INTRODUCTION

Flexor tendon injuries in the hand often produce scars that can form functionally limiting adhesions. Despite modern surgical approaches and aggressive rehabilitation protocols, tendon adhesions are still an unsolved problem for hand surgeons.\(^{[3]}\)

Advancement of siRNA technology has led to development of antisense-oligonucleotides (ASO) that are resistant to in vivo degradation. TGF-β signaling regulates scarring in tendons. Thus, inhibition of specific molecules in this pathway may reduce scar and subsequent adhesion formation in healing tendons. In the previous study, deletion of Smad3, a downstream target of TGF-β signaling pathways, reduced adhesions after tendon repair in mice.\(^{[5]}\) The current study investigates local delivery effects of specific ASOs targeting Smad3, Tgfβ1, and Ctgf on adhesion formation in a primary tendon repair animal model.

MATERIALS AND METHODS

**Experimental Animals:** C57BL/6 7- to 8-week-old male mice were obtained from Jackson Laboratories. After surgery, mice were randomly divided into four treatment groups: (1) Control (scrambled siRNA; 300µg/µl), and (2) Smad3 ASO, (3) Tgfβ1 ASO, and (4) Ctgf ASO. Treatments were delivered by local injection using micro-syringes (Hamilton) at 3 post-operative time points (Day (D) 2, D6, and/or D12), depending on the tissue harvest point (D3, D7, D10, D14, or D21).

**Animal Models:** Anesthetized mice were subjected to a full-thickness laceration of the Flexor Digitorum Longus (FDL) tendon in the hindlimb and immediately repaired using 8-0 nylon sutures in a Kessler pattern. The myotendinous junction was released following procedures to avoid disruption of the repair site during early tendon healing from active forces across the injury site.

**Adhesion & Biomechanics Testing:** Six lower hindlimbs were harvested at D14 and D21 and were rigidly fixed with a clamp and stabilizing rod from a custom adhesion testing apparatus. FDL tendons were isolated and loaded with incremental static weights up to 19g, which were digital images taken at each increment. The metatarsophalangeal (MTP) range of motion (ROM) was measured using ImageJ software by two blinded independent observers. Following adhesion testing, isolated FDL tendons were mounted individually onto the 8841 DynaMight axial servo-hydraulic testing system (Instron). Ultimate failure force (tensile strength) and stiffness (slope of linear portion of load-deformation curve) were determined from force-deformation curves.

**Quantitative Real-time RT-PCR:** Four FDL tendons were harvested per group at five time points (D3, D7, D10, D14, and D21). Total RNA was extracted from each using the RNAeasy Lipid tissue kit (Invitrogen). Single-stranded cDNA was made using a reverse transcription kit (BioRad). Using murine specific primers for target genes (Smad3, Tgfβ1, Ctgf), as well as markers for scar formation (Col1a1, Col3a1) and tendon formation (Scleraxis (Scx), Tenomodulin (Tmnd)). mRNA expression was measured and standardized to β-actin. Data were normalized to un-operated wild-type tendons, which served as a pre-operative time point (D0).

**Histology:** Specimens were harvested at several time points after surgery/siRNA treatment and paraffin embedded. 3-µm sections underwent Hematoxylin & Eosin (H&E) staining.

**Statistics:** Results are shown as the mean±standard deviation. Statistical testing included a two-way ANOVA with Tukey-Kramer post hoc multiple comparisons (P <0.05 was considered significant).

RESULTS

Adhesion testing showed a significant decrease in MTP ROM in all experimental groups at D14 and D21 compared to unoperated tendon, while MTP ROM in all ASO treatment groups was higher than that in the control treatment group, consistent with reduced adhesion formation (Fig. 1A). Although no major difference in stiffness was observed between control and experimental groups, there is a significant increase in maximum tensile force achieved by the Smad3 ASO treated mice at D14 (Fig. 1B and 1C). Histology revealed decreased fibrous tissues surrounding the repair site in all ASO treatment groups when compared with control group at D7, D14, and D21 (Fig. 2). Tgfβ1 ASO treatment markedly decreased fibrous tissues between lacerated tendons, suggesting an improved tendon repair. Realtime RT-PCR at D10 showed a significant decline in Col3a1 expression, which is typically related with the development of scar tissue formation after tendon injury, in all ASO treatment groups (Fig. 3). It was also observed that between 14 and 21 days following repair, Tgfβ1 and Ctgf ASO treated groups had a delayed peak expression of Tmnd, a marker gene for mature tenocytes.

**DISCUSSION**

In the current study, we showed several changes in the tendon-healing environment after siRNA treatment targeting Smad3, Tgfβ1, and Ctgf. The implication that there may be a decrease in early scar formation after these siRNA treatments is supported by significant improvement in MTP joint flexion in all experimental groups after 2 weeks following primary tendon repair, histological evidence, and quantitative gene expression analyses. Unexpectedly, tendon healing seems to be delayed in Tgfβ1 ASO treatment by inhibition of tenocyte progenitor infiltration into the tendon repair site. Our results indicate that these treatments are potentially effective in reducing early scar formation and improving range of motion (ROM) during the healing process of injured flexor tendons. Further studies are needed to determine the effect of these ASO treatments for initial tendon healing.

**REFERENCES**


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