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LIGAMENTS AND TENDONS

M. Spector, Ph.D.

CELL-MATRIX MECHANICS

- 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?

INTRAARTICULAR ENVIRONMENT

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

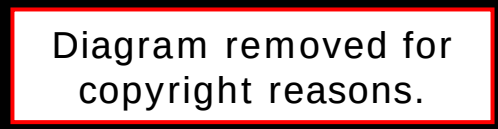
COMARISON OF JOINT TISSUES

	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

Ligament: Histology



Ligament: Polarized Light Microscopy

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

Graphs and photos removed for copyright reasons.

A Viidik, 1980

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**

α-smooth muscle actin in fibroblasts in the healing rabbit collateral ligament

Diagram and photo removed for copyright reasons.

Faryniarz, Chaponnier, Gabbiani, Yannas, and Spector; *JOR*, 14:228 (1996)

Myofibroblasts in the Healing Rabbit Medial Collateral Ligament (10 wks post-rupture)

Faryniarz, Chaponnier, Gabbiani, Yannas, and Spector; *JOR*, 14:228 (1996)

Smooth muscle actin

Photos removed for copyright reasons.

Myofibroblasts draw the ruptured ends together and tension the ligament.

SMA-containing cells in the intact human ACL

Diagram and photos removed for copyright reasons.

MM Murray, *et al*., *JOR*, 1999;17:18-27

Histologic Changes in the Human ACL after Rupture

Diagram removed for copyright reasons.

A. Inflammation

B. Epiligamentous Regeneration

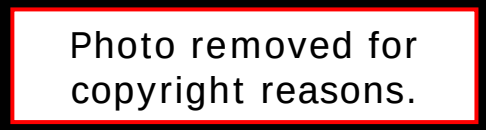
C. Proliferation

D. Remodeling

M. Meaney Murray, *et al.*, *J. Bone Jt. Surg.*, 2000;82-A:1387

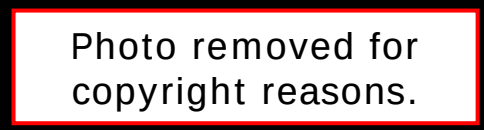
Ruptured Human Anterior Cruciate Ligaments

← **Blood Vessel**



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?



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 *In Vitro*

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

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Brigham and Women's Hospital
Harvard Medical School
Boston, MA

Graphs and diagrams removed for copyright reasons.

See results published in these two papers:

1) **Meaney Murray M, Martin SD, and Spector M. The migration of cells from human anterior cruciate ligament explants into collagen-glycosaminoglycan scaffolds. *J. Orthop. Res*. 2000;18:557-564.**

2) **Murray MM and Spector M. The migration of cells from the ruptured human anterior cruciate ligament into collagen-glycosaminoglycan regeneration templates in vitro. *Biomat.* 2001;22:2393-2402.**

Results: 2-D Culture

- Outgrowth start time
  - 10 ± 0.5 days for explants from intact ACLs
  - 8 ± 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

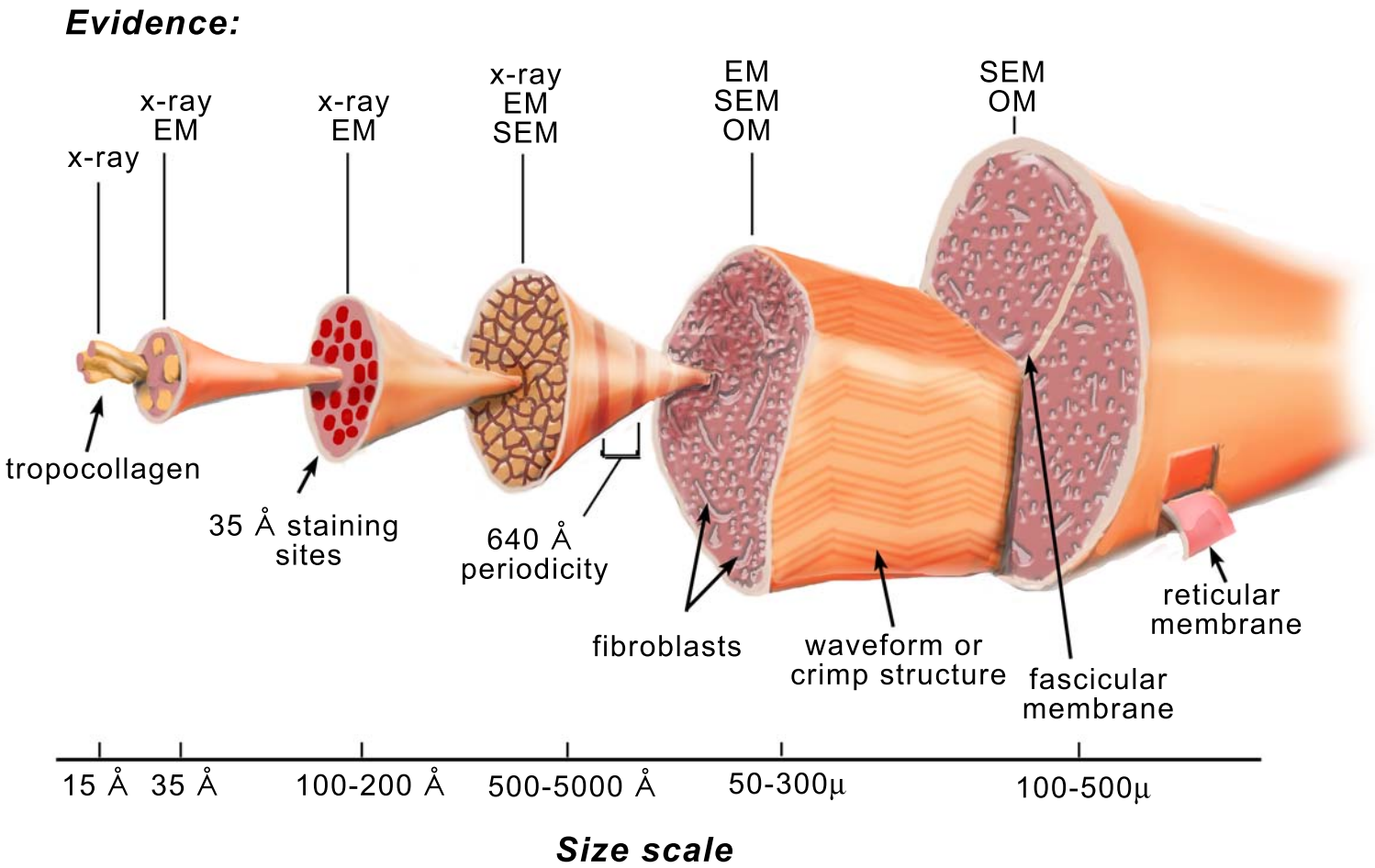

Figure by MIT OCW. After Fung.

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

Continous stress → ↑ Collagen synthesis
↑ Degradative enzyme
↑ Enzyme inhibitors

lack; In vitro

Cyclic tensile loading → ↑ Protein
↑ GAGs

Ruptured Human Rotator Cuff

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**

TENDON AND LIGAMENT

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

LIGAMENT

Stress Deprivation (Immobilization): Biochemical Changes

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