Harvard-MIT Division of Health Sciences and Technology
HST.523J: Cell-Matrix Mechanics
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Measuring Cell Contraction
Cell Force Monitor

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Clinical Application

- Wound healing following severe injury: *Repair*
  - Wound contraction
    - Myofibroblasts (α-SMA)
  - Synthesis of scar tissue
    - Anisotropic tissue
    - Significantly stiffer, reduced range of motion, pain, inferior functional properties

- Bioactive scaffolds developed to induce regeneration
  - Cell continuity is practically absent
  - Contractile cells are randomly oriented within the bioactive scaffold
  - Hypothesis: the structure of the bioactive ECM analog prevents the coordinated cell contraction that results in wound contraction and scar formation

Yannas, 2001

Development of Bioactive Scaffolds

- Bioactive scaffolds engineered to induce an appropriate or desired cell response *in vitro* or *in vivo*

- Requires critical adjustment of four physical and structural properties :
  1. Chemical composition (ligands)
  2. Average pore size
  3. Degradation rate
  4. *For induced regeneration:* Collagen fiber structure

Yannas, 2001

Bioactive Scaffold: Pore Size

- Scaffold bioactivity is significantly affected by pore structure
  - Large enough to allow cells to migrate into the structure
  - Small enough to establish a sufficiently high specific surface area

- Non-uniform scaffolds
  - Patches of scaffold are inactive

- Fabrication of a uniform scaffold
  - Uniform activity throughout scaffold
  - Study cell-scaffold interactions at a microscopic scale

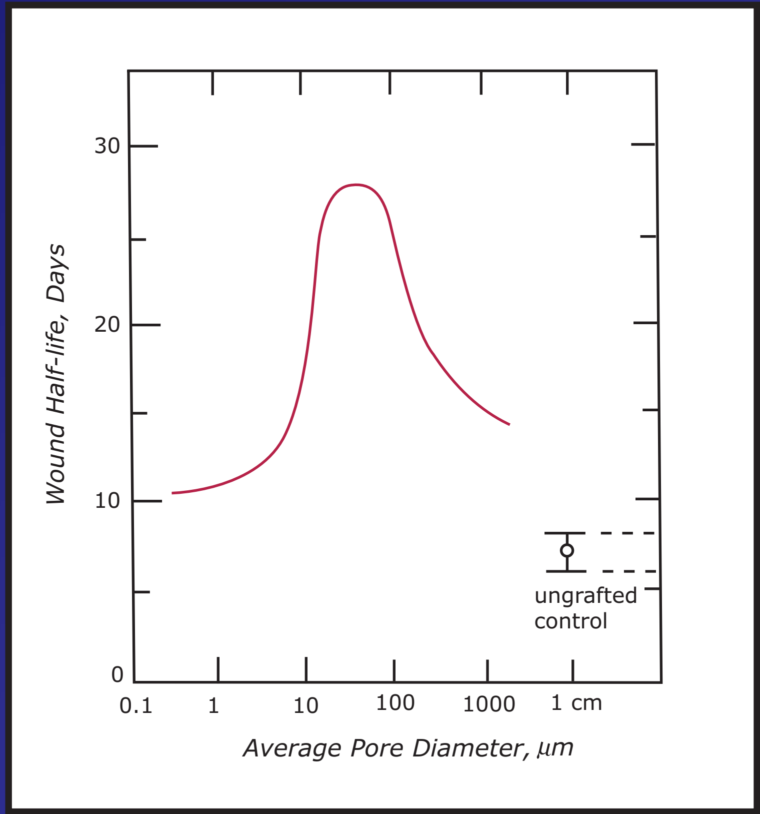


Figure by MIT OCW. After Yannas et al., 1989.

Design of Cell/Tissue Specific Biomaterials

- Material and structural properties significantly affect cell behavior
  - Different cell types respond differently to different scaffolds
    - Architecture mediated?
    - Stiffness mediated?
    - Composition mediated?
    - Degradation rate mediated?

Nehrer et al., 1997
Yannas, 2001
Salem et al., 2002
Claase et al. 2003

- How do cells detect and respond to their surrounding environments (*i.e.*, mechanical, structural, chemical) and what are the critical cues?
  - Cell-mediated contraction

Single Cell Mechanics on 2-D Substrates

- Study behavior of a single cell on a flat membrane (*i.e.*, silicone, polyacrylamide)
  - Mechanical environment (*i.e.*, system stiffness)
  - Chemical environment (*i.e.*, ligands, growth factors, cytokines)

- Experimental tools:
  - Microscopic analysis of membrane deflection

Single Cell Mechanics on 2-D Substrates

- Study cell-generated forces on flexible membranes
  - Traction forces during migration
  - Measure deflection of membranes

Photos removed for copyright reasons.

Silicone membrane

Silicone membrane with a regular dot pattern

Polyacrylamide membrane with fluorescent microspheres

Beningo and Wang, 2002

Diagrams and photo removed for copyright reasons.

Array of flexible PDMS posts

Tan et al., 2003

Single Cell Mechanics on 2-D Substrates

3T3 Fibroblast, Substrate Deformation:

Diagram showing states at t = 0 and t = 30 min
removed for copyright reasons.

Three diagrams
removed for copyright reasons.

→ →

Cell on membrane

Deformation vectors

Field of traction stresses

Pelham et al., 1999
Munevar et al., 2000

Cell Mechano-Sensitivity

Polyacrylamide gel with two distinct regions of rigidity (14 vs. 20 kPa):

Series of 16 photos (t=0:00 to t=3:50) removed for copyright reasons.

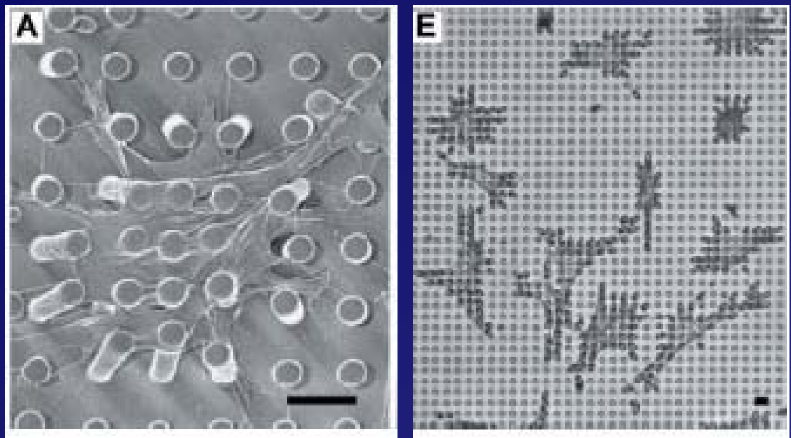
Stronger traction forces on stiff substrate (1.1 vs. 0.6 Pa)

Faster migration on soft substrate (0.44 vs. 0.23 μm/min)

Lo et al., 2000

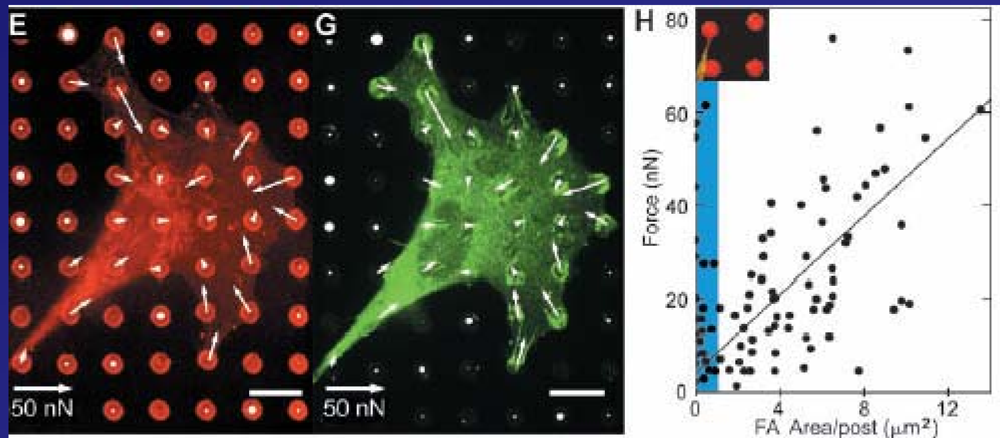
Single Cell Mechanics on 2-D Substrates

Cell deformation of an array of flexible posts:



Single cell

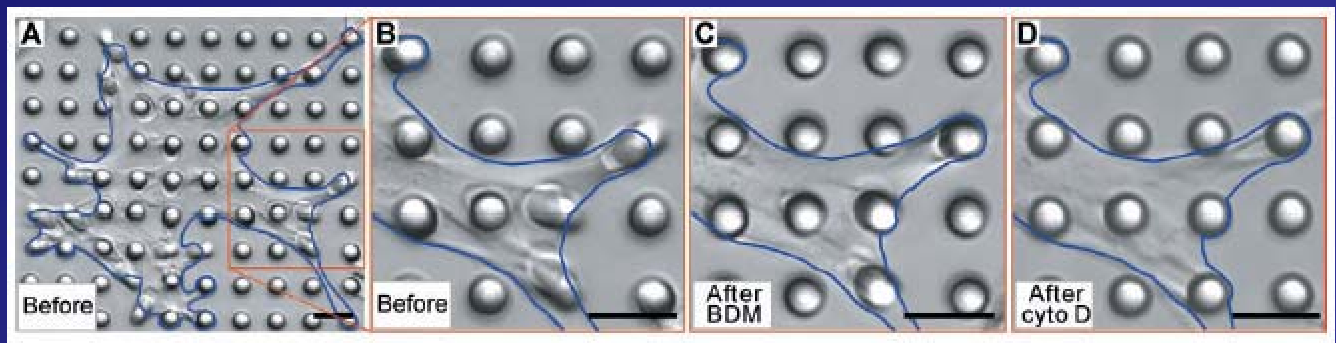
Cell population



Fibronectin on post tips

Localization of focal adhesion protein vinculin

Force/post vs. Adhesion area



Before and after treatment with 2,3-butanedione monoxime (inhibitor of myosin contractility)

Source: Figures 2 and 3 in Tan, John L. et al. "Cells lying on a bed of microneedles: An approach to isolate mechanical force." *PNAS* 100:4 (February 18, 2003) 1484-1489. Courtesy of the National Academy of Sciences. Used with permission.

