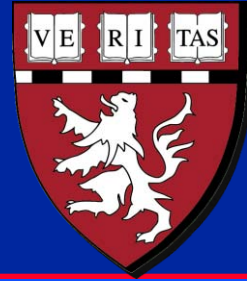


**Massachusetts Institute of Technology
Harvard Medical School
Brigham and Women's Hospital
VA Boston Healthcare System**

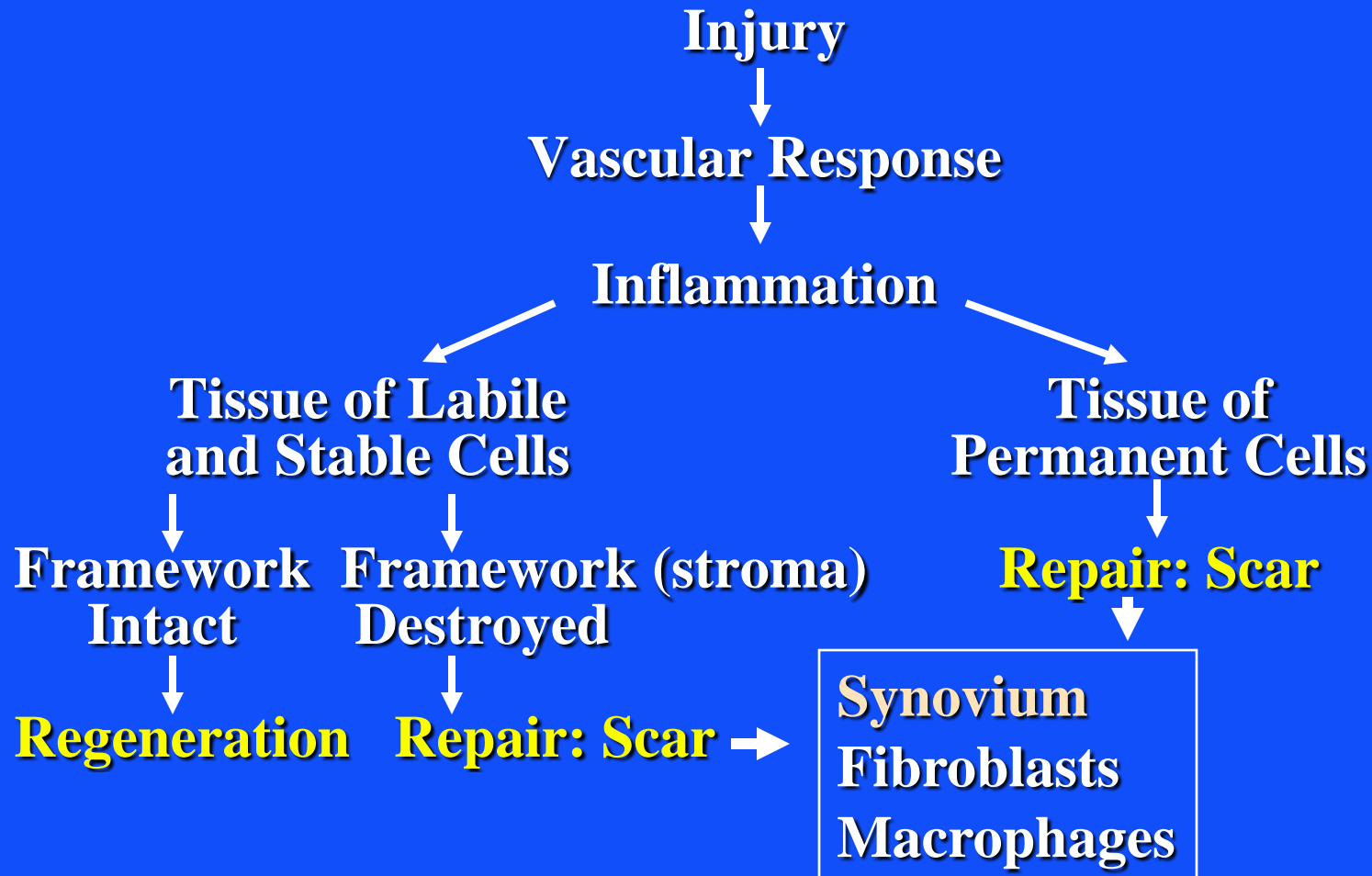


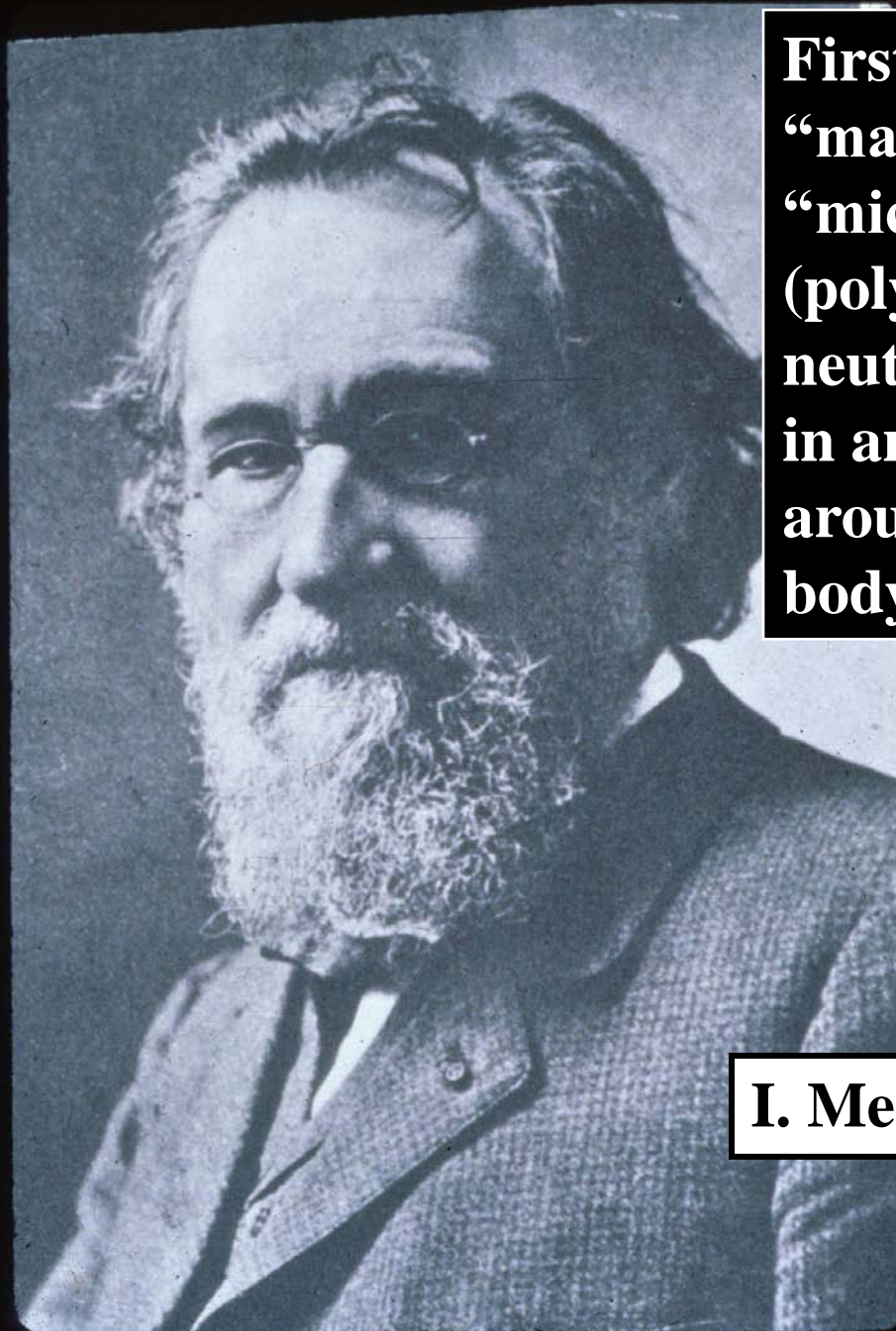
2.79J/3.96J/20.441/HST522J

CHRONIC RESPONSE TO IMPLANTS: α -Smooth Muscle Actin and Lubricin

M. Spector, Ph.D.

WOUND HEALING REGENERATION VERSUS REPAIR





**First identified
“macrophages” and
“microphages”
(polymorphonuclear
neutrophils, PMNs)
in an organism
around a foreign
body**

I. Metchnikoff



Marius Nygaard Smith-Peterson

In 1923 a piece of glass was removed from a patient's back; it had been there for a year. It was surrounded by a minimal amount of fibrous tissue, lined by a glistening synovial sac, containing a few drops of clear yellow fluid.

**J. Bone Jt. Surg.,
30-B:59 (1948)**

Rabbit Ear Chamber

IA Silver in, TK Hunt,

Diagrams removed due to copyright restrictions.

DR Knighton, *et al.*, Surg 90:62 (1981)

Healing Dead Space Wound

**Advancing
arterial
circulation**

Cellular leading edge

Rabbit Ear Chamber



Unfilled Dead Space

Photos removed due to copyright restrictions.

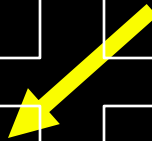
**From I. Silver, in
TK Hunt & JE Dunphy
Fund. of Wound Management (1979)**

Wound: Dead Space

Macrophages

Images removed due to copyright restrictions.
From I. Silver, in TK Hunt & JE Dunphy
Fund. of Wound Management (1979)

Fibroblasts



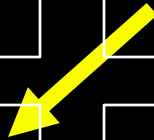
Wound: Dead Space

Macrophages

Images removed due to copyright restrictions.
From I. Silver, in TK Hunt & JE Dunphy
Fund. of Wound Management (1979)

Fibroblasts

Do these fibroblasts contract like the myofibroblasts in a skin wound?



Wound: Dead Space

Macrophages

Images removed due to copyright restrictions.
From I. Silver, in TK Hunt & JE Dunphy
Fund. of Wound Management (1979)

Fibroblasts

Synovium

**Do these
synoviocytes
express
lubricin like
those in joint
synovium?**



Image removed due to copyright restrictions.

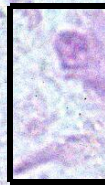
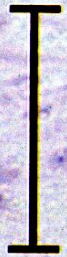
Graph of regional oxygen tension vs. distance around the foreign body.

