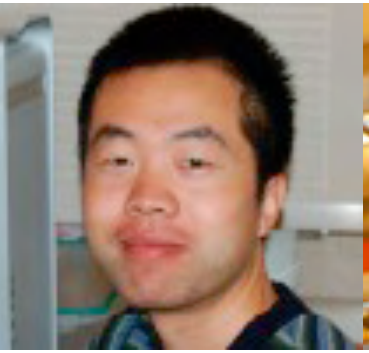
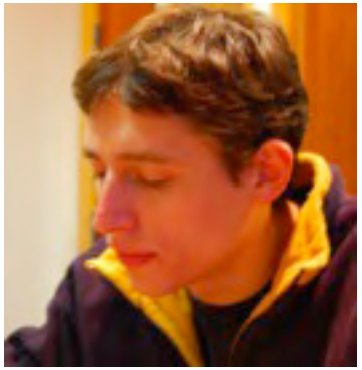
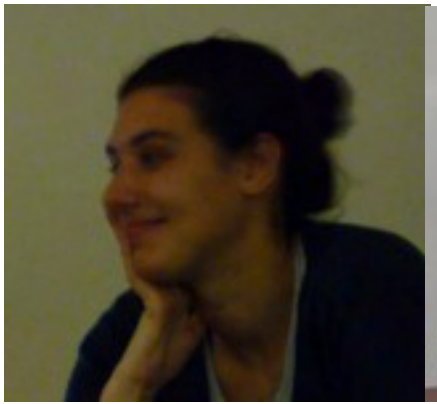
Driving cytoskeletal remodeling by extracellular matrix mechanics

Response to matrix stiffness:
Cell-type specific
Dependent on specific transmembrane receptors

How do cells measure stiffness?
Length and times scales of stiffness sensors

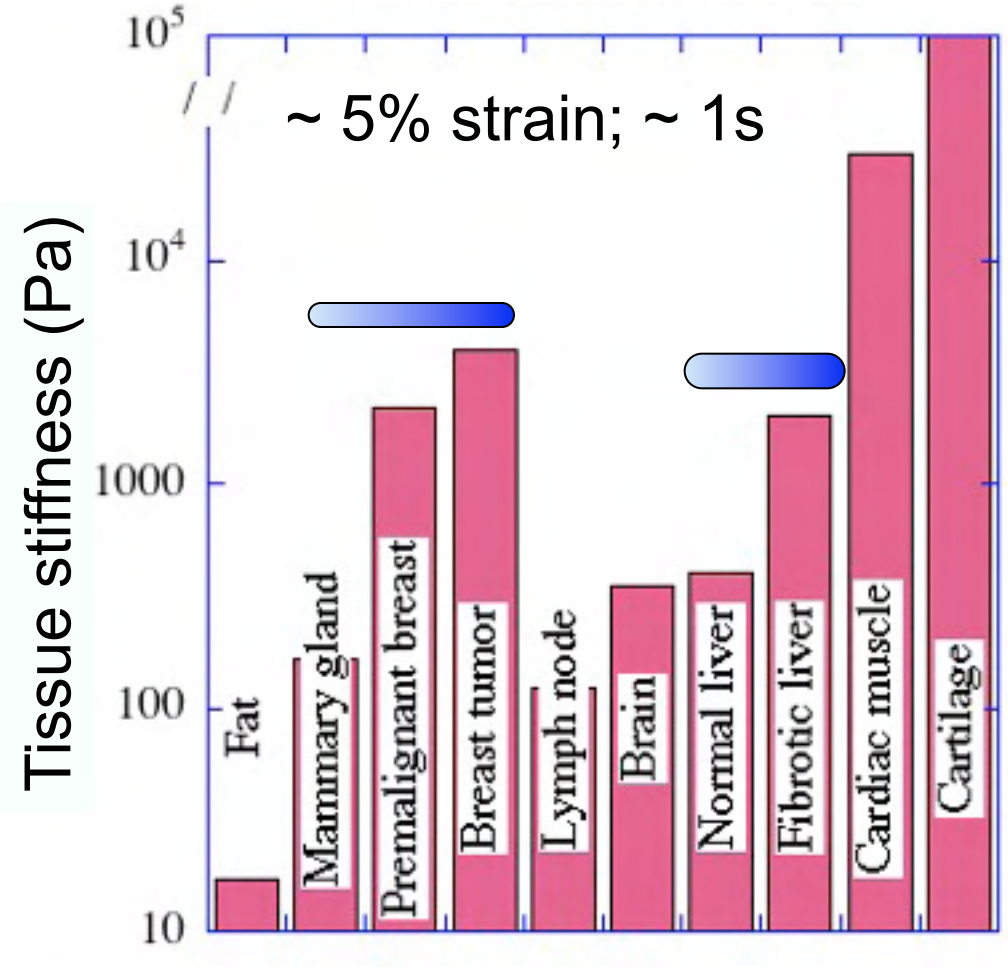
Qi Wen, Fitzroy Byfield, Ilya Levental, Maria Murray, Shang Tee



