An Integrated, Multidisciplinary Approach to Tissue Development and Engineering

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Tissue Development Occurs in the Embryo

Cell Proliferation

- Zygote
- Cleavage
- Eight-cell stage
- Blastula (hollow ball)

Cell Movement

- Blastocoelem
- Gastrulation
- Archenteron
- Endoderm
- Blastopore

Germ Layers

- Muscle and connective tissue (mostly from mesoderm)
- Nervous tissue (from ectoderm)

Key:

- Blue = Ectoderm
- Red = Mesoderm
- Yellow = Endoderm

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http://www.bio.miami.edu/dana/106/106F05_4.html
Tissue Development Also Occurs in Adults

Wound Repair

Diagram showing the process of wound repair, including stages such as fibrin clot, granulation tissue, and collagen formation, with labels for different components and processes involved in the healing process.
Why do we care to study tissue development if Mother Nature has already taken care of it?
We care about understanding and regulating tissue development because we often suffer from conditions where....

Tissue formation is *impaired*  
Chronic Ulcer

Tissue formation is *unchecked*  
Tumor

Thus, there is a huge interest in developing strategies that can enhance or suppress new tissue formation.
My Personal Interest in Tissue Development

- High School Education: Disliked Biology 1984-1996
- Freshman-Junior years in Chemical Eng 1997-2000
- Senior Undergrad in Chemical Eng Spring 2001
- Doctoral Studies in Biomedical Eng 2001-2006
- Postdoctoral Fellowship in Vascular Biology 2006-2011
- Independent Research Lab in Bioengineering 2011-

Dream Job

Growth of Biomedical Engineering & Tissue Engineering
Biomedical Engineering Defined

Biomedical engineering is a dynamic and exciting field in which engineers, scientists and clinicians collaborate to address major scientific questions in human function and pathology and develop novel technologies to improve human health.

*Duke University*

Biomedical engineers bridge the medical and engineering disciplines providing an overall enhancement of health care.

*Biomedical Engineering Society (BMES)*

Tissue Engineering Defined

Tissue engineering is a multidisciplinary field involving biology, medicine, and engineering that is likely to revolutionize the ways we improve the health and quality of life for millions of people worldwide by restoring, maintaining, or enhancing tissue and organ function.

*National Institute of Health (NIH)*
Multidisciplinary Principles Involved in Tissue Engineering

PHYSICS

CHEMISTRY

MATH / COMPUTER SCI.

BIOLOGY / PHYSIOLOGY / MEDICINE

Engineering
Multidisciplinary Principles Involved in Tissue Engineering

**PHYSICS**

**CHEMISTRY**

**MATH / COMPUTER SCI.**

**BIOLOGY / PHYSIOLOGY / MEDICINE**

Tissue Development

![Diagram of tissue development process]
Multidisciplinary Approach for Regulating Tissue Development

Application of the Principles of:

Chemistry: *Biomaterials*

Physics: *Mechanical Forces*
Multidisciplinary Approach for Regulating Tissue Development

Application of the Principles of:

Chemistry: *Biomaterials*

Physics: *Mechanical Forces*
Primary Function:

- To exhibit suitable physicochemical properties so as to ensure long-term stability in the host - *BioPassive*

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**Contemporary Definition of Biomaterial**
Nonviable material used in a medical device, intended to “interact” with biological systems

**Design Criterion:**

- *Biointeractive* materials must closely mimic extracellular matrix (ECM)
ECM provides “instructive” cues to regulate cell behavior and tissue development

- Cells actively bind to ECM molecules via their surface receptors (e.g. Integrins)
- Cell-ECM adhesive interaction triggers downstream biochemical signaling that regulates cell behavior
Evolution of Biointeractive Biomaterials

Design Criteria: must mimic both the “structural” and “instructive” cues of natural ECM

Naturally-occurring biointeractive materials:

- Collagen
- Hyaluronic acid
- Fibrin
- Chitosan (found in crab and shrimp shells)
- Alginate (extracted from seaweeds)
- Co-polymers

Adv: Very biointeractive/biocompatible

Disadv: Lack mechanical strength
Evolution of Biointeractive Biomaterials

Synthetically-produced biomaterials (using principles of organic chemistry):

- Polyesters e.g. poly-L-lactic acid (L-PLA), poly-D-lactic acid (D-PLA), poly(glycolide) (PGA), poly(lactide-co-glycolide) (PLGA)
- Polyanhydrides
- Polypeptides
- Poly(vinyl alcohol) (PVA)
- Pluronics
- Poly(ethylene oxide) (PEO)
- Co-polymers