**IA Silver in, TK Hunt,
Wound Healing and
Wound Infection (1980)**

Dead Space

Macrophages
Fibroblasts

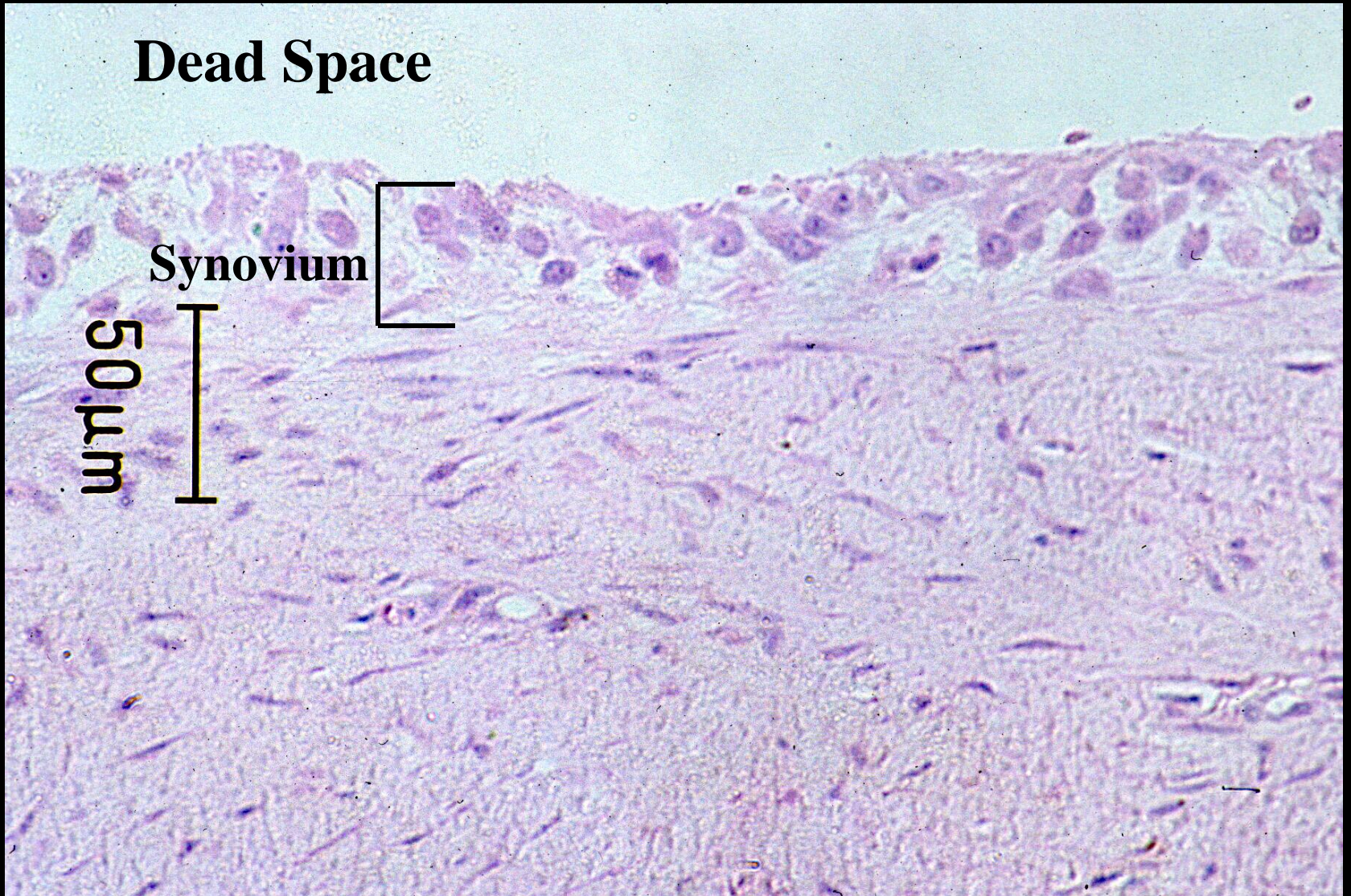
50 μ m



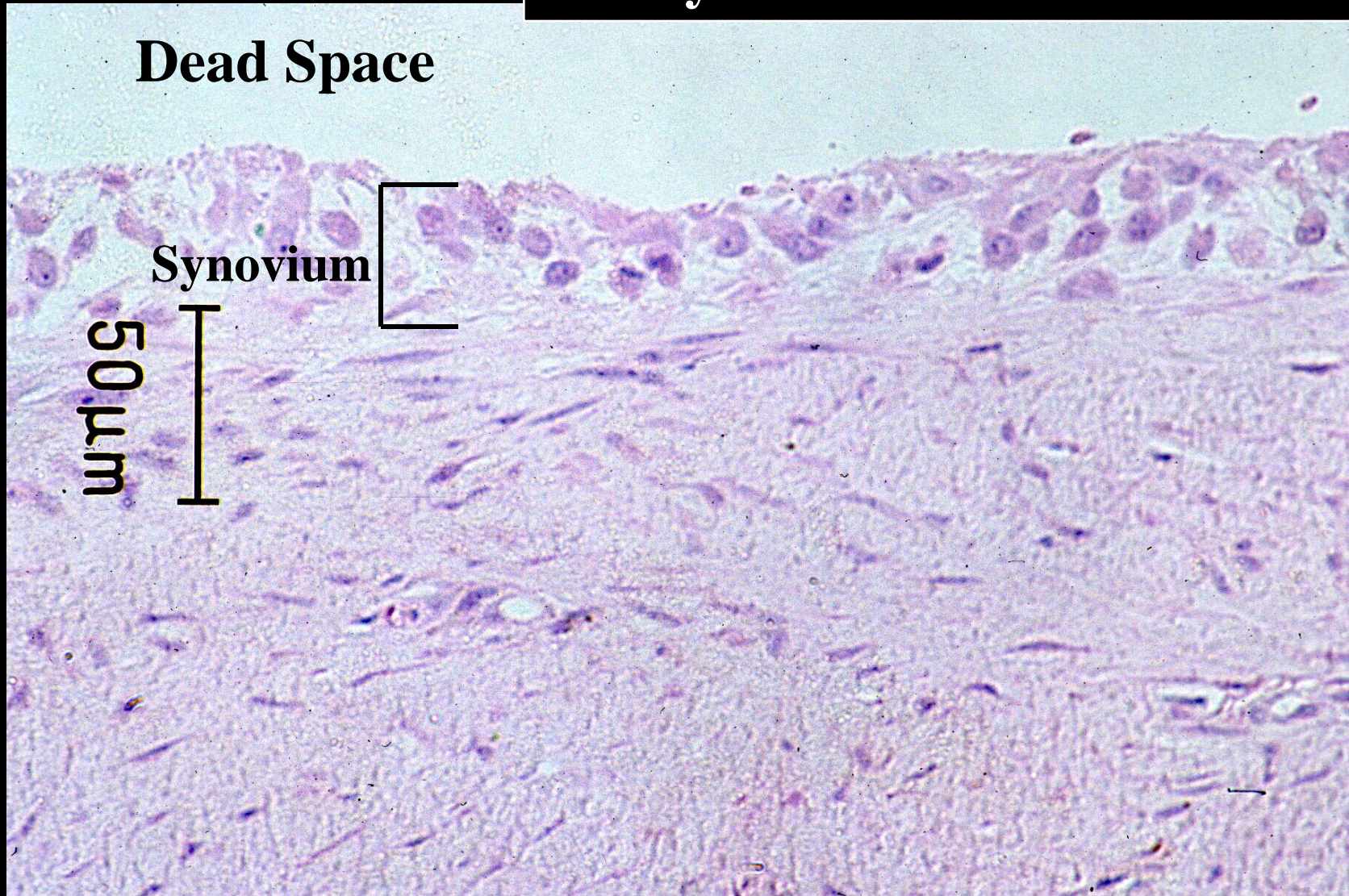
Dead Space

Synovium

50 μ m



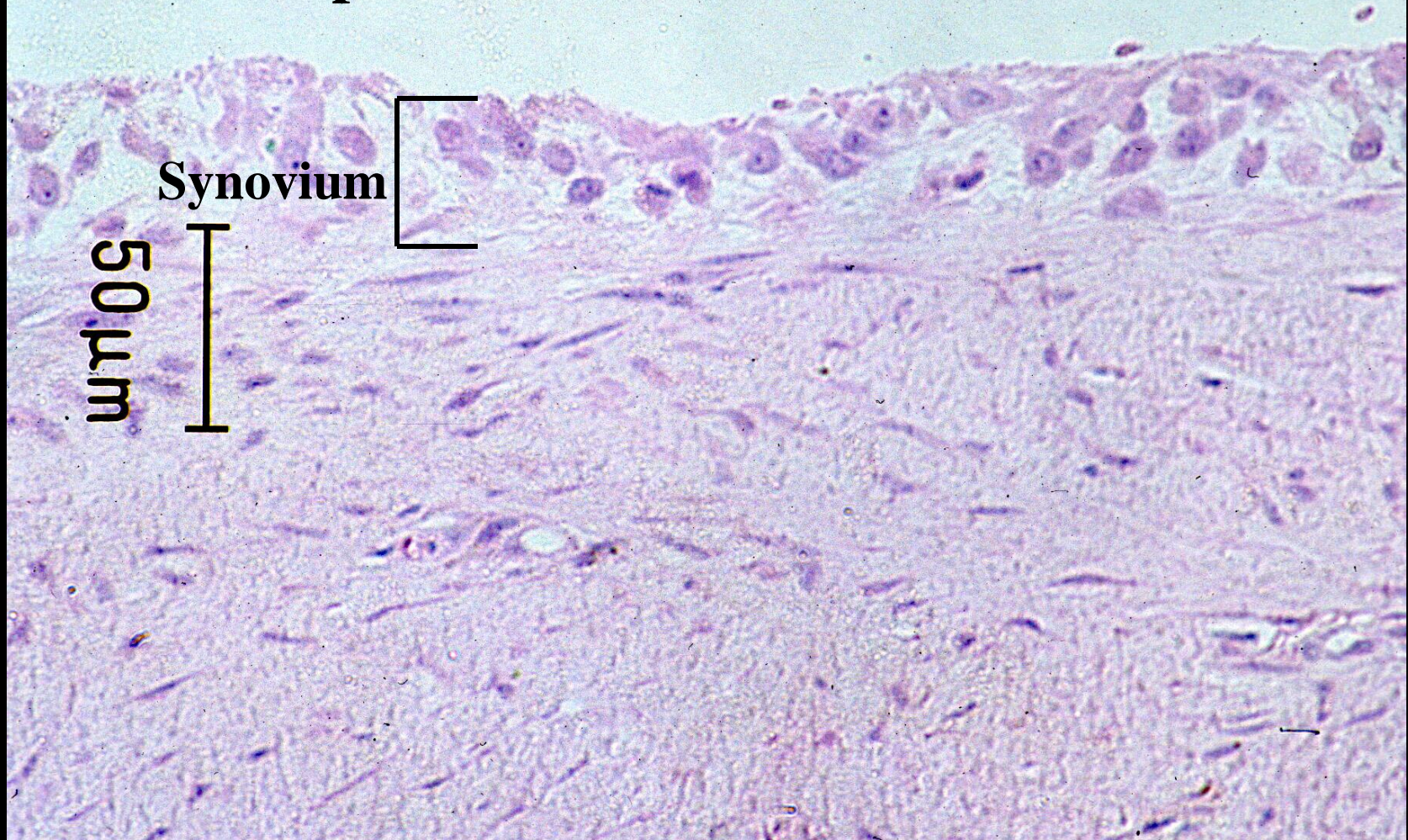
In addition to wounds, where else do dead spaces form in the body?



In addition to wounds, where else do dead spaces form in the body?

- **Joints**

Dead Space



Synovium

50 μ m

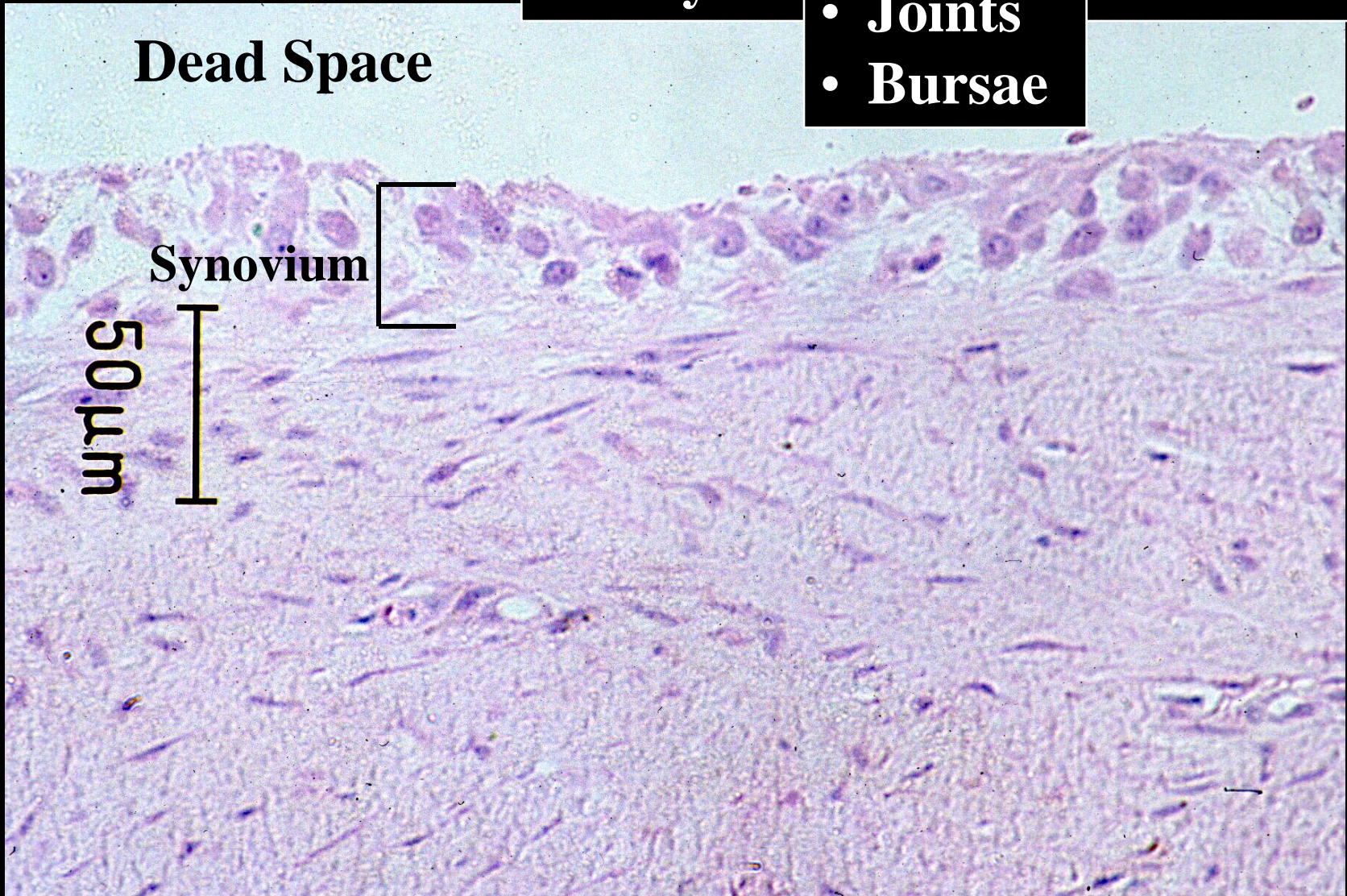
In addition to wounds, where else do dead spaces form in the body?

