Collagen III Deficiency Alters Mechanical Properties and Decreases Regulation of Fibrillogenesis Following Injury in Female Murine Tendons

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Introduction

Patients with vascular Ehlers-Danlos syndrome (*v*EDS), a rare genetic disease caused by *Col3A1* mutations, are well-known for severe vascular complications and early death. However, tendon rupture and dysfunction contribute to patient morbidity[1-4], supporting a critical role of collagen III (Col3) in tendon homeostasis and maintenance. Col3 is essential in homeostasis and healing of other collagen I (Col1)-rich tissues (e.g., skin[5], meniscus[6], and bone[7]) due to its role regulating fibrillogenesis, extracellular matrix (ECM) organization, and the formation of cross-links and scar tissue[5,6]. Therefore, the objective of this study was to define the role of Col3 in both tendon homeostasis and in response to injury, regulating collagen fibril deposition and resultant alterations in tendon mechanics. We hypothesized that a reduction in Col3 would result in a more robust, stiffer provisional matrix early in tendon healing, with smaller diameter fibrils when compared to wild-type tendons.

Methods

Female wild-type (WT) Balb/cJ and heterozygous *Col3a1*$^{+/-}$ mice at 30 days of age (n = 48) were used (IACUC approved). Injured mice underwent bilateral patellar tendon injury surgery[8] and were sacrificed 1-week (1w) post-injury in the early proliferative phase of healing. Uninjured sex, strain and age-matched mice were also examined.

Transmission Electron Microscopy (TEM)

Tendons for TEM (n = 4/group) were fixed *in situ* and processed[10] to analyze fibril structure.

Mechanics

Patella-patellar tendon-tibia complexes were prepared for mechanical testing (n = 12/group)[11]. Tendons were subjected to a viscoelastic testing protocol[10,12] consisting of: 1) preconditioning, 2) stress relaxation at strain levels of 2%, 3% and 4%, 3) a sinusoidal frequency sweep (10 cycles at 0.1, 1, 5, and 10 Hz) at each strain level, 4) return to gauge length, and 5) ramp to failure.

Statistics

Two-way repeated measures ANOVAs with post-hoc Bonferroni tests were used to assess the effects of genotype, injury and their interaction on quasistatic and viscoelastic properties. Collagen fibril diameter distributions were compared by genotype using Kolmogorov-Smirnov tests. Significance was set at $p \leq 0.05$ (solid lines) and trends at $p \leq 0.1$ (dotted lines).

Results

Following injury, tendon cross-sectional area was increased in both WT and *Col3a1*$^{+/-}$ tendons with *Col3a1*$^{+/-}$ tendons having a larger area than WT tendons (trend) following injury (Figure 1). *Col3a1*$^{+/-}$ tendons had increased failure load and stiffness (Figure 2A,B) 1w post-injury when compared to WT tendons, with no differences in uninjured tissues. Additionally, WT tendons had a lower failure load and modulus (trend) 1w post-injury when compared to uninjured, while there was no effect of injury in *Col3a1*$^{+/-}$ tendons (Figure 2A,C). Failure stress (Figure 2D) was decreased in both genotypes 1w following injury. Additionally, TEM analysis showed a shift to smaller diameter fibrils post-injury in both genotypes (Figure 3). Finally, distinctly different distributions for WT and *Col3a1*$^{+/-}$ fibrils post-injury were seen, with *Col3a1*$^{+/-}$ tendons having a larger population of smaller and larger fibrils, and WT tendons having a less pronounced peak and more flat distribution (Figure 3).

Discussion

Our study shows that Col3 deficiency alters both mechanical properties and matrix structure 1w post-injury in a murine patellar tendon injury model in novel and previously unexplored ways. Tendon area increases following injury as healing tissue is deposited into the wound site. The trend toward increased area of *Col3a1*$^{+/-}$ tendons compared to WT tendons post-injury is consistent with an increased deposition of provisional matrix, secondary to increased activation of fibroblasts in *Col3a1*$^{+/-}$ tendons, as decreased Col3 has been shown

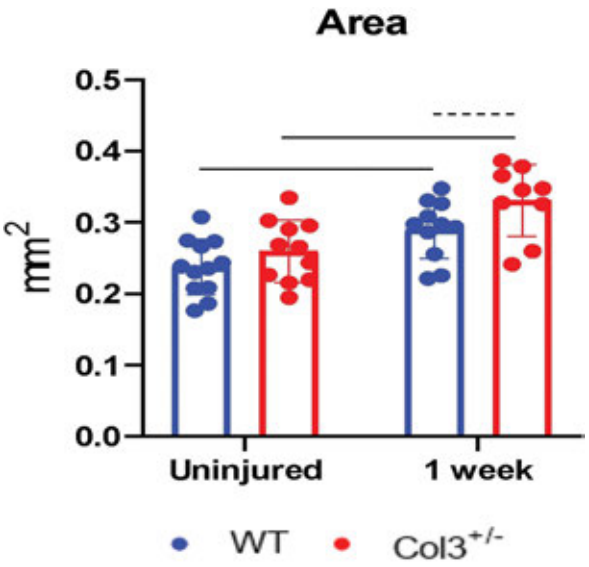

Figure 1. Tendon cross-sectional area was increased post-injury in both genotypes when compared to uninjured tendons.

