Harvard-MIT Division of Health Sciences and Technology
HST.523J: Cell-Matrix Mechanics
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MENISCUS AND INTERVERTEBRAL DISC

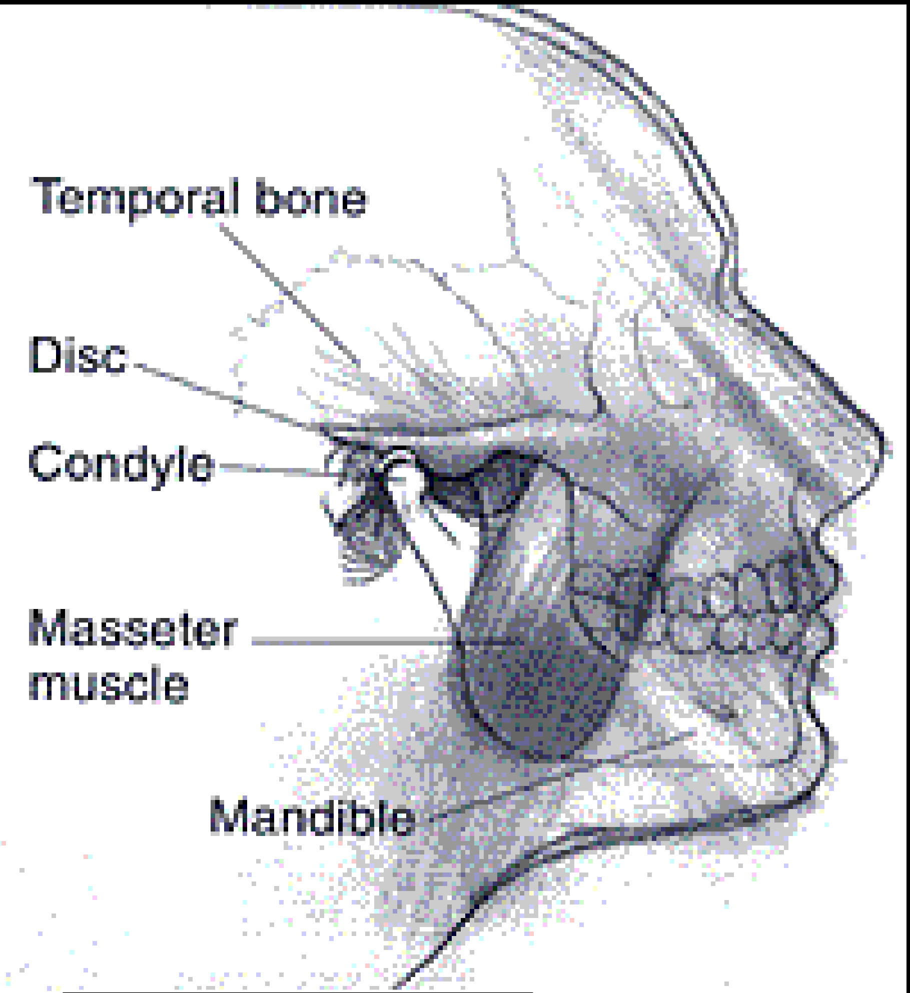
M. Spector, Ph.D.

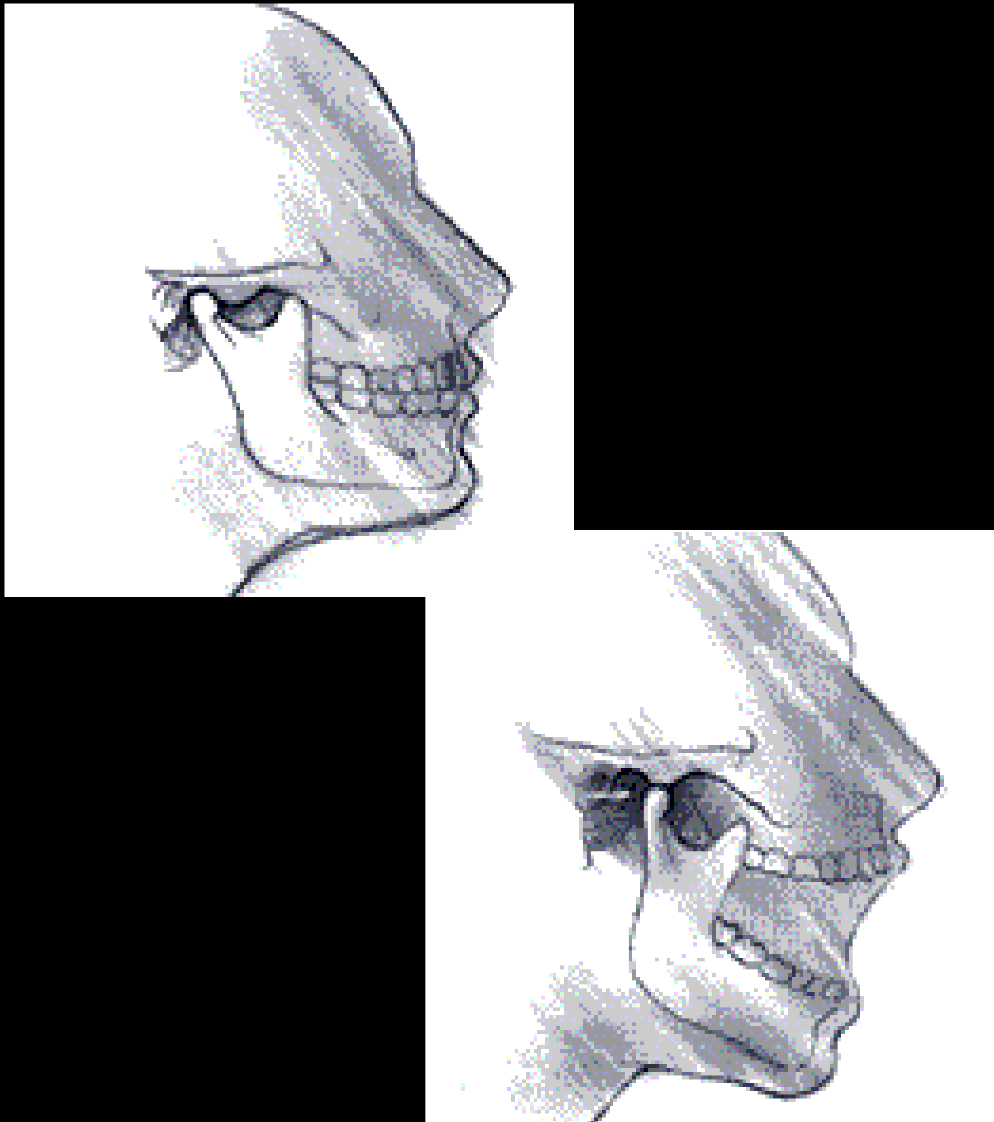
Diagrams of knee structure removed for copyright reasons.
Source: Netter drawing 3655, Cruciate and Collateral Ligaments of Right Knee Joint. (Ciba)