- **Joints**
- **Bursae**

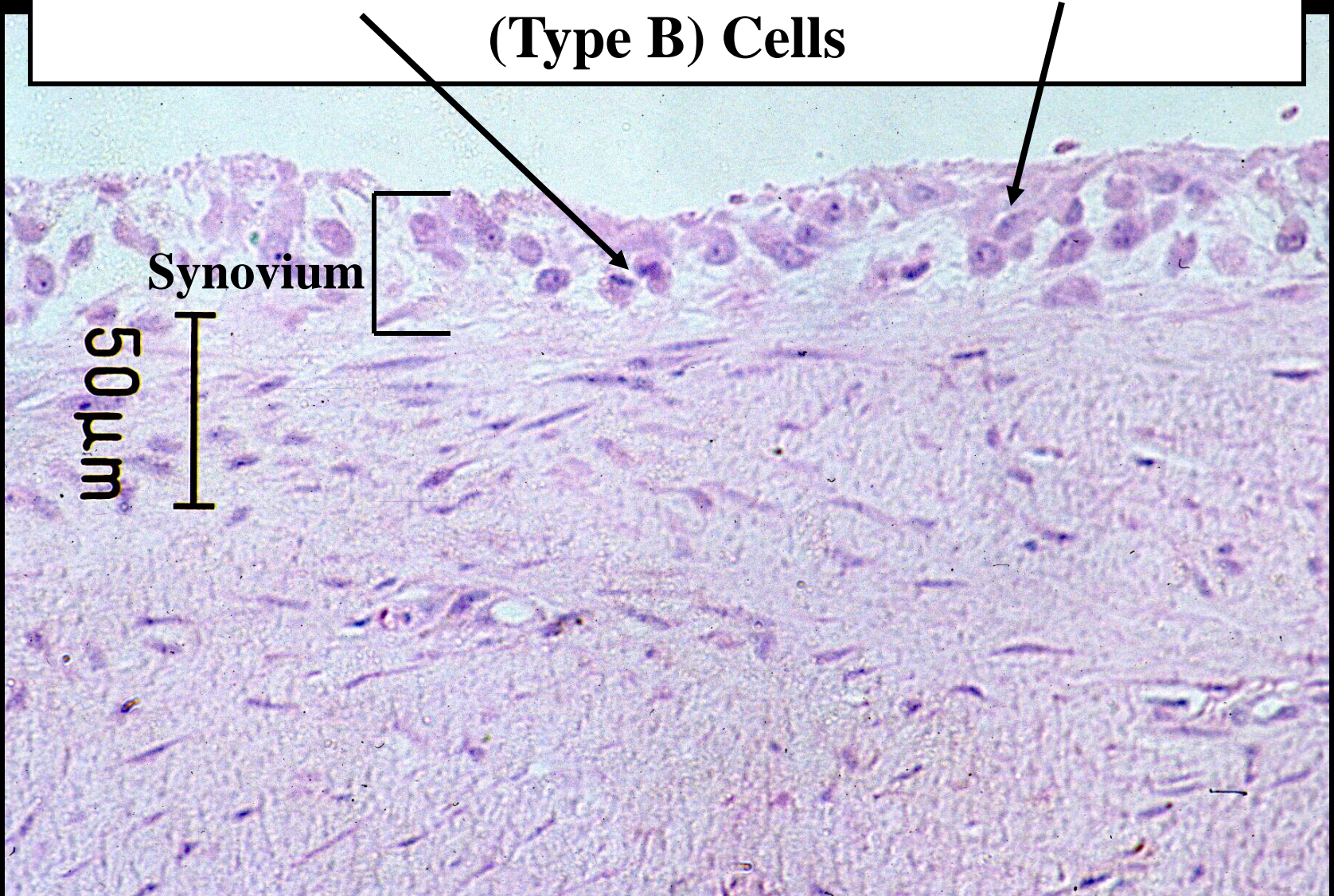
Dead Space

Synovium

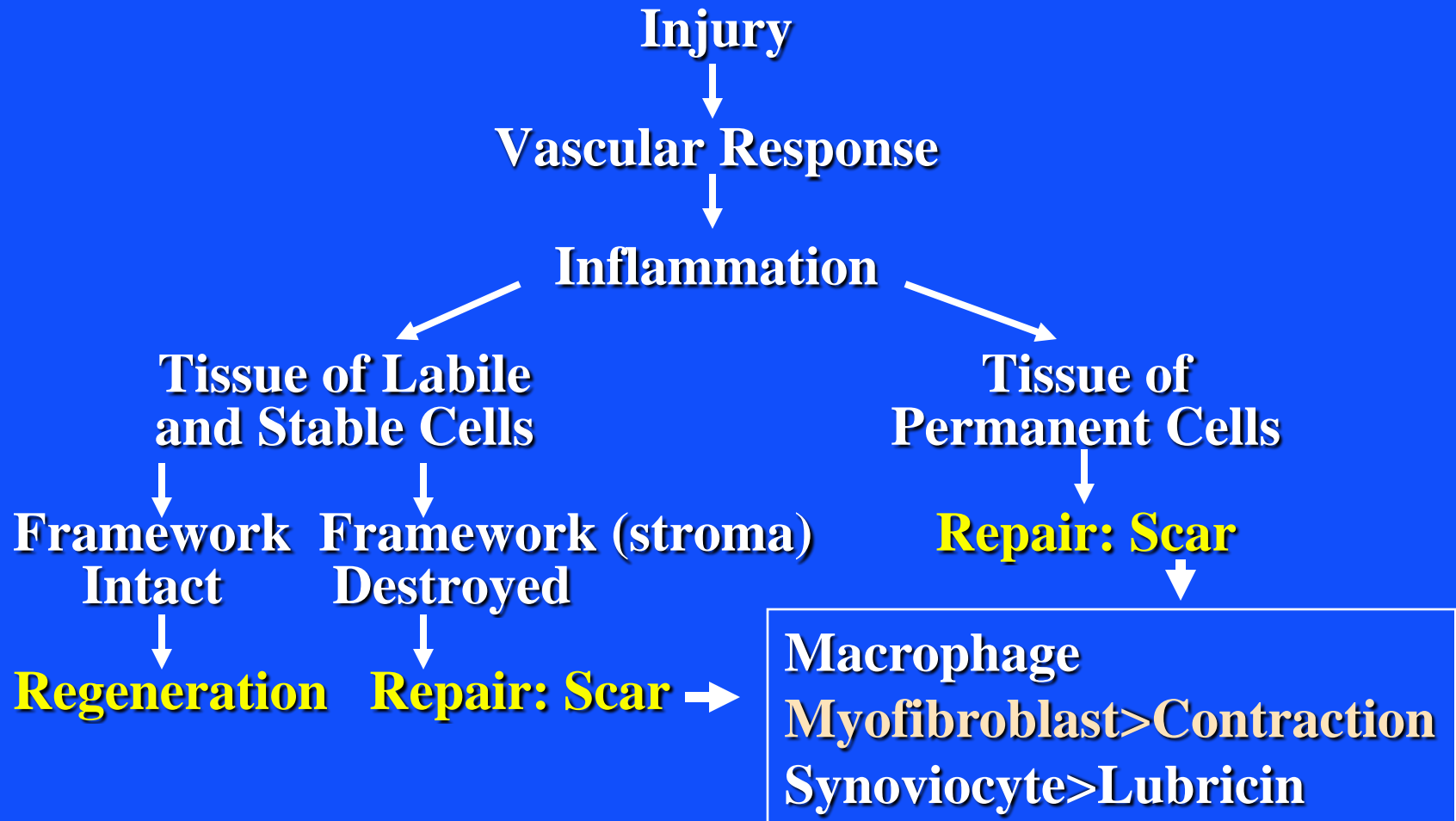
50 μ m



**Synovium:
Macrophage-like (Type A) and Fibroblast-like
(Type B) Cells**



WOUND HEALING REGENERATION VERSUS REPAIR



ACTIN ISOFORMS

- β - cytoplasmic (most cells)
- γ - cytoplasmic (most cells)

Contractile Actins

- α - skeletal muscle
- α - cardiac muscle
- α - (vascular) smooth muscle (SMA)
- γ - (enteric) smooth muscle

TISSUE CLASSIFICATION

- Muscle Cells (contractile cells)
 - skeletal α -skeletal actin
 - cardiac α -cardiac actin
 - smooth muscle α - and γ -smooth muscle actin
 - Connective Tissue Cells
 - “myofibroblasts” (SMA; contractile cells)*: dermal wound closure, fibrotic (scar) contractures, Dupuytren’s Disease
 - * G. Majno, G. Gabbiani, *et al.*, Science, 1971
-

FIBROBLAST BEHAVIOR IN FIBROUS TISSUE AROUND IMPLANTS

- **Proliferation and increased matrix synthesis of fibroblasts leads to an increase in the thickness and density of the scar tissue.**
- **Fibroblast contraction results in scar contracture.**

BREAST IMPLANTS Capsular Contracture

Removed implant: viewing the
outside of the fibrous capsule

Implant

Capsule

Inside of the
fibrous capsule

Implant

Photos removed due to copyright restrictions.
See <http://www.implantforum.com/capsular-contracture/>

CAUSE OF CAPSULAR CONTRACTION

Myofibroblasts, and the regulatory protein TGF- β , were found in the contracted capsules around silicone breast implants but not in non-contracted capsules. Mature skin scar tissue did not contain TGF- β or myofibroblasts.

Lossing C, and Hansson HA,
Plast Reconstr Surg 91:1277 (1993)

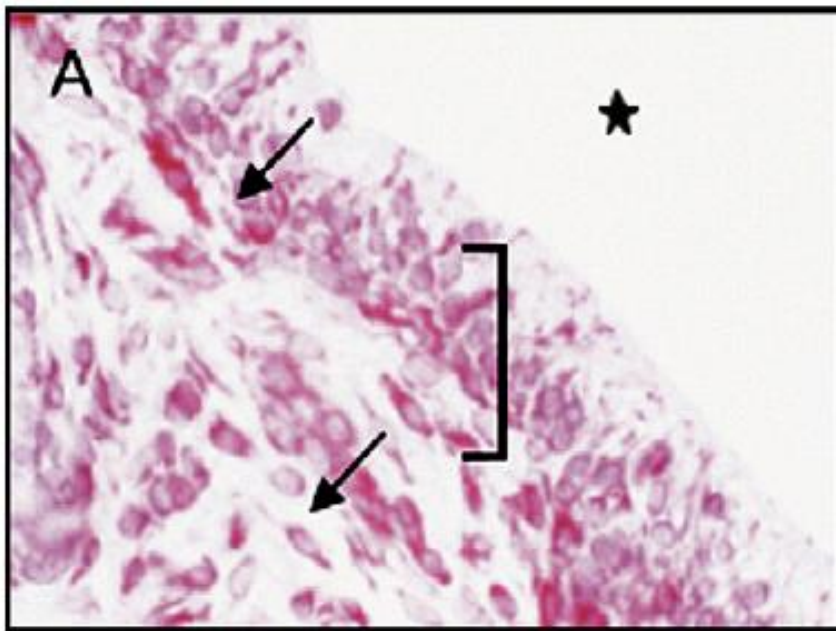
Cellular and molecular composition of fibrous capsules formed around silicone breast implants with special focus on local immune reactions[☆]

Wolfram Dolores^a, Rainer Christian^{b,c}, Niederegger Harald^a,
Piza Hildegunde^{b,c}, Wick Georg^{a,*}

^a*Institute of Pathophysiology, University Innsbruck, Medical School, Fritz-Pregl-Straße 3, 6020 Innsbruck, Austria*

^b*Department of Plastic and Reconstructive Surgery, University Innsbruck, Austria*

^c*Ludwig Boltzmann Institute for Quality Control in Plastic Surgery, Innsbruck, Austria*



Macrophages (CD68+ cells) in a capsule with an implant duration of 9 years. * Silicone implant; (arrows) macrophages; cells of the “frontier layer” in bracket. Original magnification: 400x.

