BIOMATERIALS-TISSUE INTERACTIONS:

“Tools for Understanding the Molecular, Cellular, and Physiological Bases of the Tissue Response to Implants

M. Spector, Ph.D.
BIOMATERIALS-TISSUE INTERACTIONS

Tissue* + Biomaterial**

Cell + Matrix**

* Structure comprising cells of the same type
** Solid surface
CELL-MATRIX INTERACTIONS

In Tissue
Cell + Extracellular Matrix

In Tissue Engineering Scaffolds
Cell + Biomaterial Scaffold
CONCEPTS FOR UNDERSTANDING BIOMATERIALS-TISSUE INTERACTIONS

- Control Volume
- Unit Cell Processes
- Types of Tissues
- Tissue Formation and Remodeling In Vitro
- Wound Healing In Vivo
Chondrocytes (P2 Canine) in a Type I Collagen-GAG Scaffold

B. Kinner

UNIT CELL PROCESSES

Concept of a “Control Volume” around a Cell

SOLUBLE REGULATORS

SOLUBLE REGULATORS

“Control Volume”
UNIT CELL PROCESSES

Concept of a “Control Volume” around a Cell

Cell + Matrix (Regulator) -> Product

+ Soluble Regulator

Autocrine factor

“Control Volume”

Another Cell; Paracrine

Sustainable Circulation; Endocrine
UNIT CELL PROCESSES

Concept of a “Control Volume” around a Cell

Cell + Matrix (Regulator) + Soluble Regulator A

Mechanical Loading (Strain); Subject 2.785

“Control Volume”

Cell + Matrix + Soluble Regulator B

Product
CONCEPTS FOR UNDERSTANDING BIOMATERIALS-TISSUE INTERACTIONS

- Control Volume
- Unit Cell Processes
- Types of Tissues
- Tissue Formation and Remodeling *In Vitro*
- Wound Healing *In Vivo*
UNIT CELL PROCESSES

- Mitosis
- Migration
- Synthesis
- Contraction
- Endocytosis
- Exocytosis
UNIT CELL PROCESSES

- Mitosis
- Migration
- Synthesis
- Contraction
- Endocytosis
- Exocytosis
- ?
- ?
UNIT CELL PROCESSES

- Mitosis
- Migration
- Synthesis
- Contraction
- Endocytosis
- Exocytosis
- Apoptosis
- Differentiation
COLLAGEN-GAG MATRICES: MODEL BIOMATERIALS (ANALOGS OF EXTRACELLULAR MATRIX)

Investigation of cell interactions (UCPs) in vitro

- Type I (bovine and porcine)
- Type II (porcine)
- Chondroitin 6-sulfate

- Freeze-dried
- Dehydrothermally cross-linked
- Additional cross-linking

See IV Yannas, et al. PNAS, 1989
CELL –MATRIX INTERACTIONS WITH COLLAGEN-GAG MATRICES IN VITRO

• Can provide insights into interrelationships among cell processes.
  – How do mitosis and synthesis interrelate?
  – How do mitosis and synthesis relate to contraction?
  – How does migration relate to contraction?
• Can provide insights into cell behavior in vivo.
• Can provide insights into scaffold composition and structure for improved performance in regenerative medicine.
Chondrocytes (Passage 2 Canine) in a Type I Collagen-GAG Matrix

Live cell imaging for a period of 5 hours.

J. Cheng
CELL –MATRIX INTERACTIONS

- Mitosis
- Migration
- Synthesis
- Contraction
Chondrocyte (P2 Canine) in a Type I Collagen-GAG Matrix: Mitosis

Photo removed due to copyright restrictions.

J. Cheng
Effects of Cross-Linking on Chondrocyte Proliferation in Collagen-GAG Matrices

CELL–MATRIX INTERACTIONS

- Mitosis
- Migration
- Synthesis
- Contraction
Fibroblasts Migrate Away from Soft Substrates

NIH 3T3 cells are plated on polyacrylamide substrates with a transition in flexibility. The soft side is marked with fluorescent beads (to the left). Cells turn to avoid the soft substrate as they approach the boundary from the stiff side, by retracting the leading lamellipodium that crossed the boundary.

Courtesy of Yu-Li Wang. Used with permission.

Fibroblasts Migrate Toward Stiff Substrates

NIH 3T3 cells are plated on polyacrylamide substrates with a transition in flexibility. The soft side is marked with fluorescent beads (to the left). Cells turn toward and enter the stiff side as they approach the boundary from the soft side, by expanding protrusions toward the boundary into a leading lamellipodium.

Courtesy of Yu-Li Wang. Used with permission.

