Tendinopathy, Tendon Healing & Tendon Regeneration

Alayna E. Loiselle, PhD
Associate Professor
IND 464 Musculoskeletal Basic Science Course
October 21, 2019
Outline

• Review of homeostasis
• Effects of Aging
• Disruptions in homeostasis
• Pathogenesis of tendinopathy/ tendinitis
• Mechanisms of regeneration and healing
Tendon fibroblasts are not a uniform population

Best FASEB 2019

Loiselle Lab, unpublished data

Walia et al. ORS 2019

Bautista et al. ORS 2019

Grinstein et al. ORS 2019

Swanson et al., BioRxiv. 2019
Tendon Cell Proliferation During Growth

Figure 1

A

+ DOX
Pulse E10-P0

- DOX
Chase P0-2y

B'

C'

D

E

GFP

645 days

A

Change in H2B-GFP+ cells

Rate of tendon cell turnover

Grinstein eLIFE 2019
Tendon Cell Proliferation During Growth

Grinstein eLIFE 2019
Tissue Renewal

Nuclear bomb testing between 1955-1963
Doubled atmospheric $^{14}$C
Tendon and muscle biopsies from patients born ~1940-1980
Assessment of tissue turnover rate
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

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
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
Animal Models of Tendinopathy & Healing

Supraspinatus (rotator cuff)
- Over 30 animals characterized\(^1\)
- Most common:
  - Rat
  - Mouse

Achilles Tendon
- Mechanical
- Full thickness, partial width
- Tendon to bone healing
- Complete transection
- +/- repair
- Partial transection
- Biopsy punch

Patellar Tendon
- Fatigue loading
- Biopsy punch

Flexor Tendon
- Diabetic tendinopathy
  - Complete transection
  - +/- repair
  - Partial transection
  - Biopsy punch

- Tendinopathy
- Healing

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(2) Thomopoulos et al., JOR. 2015 (review)
Zebrafish!

Figure 1: Bright field images of control (A) and injured (B) tendons. Microdissection 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.

Inflammation is a potent mediator of tendinopathy

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

Induces degenerative changes via collagen degradation

LD= low dose
HD= high dose collagenase

Mechanically Induced Tendinopathy

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

Induces matrix and cellular changes


N Andarawis-Puri et al., 2014. JOR
Tenosynovitis

- Inflammation of the sheath
- Causes: inflammatory diseases, infection, injury
- Most commonly in hand/ wrist
Developing a mouse model of pyogenic tenosynovitis
Genetic models of tendinopathy: Ehlers-Danlos Syndrome

- Joint hypermobility and frequent dislocations
- Mutation in ColV
- Tendon-specific ColV KO- EDS
  - Abnormal gait, joint laxity, altered collagen structure
- ColV\(^{+/-}\) altered tendon structure, impaired tendon healing

Sun et al., AJP. 2015, Johnston et al., JOR. 2017
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

de Jong 2014; Pennisi 2002; Beredjiklian 2003; Defranco 2004
Acquisition of Mechanical Properties

Maximum Load at Failure

- Control
- Flexor Tendon

Days Post-Surgery vs. Days of healing:
- Peak force (N)
- Intact vs. loaded and unloaded

Loiselle et al., 2009. JOR

Similarities between wound and tendon healing

Normal wound healing consists of three overlapping phases:

- Inflammation (4 – 6 days)
- Proliferation (4 – 24 days)
- Remodeling (21 days – 2 years)

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Similarities between wound and tendon healing

Nichols, Best, Loiselle. 2019
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

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

Day 3

Day 14

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
Successful Repair: A Delicate balance of deposition and remodeling

- **Tendon Injury**
  - "Scar-full" healing response
  - Impaired gliding function & strength
  - Increased tendon size/ bulk
    - Disorganized Extracellular Matrix (ECM)
      - Excessive ECM Production
        - Col3/ Col1
      - Insufficient ECM Remodeling
        - Matrix Metalloproteinases
    - ECM Producing Cells
    - MMP Producing Cells

Deposition
Remodeling
Double-edged sword of tendon healing

Matrix deposition

Mechanical properties
Fibrosis

- Thickening and/or scarring of connective tissue
- Typically in response to injury
- In response to injury fibrosis = scar tissue
- Excess matrix deposition
- Disorganized matrix
- Exuberant healing response
Impact of Diet induced Obesity and Type II Diabetes on Tendon Healing