Single Cell Mechanics: Results Summary

- Cells can sense and respond to the surrounding mechanical environment
  - Substrate flexibility/rigidity regulates cell migration and applied traction forces
  - Substrate flexibility/rigidity regulates cell growth and apoptosis
    - Cells on more flexible (4.7 vs. 14 kPa) substrates show decreased rates of DNA synthesis and increased rates of apoptosis
- Kinetics of contractile force development (smooth muscle cell):

Graph removed for copyright reasons.

Lo et al., 2000
Wang et al., 2000
Tan et al., 2003

Cell Population Mechanics in 3-D Substrates

- Study behavior of a population of cells in a scaffold (*i.e.*, collagen-GAG scaffold)
  - Mechanical environment (*i.e.*, system stiffness)
  - Chemical environment (*i.e.*, ligands, growth factors, cytokines)
- Experimental tools:
  - Live cell imaging
  - Cell Force Monitor (CFM)

Collagen-GAG Scaffold Fabrication

Production of Collagen-GAG (CG) co-Polymer Suspension:

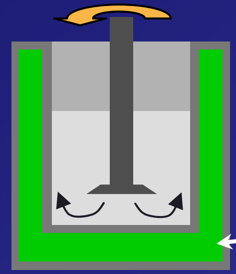
Collagen:
type I, bovine tendon, microfibrillar
Integra LifeSciences

Acetic Acid:
0.05M, pH ~3.2

GAG:
chondroitin 6-sulfate, shark cartilage
Sigma-Aldrich

Combine →

Mix CG Suspension:
- Overhead blender
- 15,000 rpm



Refrigerant-cooled flask (4°C)

Degas → P=50mtorr

Store suspension at 4°C

Fabrication of CG Scaffolds with Different Pore Sizes:

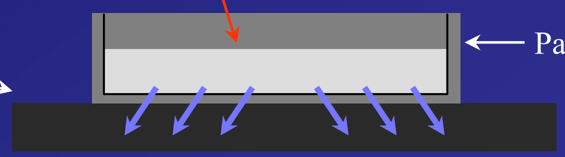
Place CG suspension into stainless steel pan

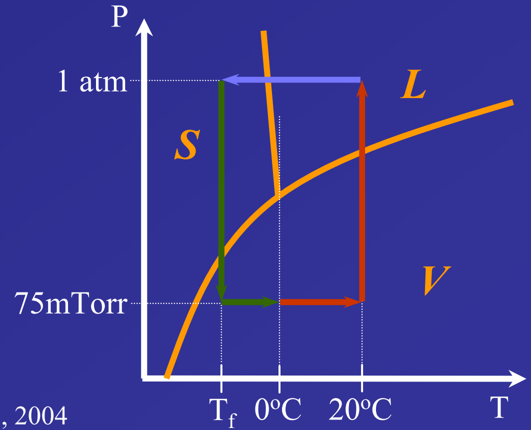
Freeze: →
T = T_f
Genesis Freeze-dryer
VirTis Co.

Ice crystals surrounded by collagen and GAG fibers

Sublimation: →
P=75mTorr, T=0°C
Removes ice content

Porous, CG scaffold





Collagen-GAG Pore Structure

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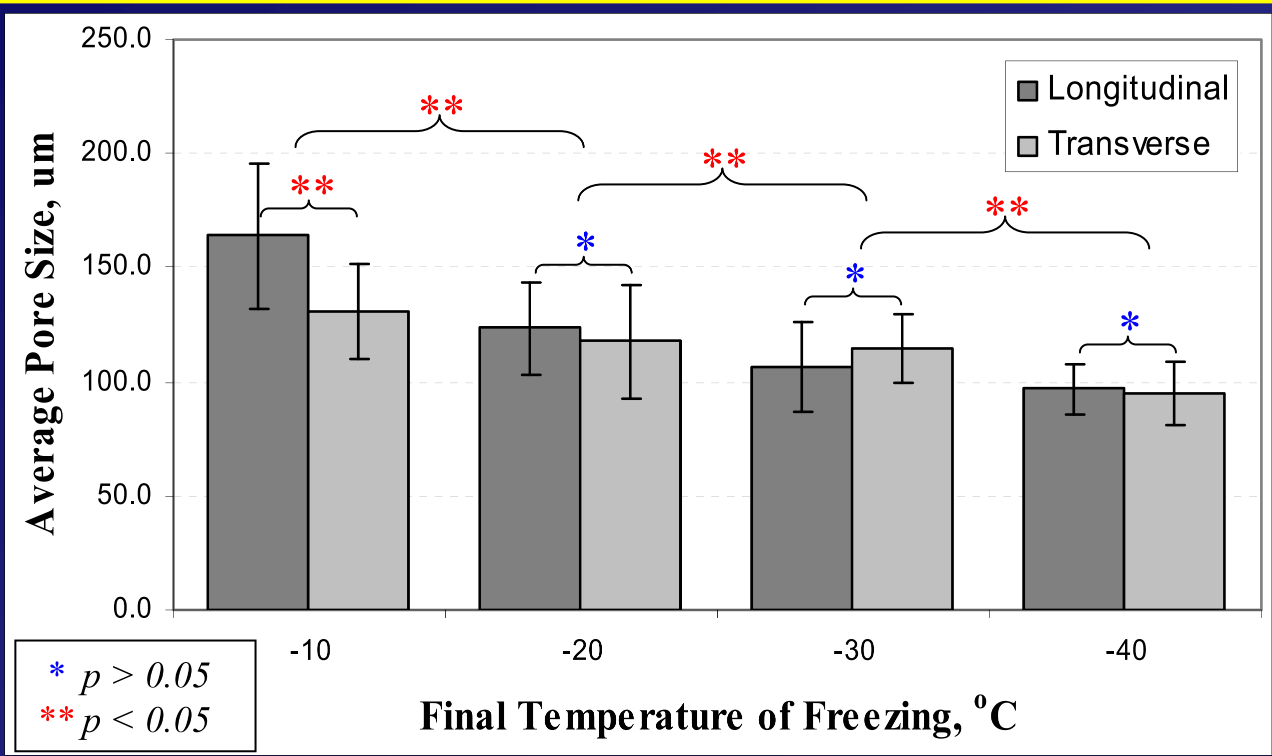
Pek et al., 2003

Effect of T_f on Mean Pore Size

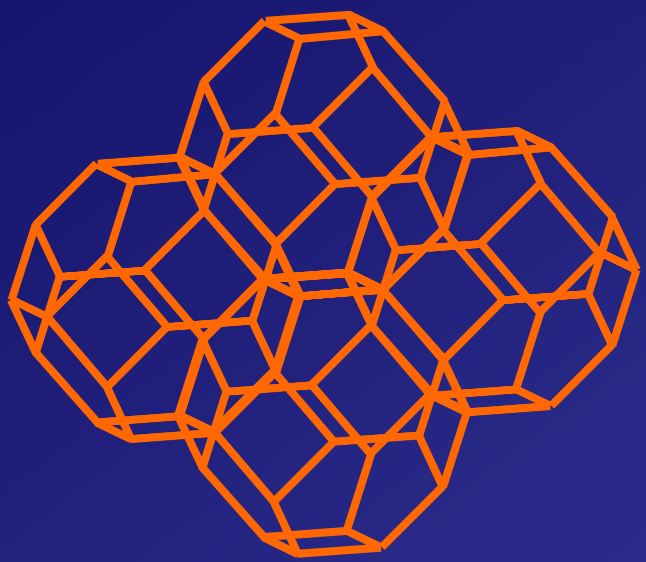


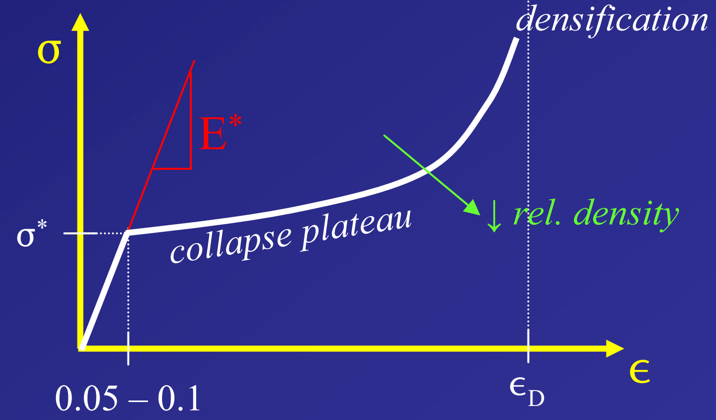
Significant effect ($p<0.05$) of T_f on mean pore size

T_f, °C	Mean Pore Size, µm	Relative Density
-10°C	150.5 ± 32.1	0.0062
-20°C	121.0 ± 22.5	0.0061
-30°C	109.5 ± 18.3	0.0059
-40°C	95.9 ± 12.3	0.0058

Mechanical Model of Open-Cell Foams

- Open-cell tetrakaidecahedron
  - Packs to fill space
  - Approximates structural features of low-density foams