to cause increased activation[5]. Decreases in failure stress in both genotypes following injury is due to increases in area without concurrent increases in failure load, indicating poor quality tissue following injury in both genotypes as would be expected 1w post-injury. Additionally, TEM analysis showed a more densely packed provisional matrix with smaller fibrils following injury in *Col3a1*$^{+/-}$ tendons likely explaining increased stiffness and further indicating a hypersecretory state of myofibroblasts for Col1 post-injury.

Lastly, the highly skewed fibril diameter distribution with an extended right tail in *Col3a1*$^{+/-}$ tendons indicates dysregulation in fibrillogenesis when compared to WT tendons post-injury. An increased population of larger fibrils reveals increased lateralization of fibrils in *Col3a1*$^{+/-}$ tendons, which is expected as Col3 presence decreases lateral growth during fibrillogenesis[13]. Notably, while *Col3a1*$^{+/-}$ tendons have increased failure load following injury compared to WT tendons at this time point, the poor quality of healing tissue quantified in this study supports the likelihood of an important role of Col3 in dictating cellular activity and healing potential.

Based on these findings, we will examine later time points to understand how fibril growth continues into later stages of healing, along with alterations to the cellular population and activity. Importantly, we will also further evaluate the role of Col3 using a novel conditional Col3 knockdown model to understand the unique temporal role of Col3 throughout

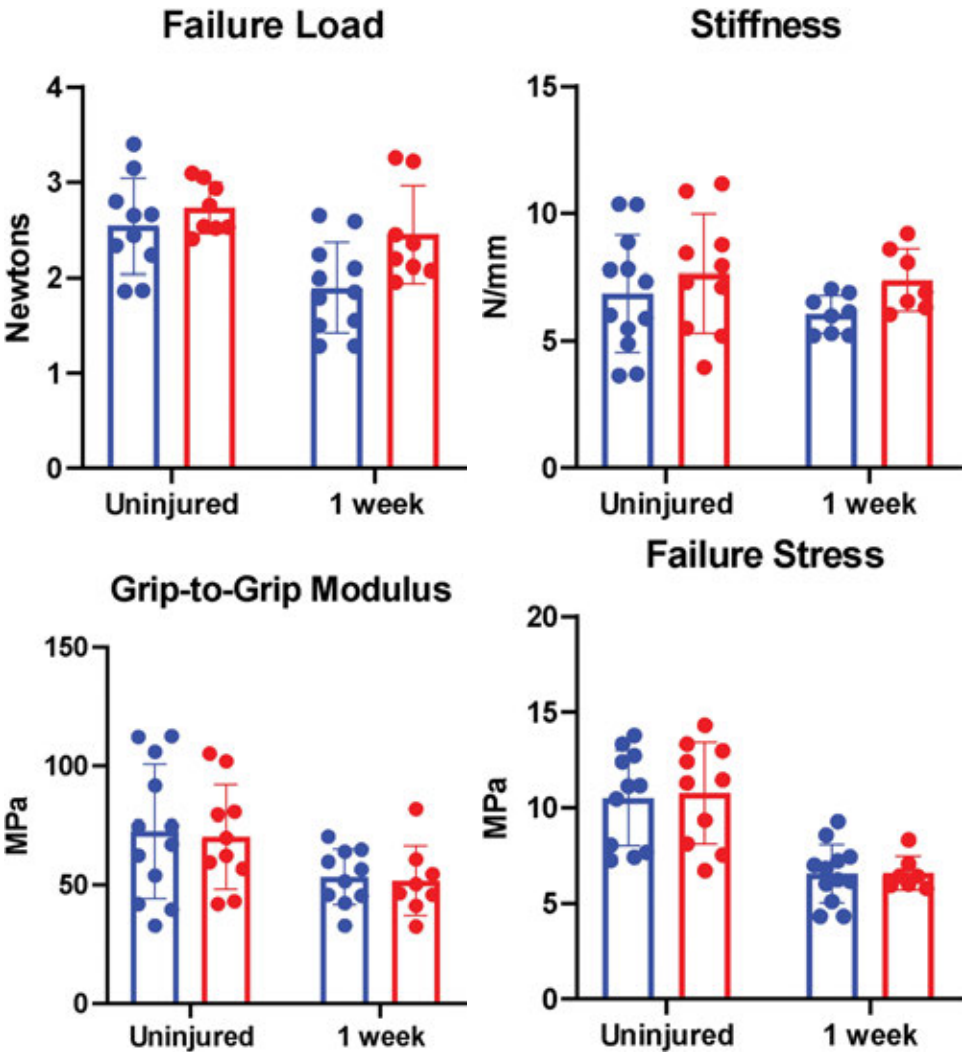

Figure 2. Failure load and stiffness was increased in *Col3a1*$^{+/-}$ tendons 1w post-injury compared to WT. Failure stress was decreased in both genotypes following injury.

