LIGAMENTS AND TENDONS

M. Spector, Ph.D.
• **Effects of mechanical forces on cells** (including the response to removing forces)
  – Load-deformation behavior of the tissue
  – Histological make-up of the tissue to derive structure-properties relationships

• **Endogenous force generated by cells**
TOPICS

• Microanatomy/Histology
• Molecular composition of the ECM
  – Hierarchical structure
• Mechanical properties
• Response to injury and healing potential
• Response to mechanical loading
JOINT TISSUES

Structure - Function Relationships

ECM Architecture - Mechanical Function
INTRAARTICULAR VERSUS EXTRAARTICULAR LIGAMENTS

• What are the unique characteristics of the joint environment?
• Why don’t these tissues heal?
• Synovial fluid
  – Dissolves the fibrin clot
• Absence of surrounding vascularized tissue
## COMPARISON OF JOINT TISSUES

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Cell Type</th>
<th>Round/</th>
<th>Lac.</th>
<th>Coll.</th>
<th>PG</th>
<th>Vasc.</th>
<th>Heal.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meniscus</td>
<td>C/T Cart.</td>
<td>Fibro-Cart.</td>
<td>Fibro-Chond.</td>
<td>Yes</td>
<td>I</td>
<td>0/+</td>
<td>0*</td>
</tr>
<tr>
<td>ACL</td>
<td>Tens. Tissue</td>
<td>Fibrous Tissue</td>
<td>Fibro-blast</td>
<td>No</td>
<td>I</td>
<td>0</td>
<td>0**</td>
</tr>
</tbody>
</table>

* Inner third
** Mid-substance
Ligament: Histology

Diagram removed for copyright reasons.
Diagram removed for copyright reasons.
Ligament: ECM Hierarchical Architecture

Diagram removed for copyright reasons.
Rat tail tendon viewed under polarized light microscopy while undergoing tensile testing

A Viidik, 1980

Graphs and photos removed for copyright reasons.
LIGAMENT

Healing

- Healing through the production and remodeling of scar
- Origin of reparative fibroblasts intrinsic or extrinsic?
- Stages
  Inflammation
  Matrix and Cellular Proliferation
  Remodeling
  Maturation

AAOS: Injury and Repair of Musculoskeletal Tissues, 1988
LIGAMENT

Stress and Motion Dependent Changes

- Stress Deprivation (Immobilization)
- Recover (Remobilization)
- Stress Increases (Exercise)
LIGAMENT

Stress Deprivation (Immobilization): Biochemical Changes

- Water content
- Total GAG
- Collagen mass
- Collagen turnover (degradation and synthesis)
- Collagen cross-linking
LIGAMENT

Stress Deprivation (Immobilization): Biomechanical Changes

↑ Joint stiffness

↓ Structural properties of the ligament–bone complex

↓ Mechanical properties of the ligament substance
LIGAMENT AND TENDON

Cell Response to Loading

Meikle; Newborn rabbit cranial sutures (ligament)

Continous stress → Collagen synthesis
                → Degradative enzymes
                → Enzyme inhibitors

Slack; In vitro (tendon cells)

Cyclic tensile loading → Protein
                        → GAGs
$\alpha$-smooth muscle actin in fibroblasts in the healing rabbit collateral ligament

10 wks

Diagram and photo removed for copyright reasons.

Faryniarz, Chaponnier, Gabbiani, Yannas, and Spector; JOR, 14:228 (1996)
Myofibroblasts draw the ruptured ends together and tension the ligament.
SMA-containing cells in the intact human ACL

Up to 50% cells SMA+

Neg. Control; no SMA antibody

Diagram and photos removed for copyright reasons.

MM Murray, et al., JOR, 1999;17:18-27
Histologic Changes in the Human ACL after Rupture

A. Inflammation

B. Epiligamentous Regeneration

C. Proliferation

D. Remodeling

Ruptured Human Anterior Cruciate Ligaments

Evidence supporting the hypothesis that SMA-enabled contraction is responsible for retraction of the ruptured ends.

Crimped morphology of SMA-containing (red) cells consistent with contraction. Imparting crimp to matrix?

Photo removed for copyright reasons.

M. Meaney Murray, et al.  
_J. Bone Jt. Surg.,_ 2000;82-A:1387
The Migration of Human Anterior Cruciate Ligament Fibroblasts into Porous Collagen-GAG Matrices \textit{In Vitro}

M. M. Murray, D. Schultz-Torres, S. D. Martin, and M. Spector

Department of Orthopaedic Surgery
Brigham and Women’s Hospital
Harvard Medical School
Boston, MA
See results published in these two papers:


Results: 2-D Culture

- **Outgrowth start time**
  - $10 \pm 0.5$ days for explants from intact ACLs
  - $8 \pm 2$ days for explants from ruptured ACLs

- **Rates of outgrowth**
  - $0.23$ mm/day for explants from intact ACLs
  - $0.25$ mm/day for explants from ruptured ACLs
Results: 3-D Culture

- Maximum cell number density in scaffold at 2 weeks
  - $462 \pm 169$ cells/mm$^2$ for explants from intact
  - $333 \pm 161$ cells/mm$^2$ for explants from ruptured

- Maximum cell number density in scaffold at 4 weeks
  - $652 \pm 330$ cells/mm$^2$ for explants from intact
  - $903 \pm 360$ cells/mm$^2$ for explants from ruptured
Tendon Hierarchy

Evidence:

- Tropocollagen
- 35 Å staining sites
- 640 Å periodicity
- Fibroblasts
- Waveform or crimp structure
- Fascicular membrane
- Reticular membrane

Size scale:

- 15 Å
- 35 Å
- 100-200 Å
- 500-5000 Å
- 50-300 µ
- 100-500 µ

Figure by MIT OCW. After Fung.
TENDON

Healing

- Contribution of cells intrinsic and extrinsic to the tendon?
- Cell from peritendinous, epitendinous, and endotendinous tissues infiltrate the wound
- Collagen synthesis evident after 7–8 days
- Fibroblasts predominate after 14 days
TENDON

Cell Response to Loading

Leikle; Newborn rabbit cranial sutures

Continuous stress → Collagen synthesis
                 Degradative enzymes
                 Enzyme inhibitors

Cyclic tensile loading → Protein
                      GAGs

In vitro
Is SMA-enabled contraction responsible for retraction of the ruptured ends?

Photos removed for copyright reasons.

J. Premdas, *et al.*
*JOR, 2001;19:221-228*
Limitations to Healing

- Absence of a fibrin clot
  - Absent or low vascularity
  - Dissolution of clot in synovial fluid (ACL)
- Cell migration restricted by matrix
- Low cell density
- Low mitotic activity
- Mechanical loading disrupts stroma
Stress Deprivation (Immobilization):
Biochemical Changes

- Water content
- Total GAG
- Collagen mass
- Collagen turnover (degradation and synthesis)
- Collagen cross-linking