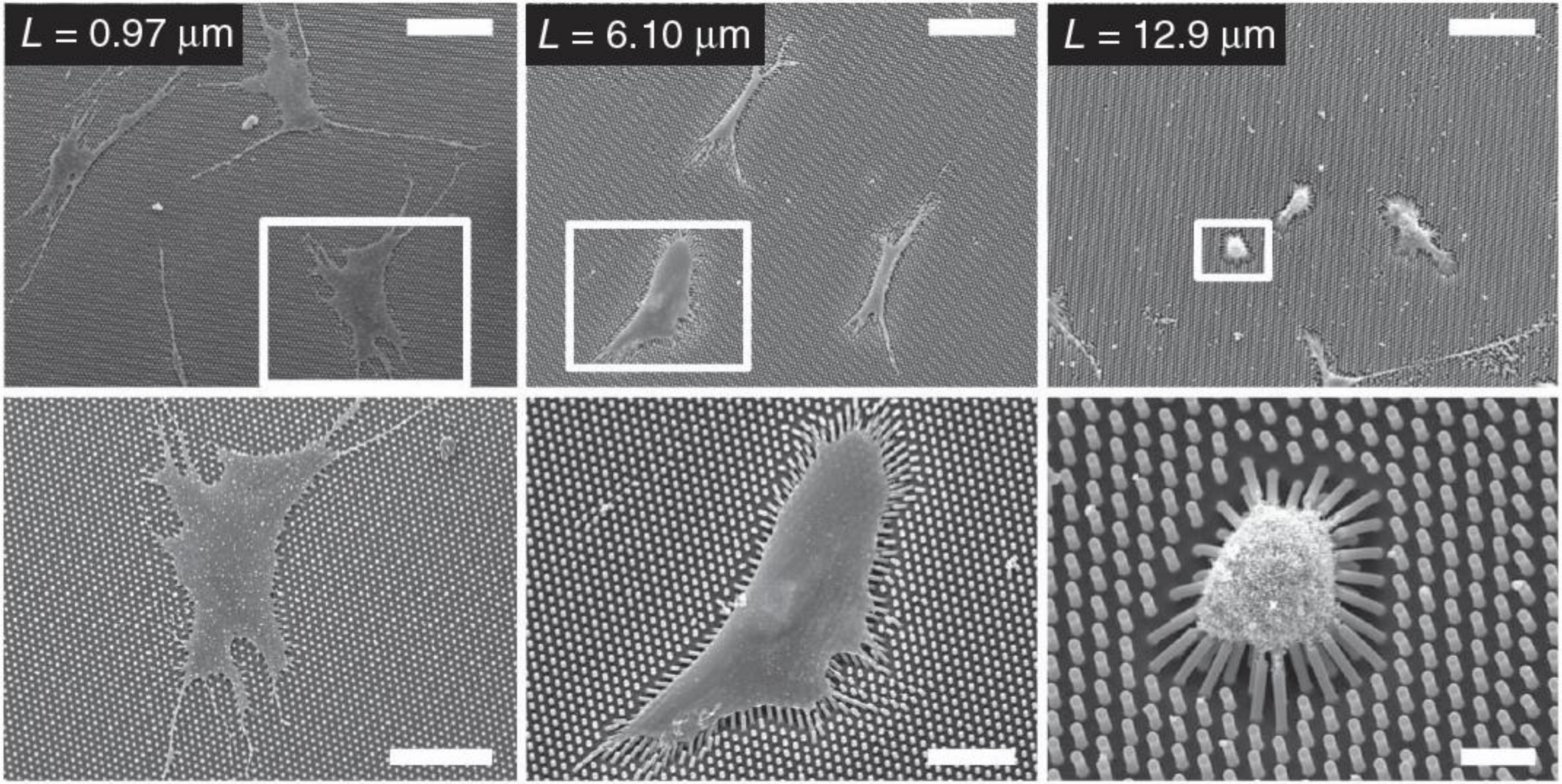
2 nN/micrometer

71 nN/micrometer

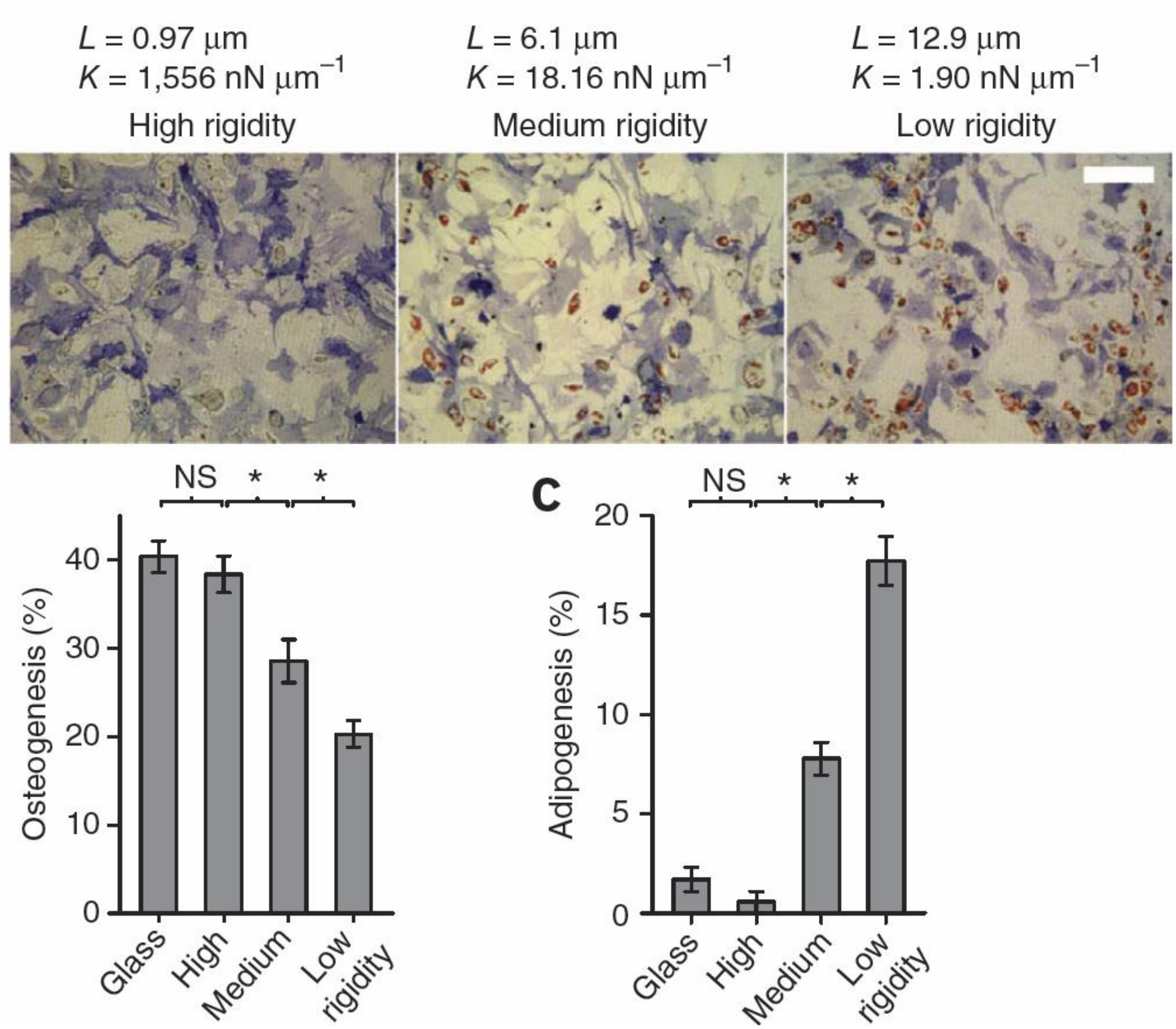


