Knockdown of Collagen V During the Inflammatory Healing Phase Significantly Affects Quasi-Static Tendon Mechanics

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Introduction

- Our established murine model of collagen V haploinsufficiency demonstrated diminished recovery of mechanical properties and altered fibril morphology following tendon injury [1].
- These studies utilized conventional mouse models of collagen V deletion and therefore the isolated role of collagen V at defined phases of tendon healing following injury remains unknown.
- **Objective:** To elucidate the specific mechanistic regulatory roles of collagen V in the late inflammatory and early remodeling phases of tendon healing in a normal matrix using inducible collagen V null and heterozygous models.
- **Hypothesis:** Decreased collagen V during the late inflammatory and early remodeling phases will result in significantly decreased dose-dependent tendon mechanical properties during both phases.

Methods

Animal Surgery

- Bilateral partial width, full thickness patellar tendon injury was performed on adult male wild-type (WT) (n=15), *Col5a1*$^{flox/+}$ (HET) (n=45), and *Col5a1*$^{flox/flox}$ (NULL) (n=45) mice with tamoxifen (TM) inducible ROSA26-Cre (IACUC approved) [2].
- Cre-induced excision of the conditional alleles of the transgenic mice was performed 5 days post-injury during the inflammatory phase (TM5) and 21 days post-injury during the remodeling phase (TM21) via IP TM injections (2mg/40g/body weight), **Figure 1.**
- The WT and TM5 mice were sacrificed at 3 and 6 weeks post-injury (TM5_3wks and TM5_6wks) and the TM21 mice were sacrificed at 6 weeks (TM21_6wks) post-injury. The WT uninjured control mice were sacrificed 30 days after TM injections.

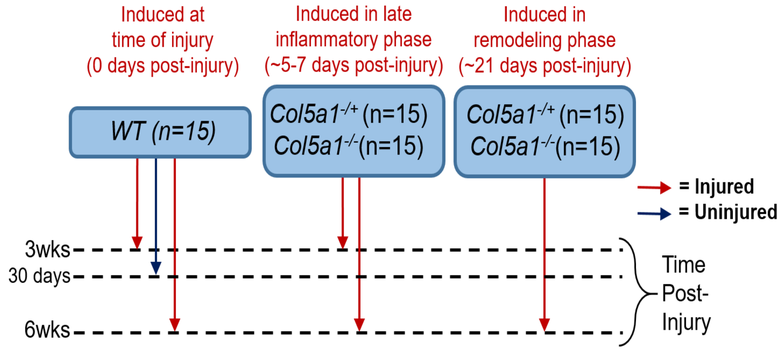

Figure 1. Study Design

Mechanical Testing

- Patellar-tendon-tibia complexes were prepared for uniaxial mechanical testing and tendon cross-sectional area was measured with a custom laser device, **Figure 2**.
- *Ex vivo* viscoelastic mechanical testing was performed as follows: 10 cycles of preconditioning, stress relaxations at 3%, 4%, and 5% strain, 10 cycles of frequency sweeps, and quasi-static ramp to failure, **Figure 3.**
- Stiffness, modulus, maximum load, and maximum stress were calculated from the ramp to failure data and percent relaxation was quantified for each percent strain level.

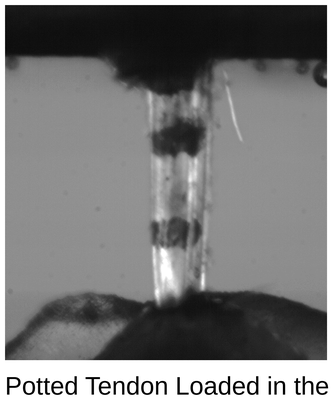

Figure 2. Potted Tendon Loaded in the Instron

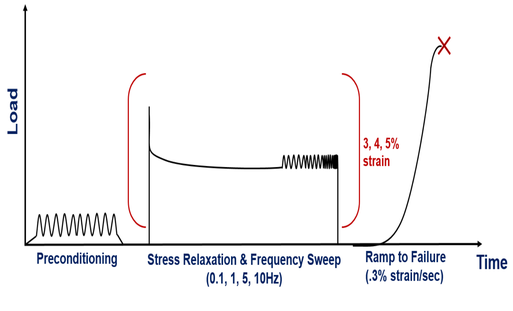

Figure 3. Mechanical Testing Protocol

Statistics

- One-way ANOVA with Bonferroni post-hoc tests were performed to compare the injured WT and injured Col5a1$^{flox/+}$ (HET) and Col5a1$^{flox/flox}$ (NULL) tendons at each Cre-induction and healing time point. Significance was set at p≤0.05 and trends at p≤0.1.

