

# Cell-Scaffold Interactions:

Scaffold Degradation

Cell Attachment

Cell Morphology

Cell Contractility

Cell Migration

Cell Differentiation

## Cell scaffold interactions

- Scaffolds also being used to characterize cell-scaffold interactions  
e.g. how cell behaviour (attachment, migration, contraction, differentiation) is affected by substrate

## Scaffold degradation

- native ECM - enzymes produced by cells resorb ECM over time;  
cells also synthesize new ECM to replace it  
e.g. bone-rates of resorption + synthesis depend on loading
- 
- cells also degrade tissue engineering scaffolds
  - length of time scaffold remains insoluble called "residence time"
  - require scaffold degradation to occur in a manner that does not interfere with new ECM synthesis
  - scaffold residence time must be approx. equal to the time required to synthesize new ECM

- degradation rate for scaffold depends on its chemical composition + crosslinking and on relative density of scaffold
- Synthetic polymers - can vary molecular weight of polymers + ratio of co-polymers eg. PLGA higher GA:LA ratio polymers degrade quicker
- collagen based scaffolds - can control degree of cross-linking

physical methods: - dehydrothermal (DHT) treatment (105°C vacuum 24 hrs)  
- removes water, forms interchain bonds through condensation

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- UV treatment

chemical methods - glutaraldehyde; carbodiimide treatments

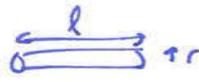
## Cell adhesion

- cells attach to ECM at focal adhesion
- at focal adhesion
  - cell has integrins - trans membrane proteins that bind to ligands on ECM
  - other end of integrin connects to sub-membrane plaque that then connects to cell's cytoskeleton (eg. to actin filaments)
- cell behaviours such as attachment, migration, proliferation, contraction

- affected by interactions between focal adhesions + integrins
- biological activity of scaffolds depends on density of ligands available for integrins to bind to
  - ligand density depends on composition of scaffold + surface area/volume of scaffold
  - Biological polymers that are constituents of native ECM (e.g. collagen) have a range of native binding sites
  - synthetic polymers don't have binding sites + need to be functionalized with adhesive proteins such as fibronectin + laminin

(4)

- specific surface area (SA/vol) of scaffold depends on pore size,  $d$ , + relative density:

- for a tetrakaidecahedral unit cell 

$$\frac{SA}{V} \propto \frac{1}{d} \left( \frac{\rho^*}{\rho_s} \right)^{1/2} \quad \left[ SA/V = \frac{2\pi r l n}{l^3} \propto \frac{r}{l^2} \propto \frac{r}{l} \frac{1}{l} \propto \left( \frac{\rho^*}{\rho_s} \right)^{1/2} \frac{1}{d} \right]$$

- dependence of cell attachment on specific surface area was measured by seeding cells (MC3T3-E1 mouse osteogenic) onto collagen-GAG scaffolds of constant relative density ( $\rho^*/\rho_s = 0.006$ ) + varying pore size

$$d = 96, 110, 121, 151 \mu\text{m}$$

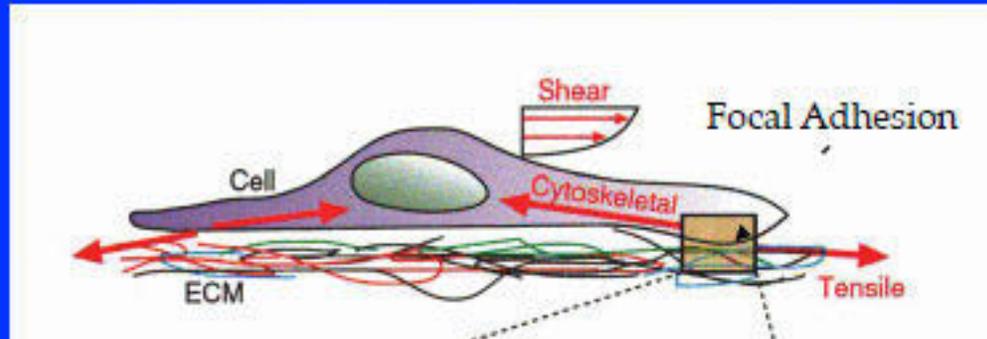
- number of cells attached measured at 24, 48 hours
- fraction of cells attached increased linearly with specific surface area

### Cell Morphology

- cell orientation follows scaffold pore orientation
- cell morphology can depend on substrate stiffness

Cell contraction } see slides.  
Cell migration }

# Cell Adhesion



Gibson, L. J., M. Ashby, et al. *Cellular Materials in Nature and Medicine*. Cambridge University Press. © 2010. Figure courtesy of Lorna Gibson and Cambridge University Press.

Figure removed due to copyright restrictions. See Figure 9.1: Gibson, L. J., M. Ashby, et al. *Cellular Materials in Nature and Medicine*. Cambridge University Press, 2010.  
<http://books.google.com/books?id=AKxiS4AKpyEC&pg=PA255>

Gibson, Ashby and Harley, 2010

# Cell Attachment

$$\frac{SA}{V} = \frac{3.65}{l} \left( \frac{\rho^*}{\rho_s} \right)^{1/2} = \frac{0.718}{d}$$

Open-cell tetrakaidecahedron

Circular cross-section edges

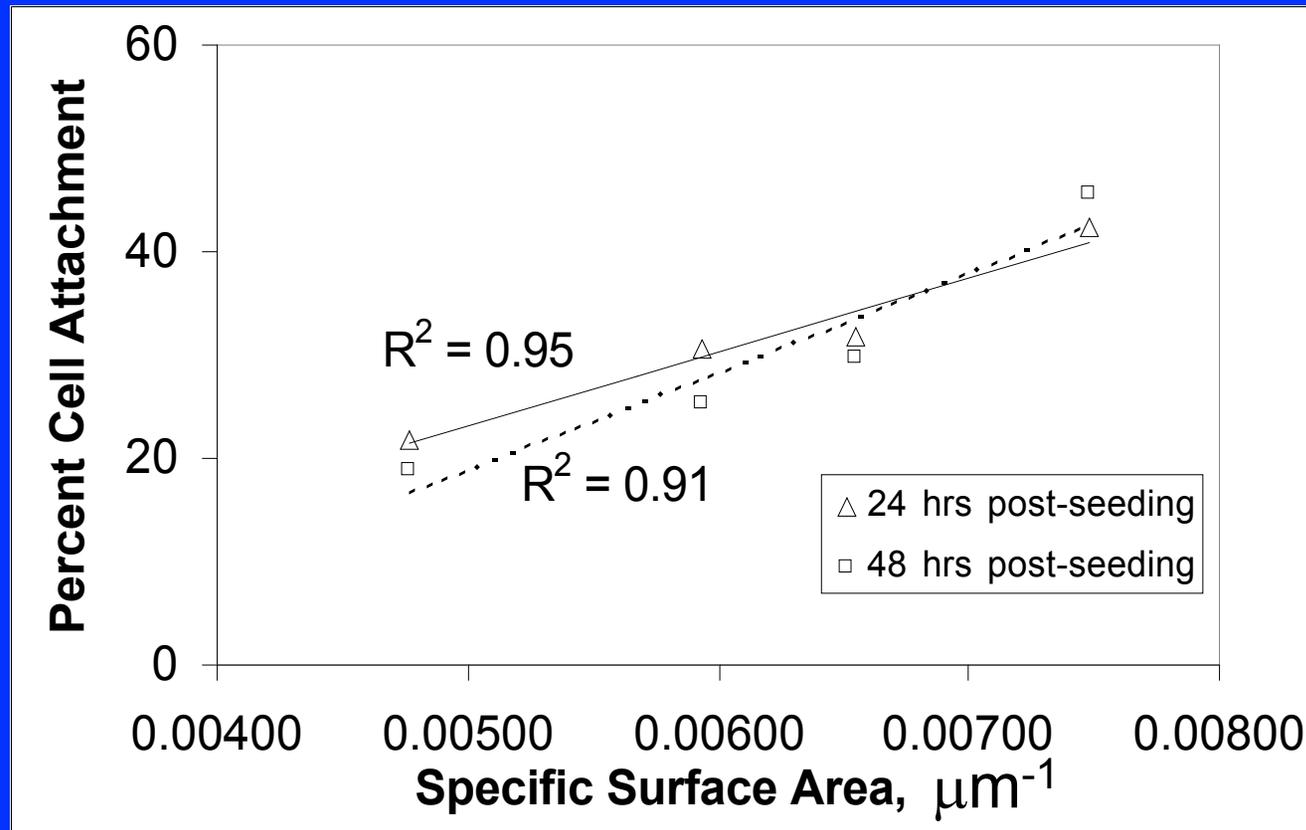
$l$  = edge length

$d$  = pore size

Collagen-GAG scaffold:

$\rho^*/\rho_s = 0.005$ ,  $d = 96, 110, 121,$   
 $150\mu\text{m}$

# Cell Attachment



O'Brien, B. A. Harley, I. V. Yannas, et al. *Biomaterials* 26 (2005): 433-41.

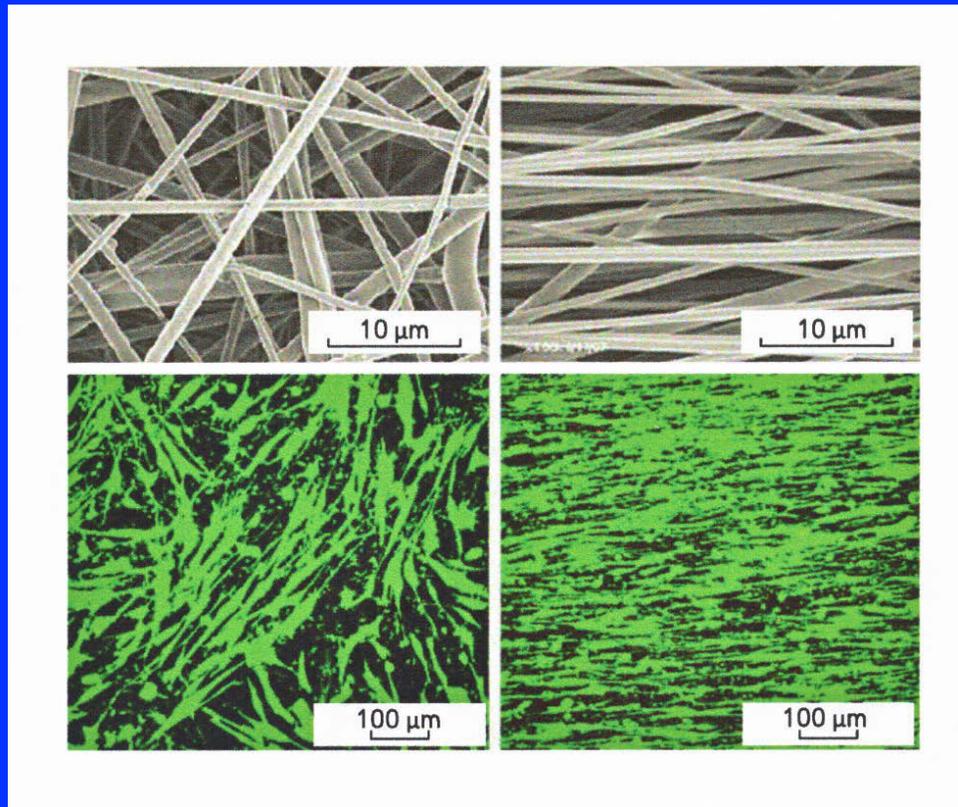
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<http://www.sciencedirect.com/science/article/pii/S0142961204002017>

O'Brien

Mouse MC3T3 osteogenic cells  
on collagen-GAG scaffold

# Cell Morphology



PLGA scaffolds

Seeded with  
rotator cuff  
fibroblasts

Random

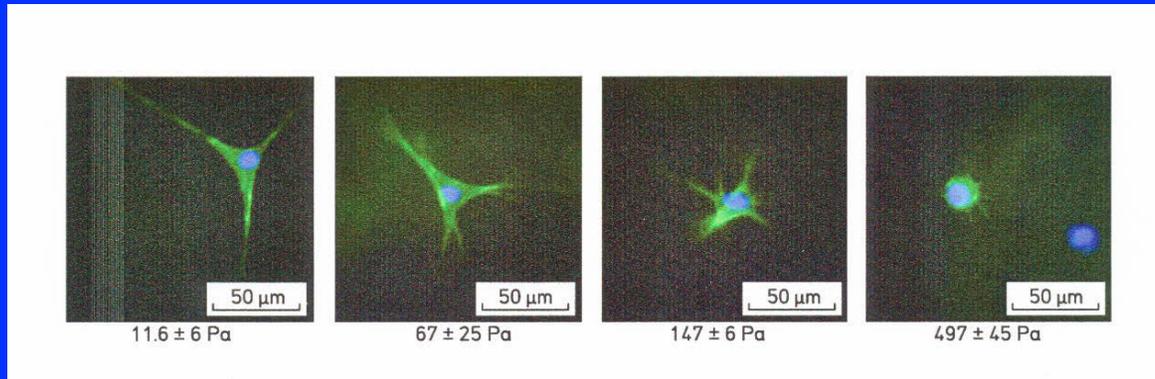
Aligned

Moffat, K. L., et al. *Clinics in Sports Medicine* 28 (2009): 157-76.  
Courtesy of Elsevier. Used with permission.

