Engineering Tissue-to-Tissue Interfaces and the Formation of Complex Tissues

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Tissue Engineering

Skalak 1988, Langer and Vacanti 1993
Tissue Engineering: the Next Generation
Engineering Complex Tissues

• Assemble or connect more than one type of tissue

• Interfaces between these different tissue types are critical for engineering collective functionality

Tissue Engineering, 2006
Challenges in Orthopedic Tissue Engineering

• Soft tissues
  - Articular cartilage
  - Ligaments
  - Tendons

• Lack of graft-bone integration
  – Compromises long term functionality

• Challenge
  – How to achieve BIOLOGICAL FIXATION of soft tissue to bone?
Interface Tissue Engineering

Tissue-to-Tissue Interfaces

- Soft tissues have limited regeneration potential
- Ligament-to-bone interface
  - Anterior cruciate ligament (ACL) graft-to-bone integration
- Tendon-to-Bone interface
- Bridging tissues to form organ systems
- Osteochondral interface

Clinical Challenge

Interface Characterization

Cell-Cell Interactions

Scaffold Design

In Vitro Testing

In Vivo Testing
How to Connect a Rope to the Wall?

- **Multiple Tissue and Cell Types**
  

  - Ligament (L) – Fibroblasts
  - Fibrocartilage – Fibrochondrocytes
    - Non-Mineralized Fibrocartilage (FC)
    - Mineralized Fibrocartilage (MFC)
  - Bone (B) – Osteoblasts

- **A gradient of cellular, chemical and mechanical properties**

  - Minimize the formation of stress concentrations
    (Butler et al., 1978, Woo et al., 1988, Matyas et al., 1995, Gao et al., 1996)

  - Load transfer between soft and hard tissues (Woo SL, et al. 1988)
Age-Related Changes

**Neonatal**

- L
- NFC
- MFC
- B

**Immature**

**Mature**

Goldner’s Trichrome Stain, 50x.

Picro Sirius Stain under polarized light, 50x.

_Wang et al., JOR 24:1745-1755, 2006_
Mechanical Properties

• Mechanical properties of the interface is not known

• Ultrasound Elastography
  
  (Konofagou et al, Ultrasound in Medicine and Biology, 1998)

  – Provides information on tissue mechanical properties
  – Permits the imaging of strain distribution within tissues
  – RF data acquired at 5 MHz, 54 frames/s during loading
  – Speckle tracking analysis

Gradient of Mechanical Response

- **Displacement Map**
  - Variations in displacement from ligament to bone

- **Strain Map**
  - Yellow – Red $\rightarrow$ Tensile Strain ($+\varepsilon_{yy}$)
  - Light Blue – Dark Blue $\rightarrow$ Compressive Strain ($-\varepsilon_{yy}$)

Spalazzi et al., JOR, 2006
• Unconfined compression and image analyses (Schinagl et al., 1999, Wang et al., 2002)

• Region-dependent variations in displacement and incremental strain, across insertion
  – Strain: FC > B

(Moffat et al., ASME 2005 Masters Research Award)
Axial stress and Young’s modulus exhibit depth-dependent variations for tibial and femoral samples:

\[ \sigma_{\text{MFC}} > \sigma_{\text{NFC}} \text{ and } E_{\text{MFC}} > E_{\text{NFC}} \]

\[ (n=4) \]

Moffat et al., PNAS, 2008
Structure-Function: Mineral

NFC

MFC

Bone
How to Regenerate the Interface?

