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 behavior (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 and 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 approximately equal to the time required to synthesize new ECM

- Degradation rate for scaffold depends on its chemical composition and cross-linking, and on relative density of scaffold
- Synthetic polymers — can vary molecular weight of polymers and ratio of co-polymers; e.g. PLGA higher GA:LA ratio polymers degrade quicker
- Collagen-based scaffolds — can control degree of cross-linking

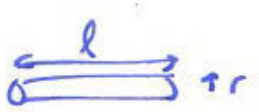
Physical methods:
— dehydrothermal (DHT) treatment (105°C vacuum 24 hours)
— removes water, forms interchain bonds through condensation
— UV treatment

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 (e.g. to actin filaments)
- Cell behaviors such as attachment, migration, proliferation, contraction affected by interactions between focal adhesions and integrins
- Biological activity of scaffolds depends on density of ligands available for integrins to bind to
- Ligand density depends on composition of scaffold and 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 and need to be functionalized with adhesive proteins such as fibronectin and laminin

- Specific surface area (SA/vol) of scaffold depends on pore side d and relative density:



- For a tetrakaidecahedral unit cell:

$$\frac{\text{SA}}{v} \propto \frac{1}{d}\left(\frac{\rho^*}{\rho_s}\right)^{1/2} \qquad \left[\frac{\text{SA}}{v} = \frac{2\pi rln}{l^3} \propto \frac{r}{l^2} \propto \frac{r}{l}\frac{1}{l} \propto \left(\frac{\rho^*}{\rho_s}\right)^{1/2}\frac{1}{d}\right]$$

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

  $d = 96, 110, 121, 151\mu m$

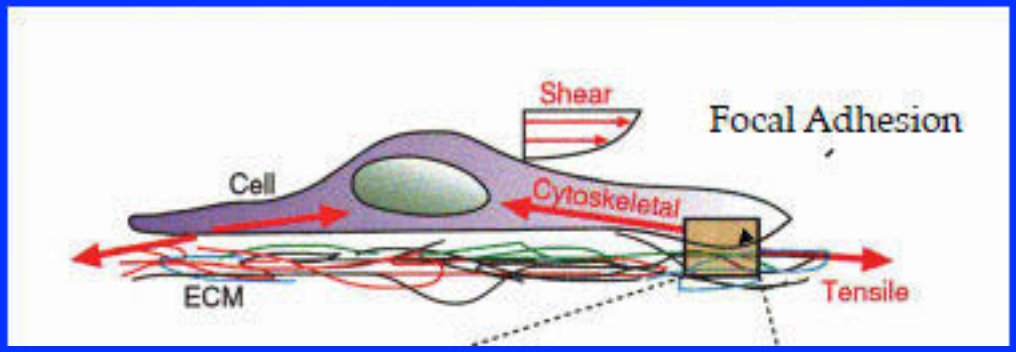
- Number of cells attached measured at 24, 48 hours
- Fraction of cells attached increased linearly with specific surface area

Cell morphology

- Cell orientation follows scaffold pore orientation
- Cell morphology can depend on a substrate stiffness

Cell contraction
Cell migration } see slides

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

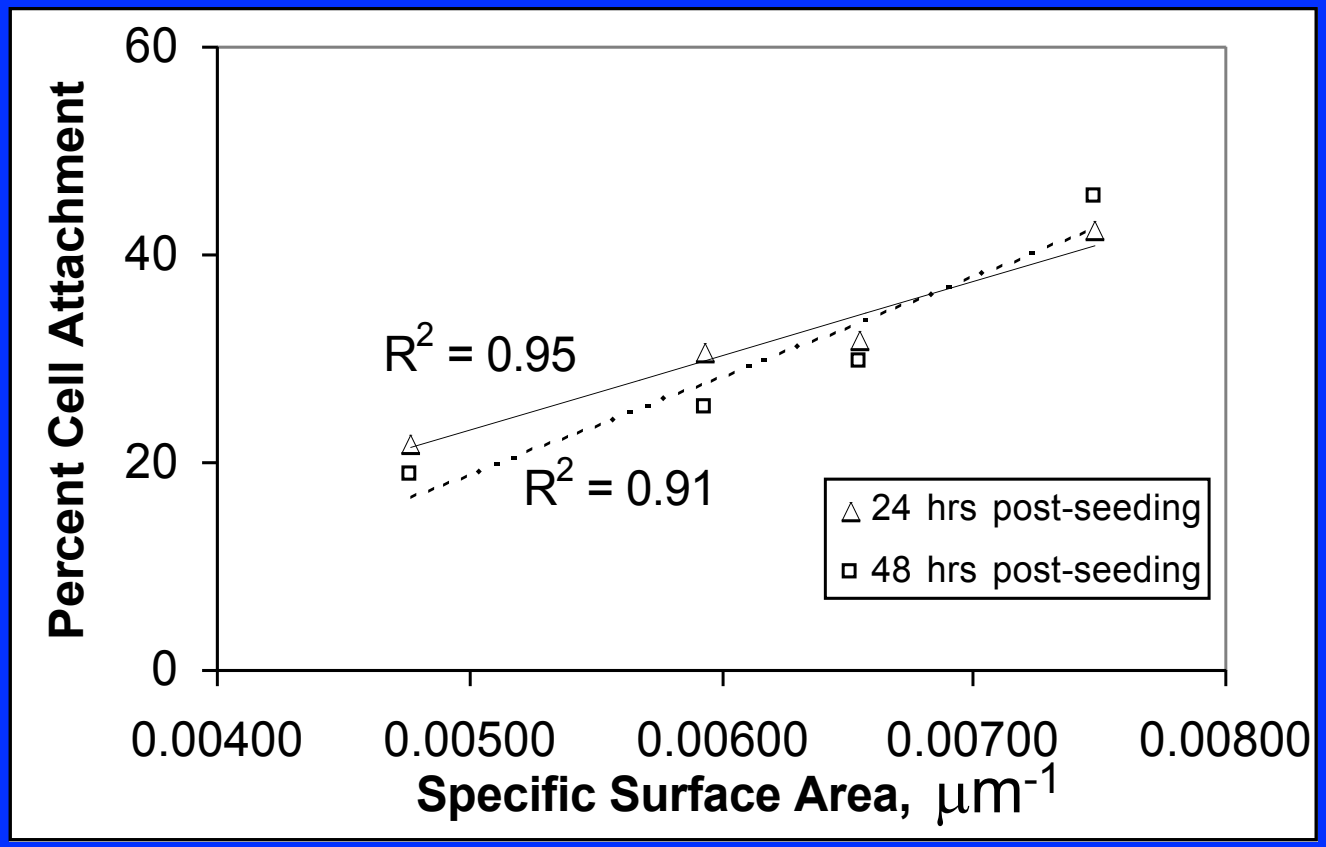
Gibson, Ashby and Harley, 2010

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:
ρ^*/ρ_s = 0.005, d = 96, 110, 121, 150μm

Cell Attachment

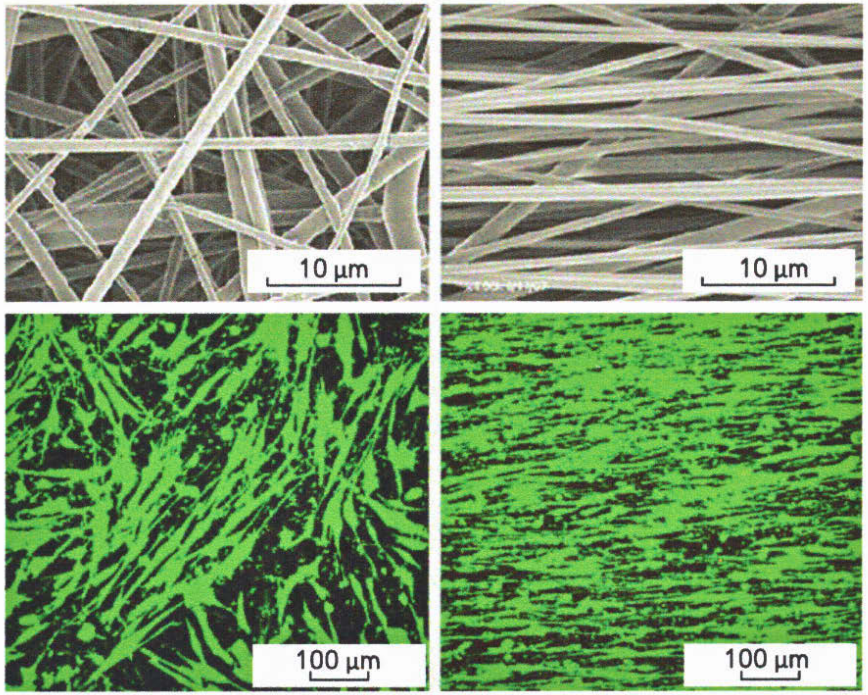


O'Brien, B. A. Harley, I. V. Yannas, et al. *Biomaterials* 26 (2005): 433-41.
Courtesy of Elsevier. Used with permission.
http://www.sciencedirect.com/science/article/pii/S0142961204002017