**Adv:** Excellent physicochemical properties

**Disadv:** Biologically inert
Biomaterial Optimization through Chemical Modifications

X-Linking “natural” biomaterials
to improve strength:

Rendering “synthetic” biomaterials
*cell-adhesive*:
1: ‘In situ’ cell delivery\textsuperscript{A} or development of bioengineered tissue ex vivo\textsuperscript{B}
Apligraf® - Tissue Engineered Skin Graft

**Limitations**

1. Stringent quality control owing to the presence of living cells
2. Large-scale production is slow and expensive
2: Injectable biomaterials for *in situ* tissue formation

Injectable, Biointeractive material

Epidermal Cell
Stromal Fibroblast
Blood Vessel
Native ECM
Injectable biomaterials for \textit{in situ} tissue formation

- Injectable, Biointeractive material
- Epidermal Cell
- Stromal Fibroblast
- Blood Vessel
- Native ECM
Biomaterials for Tissue Repair and Engineering

2: Injectable biomaterials for *in situ* tissue formation

- Injectable, Biointeractive material
- Epidermal Cell
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Biomaterials for Tissue Repair and Engineering

2: Injectable biomaterials for *in situ* tissue formation

- Injectable, Biointeractive material
- Epidermal Cell
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- Blood Vessel
- Native ECM
Chemical Modification of HA to Facilitate In Situ Crosslinking

HA (hyaluronic acid) is a naturally-occurring biomaterial.

Free thiol on HA serves 2 key roles:
1. Allows intramolecular X-linking of HA to itself
2. Allows the tethering of cell-adhesive ligands to HA

Molecular structure of repeat unit showing a free -SH group on HA
Biomacromolecules. 2002:3, 1304-1311
In Situ Crosslinkable HA Hydrogel

HA-SH + PEG crosslinker

pH 7.4 / RT

~ 5 min

0 min

1 min

3 min

5 min

Suitable for injectable use

Cell-adhesive Ligand

Bioactive HA Hydrogels Significantly Accentuate Porcine Wound Healing

- HA hydrogels on the market for animal wound care
- In Clinical Trials for humans

Control Wounds

Wounds + Hydrogels

Area between arrows and dotted line indicates the area covered by newly-formed tissue
Array of 16 different chemically-modified biomaterials →

Single-cell sort transgenic GFP⁺ hES cells
Seed-sorted cells onto diverse polymer array and culture for 7 days

Stain for hES cell-colony growth and quantify cellular response by high-content imaging

Map out the relationship between hES cell expansion and materials properties

Charaterize each polymer using high-throughput methods

Bone Marrow is the Endogenous Source of Adult Regenerative Stem Cells
“Smart” Biomaterial Scaffolds for *In Situ* Tissue Regeneration

**Conductive** and **Inductive** Scaffolds to **Recruit** and **Activate** Endogenous Bone Marrow-derived Stem Cells

- Soluble factors for *recruiting* bone marrow stem cells (*conductive factors*)
- Soluble factors for *activation* of bone marrow stem cells (*inductive factors*)
“Smart” Biomaterial Scaffolds for *In Situ* Tissue Regeneration

**Conductive** and **Inductive** Scaffolds to **Recruit** and **Activate** Endogenous Bone Marrow-derived Stem Cells

- Soluble factors for **recruiting** bone marrow stem cells (*conductive factors*)
- Soluble factors for **activation** of bone marrow stem cells (*inductive factors*)

Conductive factors released from injected scaffold
“Smart” Biomaterial Scaffolds for *In Situ* Tissue Regeneration

Conductive and Inductive Scaffolds to Recruit and Activate Endogenous Bone Marrow-derived Stem Cells

Soluble factors for recruiting bone marrow stem cells (*conductive factors*)

Soluble factors for activation of bone marrow stem cells (*inductive factors*)

Bone marrow-derived stem cells recruited to the injected scaffold
“Smart” Biomaterial Scaffolds for *In Situ* Tissue Regeneration

**Conductive and Inductive** Scaffolds to **Recruit** and **Activate** Endogenous Bone Marrow-derived Stem Cells

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**Scaffold Design:**

- Faster-degrading Bulk Scaffold Biomaterial
- Slower-degrading Micro-/nano-particles
Site-Targeting Polymeric Nanoparticles for *In Situ* Tissue Normalization

Drug-loaded Polymer nanoparticles (10-200 nm dia.)  
surface-modified with  

Tissue-targeting moiety  
(*peptides, antibodies, RNA aptamers*)