<http://www.sciencedirect.com/science/article/pii/S0278591908000707>

Moffat et al, 2009b

# Cell Morphology



$E =$     11.6                      67                      147                      497 Pa

Dikovsky, D. H., et al. *Biophysical Journal* 94 (2008): 2914-25.  
Courtesy of Elsevier. Used with permission.  
<http://www.sciencedirect.com/science/article/pii/S0006349508705411>

Smooth muscle cells encapsulated  
in a PEG-fibrinogen hydrogels of varying modulus

Dikovsky et al., 2008

# Cell Contractility: Wound Contraction and Scar Formation



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Wound contraction associated with scar formation

Use of collagen-GAG matrix inhibits wound contraction and scar formation; results in synthesis of normal dermis

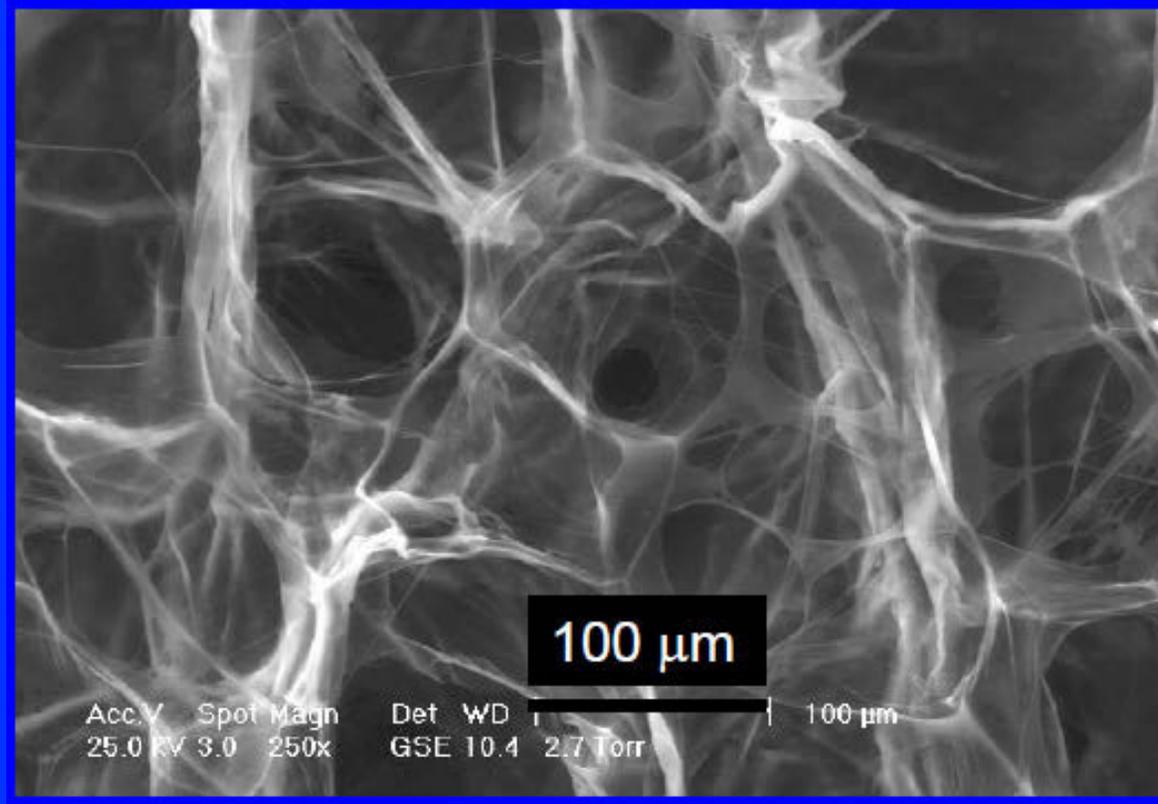
Photo courtesy of IV Yannas

This observation has led to interest in contractile response of cells on the scaffold

# Contractility of Cells

- Biological cells can contract a scaffold
- Free-floating tests
  - Measure diameter change
- Developed cell force monitor (CFM) to measure forces

# Collagen-GAG Scaffold



Pek et al., 2004

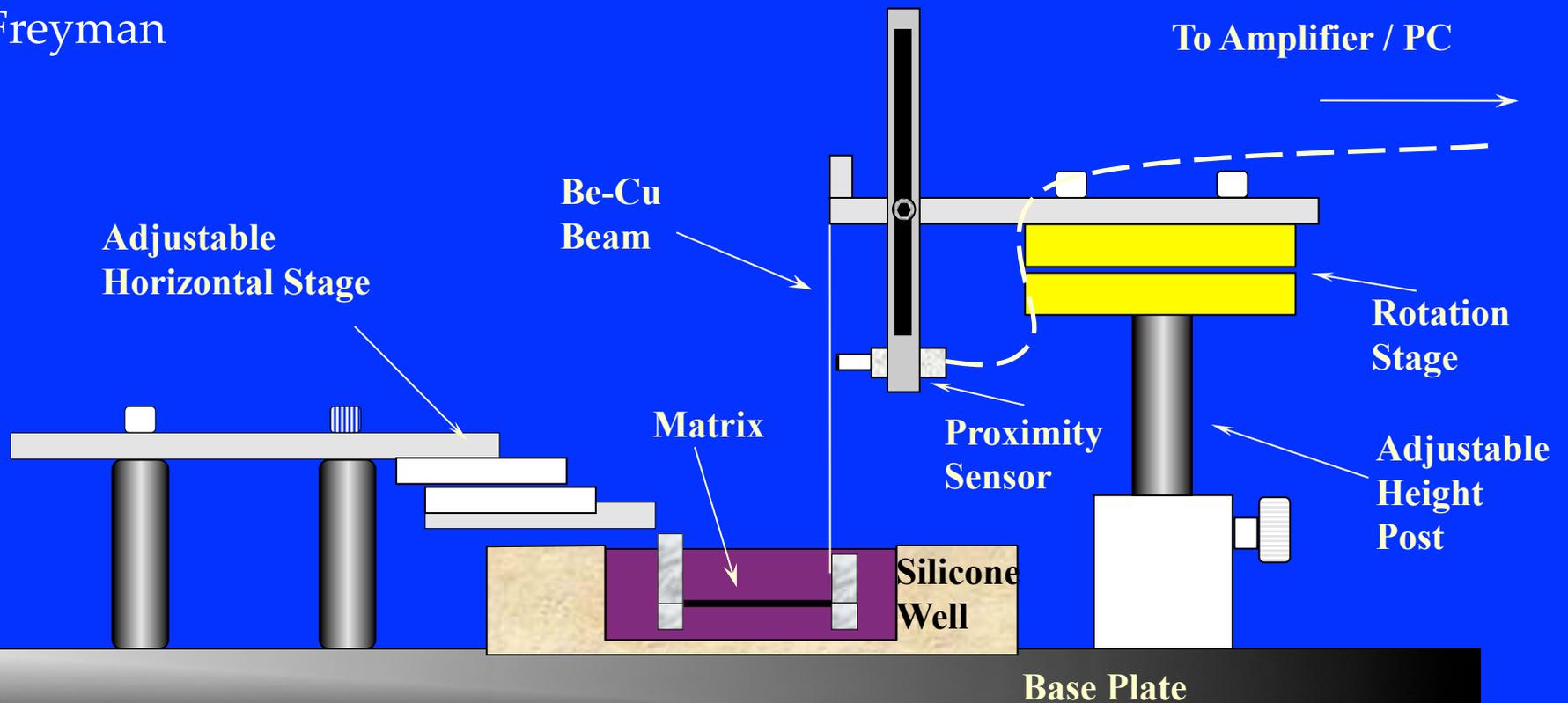
Fig. 1: Pek, Y. S., M. Spector, et al. *Biomaterials* 25 (2004): 473-82.  
Courtesy of Elsevier. Used with permission.  
<http://www.sciencedirect.com/science/article/pii/S0142961203005416>

Scaffold developed by IV Yannas (MIT)

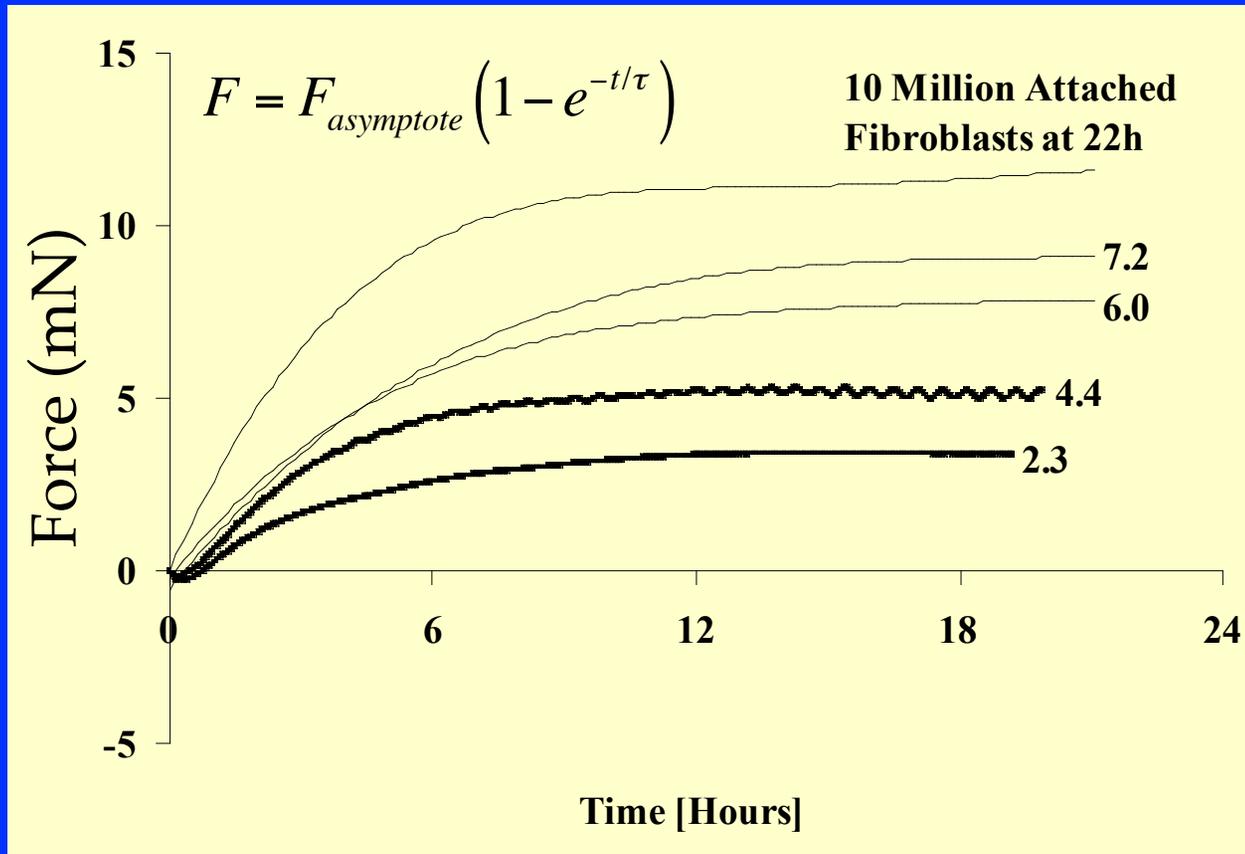
# Cell Force Monitor (CFM)