Mouse MC3T3 osteogenic cells
on collagen-GAG scaffold

O' Brien

Cell Morphology



Random Aligned

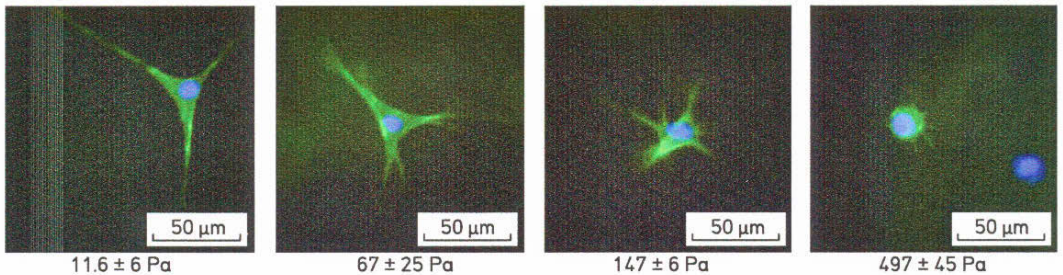
PLGA scaffolds

Seeded with rotator cuff fibroblasts

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

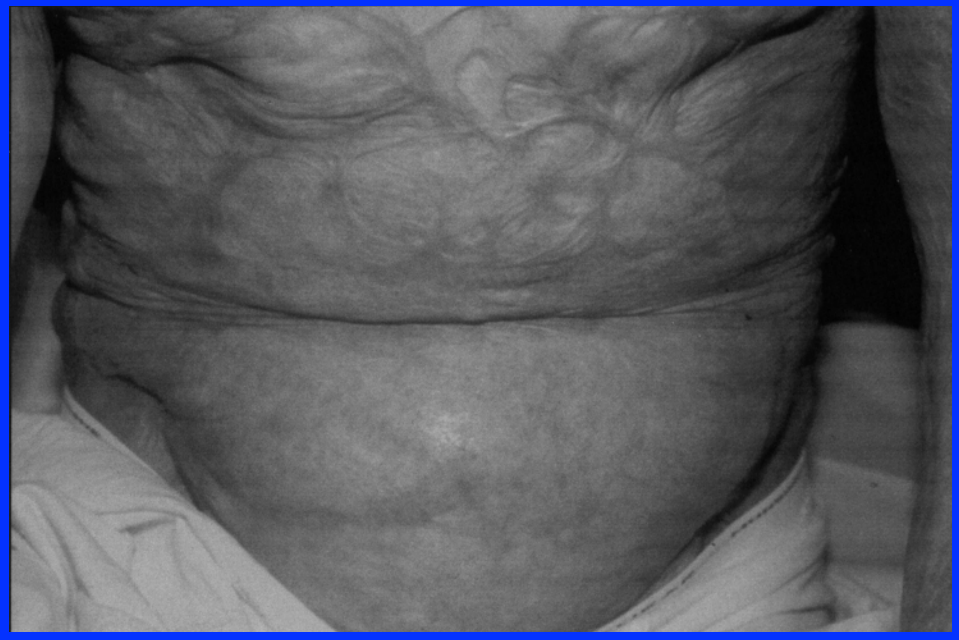


Photo courtesy of IV Yannas

Wound contraction associated with scar formation

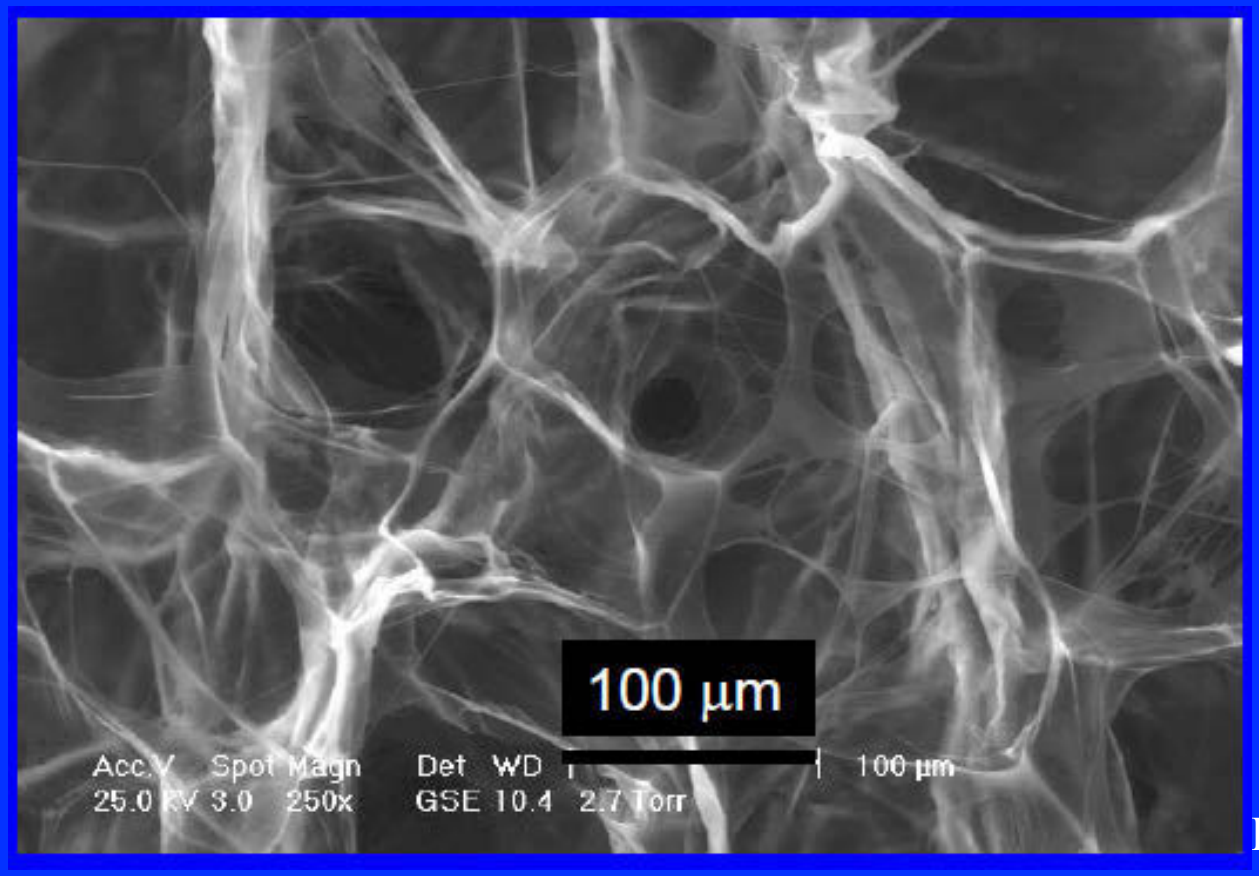
Use of collagen-GAG matrix inhibits wound contraction and scar formation; results in synthesis of normal dermis

