Identification and Modulation of Biophysical Signals that Control Stem Cell Function and Fate

David Schaffer, Ph.D.

Professor, Chemical and Biomolecular Engineering, Bioengineering, & the Helen Wills Neuroscience Institute
Director, Berkeley Stem Cell Center
University of California at Berkeley
United States Healthcare Budget

U.S. Health Care Expenditures as a % of GDP

Centers for Medicare & Medicaid Services, Office of the Actuary
Chronic Health Conditions

Alzheimer’s
Parkinson’s
Stroke

Spinal injury

Obesity

Macular degeneration
Glaucoma

Cardiovascular disease

Diabetes

Cancer

Alzheimer’s Disease: 5 million (in US)
Parkinson’s Disease: 1.5 million
Heart Disease: 27 million
Diabetes: 19 million
Historical Timeline in Molecular Medicine Development

1900 1920 1940 1960 1980 2000

- Small Molecules
- Protein Therapies
- Gene Therapy
- Stem Cells
A Stem Cell is ...

Two defining properties of stem cells:
1) self-renewal
2) differentiation
Challenges with Stem Cell Therapeutics

Functional integration into tissue
Low viability during engraftment
Functional validation of cells
Difficult engineering into functional tissues
Complex and lengthy cell differentiation
  • Yield heterogeneous cultures
  • Contaminating cells pose risks
Cell stability during expansion and differentiation
Poorly controlled culture systems with animal and human-derived proteins
  • Immunogenicity and pathogen transfer
Reproducibility
Low efficiency and reproducibility of stem cell isolation and derivation
Stem Cells

Must learn how to control self-renewal and differentiation.
Division and differentiation are hardwired during development – 959 cells
Intracellular signaling and genetic networks "compute" microenvironmental information and make decisions.

Stem Cells

Must learn how to control mammalian stem cell self-renewal and differentiation.

Ligands
Receptors
Signaling Networks
Genetic Networks
Stem Cell Microenvironment

Signal Inputs

Growth Factors & Morphogens

Extracellular Matrix

Small Molecules

Other Cells

Complex input signals interact to regulate stem cell function

Cell Behavior Outputs
Stem Cell Microenvironment is a Structurally Complex Region

“Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain” Mirzadeh et al., Cell Stem Cell (2009)
Microenvironment Exposes Cells to Many Biochemical and Biophysical Cues

Growth Factors & Morphogens: Spatial Organization, Transport

Extracellular Matrix: Mechanical Properties, Topographical Properties, Spatial Organization

Small Molecules: Transport, Electrostatics

Other Cells: Spatial Organization of Ligands

Shear Flow, Spatiotemporal Variation

Much complexity resides in the solid phase.
Study of Biophysical Properties Poses Many Challenges

- Physical properties are not encoded by single genes in a straightforward manner
  - Tissue mechanical properties depend on constituent extracellular matrix and cells
  - Shear rate is property of circulatory system, local geometry, etc.
  - Macromolecular transport – i.e. diffusion and convection – varies with tissue composition, interstitial space, fluid flow field
  - Topography is due to the identities of matrix proteins and polymers, as well as the history of their assembly

- These properties …
  - Are difficult to manipulate genetically
  - Are challenging to vary and study in vitro and in vivo.

Need novel materials systems to systematically investigate these properties, i.e. “analysis by synthesis”
Microenvironment Exposes Cells to Many Biochemical and Biophysical Cues

Growth Factors & Morphogens:
- Spatial Organization, Transport

Extracellular Matrix:
- Mechanical Properties,
- Topographical Properties,
- Spatial Organization

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Role of mechanoregulation is increasingly appreciated
Range of Cell Substrate Elasticity

Does $E$ affect cell self-renewal and/or 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@seas.upenn.edu
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SUMMARY

Microenvironments appear important in stem cell lineage specification but can be difficult to adequately characterize or control with soft tissues. Naive mesenchymal stem cells (MSCs) are shown here to specify lineage and commit to phenotypes with extreme sensitivity to tissue-level elasticity. Soft matrices that mimic brain are neurogenic, stiffer matrices that mimic muscle are myogenic, and comparatively rigid matrices that mimic collagenous bone prove osteogenic. During the initial week in culture, reprogramming of these lineages is possible, differentiate into various anchorage-dependent cell types, including neurons, myoblasts, and osteoblasts (respectively, [Deng et al., 2005; Hofstetter et al., 2002; Kondo et al., 2005], [Pittenger et al., 1999], and [McBeath et al., 2004; Pittenger et al., 1999]). For differentiated cells such as fibroblasts, it is well known that responses to the typical soluble inducers such as growth factors couple to matrix anchorage (Nakagawa et al., 1989). However, with naive stem cells, direct effects of matrix physical attributes such as matrix stiffness have yet to be examined.