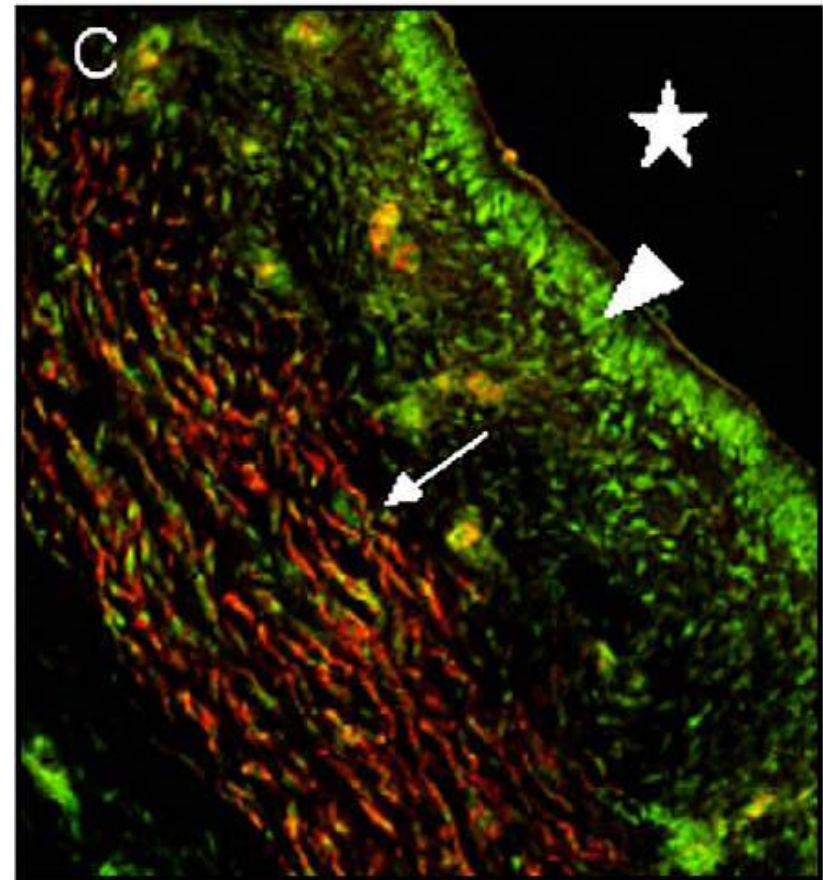
Courtesy of Elsevier, Inc., <http://www.sciencedirect.com>.

Used with permission.

Double staining for “heat shock protein,”* HSP60, (arrowhead) and for smooth muscle actin (arrow). Cells expressing both, yellow. The implant duration was 14 months. Original magnification: 250x.

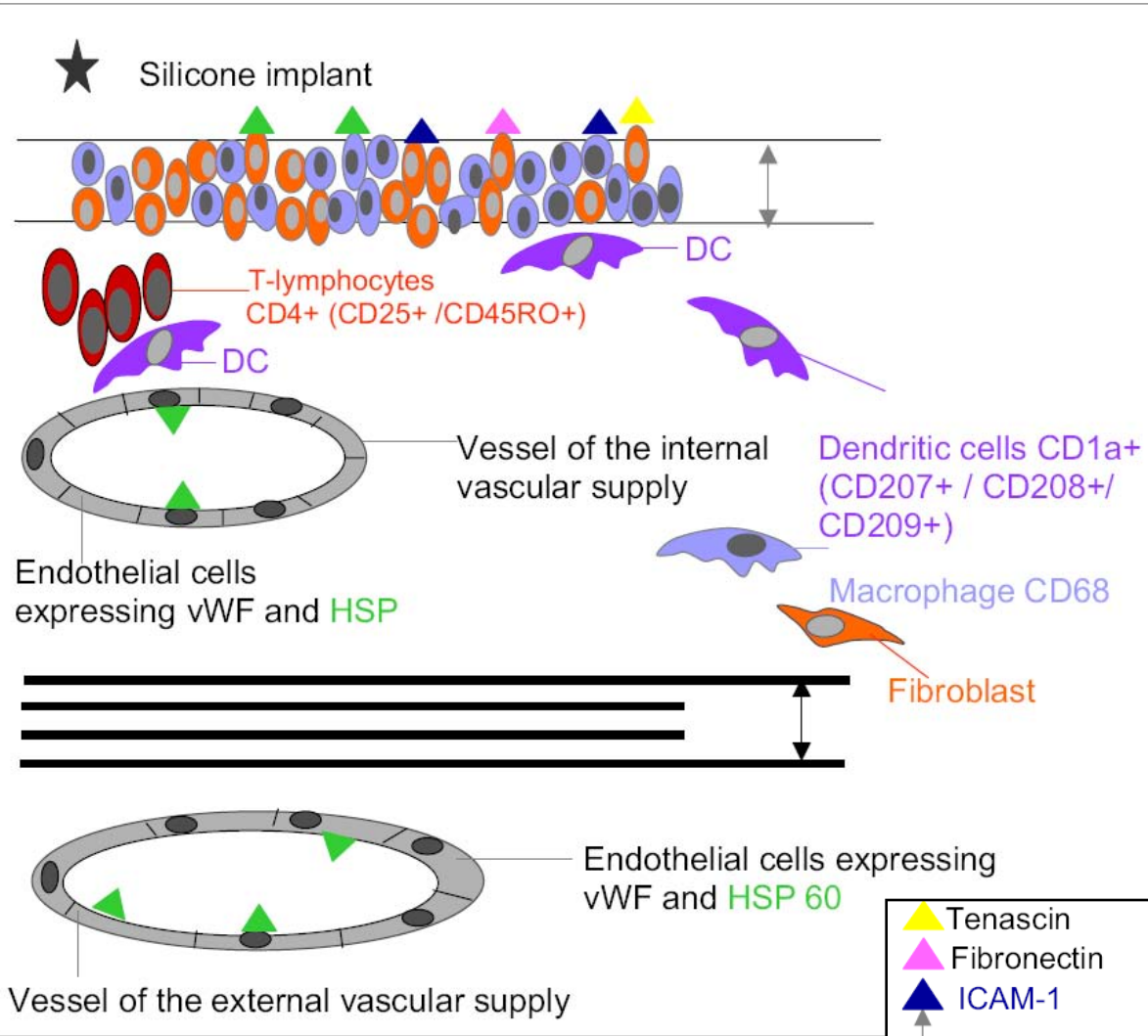
*** HSP60 (heat shock protein expression, reflects the effect of mechanical or other forms of stress exerted on the implant and capsule.**

D. Wolfram, J. Autoimmunity 23:81 (2004)



Courtesy of Elsevier, Inc., <http://www.sciencedirect.com>.

Used with permission.



The fibrous capsules show a three-layered composition:

- (1) The internal layer abutting the silicone surface formed by macrophages and fibroblasts,**
- (2) the layer of loosely arranged connective tissue including the internal vascular supply, and**
- (3) the outer layer of dense connective tissue with the external vascular supply.**

Courtesy of Elsevier, Inc., <http://www.sciencedirect.com>.
Used with permission.

D. Wolfram, J. Autoimmunity 23:81 (2004)

▲ Tenascin	} ECM proteins with high affinity for silicone
▲ Fibronectin	
▲ ICAM-1	
↕	Frontier layer formed by macrophages and fibroblasts
▲ HSP 60	expressed by stressed cells
● T-lymphocytes	CD4+ (CD25+ /CD45RO+)
● Dendritic cells	CD1a+ (CD207+ / CD208+ / CD209+)
● Macrophages	CD68
● Fibroblast	
↕	Layer of dense connective tissue (Actin+ smooth muscle cells)

UNCEMENTED GLENOID COMPONENT IN TOTAL SHOULDER ARTHROPLASTY

SURVIVORSHIP AND OUTCOMES

BY SCOTT DAVID MARTIN, MD, DAVID ZURAKOWSKI, PhD, AND THOMAS S. THORNHILL, MD
Department of Orthopedics, Brigham and Women's Hospital, Boston, Massachusetts

Photos removed due to
copyright restrictions.

White arrows:

**Radiolucencies due to
osteolysis associated with
particulate wear debris
and movement of the
prosthesis (loosening)**

- **What is the make-up of the periprosthetic tissue?**
- **Why is it so persistent?**

Fig. 1 Kaplan-Meier survival curves with clinical and radiographic failure as the end points.

Graph removed due to
copyright restrictions.

Fig. 3 Prevalence of radiolucent lines around the glenoid components.

Graph removed due to
copyright restrictions.

α -Smooth muscle actin-expressing cells and lubricin in periprosthetic tissue

Tadanao Funakoshi,^{1,2} Scott D. Martin,¹ Bryce T. Wolf,¹ Thomas M. Schmid,³
Thomas S. Thornhill,¹ Myron Spector^{1,2}

¹Department of Orthopaedic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115

²Tissue Engineering, VA Boston Healthcare System, Boston, Massachusetts 02130

³Rush University Medical Center, Chicago, Illinois

Abstract: The objective of the study was to evaluate the distributions of the following: (1) cells expressing the contractile actin isoform, α -smooth muscle actin (α -SMA) and (2) a lubricating and antiadhesion glycoprotein, lubricin, in the tissue around loose joint replacement prostheses in human subjects: periprosthetic tissue resected at revision arthroplasty of noncemented glenoid components of total shoulder arthroplasties from 10 patients. Samples of periprosthetic tissue were stained with monoclonal antibodies to α -SMA and lubricin. α -SMA was found in cells, principally of fibroblast morphology, in many of the fields of view (FOVs) in samples from all patients. Moderate correlations were observed between the percentage of FOVs containing α -SMA-expressing cells and the percentages of FOVs containing polyethylene ($R^2 = 0.79$) and metallic ($R^2 = 0.75$) particles. Lubricin was identified (1) as a discrete layer on the surface, (2) within the extracellular matrix, and (3) intracellularly. These