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

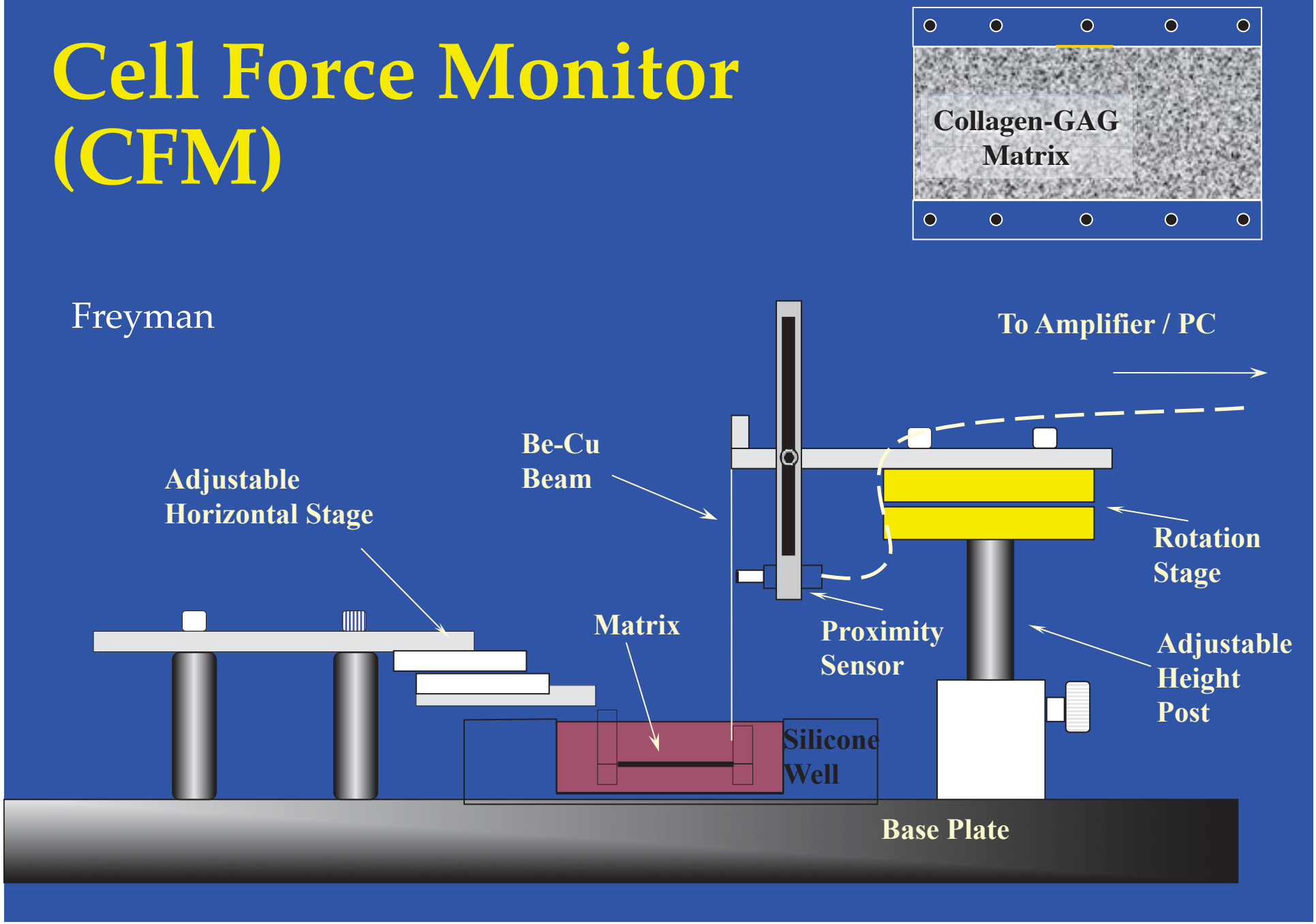


Pek et al., 2004

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

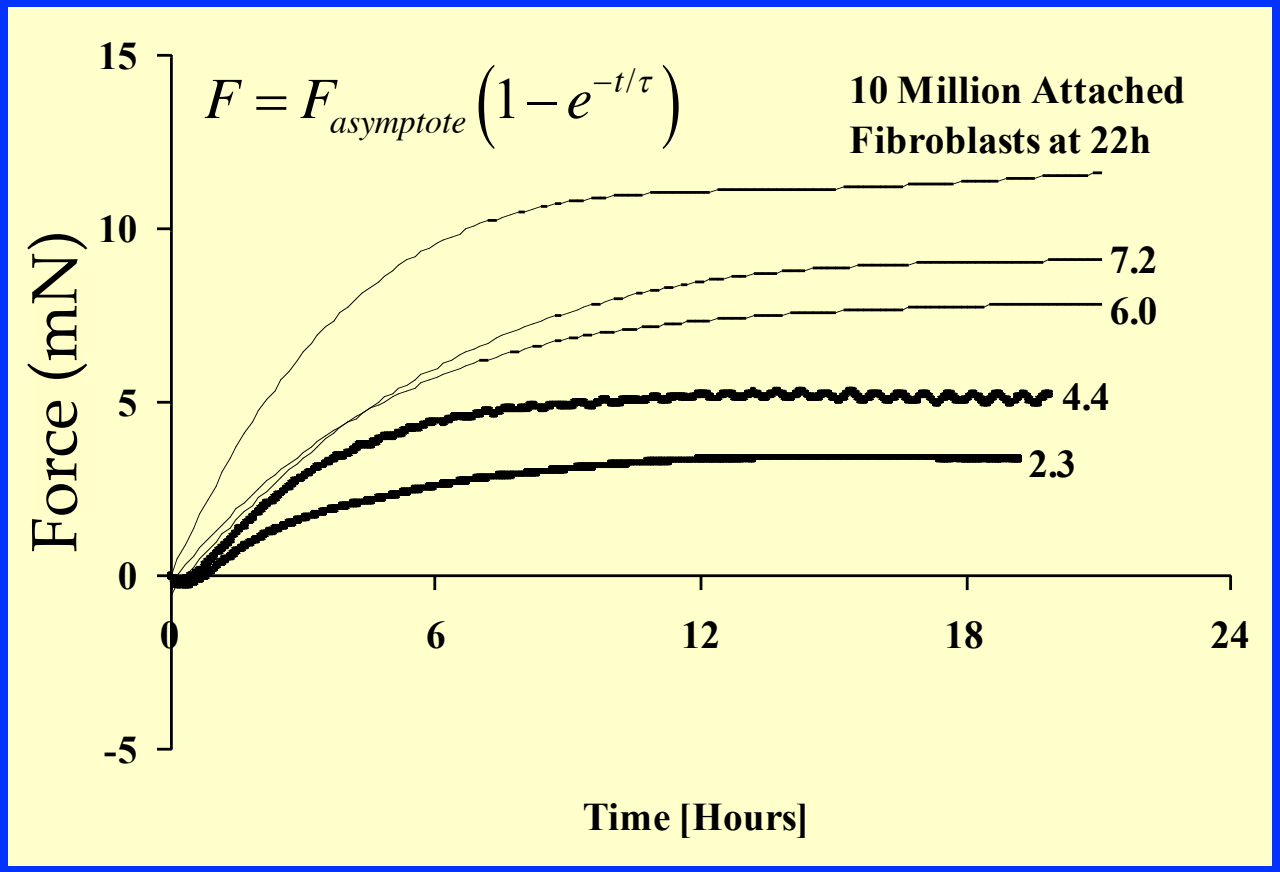
Scaffold developed by IV Yannas (MIT)

Cell Force Monitor (CFM)



Source: 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 Academic Press/Elsevier. Used with permission.

