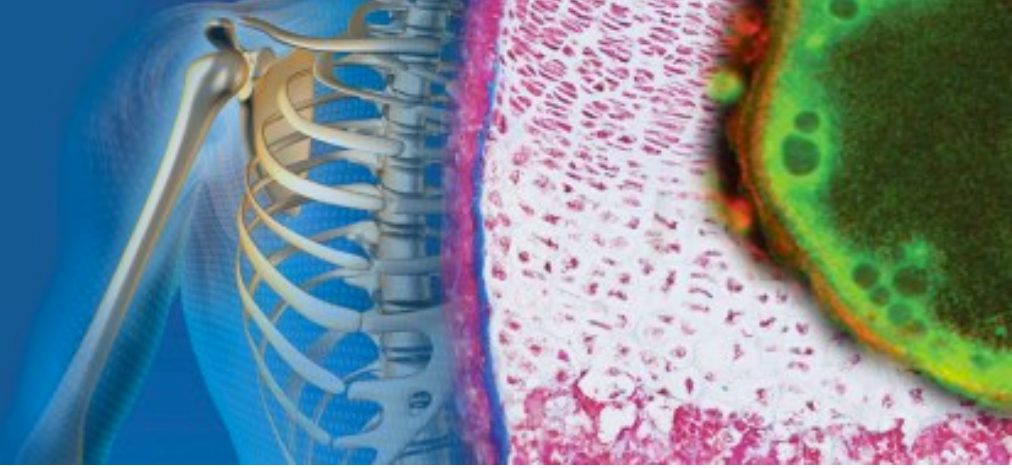
CENTER *for* MUSCULOSKELETAL RESEARCH



Tendon Homeostasis, Tendinopathy and Healing

Alayna E. Loiselle, PhD

CMSR T32 Musculoskeletal Basic Science Course

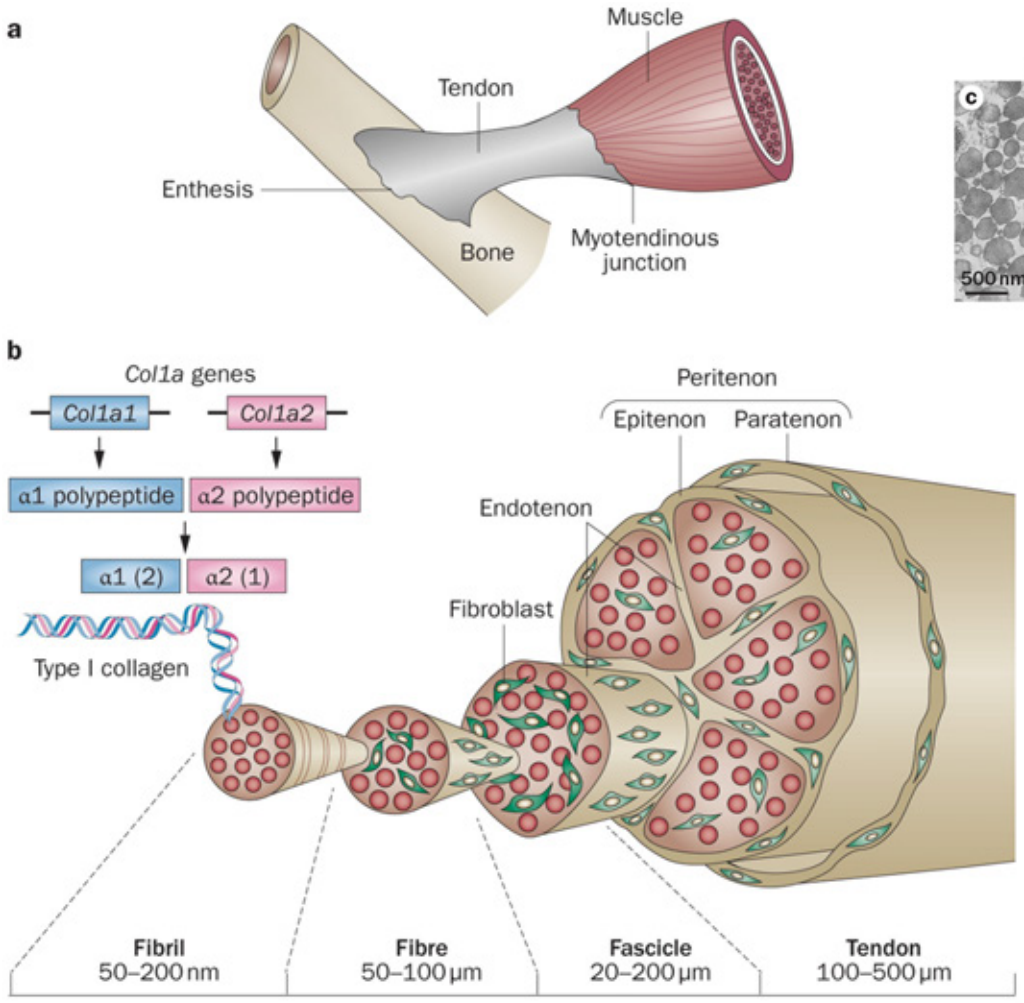
April 17th, 2017

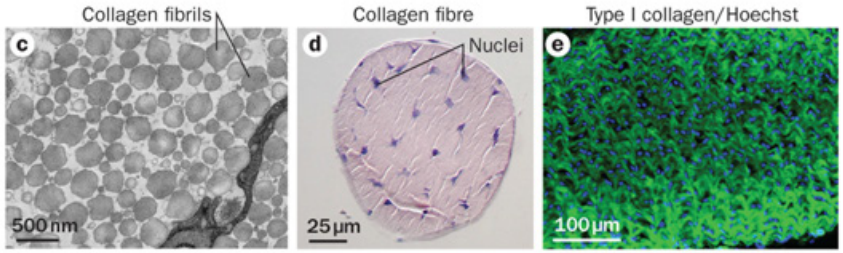
Outline

- Overview of tendon structure/ function
- Effects of Aging
- Disruptions in homeostasis
- Pathogenesis of tendinopathy/ tendinitis
- Mechanisms of regeneration and healing

JOR: New Frontiers in Tendon Research
June 2015
7 Review Articles
15 Primary Research Papers

Tendon Structure & Function





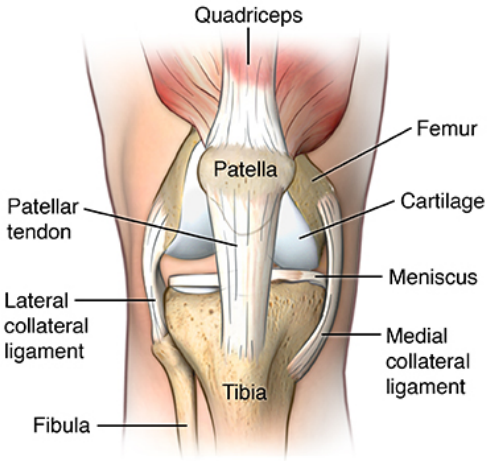
Nature Reviews | Rheumatology

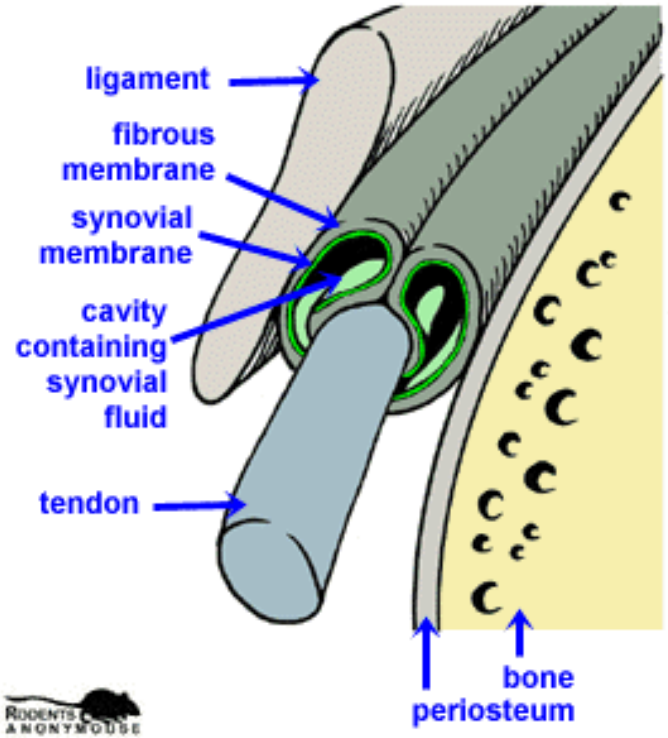
- Transmission of forces from muscle to bone
- Movement of nearly the entire skeleton
- Crimp structure increases elasticity
- Hierarchical nature allows force conduction disproportionate to size

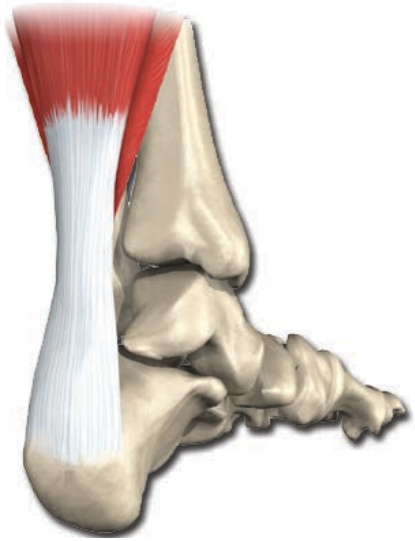
Nourissat, G. *et al.* (2015) Tendon injury: from biology to tendon repair
Nat. Rev. Rheumatol. doi:10.1038/nrrheum.2015.26

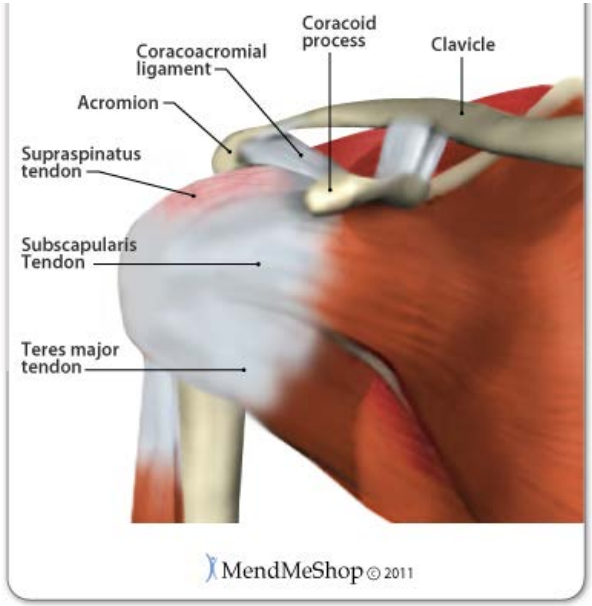
Types of Tendon

- Positional vs. energy storing
- Intrasynovial vs. extrasynovial



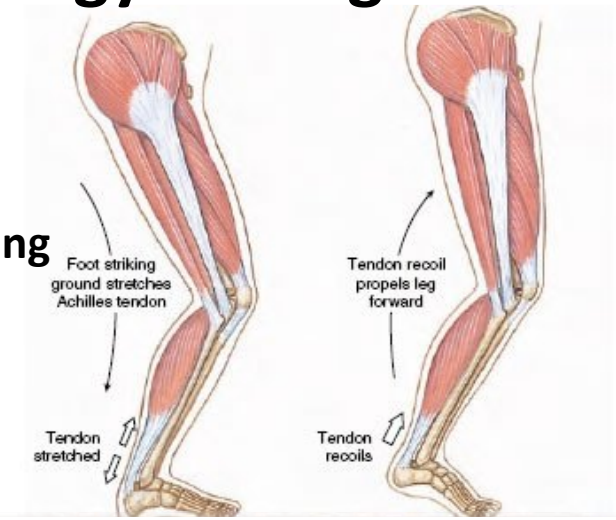






Energy Storing Potential

Humans store 20% during running



Quora.com

Animal Models of Tendinopathy & Healing

Supraspinatus (rotator cuff)

Over 30 animals characterized[1]

most common:
Rat
Mouse

Achilles Tendon

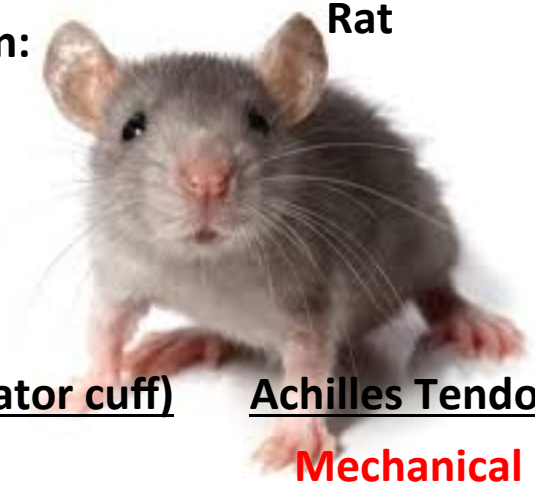
Mouse
Rat

Patellar Tendon

Mouse
Rat
Rabbit

Flexor Tendon

Mouse
Canine
Chicken
Rat
Rabbit
Horse
Pig



Supraspinatus (rotator cuff)

Mechanical

Full thickness, partial width
Tendon to bone healing

Achilles Tendon

Mechanical
chemical

Complete transection
+/- repair
Partial transection
Biopsy punch

Patellar Tendon

Fatigue loading

Biopsy punch

Flexor Tendon

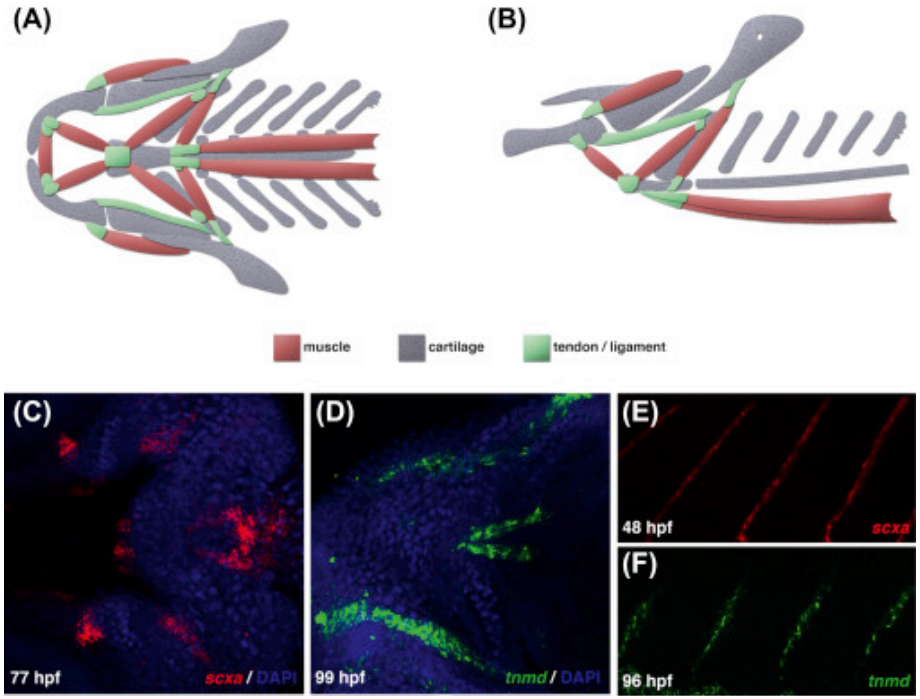
Complete transection
+ repair
Partial transection
Biopsy punch

Tendinopathy

Healing

(1) Soslowsky LJ, et al., J Shoulder Elbow Surg 1996;5:383–392
(2) Thomopoulos et al., JOR. 2015 (review)

Zebrafish!