10 microns

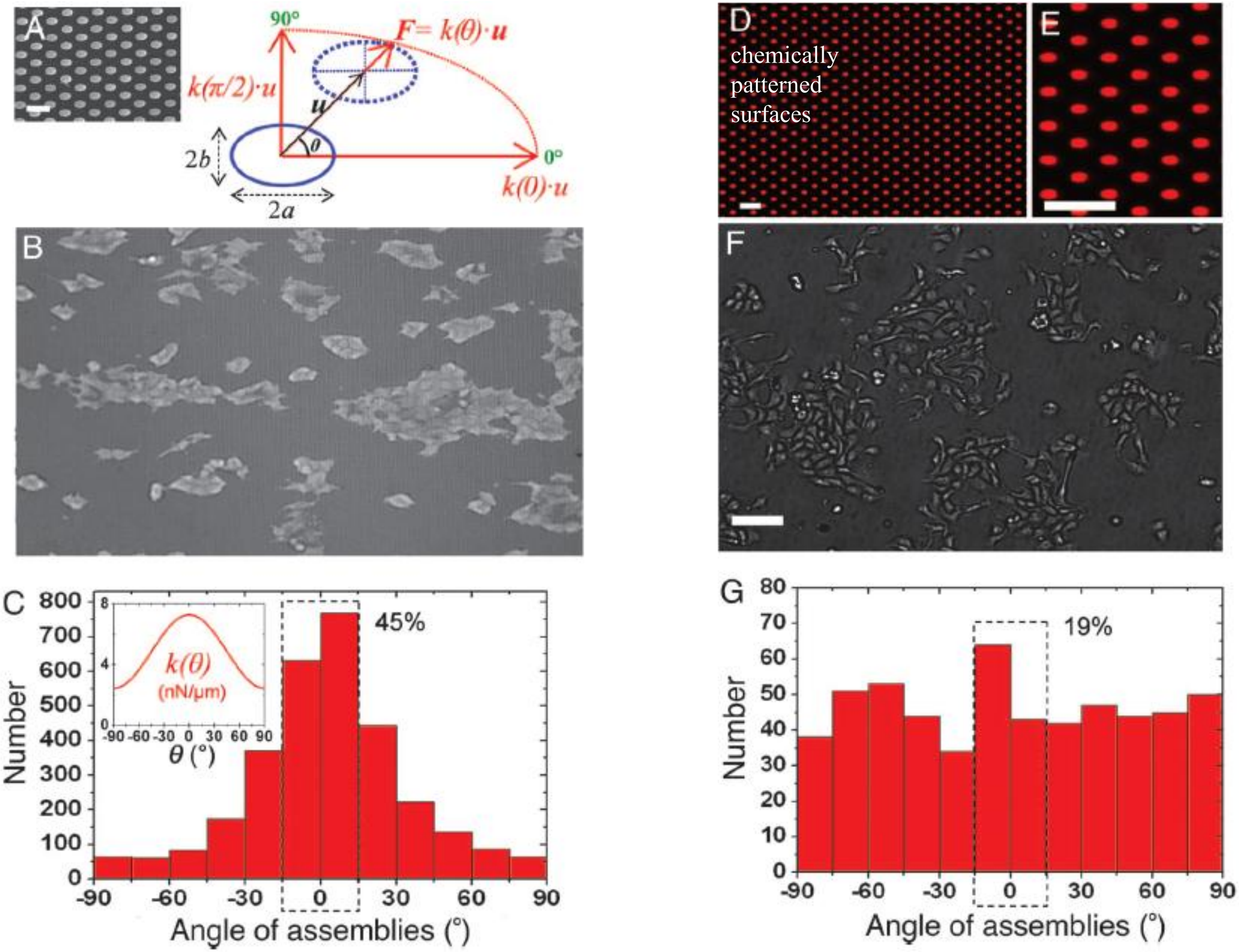
Fu, J., *et al*., *Nature Methods*, **7**:733-6 (2010).



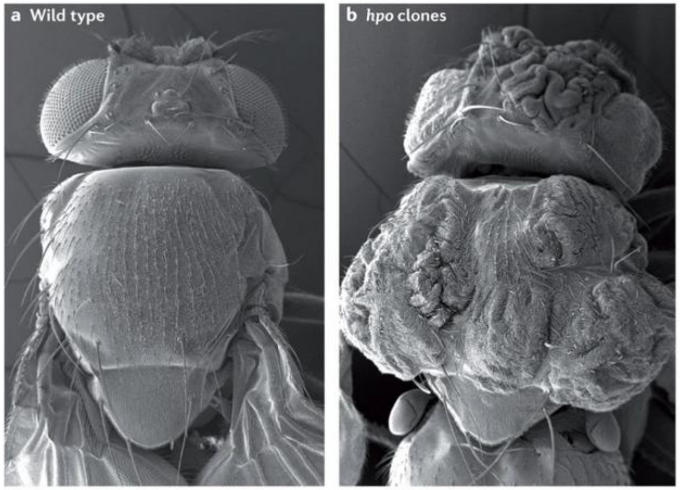
Fu, J., *et al.*, *Nature Methods*, **7**:733-6 (2010).