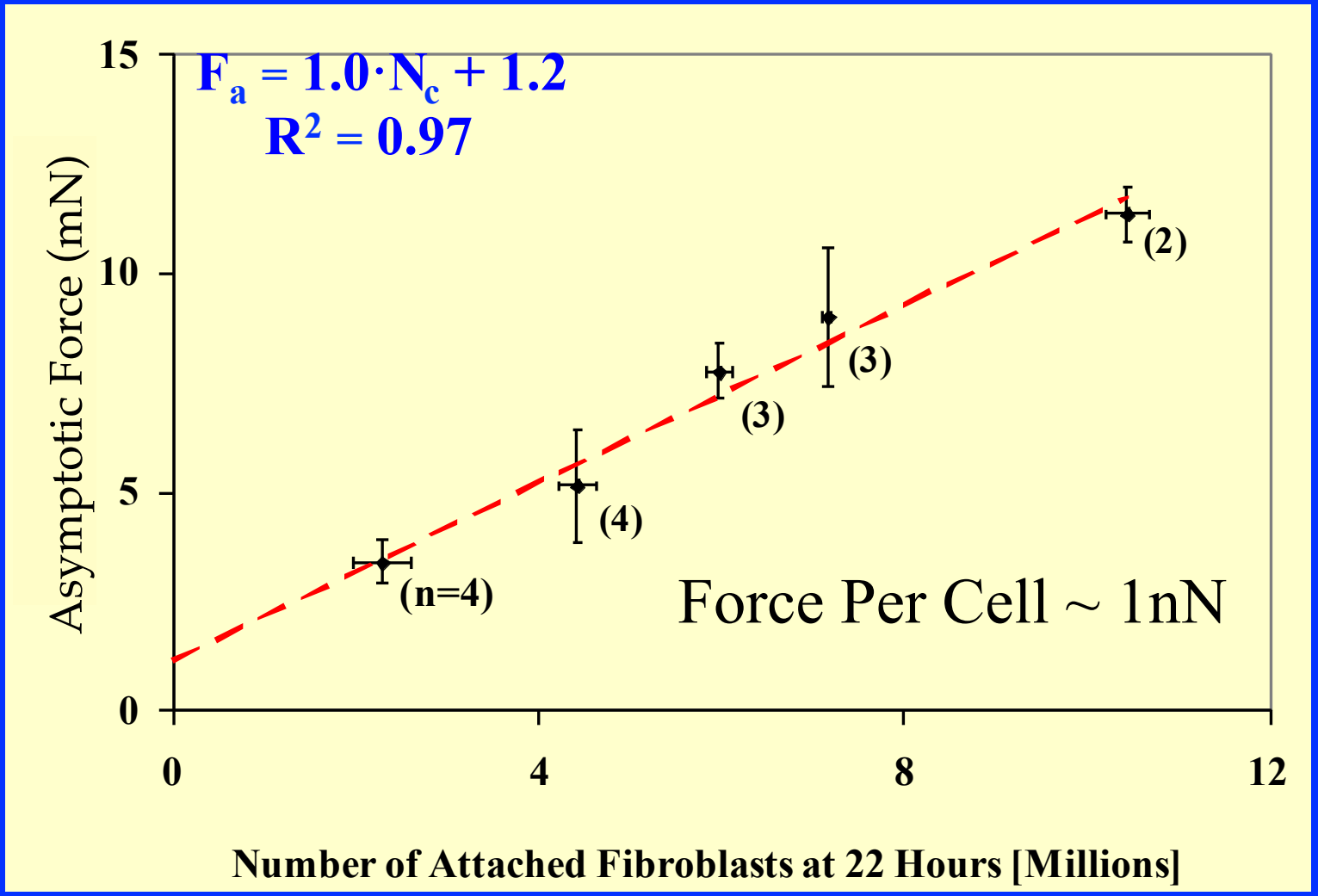
CFM: Effect of Cell Number



Time constant 5.7 hours

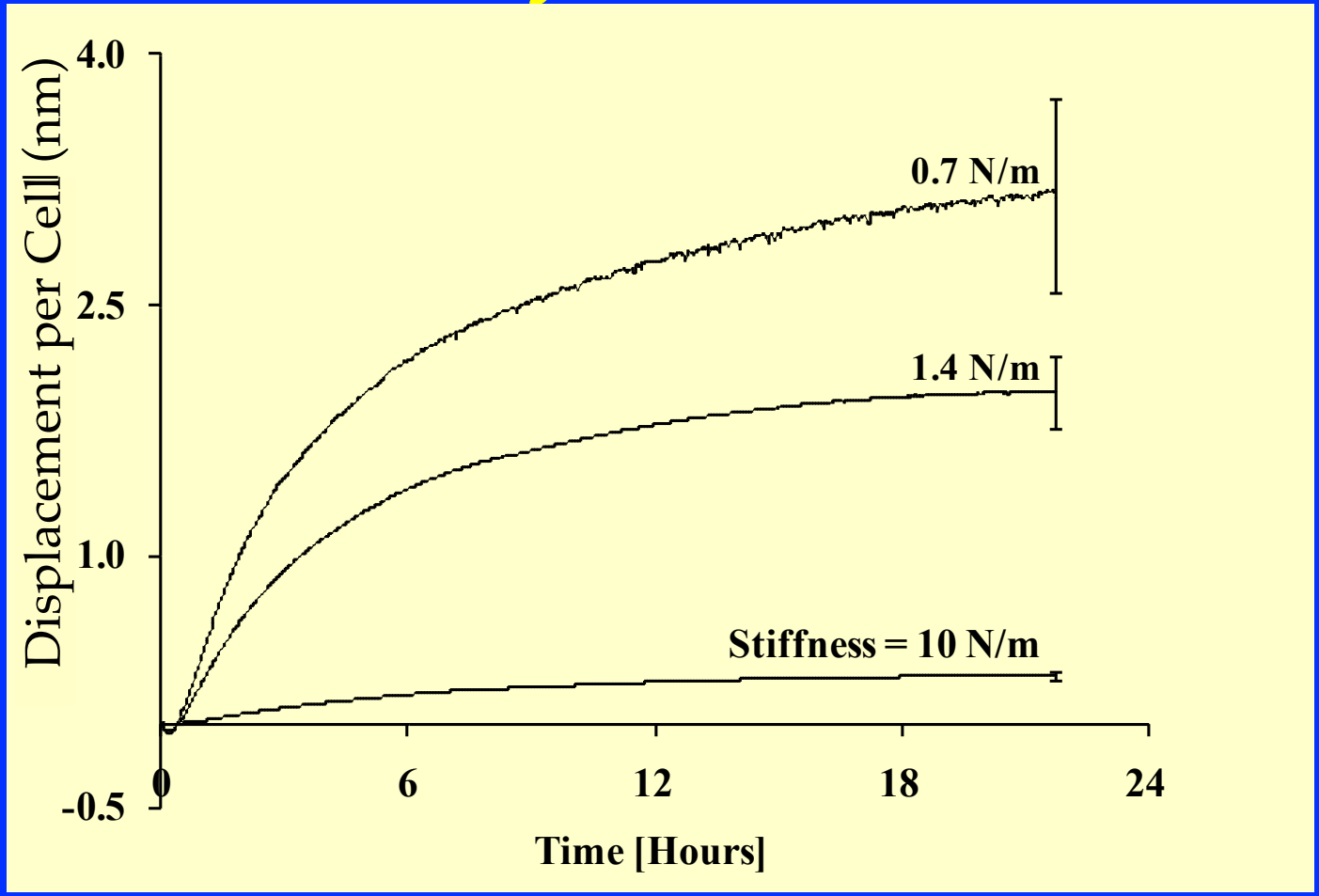
Freyman

Effect of Cell Number



Freyman

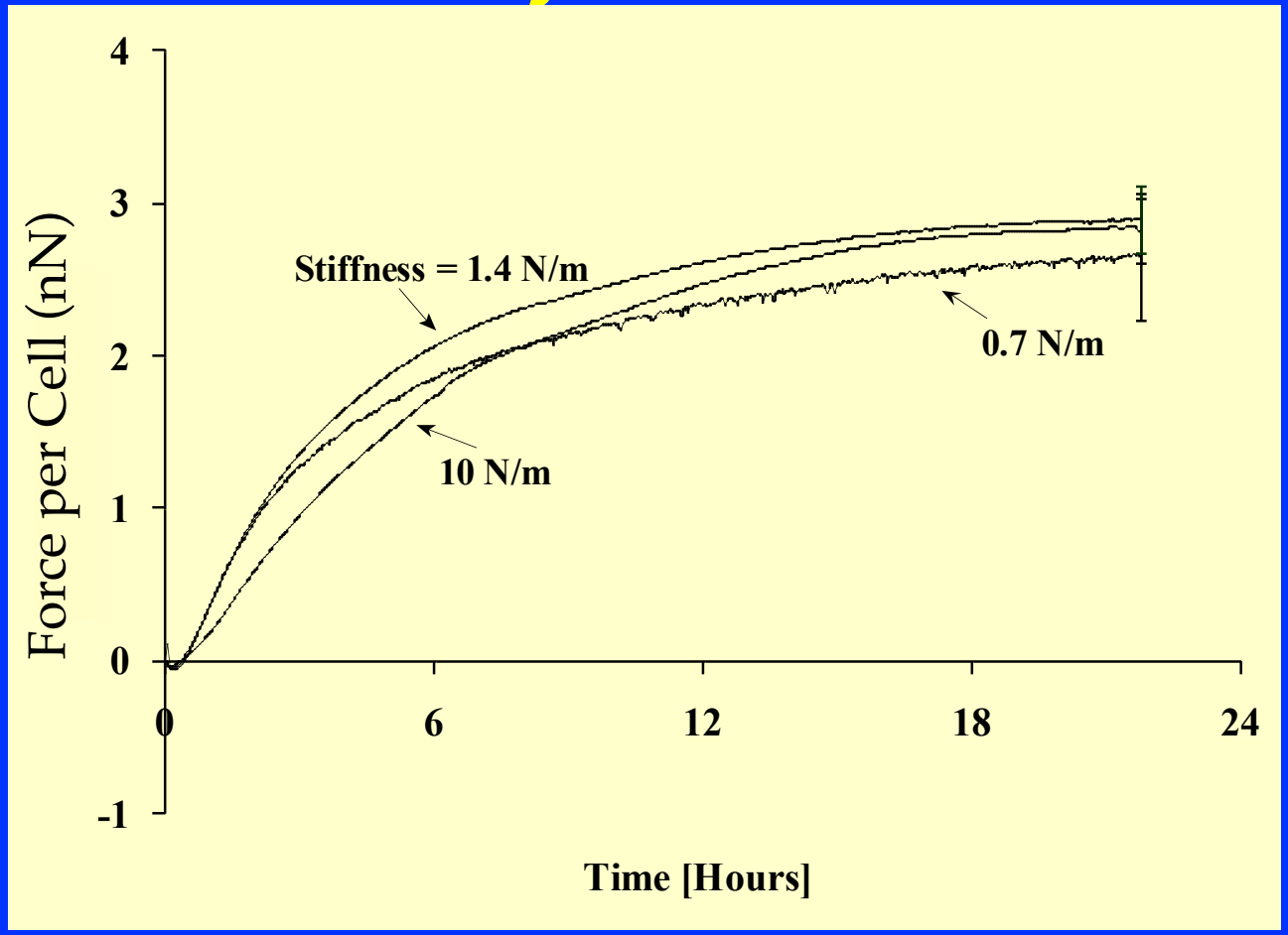
Effect of System Stiffness



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.

Freyman

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.

Freyman

Methods: Cell Elongation

Average aspect ratio of cells

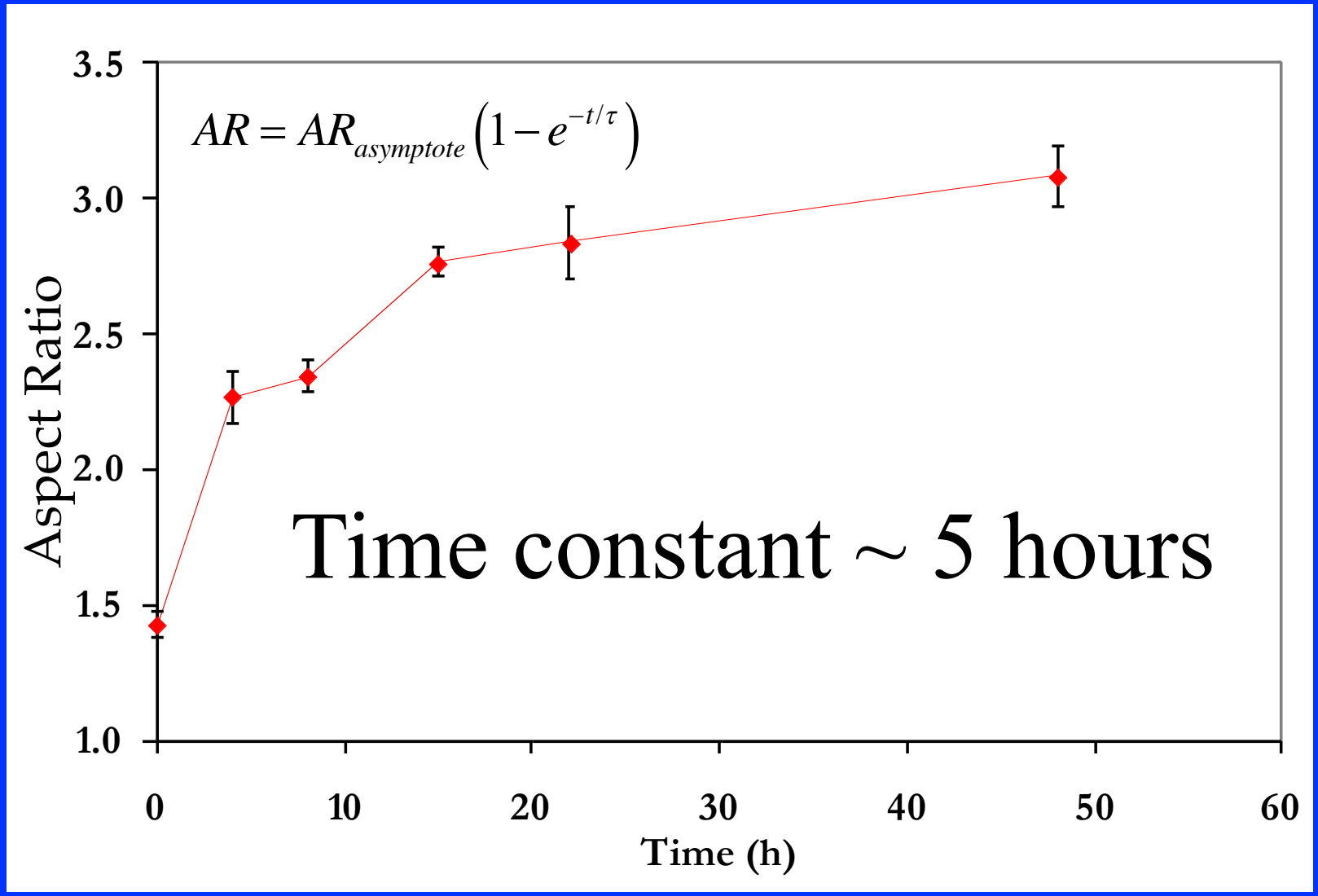
- Time points 0, 4, 8, 15, 22, and 48 h **(n=3)**
- Hematoxylin & eosin (H&E) stained glycomethacrylate (GMA) sections (5mm)
- Digital image analysis **(~200 cells per sample)**

Fibroblast Morphology

Figure removed due to copyright restrictions. See Figure 3: Freyman, T. M., et al. Micromechanics of Fibroblast Contraction of a Collagen–GAG Matrix. *Experimental Cell Research* 269 (2001): 140-53.