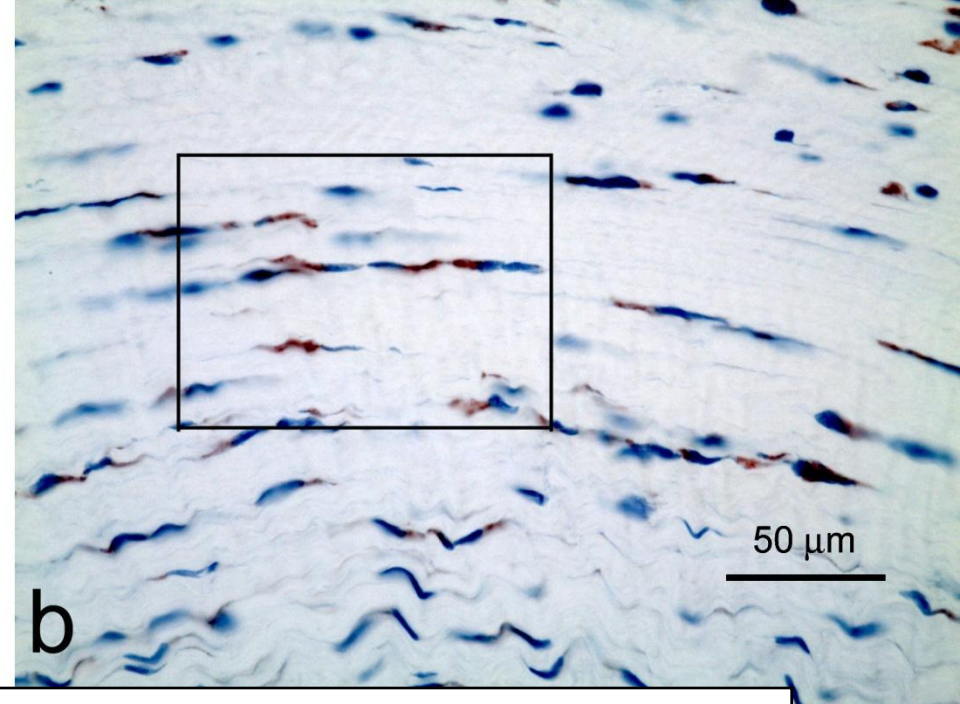
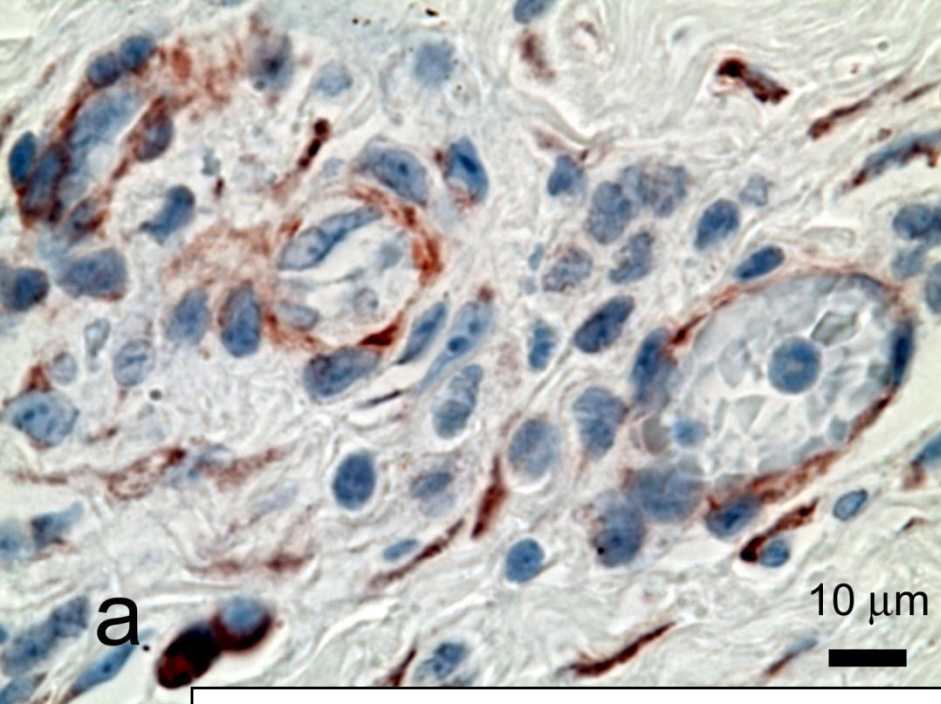
lubricin-positive features were found in samples from all patients. Strong correlations were noted between the percentage of FOVs with matrix and intracellular lubricin staining ($R^2 = 0.97$) and between the percentages of FOVs with surface and matrix staining for lubricin ($R^2 = 0.96$). Having established the presence of α -SMA and lubricin in periprosthetic tissue, hypotheses regarding their role in the development and persistence of periprosthetic tissue can be synthesized for future study, for example, α -SMA-enabled contracture of the fibrous periprosthetic tissue results in its densification, and lubricin-coated surfaces interfere with integrative repair processes necessary for resorption and remodeling. © 2009 Wiley Periodicals, Inc. J Biomed Mater Res 00A: 000–000, 2009

Key words: periprosthetic tissue; lubricin; smooth muscle actin; prosthesis

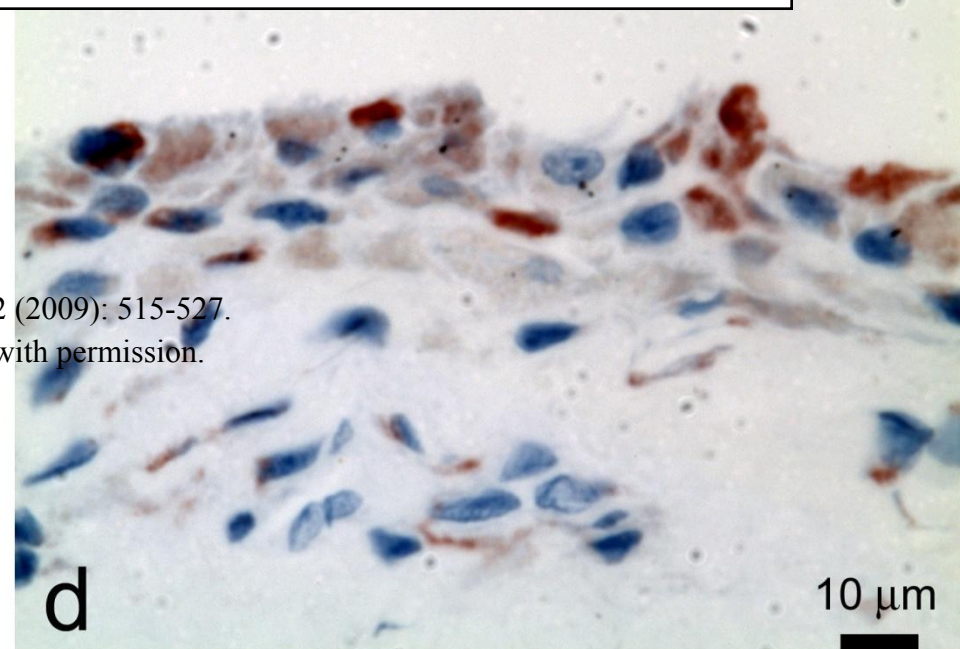
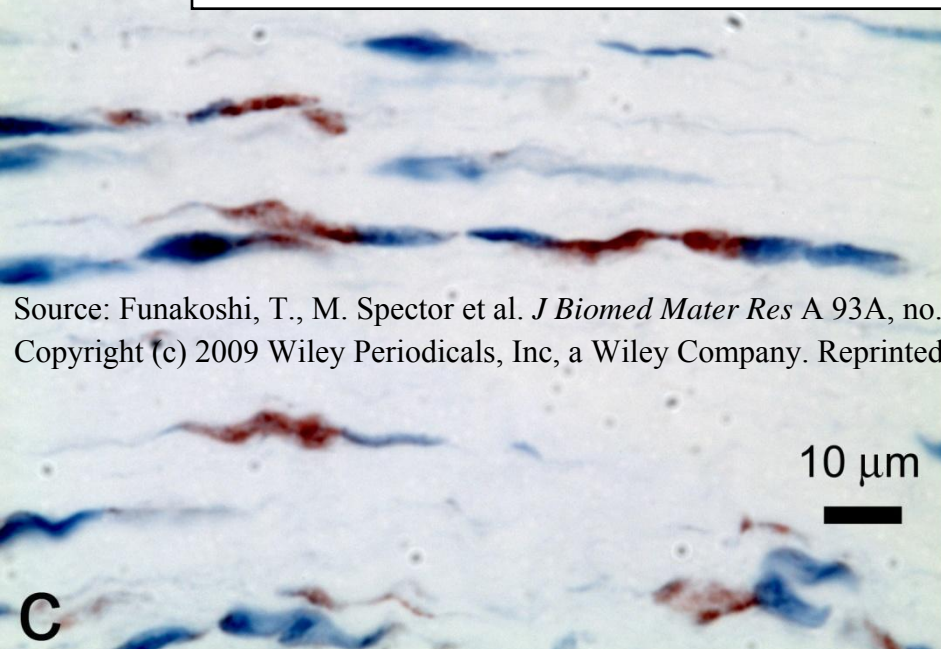
Shoulder

Tissue was resected during revision of symptomatic, non-cemented, glenoid components of Kirschner-IIc total shoulder arthroplasty



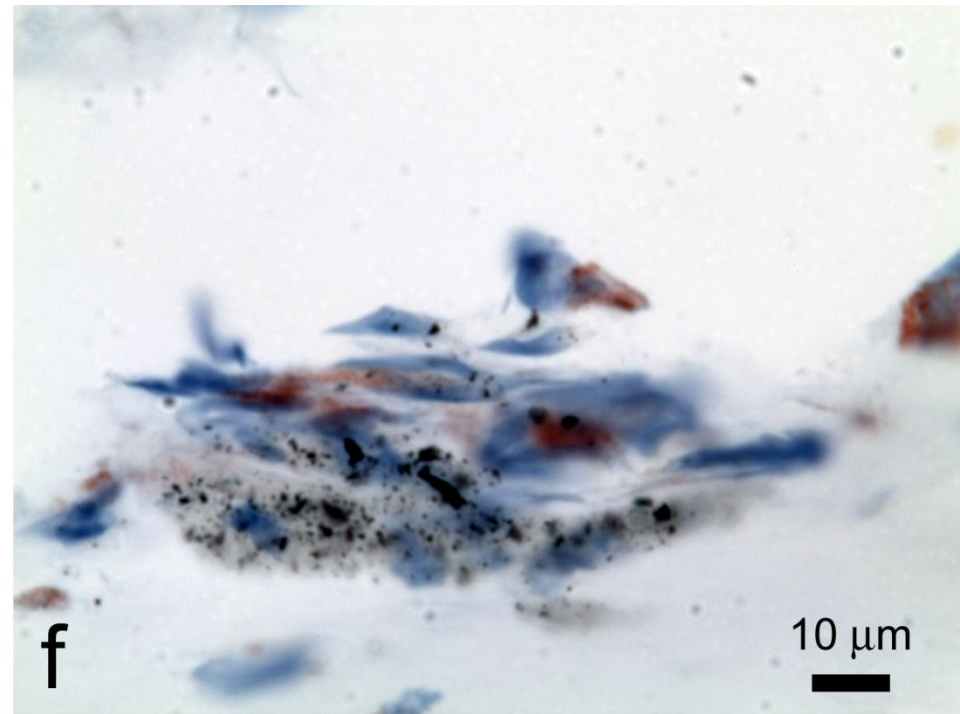
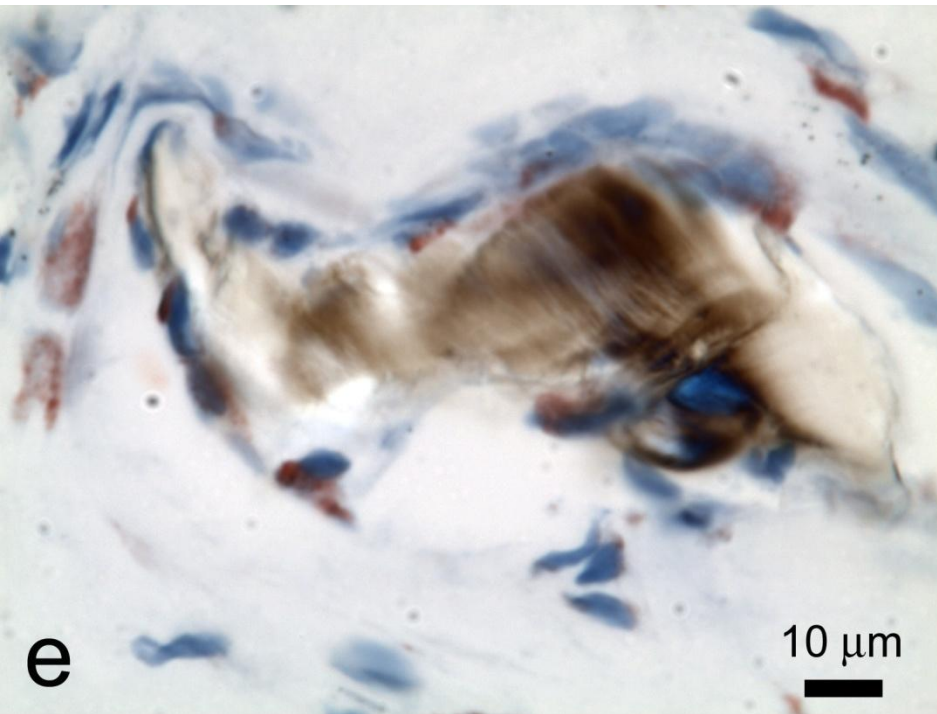


α -Smooth Muscle Actin Immunohistochemistry



Source: Funakoshi, T., M. Spector et al. *J Biomed Mater Res A* 93A, no. 2 (2009): 515-527.
Copyright (c) 2009 Wiley Periodicals, Inc, a Wiley Company. Reprinted with permission.