- Mechanism of interface regeneration is not known

- Neo-fibrocartilage formation was observed where soft tissue and bone are in direct contact (Rodeo et al., 1993; Weiler et al., 2002; Liu et al., 1997; Yoshiya et al., 2000; Anderson et al., 2001; Panni et al., 1997; Chen et al., 2003; Grana et al., 1994)
  - Non-anatomical
  - Fibrocartilage can be regenerated

- Examine the role of fibroblast-osteoblast interactions in initiating fibrochondrogenic differentiation

- Stem cell niche at various tissue-to-tissue junctions
Osteoblast-Fibroblast Co-Culture

- Homotypic and heterotypic interactions
- Paracrine/Autocrine interactions

Microchannel design

(Wang et al., JOR, 2007)
Cellular Interactions

- **Fibroblast** Osteoblast interactions during co-culture

**Day 0, 5x**

**Day 1, 5x**

**Day 2, 5x**
Osteoblast-Fibroblast Interactions

- Co-culturing osteoblasts and fibroblasts results in changes in respective phenotypes (Wang et al., JOR, 2007)
  - Suppression of cell proliferation
  - Suppression of osteoblast ALP activity
  - Suppression of mineralization
  - Increased ectopic fibroblast mineralization and ALP activity
  - Expression of fibrocartilage interface-related markers

- Triculture studies revealed that osteoblast-fibroblast interaction promoted the fibrochondrogenic differentiation of mesenchymal stem cells (Wang et al. IEEE, 2007)

A co-culture well after cell seeding (Wang et al. 2007)

Tri-Culture Model
(Wang et al., 2007)
Cellular Interaction Mechanism

• **Mode of cellular interactions**
  - Secreted soluble factors and cytokines
    - Chemical messengers directing both local and systemic cellular communications
      *(Canalis et al., 1988; Bhatia et al., 1999; Lu et al., 2007)*
  - Direct physical contact between cells
    - Important in the dynamic regulation of cell-cell adhesion, communication and tissue development
      *(Gumbiner, 2005; Kii et al., 2004; Leckband et al., 2006)*

• **Maintenance and repair of tissue could be tightly controlled by these cellular interactions**
  *(Waldman et al., 2003; Alsberg et al., 2002; Lu & Jiang, 2005)*
Bioinspired Design Criteria

- Three phases to support ligament-, fibrocartilage- and bone-like tissues
  - Controlled matrix heterogeneity
- Continuous & interconnected phases to support heterotypic interactions of interface-relevant cell populations
- Gradient of mechanical properties comparable to those of the ligament insertion site
- Biodegradable for host-mediated interface regeneration

Spalazzi et al, 2006, 2008; Moffat et al., 2008; Wang et al, 2006, 2007, Tsai et al., 2005, Lu and Jiang, 2005
**Scaffold for Interface Regeneration**

- **Biomimetic Triphasic Scaffold**
  - Phase A: Polylactide-co-glycolide (PLGA)
  - Phase B: PLGA Microspheres
  - Phase C: PLGA–Bioactive Glass Composite
  
  *(Lu et al, JBMR 2003; Lu et al., Biomaterials, 2005)*

- **Co-culture of human osteoblasts and fibroblasts on 3-D scaffolds**

  - **Phase A**
  - **Phase B**
  - **Phase C**

  **Seeding Density:** 5x10^4 cells/cm^2

  *Spalazzi et al, Tissue Engineering, 2006*
In Vivo Cell Tracking

<table>
<thead>
<tr>
<th>Phase A</th>
<th>Phase B</th>
<th>Phase C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Week 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fibroblasts</td>
<td>chondrocytes</td>
<td>osteoblasts</td>
</tr>
</tbody>
</table>

| **Week 4** |
| fibroblasts | chondrocytes | osteoblasts |
In Vivo

Mechanical Properties

**Mechanical Properties**

- *p* < 0.05
- *n* = 6

**In Vivo**

- Compressive Modulus (MPa)
  - Acellular
  - Co-Cultured

- Bar graph showing compressive modulus at Week 0 and Week 4:
  - Week 0: Acellular and Co-Cultured have similar values.
  - Week 4: Acellular has a higher compressive modulus compared to Co-Cultured.
Phase-specific Mineral Deposition

- Mineralized matrix formation observed in all groups
- Zonal distribution of mineral confined to Phase C
Multi-Tissue Formation
Compositionally Distinct & Structurally Contiguous