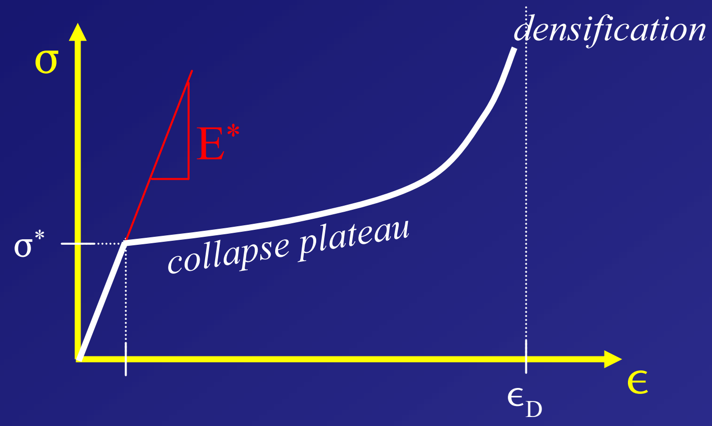


$$E^* = \left(\frac{\rho^*}{\rho_s}\right)^2 \cdot E_s$$

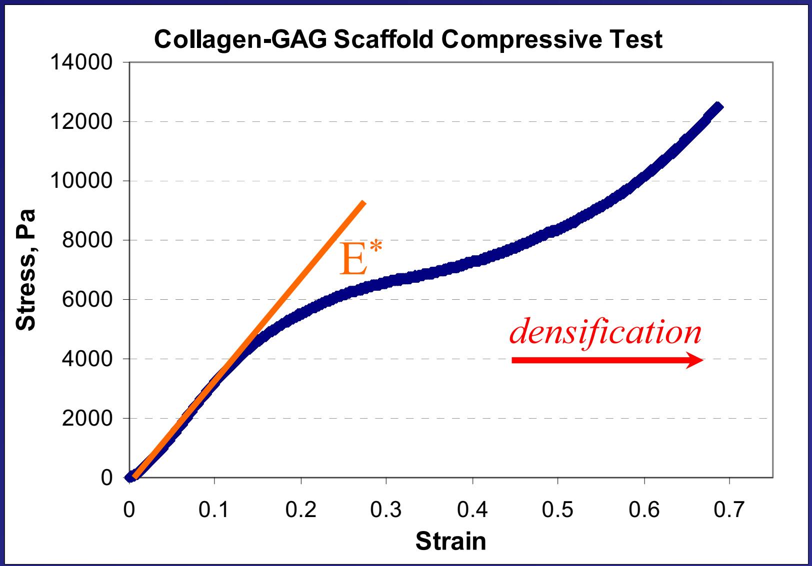
Strut bending dependence

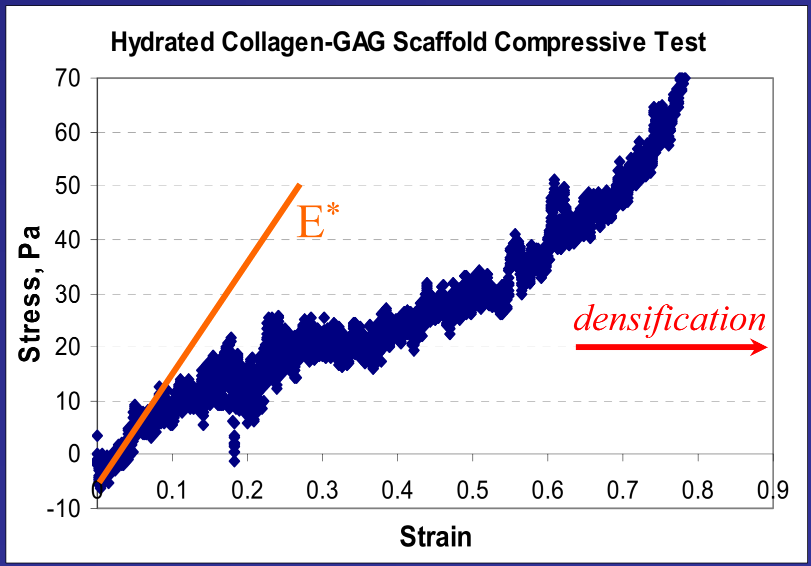
CG Scaffold Mechanics: Results

Cellular Solids Model Prediction:



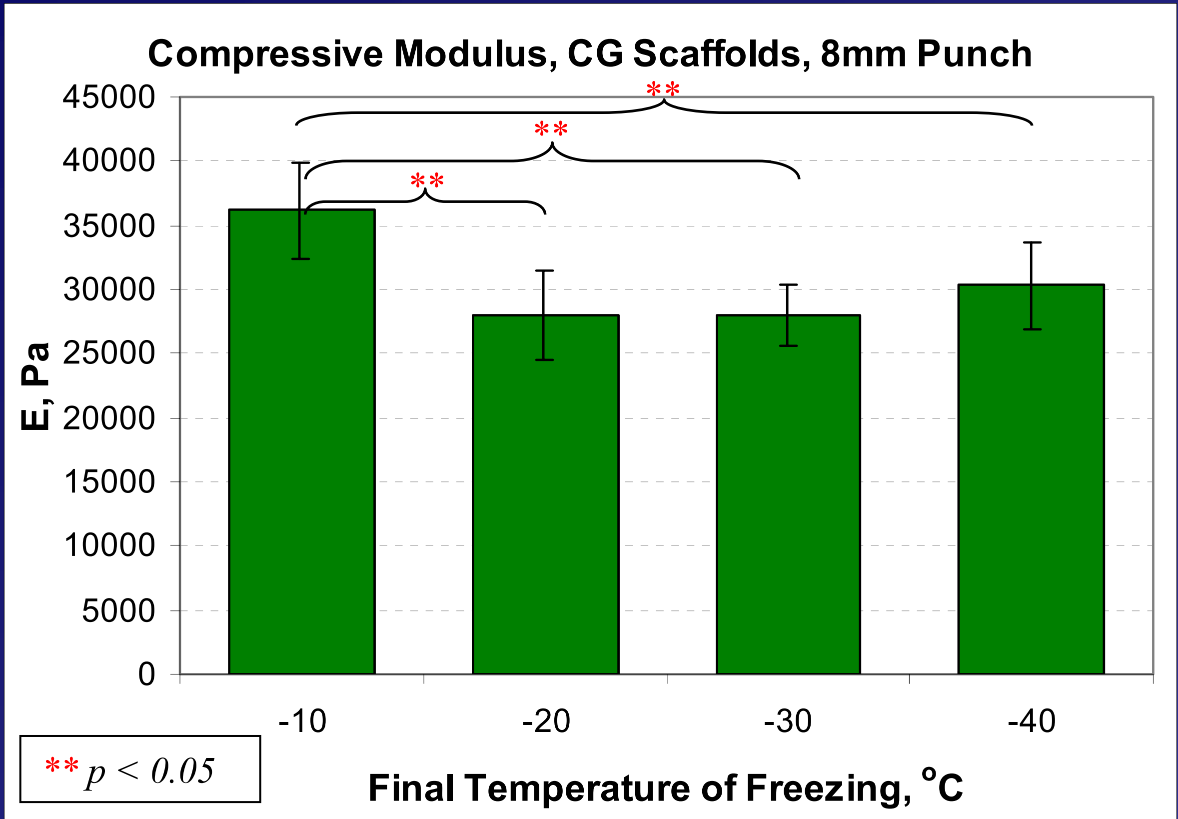
Experimental Results:





CG Scaffold Mechanics: Results

Non-hydrated Scaffold Mechanical Properties:



T_f, °C	E, kPa
-10	36.2 ± 3.8
-20	28.0 ± 3.5
-30	28.1 ± 2.4
-40	30.3 ± 3.3

Modulus determined from linear fit of data between 0.05 and 0.12 strain

CG Scaffold Mechanics: Results

Non-hydrated Scaffold Modulus determined through Experiment:

T_f, °C	$E_{non\text{-}hydrated}$, kPa	$E_{hydrated}$, Pa
-10	36.2 ± 3.8	200
-20	28.0 ± 3.5	175
-30	28.1 ± 2.4	175
-40	30.3 ± 3.3	175

