Distinct and Synergistic Roles of Collagens V and XI in Structural and Mechanical Recovery during Tendon Healing

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INTRODUCTION: The hierarchical organization of tendon during healing depends on the coordinated assembly of extracellular matrix proteins, including minor fibril-forming collagens such as types V and XI[1]. Collagen V regulates fibrillogenesis by nucleating fibril formation and co-assembling with collagens I and II, while collagen XI plays a similar regulatory role and forms heterotypic fibrils in association with collagen V[2,3]. Following tendon injury, collagen XI expression exhibits a pronounced but transient increase, whereas collagen V expression remains persistently elevated, suggesting that both collagens play essential roles in the restoration of tendon structure and function[3,4]. However, the mechanisms by which coordinated expression of collagens V and XI influence the acquisition of tendon structure and mechanical integrity during healing remain unclear. Therefore, the present study aimed to elucidate the regulatory roles of collagens V and XI and their potential synergistic interactions in tendon healing. We hypothesized that targeted alteration of collagen V and/or XI expression would impair tendon healing and compromise mechanical properties in a synergistic manner.

METHODS: *Animal model:* One hundred and fifty C57BL/6 (n=68 male, n=82 female) mice including 5 genotypes (Wild-type (WT), *Cre* negative littermates), Col XI heterogeneous knockdown (XI HET, *Rosa-CreER^{T2};Col11a1$^{f/+}$*), Col XI knockdown (XI KD, *Rosa-CreER^{T2};Col11a1$^{f/f}$*), Col V knockdown (V KD, *Rosa-CreER^{T2};Col5a1$^{f/f}$*), and Col V/XI double knockdown (DBL KD, *Rosa-CreER^{T2};Col5a1$^{f/f}$;Col11a1$^{f/f}$*) were included (n = 12–16 per group, IACUC-approved). Knockdown was induced by tamoxifen (100 mg/kg) on days −3, 0, 3, and 6. Mice at 90 days of age underwent bilateral patellar tendon punch injuries (0.75 mm) on day 0 and were euthanized at either 3- or 6-weeks post-injury. *Mechanics:* The cross-sectional area (CSA) was determined using a custom laser measurement device and patellar tendons were then stamped bilaterally into a dog-bone geometry. Verhoeff stain lines were applied to facilitate optical strain analysis. For mechanical testing, the patella was fixed in rubber and clamped in a custom grip, while the distal tibia was potted in polymethyl methacrylate (PMMA) and immersed in a 37 °C 1× phosphate-buffered saline (PBS) bath. The testing protocol comprised: (1) preconditioning; (2) static holds at 2% and 4% axial strain followed by a 10-minute hold and subsequent dynamic frequency sweeps at 0.1, 1, 5, and 10 Hz; (3) a 5-minute unloaded rest period; and (4) a ramp-to-failure at a rate of 0.1% strain/s. Elastic and viscoelastic properties (dynamic modulus (E*) and phase shift (tan δ)) were calculated[5]. Fiber alignment within the injury region was assessed and quantitatively analyzed using reflectance-mode polarized light imaging. *Statistics:* All datasets were screened for statistical outliers using the 2.2× interquartile range (IQR) criterion. Group comparisons were performed using one-way ANOVA followed by Bonferroni post hoc tests. Significance was set at $p<0.05$ and trends at $p<0.10$.

RESULTS: The CSA remained comparable across all groups at both post-injury time points. At 3 weeks, the V KD and XI KD groups exhibited trends toward reduced maximum load compared with WT, whereas the DBL KD group showed a significant reduction. In addition, the DBL KD group demonstrated significant decreases in stiffness and modulus relative to WT, as well as a significant decrease in stiffness compared with XI KD. A trend toward decreased stiffness was also observed in V KD relative to WT. Dynamic testing revealed significantly increased phase shift (tan δ) at 0.1 Hz and 4% strain in XI KD and XI HET compared with WT. At 6 weeks, V KD tendons exhibited significant decreases in maximum load, maximum stress, and toughness compared with WT. The DBL KD group displayed significantly reduced dynamic modulus relative to both WT and XI KD, and a trend toward lower dynamic modulus compared with V KD at 4% strain across all frequencies.