JW Chen and JL Galloway. Methods in Cell Biology, Volume 138, 2017, 299–320

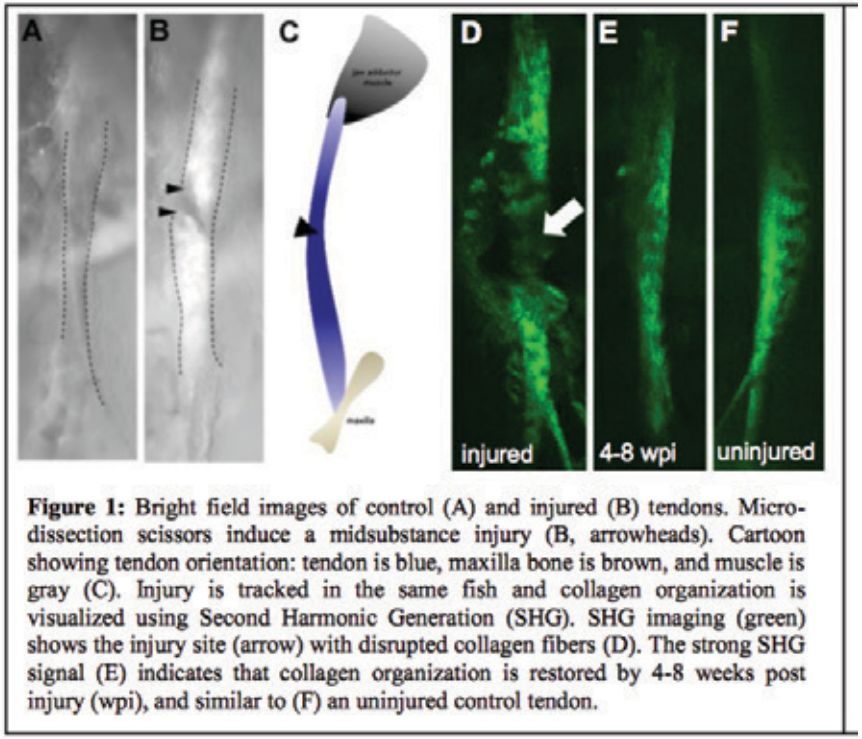
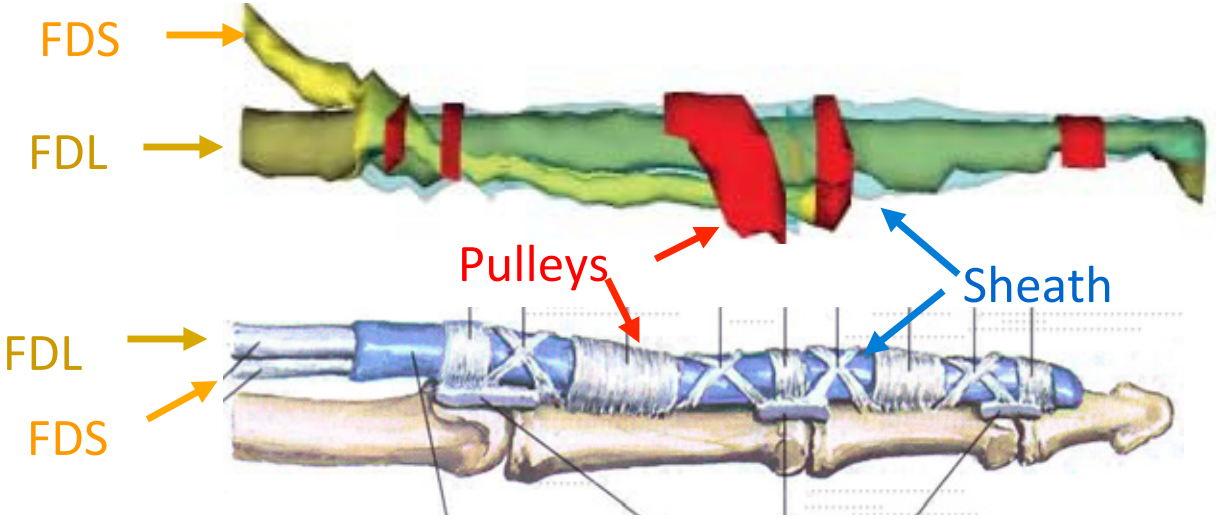


Figure 1: Bright field images of control (A) and injured (B) tendons. Micro-dissection scissors induce a midsubstance injury (B, arrowheads). Cartoon showing tendon orientation: tendon is blue, maxilla bone is brown, and muscle is gray (C). Injury is tracked in the same fish and collagen organization is visualized using Second Harmonic Generation (SHG). SHG imaging (green) shows the injury site (arrow) with disrupted collagen fibers (D). The strong SHG signal (E) indicates that collagen organization is restored by 4-8 weeks post injury (wpi), and similar to (F) an uninjured control tendon.

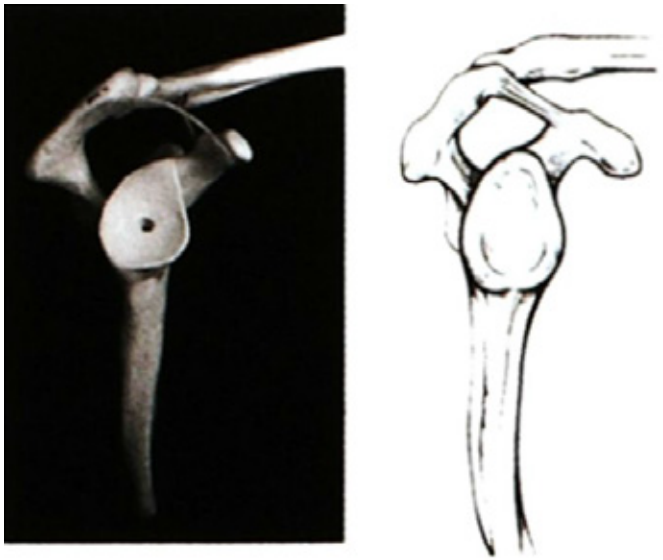
2017 ORS abstract from Galloway Lab

Comparative Anatomy

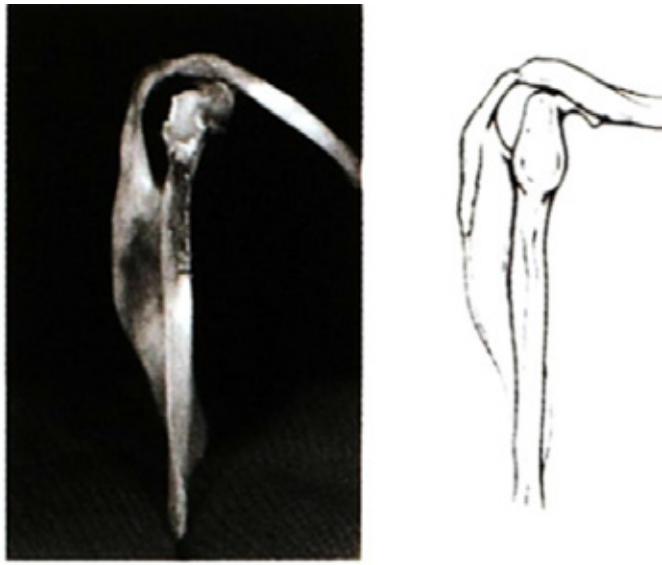


Mouse

Human



Human

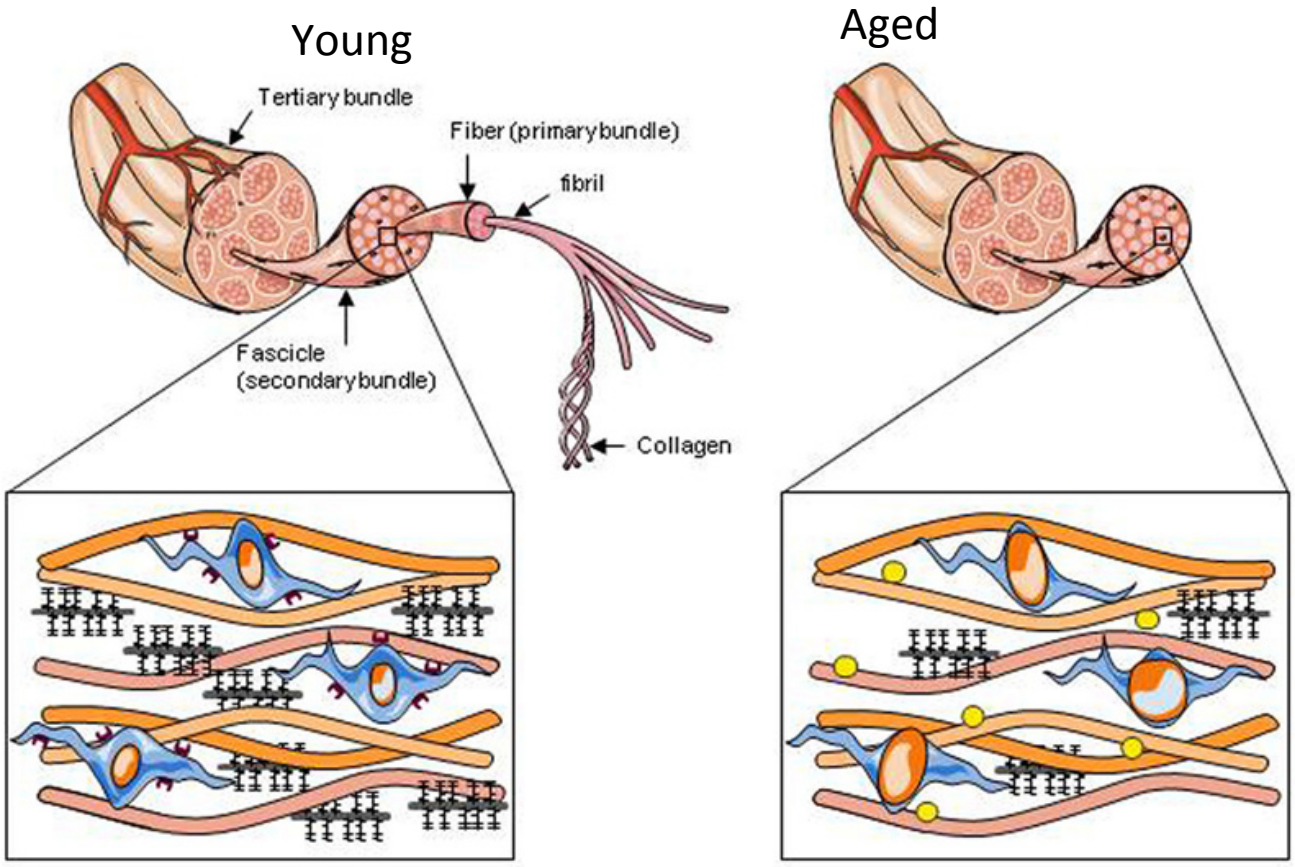


Rat

Wong et al. 2006. J. Anatomy

Hast et al., 2014 Bone & Joint

Tendon Homeostasis



- Matrix composition and organization is not dramatically different
- Decreased proteoglycan content
- Changes in cell morphology

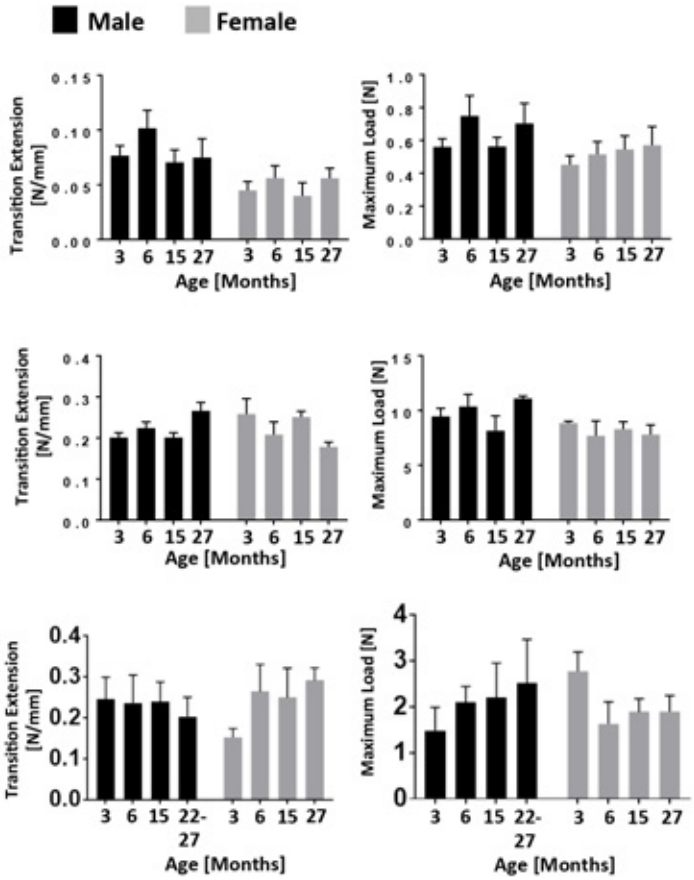
Functional Consequences of Aging?