Freyman

Fibroblast Morphology

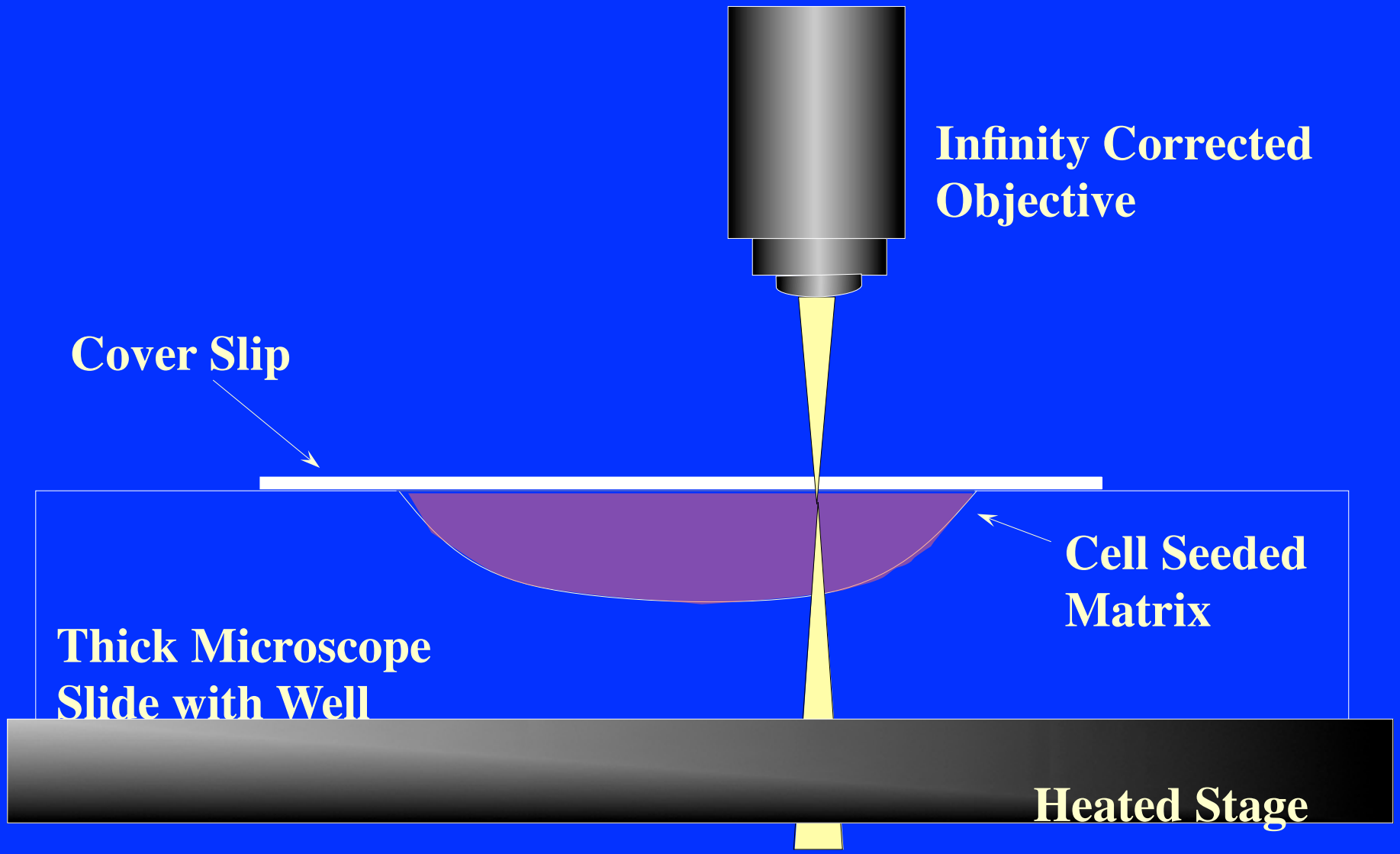


Freyman

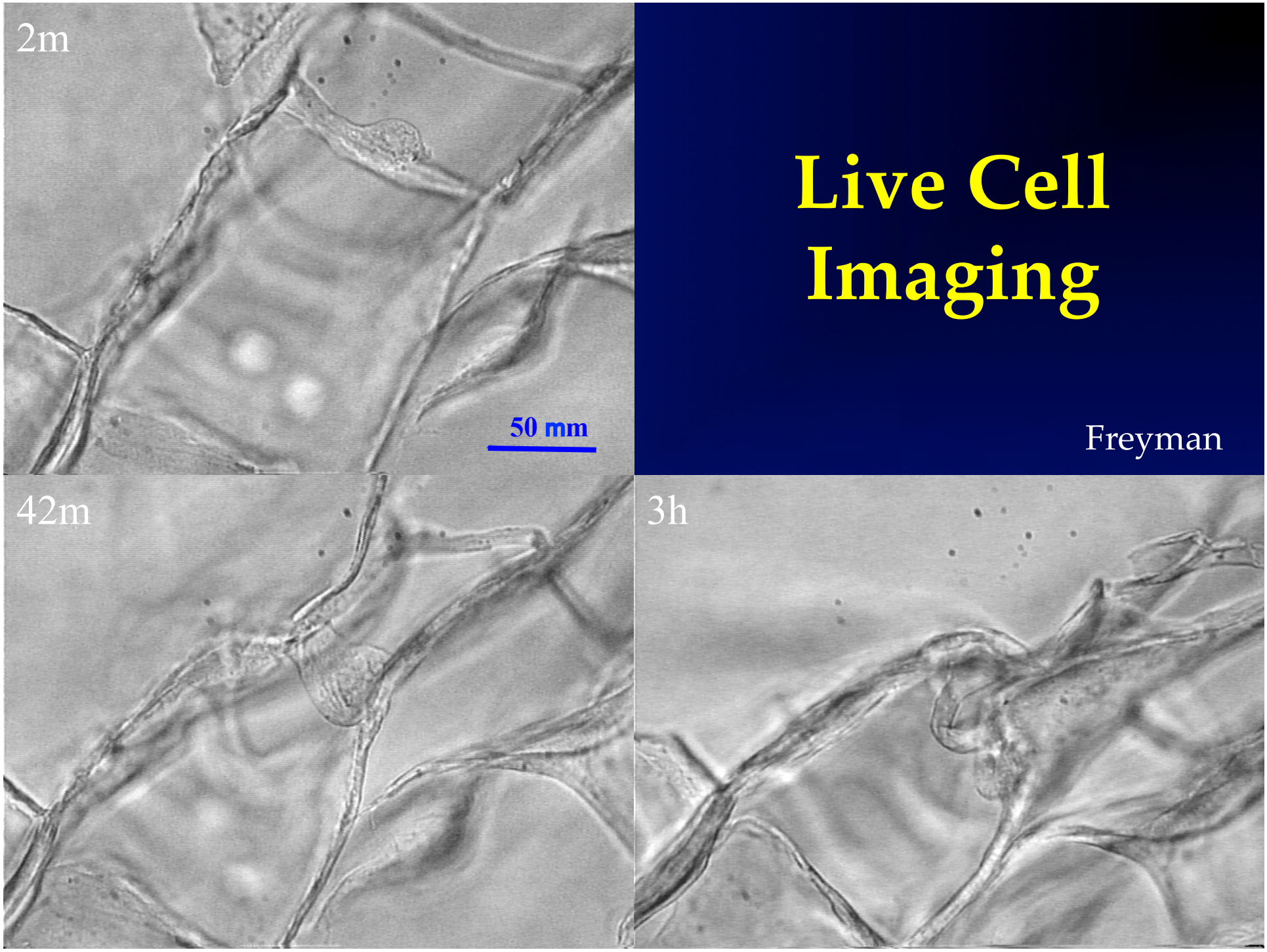
Time Constants

- 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



Source: Freyman, T. M., et al. *Experimental Cell Research* 269 (2001): 140-53. Courtesy of Elsevier. Used with permission.
http://www.sciencedirect.com/science/article/pii/S0014482701953029

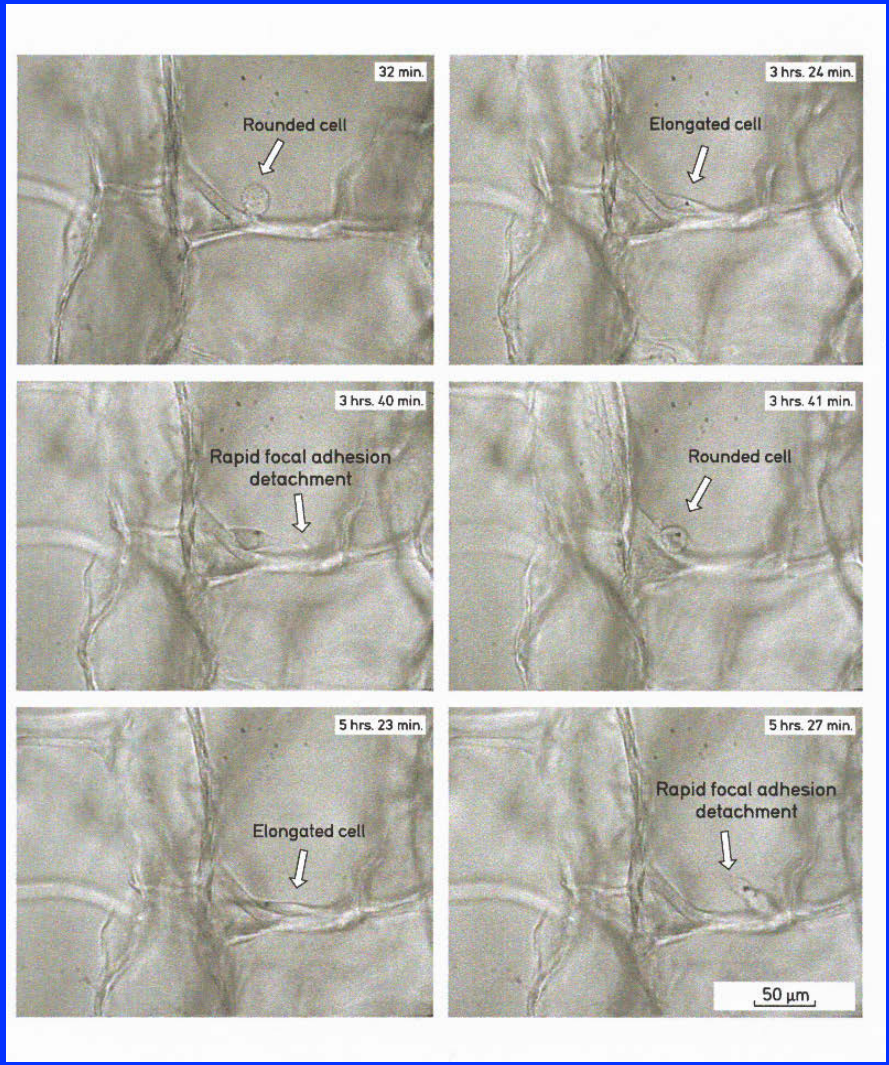

2m
50 mm
Live Cell Imaging
Freyman
42m
3h

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. *Experimental Cell Research* 269 (2001): 140-53.
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http://www.sciencedirect.com/science/article/pii/S0014482701953029

Schematic of cell elongation and matrix contraction

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

Freyman

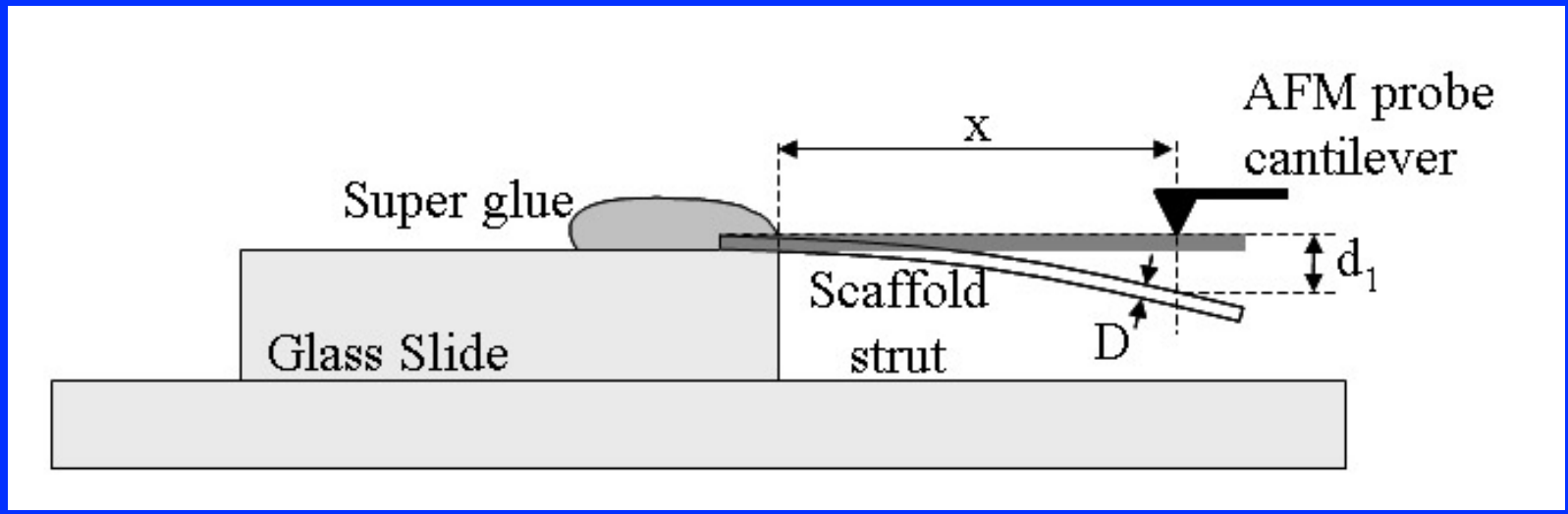
Discussion

- 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



$E_s = 762$ MPa (dry)