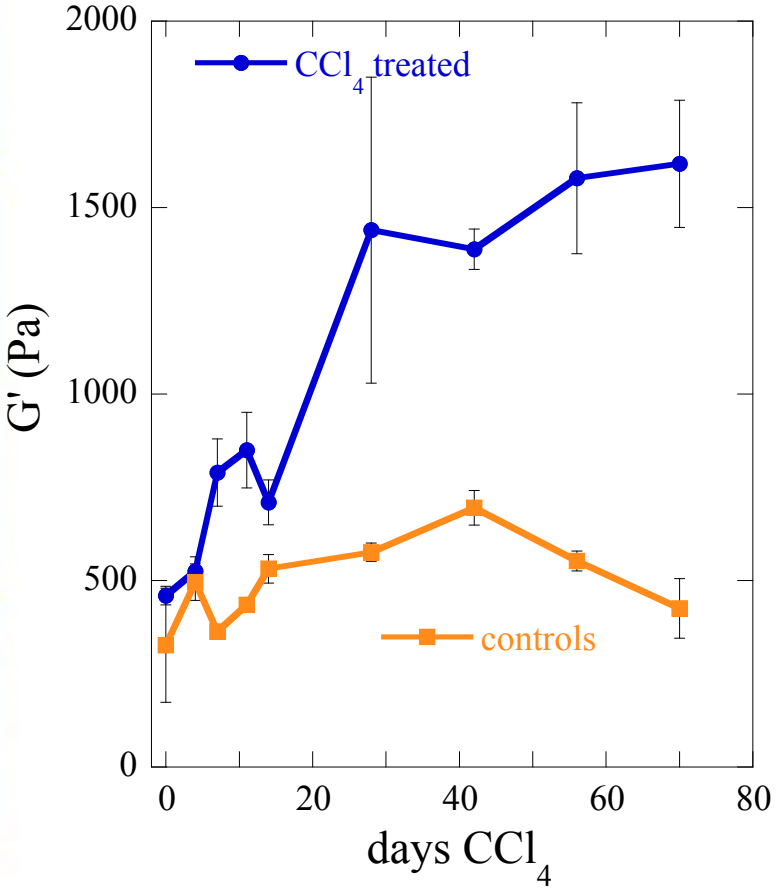




Normal tissues have well-defined stiffness

characterized by an elastic modulus*



Change in liver stiffness after inducing injury



Changes in organ stiffness often accompany disease

** time and strain-dependent*

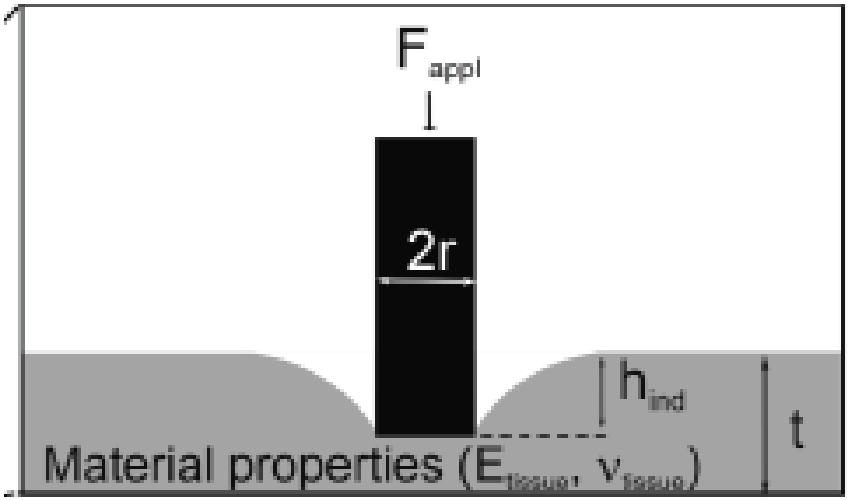
Georges et al.
Am J. Physiol, 2007

Two ways to measure soft material viscoelasticity

Oscillatory shear

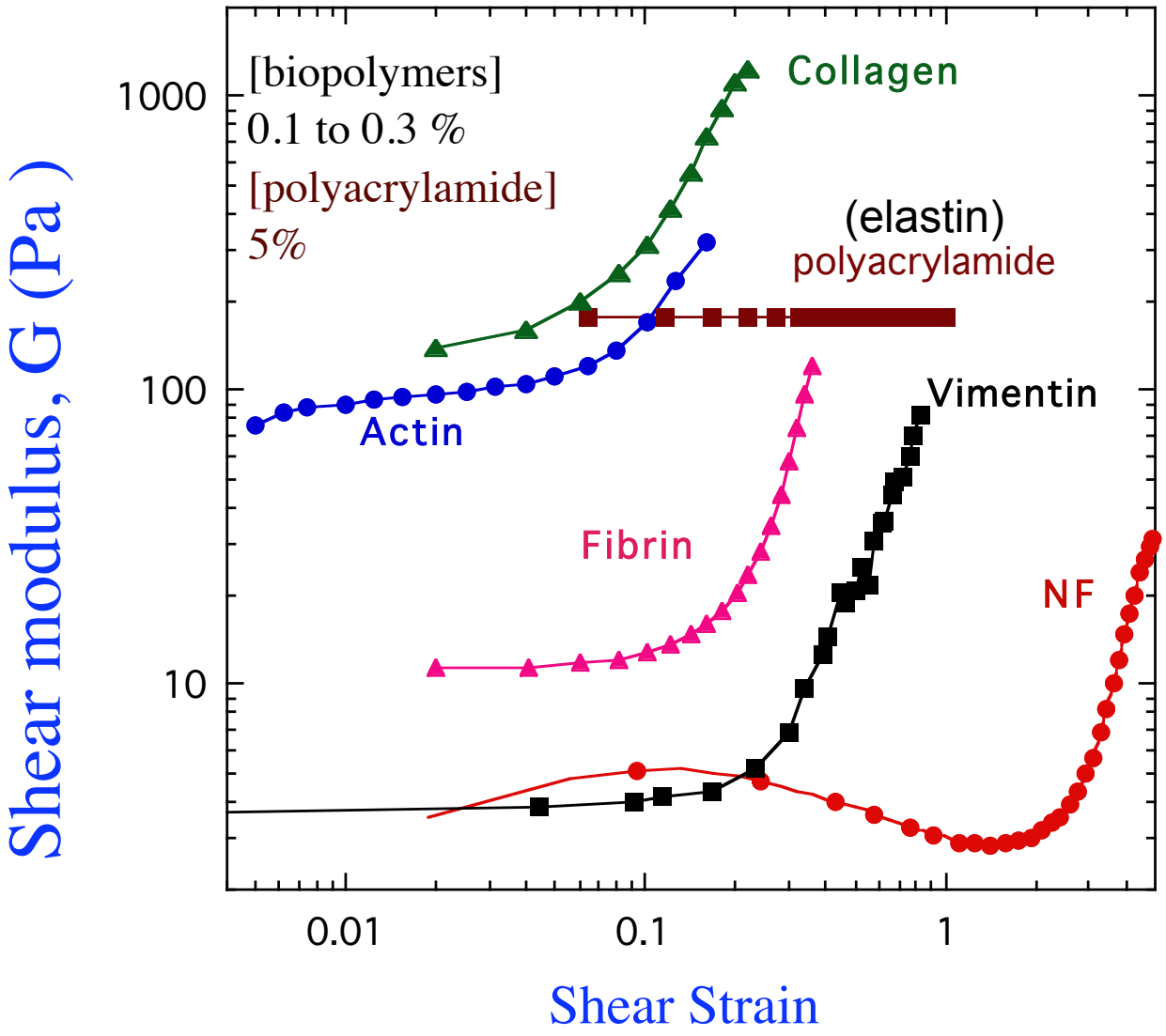
Shear modulus G

Compression or indentation



Young's modulus E

Both cytoskeletal and ECM networks are strain stiffening

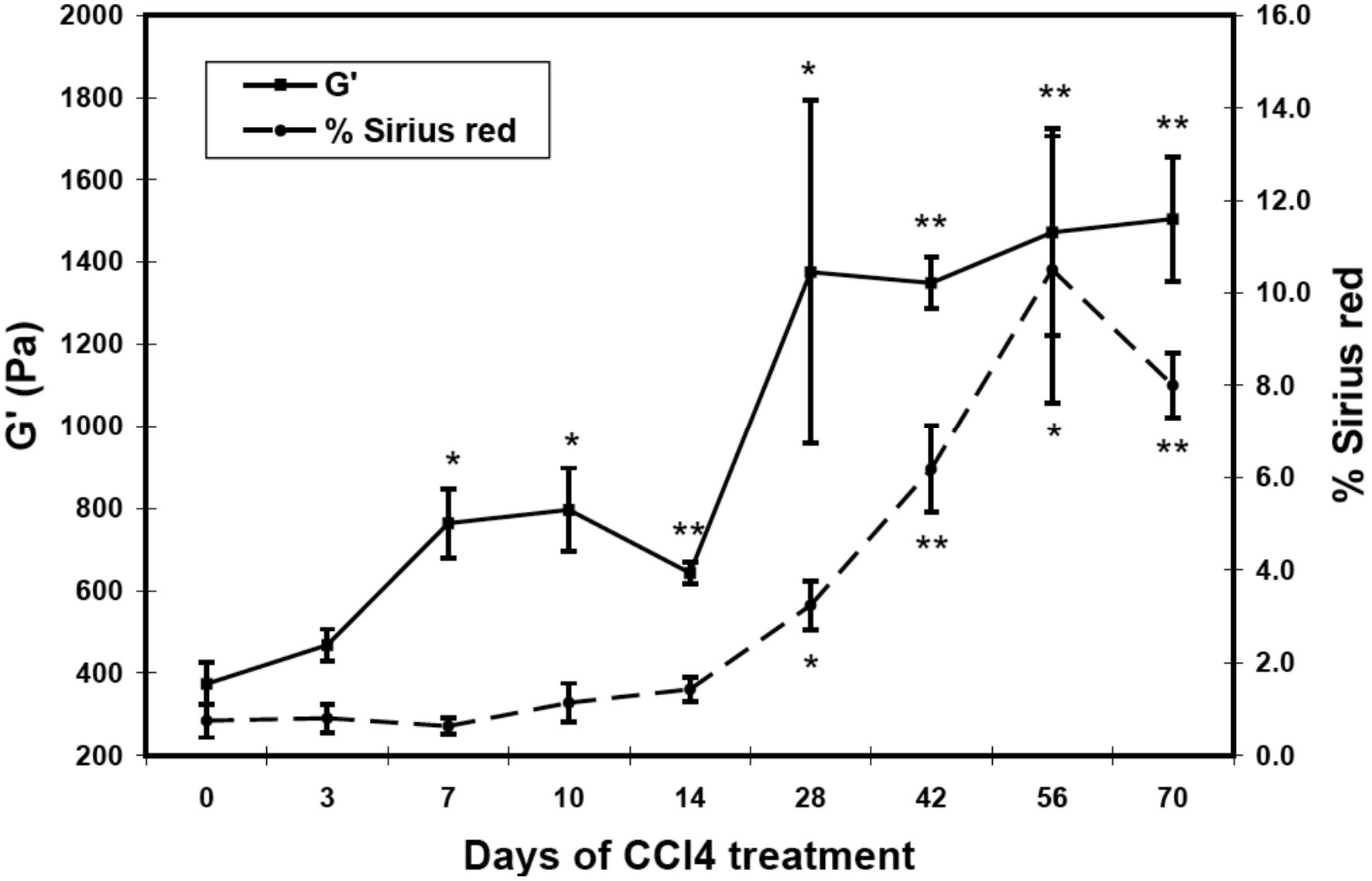


Gardel et al. Sci. 2004
Storm et al, Nat. 2005

Is it possible to infer stresses from displacement maps in these materials?

Non-linear elasticity allows cytoskeletal and ECM networks to stiffen by internal stress, without increasing polymer mass or XLs.

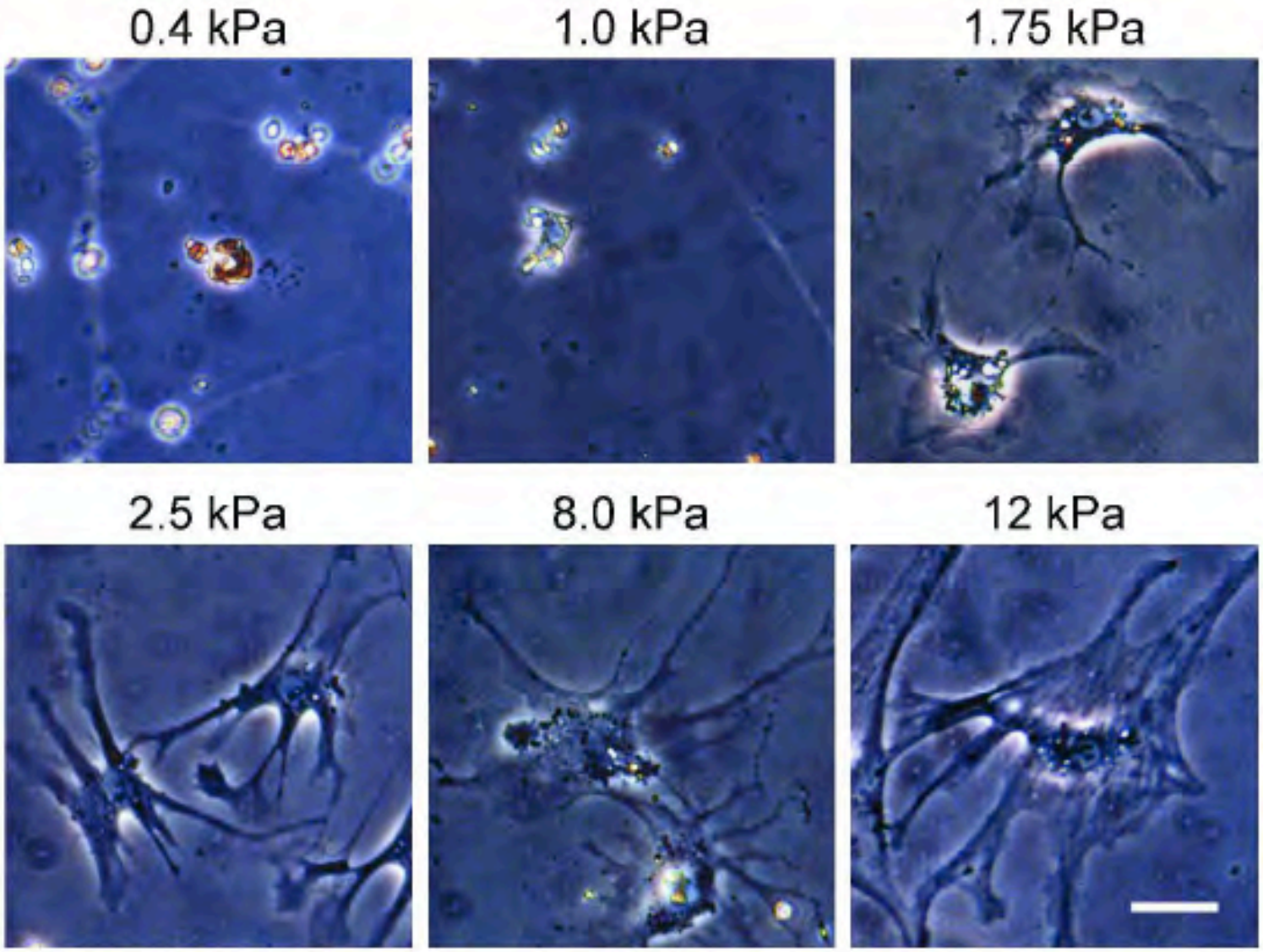
Sitffness might signal to cells in vivo:
Histological evidence of fibrosis occurs ***after*** liver stiffening



Hepatic stellate cells spontaneously activate on pathologically stiff substrates

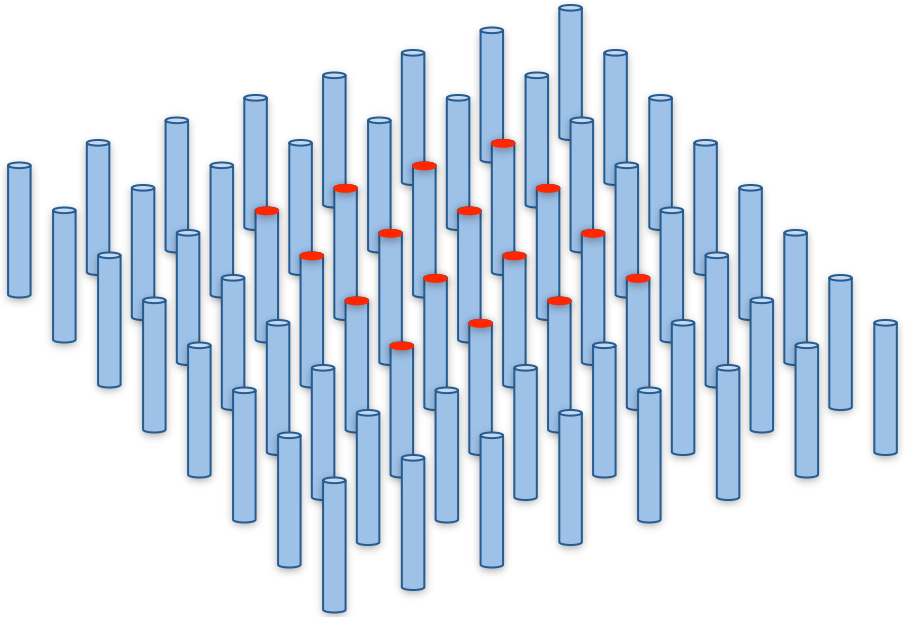
Rebecca Wells

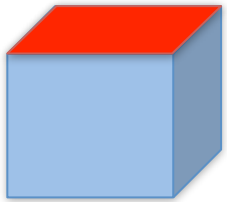
Olsen et al, Am. J. Physiol. 2011



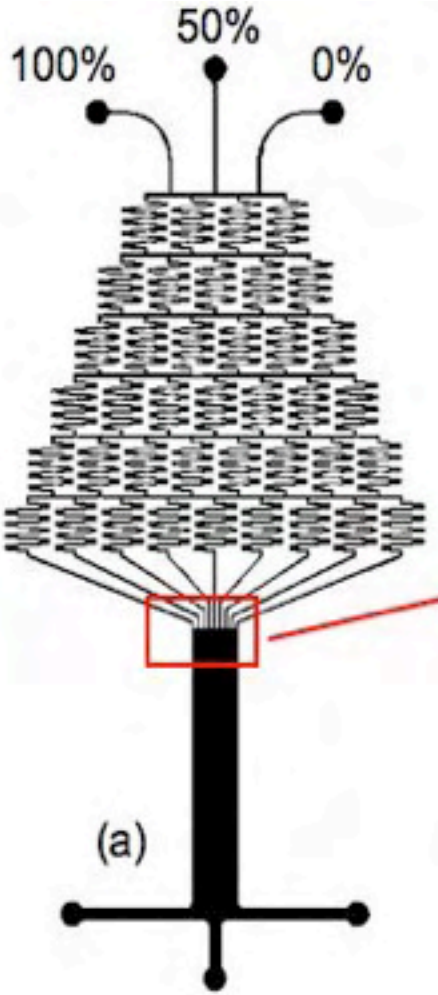
Different systems to study effects of substrate mechanics