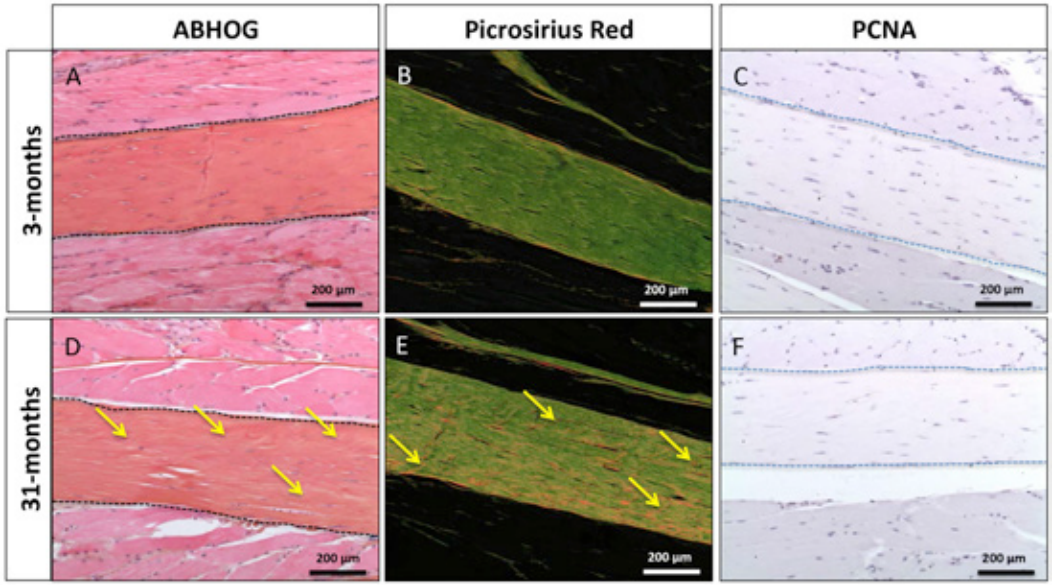
Tendon is not mechanically sensitive to aging

TT Fascicle

FDL

FCU





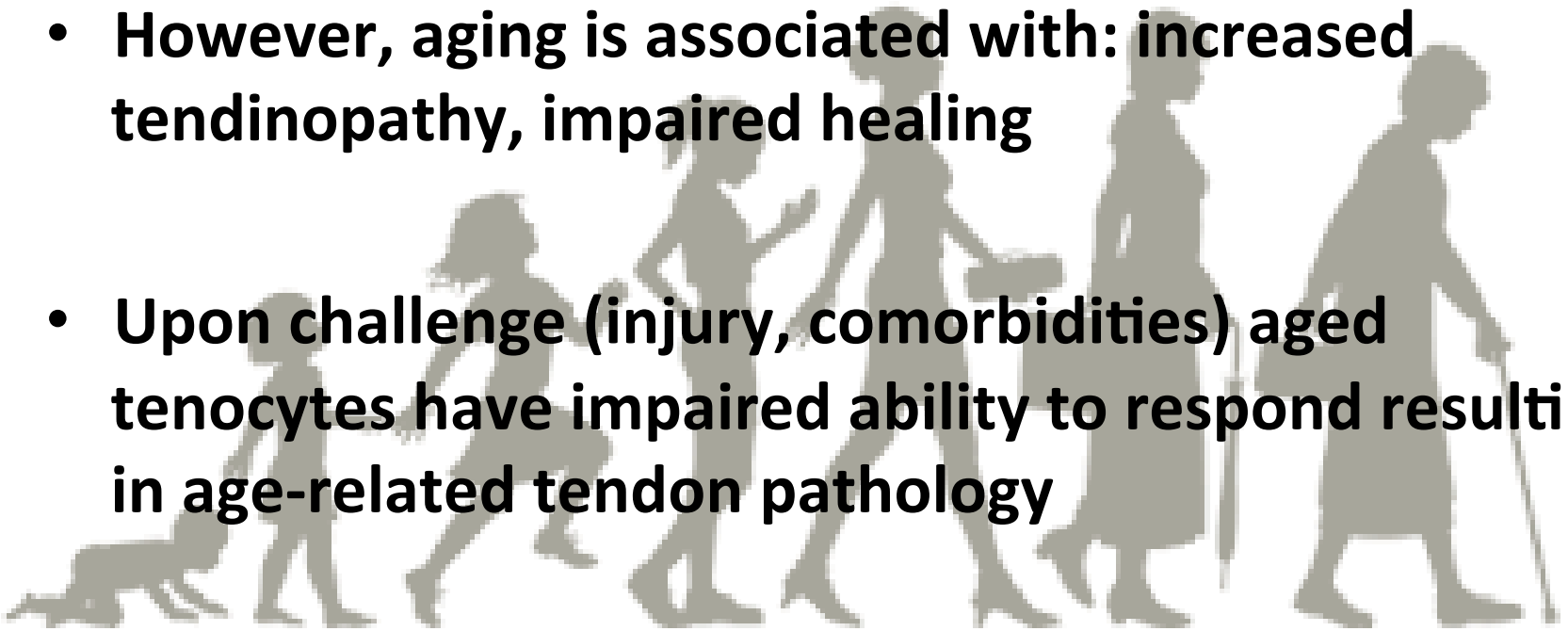
Ackerman et al., 2017. JOR

Tendon & Aging

- Tenocyte quiescence and low-frequency ECM turnover= decreased sensitivity to aging (vs. bone)

- However, aging is associated with: increased tendinopathy, impaired healing

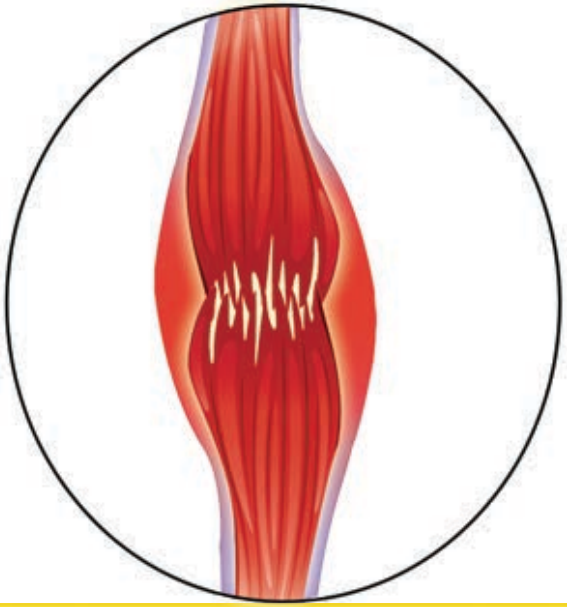
- Upon challenge (injury, comorbidities) aged tenocytes have impaired ability to respond resulting in age-related tendon pathology



Tendinopathy

Disease of the tendon

- Painful
- Exacerbated by activity
- Most common in the Achilles
- Tendonitis: acute inflammation and injury



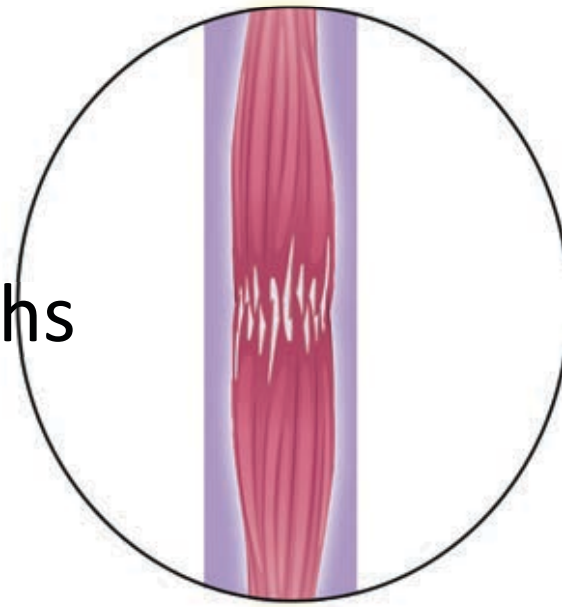
Scivet.com

Tendinopathy

Disease of the tendon

- Painful
- Exacerbated by activity

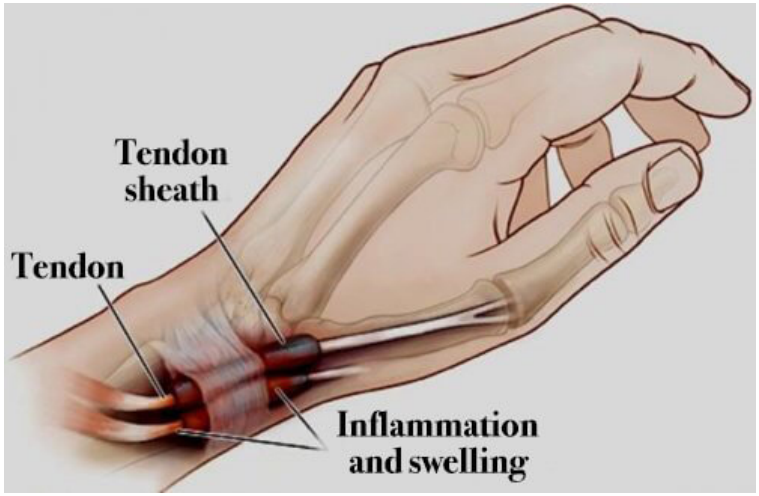
- Tendinosis: chronic with degenerative cellular changes, no inflammation
- More common than tendinitis
- Continuing for longer than 6 months

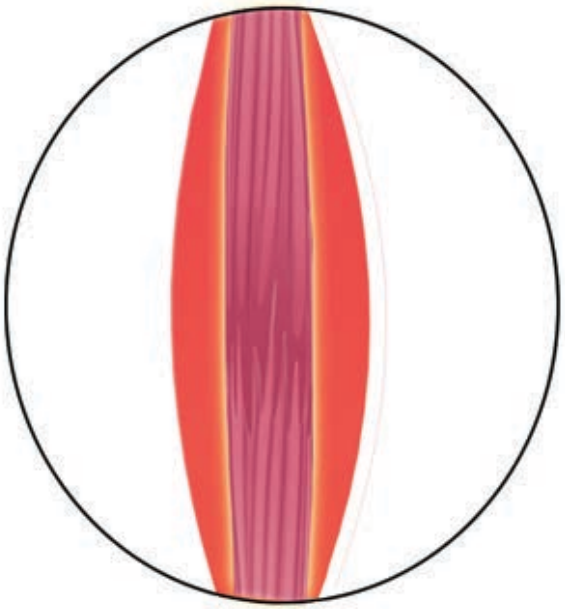


Scivet.com

Tenosynovitis

- Inflammation of the sheath
- Causes: inflammatory diseases, infection, injury
- Most commonly in hand/ wrist