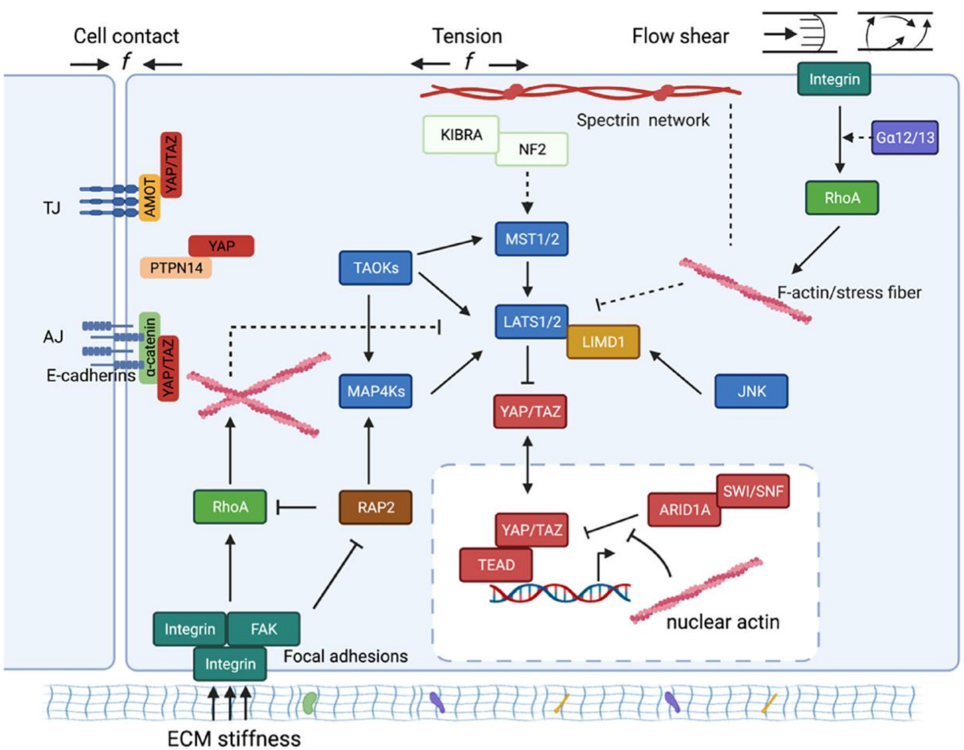


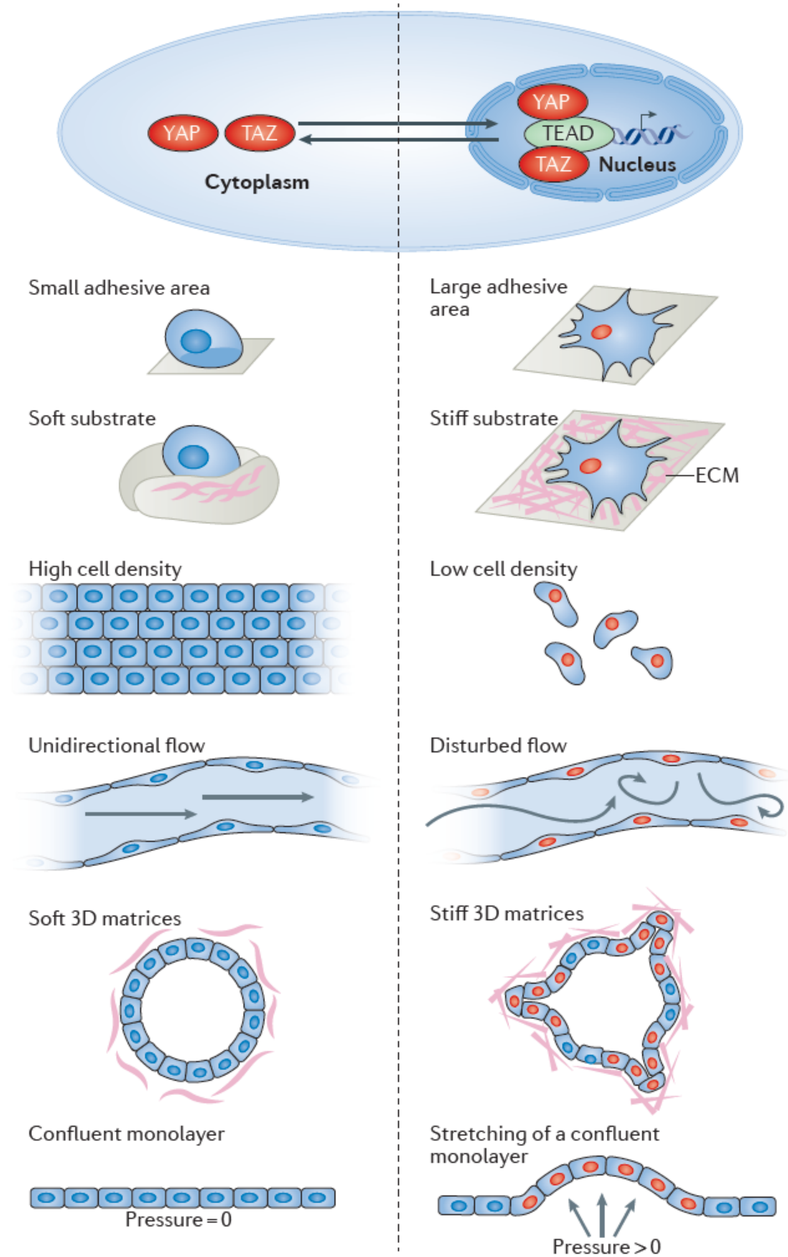
Epithelial cell response on anisotropic surfaces;
Saez *et al*. (2007) *Biophys J*, **104:8281-8286.**



