MENISCUS AND INTERVERTEBRAL DISC

M. Spector, Ph.D.
Diagrams of knee structure removed for copyright reasons. 
Source: Netter drawing 3655, Cruciate and Collateral Ligaments of Right Knee Joint. (Ciba)

Diagrams removed for copyright reasons. 
Source: Frank Netter drawing “Degeneration of lumbar intertebral discs and hypertrophic changes at vertebral margins with osetophyte formation.” (Ciba)
Temporomandibular Joint
The temporomandibular joint connects the lower jaw (mandible) to the temporal bone at the side of the head.
Morphologic Classification

- Synovial; Diarthrodial: fluid-filled
- Syndesmoses: dense connective tissue (skull)
- Synchondroses: cartilage (epiphyses)
- Synostoses: bone (from syndesmoses and synchondroses)
- Synphyses: grown together with dense fibrous tissue or cartilage (IVD)
TYPES OF FIBROCARTILAGINOUS DISCS

• Intra-articular fibrocartilaginous discs found in a few synovial joints
  – temporomandibular
  – inferior radioulnar
  – sternoclavicular

• Incomplete (crescent-shaped) discs (also called menisci or semilunar cartilages) occur in the knee joint
FIBROCARTILAGE DISCS

- Aneural
- Avascular
- Only the peripheral portion of the disc contains nerves and blood vessels
• Microanatomy/Histology
• Molecular composition of the ECM
  – Hierarchical structure
• Mechanical properties
• Response to injury and healing potential
• Response of cells to mechanical loading
• Capability of cells to generate a mechanical force
JOINT TISSUES

Structure - Function Relationships

ECM Architecture - Mechanical Function
LIGAMENT AND TENDON

Collagen:
  - X-links
  - Fiber Diam.
  - Orientation

→ Ligament Mat'l. Prop.

Ligament Mat'l. Prop. + X-sec. Area Length Shape + Bone Junction

→ Ligament Strength and Stiffness
• What are the unique characteristics of the joint environment?
• Why don’t these tissues heal?
INTRAARTICULAR ENVIRONMENT

- Synovial fluid
  - Dissolves the fibrin clot
- Absence of surrounding vascularized tissue
MENISCUS COMPOSITION

Extracellular Matrix

- Collagen fibers (75%) oriented in different directions
  - Type I (90%)
  - Type II (1-2%)
  - Type V (1-2%)
  - Type VI (1%).

- Noncollagenous protein 8-13%
## COMPARISON OF JOINT TISSUES

<table>
<thead>
<tr>
<th>Loading Type</th>
<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>
<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>
<td>0</td>
</tr>
</tbody>
</table>

* Inner third

** Mid-substance
Diagrams of knee structure and meniscus repair procedures removed for copyright reasons.
Sources: Netter drawings (Ciba), Stone Clinic, Ortho Associates.
Vascularity of the Meniscus

Photo removed for copyright reasons.
Human Meniscus: Fibrochondrocytes

Photo removed for copyright reasons.
Human Meniscus: Transmission Electron Microscopy

- Joint space

Photo removed for copyright reasons.

General matrix

Territorial matrix (fine fibrils)

FN Ghadially
Meniscus: Collagen Architecture

Diagram removed for copyright reasons.
Human Meniscus: Polarized Light Microscopy

Photo removed for copyright reasons.
MENISCUS

- Effects of mechanical forces on meniscus cells?
• Forces generated by meniscus cells?
Intact Human Meniscus 
α-Smooth Muscle Actin Immunohistochemistry

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Intact Human Meniscus
α-Smooth Muscle Actin Immunohistochemistry

Photo removed for copyright reasons.

Intact Human Meniscus
α-Smooth Muscle Actin Immunohistochemistry

Two graphs removed for copyright reasons.
a. % SMA-Containing Cells vs. Age, years
b. Bar chart with % SMA-Containing Cells

Four photos removed for copyright reasons.

Torn Native Menisci

Bar chart removed for copyright reasons.

Torn Meniscal Allografts

Bar chart removed for copyright reasons.

• Effects of mechanical forces on IVD cells?
Sequence of slides on spine functions, anatomy, injuries and therapies, removed for copyright reasons.
Source: Medtronic Somafor Danek
See http://www.medtronicsofamordanek.com/health-spinal.html for similar content
Three photos removed for copyright reasons.

D. Hastreiter, et al.

Non-seeded Implant

Annulotomy

Normal
• Response of the IVD to mechanical loading?
Biological response of the intervertebral disc to
dynamic loading

• Hypothesis: dynamic mechanical forces are important
  regulators in vivo of disc cellularity and matrix synthesis.
• A murine model of dynamic loading using an external loading
  device to cyclically compress a single disc in the tail.
• Loading
  • 50% duty cycle
  • peak stresses (0.9 or 1.3 MPa)
  • frequencies (0.1 or 0.01 Hz)
  • 6 h per day for 7 days
• Group with static compression at 1.3 MPa for 3 h/day for 7 da.
• A control group wore the device with no loading.
• Sections of treated discs were analyzed for morphology,
  proteoglycan content, apoptosis, cell areal density, and
  aggregan and collagen II gene expression.
Dynamic loading induced differential effects that depended on frequency and stress.

No significant changes to morphology, proteoglycan content or cell death were found after loading at 0.9 MPa, 0.1 Hz.

Loading at lower frequency and/or higher stress increased proteoglycan content, matrix gene expression and cell death.