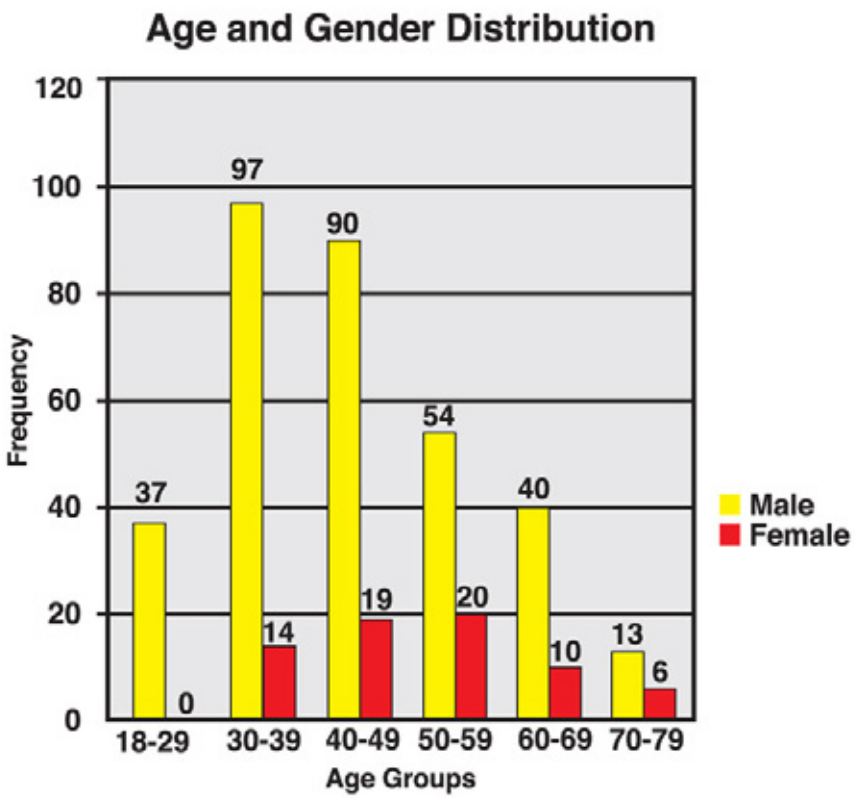




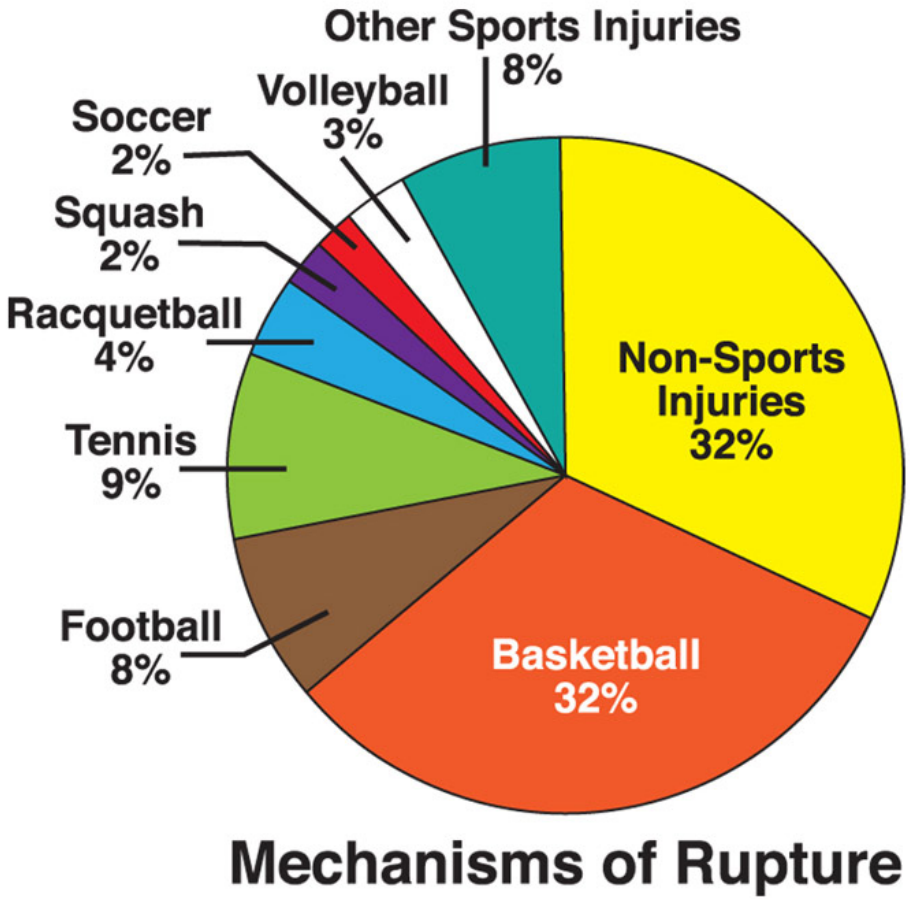
Scivet.com

Tendon Rupture

Achilles is most susceptible



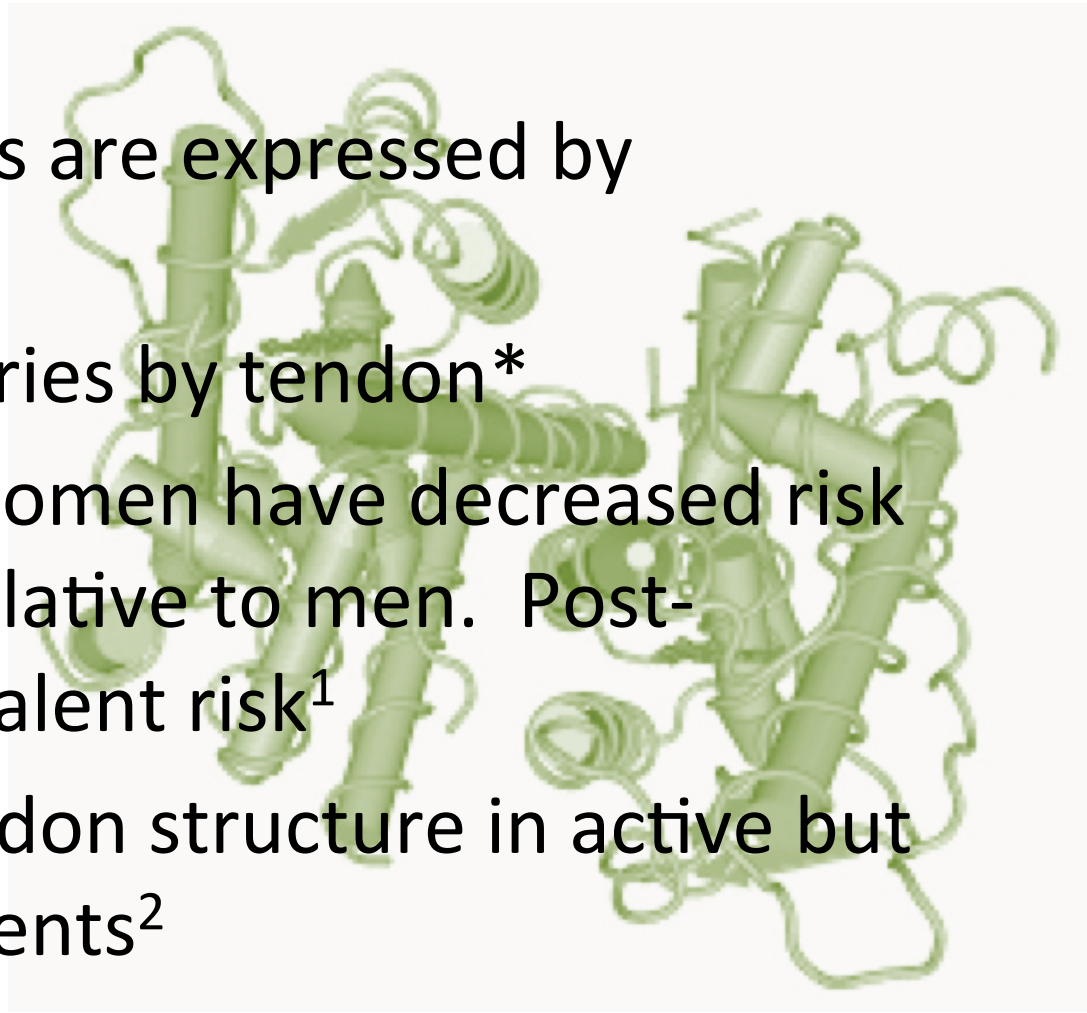
Sex-based differences



Mechanisms of Rupture

SR Raikin. Lower extremity Review. 2014

Disrupted Homeostasis: Hormonal Effects

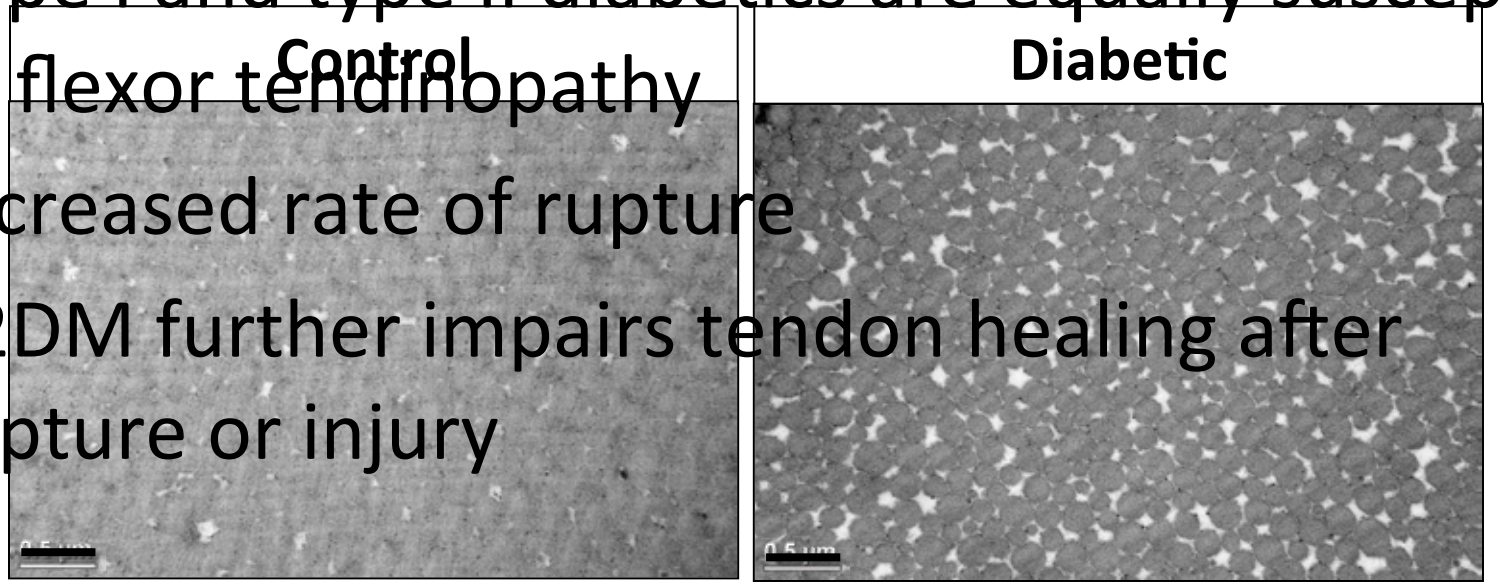


- Estrogen Receptors are expressed by tenocytes[1]
- OVX: sensitivity varies by tendon*
- Pre-menopausal women have decreased risk of tendinopathy relative to men. Post-menopause: equivalent risk[1]
- HRT: improves tendon structure in active but not sedentary patients[2]

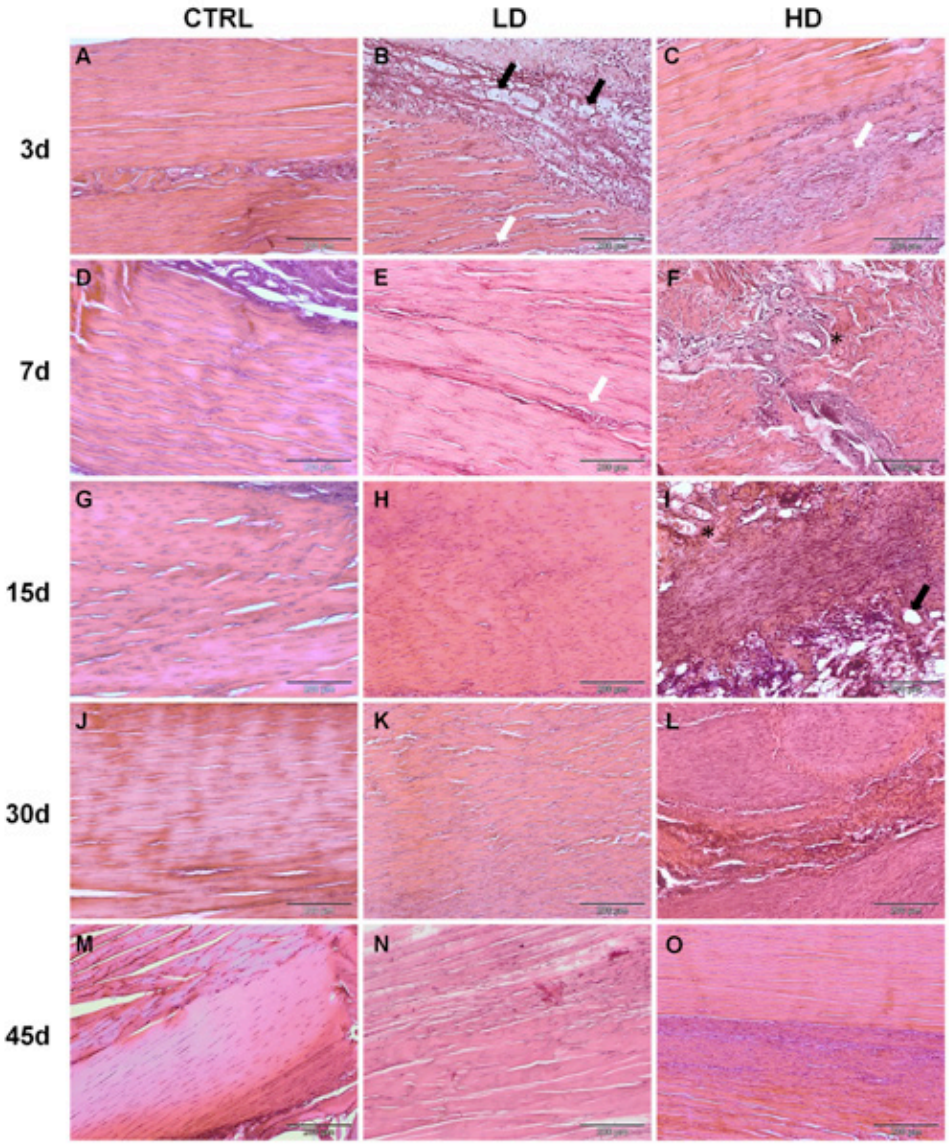
[1] Reviewed in Frizziero A et al. MLTJ 2014, [2]Cook JL et al. Scand J of Med & Sci in Sports. 2006

Disrupted Homeostasis: Diabetes

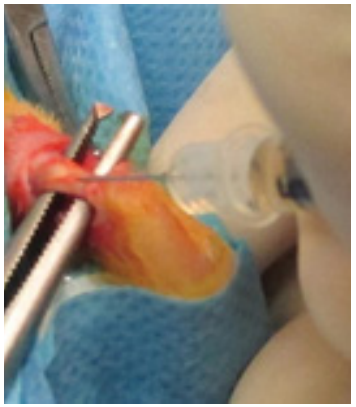
- Sensitivity varies by tendon*
- Flexors are most sensitive
- Pathological changes increase with disease duration
- Type I and type II diabetics are equally susceptible to flexor tendinopathy
- Increased rate of rupture
- T2DM further impairs tendon healing after rupture or injury



Collagenase Induced Tendinopathy



dation



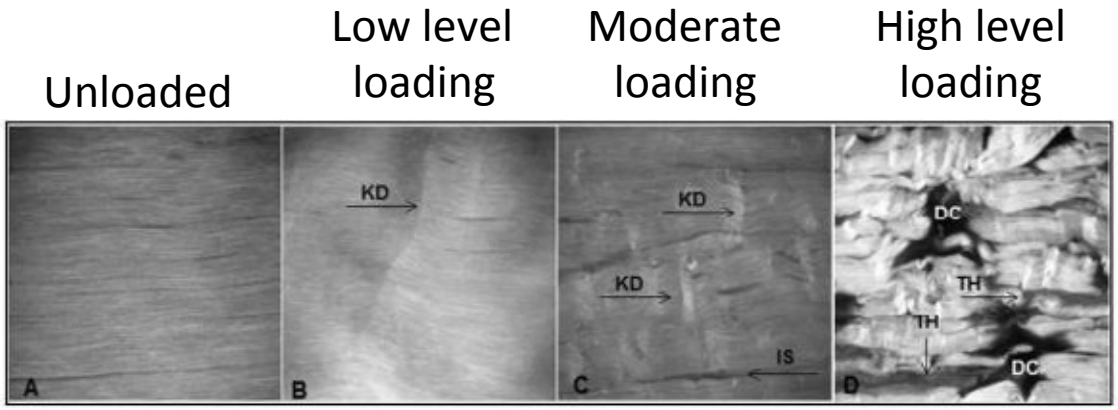
LD= low dose
HD= high dose collagenase

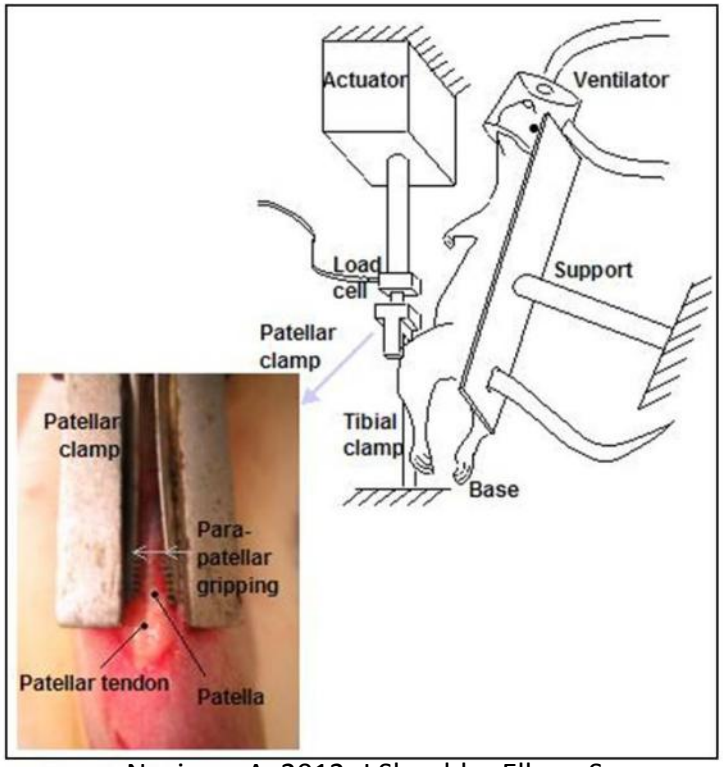
Orfei C et al. (2016) PLOS ONE 11(8): e0161590.

Mechanically Induced Tendinopathy

- Fatigue loaded under anaesthesia
- Uphill treadmill running
- Downhill treadmill running

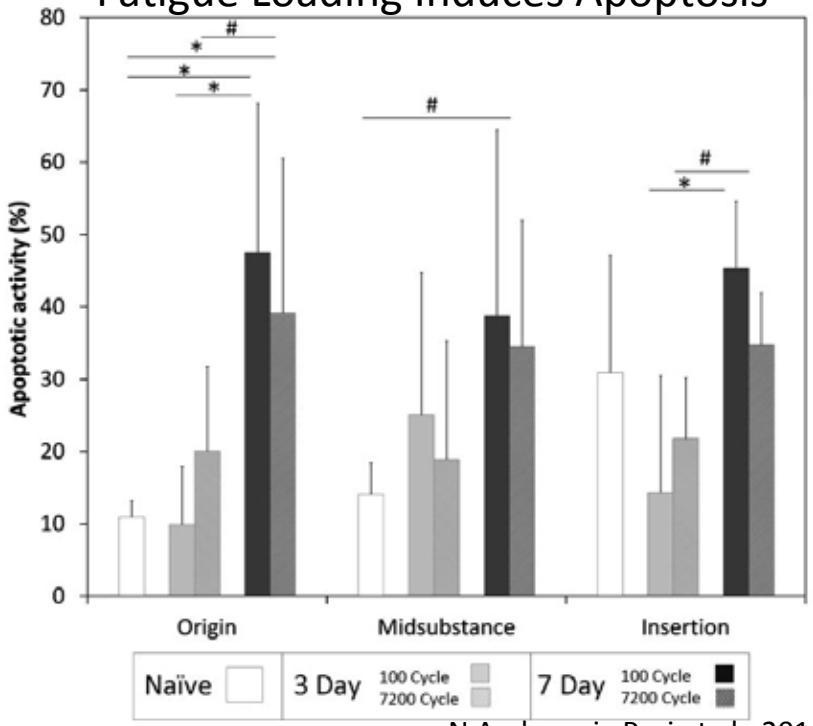
Induces matrix and cellular changes





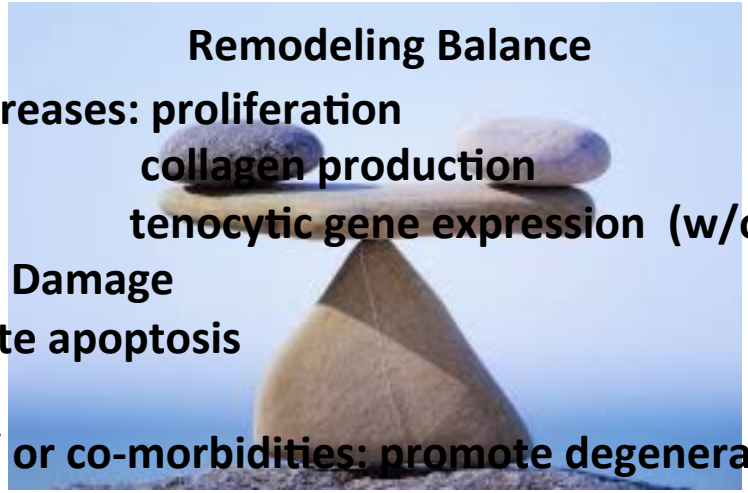
Neviaser A. 2012. J Shoulder Elbow Surg.