Gels and pillar arrays

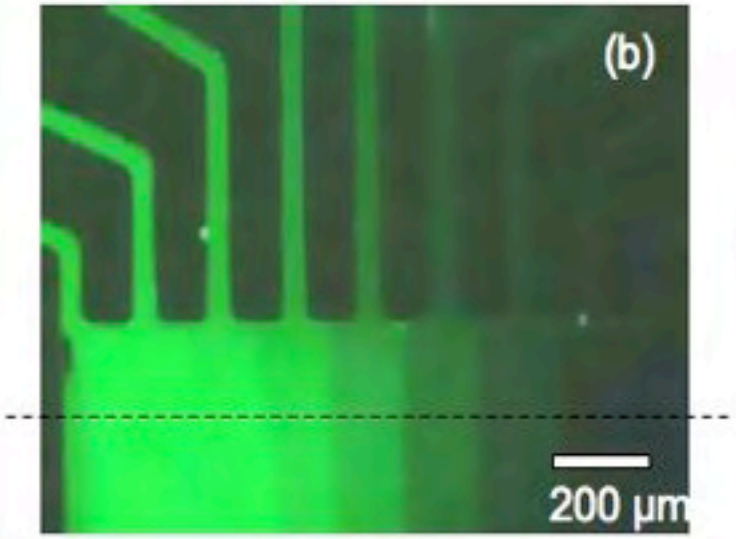


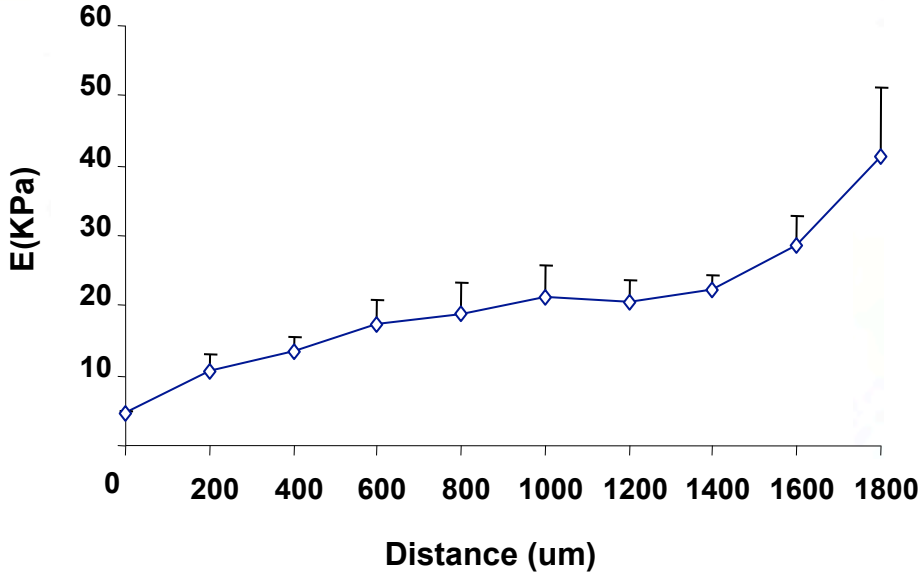


Microfuidics method to make gels with stiffness gradients



Dourian, Arratia

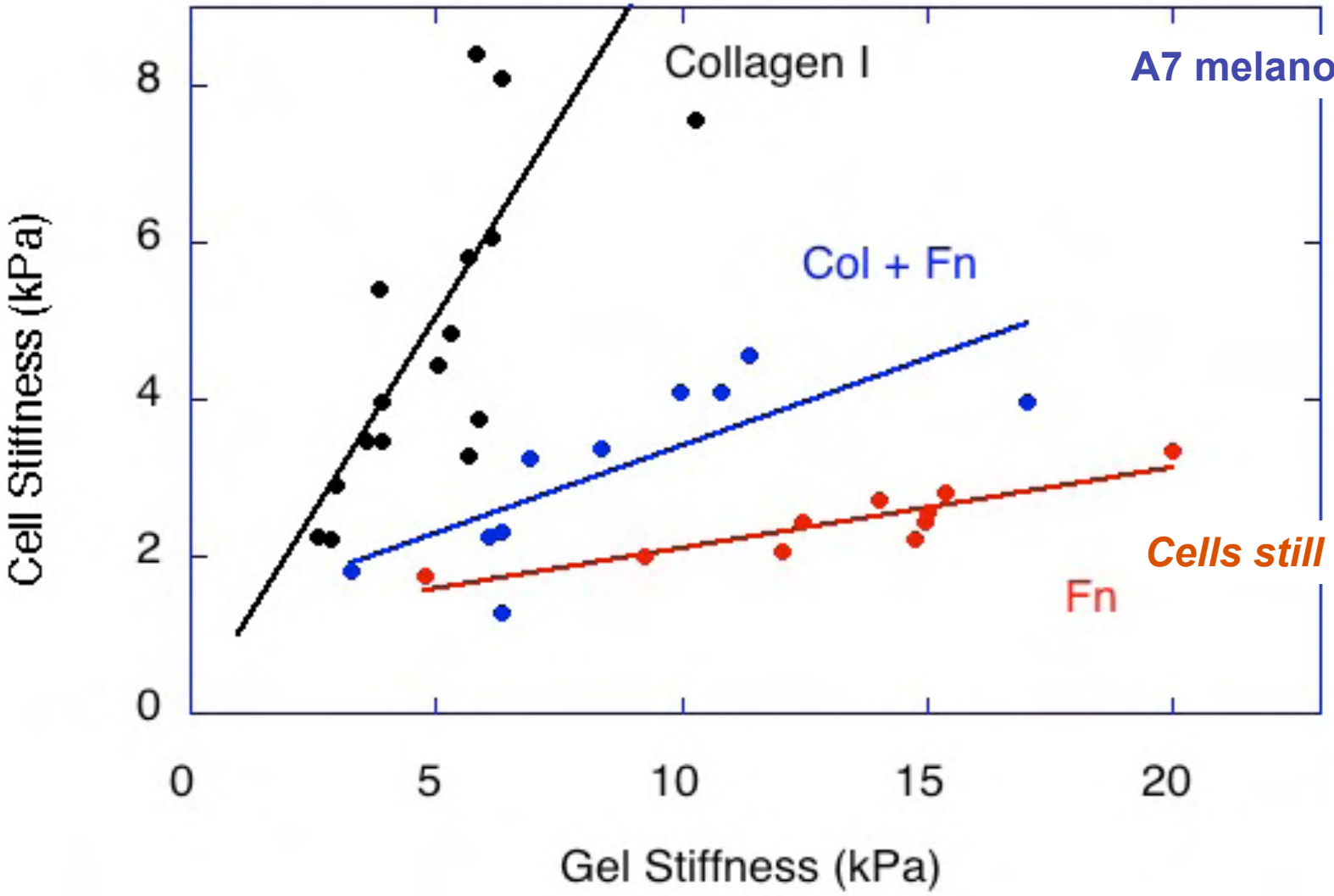




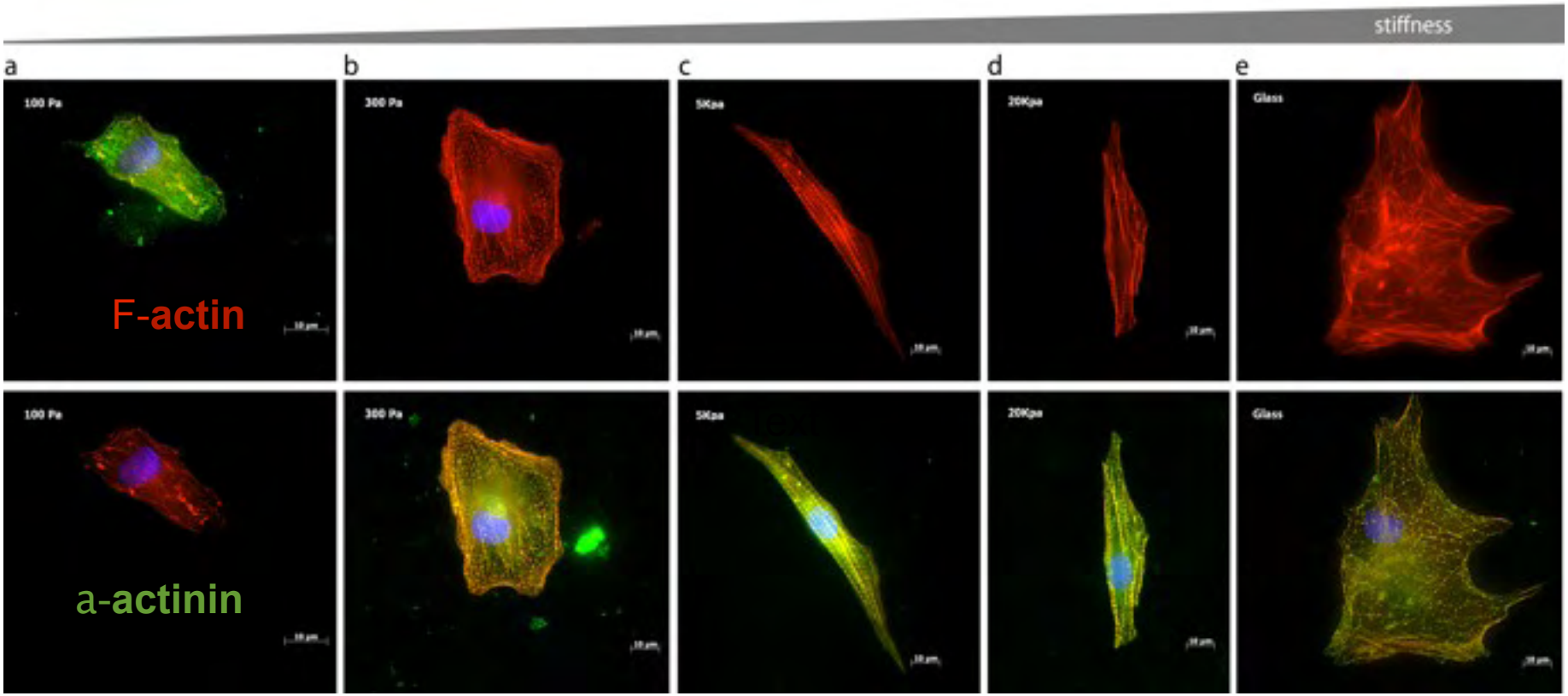
Adapted from: Zaari et al, *Advanced Materials* 2005

Byfield, Wen et al, *Biophys J* 2009

Cells tune their stiffness to match the substrate when they bind by specific adhesion receptors -