Mechanosignalling – YAP/TAZ

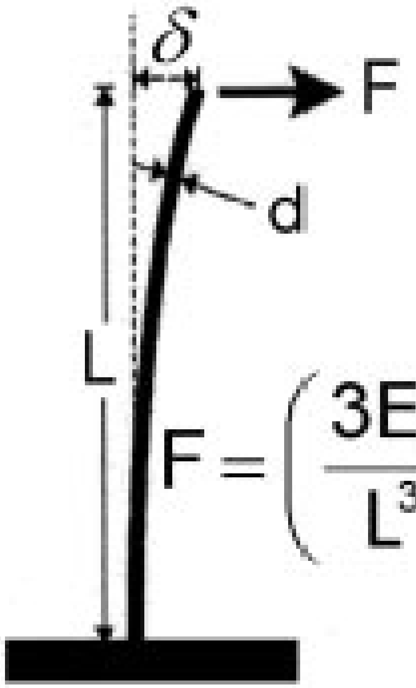




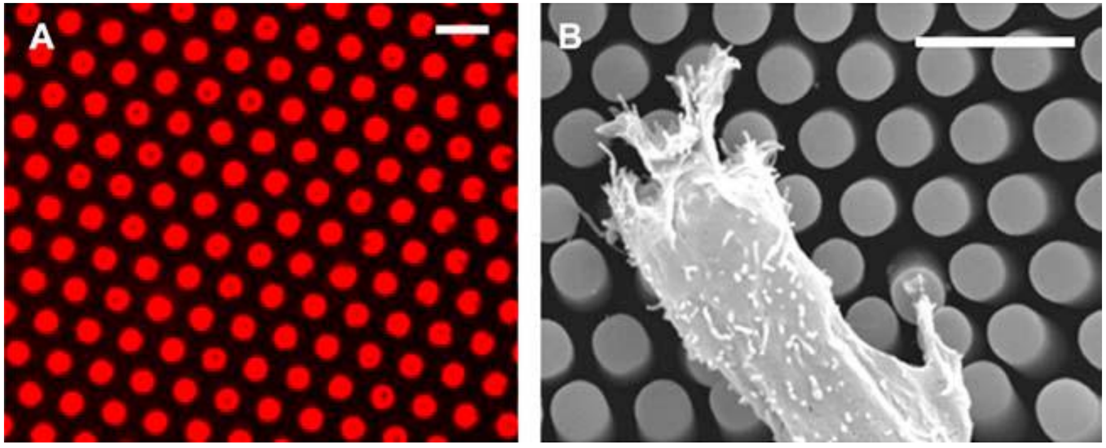


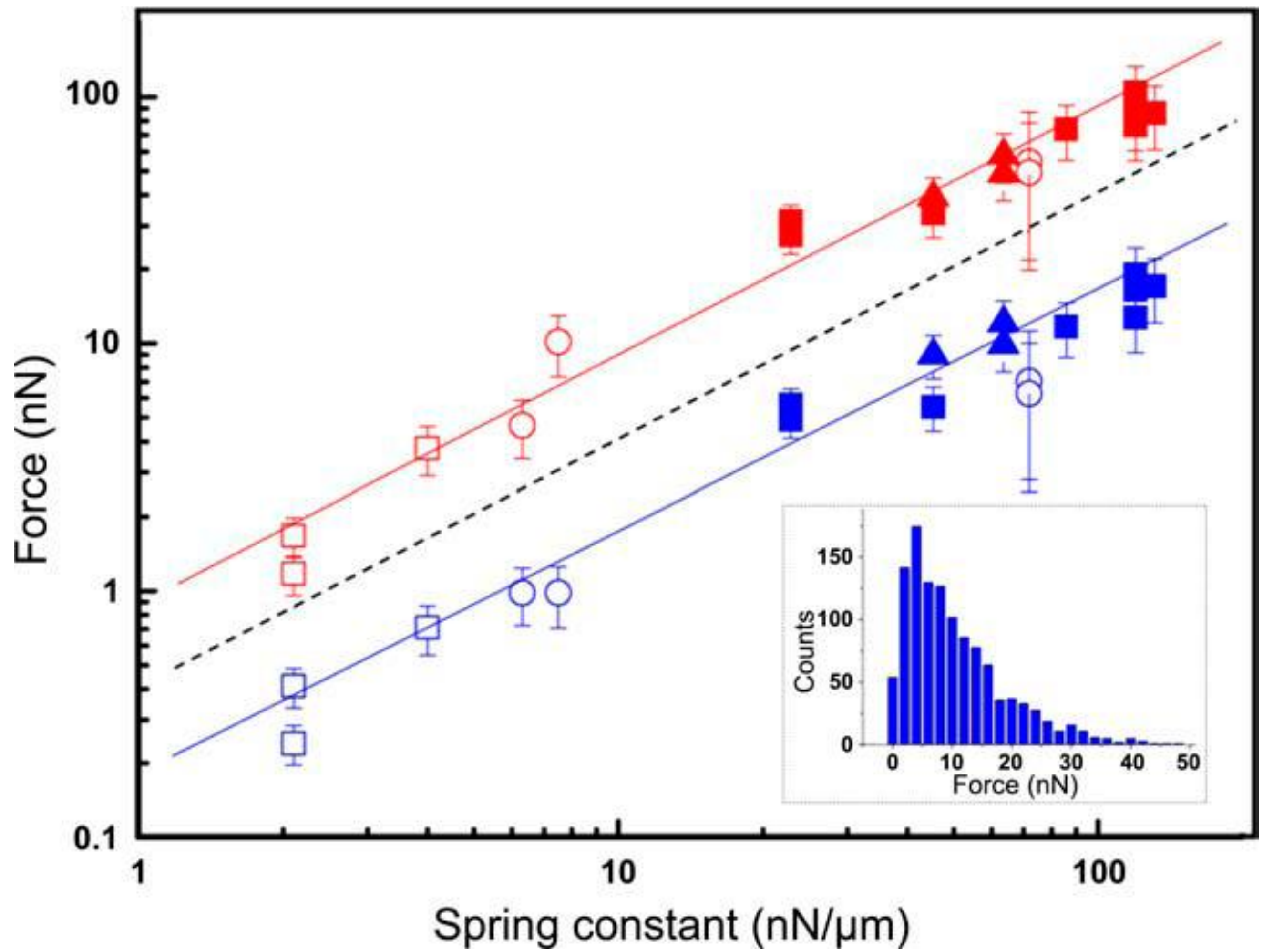
Modulating cell response; Saez *et al*. (2005) *Biophys Lett*, L52

- Do cells respond directly to rigidity? Test by changing spring constant
- Pillar arrays
  - 2 – 130 nN/μm spring constants; 1 – 100 kPa
  - 1 – 2 μm diameter pillars
  - 1.6 – 6 μm heights
  - Tops coated with fibronectin
  - Cells insensitive to surface coverage (10-40%)
- MDCK cells, epithelial. Easy, fast to grow, good cell-cell and cell-substrate interactions.



$$F = \left(\frac{3EI}{L^3}\right)\delta$$





- average and maximum forces; max at periphery of cell clusters
- ratio of post to total surface: ○=10%, □=22%, Δ=44%
- diameter: open=1 μm, closed=2 μm
- dashed line is of slope 1
- inset = typical force distribution, 64 nN/ μm
- ave deflection ~ 130 nm; max deflection ~ 1.3 μm