Fatigue Loading Induces Apoptosis



N Andarawis-Puri et al., 2014. JOR

Summary of Tendinopathy



Remodeling Balance

Physiological exercise increases: proliferation
collagen production
tenocytic gene expression (w/o chondro/ osteo/adipo)

Overuse/ Fatigue: Matrix Damage
Tenocyte apoptosis

Effects of Pathology and/ or co-morbidities: promote degeneration +/- inflammation
Smoking
obesity
high cholesterol

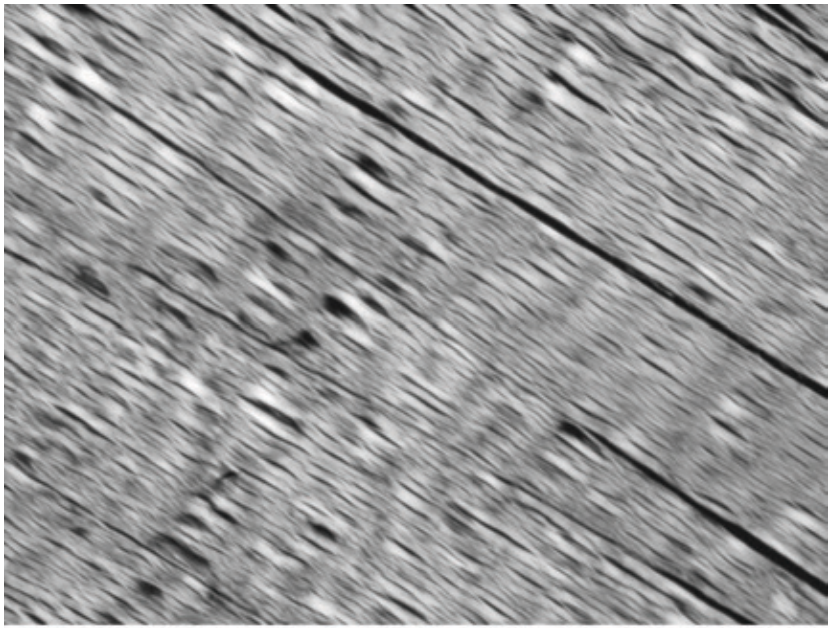
Future Directions

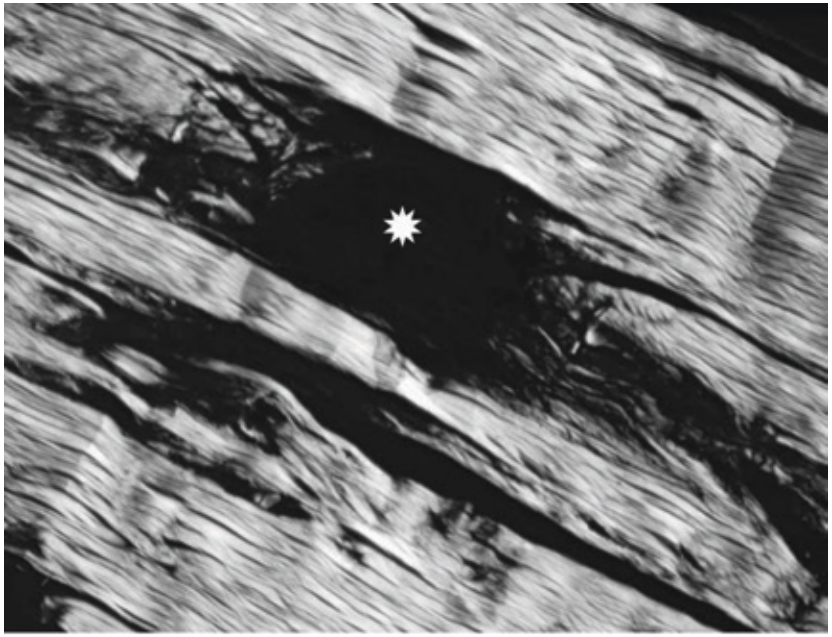
- **Continue to identify co-morbidities that predispose or accelerate tendinopathy**
- **Most clinical data are from late stage pathology**
- **Beginning to use genetic animals models to better understand tendinopathy**

Tendon Healing

- ~300,000 tendon repair procedures per year
- Over $20 Billion in associated health care costs
- Healing is complicated by scar formation

Normal tendon	Scar Tissue

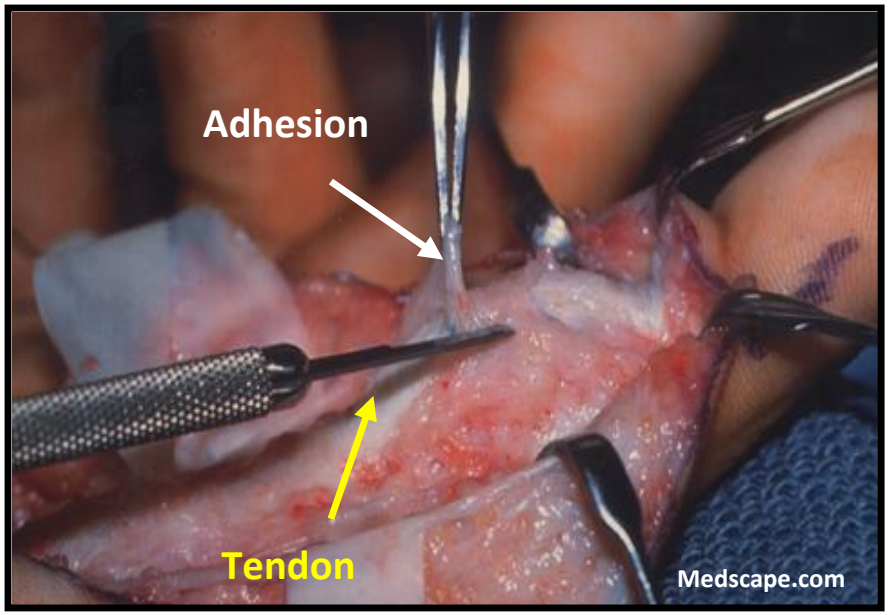




de Jong 2014; Pennisi 2002; Beredjiklian 2003; Defranco 2004

Complications of Flexor Tendon Healing

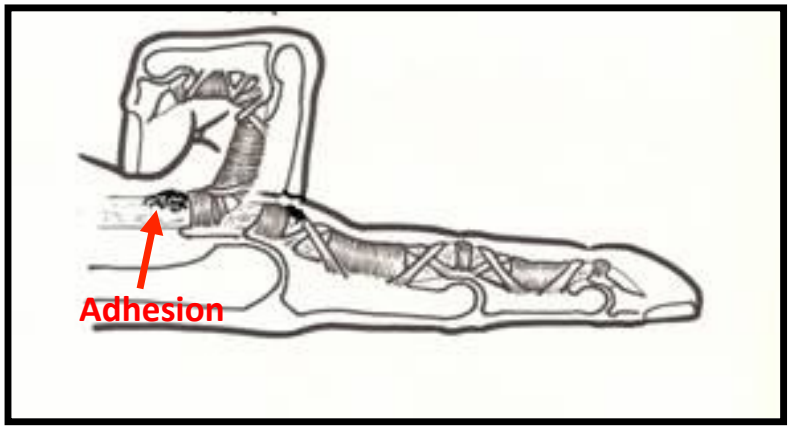
- Flexor and extensor tendons of the hand are the most commonly injured tendons



- Fibrotic Healing:
  Exuberant ECM deposition attaches tendon to synovial sheath and surrounding tissues

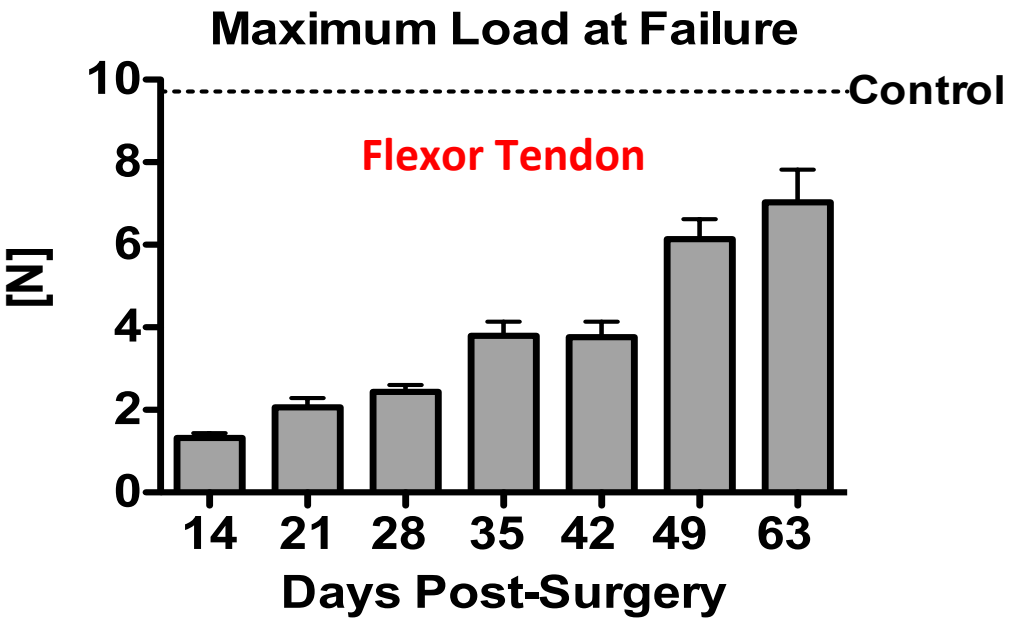
- Healing of flexor tendons is complicated by ROM-limiting scar in up to 40% of repairs

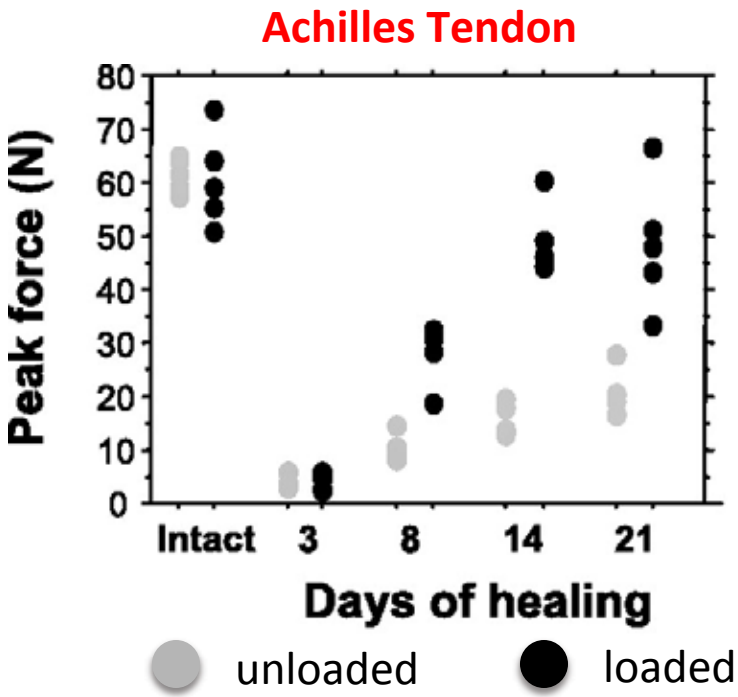
- Often require a secondary surgical procedure to lyse adhesions



Centers for Disease Control and Prevention. (2015, January).
Strickland, J.W. *et al.*, J Hand Surg [Am] **25**(2): p.214-235, (2000).
Aydin, A. *et al.*, AOTT **28**(1): p.54-9, (2004).

Acquisition of Mechanical Properties

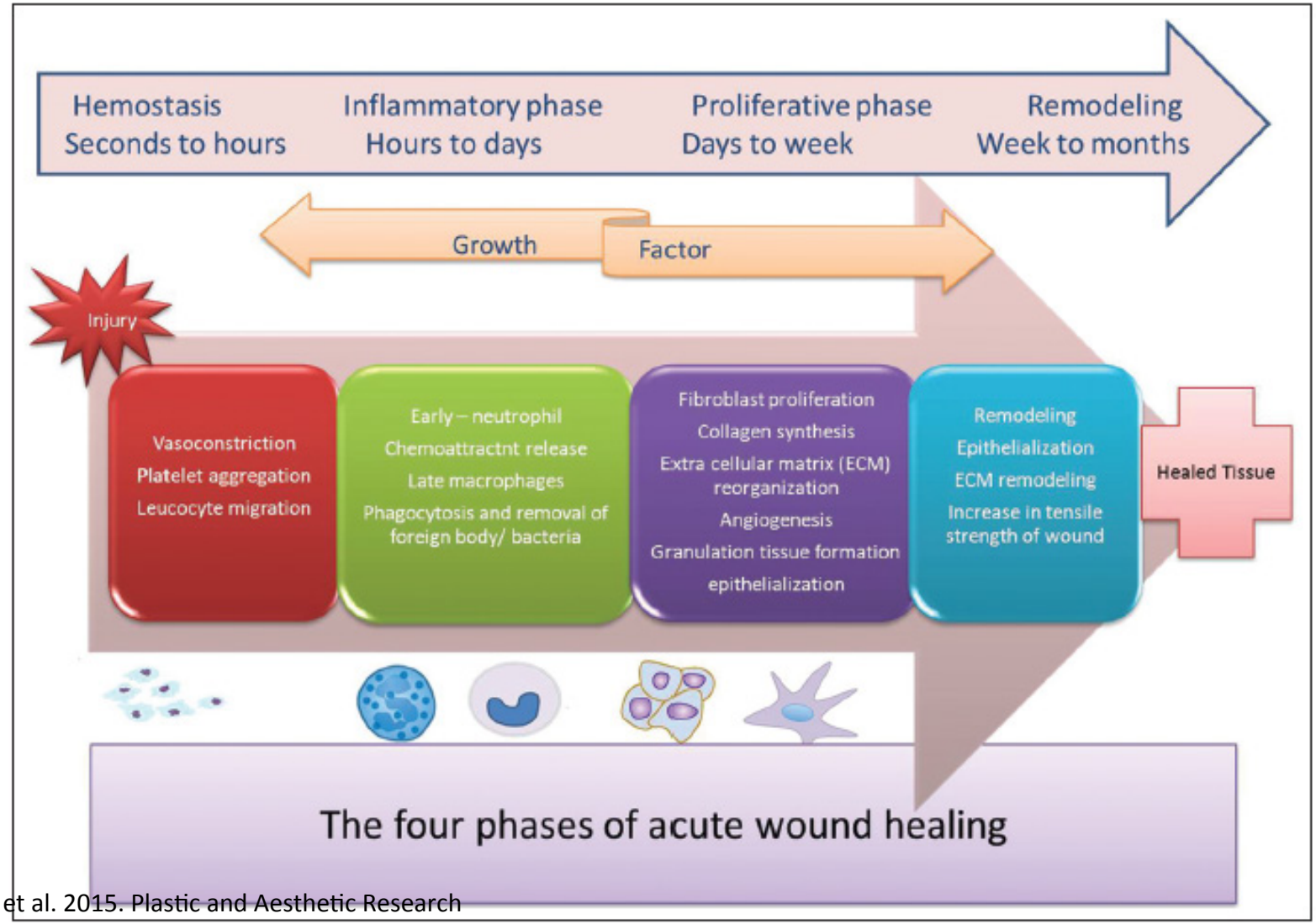




Loiselle et al., 2009. JOR

P Eliasson et al., 2009. J Appl Phys.