Cardiac myocytes on collagen-I and FN-coated PA gels



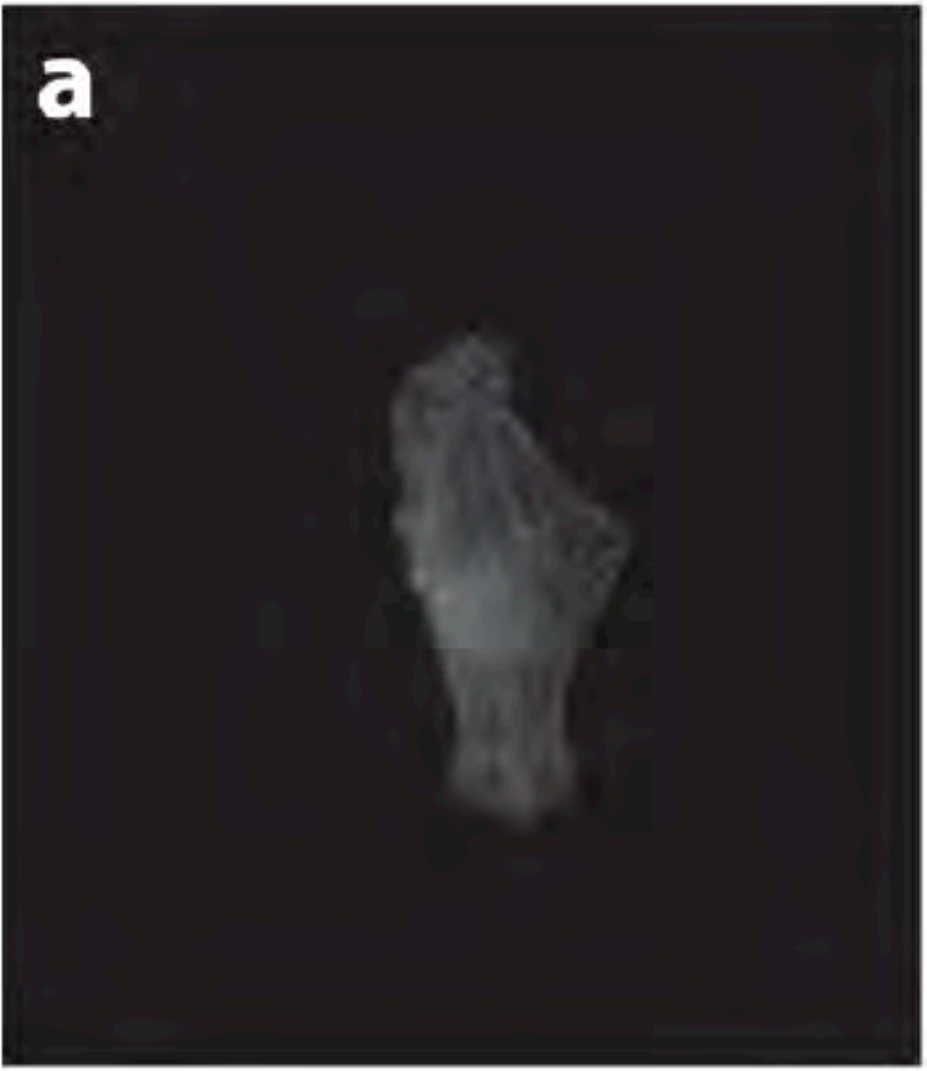
100 Pa 300 Pa 5 kPa 25 kPa glass

Anant Chopra,Erdem Tabdanov, **J. Yasha Kresh**

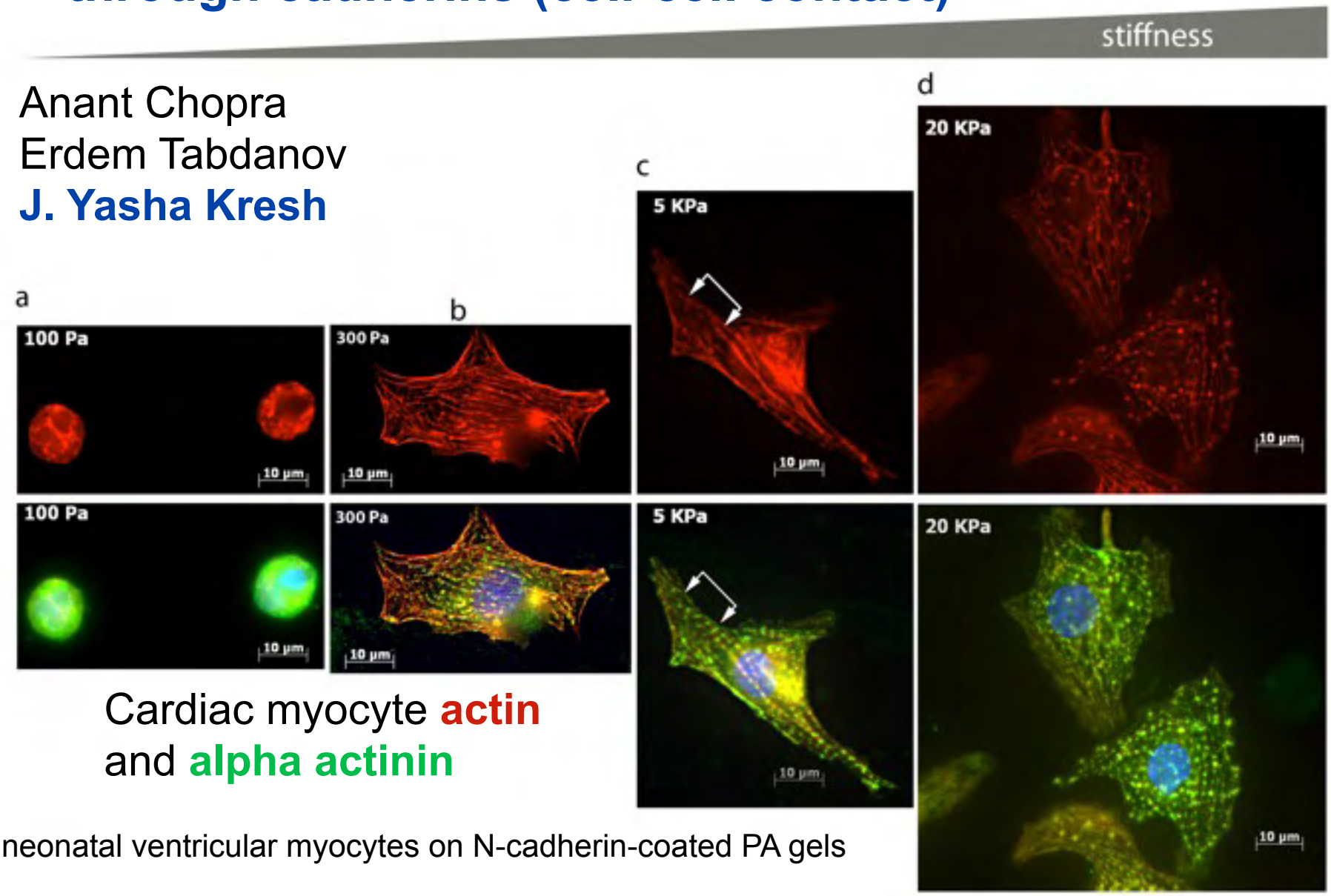
Chopra et al. Am J. Physiol. 2011

Collagen I

Fn



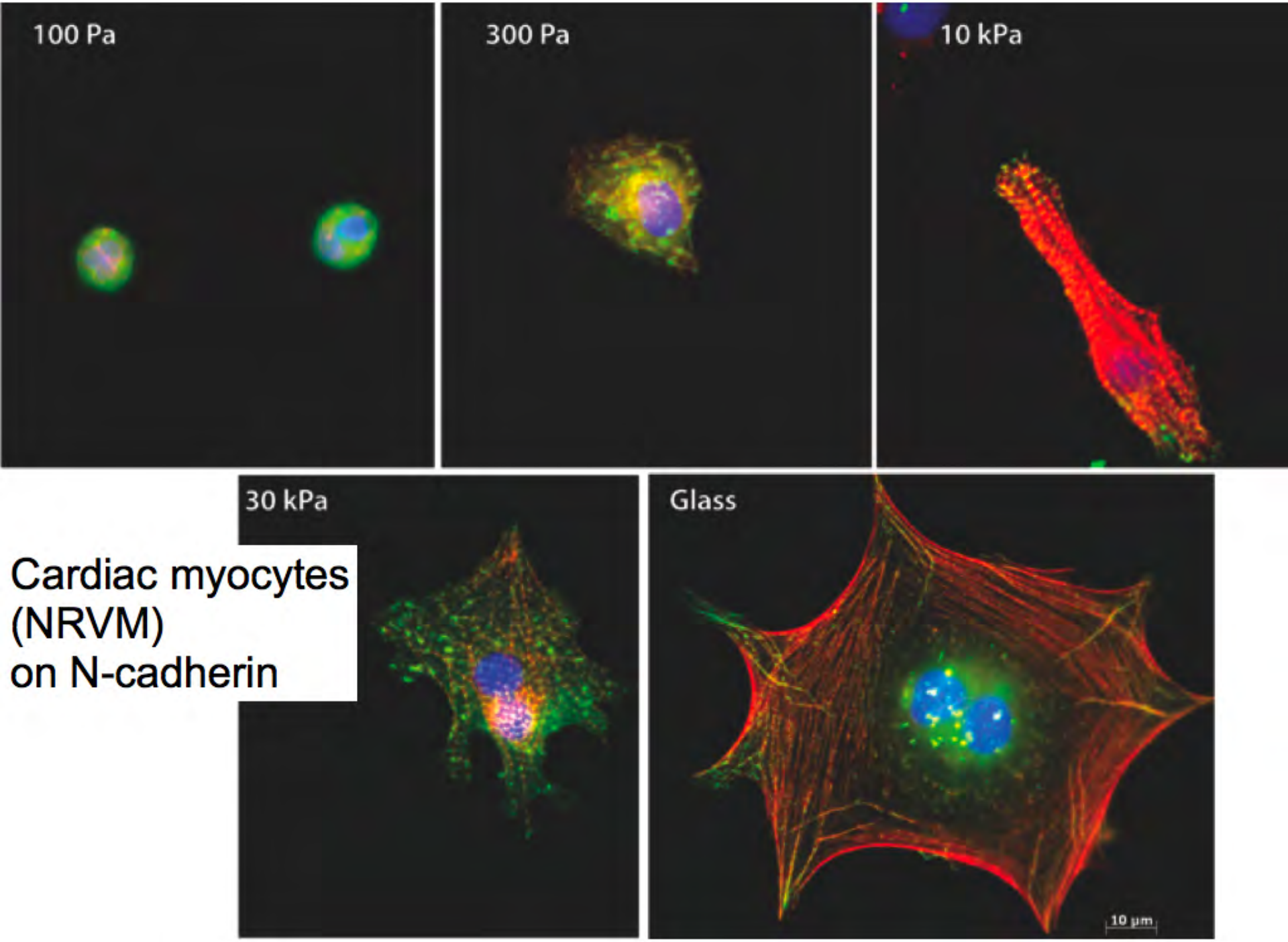
Stiffness sensing is preserved when signaling is through cadherins (cell-cell contact)



Anant Chopra
Erdem Tabdanov
J. Yasha Kresh

Cardiac myocyte **actin** and **alpha actinin**

rat neonatal ventricular myocytes on N-cadherin-coated PA gels



Cardiac myocytes
(NRVM)
on N-cadherin

How do cells sense or respond to stiffness?

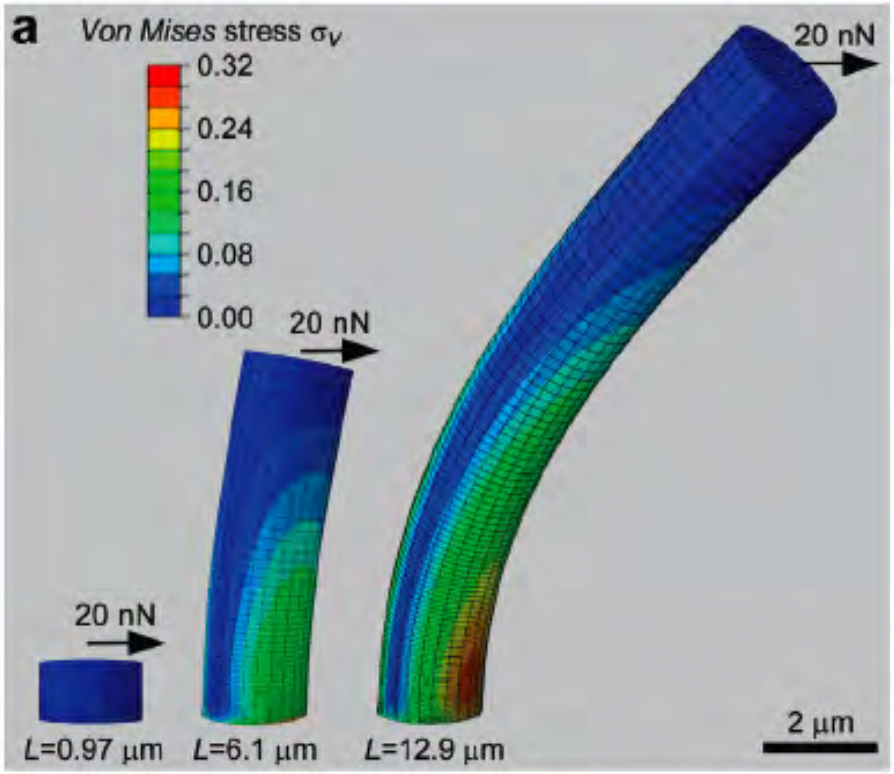
What is the range of stiffness that can be probed?
    100 - 100,000 Pa

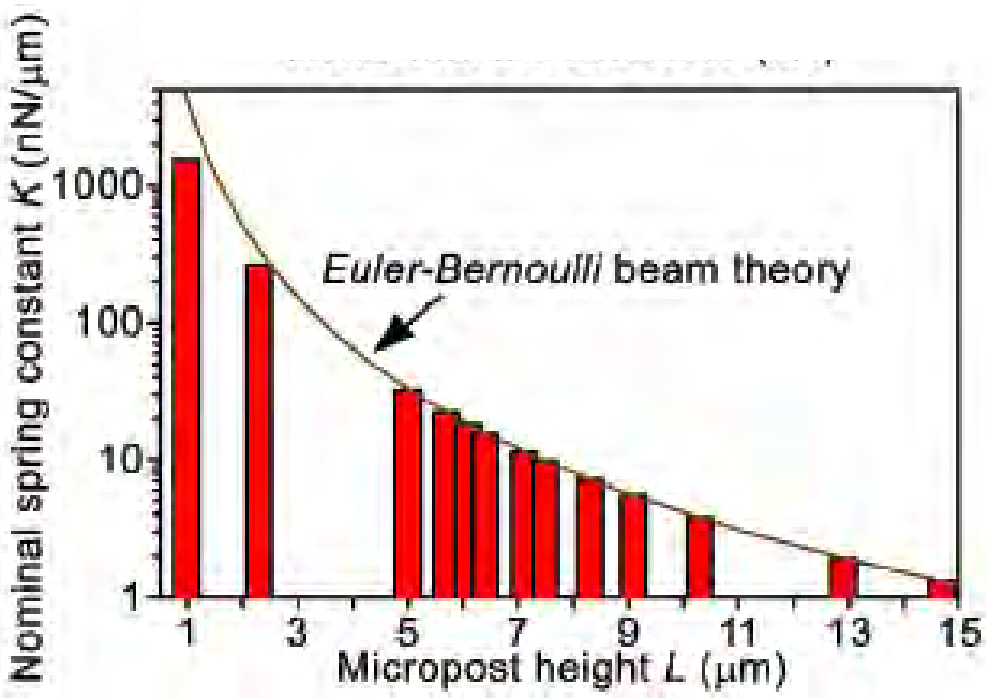
How much do cells deform their matrix?
How large an area do they probe?
How long do they integrate signal?

Is stress or strain controlled, and the other parameter measured?

Is the measurement static or dynamic?

Micropillars of different lengths to vary stiffness

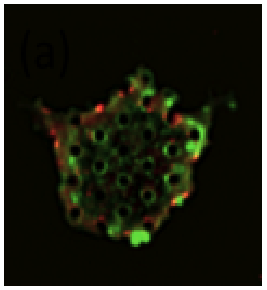




PDMS posts of identical composition (>100 kPa) and diameter (1.8μm), but different length.

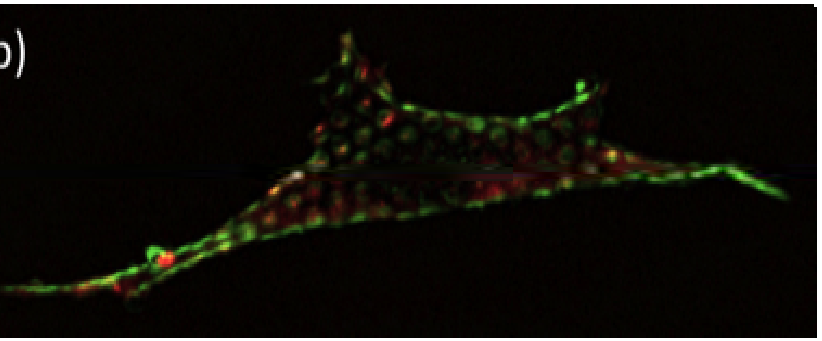
Jianping Fu et al. Nat. Meth. 2010
Saez et al, Soft Matter, 2009

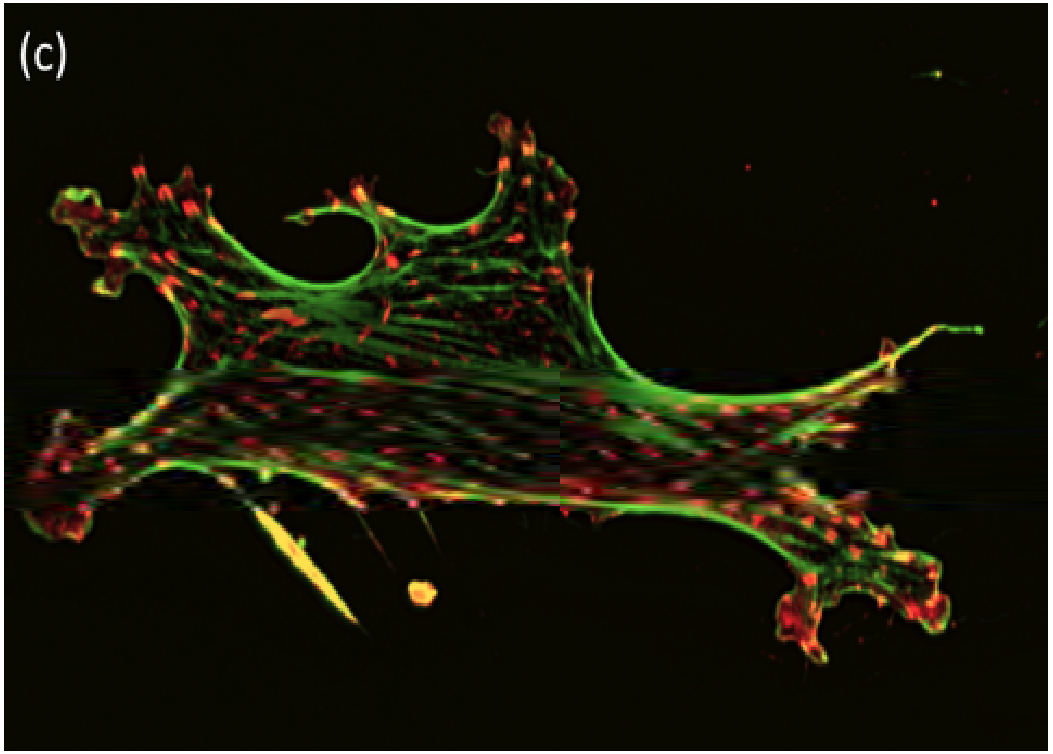
Cells can tell the difference between tall and short posts, even though the material and the pattern to which they bind is the same



