

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 characterized by genetic mutations of collagen V, a matrix protein present in tendon.¹ Two hallmarks of cEDS are connective tissue hyperelasticity and poor wound healing, and a murine model of cEDS demonstrates impaired tendon healing.^{2,3} 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. Therefore, the objective of this study was to determine the isolated role of collagen V on healing tendon mechanics. Due to its role in fibrillogenesis, we hypothesized that acute knockout of collagen V following injury would result in decreased tendon mechanical properties at both intermediate and late healing time points.

Methods

Animals

Male wild-type (WT) (n = 45) and bitransgenic *Col5a1^{fllox/+}* (n = 30) and *Col5a1^{fllox/fllox}* (n = 30) mice with a tamoxifen (TM)-inducible Cre were used in this study (IACUC approved). At 120 days old, mice received bilateral, full thickness, partial width patellar tendon injuries under sterile conditions.⁴ For Cre-mediated excision of the *Col5a1* gene, mice received two consecutive daily doses of TM (2mg/40g body weight) beginning on the day of injury. Mice were sacrificed at 3 or 6 weeks post-injury. Healthy WT control mice received TM doses (3 days of 4mg/40g body weight) at 120 days old and were sacrificed 30 days later. Tibia-patellar tendon-patella complexes were harvested and prepared for mechanical testing as previously described.⁵

Mechanical Testing

Uniaxial, viscoelastic testing was performed with an Instron 5848. The testing protocol consisted of 10 cycles of preconditioning, followed by stress relaxations at 3%, 4%, and 5% strain. Following each stress relaxation, frequency sweeps of 10 cycles at 0.1, 1, 5, and 10Hz were performed. A ramp-to-failure followed the 5% stress relaxation. Percent relaxation, dynamic modulus (E^*), and phase shift (δ) were quantified for each stress relaxation and frequency sweep. Stiffness, modulus, maximum

load, and maximum stress were quantified from the ramp-to-failure data.

Statistics

For all mechanical properties, one-way ANOVAs with Bonferroni post-hoc tests were used to compare across genotypes and uninjured controls at each healing time point. Significance was set at $p \leq 0.05$, and trends were set at $p \leq 0.1$.

Results

Injury effects

Compared to uninjured tendons, injured WT tendons had increased cross-sectional area (CSA) (Fig 1).

Quasi-Static Mechanics

Compared to uninjured tendons, injured WT tendons were less stiff (Fig 2A), had no differences in max load (data not shown), had decreased modulus (data not shown), and lower max stress (Fig 2B).

Stress Relaxation

Compared to uninjured tendons, injured WT tendons had greater stress relaxation at 3%, 4%, and 5% strains (data not shown).

Dynamic Mechanics

Compared to uninjured tendons, injured WT tendons had decreased dynamic moduli at all

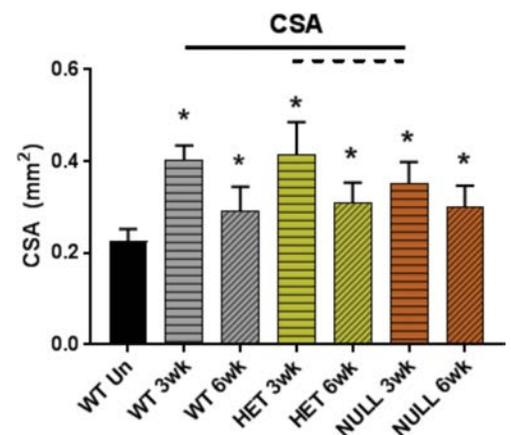


Figure 1. Cross-sectional area. Injured tendons across all genotypes and healing time points had larger CSA than uninjured tendons. * indicates $p < 0.05$ compared to uninjured tendons. Solid bars indicate $p < 0.05$, and dashed bars indicate $p < 0.1$.