Fibroblasts Migrate Toward Stretching Forces

NIH 3T3 cells are plated on polyacrylamide substrates. Pulling forces are exerted by inserting a blunted needle in the substrate near the trailing end of the cell and dragging the needle away from the cell. Cells switch the direction of migration by expanding secondary protrusions toward the needle into a leading lamellipodium.

Fibroblasts Migrate Away from Compressing Forces

NIH 3T3 cells are plated on polyacrylamide substrates. Pushing forces are exerted by inserting a blunted needle in the substrate near the leading edge of an approaching cell and moving the needle toward the cell. Cells switch the direction of migration by retracting the leading lamellipodium.

Monolayers were ""wounded"" by scraping.

Chondrocytes (P2 Canine) in a Type I Collagen-GAG Matrix: Migration and Contraction

Diagram removed due to copyright restrictions.

Fig. 2 in Madri, Kidney Int. 41 (1992): 562.
Schematic of the modulation of microvascular endothelial cell phenotype during angiogenesis.

Madri, Kidney Int. 41:562 (1992)
CELL – MATRIX INTERACTIONS

- Mitosis
- Migration
- Synthesis
- Contraction
Effects of Cross-Linking on Chondrocyte Biosynthesis in Collagen-GAG Matrices

Protein Synthesis; Proline Incorporation

Proteoglycan Synthesis; Sulfate Incorporation

CELL - MATRIX INTERACTIONS

- Mitosis
- Migration
- Synthesis
- Contraction
α-smooth muscle actin-fusion peptide (SMA-FP) inhibits the tension exerted by lung fibroblasts on silicone substrates. After washing our of the FP, cells contract again.

See video at
http://jcb.rupress.org/content/suppl/2002/05/03/jcb.200201049.DC1/1.html

Chondrocytes (P2 Canine) in a Type I Collagen-GAG Matrix: Contraction


40 min

Non-Seeded: 8 days

Cell-Seeded: 8 days

10mm

21 days

Non-Seeded and Cell-Seeded Collagen-GAG Scaffolds

Courtesy of Scott Vickers. Used with permission.
Adult canine articular chondrocytes (passage 3) contract a type I collagen-GAG matrix, reflected in the decrease in diameter.
Chondrocytes express the gene for $\alpha$-smooth muscle actin and this enables them to contract.

α-Smooth Muscle Actin Immunohistochemistry of Human Articular Cartilage

Kim and Spector, JOR 2000;18:749

MUSCULOSKELETAL CELLS THAT CAN EXPRESS α-SMOOTH MUSCLE ACTIN AND CAN CONTRACT

- Articular chondrocyte
- Osteoblast
- Meniscus fibroblast and fibrochondrocyte
- Intervertebral disc fibroblast and fibrochondrocyte
- Ligament fibroblast
- Tendon fibroblast
- Synovial cell
- Mesenchymal stem cell

POSSIBLE ROLES FOR α-SMOOTH MUSCLE ACTIN-ENABLED CONTRACTION

Musculoskeletal Connective Tissue Cells

- Tissue engineering
- Healing
- Disease processes
- Tissue formation and remodeling

Contracture of scaffolds
Closure of wounds (skin wounds and bone fractures)
Contracture (Dupuytren’s)
Modeling of ECM architecture (e.g., crimp in ligament/tendon?)
CONCEPTS FOR UNDERSTANDING BIOMATERIALS-TISSUE INTERACTIONS

- Control Volume
- Unit Cell Processes
- Types of Tissues
- Tissue Formation and Remodeling *In Vitro*
- Wound Healing *In Vivo*
<table>
<thead>
<tr>
<th>TYPES OF TISSUES</th>
<th>Yes</th>
<th>No</th>
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</thead>
<tbody>
<tr>
<td><strong>Connective Tissues</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Bone</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>• Articular Cartilage, Ligament, Intervertebral Disc, Others</td>
<td></td>
<td>√</td>
</tr>
<tr>
<td><strong>Epithelia (e.g., epidermis)</strong></td>
<td>√</td>
<td></td>
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<tr>
<td><strong>Muscle</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Cardiac, Skeletal</td>
<td></td>
<td>√</td>
</tr>
<tr>
<td>• Smooth</td>
<td></td>
<td>√</td>
</tr>
<tr>
<td><strong>Nerve</strong></td>
<td></td>
<td>√</td>
</tr>
</tbody>
</table>
BIOMATERIALS-TISSUE INTERACTIONS

Cell + Matrix

Connective Tissue
Epithelia
Muscle
Nerve
UNIT CELL PROCESSES

Concept of a “Control Volume” around a Cell

Cell + Matrix $\rightarrow$ Product + Soluble Regulator

Soluble Regulator A

Mechanical Loading (Strain)