α -Smooth Muscle Actin Immunohistochemistry



Source: Funakoshi, T., M. Spector et al. *J Biomed Mater Res A* 93A, no. 2 (2009): 515-527.

Copyright (c) 2009 Wiley Periodicals, Inc, a Wiley Company. Reprinted with permission.

The Synovial-Like Membrane at the Bone-Cement Interface in Loose Total Hip Replacements and Its Proposed Role in Bone Lysis*

BY STEVEN R. GOLDRING, M.D.†, ALAN L. SCHILLER, M.D.†, MERRILEE ROELKE, M.S.†, CAROL M. ROURKE†,
DONALD A. O'NEILL, M.D.‡, AND WILLIAM H. HARRIS, M.D.†, BOSTON, MASSACHUSETTS

*From the Departments of Medicine, Orthopaedic Surgery, Pathology, and Medical Services (Arthritis Unit),
Massachusetts General Hospital and Harvard Medical School, and New England Deaconess Hospital, Boston*

This membrane, rather than being a nondescript so-called fibrous membrane, has the histological and histochemical characteristics of a synovial-like lining. The synovial-like cells are adjacent to the cement layer. Deep to them macrophages predominate. Inflammatory cells are absent. Cell cultures of this membrane contain stellate cells similar to those found in cell cultures of normal and rheumatoid synovial tissue. This membrane has the capacity to produce large amounts of prostaglandin E₂ and collagenase.

Four photos removed due to copyright restrictions.

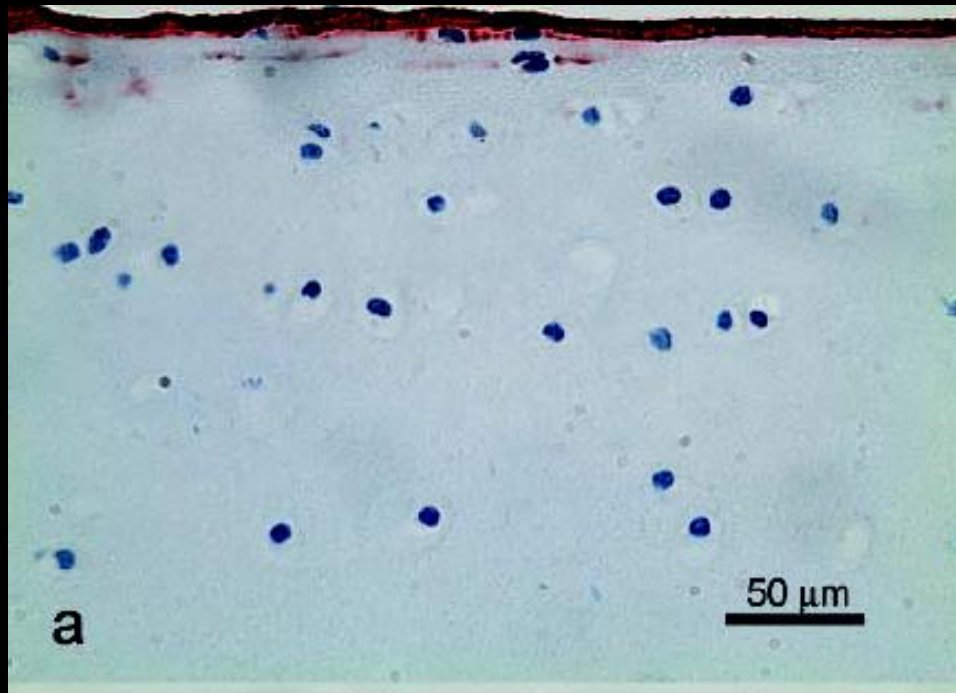
**S. Goldring, *et al.*,
J. Bone Joint Surg. 65A:575 (1983)**

Note the absence of any basement membrane

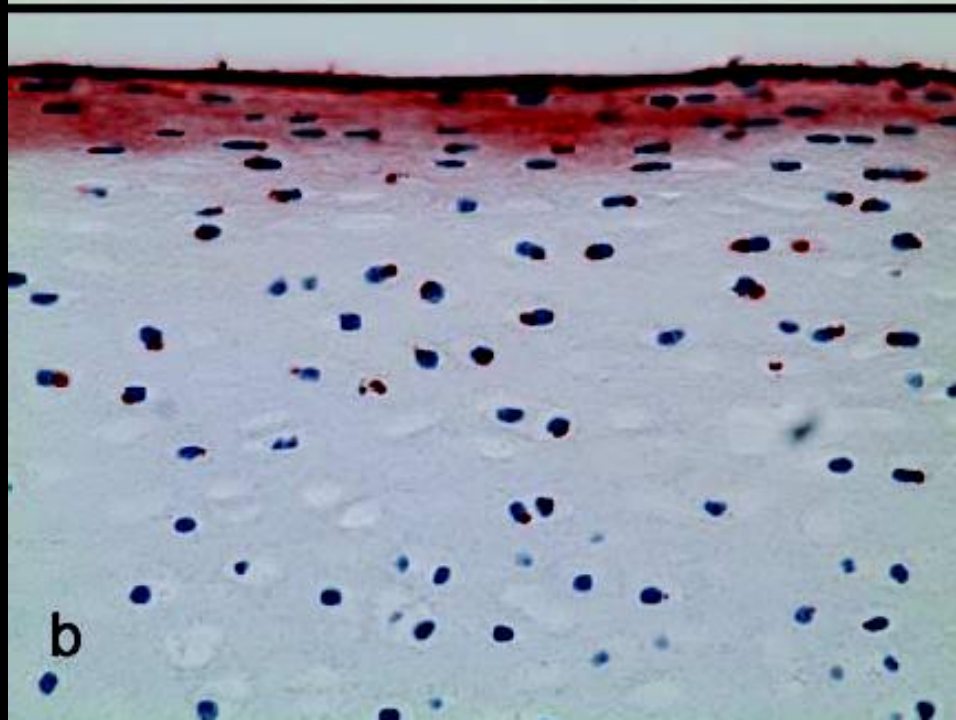
Lubricin/Superficial Zone Protein/PRG4

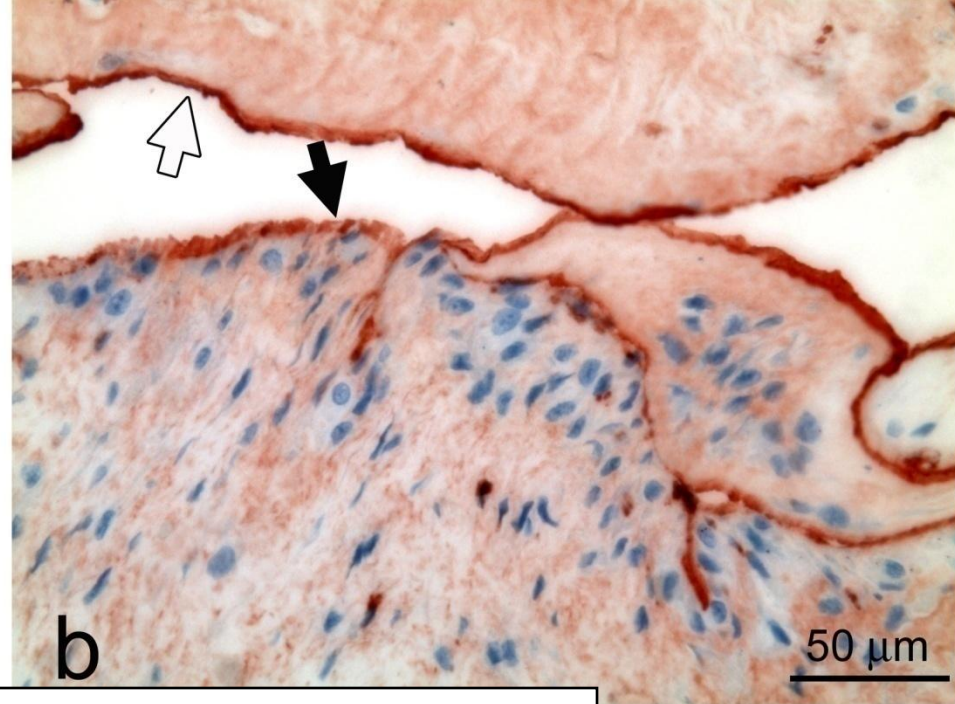
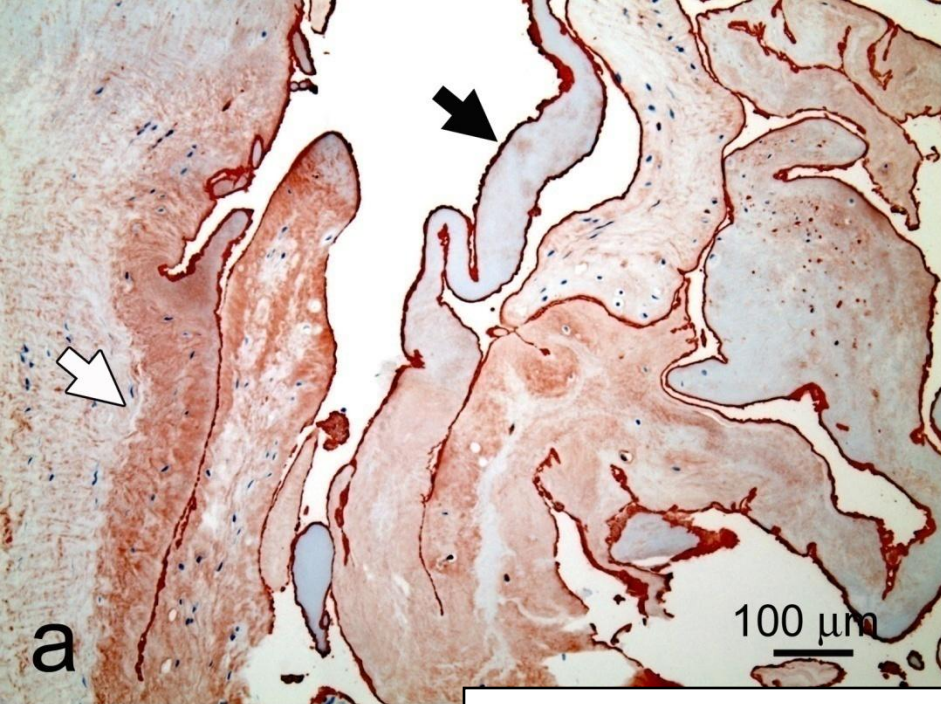
- A glycoprotein synthesized by synovial cells and identified in synovial fluid was found to provide the principal boundary lubrication for articular cartilage of joints (Swann 1977).
- Later a protein synthesized by chondrocytes in the superficial zone of articular cartilage (named superficial zone protein; Schumacher 1994) was found to be homologous with lubricin.
- *Prg4* is the name given to the gene that was found to encode these homologous glycoproteins (Ikegawa 2000).
- Proteins with various structures, properties and functions may result from this same gene as a result of alternative exon splicing during gene expression, and post translational modification of the protein.
 - Four such splice variants have been found for human and mouse.
- Lubricin/SZP has been found on the surface of meniscus, and in tendons and ligaments.