Freyman



# CFM: Effect of Cell Number

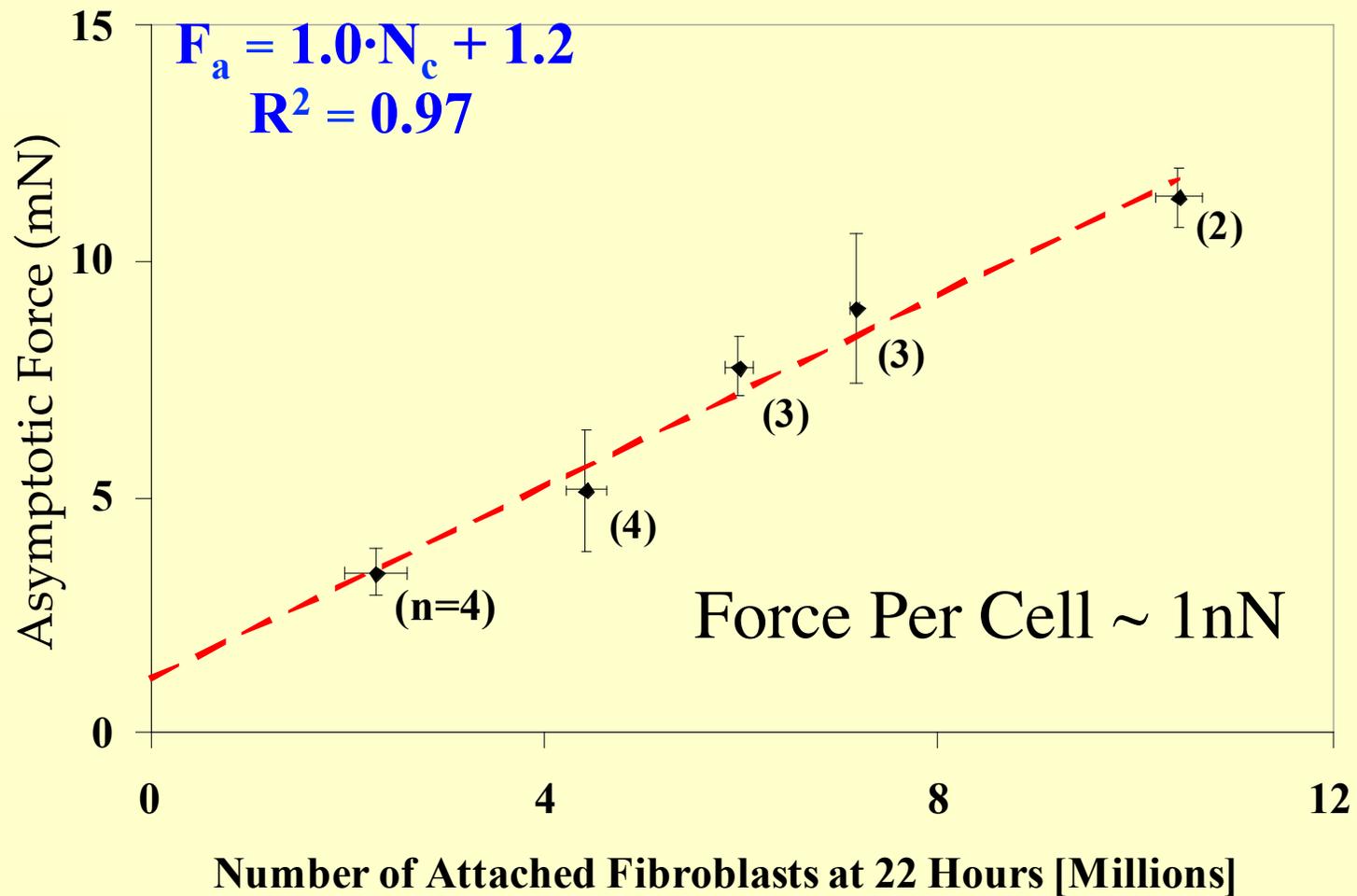


Time constant 5.7 hours

Freyman

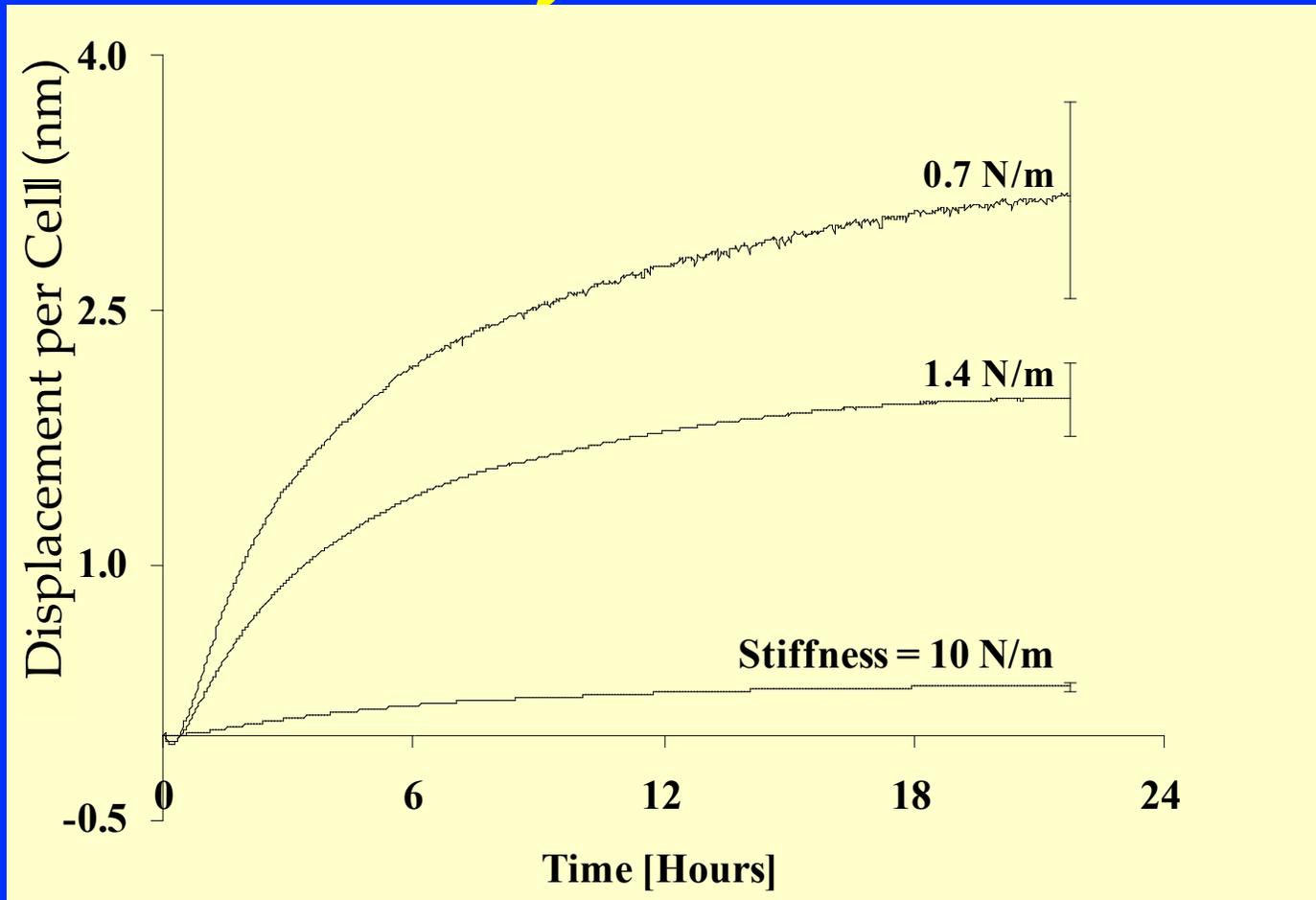
Freyman, T. M., I. V. Yannas, et al. "Fibroblast Contraction of a Collagen-GAG Matrix." *Biomaterials* 22 (2001): 2883-91. Courtesy of Elsevier. Used with permission.

# Effect of Cell Number



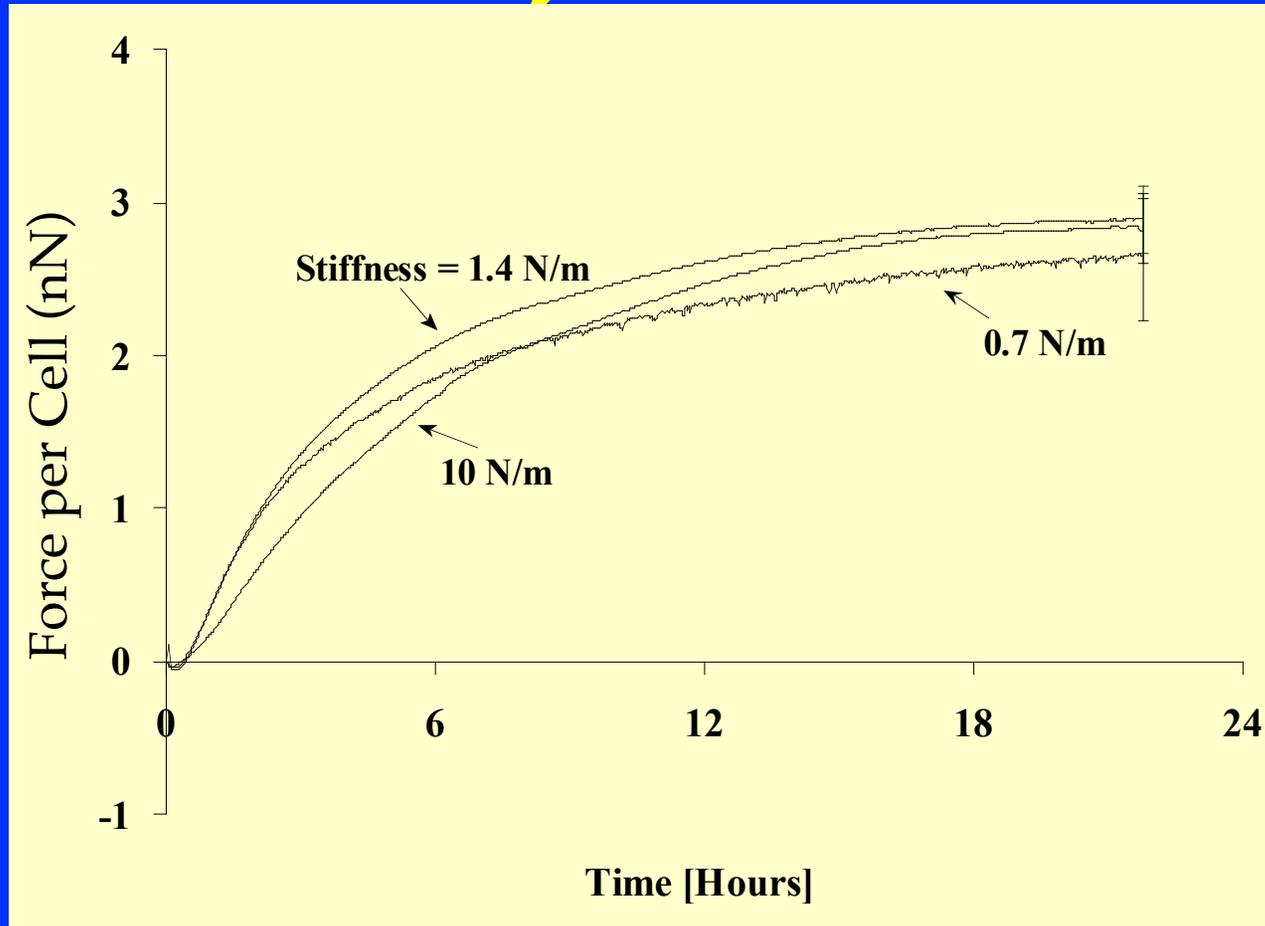
Freyman, T. M., I. V. Yannas, et al. "Fibroblast Contraction of a Collagen-GAG Matrix." *Biomaterials* 22 (2001): 2883-91. Courtesy of Elsevier. Used with permission.