hMSC
on Fn

paxillin
F-actin

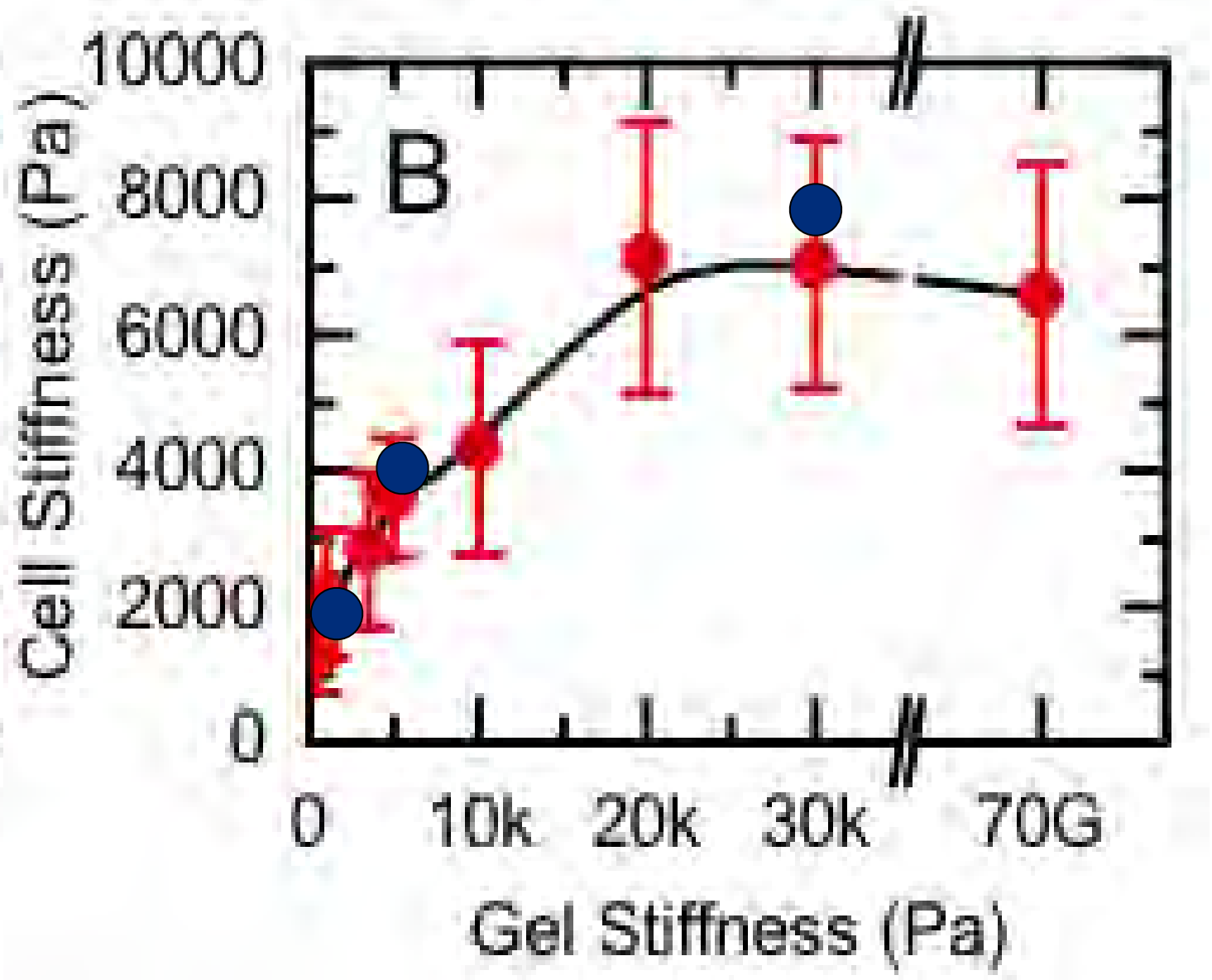




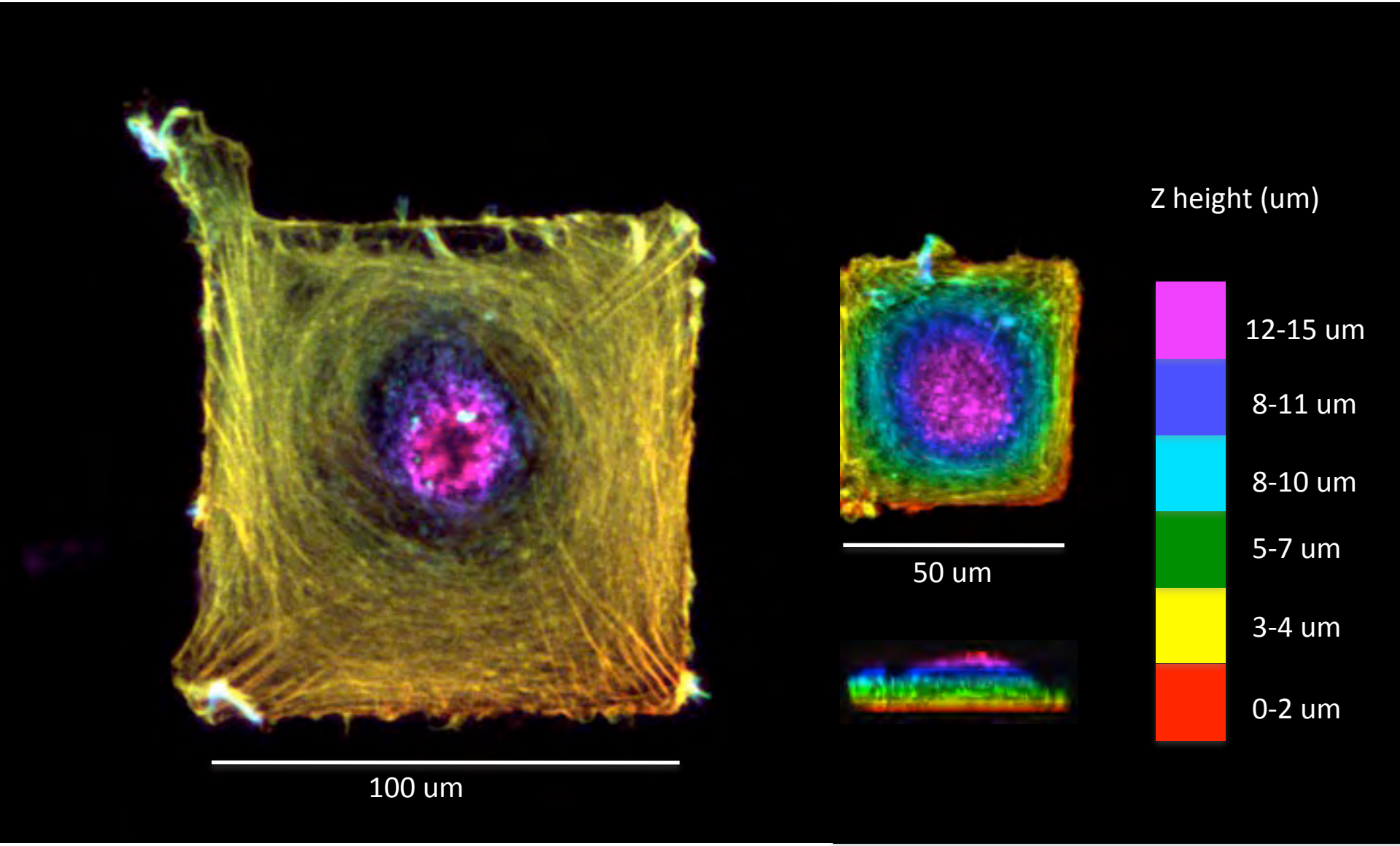
1.8 μm PDMS posts (>100 kPa) of different length spaced 6 μm apart. Long posts look soft, short posts look stiff

S Tee, J. Fu et al, Biophys J 2011

Effective stiffness of micropost arrays and gels have similar effects on cell stiffness

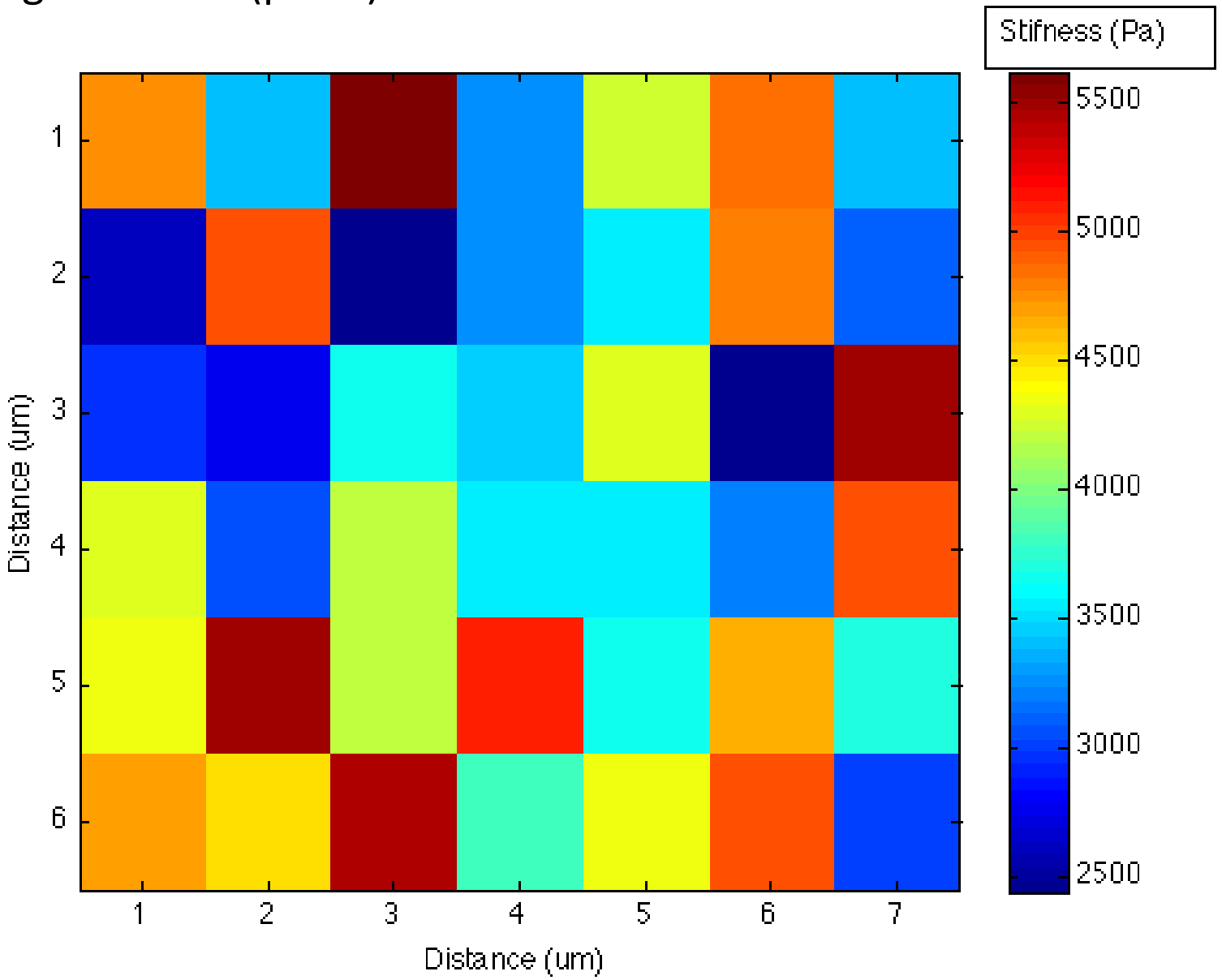


What happens when adherent size is constrained on short and tall pillars?

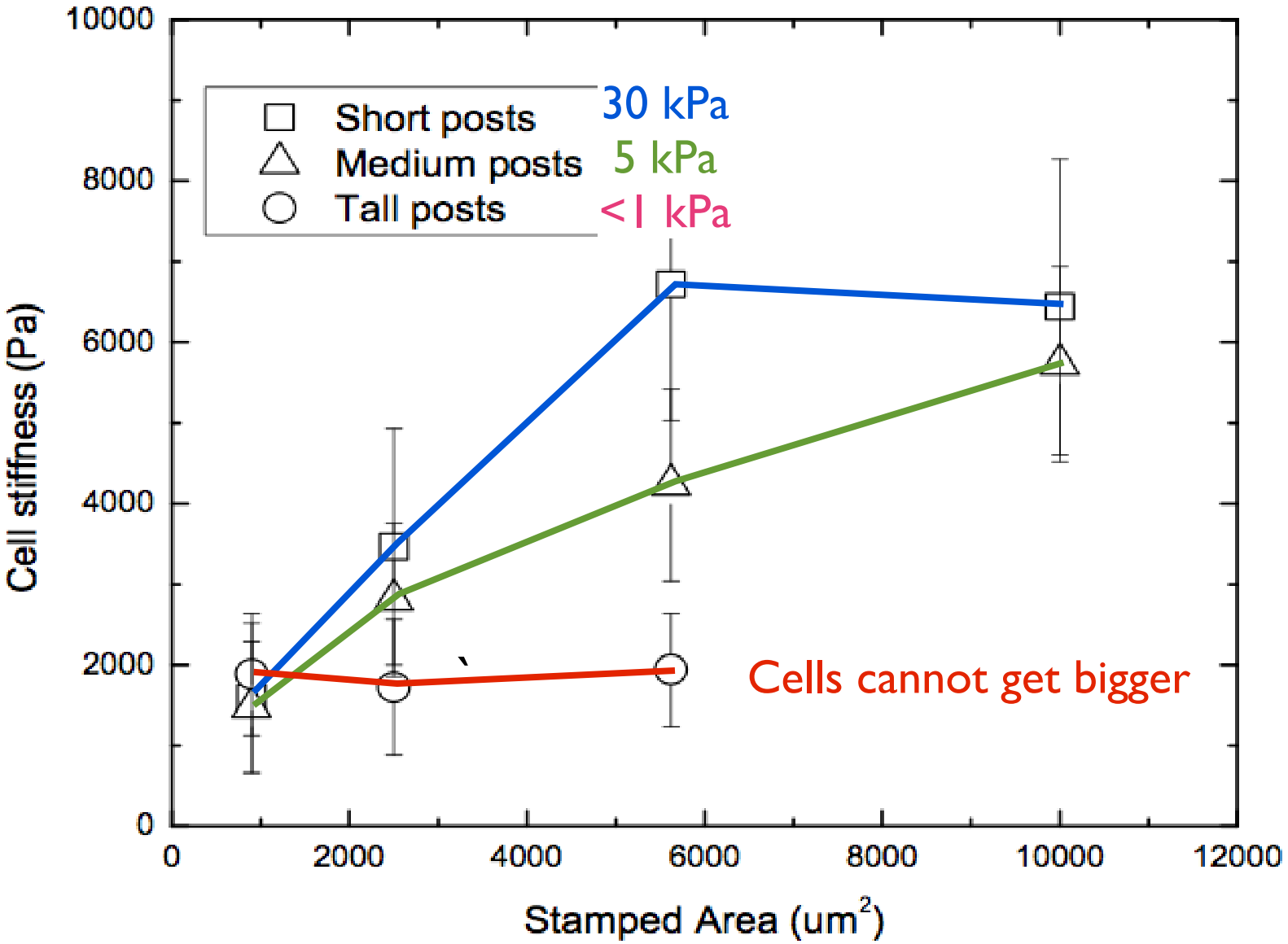


hMSC on stamped microposts stained with phalloidin

Small amplitude AFM poking measured cortical stiffness, not stress fibers or underlying substrate (posts)