Adult Bovine

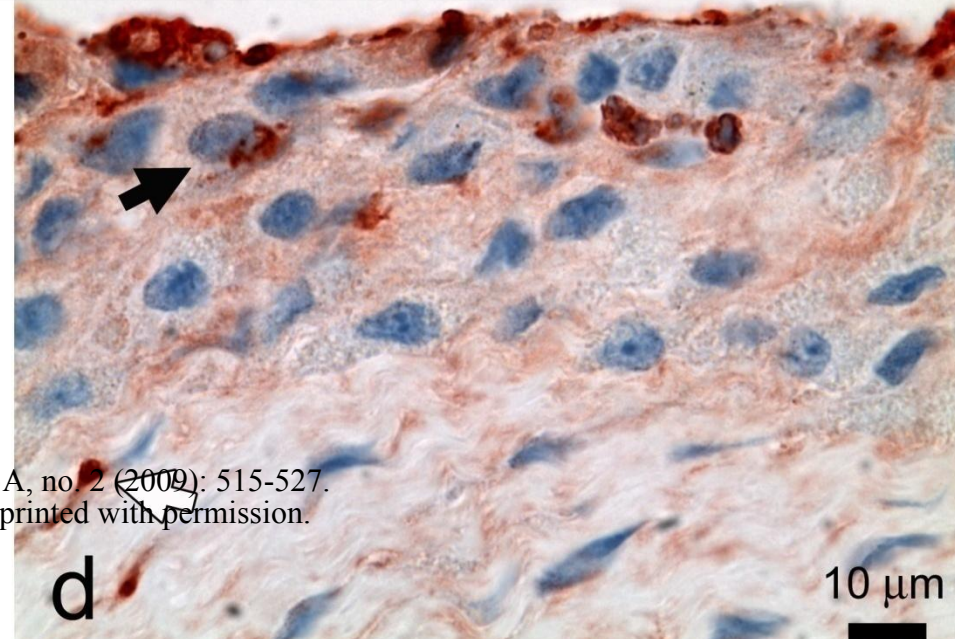
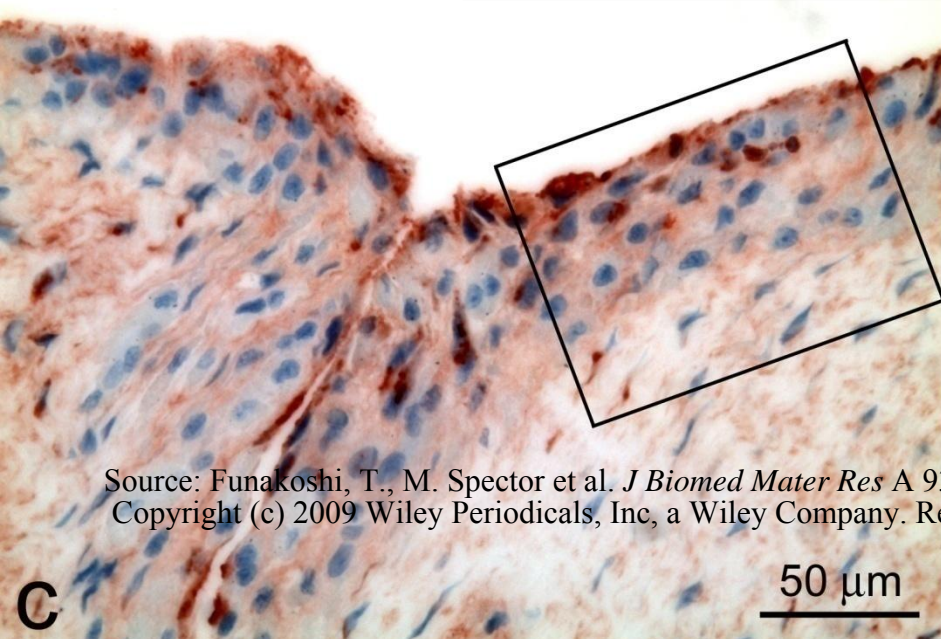


Calf

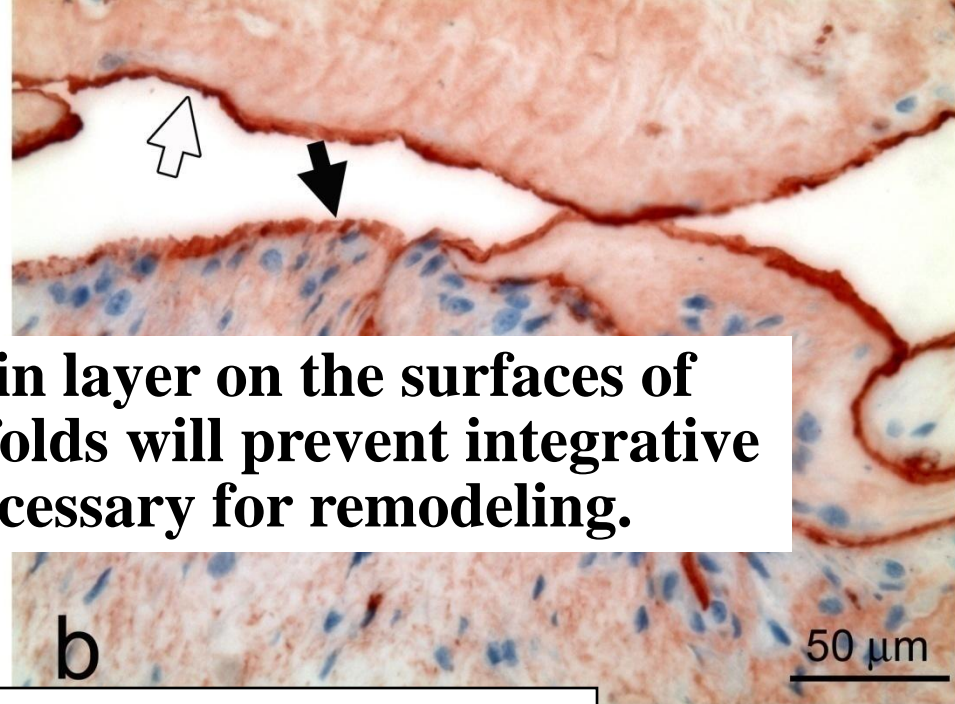
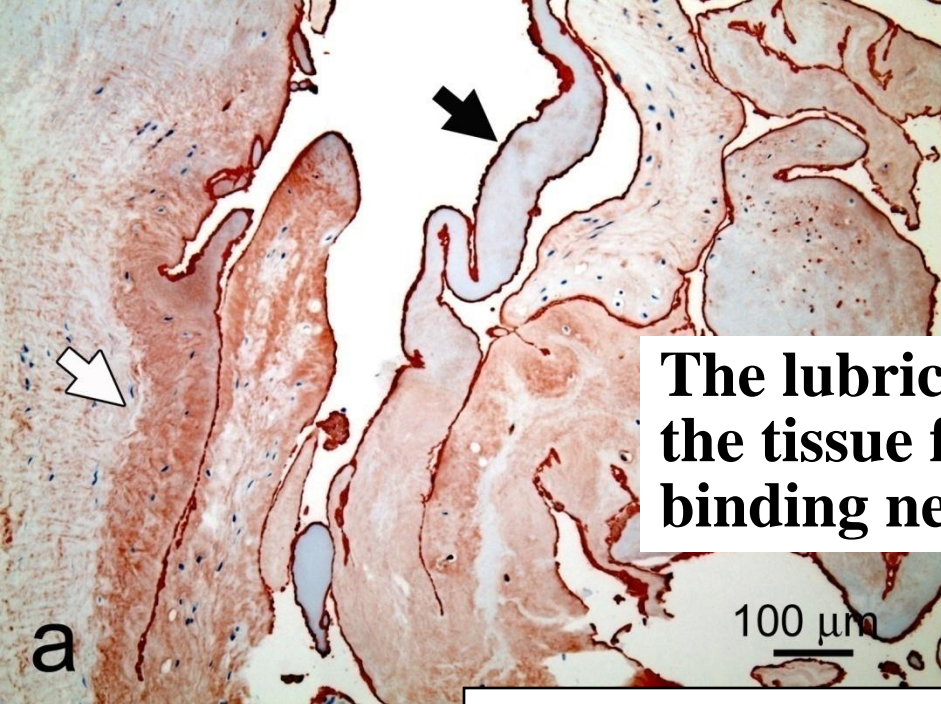




Lubricin Immunohistochemistry

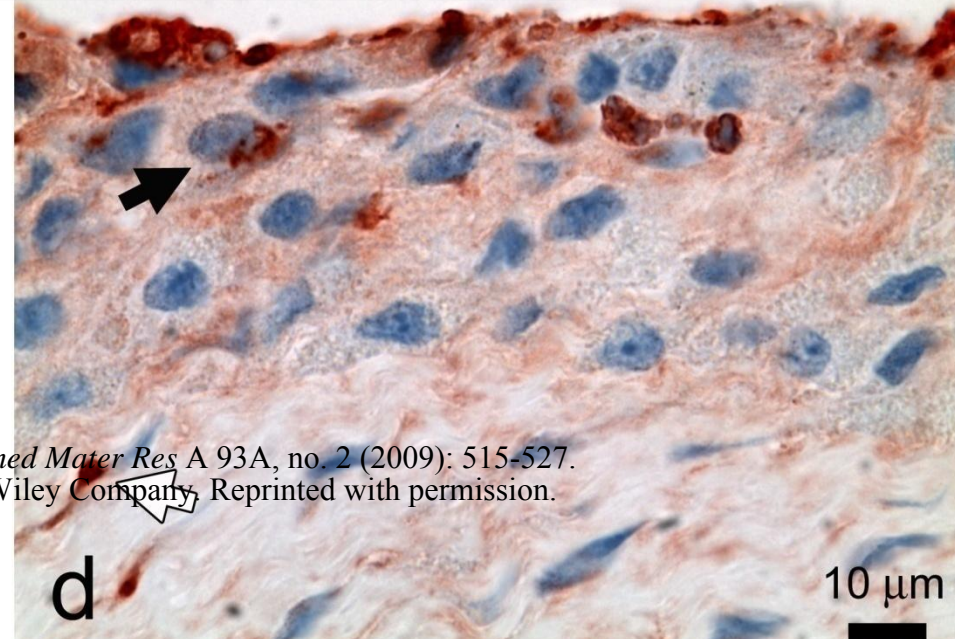
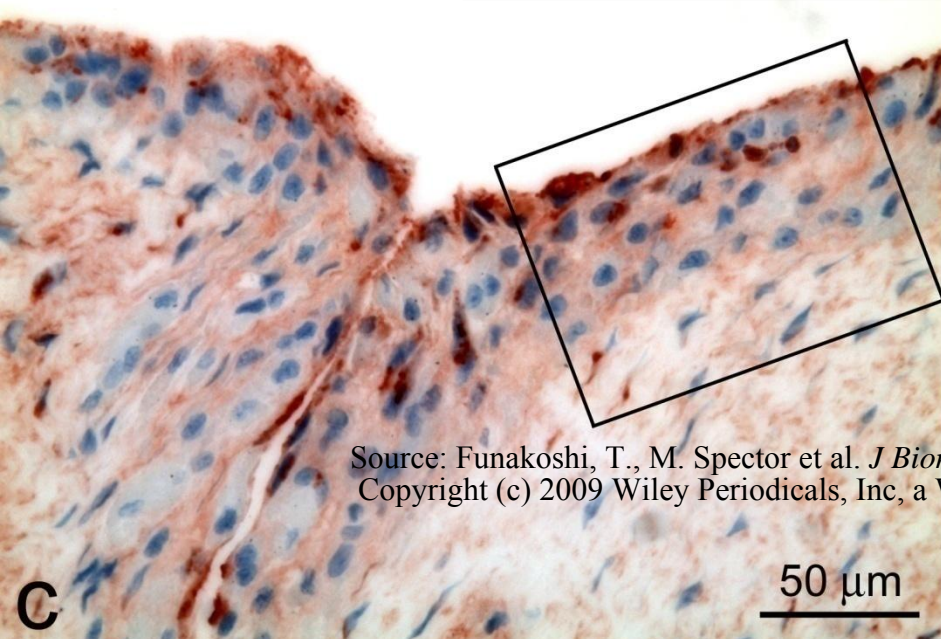


Source: Funakoshi, T., M. Spector et al. *J Biomed Mater Res A* 93A, no. 2 (2009): 515-527.
Copyright (c) 2009 Wiley Periodicals, Inc, a Wiley Company. Reprinted with permission.