The results have implications in the prevention of intervertebral disc degeneration, suggesting that loading conditions may be optimized to promote maintenance of normal structure and function.
Biological response of the intervertebral disc to dynamic loading

<table>
<thead>
<tr>
<th>Group</th>
<th>Frequency (Hz)</th>
<th>Peak Stress (MPa)</th>
<th>Loading Duration/Day (h)</th>
<th>Number of Days of Loading</th>
<th>Number of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>0.9</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>0.01</td>
<td>0.9</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>1.3</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>0.01</td>
<td>1.3</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>Static</td>
<td>1.3</td>
<td>3</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>Sham</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>13</td>
</tr>
</tbody>
</table>

Table by MIT OCW.
Proteoglycan content in the nucleus as a percentage of the area of the nucleus. *Statistically significant difference compared with sham, **significant compared with 0.1 Hz, 0.9 MPa. Proteoglycan content was unchanged by loading at low stress, high frequency but increased with decreasing frequency and/or increasing stress.
% of apoptotic cells in the nucleus as a function of loading condition. *Statistically significant difference compared with sham, **significant compared with 0.1 Hz, 0.9 MPa. Apoptosis in the nucleus was unchanged by loading at low stress, high frequency but increased with dec. frequency and/or inc. stress.
Percentage of apoptotic cells in the annulus as a function of loading condition. *Statistically significant difference compared with sham, **significant compared with 0.1 Hz, 0.9 MPa. Apoptosis in the annulus was unchanged by loading at low stress, high frequency but increased with decreasing frequency and/or increasing stress.
Effect of dynamic hydrostatic pressure on rabbit intervertebral disc cells

Piston–chamber assembly installed in an Instron servo-hydraulic mechanical testing system. A haversine compressive cyclic load was applied by the machine actuator on the piston. The piston transferred the load to the cells placed in the chamber filled with medium.
Effect of dynamic hydrostatic pressure on rabbit intervertebral disc cells

High frequency and high amplitude hydrostatic stress stimulated collagen synthesis in cultures of outer annulus cells whereas the lower amplitude and frequency hydrostatic stress had little effect.
Total 3H-proline incorporated by monolayer annulus cells under no loading (group I: control), low level loading (group II: 0.3 MPa, 1 Hz), and high level loading (group III: 1.7 MPa, 20 Hz). Incorporation was measured after three and nine days of loading.
Variation of ratio of released collagen ($R$) versus loading amplitude ($A$) (MPa) within the frequency range of 1–20 Hz and loading amplitude range of 0.75–3.0 MPa. The ratio decreases significantly by increasing the loading amplitude ($p<0.05$).
INTERVERTEBRAL DISC

• Forces generated by IVD cells?
Autopsy specimens

41 L4-L5 and L5-S1 discs were retrieved from 21 autopsies via anterior approach (11 male and 10 female).

The time after death was 15 ± 9 hours with a maximum of 22 hours.

The subject age range was 32-82 years, 63 ± 13 years (mean ± standard deviation).

The discs were scored as to Thompson² grade.
Results: $\alpha$-Smooth Muscle Actin

Some discs had no $\alpha$-SMA positive cells.

Within each disc, $\alpha$-SMA staining percentages were highest in the nucleus and lowest in the outer annulus.

Student’s $t$-test revealed that $\alpha$-SMA-positive cells were preferentially round in shape ($p = 0.0025$).

Heterogeneity of staining within clusters.

## Results

**Average ± Std. Error (Range)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Nucleus Pulposus n = 38</th>
<th>Inner Annulus n = 39</th>
<th>Outer Annulus n = 41</th>
<th>p Value for Regional Dependence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell density (cells/mm²)</td>
<td>28 ± 5 (2-140)</td>
<td>47 ± 8 (9-270)</td>
<td>121 ± 14 (25-510)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>% in clusters of total cells</td>
<td>29 ± 3 (3-73)</td>
<td>13 ± 2 (0-53)</td>
<td>5 ± 1 (0-18)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Average number of cells per cluster</td>
<td>2.5 ± 0.1 (2.0-4.9)</td>
<td>2.2 ± 0.1 (2.0-2.9)</td>
<td>2.2 ± 0.0 (2.0-3.0)</td>
<td>0.0457</td>
</tr>
<tr>
<td>% Round of total cells</td>
<td>96 ± 1 (71-100)</td>
<td>82 ± 3 (45-100)</td>
<td>39 ± 3 (6-85)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>% + α-SMA of total cells</td>
<td>15 ± 3 (0-63)</td>
<td>12 ± 3 (0-81)</td>
<td>4 ± 1 (0-39)</td>
<td>0.0019</td>
</tr>
<tr>
<td>% Round of + α-SMA cells</td>
<td>98 ± 1 (71-100)</td>
<td>88 ± 4 (29-100)</td>
<td>54 ± 6 (0-100)</td>
<td>0.0099</td>
</tr>
</tbody>
</table>

α-SMA + cells in the (a) nucleus pulposus, and (b) inner annulus of one disc.

Two photos removed for copyright reasons.

DISCUSSION

α-smooth muscle actin

Increased expression of α-SMA in round cells

The significant difference in the percentage of α-SMA-containing cells among the 3 regions in the IVD might reflect different functional requirements.

Perhaps a necessity to maintain rounded shape?

Specific role of α-SMA in the IVD needs to be investigated

POSSIBLE ROLES FOR SMA-ENABLED CONTRACTION OF MS CELLS

- **Healing**
  - Closure of wounds
  - Tensioning of a healing ligament
  - Retraction of the ends of torn ligaments/tendons that do not heal

- **Disease processes**
  - Contracture

- **Tissue formation and remodeling**
  - Modeling of ECM architecture (e.g., crimp in ligament/tendon?)

- **Tissue engineering**
  - Contracture of scaffolds