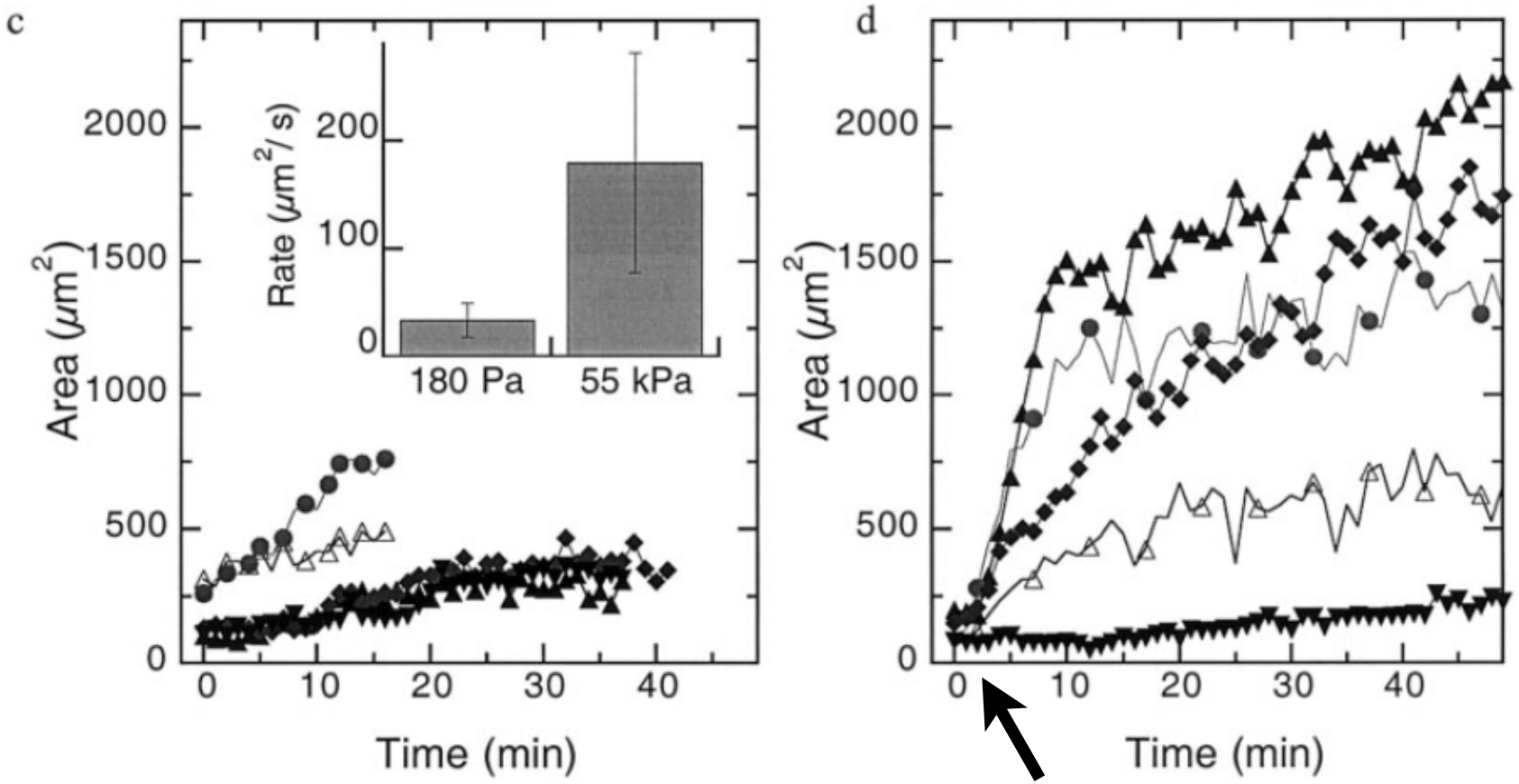
MSC stiffness depends on both substrate stiffness and adherent area



What is the time needed for mechanosensing?

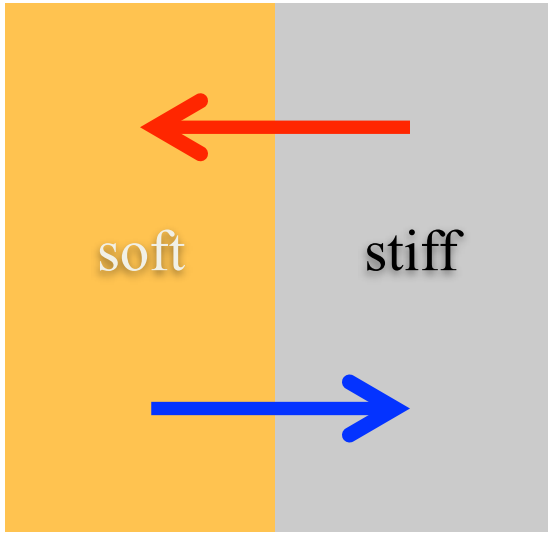
Experimentally what is measured is a cell's response, which is an upper limit

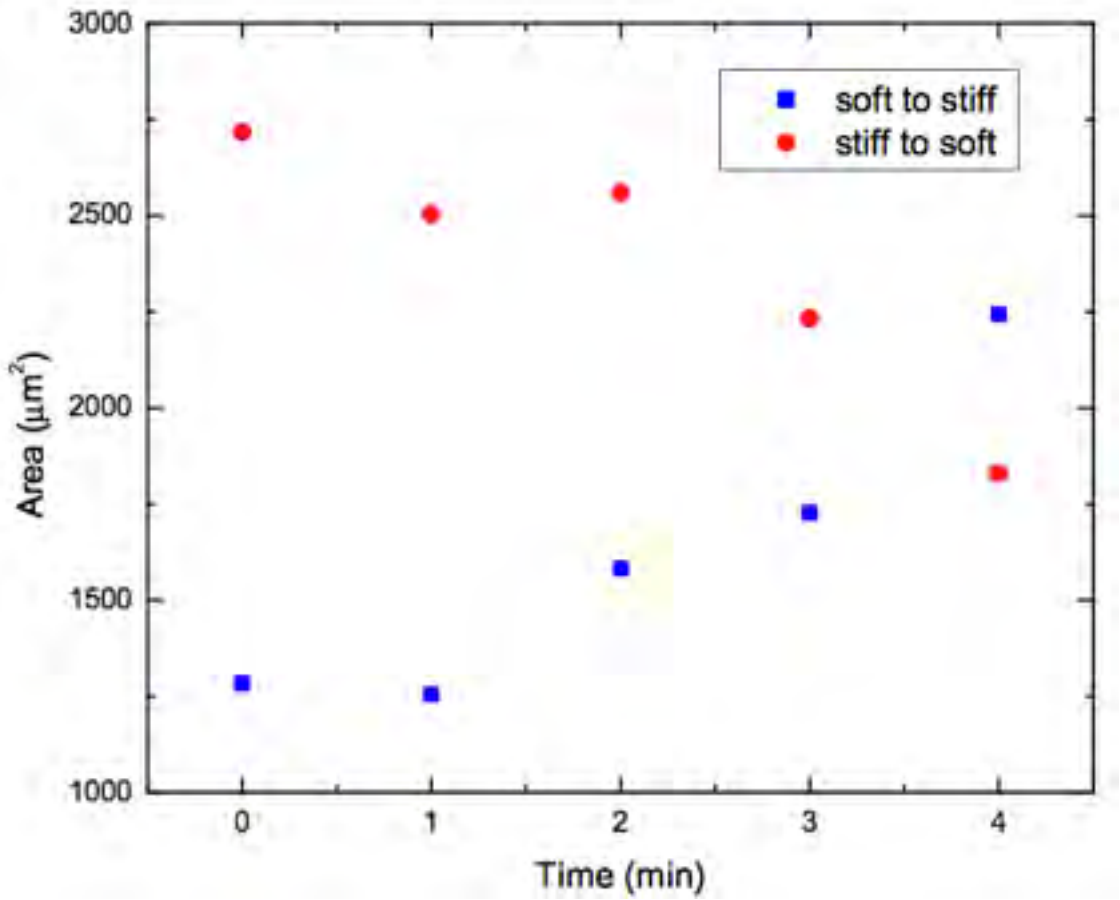
Initial spreading of fibroblasts settling from suspension onto Fn-coated gels with same adhesion ligand density but different stiffness



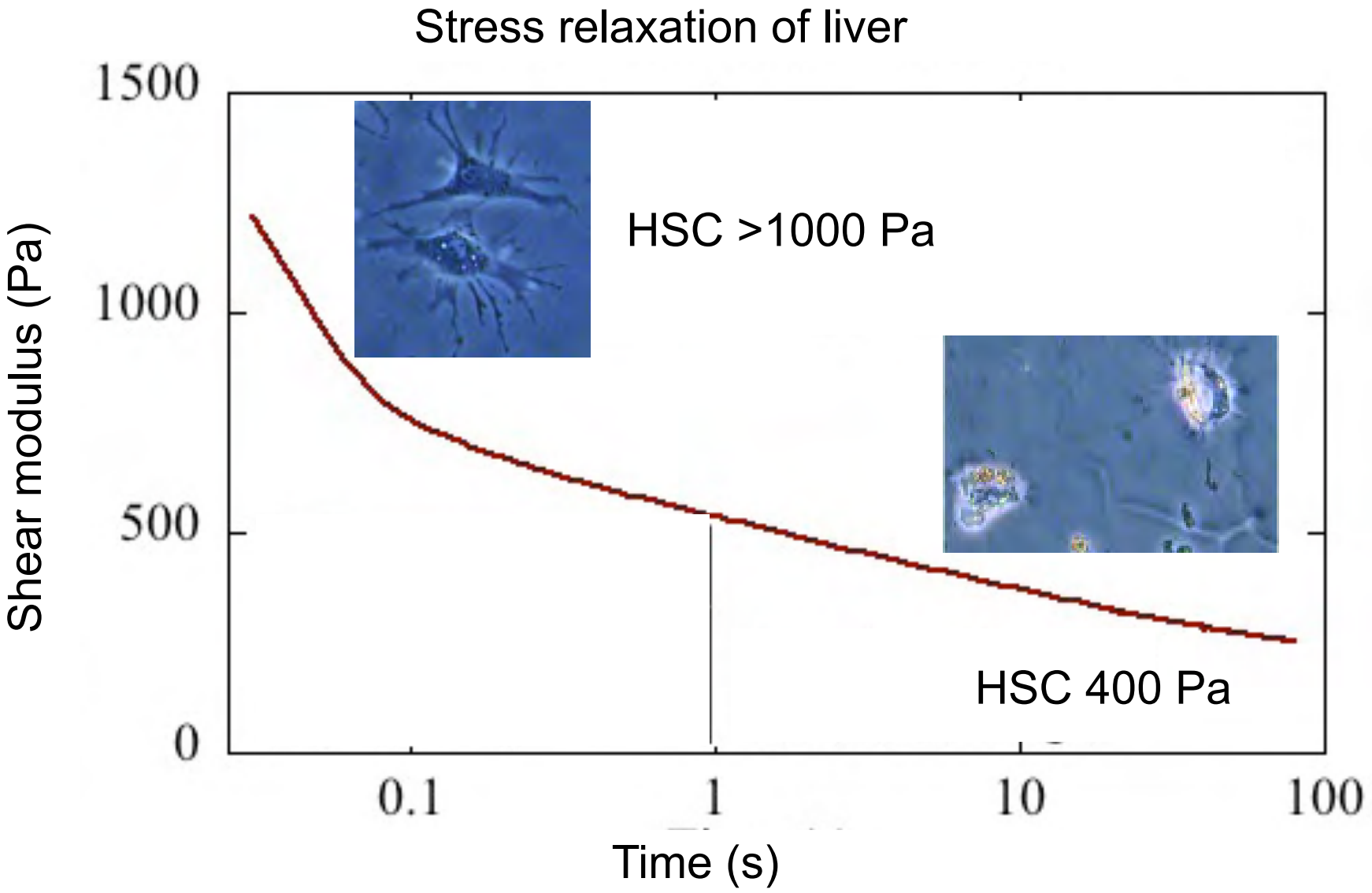
Two minutes is enough to measure stiffness