$\rho^*/\rho_s = 0.006$

Cellular Solids Model Predictions:

$$E^* = \left(\frac{\rho^*}{\rho_s}\right)^2 \cdot E_s$$

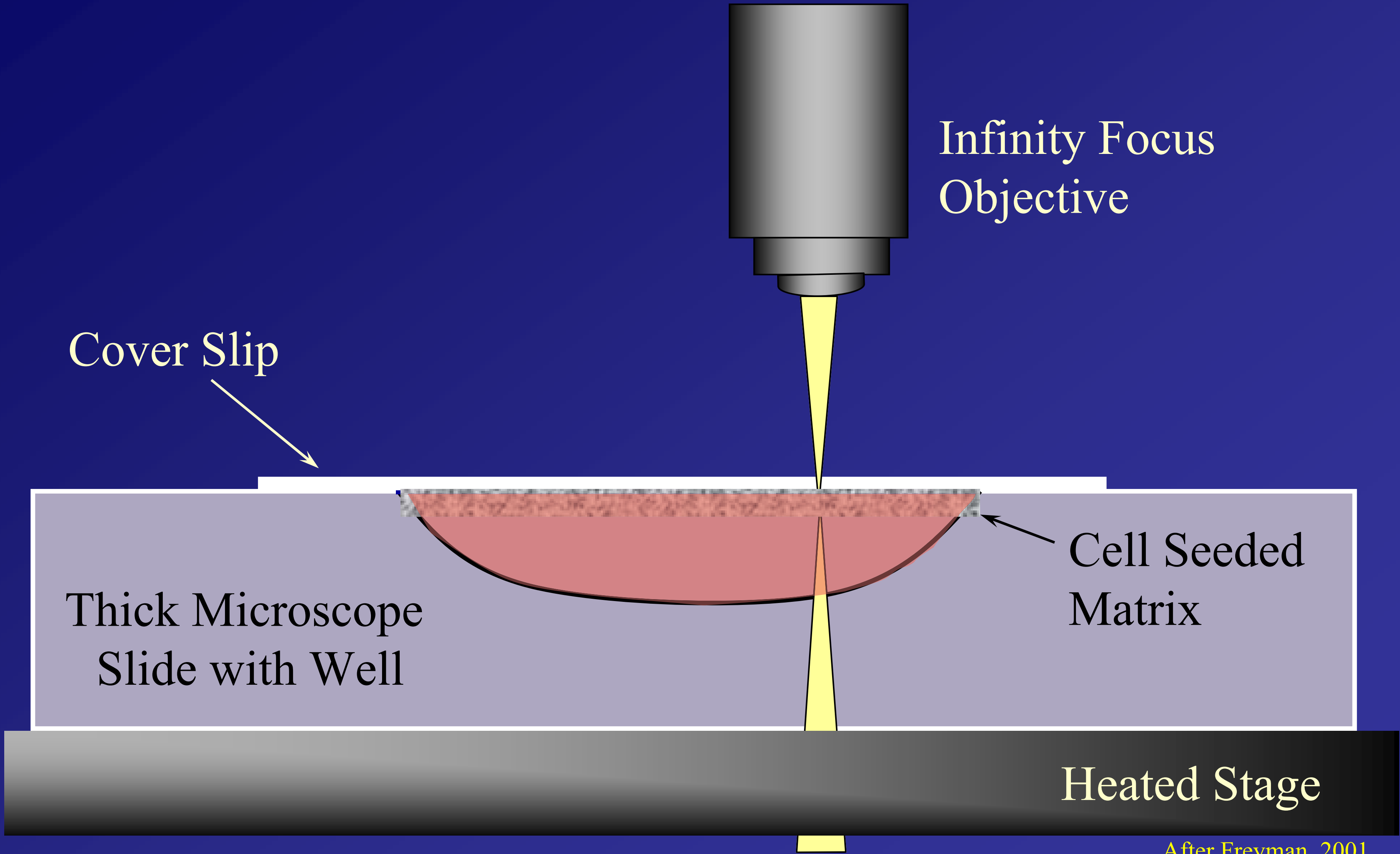
No dependence on mean pore size

Relative density = 0.006
Non-hydrated Collagen modulus:
$(E_s) \sim$ 1GPa

E ~ 36kPa

Agreement between cellular solids model estimated and experimentally measured mechanical properties

Live Cell Imaging



After Freyman, 2001

Live Cell Imaging

Freyman, 2001

Photos removed for copyright reasons.

Fibroblast Morphology in CG Scaffold

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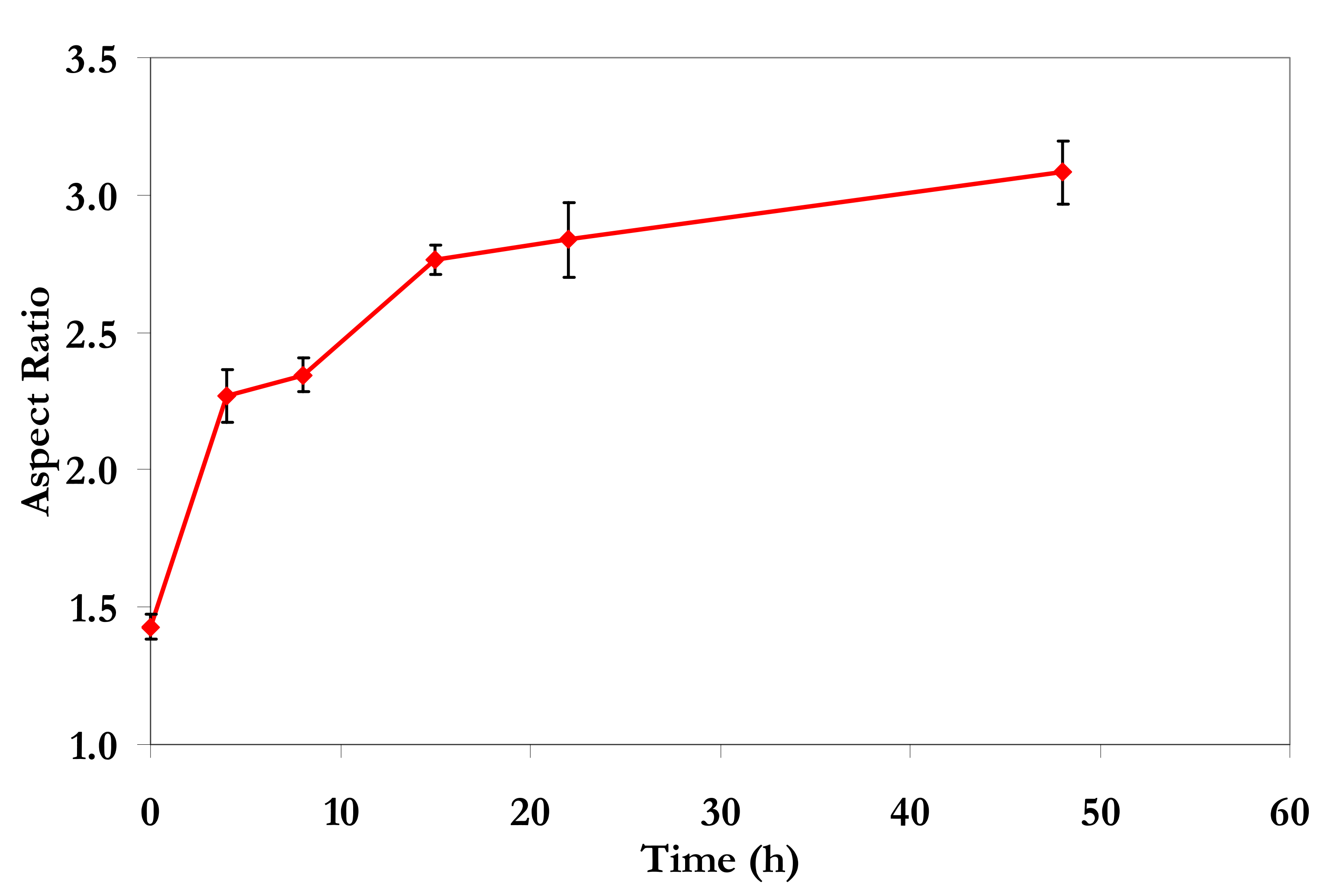
Freyman, 2001

Cell Aspect Ratio:

