Lecture #16: Cell Mechanics
Mechanical Compression

(a) Unconfined compression,
Mechanobiology is Fundamental

• Not restricted to muscle or bone cells

• Most cells can generate force
  – Comes from within the cell
  – Exerts contraction on surrounding ECM and cells

• These forces are essential to cellular function
  – Migration and mitosis
  – Proliferation and differentiation
Mechanotransduction

- Tugging on ECM can deform proteins (e.g. fibronectin) to reveal previously hidden (cryptic) binding sites
  - Release of bound growth factors also possible

- Stretch-activated ion channels
  - Cardiac myocytes

- Integrins
  - Integrin binding and tension cause the formation of focal adhesions, which trigger further intracellular signaling
Cell-Cell: Adherens Junctions
Cell Membrane

- Fluid Flow
- Glycocalyx
- Cilia
- GR
- Ion channel
- Microtubules
Nucleus
Cell-Matrix: Focal Adhesion
Focal Adhesion Complex

Composition of a focal adhesion

- Actin filament
- Vinculin
- Tensin
- Talin
- Integrin
- Src
- Focal adhesion kinase
- a-actinin
- Paxillin
- Extracellular Matrix

Cell Migration Lab, University of Reading
Focal Adhesions

Red: Actin fibers
Green: Focal Adhesion Complexes

Integrins Must be Activated to Bind

(A) inactive integrin
\[ \alpha \text{ subunit} \quad \beta \text{ subunit} \]
OUTSIDE-IN ACTIVATION

(B) strong binding to extracellular matrix

\[ \text{inactive integrin} \quad \text{active integrin} \]

\[ \text{inside-out activation} \]

\[ \text{strong binding to cytoskeleton} \]

Figure 20-15 Essential Cell Biology 3/e (© Garland Science 2010)
Signaling Happens In Both Directions

FA grows in response to external tugging (outside-in)

Internal contraction also results in FA growth and integrin activation (inside-out)

Figure 4.7 Schematic overview of integrin-mediated activation leading to inside-out and inside-out signaling. Integrin engagement leads to PKC activation and autophosphorylation of focal adhesion kinases (FAK), these outside-in signals will among others, result in the activation of the MAPK pathway, which finally leads to cell proliferation and cell spreading. Inside-out signaling occurs when changes within the cell lead to changes in the affinity of the integrin pair for its extracellular target. PKC, Protein kinase-c; FAK, focal adhesion kinases; MAPK, mitogen-activated protein kinase.
Mechanotransduction Directs Differentiation

Matrix Elasticity Directs Stem Cell Lineage Specification

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Figure 1
Pelham and Wang, PNAS, 1997

0.26% Bis-

0.03% Bis-

Pelham and Wang, PNAS, 1997
Figure 2
Figure 3
Figure 5

A. Image showing myosin expression with and without Blebbistatin treatment.

B. Table showing protein expression for different treatments.

C. Graph showing NMM IIB fluorescence normalized to naive MSC as a function of substrate elasticity.

Figure 5
Figure 6
Figure 7

Graph showing the relationship between prestress (σ) and cortical stiffness (κ) with substrate elasticity (E) as the independent variable. The graph includes data for different cell types (hFOB, C2C12, MSC) and treatments (Stiff Matrix, Soft Matrix, Blebbistatin). The graph illustrates a linear increase in both prestress and cortical stiffness with increasing substrate elasticity.
The Protein Tethering Question

Extracellular-matrix tethering regulates stem-cell fate

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Follow-Up

Interplay of matrix stiffness and protein tethering in stem cell differentiation

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Adipose Stromal Cells

Stain For-Osteogenic Differentiation

Stain For-Adipogenic Differentiation
Polyacrylamide Conjugation with ECM, with sulfo-SANPAH

Figure 10.16.4  Schematic of the functionalization procedure for PA hydrogels. (A) The surface becomes activated upon addition of sulfo-SANPAH to the PA hydrogel, a reaction catalyzed with 365-nm UV light. (B) Overnight attachment of your favorite ECM protein in a 50 mM HEPES solution, pH 8.5. (C) Completed functionalization of your favorite ECM protein to the PA hydrogel. (D) Confocal cross-sectional fluorescence image of a 34-kPa PA hydrogel functionalized with rat fibronectin, demonstrating that the ECM protein represents a relatively uniform, thin layer of the PA hydrogel. For the color version of this figure go to http://www.currentprotocols.com/protocol/cb1016.
Figure 2

No Significant Effect of SS Concentration on Differentiation