Similarities between wound and tendon healing

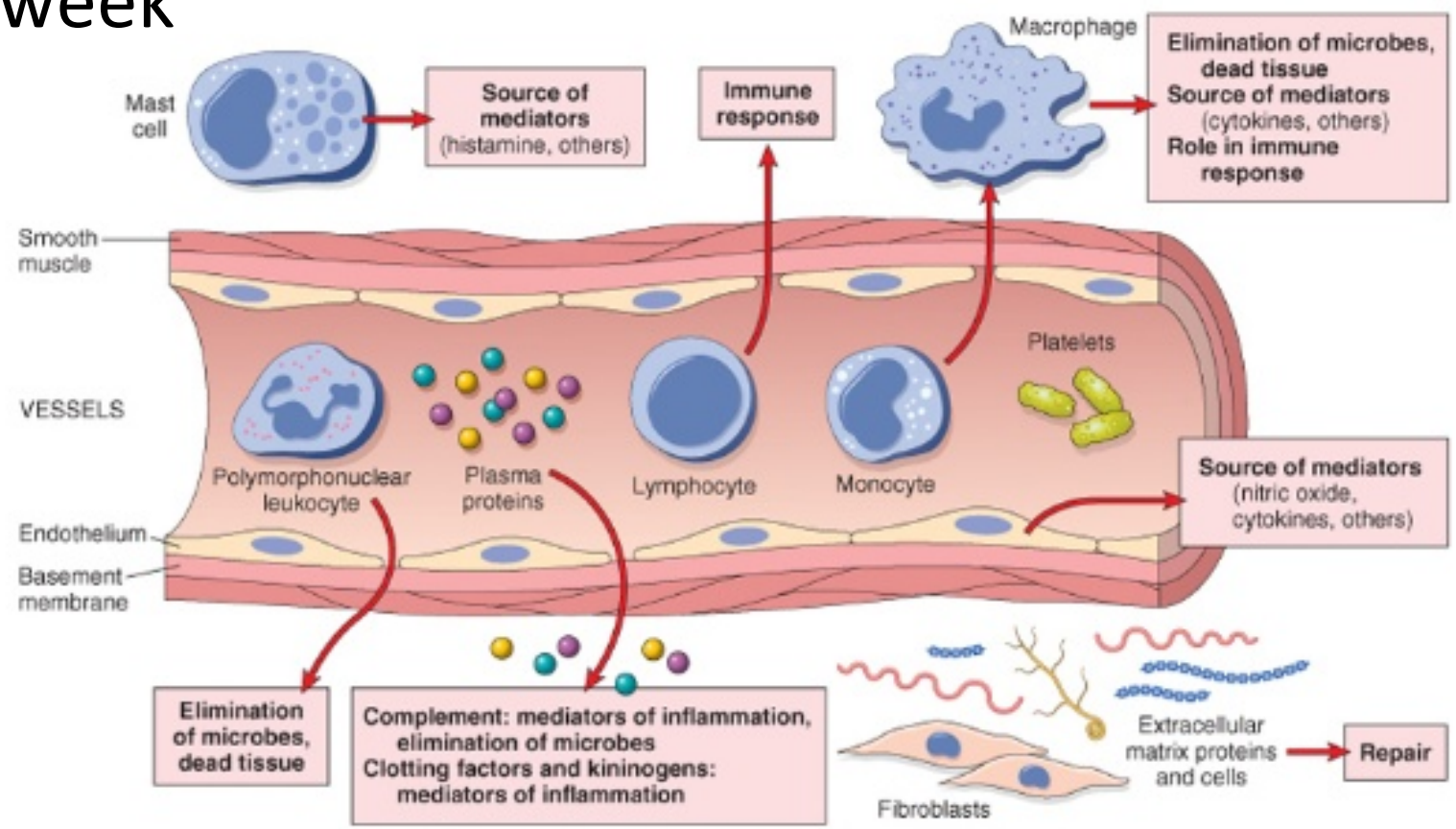


Thiruvoth et al. 2015. Plastic and Aesthetic Research

AJ Jomas et al. 2014. Advanced Drug Delivery Review

Inflammation

- Begins immediately after injury, lasts for ~ 1 week



Inflammation

Well-regulated inflammation is beneficial

-activates healing cascade

-recruitment/activation of cells

Excessive/ Chronic inflammation is pathological

-degenerative matrix changes

-fibrotic healing

Benefits of Anti-inflammatory therapy is controversial

Timing may be key!

Generally effective at preventing excess scar formation

Early inhibition decreases mechanics

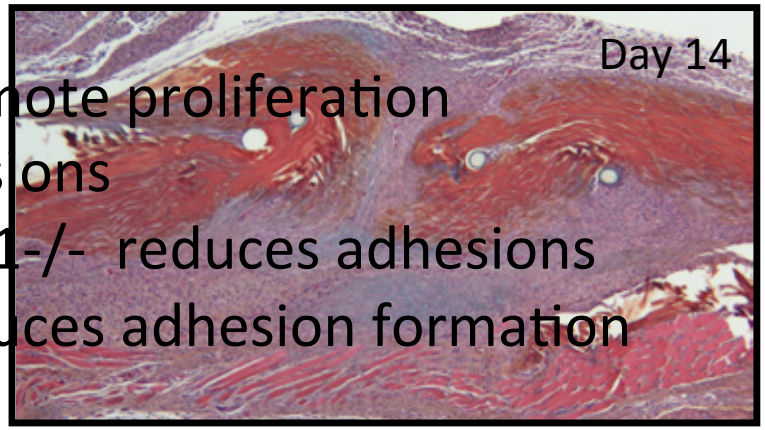
Delayed healing maintains mechanics

Cell-type specific considerations

Virchenko et al., 2004. Am J Sports Med, Geary et al. 2015. PlosOne

Proliferative/ Granulation Phase

- Lasts a few weeks
- Begins ~day 7 in mouse model
- Proliferation of 'fibroblasts'
- Bridging on injury site
- Production of ECM components (Col1/ Col3)
- Rapid deposition of disorganized ECM

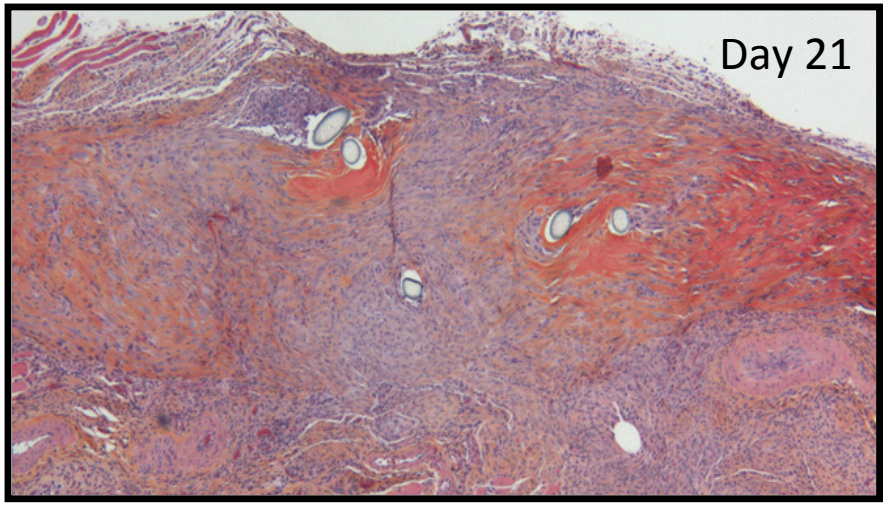


PDGF and bFGF: promote proliferation
-can also cause adhesions
TGFB: Smad3-/-, PAI-1-/- reduces adhesions
S100a4: Ablation reduces adhesion formation

Thomopoulos et al., 2009. JOR, Thomopoulos et al., 2010. JBJS, Katzel et al. 2010. JOR, Awad Lab, Loiselle Lab

Remodeling Phase

- Lasts many months
- Begins ~day 21 in mouse model
- Reorientation of ECM
- Mmp-mediated remodeling

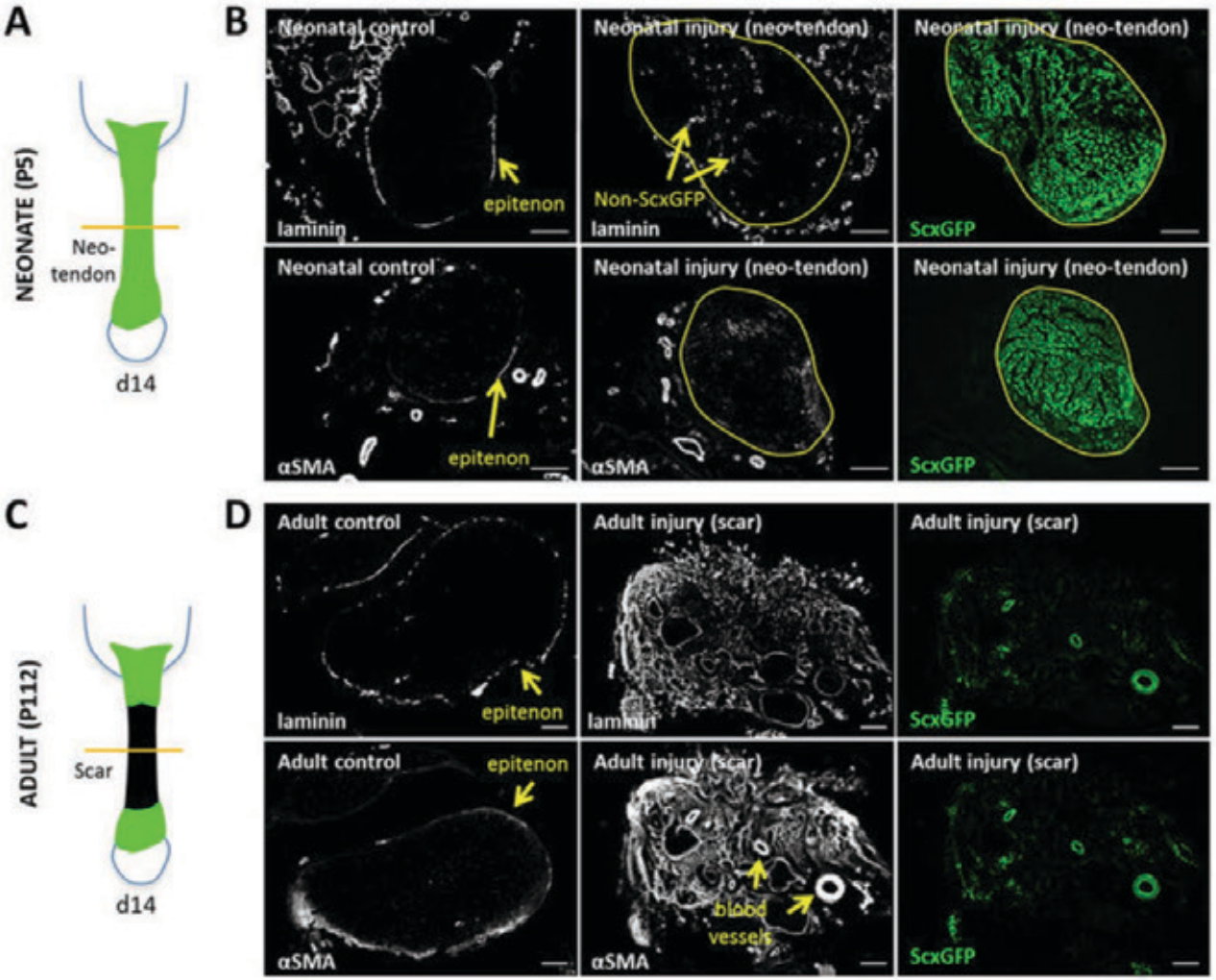


Cellular Basis of Healing

- Time-dependent dynamic cellular environment
- Involves multiple cell types
- Tenocyte apoptosis
- Extrinsic inflammatory cells
- Resident tenocytes, epitenon cells, sheath cells, tendon basement membrane cells
- Bone marrow derived cells

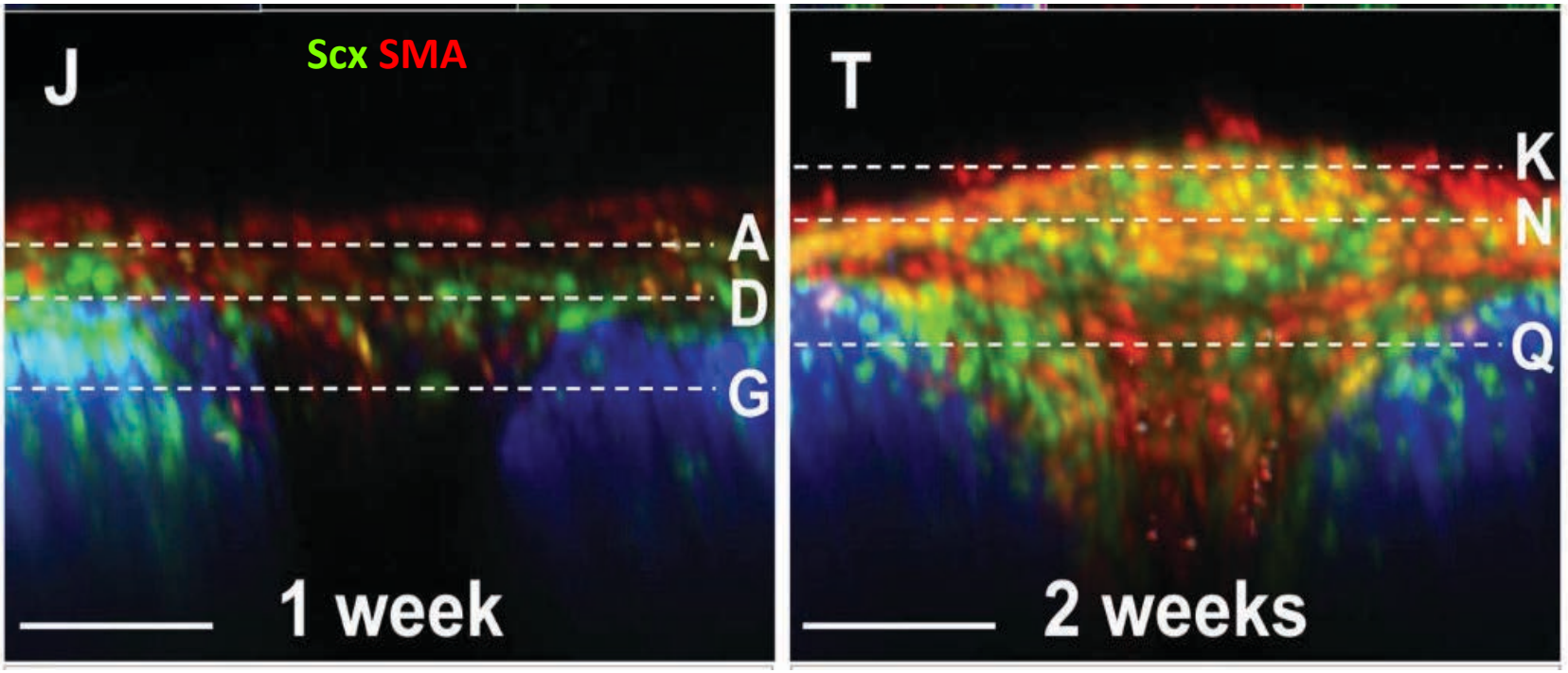
Cellular Basis of Healing: Intrinsic Cells

