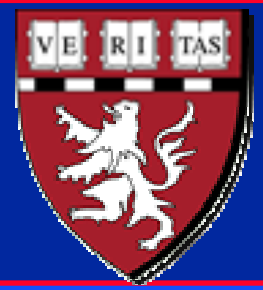
Massachusetts Institute of Technology
Harvard Medical School
Brigham and Women's Hospital
VA Boston Healthcare System



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IN VIVO EXPRESSION OF α-SMOOTH MUSCLE ACTIN AND CELL CONTRACTION

M. Spector, Ph.D.

TISSUE CLASSIFICATION

- **Connective Tissue**
  - **Synthesize and maintain a structurally competent ECM (including a supporting and connecting framework for all other tissue types); matrix and cell continuous**
- **Muscle Cells**
  - **Contraction; cell continuous, BM**
- **Epithelia**
  - **Lining and secretory cells; cell continuous, BM**
- **Nerve**
  - **Voltage conduction; cell continuous, BM**

FORCES GENERATED BY CELLS

All Cells	Actin Isoforms
• Migration	β- and γ- cytoplasmic
• Maintain cell shape	β- and γ- cytoplasmic
Muscle Cells	
• Contraction	α-smooth muscle (vascular)
	γ-smooth muscle (enteric)
	α-skeletal muscle
	α-cardiac muscle

TISSUE CLASSIFICATION

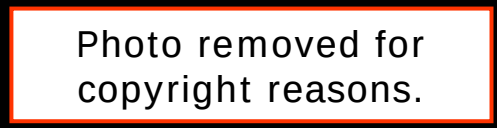
- Connective Tissue Cells
- Muscle Cells (contractile cells)
  - skeletal — α-skeletal actin
  - cardiac — α-cardiac actin
  - smooth muscle — α- and γ-smooth muscle actin
- Epithelial Cells
- Nerve Cells

TISSUE CLASSIFICATION

- **Connective Tissue Cells**
  - **– "myofibroblasts" (α-SMA; contractile cells)**
- **Muscle Cells (contractile cells)**
  - **– skeletal** **α-skeletal actin**
  - **– cardiac** **α-cardiac actin**
  - **– smooth muscle** **α- and γ-smooth muscle actin**
- **Epithelial Cells**
- **Nerve Cells**

α-Smooth Muscle Actin-Containing Fibroblasts Myofibroblasts (day 10)

Ungrafted



Grafted

IV Yannas, *et al.*

Summary: mechanism

It has been demonstrated by other investigators that wound contraction in connective tissues is caused by the cooperative pulling force offibroblasts that adopt a contractile phenotype and express an isofromof the protein actinfound in smooth muscle cells (alpha-SM actin). These cells have been termed myofibroblasts.

The image on the top shows an ungraftedskin wound that has been stained with an antibody for alpha-SM actin, red indicates positive stain. They are oriented parallel across the wound bed. In this particular configuration, the wound edges are moving together in this direction across the screen. MFB form a cell-continuous network and are able to transmit the force across the wound.

In the grafted wound at the bottom, MFB are present, but due tothe random pore walls of the matrix, they are not able to form a continuousaligned network across the wound, and contraction does not take place. Once again, this inhibition of contraction does not happen if the pore size and contact surface are not right and if the chemistry for cell attachment and pulling is not right.

The interruption of the MFB network is the proposed mechanism ofaction of the ECM analog in preventing contraction. Maybe also a statementabout MFB imparting the alignment of collagen in scar.