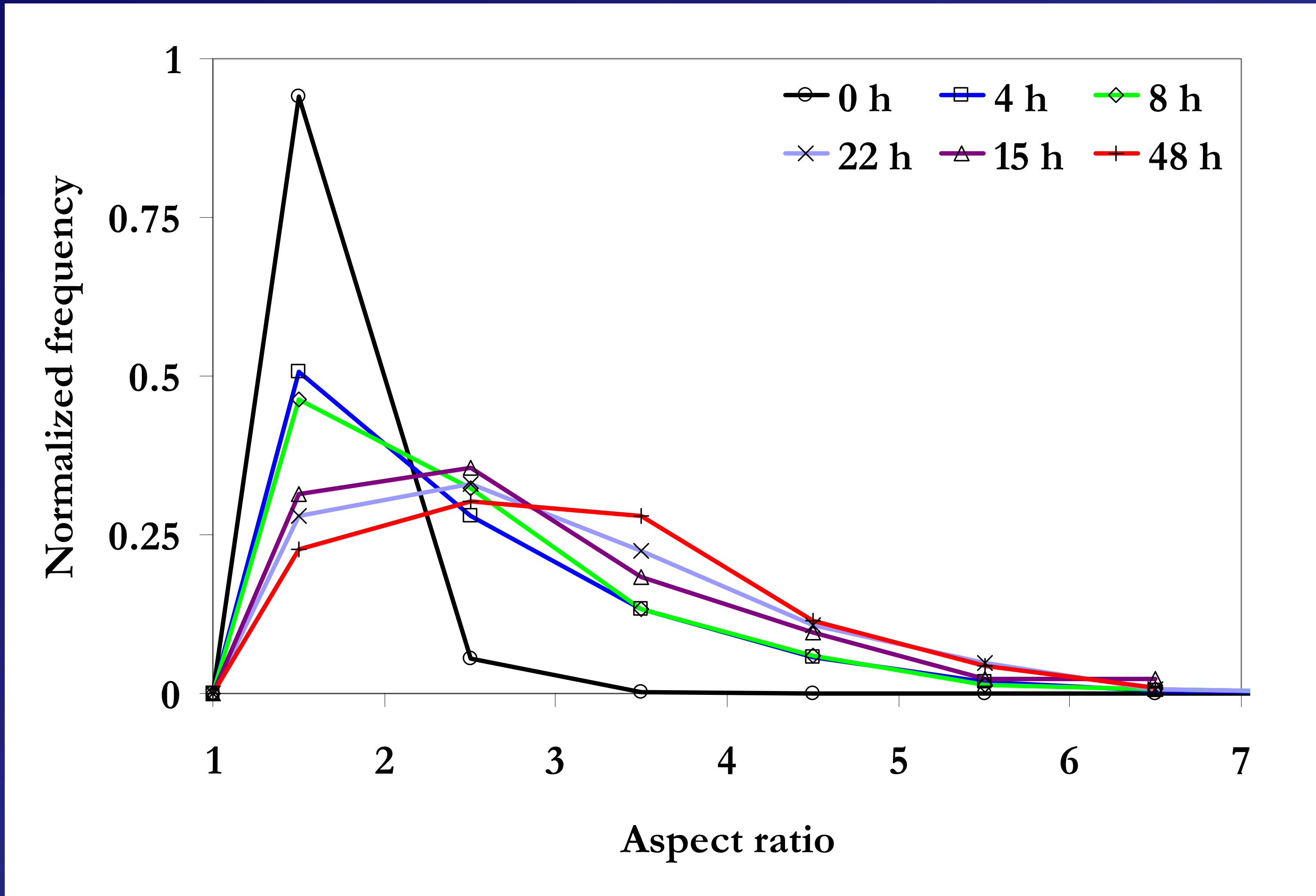
$$AR = \frac{l}{w}$$

Fibroblast Aspect Ratio vs. Time in CG Scaffold



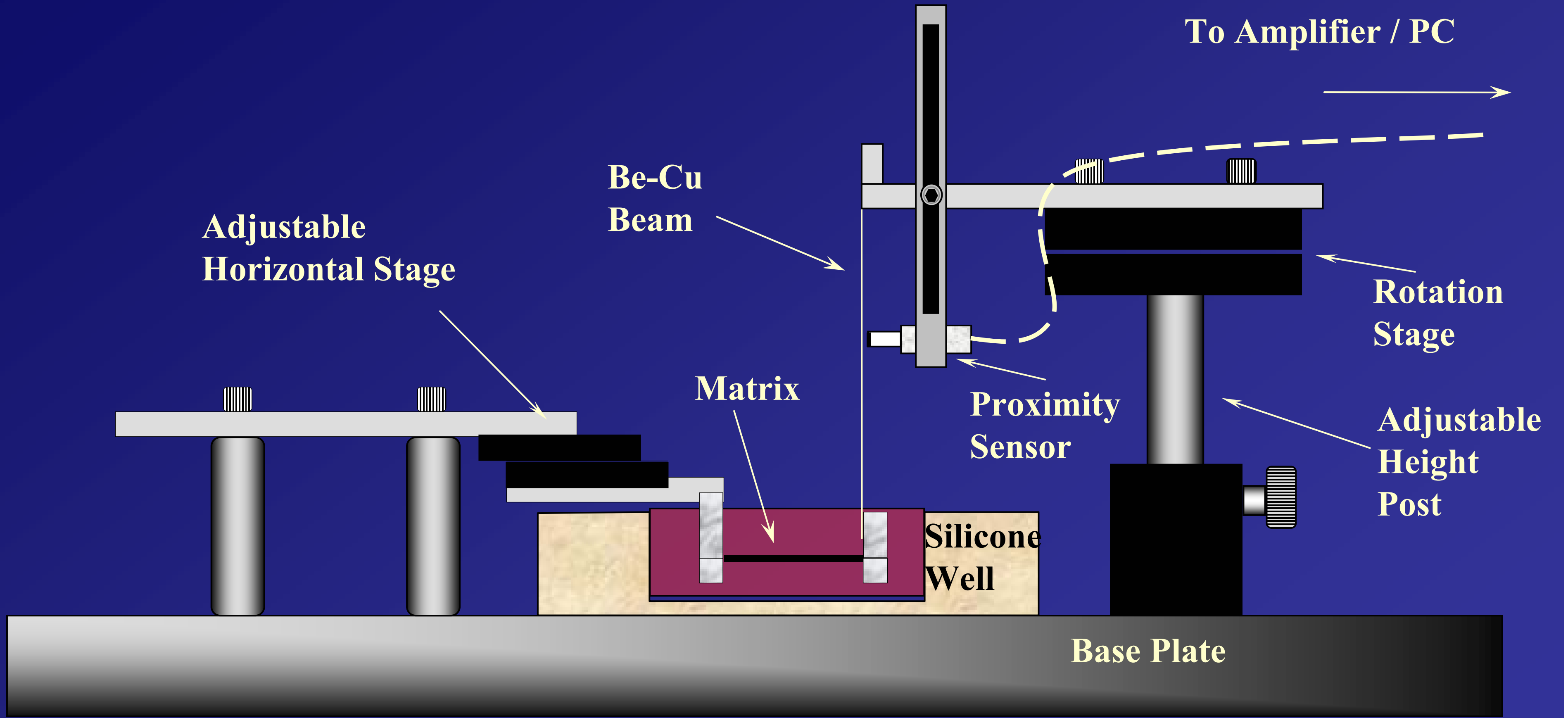
After Freyman, 2001

Fibroblast Aspect Ratio Frequency vs. Time



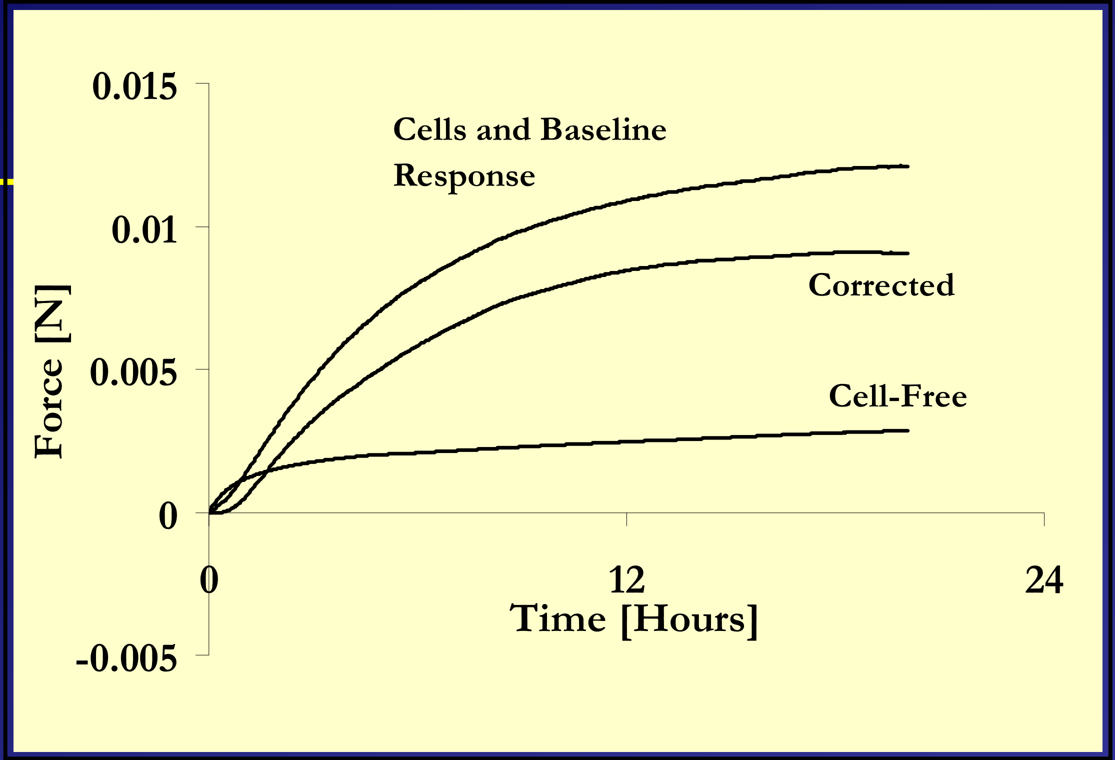
After Freyman, 2001

Cell Force Monitor (CFM)



After Freyman, 2001

Force Calculation



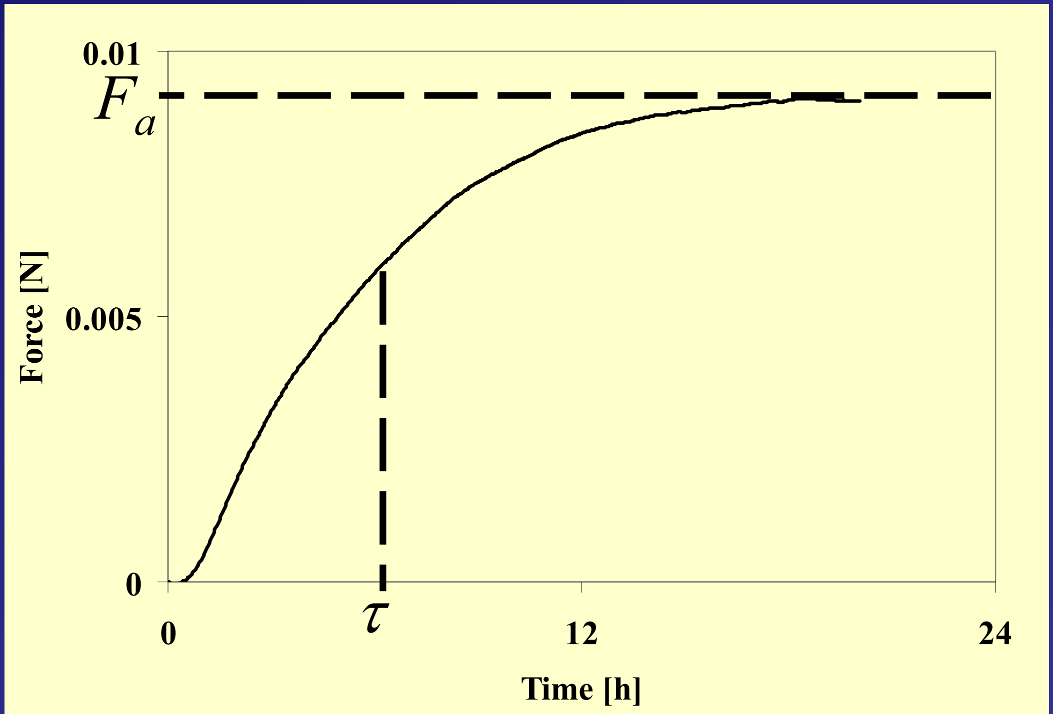
$$F = F_{beam} + F_{matrix} = V \cdot C_{force} + V \cdot C_{displ} \cdot K_{matrix}$$