Cell Death  

Cell Proliferation  

Normalized Tissue
Pancreatic Islet-targeting Polymeric Nanoparticles

[Chemical structure of the nanoparticles]

PLGA-b-PEG-COOH

EDC/NHS

(Islet homing peptide)

Am J Pathol, 2005

Spontaneous self-assembly in aq. solution

Dynamic Light Scattering

Transmission Electron Microscopy

Islet-targeting NPs Preferentially Bind to and Enter Islet Cells

Islet CE NP-Pep I
Skin CE NP-Pep I
Islet CE NP-Pep X

Fluorescence Intensity (arbitrary units)

***

Islet CE NP+PepA
Skin CE NP+PepA
Islet CE NP+PepX

Correlation Plot
99% Correlation

Application of the Principles of:

**Chemistry:** *Biomaterials*

**Physics:** *Mechanical Forces*

Application of the principles of chemistry have a profound effect on the therapeutic efficacy of Tissue Engineering Biomaterials.
Multidisciplinary Approach for Regulating Tissue Development

Application of the Principles of:

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Application of the principles of chemistry have a profound effect on the therapeutic efficacy of Tissue Engineering Biomaterials.
Tissue Development is a Highly Orchestrated Mechanical Process

Morphogenetic Movements in Early Human Embryo

Wikipedia
Physical Interactions Also Drive Late-stage Tissue and Organ Patterning

Branching Morphogenesis in Late Stage Embryos


Terminal differentiation to adopt organ-specific function

Lung

Kidney

Mammary Gland
Cell-generated Tensional Forces Influence Embryonic Lung Branching

Control

Y27632
(Lower Cell Tension)

CNF-1
(Higher Cell Tension)

Fewer branches

More Branches

Moore et al. (2002) J Surg Res. 104; 95-100
Adult Tissues and Organs Also Experience Physiological forces

Articular Cartilage

Bone

Vascular Hemodynamic Forces
Abnormal Physiological forces Lead to Disease

Lack of gravity in space renders astronauts and cosmonauts very susceptible to “Osteoporosis” (bone loss)

Branch points and bends in blood vessels, which experience chaotic blood flow, are most susceptible to “Atherosclerosis”

“Tumors” are stiffer than the surrounding normal tissue

http://archives.focus.hms.harvard.edu/2001/May4_2001/pathology.html
Analyze Disease Pathophysiology
Design & Develop Effective Biomaterials

Organ-level Biomechanics

Cellular Biomechanics

ECM
BIOMECHANICS

MECHANICAL FORCES

BIOLOGY & MEDICINE

BIOMECHANICS

Analyze Disease Pathophysiology
Design & Develop Effective Biomaterials

Organ-level Biomechanics
Cellular Biomechanics

ECM
Cell
Classical “Deformable” Body Mechanics For Study of Organ-Level Biomechanics

- **Elasticity** (of a solid body) - a measure of the *resistance* to any deformation, and if deformed, its ability to *completely* regain its original dimensions

- **Plasticity** (of a solid body) – a measure of the extent of *permanent* deformation under the application of force

- **Viscosity** (of fluids) - measure of the resistance to flow

  ➢ *Most biological tissues are a heterogeneous mix of the solid and fluid phases.*  
  *As such, their physical properties are more appropriately described by the term*

- **Viscoelasticity** - employs the principles of both solid body and fluid mechanics
P: Proportionality Limit (*stress proportional to strain*)

E: Elastic Limit

Y: Yield Point (*beginning of plastic deformation*)

U: Ultimate Strength (*beginning of “necking”*)

R: Rupture Strength

\[ \sigma = E \varepsilon \]

where \( E \) = Young’s modulus

→ **Comparisons of stress-strain diagrams of different tissues/biomaterials indicate which one is relatively stiffer, harder, more ductile or brittle**
Classical Viscoelastic Models As Applied to Biological Tissues

Maxwell Model (Series)

Kelvin Voight Model (Parallel)

Standard Model
Analyze Disease Pathophysiology
Design & Develop Effective Biomaterials

Organ-level Biomechanics

Cellular Biomechanics
Mechanical Forces Acting on Cells

- **Active Mechanical Stimuli** – Tensile, Compressive and Shear forces

- **Passive mechanical Stimuli** – Extracellular Matrix (ECM) stiffness

⇒ The cytoskeleton and ECM adhesions are the key players in cell mechanosensing

**Analogy**

- Canvas = Cytoplasm
- Beams = Cytoskeleton
- Pegs = ECM Adhesions
Cell response to mechanical forces