Tenocytes: Scx remains best marker with genetic tools



Howell et al., 2017. SciRep

Contribution of Scleraxis Cells to Healing, Regeneration and Scar Formation is Unclear

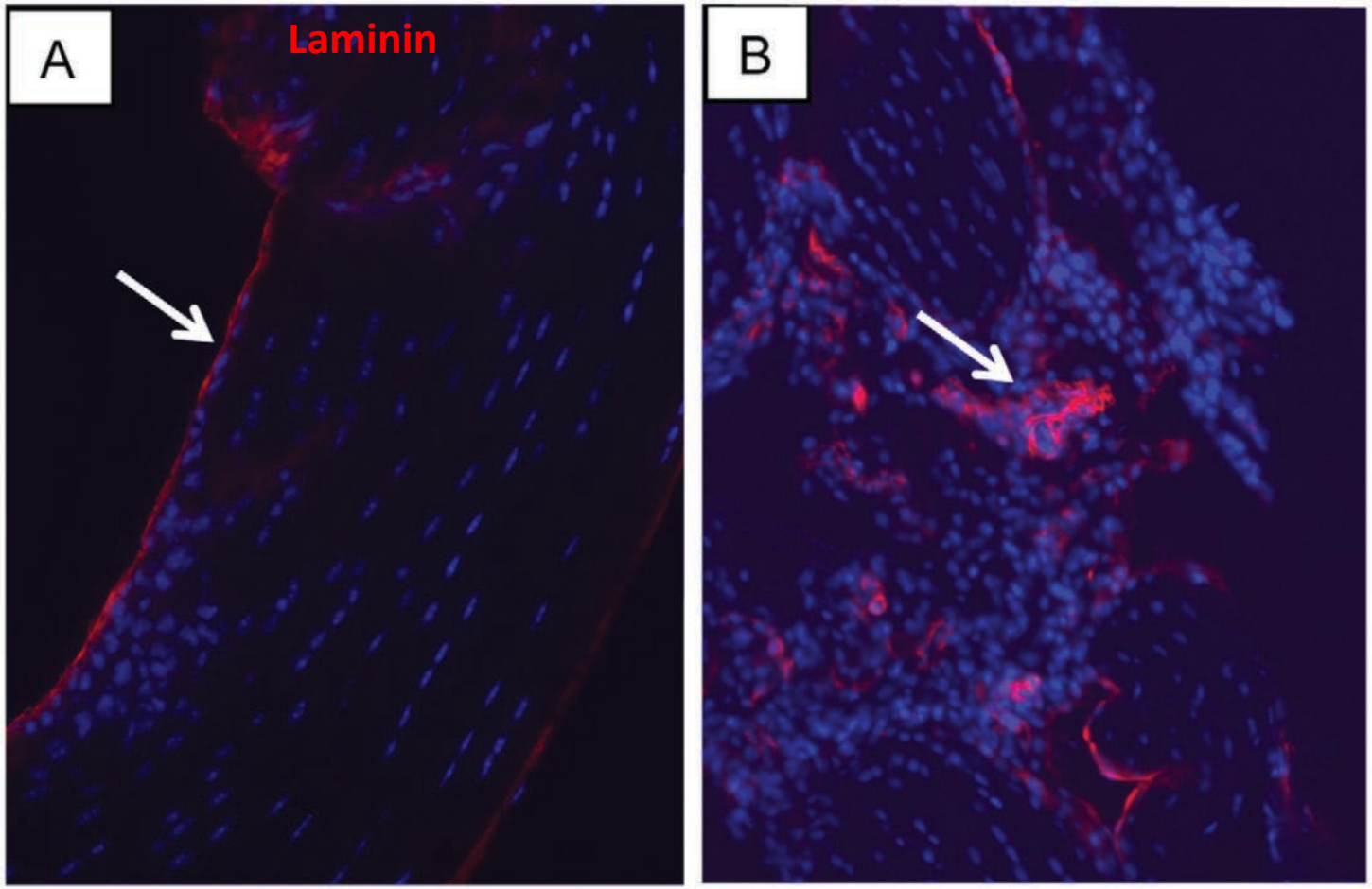


Function is likely to be context dependent

Dyment et al., 2014. PlosOne

Cellular Basis of Healing: Intrinsic Cells

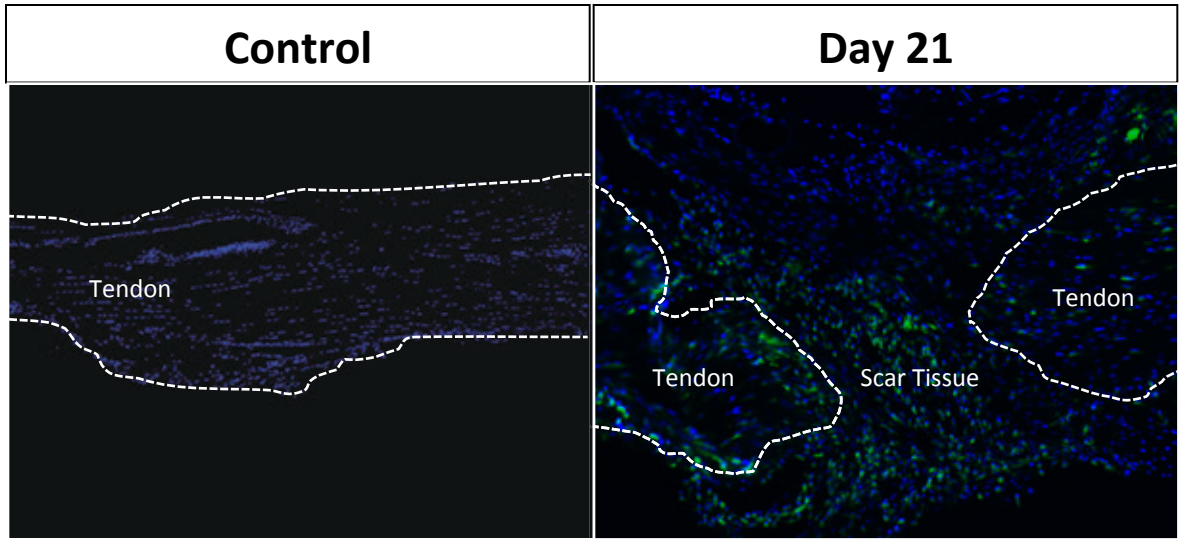
Contribution of basement membrane (laminin^{+}) cells to healing and adhesion formation



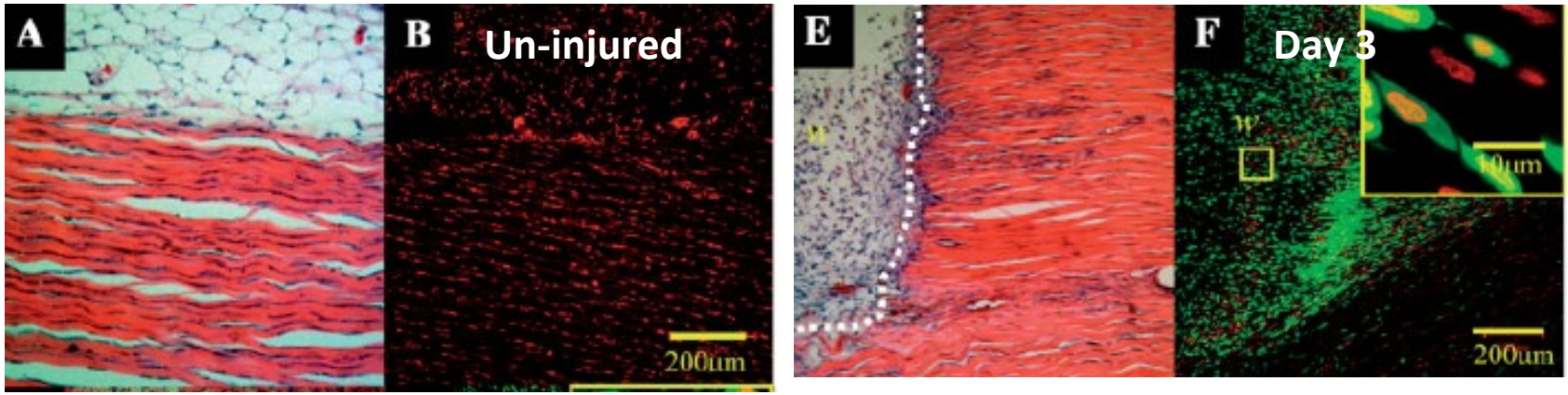
Taylor et al., 2011. PlosOne

Cellular Basis of Healing: Extrinsic Cells

Bone Marrow Derived Cells Migrate Specifically to the Repair Site



- Specific Sub-populations remain unknown
- Function not ~clear
- BM-Mmp9 sufficient for adhesion formation

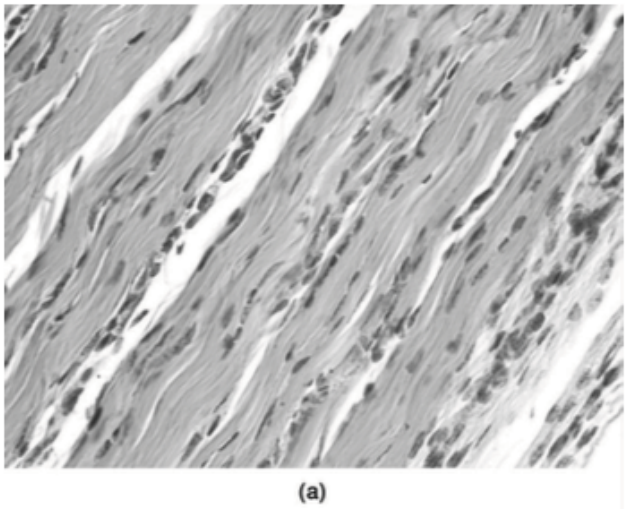


Loiselle et al., 2012. PlosOne, Kajikawa, Y. et al. Journal of Cell Physiology. 2007

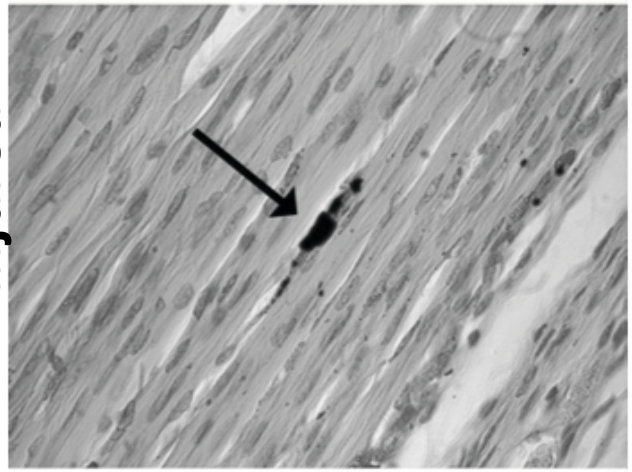
Regenerative Healing: Embryonic

Fetal Sheep

Un-injured

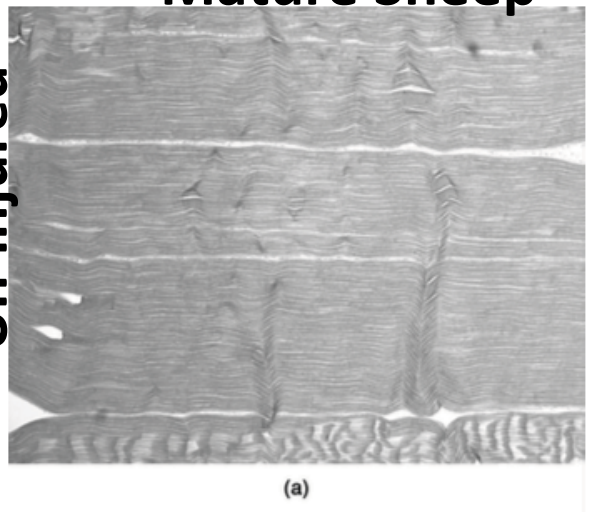


Injured

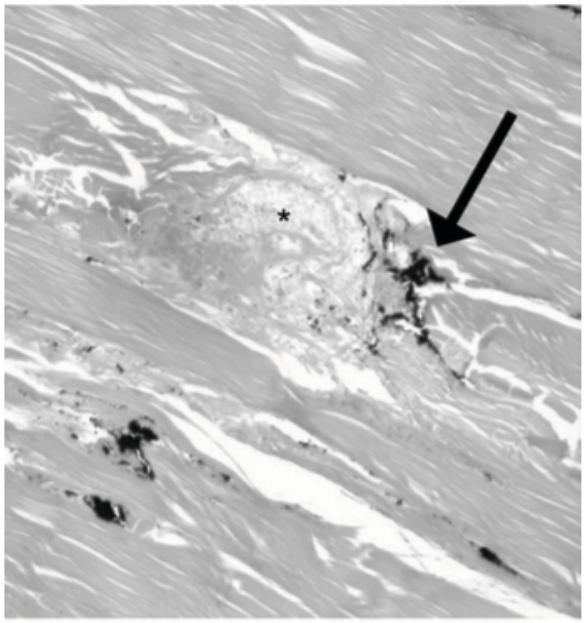


Mature Sheep

Un-injured



Injured



Fetal:
Low levels of TGFB1
and TGFB2
High levels of TGFB3

Beredjiklian PK et al., 2003. Annals of Biomedical Engineering

Regenerative Healing: Embryonic

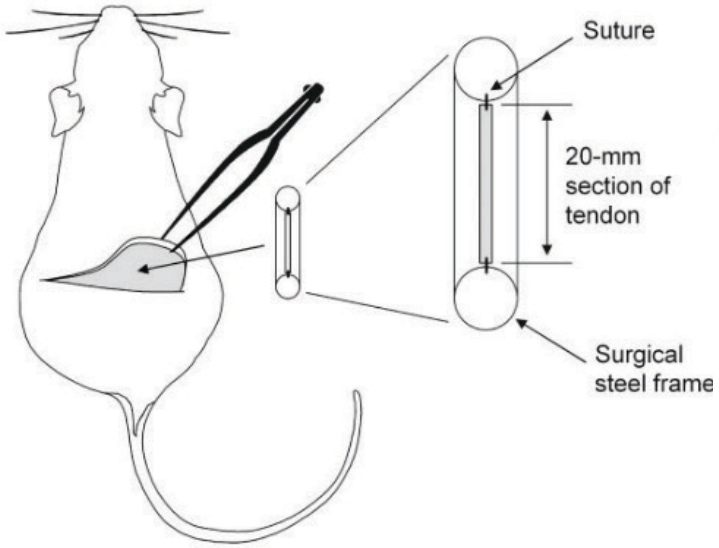


Figure 1. Schematic of subcutaneous transplantation procedure. Each mouse received one graft of either adult or fetal tendon.