Cells can change area within minutes after crossing soft/stiff boundary



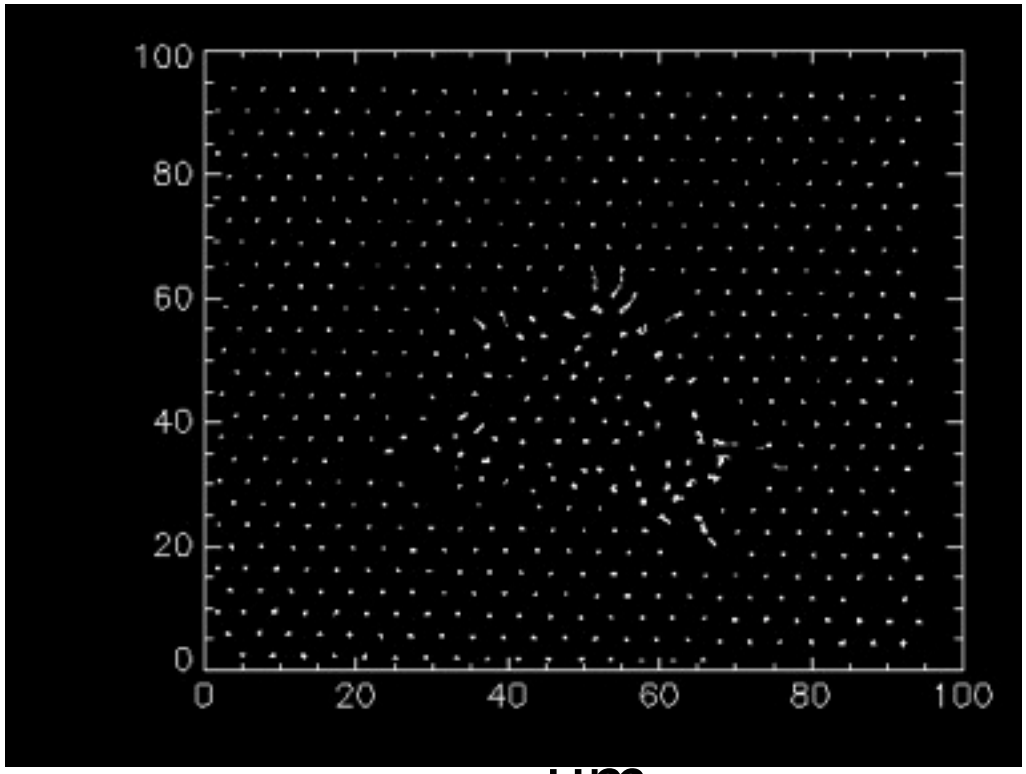


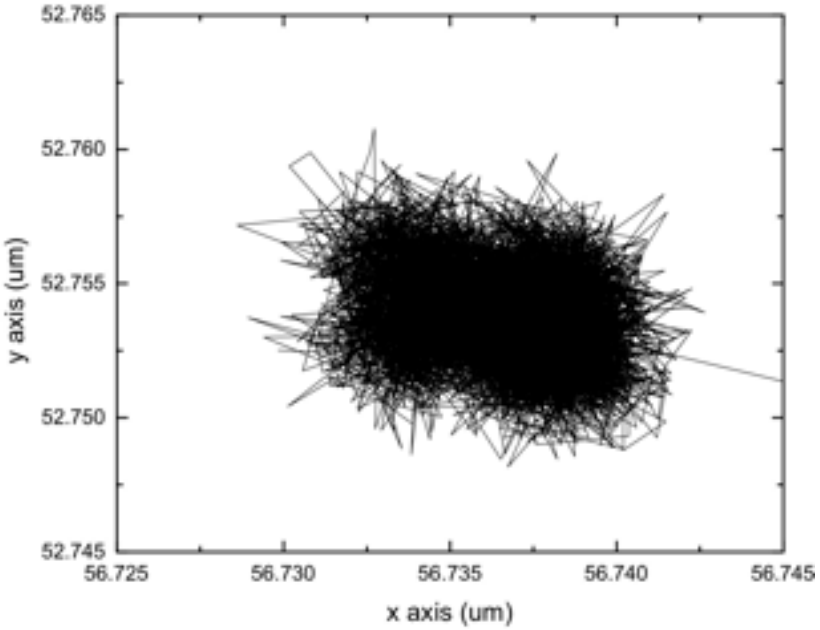
Tissues are not simple solids: flow on long time scales
What level of stiffness do cells use as a guide?



What happens locally?

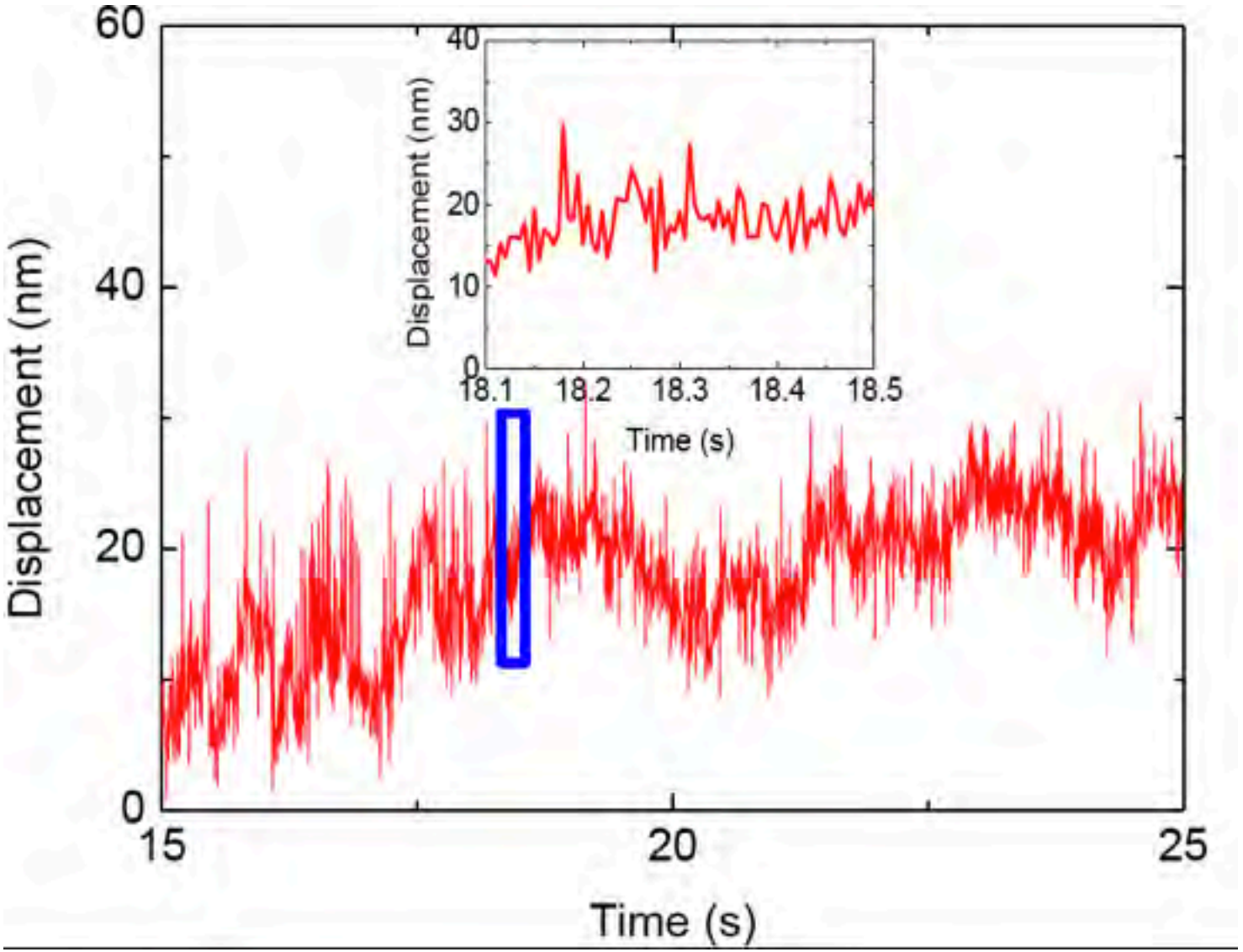
Force fluctuations on a single post, superimposed on mean deflection.





Shang Tee, John Crocker

Rapid ~10 nm and ~10 pN scale fluctuations might be the basic element of the stiffness sensor



Conclusions

Soft tissues have well-defined and controlled elastic moduli

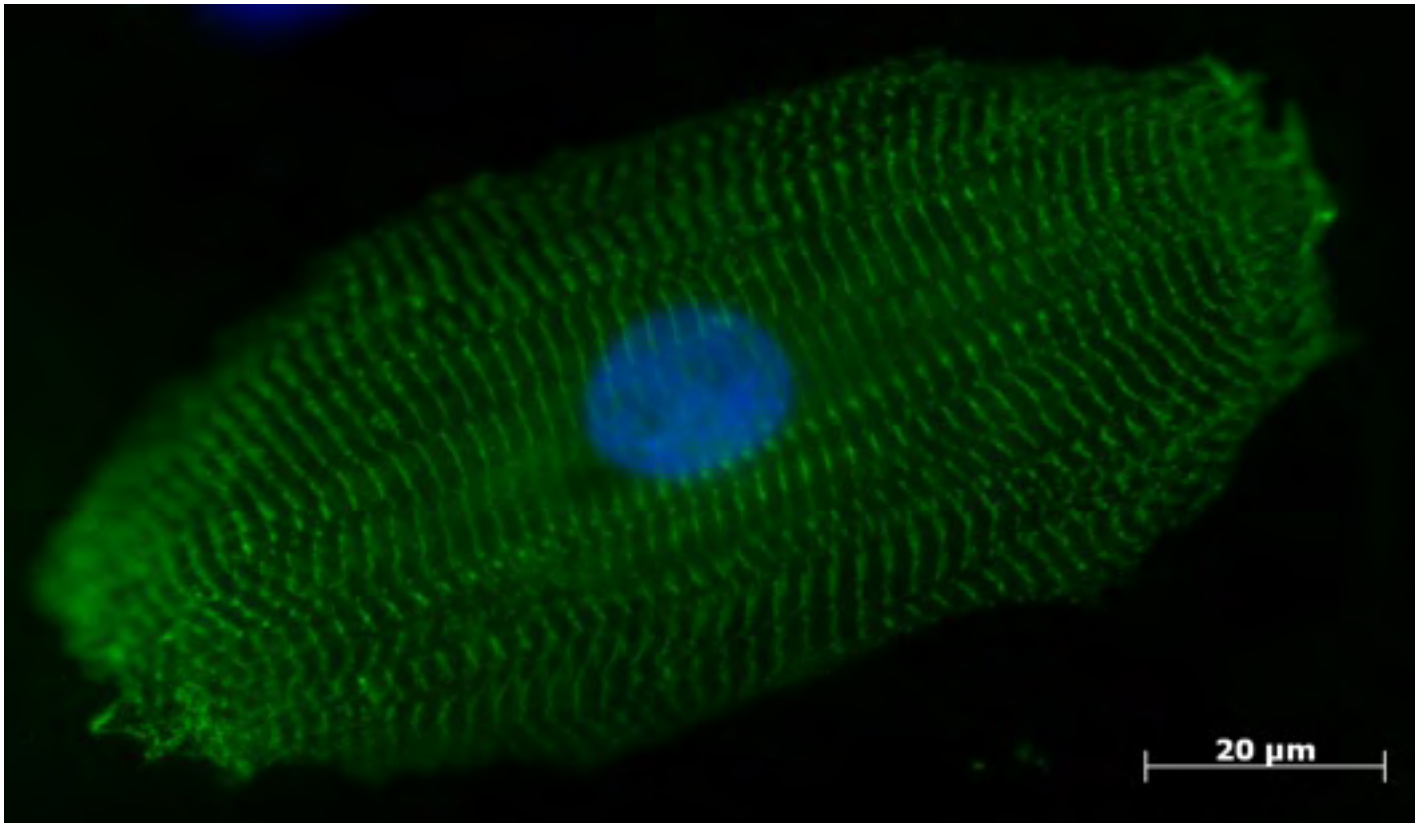
Cells in vitro do not: their stiffness depends on environment

Substrate stiffness affects each cell type differently, and manipulating matrix stiffness can influence cell fate.

Stiffness is sensed at both cell-ECM and cell-cell interfaces by distinct mechanisms

Stiffness sensing appears to operate on a length scale >1 μm and a time > 1 s.

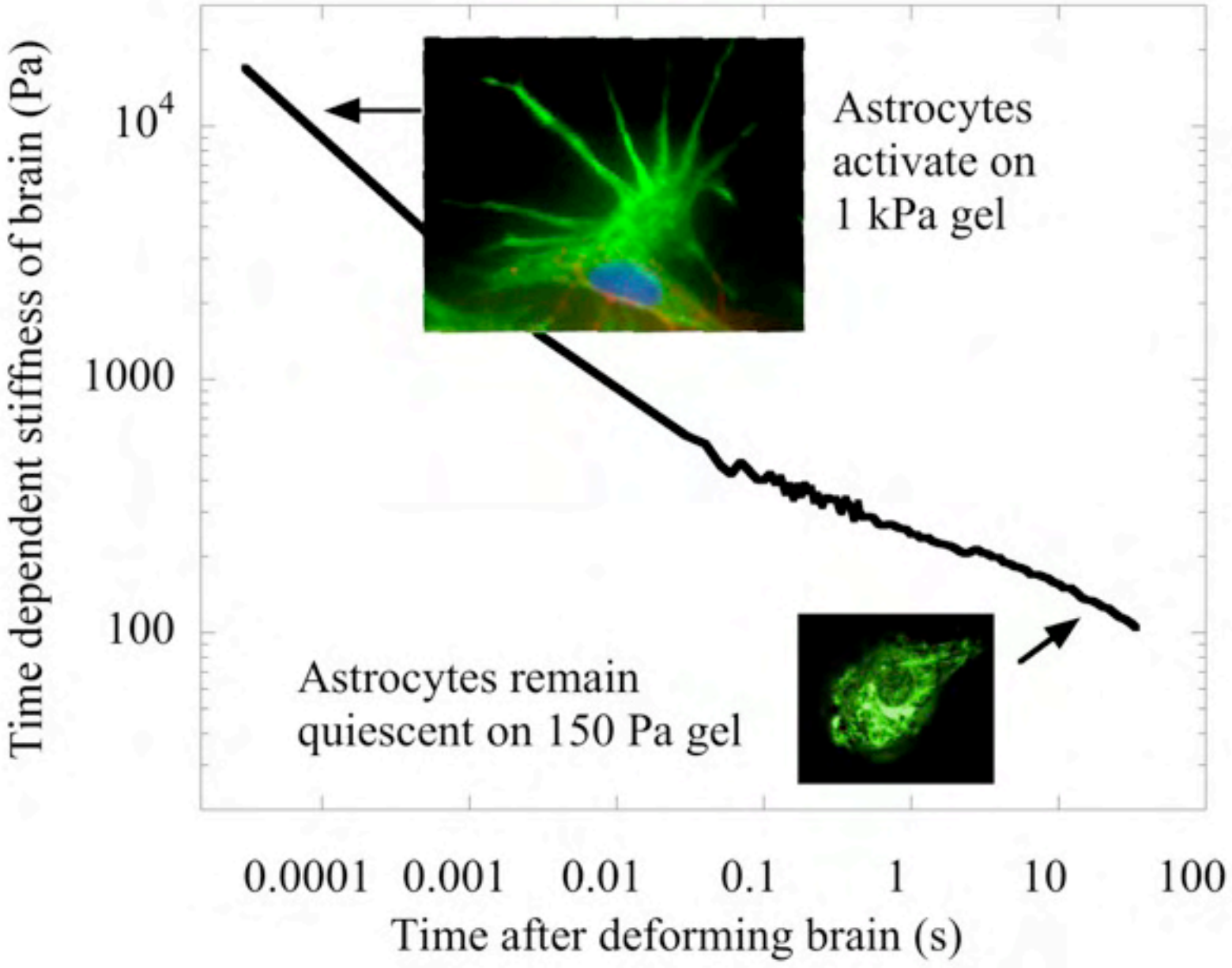
Elastic modulus of matrix and neighboring cells seems to be key to producing native phenotype.
But not quite:



Cardiac myocyte on Fn-laminated gel of hyaluronic acid instead of Fn-laminated polyacrylamide. E < 900 Pa

Sarah Atzet, Glenn Prestwich, Glycosan, Inc.