Tensegrity (Tensio\*nal Integrity) Model of Cellular Force Balance

\[ \text{Tension in Actin Microfilaments (MFs)} = \text{Microtubule Compression} + \text{Cell-ECM Adhesion Strength} \]

Cell Shape Equilibrium

Demonstration of Residual Tension in Actin Microfilaments (MFs)

Tensegrity is an Architectural Design Principle
Proposed by Architect Buckminster Fuller

Examples of tensegrity-based engineered structures
Force-dependent Control of Cell Shape and Fate

Cell Shape Equilibrium

Actin Cytoskeletal Tension = Microtubule Compression + Cell-ECM Adhesion Strength

Cell Shape Alone Can Control Cell Fate

Focal Adhesions are the sites for Mechanotransduction – the conversion of adhesive/mechanical signals into intracellular biochemical response

Chen et al. Science, 1997, 276; 1425
Dike et al. In Vitro Cell Dev Bio Anim, 1999, 35; 441
Ingber DE. J Cell Sci. 2003
Actin cytoskeleton is more organized (tensed) on stiffer substrates

Ghosh, K et al. (2007) Biomaterials
Using Atomic Force Microscope to Measure Cell Stiffness

Cell indentation by AFM tip

Force applied on the cell ~ 10-25 nN

Cell Stiffness Adapts to the Stiffness of the Underlying Substrate

Response Amp (mV) (Cell compliance)

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Ghosh, K et al. (2007) Biomaterials
Stiffer Hydrogels Preferentially Accentuate Tissue Repair

Tissue Formation Begins at the Nanoscale

Nanofibrous scaffolds may interact more effectively with cells

Electrospun HA Nanofibrous Scaffolds Mimic ECM Architecture and Enhance Cell Function

*Dendritic* Cell Morphology in *Native ECM*

**Electrospinning**

HA/PEO blend

Nanofibrous HA scaffold

Dendritic cell morphology on Nanofibrous HA Scaffold

Cells Proliferate Preferentially on Nanofibers

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Biomaterials (2007) 27: 3782-3792

Biomaterial spray as an alternative approach?

**Conventional delivery of biomaterials for tissue engineering**

**Alternative delivery strategy that enables the use of nanofibrous biomaterial scaffold**
Multidisciplinary Approach for Regulating Tissue Development

Application of the Principles of:

Chemistry: *Biomaterials*

Physics: *Mechanical Forces*

Application of the principles of Chemistry have a profound effect on the therapeutic efficacy of contemporary Biomaterials

Mechanical forces and biomaterial stiffness/structure actively regulate cell function and tissue formation
How do we inspire students for a career in multidisciplinary Biomedical Engineering?

By making them aware of various career opportunities

- Science/Engineering Doctorates
- Patent Law
- Presidents/Chief Scientific Officers in Biomedical Companies
- University Professors
- MD/Ph.D.
California has the highest concentration of Biotech/Biomedical Companies in the US
How do we inspire students for a “RESEARCH” career in multidisciplinary Biomedical Engineering?

Know the essential traits for success in “multidisciplinary” Biomedical Engineering research

Unfortunately, no standardized test truly tests the level of fundamental curiosity of a student.

Perhaps that may explain why it’s often the B-grade students who end up doing the really good research

*Thomas Cech, Nobel Laureate and Past President of HHMI*
Identify “curious” students and cultivate their interests in biomedical engineering

Past Siemens Winners from Stony Brook

Prized students

Four more from LI win respect, money in Siemens science competition; teacher also earns award

BY JOHN HILDEBRAND
STAFF WRITER

From left, Long Island winners Abhinav Khanna and Benjamin Pollack, both of JFK Plainview-Old Bethpage; Ran Li, of Valley Stream Central; and Adam Solomon, of Bellmore-Merrick JFK, yesterday in Manhattan.

From BM-M graduates, Ezra Katz, last year’s finalist in the Siemens-Westinghouse Competition in Math, Science and Technology, has again been selected as a Regional Semi-Finalist in the 2004-2005 competition. Ezra, currently a 12th grade student in the Mesivta, is the son of Dr. Michael and Sherry Katz, who resides in Lawrence, N.Y.

The Siemens Foundation provides more than $1 million in college scholarships and awards each year to talented high school students in the United States. Its signature programs, the Siemens-Westinghouse Competition in Math, Science and Technology, recognize remarkable talent early on, fostering individual growth for high school students who are willing to challenge themselves through science research. Through this competition, students have an opportunity to achieve national recognition for science research projects that they complete in high school.

Siemens-Westinghouse selected Ezra as one of just 332 semifinalists through-
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