4-wk old Male C57BL/6J Mice

Low Fat Diet (LFD): 10% Kcal from fat; lean control

High Fat Diet (HFD): 60% Kcal from fat; T2DM

Body Weight

Diet initiation

Surgery

Harvests

Days Post-Surgery

Weeks

Body Weight (g)

Diet

LFD

HFD

Fasting Blood Glucose

LFD

HFD

Blood Glucose [mg/dL]

Time Post-Glucose Bolus

LFD

HFD

Percent Body Fat

Diet

LFD

HFD

* * * *

T2DM Impairs Tendon Healing

**MTP Flexion Angle**

Days Post-Repair: 10, 14, 21, 28

**Gliding Resistance**

Days Post-Repair: 10, 14, 21, 28

**Max Load at Failure**

Days Post-Repair: 10, 14, 21, 28

**Col3a1**

Days Post-Repair: 3, 7, 10, 14, 21, 28

**Col1a1**

Days Post-Repair: 3, 7, 10, 14, 21, 28

T2DM Prolongs and Alters Inflammation

Increased macrophage content
Prolonged macrophage presence
Increased, prolonged pro-inflammatory M1 macs
Increased, early anti-inflammatory* M2 macs

Aging decreases fibrotic tendon healing (too much)

Ackerman et al., JOR. 2017.
Aging decreases fibrotic tendon healing (too much)

No change in proliferative capacity

Decreased matrix production on a per cell basis

Is the cellular environment the same?

Ackerman et al., JOR. 2017.
Origin of Cells During Tendon Healing: Intrinsic & Extrinsic

The cellular components of tendon healing are not well characterized

Extrinsic Healing
- Resident Tenocytes
- Macrophages/Inflammatory cells
- Scx-lineage

Intrinsic Healing
- Bone marrow/circulating cells

Undefined Milieu
- Myofibroblasts (α-SMA)
- Sheath (Prg4)
- Basement membrane (laminin)
- ?
Cellular Basis of Healing: Intrinsic Cells

Tenocytes: Scx remains best marker with genetic tools

Howell et al., 2017. SciRep
Contribution of Scx-cells to healing is controversial and context dependent.

Howell et al., 2017. Scientific Reports
Dyment et al., 2013. PlosOne.
Loiselle et al., 2009. JOR
Sakabe et al., 2018. JBC.
Contribution of Scleraxis Cells to Healing, Regeneration and Scar Formation is Unclear

Function is likely to be context dependent

Dyment et al., 2014. PlosOne
**Scx\(^{\text{Lin}}\) Cells Contribute to Bridging Population by Day 14**

**A- Scx\(^{\text{Lin}}\) - Tmx Washout**

- Repair
- Harvests

<table>
<thead>
<tr>
<th>Tmx</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
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<tbody>
<tr>
<td>-6</td>
<td>-5</td>
<td>-4</td>
<td>0</td>
<td>7</td>
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</table>

**Scx\(^{\text{Lin}}\) DAPI**

<table>
<thead>
<tr>
<th>Un-injured</th>
<th>Day 7 Post-Repair</th>
</tr>
</thead>
</table>

**Best & Loiselle FASEB 2019**
Scx+ Cell Death Improved Healing Tendon Strength

Scx-Cre; ROSA-DTR+/−

> Loss of Scx cells is beneficial to late healing

A

Stiffness

<table>
<thead>
<tr>
<th></th>
<th>Cre-</th>
<th>Cre+</th>
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<tbody>
<tr>
<td>Day 14</td>
<td></td>
<td></td>
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<tr>
<td>Day 28</td>
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B

Maximum Load at Failure

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<thead>
<tr>
<th></th>
<th>cre-</th>
<th>cre+</th>
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<tbody>
<tr>
<td>Day 14</td>
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<td>Day 28</td>
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MTP ROM

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<tr>
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<tr>
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<tr>
<td>Day 28</td>
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Gliding Resistance

<table>
<thead>
<tr>
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<th>cre-</th>
<th>cre+</th>
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<tbody>
<tr>
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Cellular Basis of Healing: Intrinsic Cells

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

Taylor et al., 2011. PlosOne
S100a4 and Scx cells have distinct spatial expression profiles during healing

Scx-Cre^{ERT2}; Rosa-Ai9; S100a4-GFP^{promoter}

Day 21 Post-Repair

Best & Loiselle FASEB 2019
Cellular Basis of Healing: Extrinsic Cells

Bone Marrow Ablation

C57BL/6

Bone Marrow Transplant

GFP Bone Marrow Chimeric Mouse

Bone Marrow Harvest

GFP

Tendon Injury and Repair

Fluorescence
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

Future Work

• Delineate intrinsic vs. extrinsic contributions of cell types
  – Macrophages
  – S100a4
• Define functions of intrinsic & extrinsic populations
• Understand how homeostatic populations change in response to injury
Regenerative Healing: Embryonic