- Parallel - sum of forces
- Voltage, C_{force}, C_{displ}, *and* K_{matrix}
- Correct for matrix only

Freyman, 2001

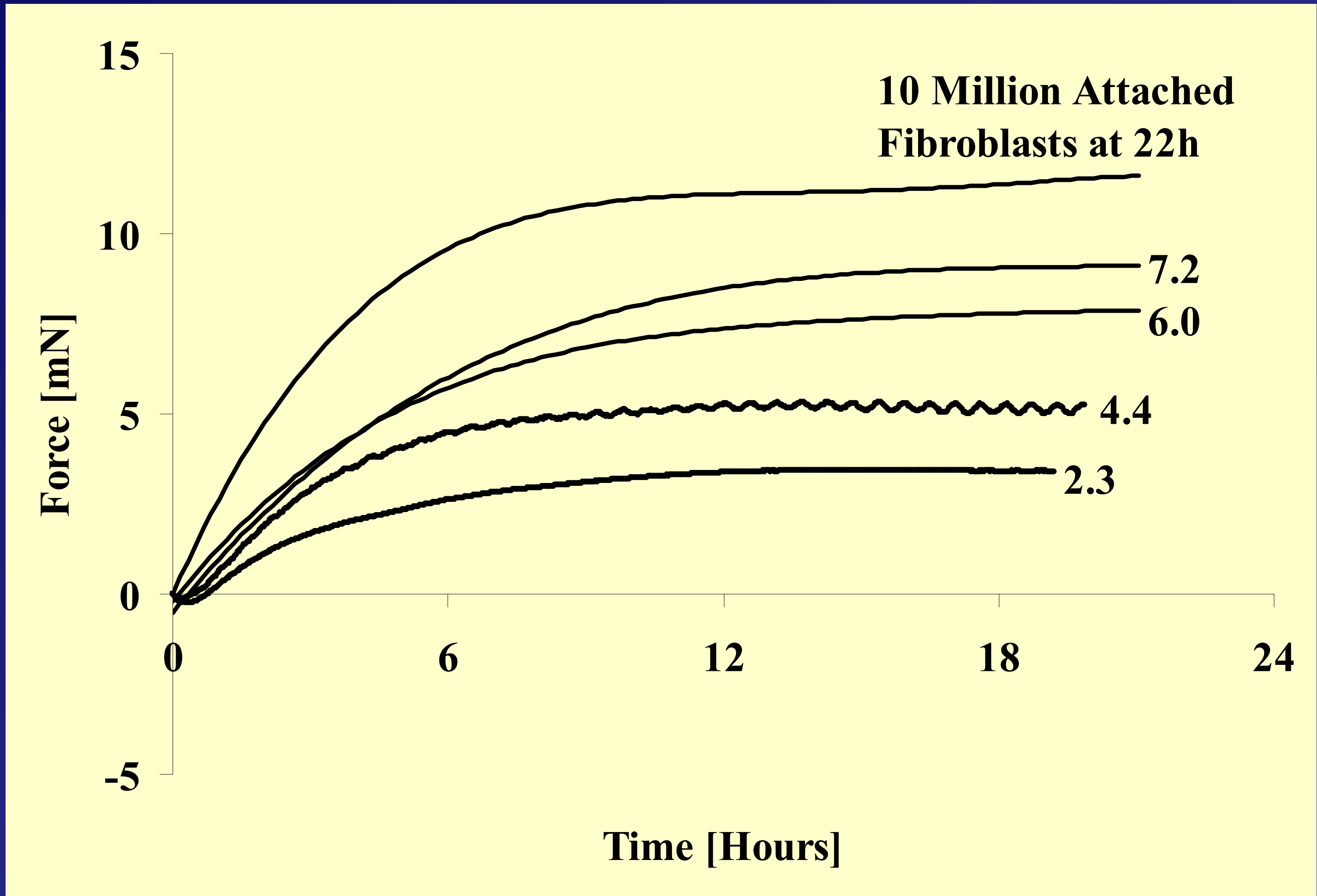
Cell Force Monitor: Interpretation of Data

- Fit data for each sample to: $$F = F_a \cdot \left(1 - e^{-t/\tau}\right)$$
  - Each data set defined by N_c, F_a, and τ



After Freyman, 2001

Effect of Cell Number on Contractile Force



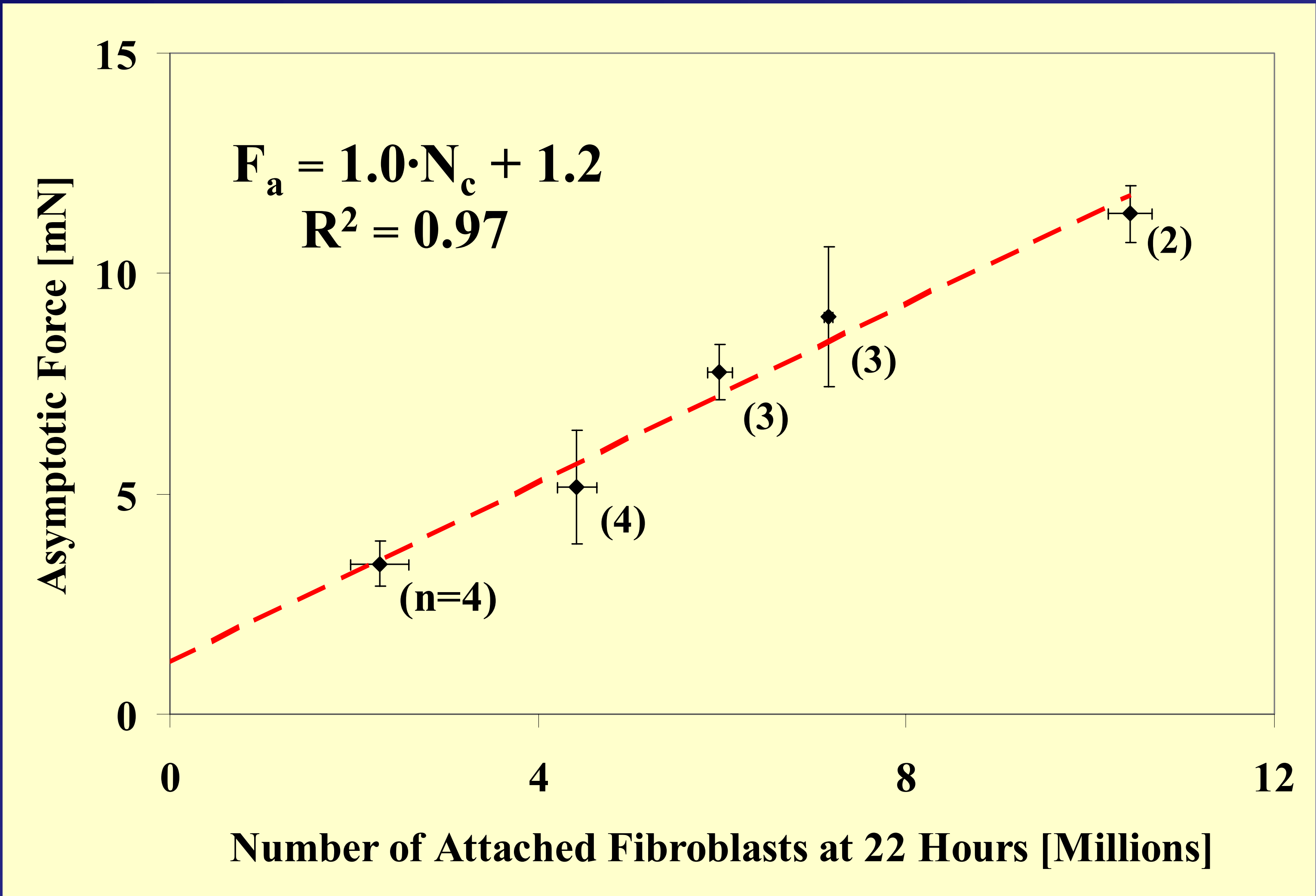
After Freyman, 2001

Effect of Cell Number: Fitting Parameters

Number of Attached Cells, N_c [x 10^6]	Time Constant, τ [hr.]	Asymptotic Force, F_a [mN]
2.3 ± 0.31	5.0 ± 1.3	3.7 ± 0.6
4.4 ± 0.21	4.0 ± 0.5	5.4 ± 1.4
6.0 ± 0.13	5.0 ± 0.4	8.1 ± 0.5
7.2 ± 0.05	7.0 ± 1.5	10.0 ± 1.9
10.0 ± 0.23	4.0 ± 0.5	12.0 ± 0.7

