Collagen V Deficiency During Healing
Mitigates the Quasi-Static Mechanical Deficits of Injured Tendons

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Introduction

- Classic Ehlers-Danlos Syndrome (cEDS) is associated with mutations in the genes encoding collagen V, a fibrillagenic collagen present in tendon.¹
- Hallmarks of cEDS are connective tissue hyperelasticity and poor wound healing.²
- A murine model of cEDS demonstrates impaired tendon healing.³ It is unknown whether this impaired healing response is due to the regulatory role of collagen V during tendon healing or due to pre-existing differences in collagen V-deficient tendons.

**Objective:** Determine the effect of acute knockout of collagen V on healing tendon mechanics and tendon fibril structure.

Materials and Methods

**Animals and Injury:** Study design and injury model are shown in Figure 1 (IACUC approved). At time of injury, mice received two daily doses of tamoxifen (TM) and were sacrificed at 3 or 6 weeks post-injury. WT uninjured control mice (n=15) received 3 daily TM doses at 120 days old and were sacrificed 30 days later.

**Mechanical Testing:** Tibia-patellar tendon-patella complexes were prepared for mechanical testing.⁴ Patellar tendons were uniaxially tested (Instron 5848) with the protocol: preconditioning, stress relaxation and frequency sweeps at 3, 4, and 5% strain, and a ramp to failure. Stress relaxation, dynamic modulus, phase shift, stiffness, max load, modulus, and max stress were computed.

**Transmission Electron Microscopy (TEM) Imaging:** At sacrifice, patellar tendons (n=4/group) were fixed, prepared, and sectioned.⁴ ~90nm thick sections were imaged (JEOL 1400 TEM), 12 regions of interest per tendon were analyzed within the healing tissue. Fibril diameters were measured via Bioquant along the minor axis of the fibril cross section.

**Statistics:** For mechanical properties, one-way ANOVAs with Tukey post-hoc tests were used to compare across genotypes and uninjured controls at each healing time point. Significance was set at p<0.05, and trends at p<0.1.

![Figure 1. Study Design and Injury Model Schematic. At 120 days of age, mice received injuries and TM injections for excision of the Col5a1 gene (A). Injuries were bilateral, full thickness, partial width patellar tendon defects as described (B).](*p<0.05 and *p<0.1)

Discussion

- Injured tendons exhibited decreased mechanical properties through healing (Fig 2)
  - Collagen V haploinsufficiency during tendon healing led to better mechanical outcomes
  - Collagen V deficiency during tendon healing altered collagen fibril structure (Fig 3 & 4)
  - Collagen V knockout tendons contained larger and less regular fibrils than WT tendons
  - Collagen V plays a complex role in the healing tendon
  - WT expression may be too restrictive on fibril size in the healing matrix, resulting in smaller fibrils and a likely weaker matrix
  - NULL expression leads to irregular fibrils, which are likely mechanically inadequate
  - HET expression may provide balance of larger and more regular collagen fibrils
  - Poor healing in cEDS patients is likely due to pre-existing differences in collagen V-deficient matrix
  - Fibroblasts from murine model of cEDS demonstrated decreased proliferation, migration, and wound healing compared to WT fibroblasts⁶
  - cEDS may cause intrinsic changes to cellular capacity for proper healing

Conclusions

This study surprisingly reveals that collagen V deficiency during tendon healing leads to improved mechanical function from what is normally seen following injury. This work provides further understanding of the role of collagen V in regulating tendon function.

![Figure 2. Cross-Sectional Area (CSA) and Quasi-Static Properties. Tendon CSA (A) increased with injury, while stiffness (B) decreased with injury. HET tendons did not exhibit this decrease in stiffness with injury. Max load was not altered by injury (C). WT tendon modulus (D) and max stress (E) decreased with injury. (*p<0.05 and *p<0.1 relative to WT uninjured, ---p<0.05, ---p<0.1)

![Figure 3. Fibril Diameter Distributions. HET and NULL tendons had a shift towards larger diameter fibrils compared to WT tendons at both healing time points. NULL tendons contained more large diameter fibrils than HET tendons at both healing time points. (Inset plots: Cumulative distributions of fibril diameters)

![Figure 4. Representative TEM Images. Fibril irregularity was observed in 6-week HET tendons and was pervasive in 6-week NULL tendons (white arrows). (Scale bar = 200nm)

References & Acknowledgments