# Effect of System Stiffness



Freyman, T. M., et al. "Fibroblast Contractile Force is Independent of the Stiffness which Resists the Contraction." *Experimental Cell Research* 272 (2002): 153-62. Courtesy of Elsevier. Used with permission.

# Effect of System Stiffness



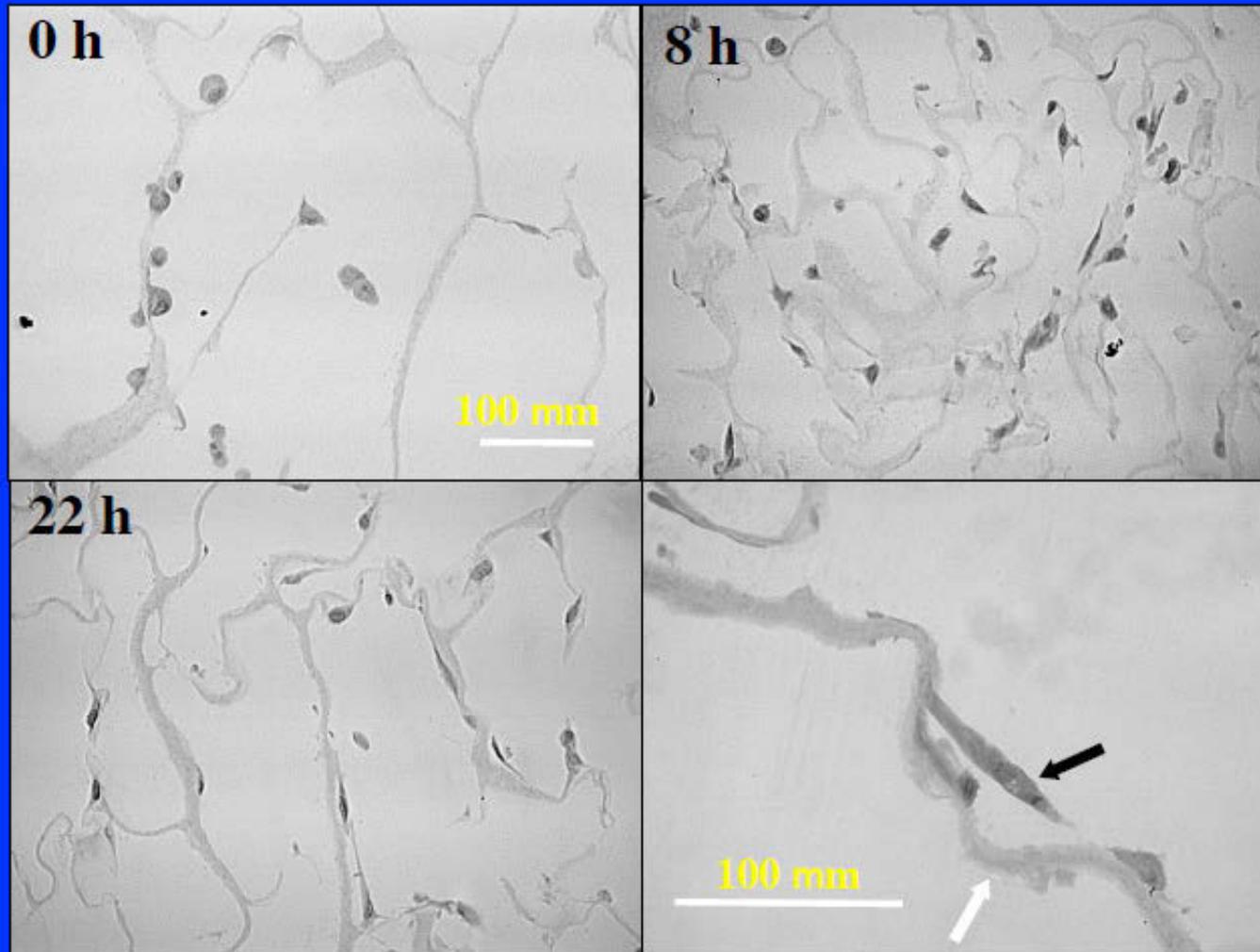
Freyman, T. M., et al. "Fibroblast Contractile Force is Independent of the Stiffness which Resists the Contraction." *Experimental Cell Research* 272 (2002): 153-62. Courtesy of Elsevier. Used with permission.

# Methods: Cell Elongation

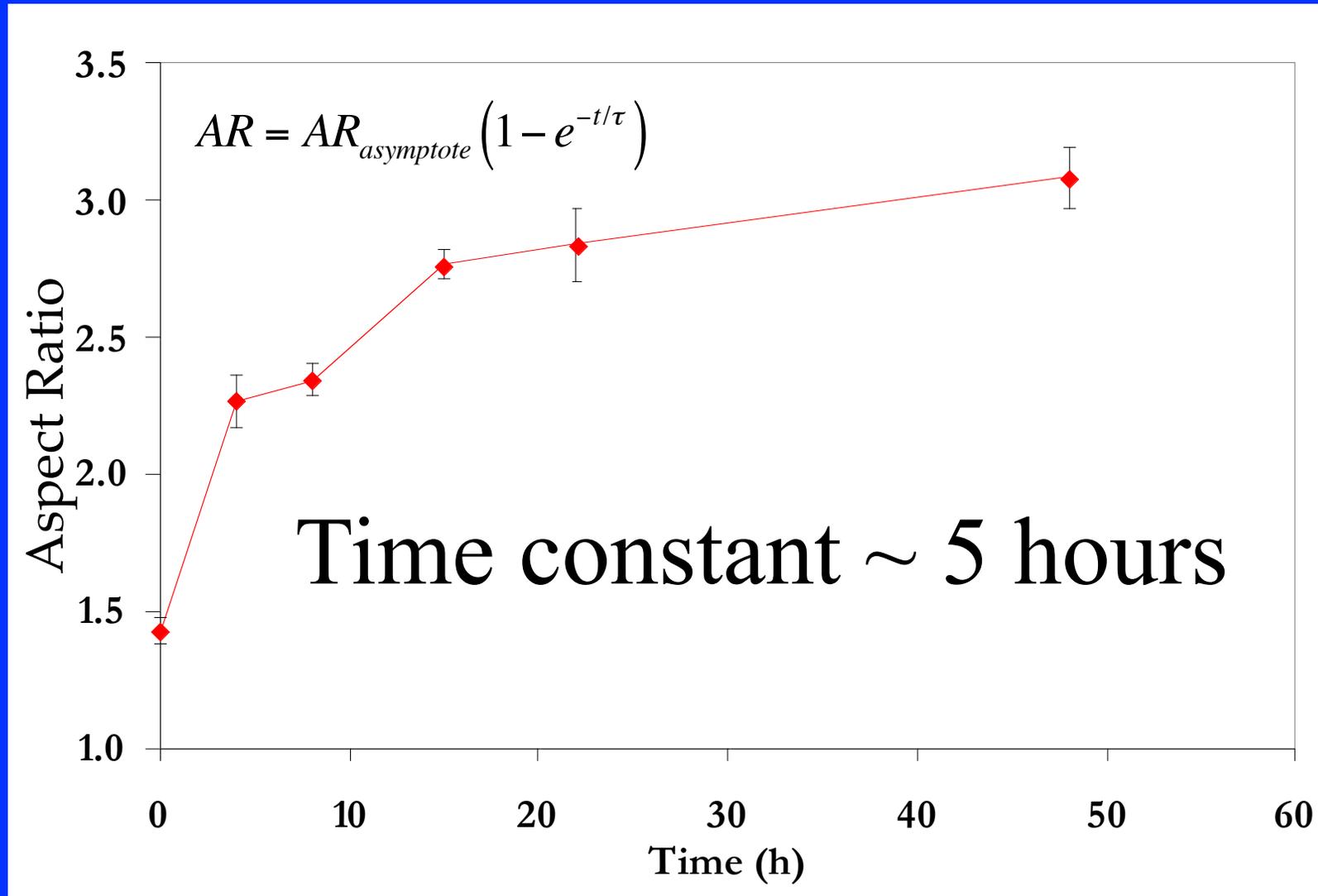
## Average aspect ratio of cells

- Time points 0, 4, 8, 15, 22, and 48 h (n=3)
- Hematoxylin & eosin (H&E) stained glycomethacrylate (GMA) sections (5mm)
- Digital image analysis (~200 cells per sample)