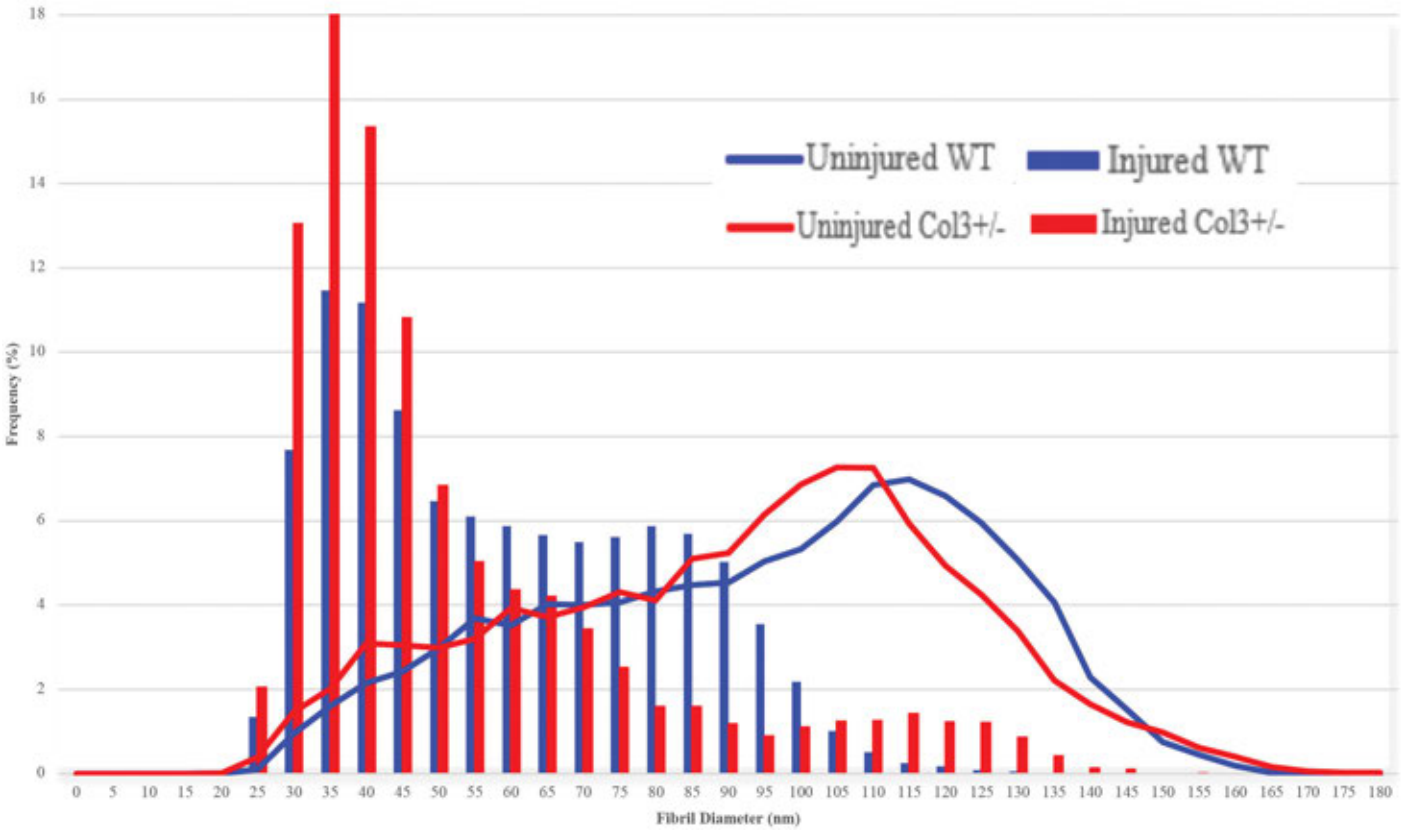

Figure 3. Smaller fibrils were seen in both genotypes following injury. *Col3a1*$^{+/-}$ tendons post-injury had a larger population of smaller fibrils and larger fibrils when compared to WT. Uninjured: line graph, Injured: bar graph.

healing and more specifically, to rigorously analyze the targeted role of Col3 by evaluating the dose response in an otherwise normal matrix.

Significance

Col3 is crucial during early wound healing, affecting matrix structure and function, likely influencing long-term healing. Elucidating the mechanistic role of Col3 throughout healing will provide the necessary foundation for developing Col3-inspired therapies that optimize tendon healing and will ultimately have a profound impact on tendon healing, thereby decreasing healthcare expenditures and improving patient quality of life.

Acknowledgments

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