After Freyman, 2001

Asymptotic Force Generation vs. Cell Number



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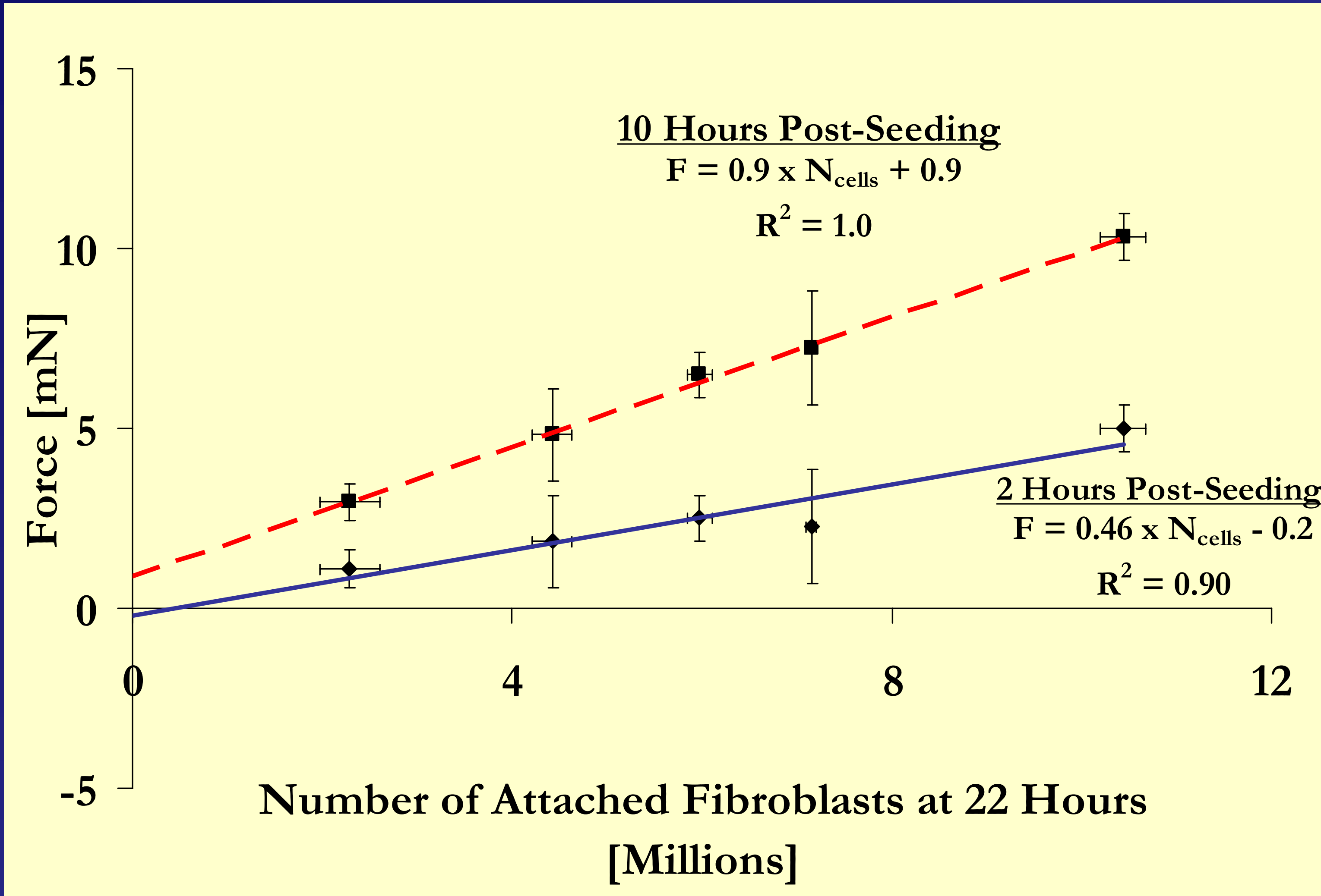
Asyptotic Force Generation vs. Cell Number, Time



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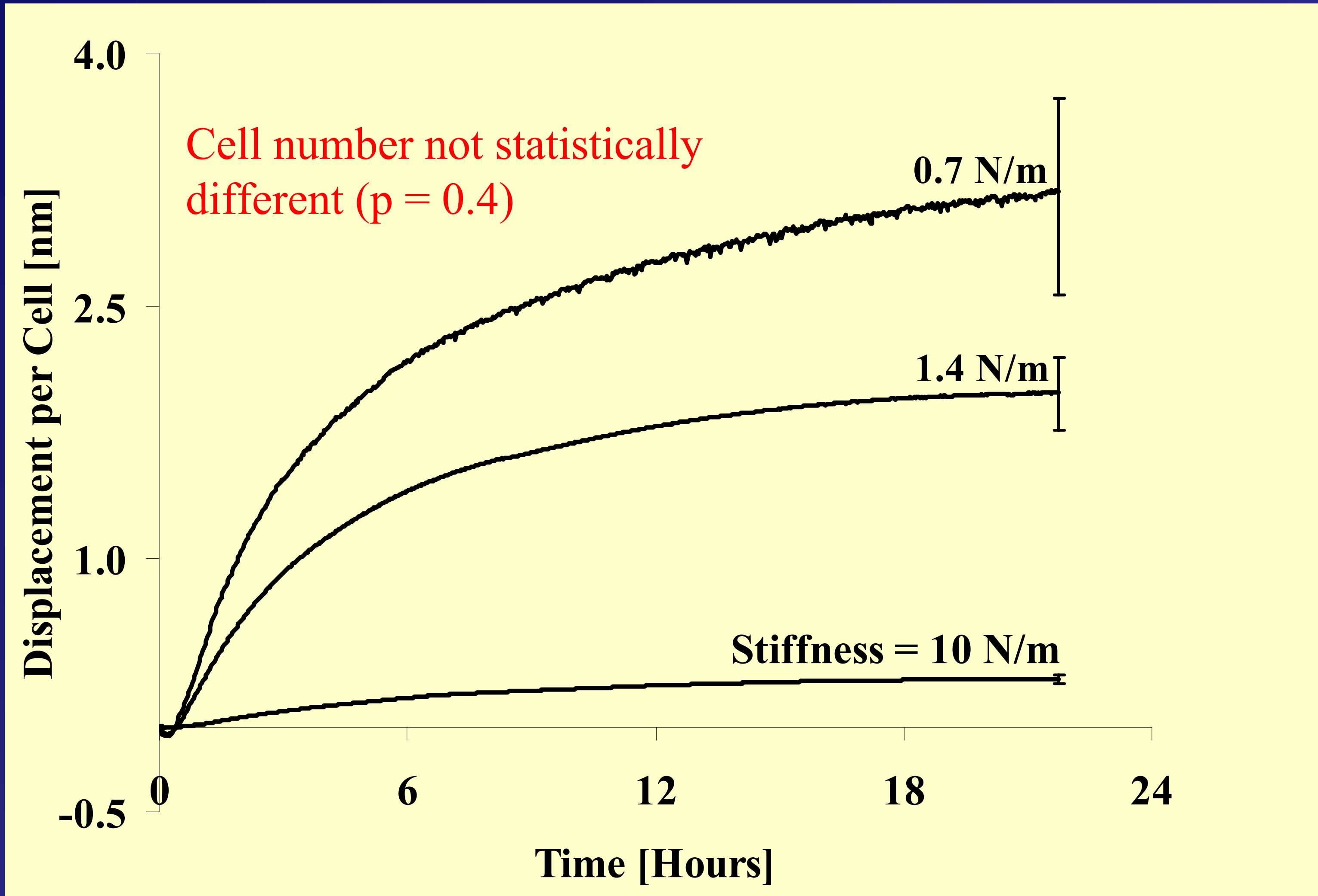
Effect of System Stiffness on Contractile Force