# Fibroblast Morphology



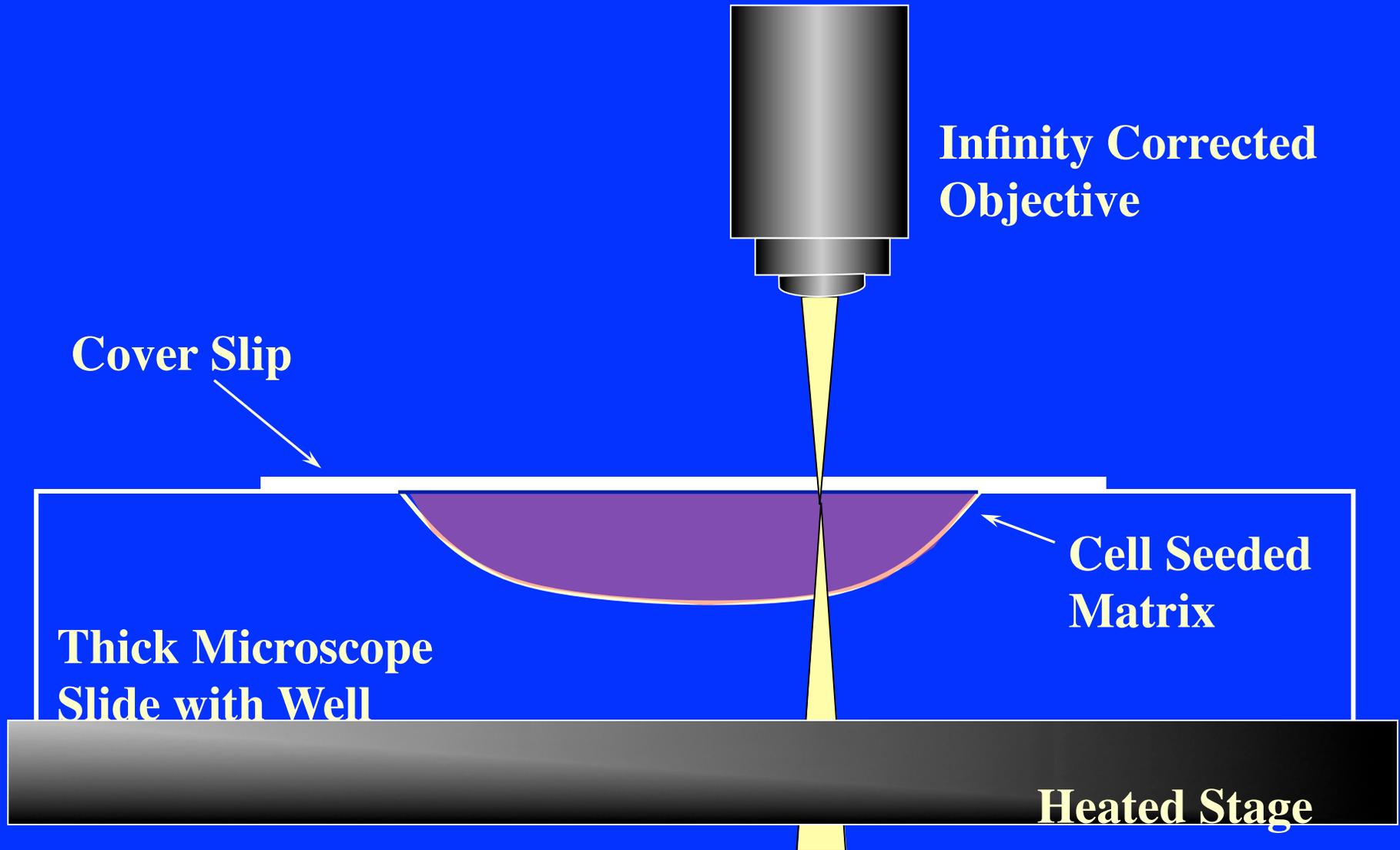
# Fibroblast Morphology

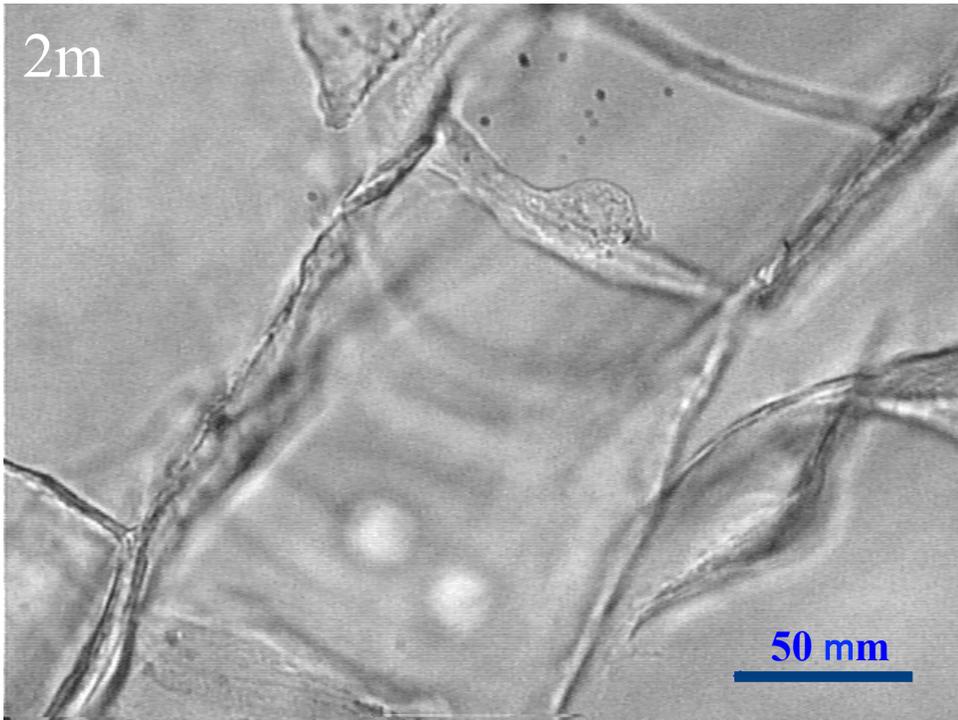


# Time Constants

- Time constant for contraction ~ 5.7 hours
- Time constant for elongation ~ 5 hours
- Suggests a link between the average elongation of the cell population and the macroscopic contraction of the population

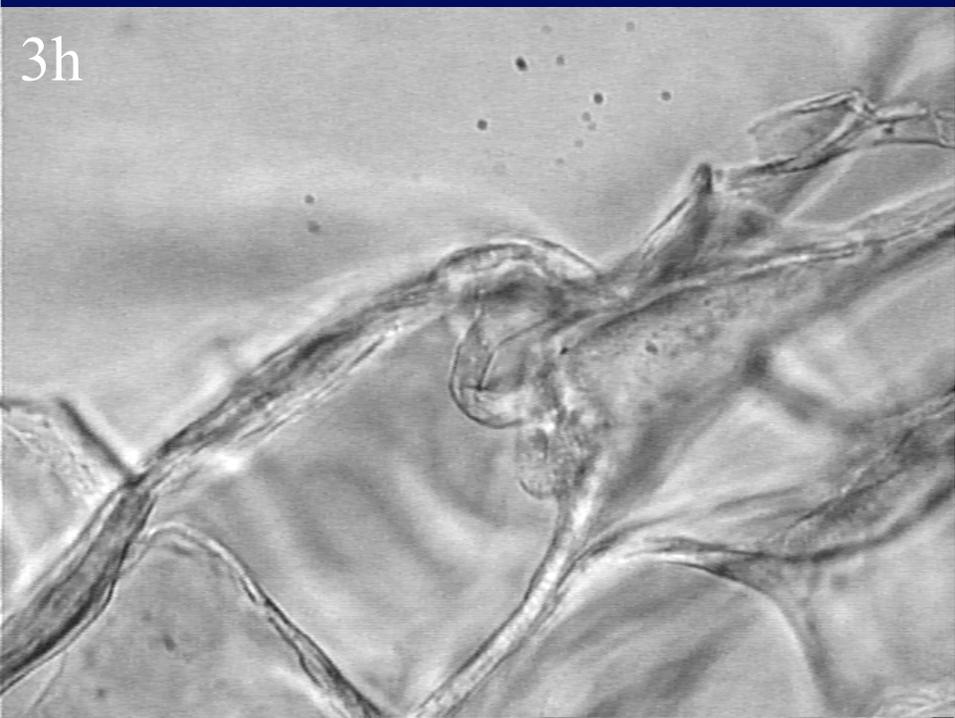
# Methods: Live Cell Imaging





# Live Cell Imaging

Freyman

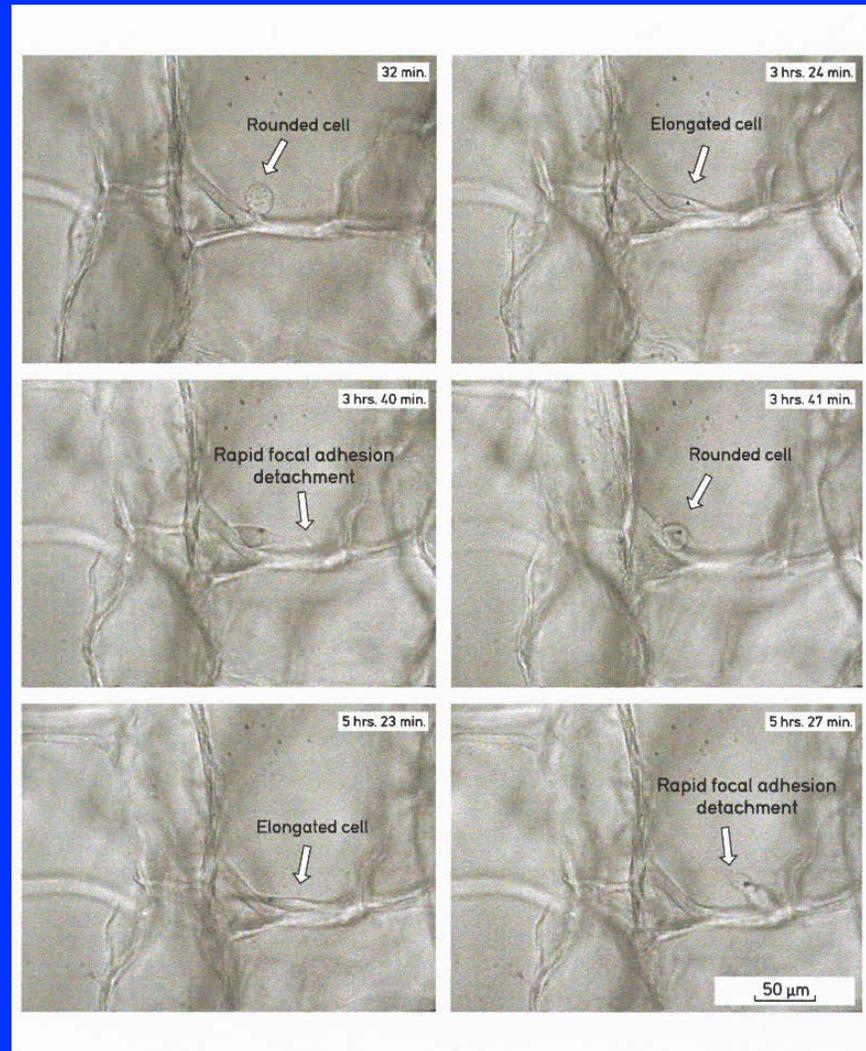


# Live Cell Imaging

Figure removed due to copyright restrictions. See Figure 7: Freyman, T. M., et al. "[Micromechanics of Fibroblast Contraction of a Collagen-GAG Matrix](#)." *Experimental Cell Research* 269 (2001): 140-53.