CONNECTIVE TISSUE CELLS THAT CAN EXPRESS α-SMOOTH MUSCLE ACTIN

- Articular chondrocyte
- Osteoblast
- Meniscus fibroblast and fibrochondrocyte
- Intervertebral disc fibroblast and fibrochondrocyte
- Ligament fibroblast
- Tendon fibroblast
- Synovial cell
- Mesenchymal stem cell

M. Spector, *Wound Repair Regen.* 9:11-18(2001)

CONTRACTILE CONNECTIVE TISSUE CELLS

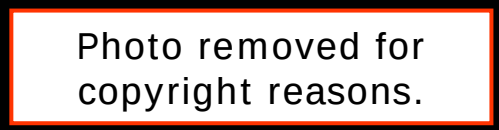
- Express SMA *in vivo*
- Capable of contracting collagen-GAG matrices *in vitro*
- SMA-positive cells retain differentiated phenotype
- SMA trait derived from the stem cell
- Amount of contraction correlated with the SMA content
- SMA and contraction up-regulated by TGF-β1
- Roles have yet to be determined, but may be both positive and negative

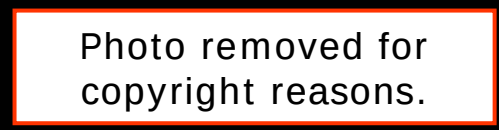
Canine Articular Cartilage

Normal top zone; 50 ± 7%

9-wk repair

SMA Neg. Control





Normal basal zone; 23 ± 5%

Q. Wang, *et al.*, *Wound Rep. Regen.*, 2000;8:145-158

Human Articular Cartilage

Kim and Spector,
***JOR* 2000;18:749-755**

Photos removed for copyright reasons.

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

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

Photo and diagram 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)

Photo removed for copyright reasons.

Smooth muscle actin

Myofibroblasts draw the ruptured ends together and tension the ligament.

Photo removed for copyright reasons.

SMA-containing cells in the intact human ACL

SMA (red)

Up to 50% cells SMA+

Photos removed for copyright reasons.

Neg. Control; no SMA antibody

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

SMA-expressing cells

"Retraction"

C. Proliferation

D. Remodeling

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

Ruptured Human Anterior Cruciate Ligaments

← **Blood Vessel**

Photo removed for copyright reasons.

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**

Ruptured Human Rotator Cuff

Photos removed for copyright reasons.

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

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

Osteoblasts Expressing SMA

Canine trabecular bone

Photo removed for copyright reasons.

C. Menard, *et al.*, Biomat. 2000;21:1867

Human trabecular bone

Photo removed for copyright reasons.

B. Kinner, *et al. JOR* 2002;20:622

Graph of %SMA+ vs. Patient Age
removed for copyright reasons.

B. Kinner, *et al. JOR* 2002;20:622

Osteoblasts Expressing SMA in Human Bone Explants 6 wks

Photo removed for copyright reasons.

B. Kinner, *et al. JOR* 2002;20:622

Osteoblastic cells (MC3T3-E1) contracting a collagen-GAG matrix

Pores compressed as specimens decrease in size (no evident dissolution)

Photo removed for copyright reasons.

1 wk

Loss of SMA

Photo removed for copyright reasons.

Photo removed for copyright reasons.

2 wk

4 wk

C. Menard, *et al.*, *Biomat.* 2000;21:1867

Mouse Tibia (Closed) Fracture Model

B. Kinner, *et al.*, *Bone* 2002;30:738

Photos removed for copyright reasons.

3 weeks post-fracture

Mouse Tibia (Closed) Fracture Model

3 weeks post-fracture

Photos removed for copyright reasons.

B. Kinner, *et al.*, *Bone* 2002;30:738

Distraction Osteogenesis; Rat Model

Photo removed for copyright reasons.

2 latent+13 distraction +3 consolidation (days)

B. Kinner, *et al*. *JOR* 2003;21:20

Distraction Osteogenesis; Rat Model

Photo removed for copyright reasons.

2 latent+10 distraction (days)

B. Kinner, *et al. JOR* 2003;21:20

SMA AND CONTRACTION OF MUSCULOSKLETAL CELLS

Many Questions to be Answered

- What are the roles of SMA-enabled contraction in normal and pathological processes?
- What therapeutic approaches can be taken for its regulation?
- How does the SMA-enabled contraction impact musculoskeletal tissue engineering?

TISSUE CLASSIFICATION

- **Connective Tissue**
  - **Synthesize and maintain a structurally competent ECM for all tissue types**
  - **Employ SMA-enabled contraction to model the ECM and to close wounds**
- **Muscle Cells**
- **Epithelia**
- **Nerve**