What stiffness do astrocytes and neurons feel in the brain?



Conclusion 1

Stiffness (elastic modulus) is a well-conserved property of soft tissues

Abnormal matrix stiffness is sufficient to trigger cellular reponses

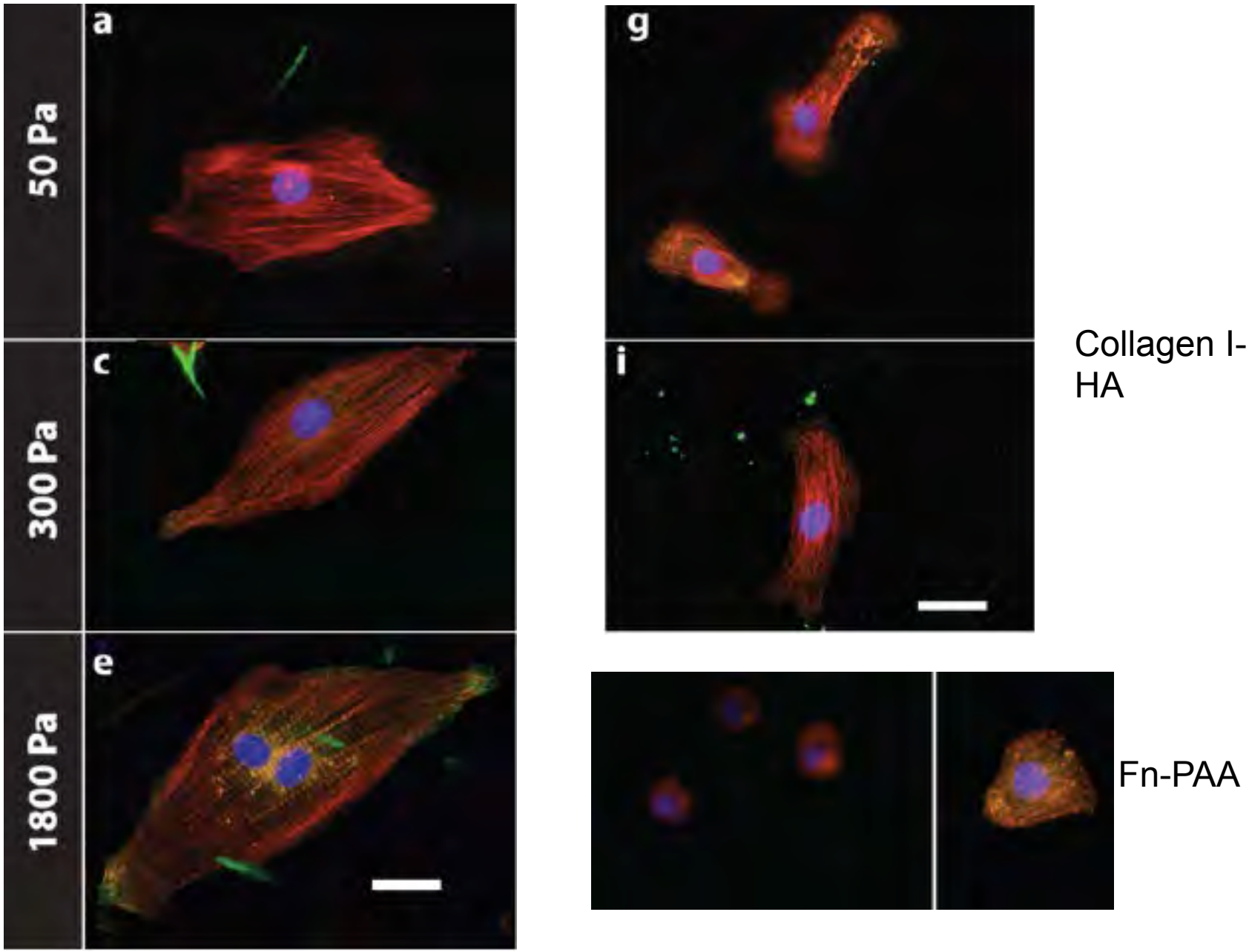
Stiffness sensing works through both cell-cell and cell-matrix attachments

Mechanosensing depends on type of integrin engaged

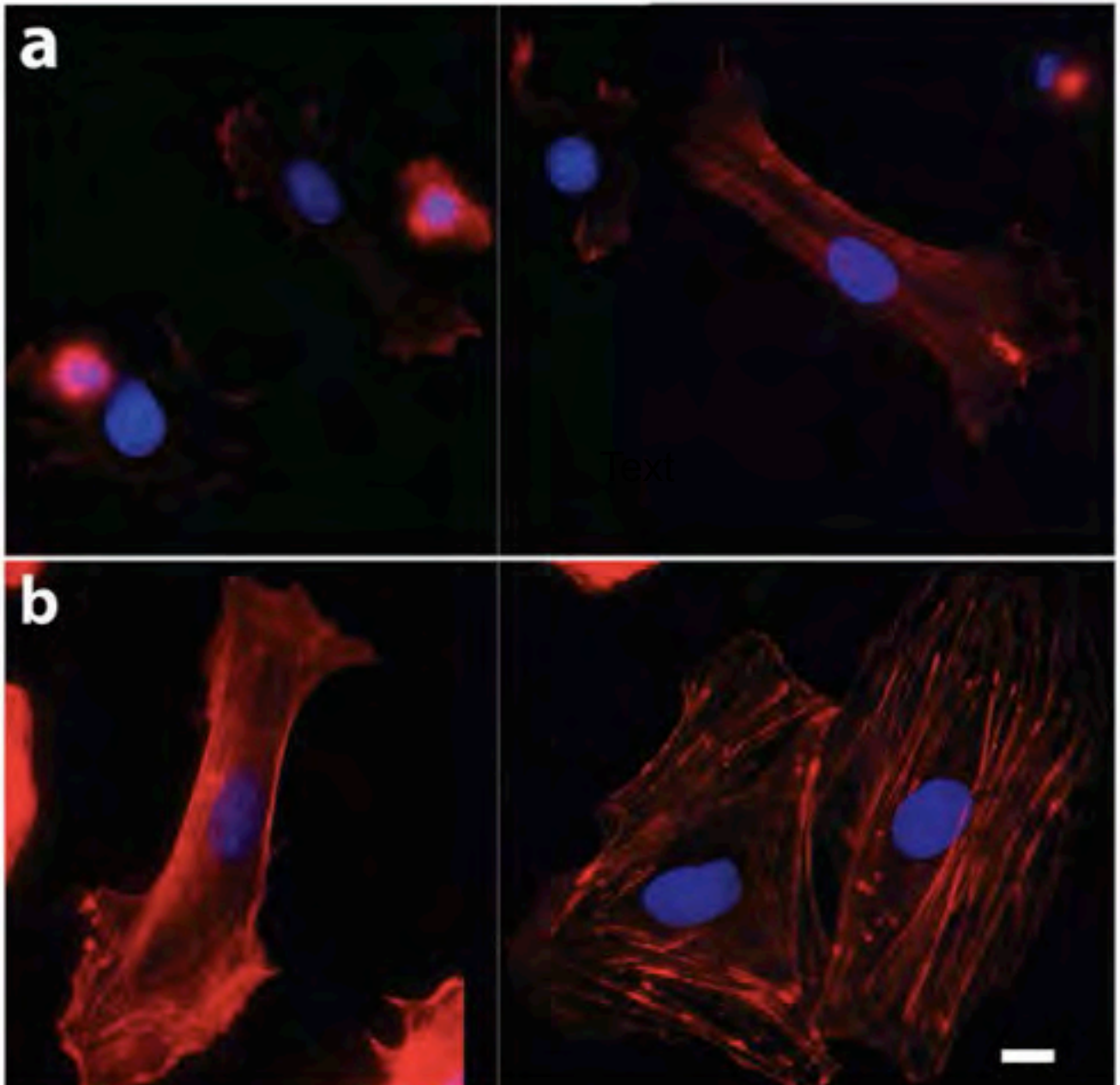
- Fn seems to be more efficient than collagen I or N-cadherin for driving native myocyte structure

Stiffness sensing appears to occur on a >> molecular scale

Hyaluronic acid reprograms mechanosensing by cardiomyocytes



Cardiac fibroblasts form large actin fibers and adhesion complexes on very soft Fn-HA gels

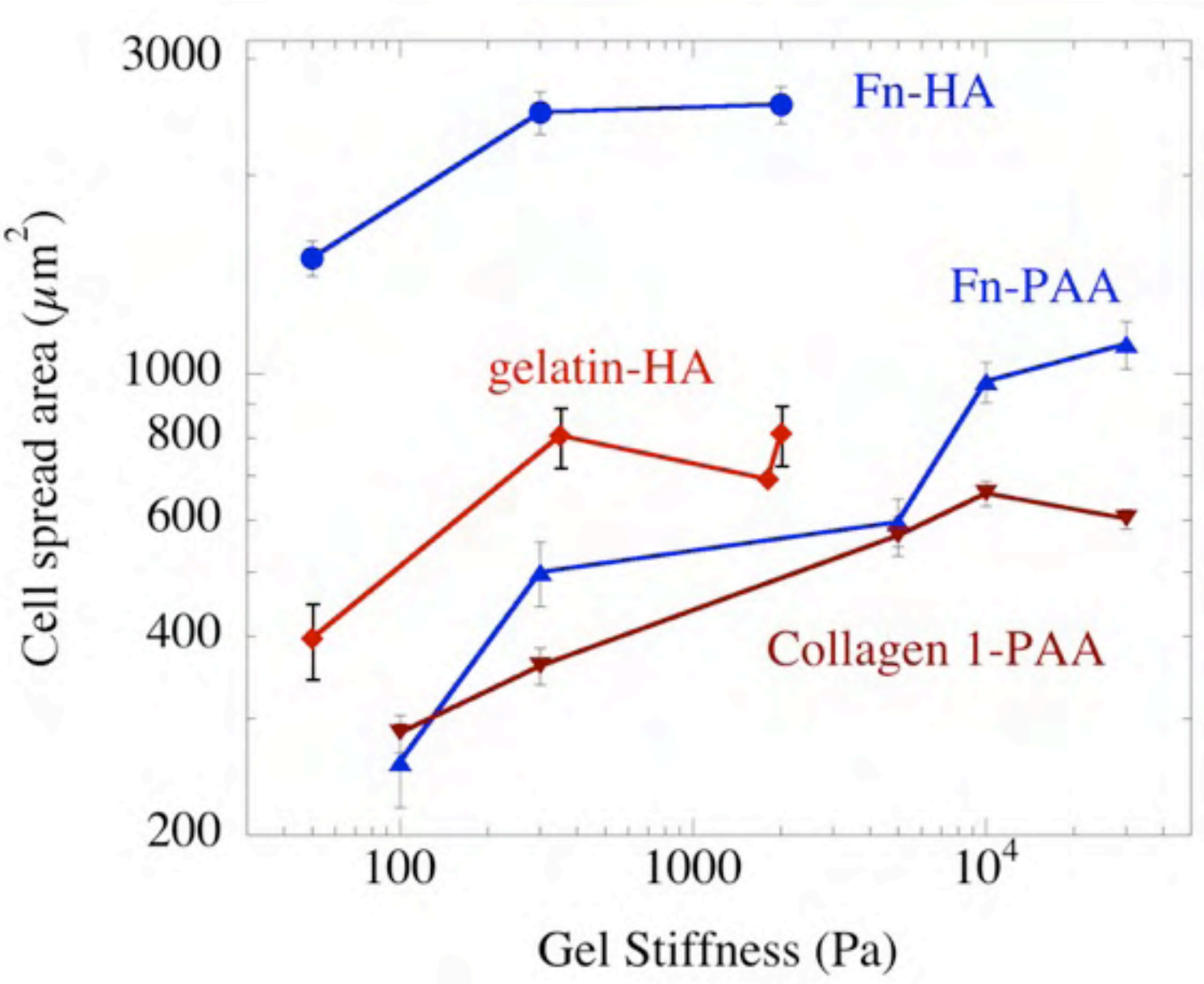


300 Pa Fn-PAA

300 Pa FN-HA

"**stress fibers**" without stress

Hyaluronic acid reprograms mechanoresponse by cardiomyocytes



Conclusions

Tissue stiffness is well-regulated and changes in disease

A cells properties depend on the physical properties of its environment

The mechanism by which a cell senses extracellular matrix depends on the type of transmembrane complex by which it binds

Receptors for HA can reset the stiffness sensitivity of cells that adhere through integrins