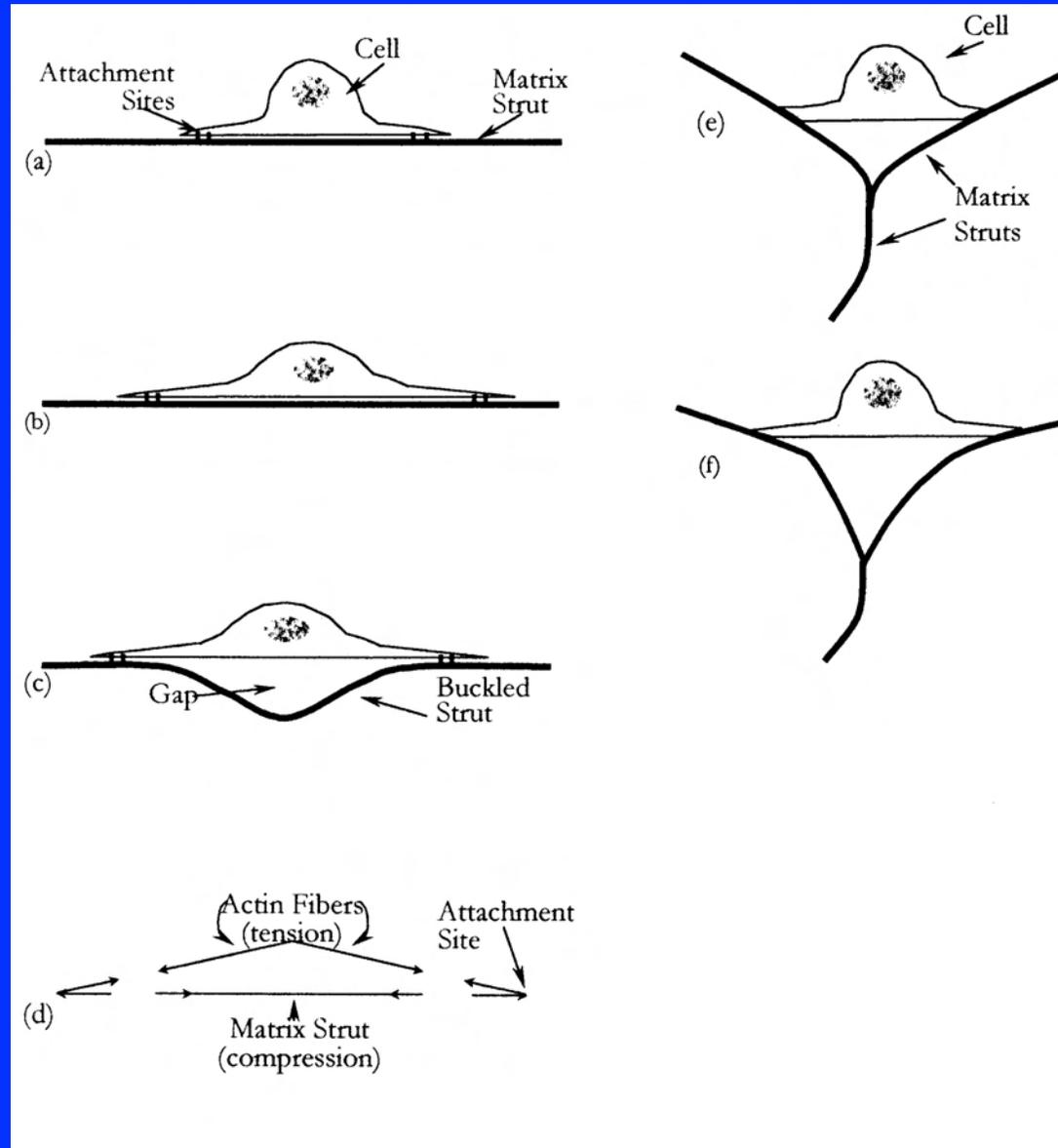
Freyman

# Live Cell Imaging



Source: Freyman, T. M., et al. "Micromechanics of Fibroblast Contraction of a Collagen-GAG Matrix." *Experimental Cell Research* 269 (2001): 140-53. Courtesy of Academic Press/Elsevier. Used with permission.

# Schematic of cell elongation and matrix contraction



Freyman

Source: Freyman, T. M., et al. "Micromechanics of Fibroblast Contraction of a Collagen-GAG Matrix." *Experimental Cell Research* 269 (2001): 140-53. Courtesy of Academic Press/Elsevier. Used with permission.

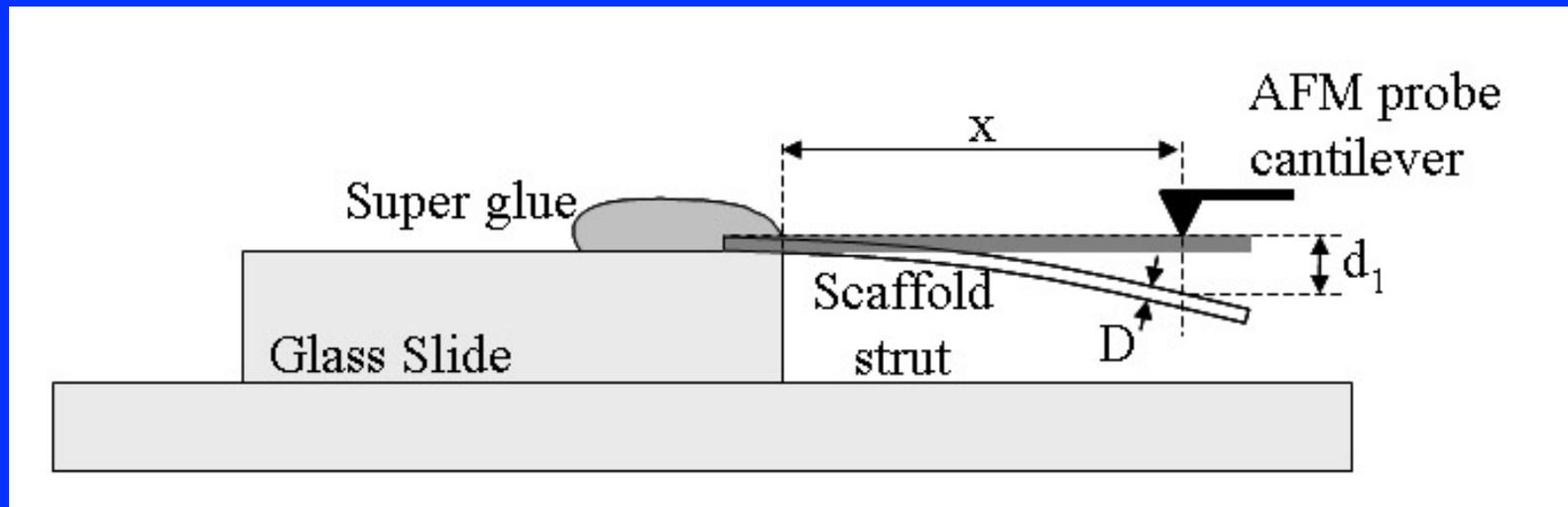
# Discussion

- Cell elongation linked to contraction
  - time constants for cell elongation and contractile force development similar ( $\tau \sim 5h$ )
  - as cell elongates, observe gap between central portion of cell and matrix
  - adhesion points at periphery of cell
  - tensile forces in actin filaments induce compression in the matrix => buckling

# Single Cell Contractile Force

- Contraction: cell buckling
- Measure  $E_s$  from AFM bending test
- Allows calculation of contractile force of single fibroblast

# Single Cell Contractile Force



$E_s = 762 \text{ MPa}$   
(dry)

$E_s = 5.28 \text{ MPa}$   
(wet)

Source: Harley, B. A., et al. *Acta Biomaterialia* 3 (2007): 463-74.  
Courtesy of Elsevier. Used with permission.

<http://www.sciencedirect.com/science/article/pii/S1742706107000025>

Harley, Silva

# Single Cell Contractile Force

- Euler buckling: 
$$F = \frac{n^2 \pi^2 E_s I}{l^2}$$
 
$$I = \frac{\pi d^4}{64}$$

$n^2 = 0.34$  (hydrostatic loading of tetrakaidecahedral cells (Triantafillou))

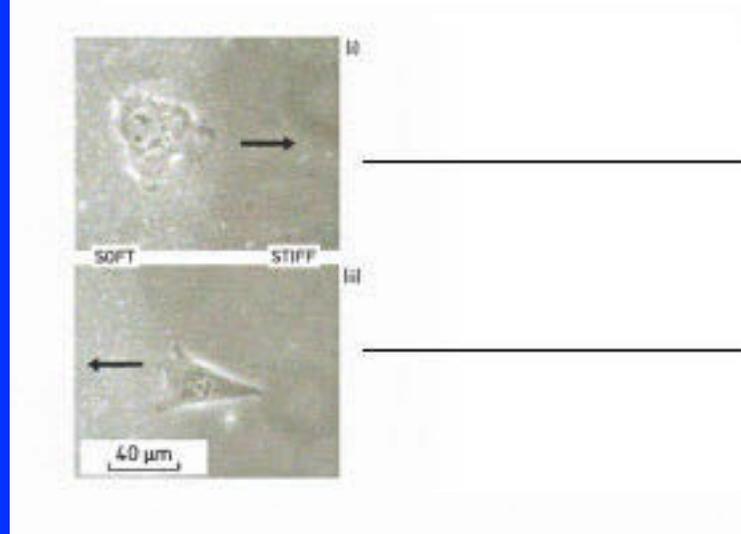
$d = 3.9 \pm 0.8 \mu\text{m}$ ;  $l$  from live cell imaging

$$F_c = 11 \text{ to } 41 \text{ nN (average } 26 \text{ nN)}$$

Harley, Wong

# Cell Migration

Figure removed due to copyright restrictions. Figure 3: Cornwell, K. G., et al. *Journal of Biomedical Material Research A* 80 (2007): 362-71. <http://onlinelibrary.wiley.com/doi/10.1002/jbm.a.30893/abstract>



Migration speed on one-dimensional fiber constructs

NIH 3T3 cells on 2D flat substrate:

Cells on soft substrate cross to stiff substrate

Cells on stiff substrate will not cross onto soft substrate; instead spread out at boundary

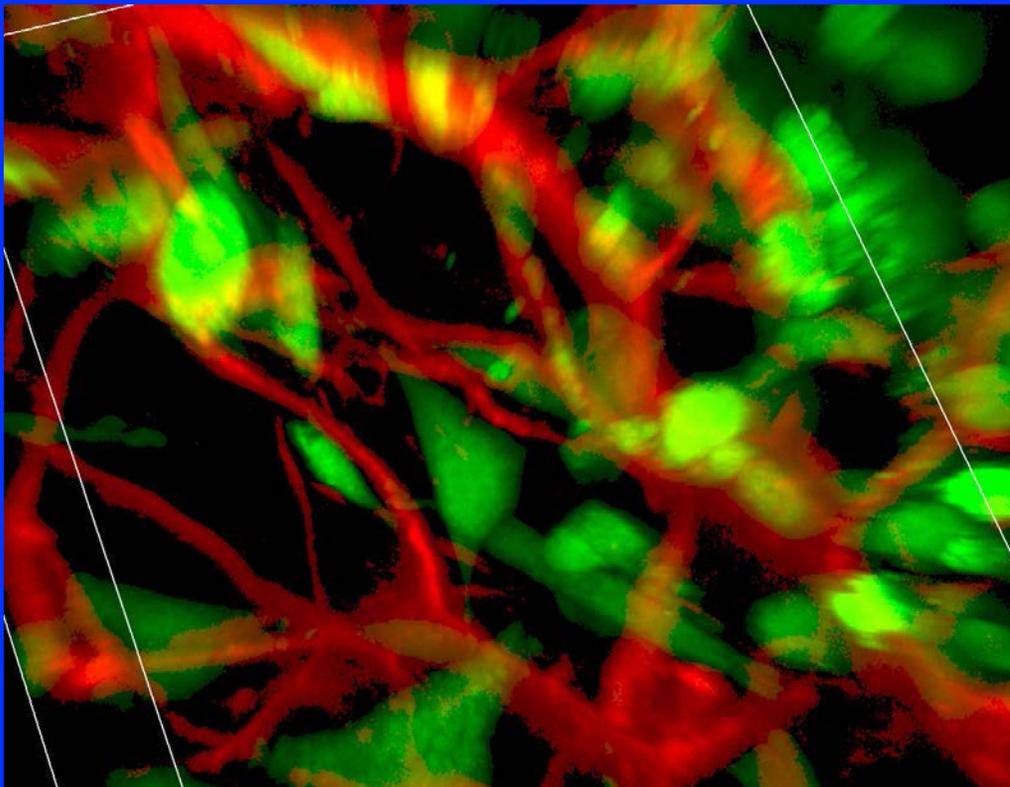
Source: Lo, et al., *Biophysical Journal* 79 (2000): 144-52.

Courtesy of Elsevier. Used with permission.