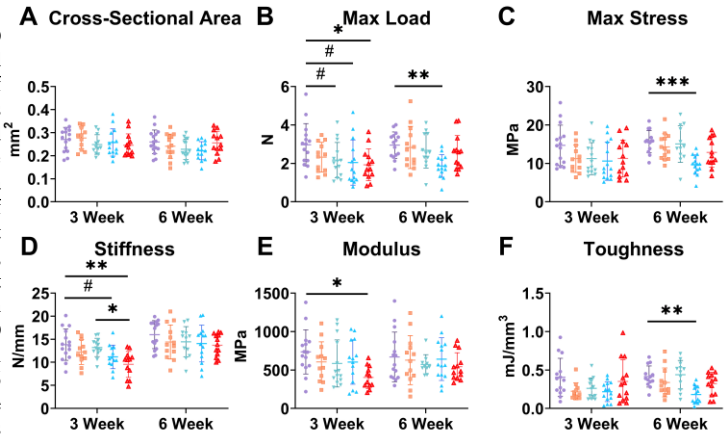

Figure 1. The CSA remained comparable across all groups at both time points (A). At 3 weeks, V KD and XI KD showed trends toward reduced max load compared with WT, while DBL KD was significantly lower (B). The DBL KD showed significant decreases in stiffness (D), and modulus (E) compared with WT, and lower stiffness than XI KD (D). A trend toward decreased stiffness was also observed in V KD relative to WT (D). At 6 weeks, V KD tendons had significantly reduced max load (B), max stress (C), and toughness (F) compared with WT. (#p≤0.1, *p<0.05, **p<0.01, ***p<0.001).

DISCUSSION: At 3 weeks post-injury, the observed trends in V KD and XI KD groups, along with the significant decrease of maximum load in the DBL KD group, support the hypothesis that reduced expression of collagens V and XI compromises tendon healing[6]. The marked decreases in stiffness and modulus in DBL KD tendons suggest a more pronounced impairment of the healing process in the absence of both collagens. The lack of comparable deficits in modulus in other genotypes may imply a synergistic interaction between collagens V and XI, in which the loss of either alone is insufficient to produce evident mechanical impairment during tendon healing. The trend toward reduced stiffness in V KD, along with its preservation in XI KD and XI HET, may further suggest that collagen V plays a comparatively dominant role in early tendon healing[7]. The increased phase shift observed in XI HET and XI KD indicates that collagen XI contributes more prominently to the regulation of tendon viscoelastic behavior during the early post-injury phase[8]. By 6 weeks post-injury, maximum load, maximum stress, and toughness were significantly reduced in V KD tendons but were comparable to WT levels in DBL KD tendons, pointing to a complex regulatory interplay between collagens V and XI during tendon remodeling[6]. The preservation of stiffness and modulus across all groups highlights a dissociation between bulk elastic properties and ultimate tensile strength. In the absence of the functionally dominant collagen V[7], the presence of collagen XI alone may provoke a maladaptive remodeling response that compromises failure properties. Alternatively, the additional knockdown of collagen XI may induce a compensation response that salvages tendon healing. However, DBL KD tendons exhibited significantly reduced dynamic modulus compared with WT and XI KD, and trended lower than V KD, indicating that collagens V and XI act synergistically to regulate viscoelastic behavior, with collagen V assuming a more prominent role in later stages of remodeling. Collectively, these results indicate that collagens V and XI contribute both interactively and independently in different phases of tendon healing[1], and that the absence of one collagen type can provoke a distinct remodeling trajectory compared with the absence of both.

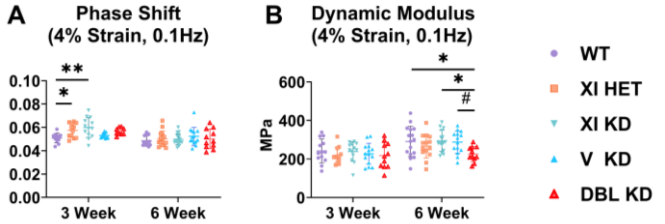

Figure 2. At 3 weeks post-injury, XI KD and XI HET group exhibited a significantly increased phase shift (tan δ) at 0.1 Hz under 4% strain compared with WT (A). At 6 weeks, the DBL KD group demonstrated a significantly reduced dynamic modulus relative to WT and XI KD, with a trend toward reduction compared with V KD, across all frequencies at 4% strain (B). (#p≤0.1, *p<0.05, **p<0.01, ***p<0.001).

SIGNIFICANCE: Collagens V and XI exhibit both distinct and synergistic functions in restoring tendon structure and mechanics during healing and remodeling. Elucidating the mechanisms underlying their contributions will provide a critical foundation for the development of targeted therapeutic strategies aimed at modulating these collagens in pathological conditions or after injury.

REFERENCE: [1] Wenstrup et al., *J Biol Chem*, 2011. [2] Connizzo et al., *J Orthop Res*, 2015. [3] Sun et al., *Matrix Biol*, 2020. [4] Johnston et al., *J Orthop Res,* 2017. [5] DiStefano et al., *PLoS ONE,* 2025. [6] Leiphart et al., *J Biomech,* 2022. [7] Sun et al., *J Cell Sci*, 2011. [8] Ye et al., *J Biomech Eng,* 2025.

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