“Control Volume”
BIOMATERIALS-TISSUE INTERACTIONS

Cell + Matrix

Connective Tissue
Epithelia
Muscle
Nerve

Adhesion Protein
Collagen Biomaterial
BIOMATERIALS-TISSUE INTERACTIONS

Cell + Matrix

Connective Tissue
Epithelia
Muscle
Nerve

Adhesion Protein
Collagen Biomaterial

Integrin
Chinese hamster ovary cell migration in a wound-healing assay

CHO cells express the α5β1 but not α4β1 integrin

Photos removed due to copyright restrictions.
Figure 3 in Watters, C. Cell Biol. Ed. 2:210 (2003)

Image and links to associated videos at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC256980/figure/F3/

Cells transfected with plasmid DNAs for α4 and α4/GFP
Migration of fibroblast-like fibrosarcoma cells in a 3-D collagen lattice


HT1080/MT1 cell - spontaneous mesenchymal migration

Reduction of migration speed and induction of detached, nonmobile spherical morphology by adhesion perturbing anti–α1 integrin antibody, as a consequence of impaired collagen fibril binding.

HT1080/MT1 cell-blocked β1 integrins (mAb 4B4)
“UNIT CELL PROCESSES”

Cell + Matrix → UCP

Connective Tissue
Epithelia
Muscle
Nerve

Mitosis
Synthesis
Migration
Contraction
Endocytosis
Exocytosis
“UNIT CELL PROCESSES”

Cell + Matrix → Product

UCP

Connective Tissue
Epithelia
Muscle
Nerve

Mitosis
Synthesis
Migration
Contraction
Endocytosis
Exocytosis

Cell proliferation
Matrix molecules, enzymes, cytokines
Translocation
Strain
Solubilized fragments
Regulators
“UNIT CELL PROCESSES”

Regulator

Connective Tissue
Epithelium
Muscle
Nerve

Cell + Matrix ➔ Product + Regulator

UCP

Mitosis
Synthesis
Migration
Contraction
Endocytosis
Exocytosis

Cytokines (Growth Factors)
REGULATORS

• Cytokines/Growth Factors
  – (previously: http://web.indstate.edu/thcme/mwking/growth-factors.html)

• http://www.copewithcytokines.de/
“UNIT CELL PROCESSES”

Regulator \[\rightarrow\] Mechanical Force (Strain) \[\rightarrow\] UCP

Cell + Matrix \[\rightarrow\] Product + Regulator

Connective Tissue
Epithelia
Muscle
Nerve

Adhesion Protein
Collagen Biomaterial
Integrin

Mitosis Synthesis
Migration Contraction
Endocytosis Exocytosis

Cytokines (Growth Factors)
“UNIT CELL PROCESSES”

Regulator + Mechanical Force (Strain) → UCP → Product + Regulator

Connective Tissue
Epithelia
Muscle
Nerve

Adhesion Protein
Collagen Biomaterial

Mitosis
Synthesis
Migration
Contraction
Endocytosis
Exocytosis

Cytokines (Growth Factors)

Integrin
Regulator (TGF-β1)

Cell + Matrix → UCP → Product + Regulator

Connective Tissue
Epithelia
Muscle
Nerve
Integrin

Adhesion Protein
Collagen Biomaterial

Mitosis Synthesis Migration
Contraction
Endocytosis Exocytosis

Matrix strain (contracture/shrinkage)

Cytokines (Growth Factors)
“UNIT CELL PROCESSES”

TGF-β1

Fibroblast + Collagen → Contraction → Contracture + Reg.
CONCEPTS FOR UNDERSTANDING BIOMATERIALS - TISSUE INTERACTIONS

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Canine chondrocytes grown in a type II collagen-GAG scaffold for 2 weeks. (Safranin O stain for GAGs)

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WOUND HEALING

Roots of Tissue Engineering

Injury → Inflammation (Vascularized tissue) → Reparative Process

Regeneration*
- CT: bone
- Ep: epidermis
- Muscle: smooth

*spontaneous

Repair (Scar)
- CT: cartilage
- Nerve
- Muscle: cardiac, skel.
RESPONSE TO IMPLANTS: WOUND HEALING

Surgical Implantation → Vascular Response
  Clotting
  Phagocytosis
  Neovascularization
  New Collagen Synthesis

Acute Inflammation

Tissue of Labile and Stable Cells
  Framework
    Intact
    Regen. (incorp. of implant)
    Chronic Inflammation
  Framework
    Destroyed
    Scarring
    Chronic Inflammation

Tissue of Permanent Cells
  Implant Movement
  Scarring
    (fibrous encapsulation; synovium)
    Chronic Inflammation

Granulation Tissue