$E_s = 5.28$ 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)

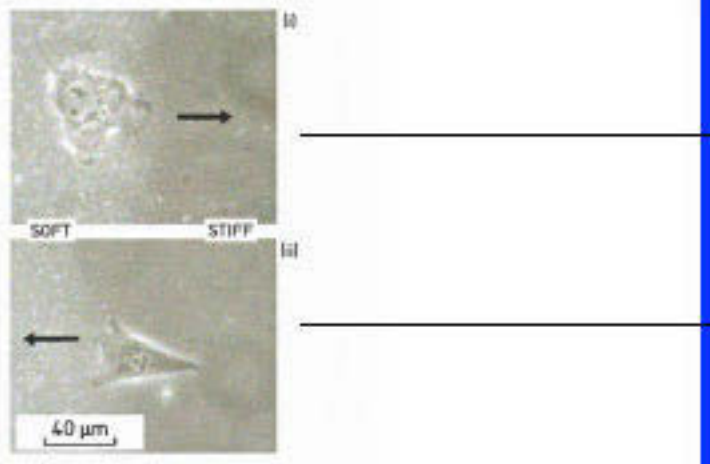
d = 3.9 +/- 0.8 μm; l from live cell imaging

F_c = 11 to 41 nN (average 26 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



Source: Lo, et al., *Biophysical Journal* 79 (2000): 144-52. Courtesy of Elsevier. Used with permission. http://www.sciencedirect.com/science/article/pii/S0006349500762795

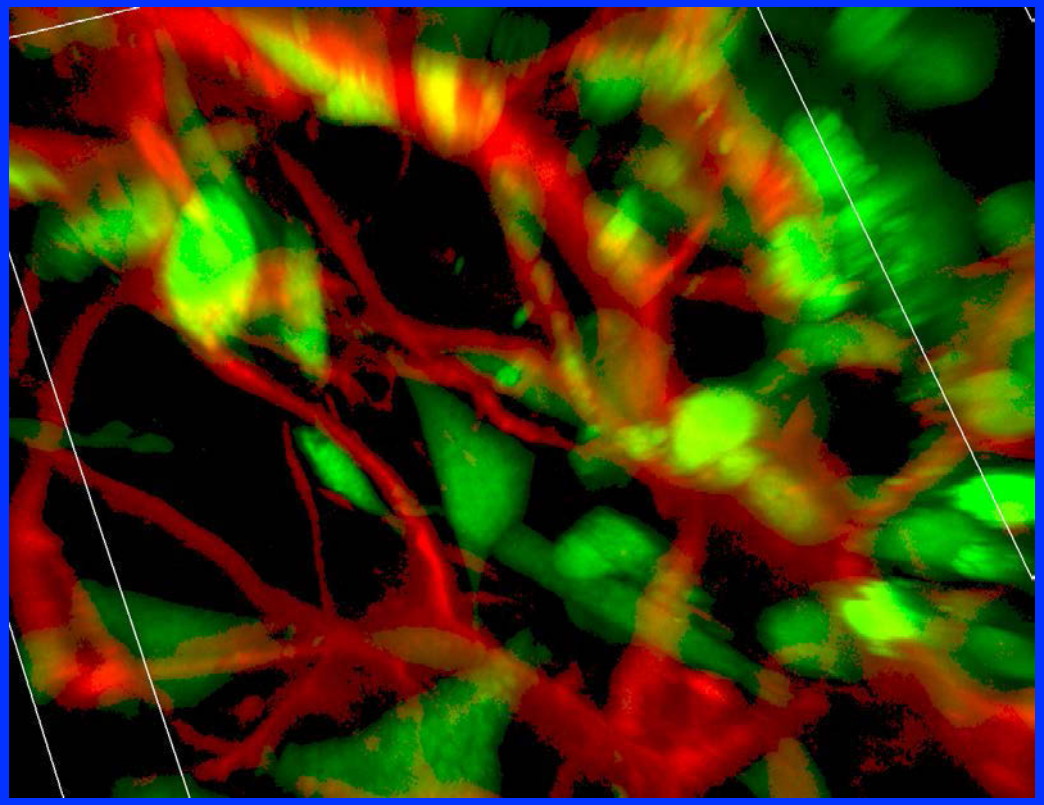
Migration speed on one-dimensional fiber constructs

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

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

Cell Migration: Fibroblasts in CG Scaffold



Courtesy of Brendan Harley. Used with permission.

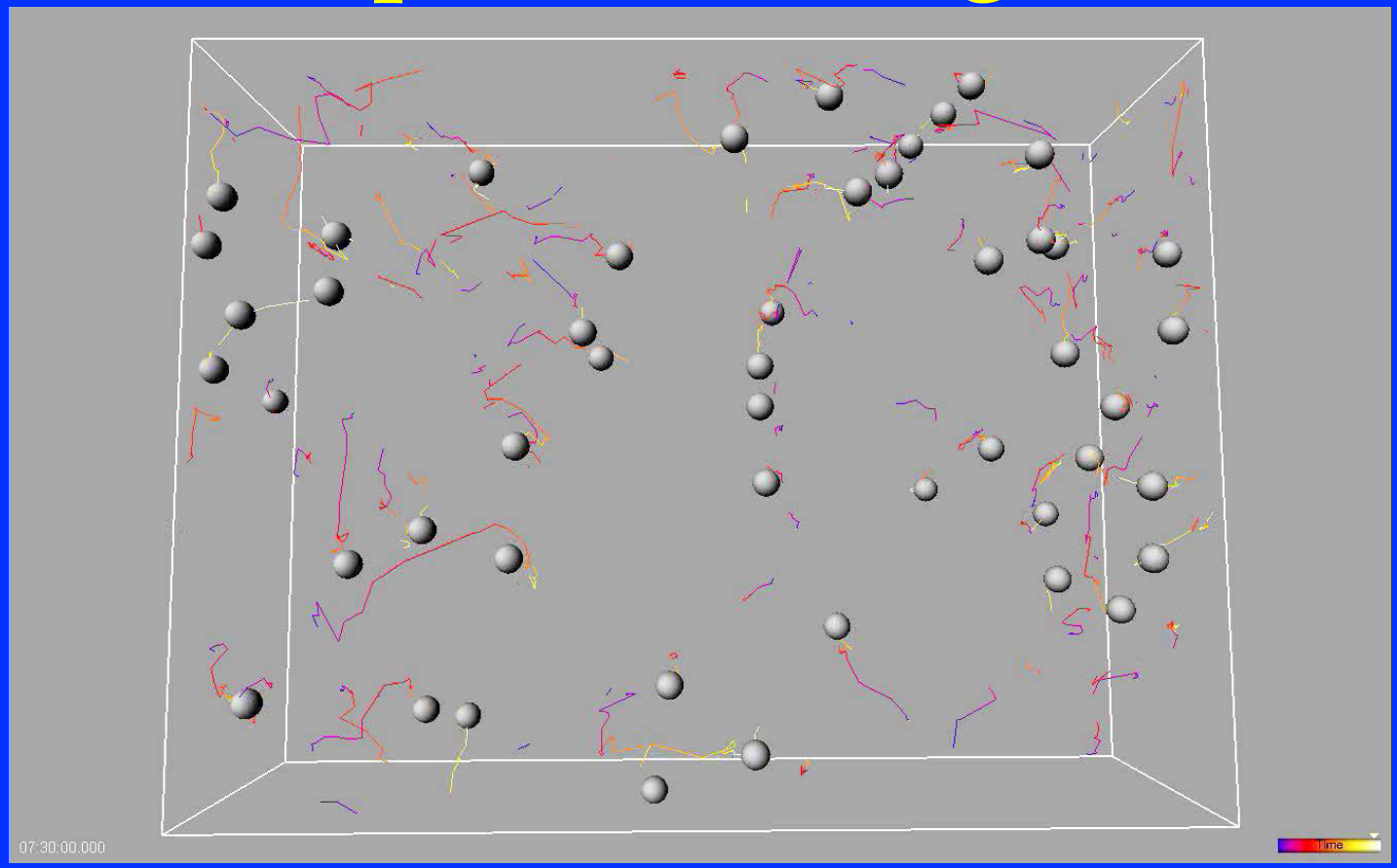
Confocal Microscopy

NR6 Fibroblasts
CMFDA Live Cell Tracker

CG Scaffold
Alexa Fluor 633 Stain

Harley

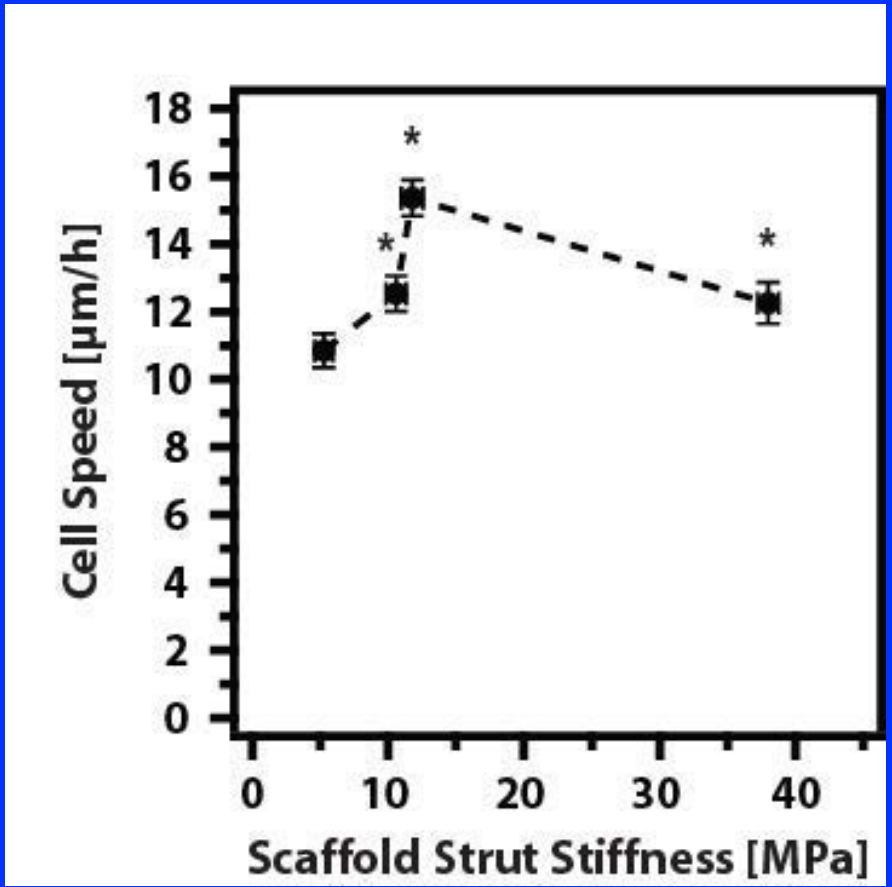
Fibroblast Migration: Spot Tracking



Courtesy of Brendan Harley. Used with permission.