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.





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TYPES OF JOINTS

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

TOPICS

- 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

INTRAARTICULAR VERSUS EXTRAARTICULAR LIGAMENTS

- **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%

COMARISON OF JOINT TISSUES

	Loading	Tissue Type	Cell Type	Round/ Lac.	Coll.	PG	Vasc.	Heal.
Art. Cart.	Comp.	Hyal. Cart.	Chond.	Yes	II	+++	0	0
Meniscus	C/T	Fibro-Cart.	Fibro-Chond.	Yes	I	0/+	0*	0
ACL	Tens.	Fibrous Tissue	Fibro-blast	No	I	0	0**	0

* 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

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?**

MENISCUS

- **Forces generated by meniscus cells?**

Intact Human Meniscus
α-Smooth Muscle Actin
Immunohistochemistry

Photos removed for copyright reasons.

S Ahluwalia, et al, *JOR* 19:659;2001

Intact Human Meniscus
α-Smooth Muscle Actin Immunohistochemistry

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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

S Ahluwalia, et al, *JOR* 19:659;2001

Torn Human Meniscus SMA IH

Four photos removed for copyright reasons.

BY Lin, *et al*, *WRR* 10:25 (2002)

Torn Native Menisci

Bar chart removed for copyright reasons.

BY Lin, *et al*, *WRR* 10:25 (2002)

Torn Meniscal Allografts

Bar chart removed for copyright reasons.

BY Lin, *et al*, *WRR* 10:25 (2002)

INTERVERTEBRAL DISC

- 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

Annulotomy

Three photos removed for copyright reasons.

Normal

D. Hastreiter, *et al.*

Non-seeded Implant

INTERVERTEBRAL DISC

- **Response of the IVD to mechanical loading?**

Biological response of the intervertebral disc to dynamic loading

AJL Walsh and JC Lotz *J. Biomech* 37:329 (2004)

- **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 aggrecan and collagen II gene expression.**

Biological response of the intervertebral disc to dynamic loading

AJL Walsh and JC Lotz *J. Biomech* 37:329 (2004)

- **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

AJL Walsh and JC Lotz *J. Biomech* 37:329 (2004)

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

Table by MIT OCW.

mean±sd

Bar chart removed for copyright reasons.

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.

Bar chart removed for copyright reasons.

% 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.

Bar chart removed for copyright reasons.

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

Kasra, *et al*., *JOR* 21:597 (2003)

Photo removed for copyright reasons.

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

Kasra, *et al*., *JOR* 21:597 (2003)

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.

Bar chart removed for copyright reasons.

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.

Graph removed for copyright reasons.

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?**

METHODS

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 Thompson[2] grade.

D Hastreiter, et al, *JOR* 19:597;2001

Results: α-Smooth Muscle Actin

Bar chart removed for copyright reasons.

- Some discs had no α-SMA positive cells
- Within each disc, α-SMA staining percentages were highest in the nucleus and lowest in the outer annulus.
- Student's *t*-test revealed that α-SMA-positive cells were preferentially round in shape ($p = 0.0025$).
- Heterogeneity of + staining within clusters

D Hastreiter, et al, *JOR* 19:597;2001

Results

Average ± Std. Error (Range)

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

D Hastreiter, et al, *JOR* 19:597;2001

Two photos removed for copyright reasons.

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

D Hastreiter, et al, *JOR* 19:597;2001

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

D Hastreiter, et al, *JOR* 19:597;2001

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