Results: COLV Knockdown in the Late Inflammatory Phase

The effects of COLV knockdown in the late inflammatory phase were diminished with healing time, **Figure 4.**

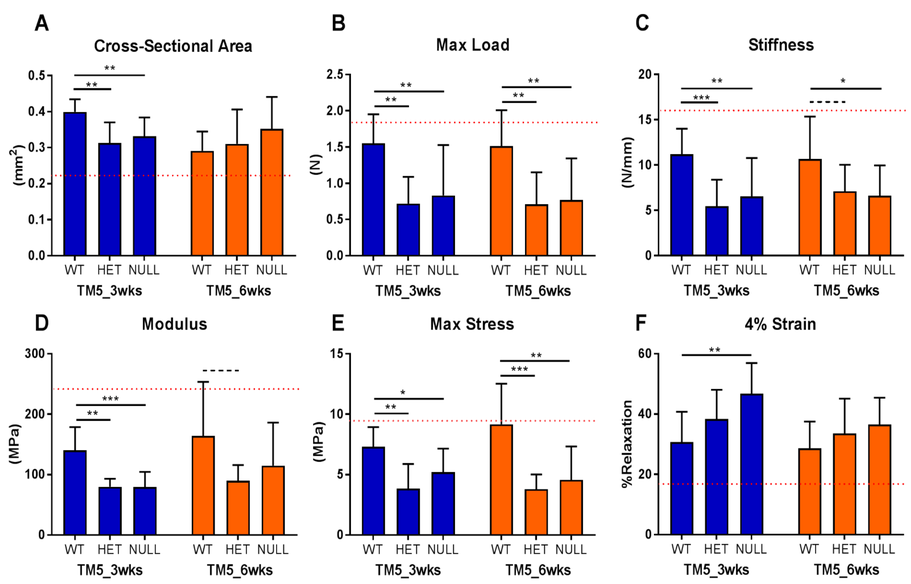

Figure 4. (A) Cross-sectional area, **(B)** max load, **(C)** stiffness, **(D)** modulus, and **(E)** max stress of the WT tendons were significantly increased compared to the TM5-induced HET and NULL tendons after 3 weeks of healing. **(F)** NULL tendons exhibited increased stress relaxation at 4% strain than the WT and HET tendons. These differences were not as pronounced after 6 weeks of healing. Uninjured WT tendons are represented with the red dotted line. Data presented as mean+SD. (*p<0.05, **p<0.01, ***p<.001, trend ---).

Results: COLV Knockdown in the Early Remodeling Phase

COLV knockdown in the late inflammatory phase (TM5) had a greater effect on tendon mechanics than knockdown during the early remodeling phase (TM21) after 6 weeks of healing, **Figure 5**.

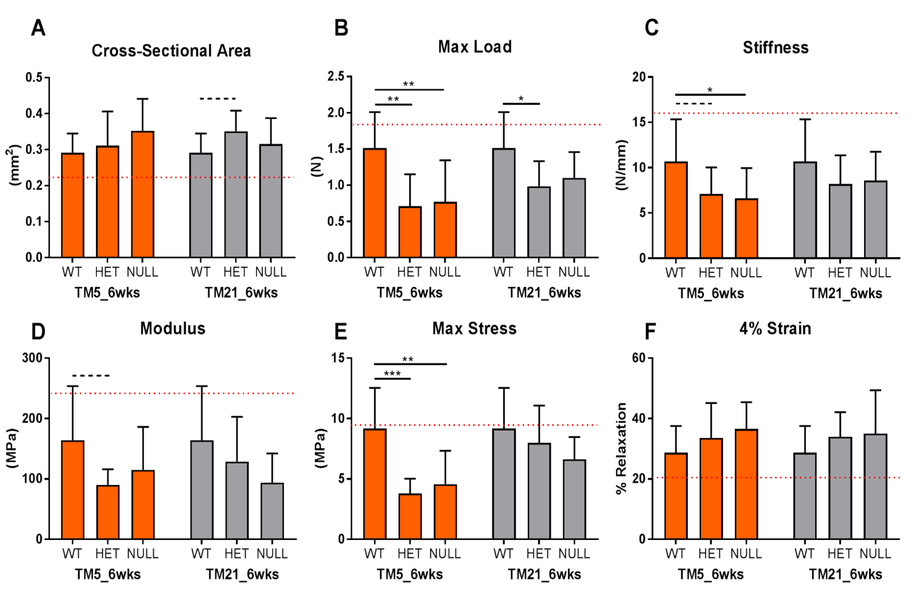

Figure 5. (A) Cross-sectional area and **(B)** max load were significantly different between the WT and HET tendons with TM21-induction after 6 weeks of healing. TM5-induced WT tendon exhibited greater **(B)** max load, **(C)** stiffness, **(D)** modulus, and **(E)** max stress than the HET and/or NULL tendons. These differences were not observed between the TM-21 induced tendons after 6 weeks of healing. **(F)** No differences in stress relaxation. Uninjured WT tendons are represented with the red dotted line. Data presented as mean+SD. (*p<0.05, **p<0.01, ***p<.001, trend ---).

Discussion

- Contrary to our hypothesis, the degree of collagen V deficiency did not have a significant effect on the healing response in the late inflammatory and remodeling phases.
- Interestingly, knocking down collagen V during the late inflammatory phase resulted in more substantial deficits in tendon mechanics than collagen V knockdown during the early remodeling phase.
- The observations support the direct correlation between collagen V production and tendon inflammation as concluded in a study where collagen V was substantially increased in chronically inflamed connective tissue [3]
- Further investigation is required to elucidate the mechanistic role of collagen V at the gene and protein level and to define the pathologic and functional significance of collagen V.

References & Acknowledgments

[1] Johnston et al., *J Orthop Res*, 35(12), [2] Dunkman et al., *Ann. Biomed Eng,* 42(3), [3] Harayanan et al., *Collagen Rel Res,* 3(4)

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