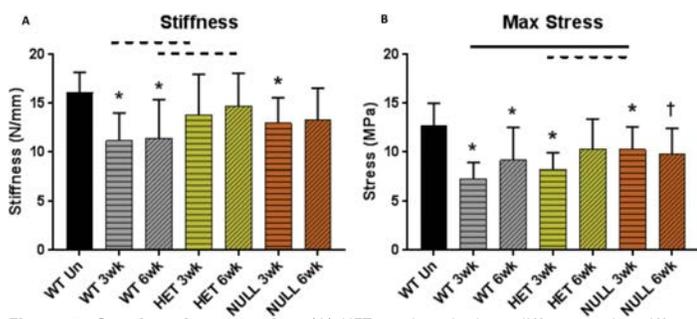


Figure 2. Quasi-static properties. (A) HET tendons had no differences in stiffness compared to uninjured tendons but trended towards higher stiffness compared to WT at both healing time points. (B) 6-week HET tendons had no differences in max stress compared to uninjured tendons. * indicates $p < 0.05$, and † indicates $p < 0.1$, compared to uninjured tendons. Solid bars indicate $p < 0.05$ and dashed bars indicate $p < 0.1$.

strains and frequencies, larger $\tan(\delta)$ values at 3 and 4% strain and for most frequencies at 5% strain for 6-week WT tendons (data not shown).

Genotype effects

Injured *Col5a1*^{+/-} (HET) and *Col5a1*^{+/+} (NULL) tendons had increased CSA relative to uninjured (Fig 1). At 3 weeks post-injury, NULL tendons had decreased CSA relative to WT and a trend towards smaller CSA relative to HET.

Quasi-Static Mechanics

No differences in stiffness were observed between HET and uninjured tendons (Fig 2A). HET tendons trended towards higher stiffness relative to injured WT tendons at both healing time points. 3-week NULL tendons were less stiff than uninjured tendons, but this decrease did not persist at 6-weeks. 3-week HET tendons had lower max stress than uninjured tendons, but this decrease did not persist at 6-weeks (Fig 2B). 3-week NULL tendons had lower max stress than uninjured tendons, and this difference persisted as a trend at 6-weeks. 3-week NULL tendons had higher max stress than 3-week WT tendons and trended towards higher max stress relative to 3-week HET tendons. HET and NULL tendons had decreased modulus compared to uninjured. No differences in max load were observed between knockout and uninjured tendons. No differences in max load or modulus were observed between injured genotypes.

Stress Relaxation

HET and NULL tendons had increased stress relaxation at 3% and 4% strain relative to uninjured. 3-week HET and NULL tendons exhibited increased stress relaxation at 5% strain relative to uninjured, which persisted for 6-weeks HET but not for 6-week NULL tendons. No differences in stress relaxation were observed between injured genotypes at any strain.

Dynamic Mechanics

HET and NULL tendons had decreased dynamic moduli relative to uninjured. For most frequencies at 3% and 4% strain, HET and NULL tendons had larger $\tan(\delta)$ values than uninjured, while at 5% strain, 6-week HET tendons had larger $\tan(\delta)$ values than uninjured. No differences in $\tan(\delta)$ values were observed

between 6-week NULL and uninjured tendons. No differences in dynamic modulus or $\tan(\delta)$ values were observed between injured genotypes at any strain or frequency.

Discussion

Injured tendons exhibited substantial deficits in mechanical properties relative to uninjured tendons. Contrary to our hypothesis, however, acute knockout of collagen V did not further impair the mechanical properties of these healing tendons. Instead, stiffness did not decrease through healing in HET tendons. Injured HET tendons were stiffer than WT tendons at each healing time point. No decreases in max stress were seen with 6-week HET tendons. These results demonstrate that while healing tendons have impaired mechanical properties, collagen V deficiency during healing does not further diminish these properties. Instead, collagen V haploinsufficiency during healing mitigated the decreases in stiffness and max stress seen in WT injured tendons. Impaired tendon healing in cEDS patients may not be due to the regulatory role of collagen V during healing and may instead be due to pre-existing deficiencies of the tissue. A previous study found that fibroblasts from a murine model of cEDS demonstrated decreased proliferation, migration, and wound healing relative to WT fibroblasts.⁶ Results of the present study support the notion that poor wound healing in cEDS patients is due to differences in tissue properties that existed prior to injury. A limitation of this study is the global nature of the collagen V knockouts, which could cause confounding effects on neighboring tissues. The inducible knockout models used here lessen these effects due to the short period of knockout. Future studies will analyze the composition and gene expression of these tendons to identify other differences in healing, collagen V-deficient tendons. Overall, this study demonstrates that collagen V deficiency does not impair the mechanical properties of injured tendons beyond the normal healing response, and instead mitigates some of these mechanical deficits.

Significance

This study reveals that the quasi-static mechanical deficits of injured tendons are not worsened, and are instead mitigated, by collagen V deficiency. These results provide a further understanding of the role of collagen V in tendon healing.

Acknowledgements

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