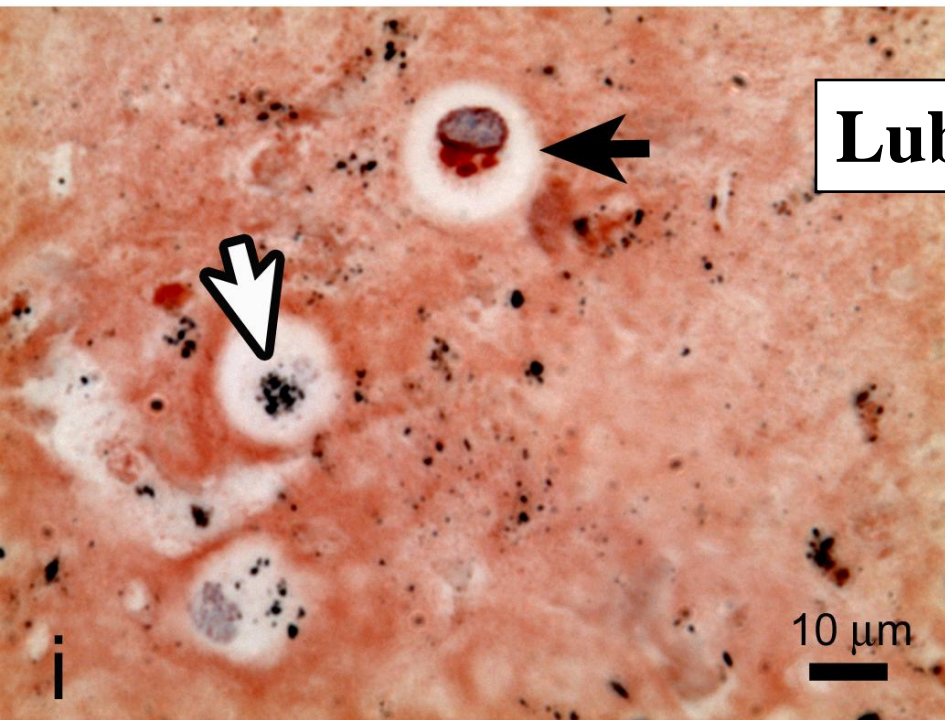
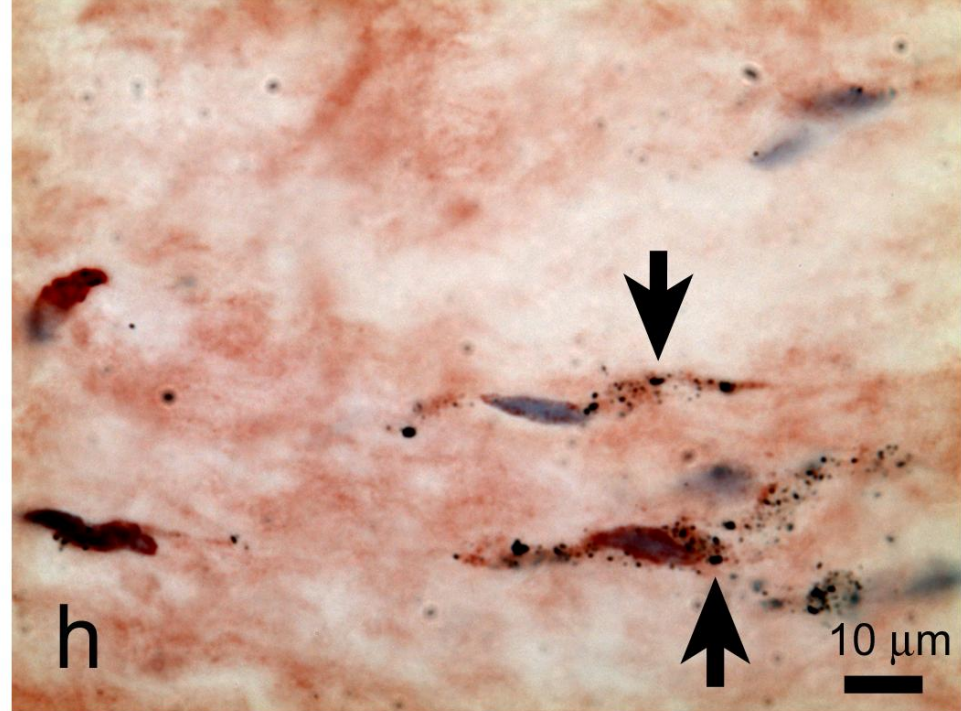
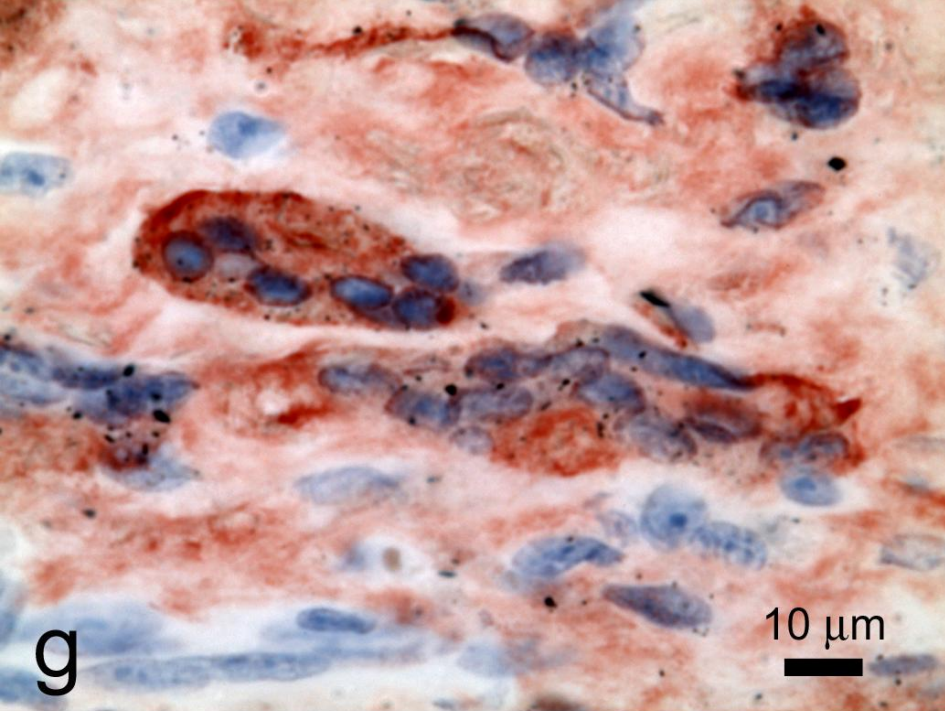


The lubricin layer on the surfaces of the tissue folds will prevent integrative binding necessary for remodeling.

Lubricin Immunohistochemistry



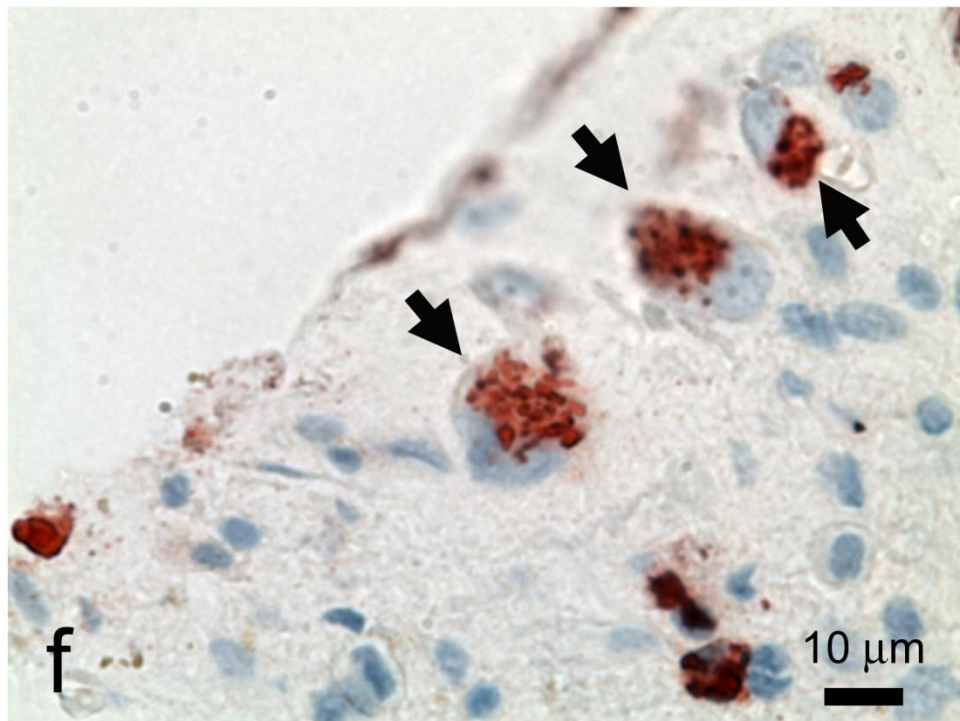
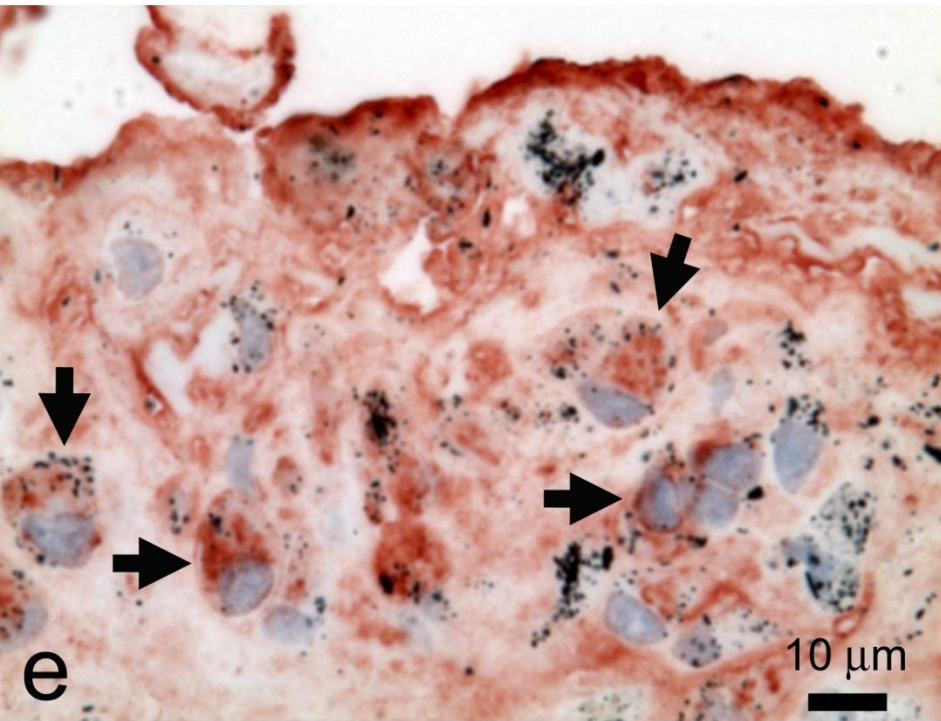
Source: Funakoshi, T., M. Spector et al. *J Biomed Mater Res A* 93A, no. 2 (2009): 515-527.
Copyright (c) 2009 Wiley Periodicals, Inc, a Wiley Company. Reprinted with permission.



Lubricin Immunohistochemistry

Source: Funakoshi, T., M. Spector et al. *J Biomed Mater Res A* 93A, no. 2 (2009): 515-527. Copyright (c) 2009 Wiley Periodicals, Inc, a Wiley Company. Reprinted with permission.

Lubricin Immunohistochemistry



Source: Funakoshi, T., M. Spector et al. *J Biomed Mater Res A* 93A, no. 2 (2009): 515-527. Copyright (c) 2009 Wiley Periodicals, Inc, a Wiley Company. Reprinted with permission.

CHRONIC RESPONSE TO IMPLANTS IN SOFT TISSUE *

- Persistence of macrophages* at the implant surface due to dead space /hypoxia and presence of fibroblasts* results in **synovium**
- Proliferation and increased matrix synthesis of fibroblasts can result from mechanical perturbation by the implant or by agents released by the implant, leading to an increase in the thickness and density of the scar tissue under the control of macrophages.

* and in bone in cases where bone formation has been prevented

Theory to Explain α -SMA and Lubricin in Periprosthetic Tissue

- The micromovement of a prosthesis stimulates the expression of α -SMA and lubricin
 - directly as a result of mechanical stimulation of selected cells
 - indirectly by inducing macrophages to release elevated levels of TGF- β , which up-regulates α -SMA and lubricin
- Myofibroblast (*i.e.*, α -SMA expressing fibroblast) contraction activates latent TGF- β 1 from extracellular matrix.
- Particulate debris further stimulates the release of TGF- β by macrophages during phagocytosis.

Effects of α -SMA and Lubricin on Prosthetic Performance

- **Myofibroblast contraction** can result in scar contracture and densification of the fibrous tissue.
- The “**synoviocytes**” **express lubricin** which can interfere with healing; remodeling of the scar tissue.

MIT OpenCourseWare
<http://ocw.mit.edu>

20.441J / 2.79J / 3.96J / HST.522J Biomaterials-Tissue Interactions
Fall 2009

For information about citing these materials or our Terms of Use, visit: <http://ocw.mit.edu/terms>.