<http://www.sciencedirect.com/science/article/pii/S0006349500762795>

Top: Cornwell et al., 2007; Bottom: Lo et al, 2000

# Cell Migration: Fibroblasts in CG Scaffold



Confocal  
Microscopy

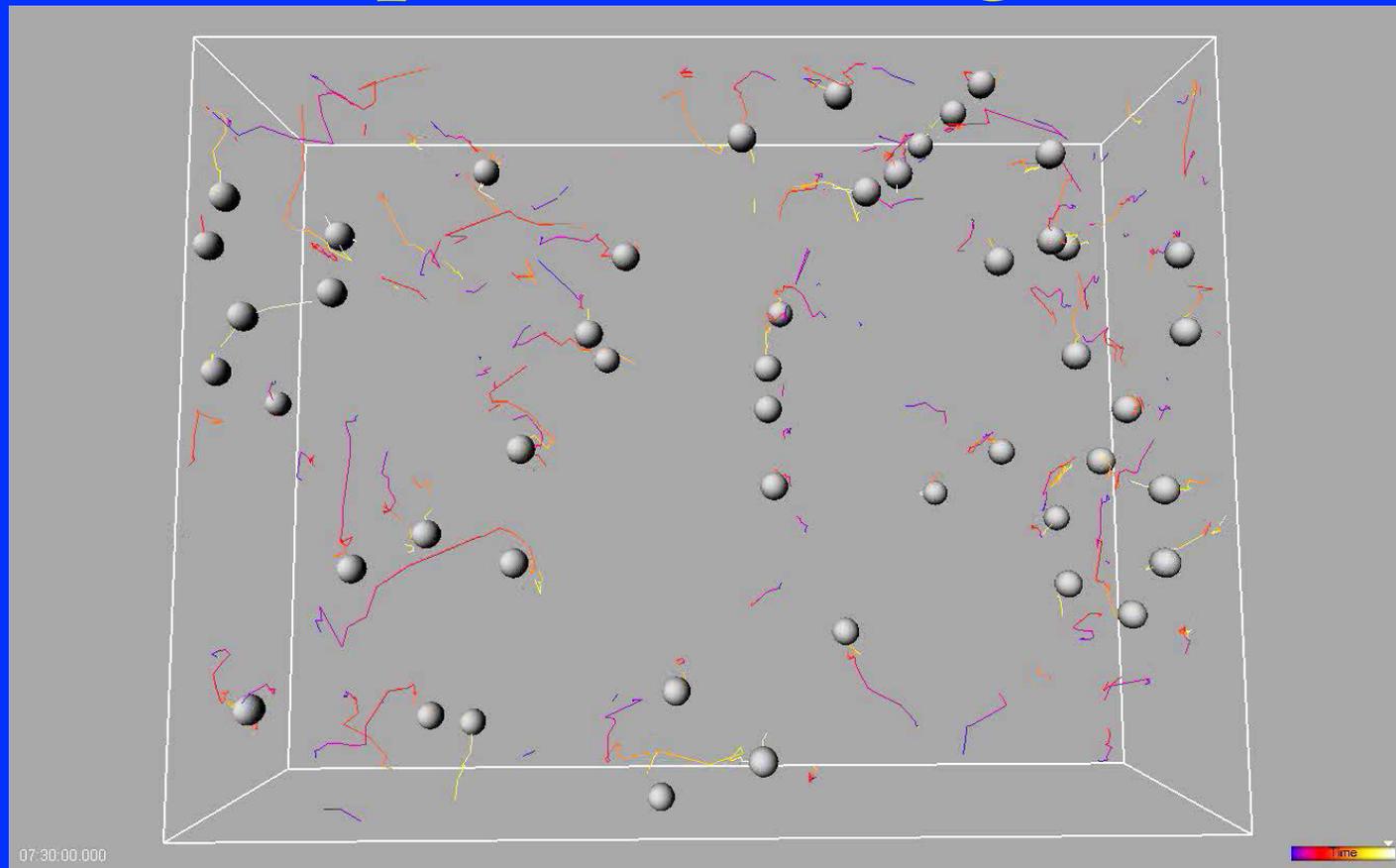
NR6 Fibroblasts  
CMFDA Live  
Cell Tracker

CG Scaffold  
Alexa Fluor 633  
Stain

Courtesy of Brendan Harley. Used with permission.

Harley

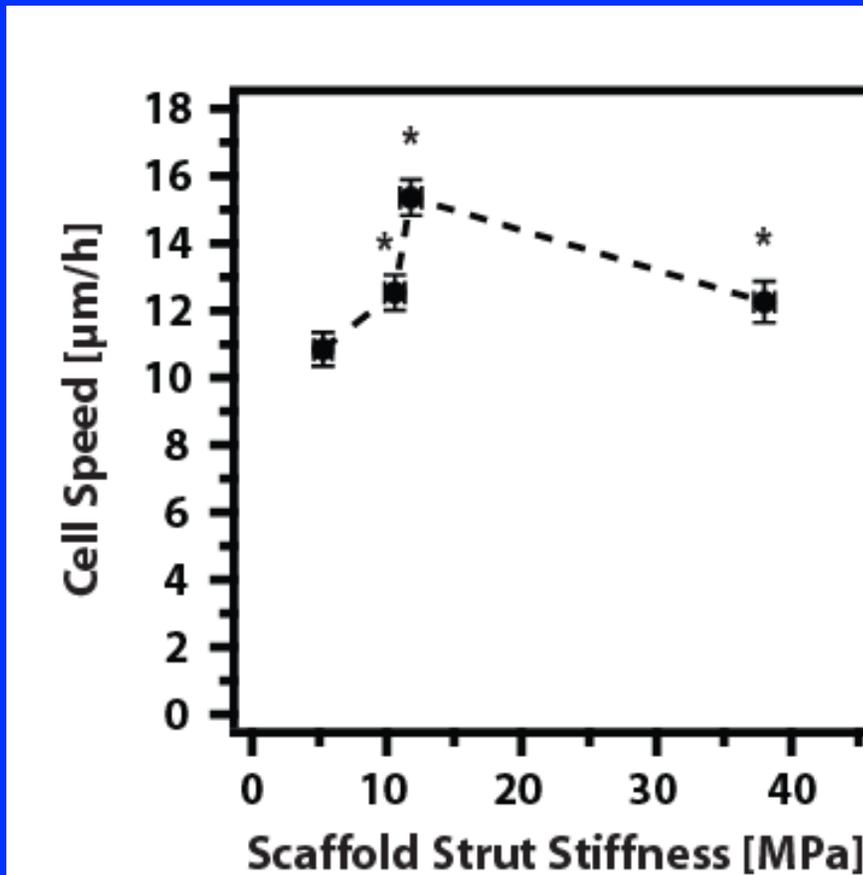
# Fibroblast Migration: Spot Tracking



Courtesy of Brendan Harley. Used with permission.

Harley

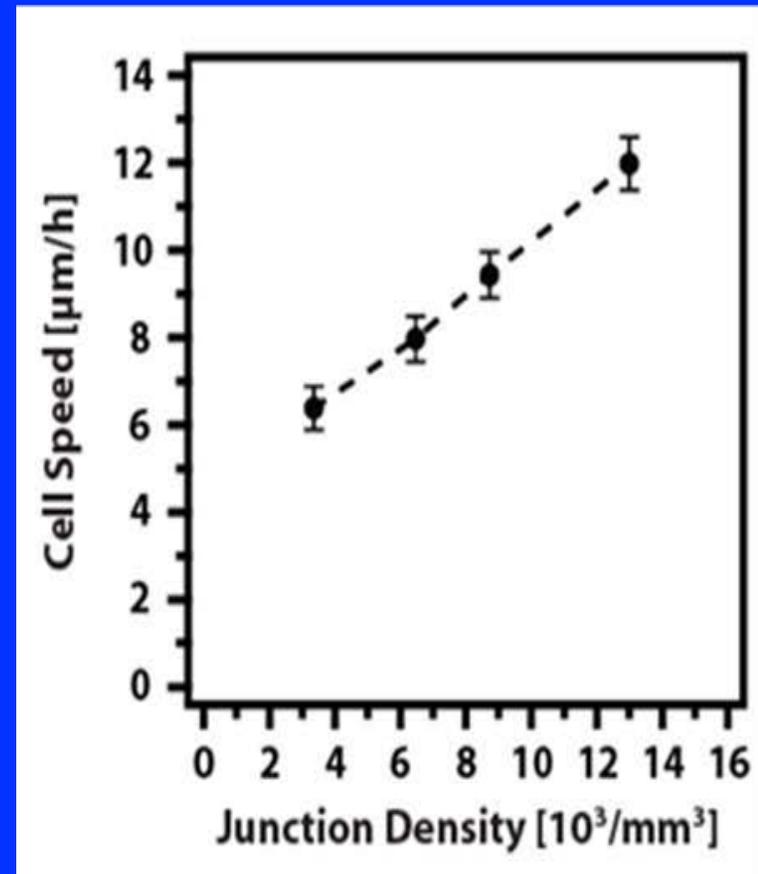
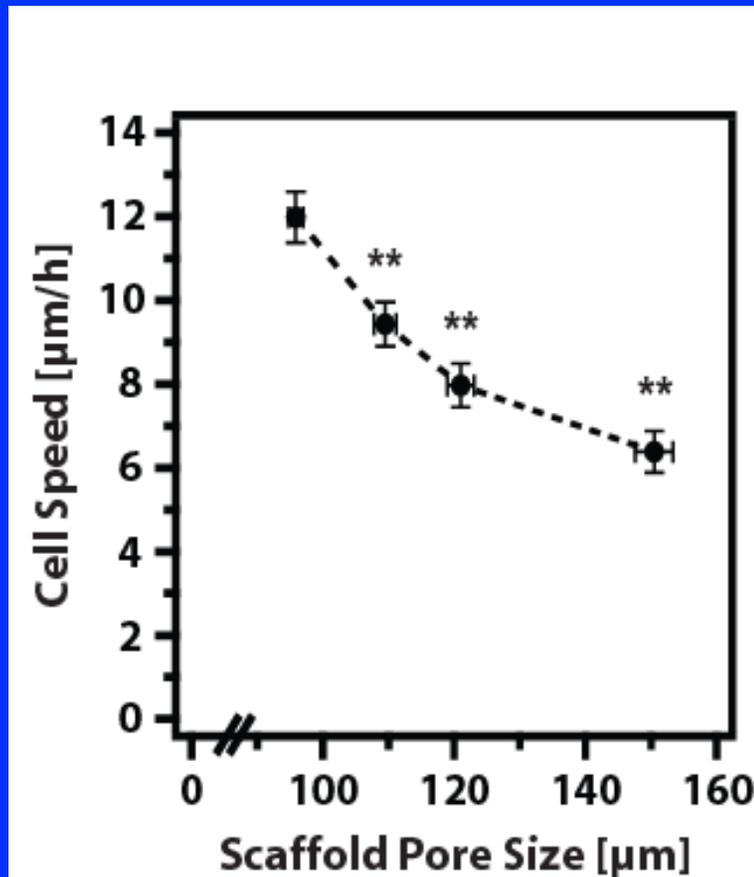
# Migration Speed vs Strut Stiffness



Source: Harley, B. A. C., et al. *Biophysical Journal* 95 (2008): 4013-24.  
Courtesy of Elsevier. Used with permission.

