Lecture #16:
Cell Mechanics
Mechanical Stretching

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
Mechanisms of Mechanotransduction

Cell-Cell: Adherens Junctions
Cell-Matrix: Focal Adhesion
Focal Adhesion Complex

Composition of a focal adhesion

- α-actinin
- Paxillin
- Vinculin
- Tensin
- Talin
- Integrin
- Focal adhesion kinase
- Src
- Actin filament

Cell Migration Lab, University of Reading
Focal Adhesions

Red: Actin fibers
Green: Focal Adhesion Complexes

Cell Attachment to ECM

- Collagen fibril
- Fibronectin
- Plasma membrane
- Integrin dimers
- Adaptor proteins
- Actin filament

Figure 20-14c Essential Cell Biology 3/e (© Garland Science 2010)
Integrins Must be Activated to Bind

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

Adam J. Engler,1,2 Shamik Sen,1,2 H. Lee Sweeney,1 and Dennis E. Discher1,2,3,4,*
1 Pennsylvania Muscle Institute
2 School of Engineering and Applied Science
3 Cell & Molecular Biology Graduate Group
4 Physics Graduate Group
University of Pennsylvania, Philadelphia, PA 19104, USA
*Contact: discher@ssas.upenn.edu
DOI 10.1016/j.cell.2006.06.044
Figure 4

**1 week cultures**

A. MyoD Fluorescence (au) vs. Substrate Elasticity, \( E \) (kPa) for C2C12, MSC + MIM, and MSC with Blebb.

B. CBF\( \alpha \)1 Fluorescence (au) vs. Substrate Elasticity, \( E \) (kPa) for hFOB, MSC + OIM, and MSC.

**Neuro-induced MSCs on 1 kPa matrix in Growth Media (GM)**

D. Media Change: Myo-Induction (MIM) showing changes in MyoD and \( \beta \)3 tubulin.

E. Media Change: Osteo-Induction (OIM) showing changes in \( \beta \)3 tubulin and CBF\( \alpha \)1.

C. Western blot analysis for MyoD1, CBF\( \alpha \)1, and Actin for Control Cells, MSC + Sup., and MSC.

Substrate: Control Cells 1 11 34 GL 1 11 34 GL 1 11 34 GL

<table>
<thead>
<tr>
<th>Substrate</th>
<th>MyoD1</th>
<th>CBF( \alpha )1</th>
<th>Actin</th>
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<td>Control Cells</td>
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<td>MSC + Sup.</td>
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<td>MSC</td>
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The Protein Tethering Question

Extracellular-matrix tethering regulates stem-cell fate

Britta Trappmann¹, Julien E. Gautrot¹,², John T. Connelly²,³, Daniel G. T. Strange⁴, Yuan Li⁵, Michelle L. Oyen⁴, Martien A. Cohen Stuart⁵, Heike Boehm⁶,⁷, Bojun Li⁸, Viola Vogel⁸, Joachim P. Spatz⁶,⁷, Fiona M. Watt²,⁹,* and Wilhelm T. S. Huck¹,¹⁰,*
Follow-Up

Interplay of matrix stiffness and protein tethering in stem cell differentiation

Jessica H. Wen, Ludovic G. Vincent, Alexander Fuhrmann, Yu Suk Choi, Kolin C. Hribar, Hermes Taylor-Weiner, Shaochen Chen and Adam J. Engler

PUBLISHED ONLINE: 10 AUGUST 2014 | DOI: 10.1038/NMAT4051
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