Fetal Sheep

Un-injured

(a)

Injured

Mature Sheep

Un-injured

(a)

Injured

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

Center for MusculoSkeletal Research
Regenerative Healing: Embryonic

Embryonic and adult tendon implanted in to the back to determine if embryonic tendons could heal scar-lessly in an adult environment

<table>
<thead>
<tr>
<th>Adult 1 week</th>
<th>Embryonic 1 week</th>
<th>Embryonic 3 weeks</th>
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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.

Beredjikian 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? (2017)

✓ Critical mass of labs studying cell lineage/fate/function
✓ Big data and single-cell RNAseq
  • Development of tendon specific tools
  • Identification of new ‘tendon-specific’ markers
Where do we go now?

- Tendon Cell Heterogeneity
- Delineate functions of subpopulations
- Need to define cell origin, lineage and fate
- Understanding of cell-cell, cell-matrix interaction ➔ phenotype

Defining Cell Lineage and Terminal Fate

Injury

S100a4\textsuperscript{lineage+}; S100a4\textsuperscript{active-}; αSMA\textsuperscript{+}
Myofibroblasts

S100a4\textsuperscript{lineage+}; S100a4\textsuperscript{active+}
Non-myofibroblasts

Understanding Cell-Cell Interactions

Resident Tenocytes

S100a4\textsuperscript{lineage+}; S100a4\textsuperscript{active+}; αSMA\textsuperscript{-}

Immune Cells

Fibrotic Healing
Tenocyte transition to myofibroblasts?

- "Specialized"/ Activated Fibroblasts
- Involved in matrix deposition
- Restoration of tissue integrity
Tendon Injury → Acute Inflammation → Excess ECM Deposition → Fibrosis

No Treatments

Tenocyte heterogeneity - differential response to injury

Chronic low-level inflammation → Macrophage Persistence → Myofibroblast Persistence → Excess ECM Production → Fibrosis

UR Medicine | Orthopaedics & Rehabilitation
Fibroblast heterogeneity at baseline with differential contributions to myofibroblast fate
Phenotypic and functional shifts over time

Uninjured

Day 2–4 after MI

Day 4–7 after MI

Day >10 after MI

Resident quiescent cardiac fibroblast

Activated cardiac fibroblast

Cardiac myofibroblast

Cardiac matrifibrocyte
## Phenotypic and functional shifts over time

<table>
<thead>
<tr>
<th>Day 14 Post-Surgery</th>
<th>Day 28 Post-Surgery</th>
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<tbody>
<tr>
<td><img src="image1.png" alt="Image A" /></td>
<td><img src="image2.png" alt="Image C" /></td>
</tr>
<tr>
<td><img src="image3.png" alt="Image B" /></td>
<td><img src="image4.png" alt="Image D" /></td>
</tr>
</tbody>
</table>

### αSMA Postn
- **Day 14 Post-Surgery**: Image A shows an increase in α-SMA expression compared to Day 28 Post-Surgery (Image C).
- **Day 28 Post-Surgery**: Image C shows a decrease in α-SMA expression.

### αSMA Hsp47
- **Day 14 Post-Surgery**: Image B shows a significant increase in both α-SMA and Hsp47 expression.
- **Day 28 Post-Surgery**: Image D shows a decrease in Hsp47 expression with a maintained α-SMA level.

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*Images and data are illustrative and do not represent actual research data.*
The controversial relationship between S100a4 and $\alpha$-SMA

Defining the myofibroblast pre-cursors during tendon healing

- ECM Deposition
- ECM Contraction

‘Fibroblast/tenocyte’ → Myofibroblasts

- Nearly all dermal fibroblasts are S100a4$^+/\alpha$-SMA$^+$
- No co-localization seen in quiescent or activated hepatic stellate cells

S100a4\(^+\) cells are not myofibroblasts in the healing tendon
But, S100a4-lineage cells become α-SMA\(^+\) myofibroblasts

Ackerman et al., eLife. 2019
Dynamic Contribution of ScxLin and Scx Expression to Myofibroblast Fate

Scleraxis^{Ai910-12} Mice

Labels tendon cells that express Scx 10-12 days post-surgery

| Scx^+ at D10-12 post-op ⇒ myofibs |

RFP (Scx^{Ai9})
αSMA
DAPI

Orthopaedics & Rehabilitation