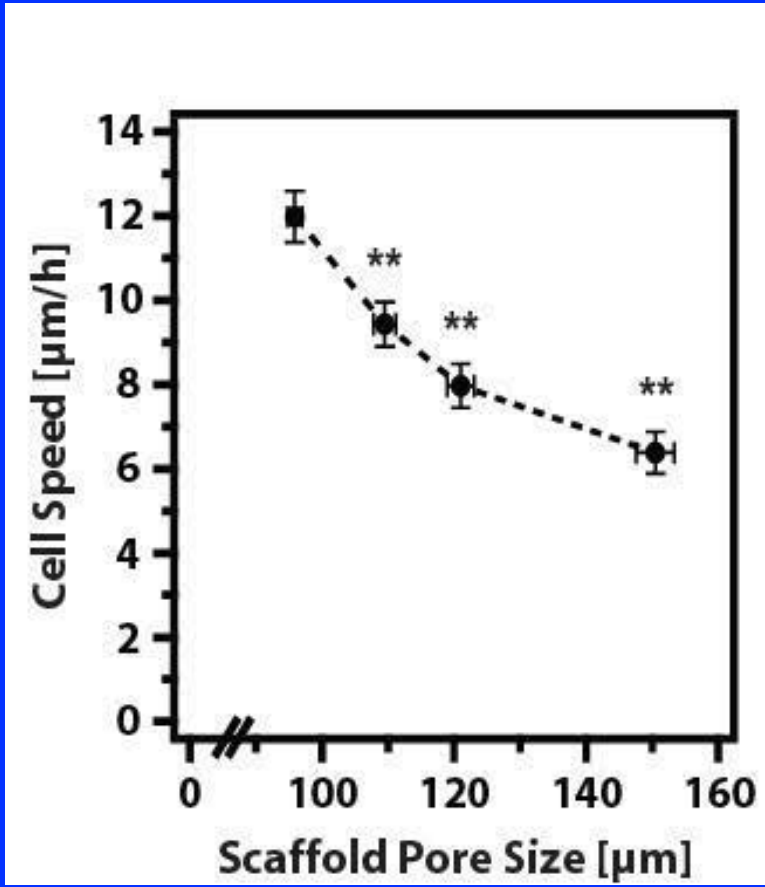
Harley

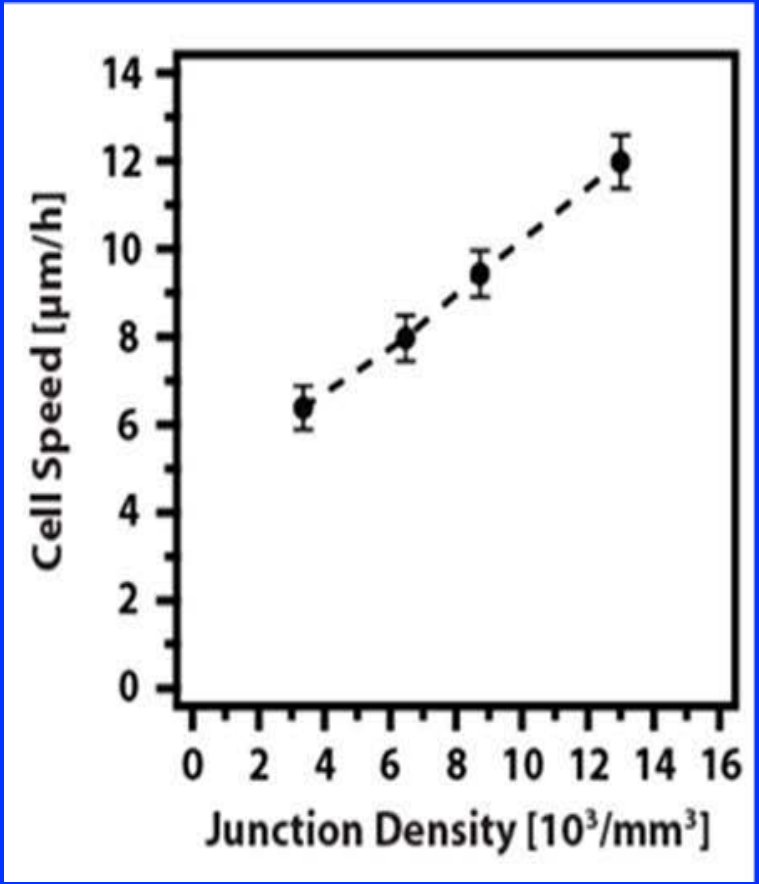
Migration Speed vs Strut Stiffness



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

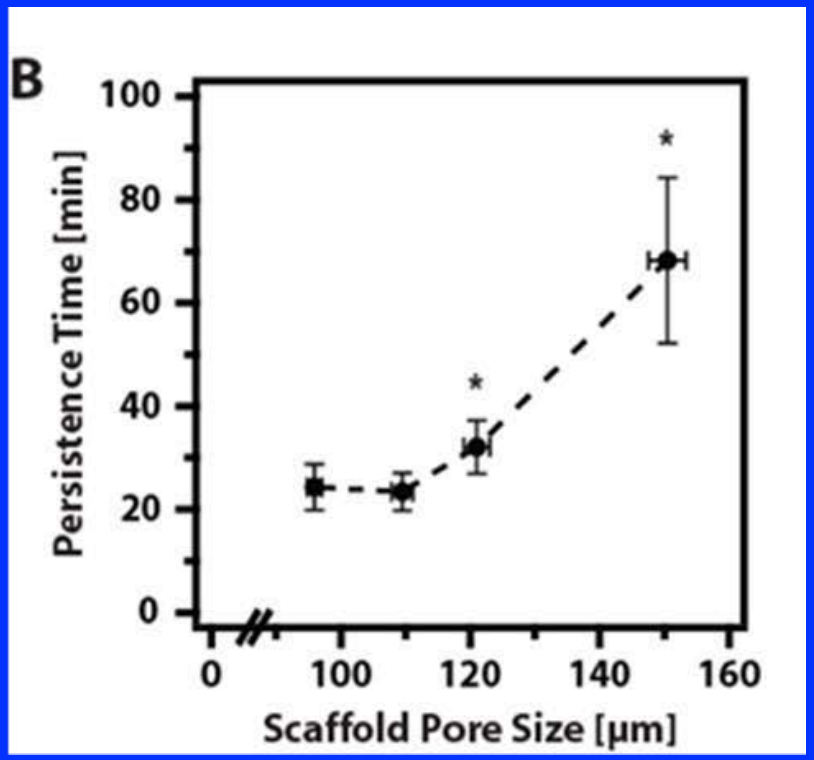
Migration Speed vs Pore Size

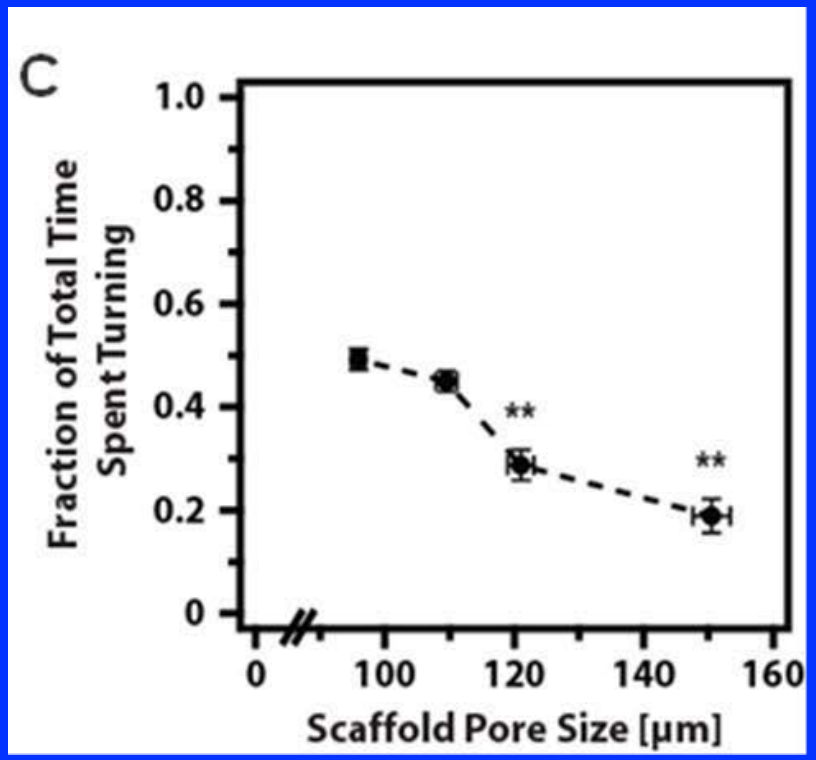




Source: Harley, B. A. C., et al. *Biophysical Journal* 95 (2008): 4013-24.
Courtesy of Elsevier. Used with permission.
http://www.sciencedirect.com/science/article/pii/S0006349508785394