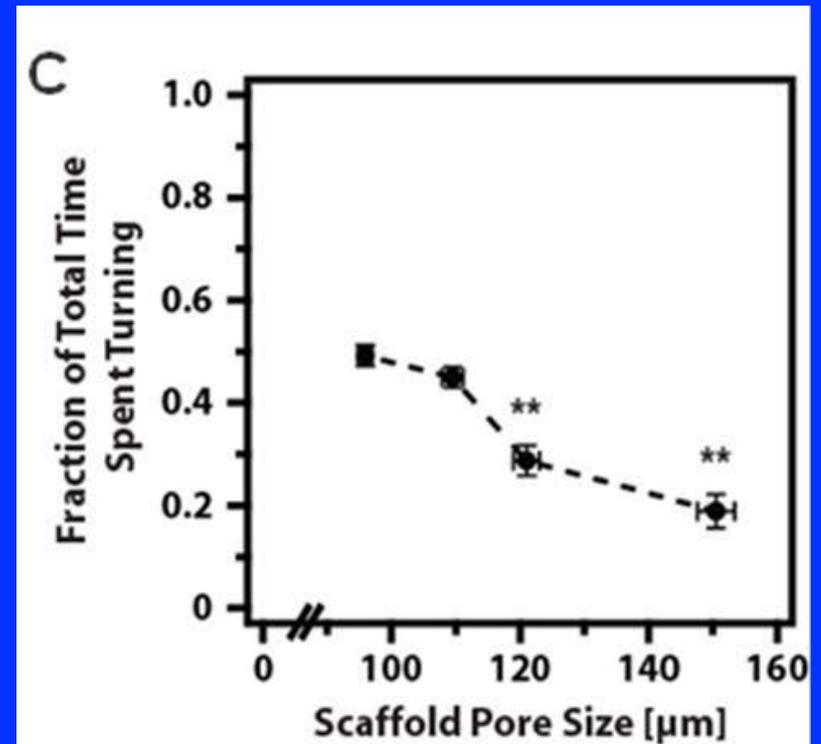
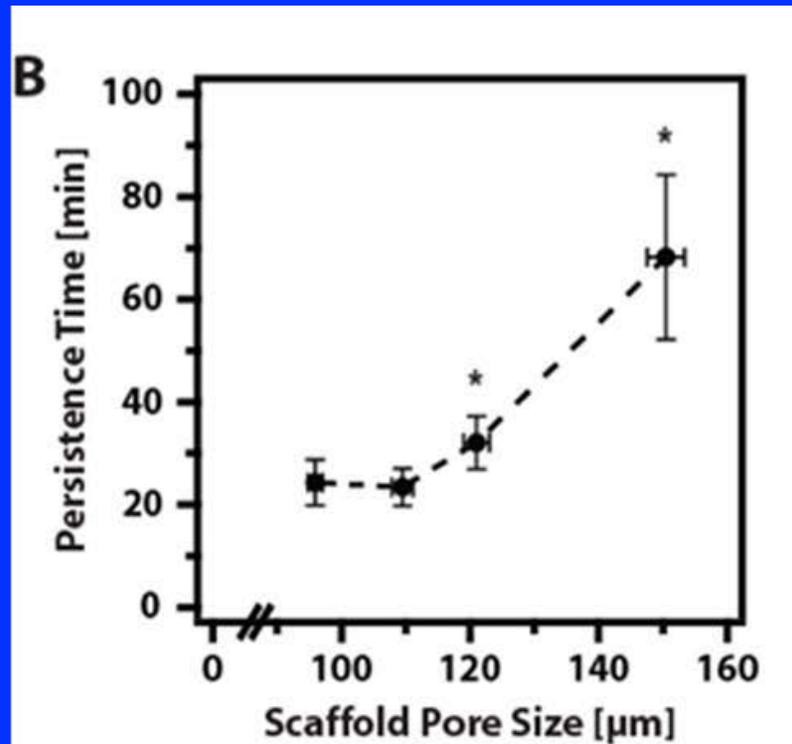
<http://www.sciencedirect.com/science/article/pii/S0006349508785394>

# Migration Speed vs Pore Size



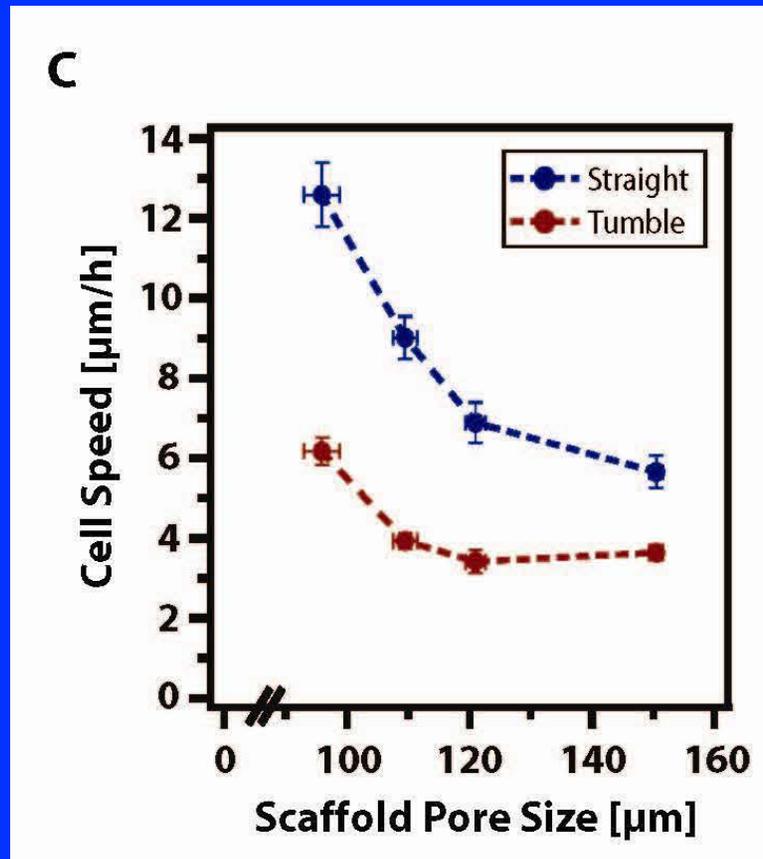
Source: Harley, B. A. C., et al. *Biophysical Journal* 95 (2008): 4013-24.  
Courtesy of Elsevier. Used with permission.  
<http://www.sciencedirect.com/science/article/pii/S0006349508785394>

# Migration Speed vs Pore Size



Source: Harley, B. A. C., et al. *Biophysical Journal* 95 (2008): 4013-24.  
Courtesy of Elsevier. Used with permission.  
<http://www.sciencedirect.com/science/article/pii/S0006349508785394>

# Migration Speed vs Pore Size



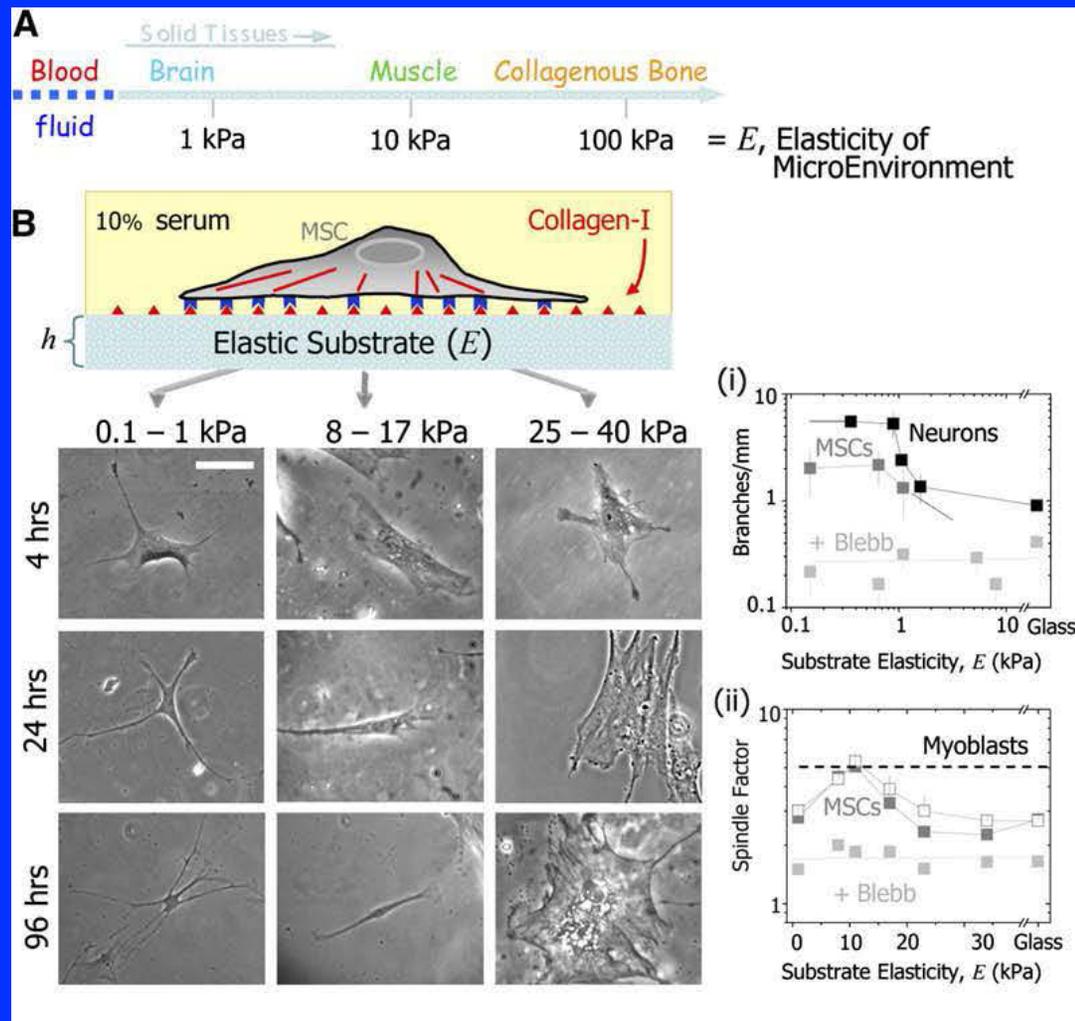
Cells on scaffolds with smaller pore sizes have a higher speed both along a strut and at a strut junction than cells in scaffolds with larger pores

As pore size decreases, specific surface area increases and # binding sites increases

Source: Harley, B. A. C., et al. *Biophysical Journal* 95 (2008): 4013-24.  
Courtesy of Elsevier. Used with permission.

<http://www.sciencedirect.com/science/article/pii/S0006349508785394>

# Cell Differentiation



Neuron-like    Myoblast-like    Osteoblast-like

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

Courtesy of Elsevier. Used with permission.

<http://www.sciencedirect.com/science/article/pii/S0092867406009615>

Engler et al., 2006



# Summary

- Cell attachment increases linearly with specific surface area (binding sites)
- Cell morphology depends on orientation of pores in scaffold and on the stiffness of the scaffold

# Summary

- Cell contractile behaviour:
  - Cells bind at periphery of cells
  - As they spread and elongate, unsupported length increases
  - Compressive force in strut reaches buckling load
  - For a population of cells in the cell force monitor, force per cell  $\sim 1\text{nN}$
  - Contractile force calculated from buckling of a strut by a single cell  $\sim 11\text{-}41\text{ nN}$

# Summary

- Cell migration speed increases with stiffness of 1D fibers
- Cells will not migrate from a stiff 2D substrate to a soft one
- In collagen-GAG scaffolds:
  - Cell migration speed increases at low scaffold stiffness and then decreases at higher scaffold stiffnesses
  - Cell migration speed increases at smaller pore sizes

# Summary

- Cell differentiation
  - Mesenchymal stem cells differentiate to different morphologies, resembling different cell lineages (neuron, myoblast, osteoblast), depending on substrate stiffness
  - Differentiated cells on substrates of different stiffness have cell markers associated with the different cell lineages (neurons, myoblasts, osteoblasts)

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- Drs. TM Freyman, BA Harley, FJ O' Brien, M Zaman
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- Drs. Spector and Germaine
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