Differentiated cells ranging from neurons to osteoblasts adhere, contract, and crawl not only within soft tissues such as that of the brain or on top of crosslinked collagen “osteoids” in remodeling bone but also in vitro on collagen-coated coverslides and glass (Figure 1A). Such
Matrix Elasticity Directs Stem Cell Lineage Specification

D
tissues have different stiffnesses, and the stiffness of a given tissue may serve as an instructive cue for stem cells to differentiate into cells of that tissue. Demonstrated the concept with mesenchymal stem cells - Engler et al., Cell (2006)
1) Play a role in learning and memory
2) Hippocampus affected by Alzheimer’s Disease
Adult Neural Stem Cells are Multipotent

Neurons  Astrocytes  Oligodendrocytes

Blue = nuclei
Hydrogel Polymer Networks

Tunable Mechanical Properties

Mixed Differentiation Conditions

13.5 Pa

13.5 Pa

97.8 Pa

431 Pa

902 Pa

5460 Pa

9580 Pa

PS

13.5 Pa

13.5 Pa

97.8 Pa

431 Pa

902 Pa

5460 Pa

9580 Pa

PS

β-tubIII+

GFAP
Mixed Differentiation Conditions

Saha et al. (2008)
Molecular Mechanisms of NSC Mechanoregulation

Matrix Stiffness

Cdc42

RhoA

ROCK

MLC

P-MLC

MLCK

Myosin Phosphatase

Cellular Stiffness

Differentiation

MLC

Differentiation

RhoA

ROCK
Mechanosensitive Signaling Regulates Neuronal Differentiation in the Adult Brain

Keung et al. (2011)
Human Embryonic Stem Cells

- Cleavage Stage Embryo
- Cultured Blastocyst
- Inner Cell Mass
- hESC Culture

Terese Winslow (2001)
Dopamine Neuron Differentiation from Human Embryonic Stem Cells

SMAD Inhibition

Neuronal Patterning (Shh, FGF-8) and Maturation (GDNF) Factors

Day 0 - 5

Day 5 - 19

Stiffness “Pulse”
Stiffness Modulates Neural Differentiation of Human Embryonic Stem Cells

Soft materials lead to higher differentiation into dopamine (TH+) neurons

700 Pa

75 kPa
Microenvironment Exposes Cells to Many Biochemical and Biophysical Cues

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Shear Flow, Spatiotemporal Variation

nm-μm scale features of microenvironment impact cell fate
Shape and Topographical Regulation of Stem Cell Function

Dorothee et al. (2011)
Does Substrate Roughness at Different Size Scales Impact Fate Decisions?

Flat control surface

7.5 μm pattern
Surface Topography Can Regulate NSC Fate Decisions

% Neuron
% Astrocyte

Pattern size (μm)

Flat control surface
Mesenchymal stem cell shape controls differentiation into bone (osteoblasts) vs. fat (adipocytes) cell fate.

Future Opportunities and Challenges for Melding Chemistry and Engineering to Study Stem Cell Biology

- Investigating the mechanistic roles of biophysical aspects of the niche in regulating cell function

- Developing new technologies and materials to systematically investigate these properties, i.e. “analysis by synthesis”
Stem Cell Microenvironment is Dynamic
Spatiotemporal Control Over Material Properties Using Light

Kloxin et al., *Science* (2009)
Future Opportunities and Challenges for Melding Chemistry and Engineering to Study Stem Cell Biology

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- Developing new technologies and materials to systematically investigate these properties, i.e. “analysis by synthesis”

- Further engineering materials to aid in translation, including cell culture applications and clinical cell replacement therapies
Questions?