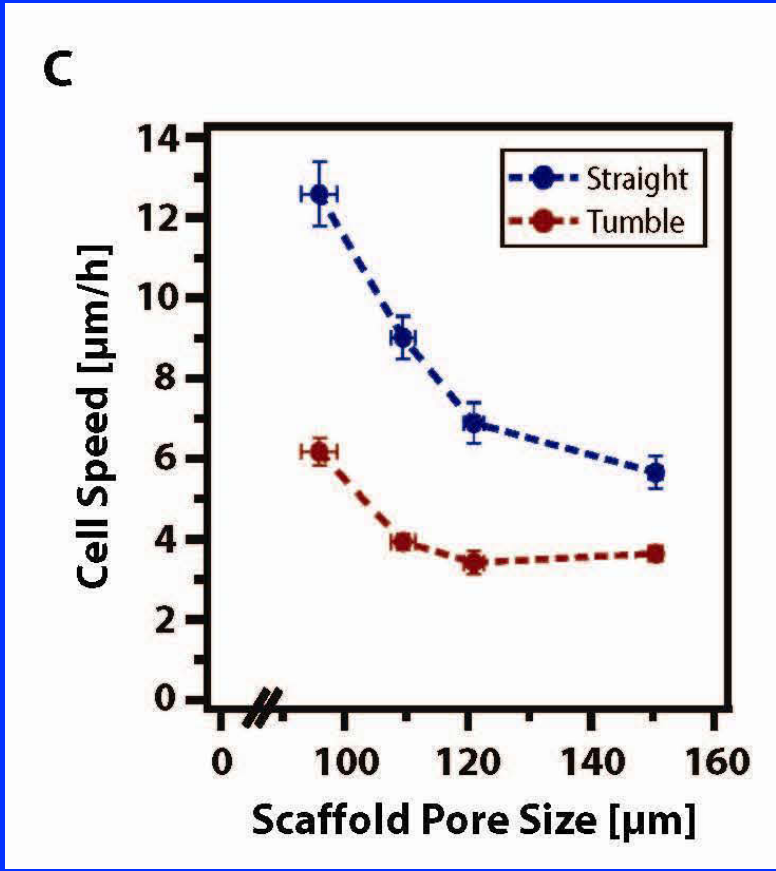
Migration Speed vs Pore Size





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



Source: Harley, B. A. C., et al. *Biophysical Journal* 95 (2008): 4013-24.
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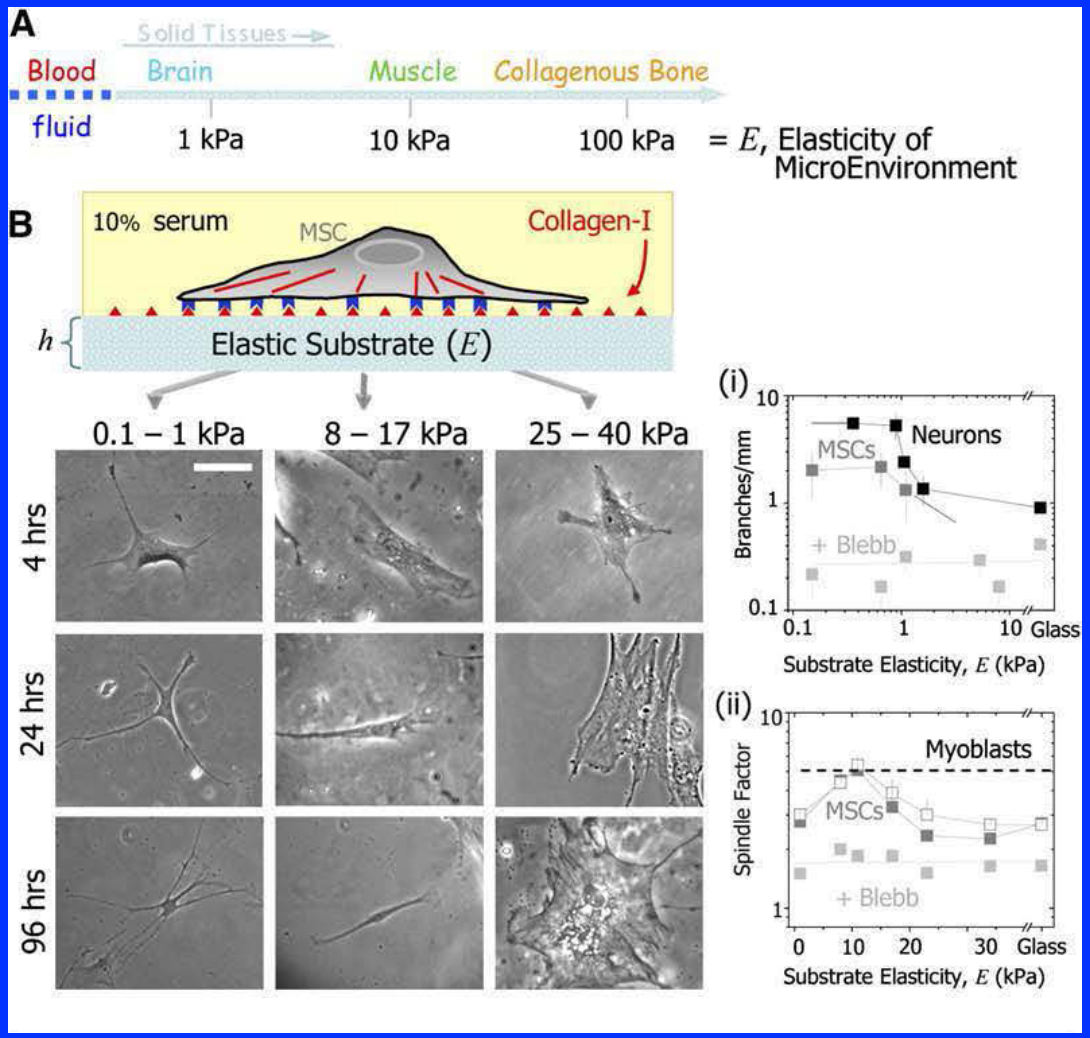
http://www.sciencedirect.com/science/article/pii/S0006349508785394

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

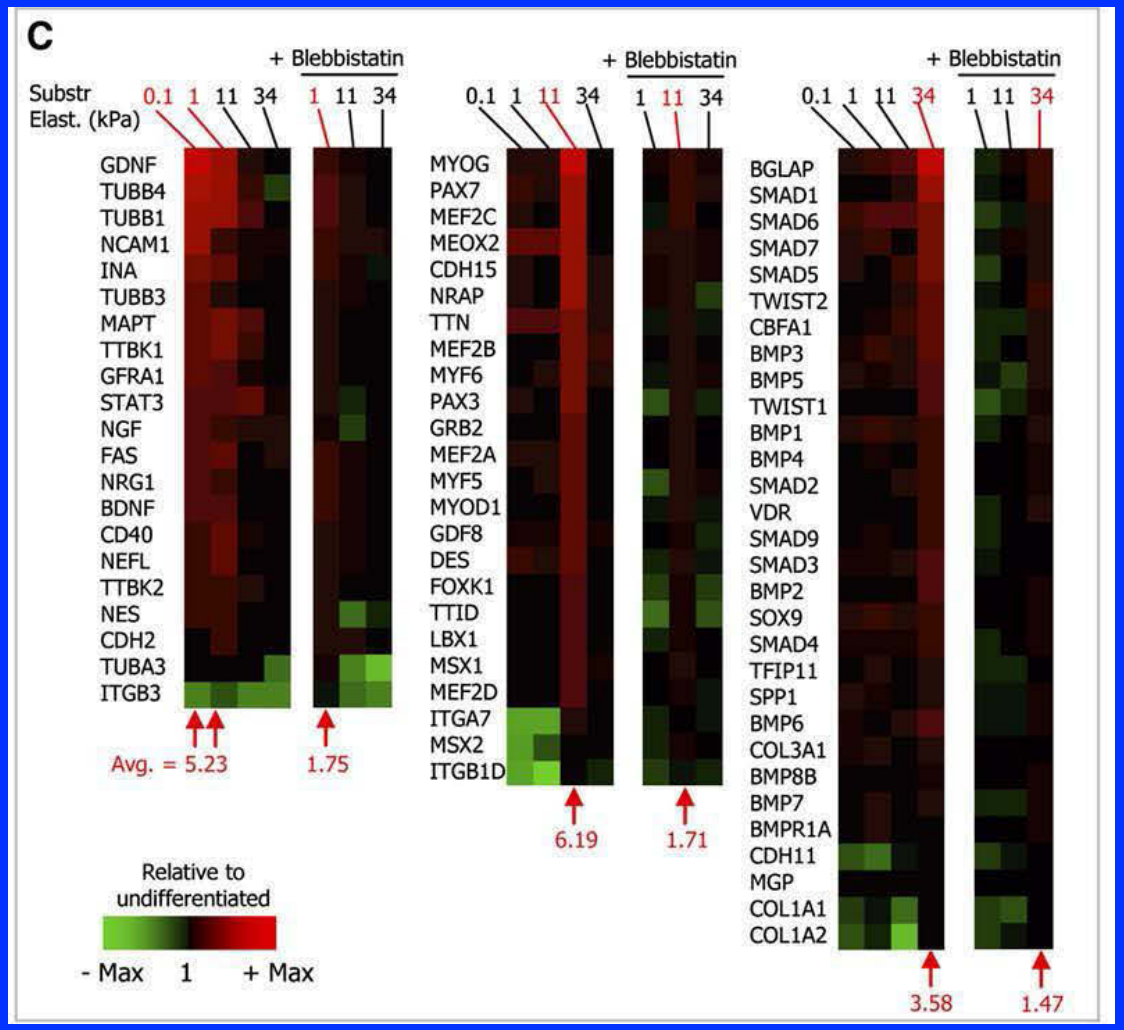
Cell Differentiation



Engler et al., 2006

Neuron-like Myoblast-like Osteoblast-like

Cell Differentiation



Engler et al, 2006

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

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 ~ 1nN
  - Contractile force calculated from buckling of a strut by a single cell ~ 11-41 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)

Acknowledgements

- Drs. TM Freyman, BA Harley, FJ O'Brien, M Zaman
- JH Leung, R Yokoo, Y-S Pek, MQ Wong, ECCM Silva, HD Kim, K Corin
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- Drs. Spector and Germaine
- NIH Training Grant, NIH grant (DE 13053), Matoula S. Salapatas Professorship, Cambridge-MIT Institute

3.054 / 3.36 Cellular Solids: Structure, Properties and Applications
Spring 2015