Lecture #9
Mechanical Forces

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http://courses.engr.illinois.edu/bioe498gu/
Stiffness of Adult Tissues

ExCellness culture surfaces

Conventional plastic culture
Bulk Tissue Measurements

ElectroForce® 3100 Test Instrument

Performance and Durability in a Compact Package

Measuring less than 20 inches tall, the ElectroForce® 3100 test instrument is the smallest in the ElectroForce product family. Like all ElectroForce test instruments, the 3100 is extremely lab friendly thanks to its practically maintenance-free operation. With its compact size, whisper-quiet operation and energy-efficient design, the 3100 test instrument will fit on any tabletop and can be plugged into a standard wall outlet. Due to its exceptional control resolution, the 3100 test instrument is well-suited for:

- Tissue mechanics research
- Micro-indentation of cartilage and soft tissue
- Mechanical stimulation of tissue-engineered constructs
- Individual fiber testing
- BioMEMS evaluation and testing
- Durability testing of medical devices
- Dynamic Mechanical Analysis (DMA)

Bose® ElectroForce Linear Motor

The ElectroForce 3100 instrument utilizes the performance and resolution of the ElectroForce linear motor. The proprietary motor utilizes a simple and durable moving-magnet design to achieve the proper performance for many low-force applications.

WinTest® Controls

WinTest® controls set the standard for dynamic mechanical fatigue testing. WinTest software provides an intuitive interface that enables the user to efficiently set up tests. The software features a fully integrated display that simplifies test operation while providing advanced test capabilities. Data acquisition, waveform generation and instrument control are all provided within this comprehensive package.

Example of Minimum Displacement and Force Control with the ElectroForce® 3100 Test Instrument

NOTE: Tests conducted with 50 g force and ±0.1 m displacement transducers to show system capability. These transducers are not included in the standard system configuration.
Biophysical Signals in the Microenvironment

Sun et al., Ann Rev Biophys, 2012
Biophysical Signals in the Microenvironment

![Diagram of cell microenvironment](image)
Mesenchymal Stem Cell Differentiation Substrates with Different Mechanical Stiffness (polyacrylamide)

Engler et al., Cell, 2006
Sun et al., Ann Rev Biophys, 2012
Substrate Rigidity

**a. Cell microenvironment**
- Soluble factors
- Surface receptors
- Cytoskeleton
- Nucleus
- Matrix rigidity
- Adhesive molecules
- Stretch force

**b. Micromechanical regulation of cell function**
1. Mechanical signal: substrate rigidity

**Images:**
- Rigid substrate
- Soft substrate

**C. Synthetic microenvironment**

Fu lab, U. Mich
In-Class Exercise
F = force, E= elastic modulus (of material), I = moment of inertia (function of width, thickness), L= height, delta = displacement

\[ F = \left( \frac{3EI}{L^3} \right) \delta \]

K (spring constant)
Mechanical Forces In Vivo?
Oil Droplets as Force Sensors

Campas et al., Nat Methods, 2014
Oil Droplets as Force Sensors - Cell Aggregates

**a**
3D cells-droplets aggregate

**b**
RGD-functionalized Mesenchymal cells

**c**
Anisotropic normal stress $\delta \sigma_{nn} \ (nN \mu m^{-2})$

**d**
E-cadherin-functionalized Epithelial cells

**e**
Anisotropic normal stress $\delta \sigma_{nn} \ (nN \mu m^{-2})$
Oil Droplets as Force Sensors - Cell Aggregates

Blebbistatin: Blocks nonmuscle myosin

Cyto D: Blocks actin polymerization

Campas et al., Nat Methods, 2014
Oil Droplets as Force Sensors - In Vivo Tissue
Substrate Rigidity

a. Cell microenvironment
- Soluble factors
- Surface receptors
- Cytoskeleton
- Nucleus
- Matrix rigidity
- Adhesive molecules
- Stretch force

b. Micromechanical regulation of cell function
1. Mechanical signal: substrate rigidity

Rigid substrate

Soft substrate

C. Synthetic microenvironment
Substrate Rigidity + External Force

a. Cell microenvironment

- Soluble factors
- Surface receptors
- Cytoskeleton
- Nucleus
- Matrix rigidity
- Adhesive molecules
- Stretch force
- Shear stress
- Focal adhesion

b. Micromechanical regulation of cell function

1. Mechanical signal: substrate rigidity
   - Rigid substrate
   - Soft substrate

2. Mechanical signal: cell stretch force
   - Membrane stretch


C. Synthetic microenvironment
Examples of Applied Stress: Shear Stress

Purple: endothelial cell marker, Brown: smooth muscle cell marker
Examples of Applied Stress: Cyclic (Bead)

Sun et al., Ann Rev Biophys, 2012
Mouse ES Cells Softer Than Differentiated Cells

Chowdhury et al., Nat Mat, 2010

Micropatterned Differentiated Cells: To Define Cell Shape
Stress-Induced Differentiation

![Image of cell differentiation over time](BF_0h_1h_24h_48h_Oct3_4_CAGGS)

![Graph showing Oct3/4 expression over time](Time_0_12_24_36_48_60_72h_Oct3_4_expression_PERCENT_CONTROL)

Chowdhury et al., Nat Mat, 2010
Biophysical Signals in the Microenvironment

Sun et al., Ann Rev Biophys, 2012
Substrate Elasticity Regulates Skeletal Muscle Stem Cell Self-Renewal in Culture


Stem cells that naturally reside in adult tissues, such as muscle stem cells (MuSCs), exhibit robust regenerative capacity in vivo that is rapidly lost in culture. Using a bioengineered substrate to recapitulate key biophysical and biochemical niche features in conjunction with a highly automated single-cell tracking algorithm, we show that substrate elasticity is a potent regulator of MuSC fate in culture. Unlike MuSCs on rigid plastic dishes (~10^6 kilopascals), MuSCs cultured on soft hydrogel substrates that mimic the elasticity of muscle (12 kilopascals) self-renew in vitro and contribute extensively to muscle regeneration when subsequently transplanted into mice and assayed histologically and quantitatively by noninvasive bioluminescence imaging. Our studies provide novel evidence that by recapitulating physiological tissue rigidity, propagation of adult muscle stem cells is possible, enabling future cell-based therapies for muscle-wasting diseases.