Week 4
5x
Clinical Application

- Incorporation of multi-phasic scaffold and onto ACL reconstruction grafts
  - Synthetic tissue engineered grafts
  - Soft tissue autografts or allografts
    - Induction of fibrocartilage formation on the tendon graft

- Biological Fixation
- Multi-tissue formation

*modified from www.nucleusinc.com
What is Next: Stem Cells

Phase A
- fibroblasts

Phase B
- chondrocytes

Phase C
- osteoblasts

- Feasibility – multiple cell sources required
- Stem Cell-Mediated Interface Regeneration
  - Fibroblasts
  - Osteoblasts
  - Chondrocytes
Growth Factor Gradient

Peret and Murphy, Adv. Funct. Mat., 2008
Gradient Scaffolds: Growth Factors

Polymer + Solvent + Growth Factor (TGF-β or BMP-2) + Nanophase Material

Aqueous non-solvent

To frequency generator

Piezoelectric transducer

Custom nozzle

Sinter Microspheres

TGF-β microspheres

BMP-2 microspheres

Axis of variation

100% Chondrogenic

100% Osteogenic
Scaffoldless Approach

What is NEXT?

- Higher mineral to matrix ratio (mineral content) in bone and mineralized fibrocartilage
- Mineral content higher in tibial and femoral bone compared to femoral insertion
Engineering Gradient Scaffolds

Hydrogels

Electrospun Nanofibers

Gas-Foamed

Salt-Leached

Freeform Fabrication

Topography Library

Combine different fabrication approaches

Reviews:
- Simon et al., Comb Chem H T Scr, 2009
- Seidi et al., Acta Biomater, 2011

- Chatterjee et al., Biomaterials, 2010
- Ramalingam et al., J Biomat Appl, in press
- Li et al., Nano Letters, 2009
- Chatterjee et al., J Funct. Biomat., in press
- Simon et al., Rev Sci Instrum, 2007
- Yang et al., Adv Mater, 2008
- Pirano et al, Lab Chip, 2012
- Kumar et al., Biomaterials, 2011
Gradations in Mineral

Li et al, Nano Letters, 2009
Cell-Mediated Gradient

- Runx-2 transfection gradient to control fibroblast mineralization

*Phillips et al, PNAS, 2008*
# Bioinspired Scaffold: Complex Tissues

<table>
<thead>
<tr>
<th>Multi-Tissue Native Interface</th>
<th>Regional Collagen Distribution</th>
<th>Biomimetic Multi-phased Scaffold</th>
<th>Multi-Tissue Formation \textit{In Vitro}</th>
<th>Multi-Tissue Formation \textit{In Vivo}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligament</td>
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<td>Phase A</td>
<td>Phase A</td>
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</tr>
<tr>
<td>Fibrocartilage</td>
<td>Fibrocartilage</td>
<td>Phase B</td>
<td>Phase B</td>
<td>Phase B</td>
</tr>
<tr>
<td>Bone</td>
<td>Bone</td>
<td>Phase C</td>
<td>Phase C</td>
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</tr>
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</table>

**C**

**Regional Collagen Distribution**

**Biomimetic Multi-phased Scaffold**

**Multi-Tissue Formation \textit{In Vitro}**

**Multi-Tissue Formation \textit{In Vivo}**

**Biological Graft Fixation and Integrative Soft Tissue Repair**

*Moffat et al, CSM, 2009*
Challenges in Interface Tissue Engineering

• **Mechanism of Interface Regeneration**
  – Biological: cell to cell interactions
  – Chemical: growth factors or proteins
  – Physical: mechanical, electrical

• **Structure-Function Relationships at the Soft Tissue-Bone Interface**

• *Biomimetic* and *Bioactive* Scaffold Design
  - Strategic Biomimicry

• **Controlled Heterogeneity on Tissue Engineered Scaffolds *In Vivo***
ACKNOWLEDGEMENTS