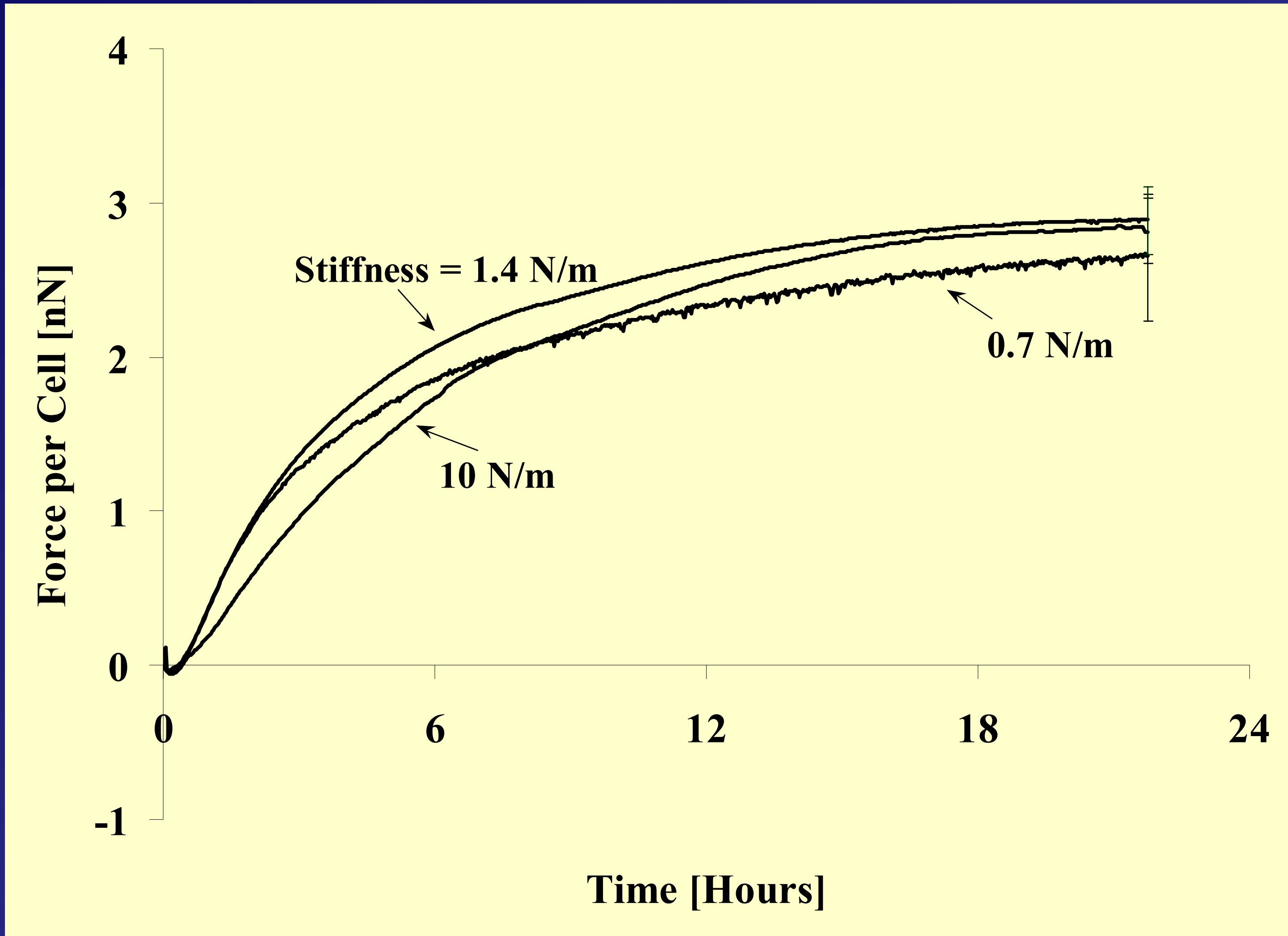
After Freyman, 2001

Effect of System Stiffness: Fitting Parameters

	Total System Stiffness			
Curve Fit Parameters	10 N/m	1.4 N/m	0.7 N/m	Trend
Mean Asymptotic Force per Cell, F_{cell} [nN]	3.2 ± 0.3	2.9 ± 0.2	2.7 ± 0.4	Const.
Mean Asymptotic Displacement per Cell, d_{cell} [nm]	0.32 ± 0.03	2.0 ± 0.2	3.2 ± 0.6	↑
Mean Time Constant, τ [hr.]	7.9 ± 1.3	5.2 ± 0.85	5.1 ± 0.60	Const.
Rate of Contraction per Cell [nm/(hr cell)]	0.04 ± 0.004	0.38 ± 0.04	0.63 ± 0.06	↑

After Freyman, 2001

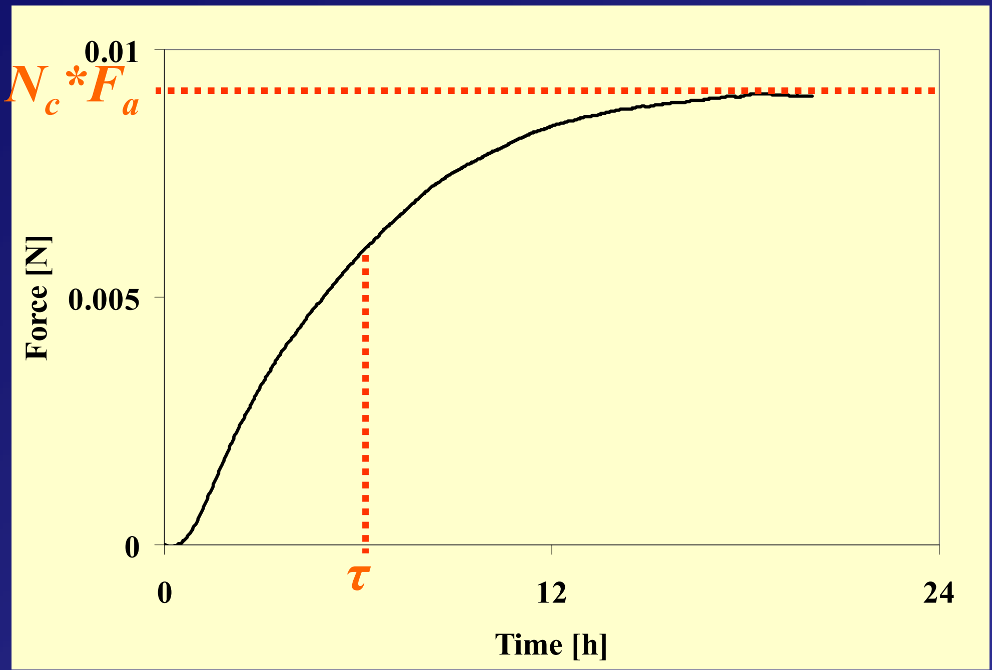
Force Generation vs. System Stiffness



After Freyman, 2001

Cell Population Mechanics: Results Summary

- Cell-generated contractile forces
  - Defined by F_a, N_c, and τ



$$F = F_a \cdot (1 - e^{-t/\tau})$$

$$\Delta \propto (1 - e^{-t/\tau})$$

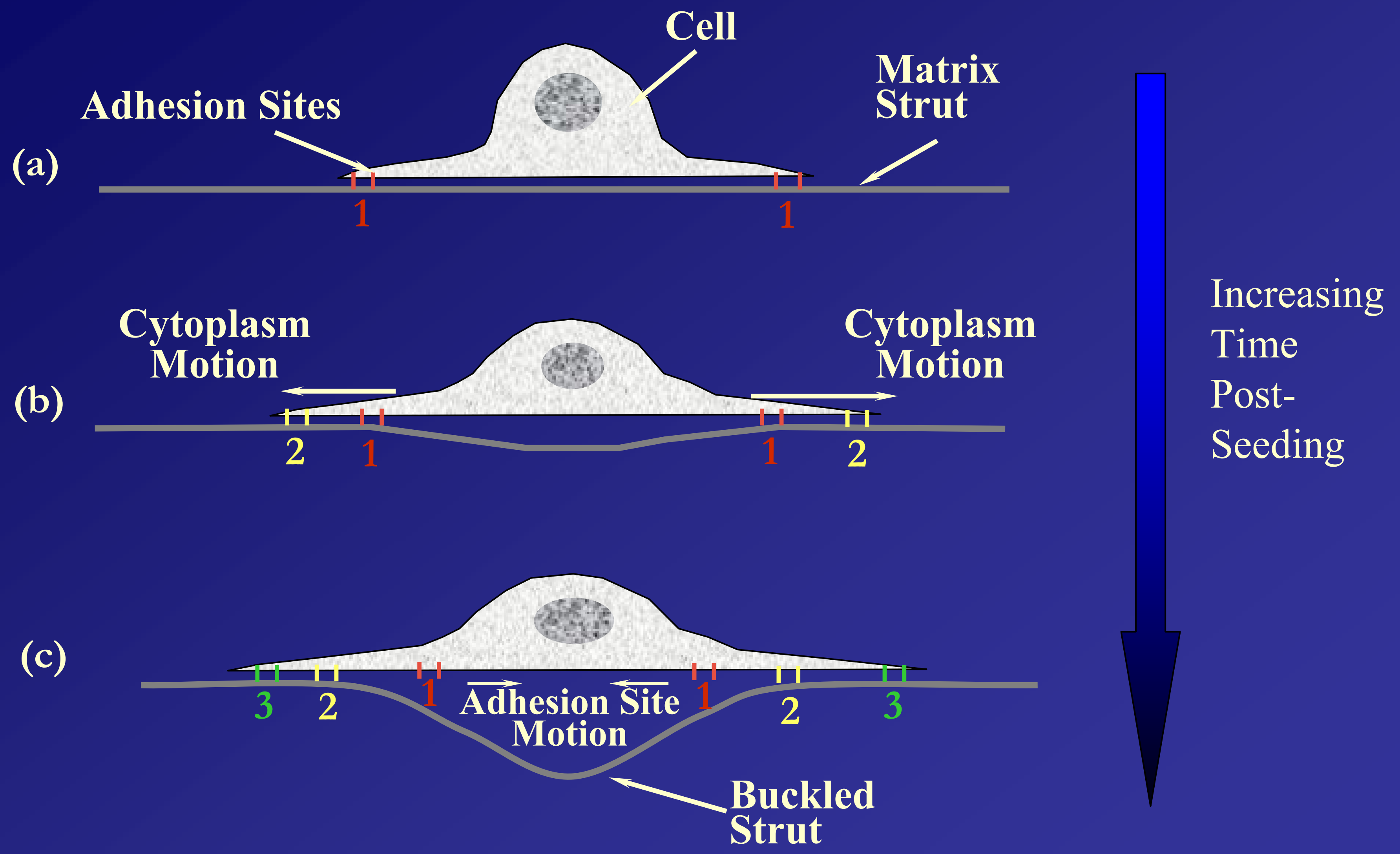
F_a = ~1 nN/cell

Δ = Cell aspect ratio

$\tau = 5.2 \pm 0.5$ hr

After Freyman, 2001

Fibroblast Model: Elongation and Contraction



After Freyman, 2001

CFM: Conclusions

- Force determined at level of individual cells (*i.e.*, not cooperatively)
  - Cell-generating contractile forces independent of cell density (1200 – 5100 cells/mm^3)
- Contraction was limited by the force which developed; not displacement
- Force measured was that required to support cell elongation

CFM: Conclusions

- Cells elongate (spread) on scaffold struts prior to generating contractile forces
  - Time for contraction to develop independent of cell density and system stiffness
- Cell-generated contractile force independent of system stiffness

Conclusions

- Cell-mediated contraction
  - Single cells on 2-D membranes
  - Cell populations in 3-D scaffolds

  - Cells are exquisite mechanotransducers
    - Cells respond to mechanical environment
      - Migration, Traction force, Apoptosis, Cell growth (2-D)
      - Cell spreading, Migration, Contractile Force (3-D)

- CFM Demonstration