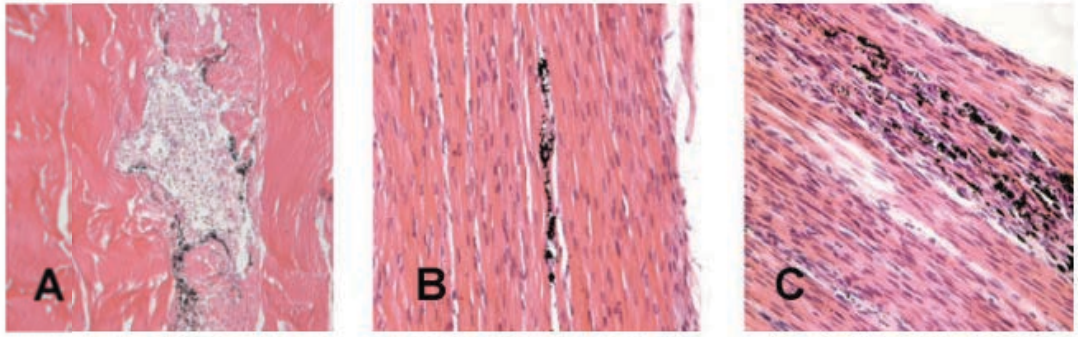
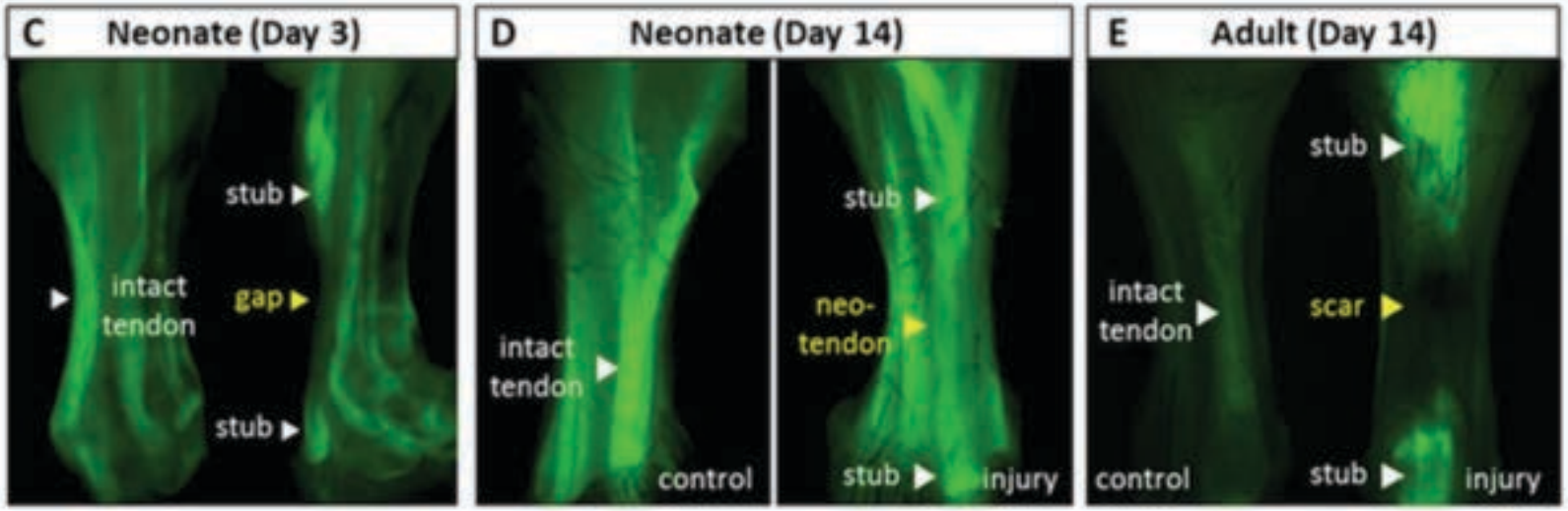
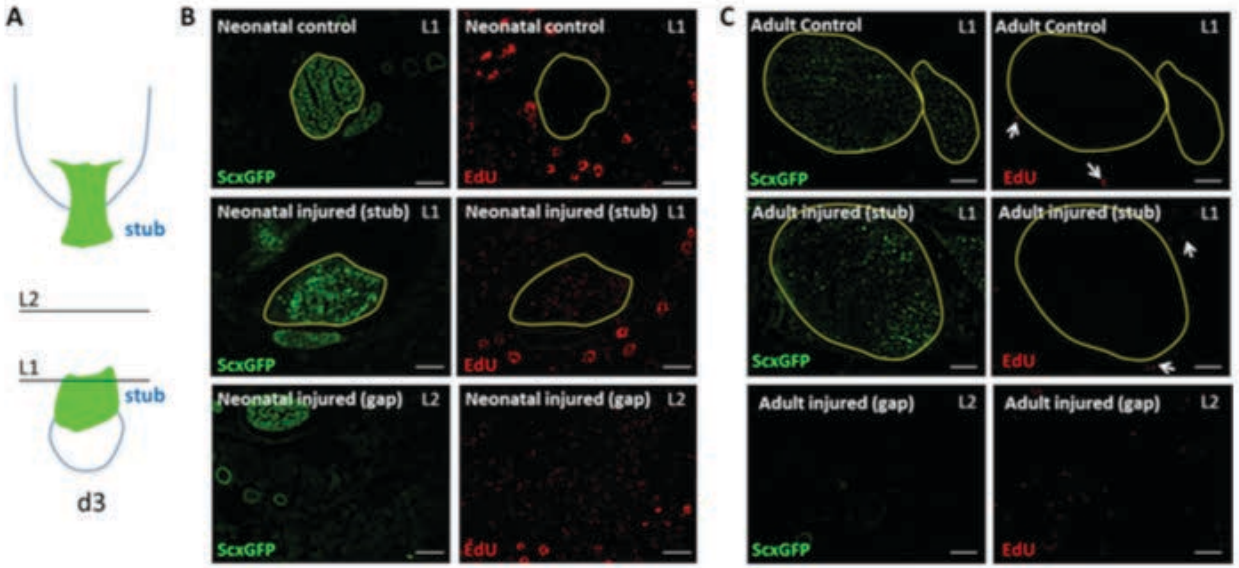


Figure 2. H&E sections of wounded tendons (black indicates wound). (A) 1-week adult, original magnification ×50; (B) 1-week fetal, original magnification ×200; (C) 3-week fetal, original magnification ×200. Note the substantial inflammatory response in the adult, but not the fetal, specimens.

Beredjiklian PK et al., 2006. JOR

Regenerative Healing: Neonatal

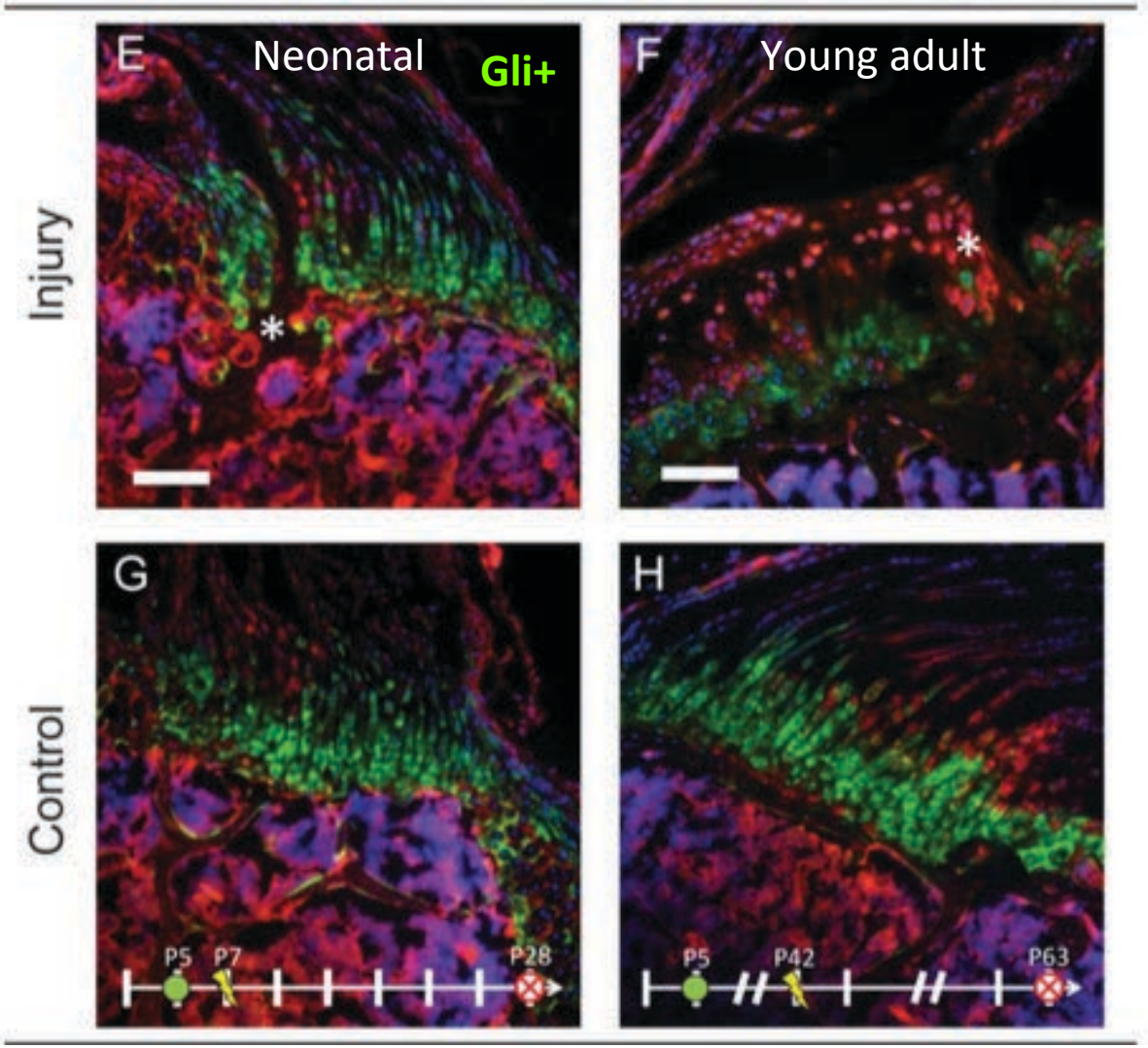




Howell et al., 2017 SciRep

Regenerative Healing: Neonatal

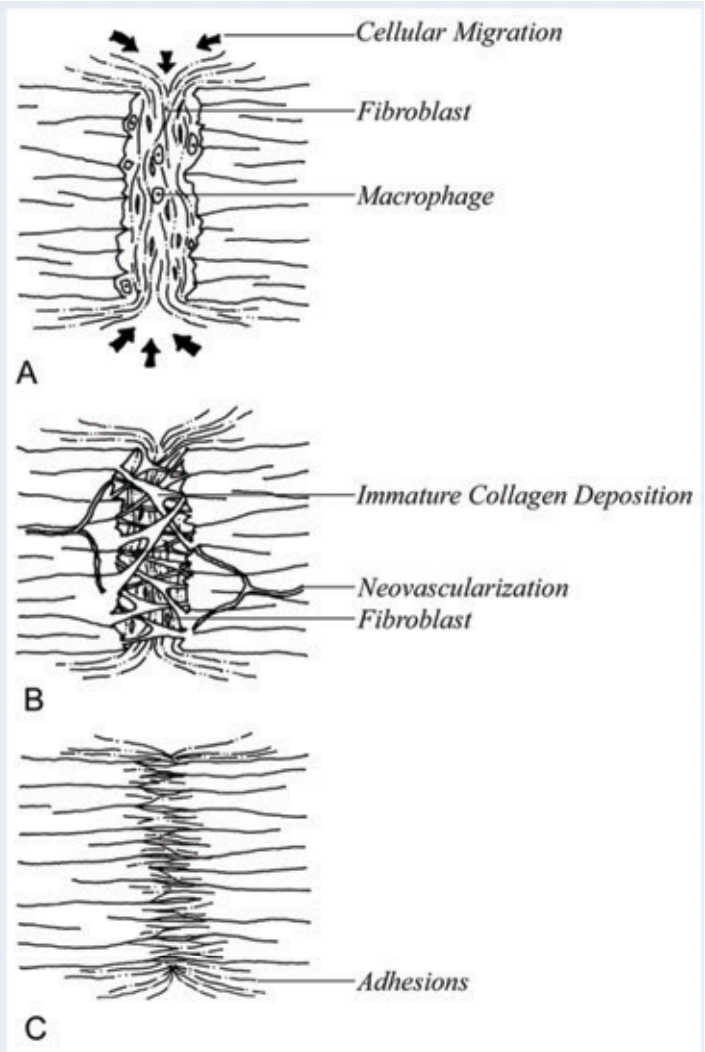
Tracing Gli1-lineage cells after enthesis injury



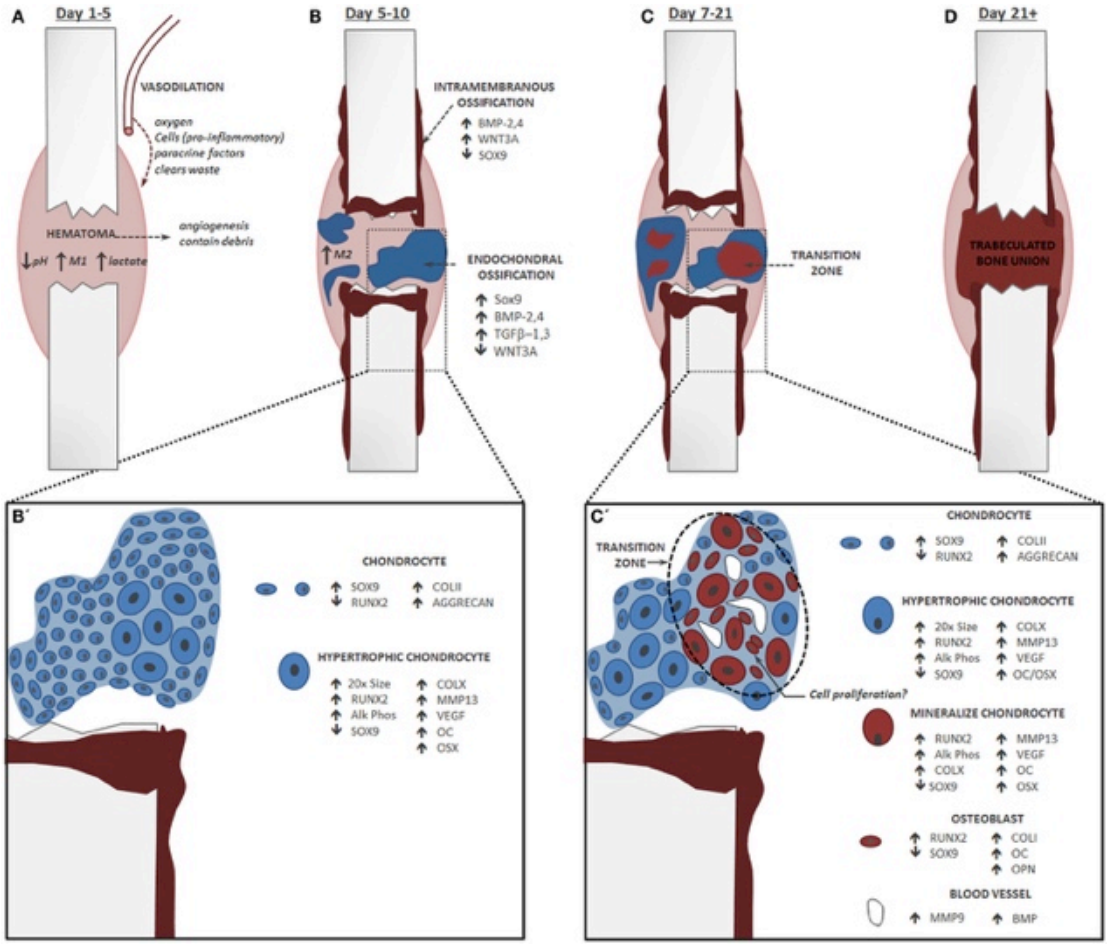
Schwartz AG et al., 2017, Development

Where do we go from here?

- Cri ate/ fun
- De
- Big
- Ide rkers



Beredjiklian 2003. JBJS



Bahney et al., 2015. Front Endocrinol.