Effects of microsurgical reconstruction on tendon and inflammatory response in a clinically relevant model of Achilles tendon repair

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DISCLOSURES: Varun Arvind (N), Alice H. Huang (N)

INTRODUCTION: Tendon injuries are common and debilitating, with limited regenerative potential in adults. Mouse models of tendon injury consist of reconstructive and non-reconstructive (partial/full thickness transections without repair) injury models. Reconstructive models often involve immobilization via external fixation, denervation, or internal bracing with cerclage to protect tendon repair; however, none of these models reflect clinical management of acute tendon rupture which includes reconstruction with transient immobilization followed by resumed activity[1–3]. In this study we perform Achilles tenotomy followed with microsurgical tendon reconstruction with transient casting and assess tendon healing via gene expression and mechanical properties. Given the notable role of macrophages in orchestrating tendon healing, we also characterize the inflammatory polarization of macrophages in response to injury and repair[4,5]. We hypothesize that repair with transient casting will result in Achilles tendon repair with improved mechanical properties and increased expression of tenogenic genes. We further hypothesize that macrophages will assert an inflammatory (Ly-6c^{hi}) phenotype due to the suture foreign body.

METHODS: Achilles tendons were transected in 4 month old skeletally mature C57BL/6J mice in accordance with IACUC. Male and female mice were randomly allocated to Achilles tenotomy, tenotomy with casting, or tenotomy with reconstruction and casting. Tendons were microsurgically reconstructed immediately after transection using a two-stranded modified Kessler technique with a 8-0 nylon suture. Following, a locked-running epitendinous repair was performed using an 8-0 nylon suture (Fig. 1A). PBS was applied during repair to prevent tissue drying. Skin was closed with 6-0 prolene. With the foot in maximal plantarflexion, Velcro-tape was wrapped circumferentially from the midfoot extending past the knee to immobilize the leg[6]. Velcro was secured with glue and 6-0 Prolene suture. Velcro casts were removed 10 days post reconstruction. Tensile testing (preload 0.05N for 1min followed by ramp to failure at 1%/s) of tendons was carried out at 28 days post injury (DPI). For tensile testing of reconstructed tendons, the suture knot was cut but left in place as to not damage the tendon. For flow cytometry, tendons were enzymatically digested (5mg/mL collagenase I, 1mg/mL collagenase IV in DMEM) and Ly-6c^{hi} (M1-like) and Ly-6c^{lo} (M2-like) monocytes were gated on CD45+, Gr1–, CD11b+ cells. Gene expression by rt-qPCR was performed at 14DPI on RNA from bulk tendon isolated using Trizol reagent. Statistics were carried out by one-way ANOVA (Graphpad Prism).

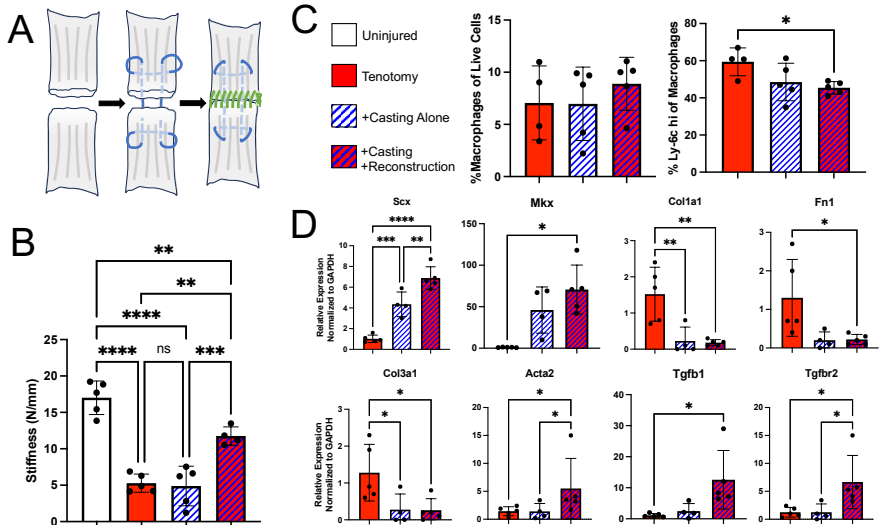

Fig. 1. Characterization of microsurgical reconstruction and casting on tendon repair. A) Schematic of repair. B) Tensile testing of tendons at 28DPI. C) Flow cytometry of tendons at 14DPI to assess macrophage recruitment and polarity. D) qRT-PCR of tendons at 14DPI to assess gene expression of tendon, matrix, myofibroblast, and TGFB associated genes.

RESULTS: In preliminary studies, reconstruction without casting led to consistent repair failure (not shown). At final time points, only 1 of the 15 (6.7%) reconstructions with casting failed grossly and was excluded from further analysis. Tenotomy alone resulted in decreased stiffness with no improvement with casting alone (Fig. 1B). Reconstruction with casting resulted in improved stiffness compared to casting or tenotomy alone but remained inferior to uninjured tendon (Fig. 1B). Surprisingly, flow cytometry of tendons at 14DPI demonstrated no difference in recruitment of macrophages across groups; however, polarization of macrophages in reconstructed and casted mice revealed decreased polarization of inflammatory (Ly-6c^{hi}) macrophages compared to tenotomy or casting alone (Fig. 1C). Gene expression of tendons at 14DPI revealed increased expression of the tenogenic transcription factors *Scx* and *Mkx* in the reconstruction group compared to casting or tenotomy alone (Fig. 1D). Expression of *Scx* was increased in casting compared to tenotomy alone (Fig. 1D). Assessment of matrix proteins (*Col1a1, Fn1, Cos3a1*) showed decreased expression in both casting with and without reconstruction compared to tenotomy alone (Fig. 1D) Interestingly, expression of *Acta2* which is expressed by myofibroblasts was increased in the reconstructed group (Fig. 1D). Given the importance of *Tgfb* signaling in tendon repair, we assessed expression of the ligand (*Tgfb1*) and receptor (*Tgfbr2*) pair and found increased expression in the reconstruction but not casting alone group compared to tenotomy (Fig. 1D).

DISCUSSION: While prior *in vivo* studies in mice have revealed deep insights into the cellular and molecular mechanisms of tendon repair, translation of such work will require further investigation in clinically relevant microsurgical repair models that more closely resemble clinical practice without permanent immobilization using internal/external fixators or denervation. Moreover, treatments that target inflammation or signaling should consider how repair strategies may alter underlying biology. Here we describe and characterize a novel model of tendon reconstruction with transient immobilization that more closely resembles clinical management. Importantly, we show that reconstruction with casting confers important changes in gene expression and macrophage polarity that may contribute to improved mechanical properties.

SIGNIFICANCE: Development of a clinically relevant preclinical model of tendon reconstruction can enable the investigation of repair mediated tendon healing to further improve the treatment and understanding of tendon injury.

ACKNOWLEDGEMENTS: This study was supported by NIH/NIAMS R56 AR076984 and R01 AR081674 to AHH. We also acknowledge the CSCI Flow Cytometry Core and Genomic Center at Columbia University.

REFERENCES: [1] Michael+, J Orthop Translat, 2020; [2] Tuzmen+, J Orthop Res, 2018; [3] Palmes+, J Orthop Res, 2002; [4] Howell+, FASEB, 2021; [5] Sunwoo+, J Orthop Res, 2020; [6] Aihara+, J Phys Therapy Sci, 2017.