The Effects of Bio-Lubricating Molecules on Flexor Tendon Allograft in A Canine Model In Vivo

Chunfeng Zhao, M.D.¹, Wei Zhuang, M.D.¹, Ramona Reisdorf, B.S.¹, Andrew R. Thoreson, M.S.¹, Gregory D. Jay, M.D., Ph.D.², Kai-Nan An, Ph.D.¹, Peter C. Amadio, M.D.¹.
¹Mayo Clinic, Rochester, MN, USA, ²Brown University, Providence, RI, USA.


Introduction: Flexor tendon graft is the primary surgical procedure for restoring hand function if direct repair cannot be performed due to severe trauma or primary repair failure. The most common flexor tendon graft is an autologous extrasynovial tendon, such as the palmaris longus. However, the flexor tendons in zone II are intrasynovial tendons. This mismatch in tissue type has been considered a major cause of poor function following tendon autograft. Although allograft intrasynovial tendons are available, allograft preparation procedures alter the tendon surface. A recent study has demonstrated that intrasynovial allograft treated with carbodiimide derivatized hyaluronic acid combined with lubricin (cd-HA-Lubricin; CHL) improves the surface quality in an in vitro model (1). Therefore, the purpose of this study was to investigate the effect of CHL on flexor tendon reconstruction using intrasynovial allograft tendon in a canine in vivo model.

Methods: Surgical Procedure: Twenty-eight flexor digitorum profundus (FDP) tendons of the 2nd and 5th digits from fourteen purpose-bred dogs were used for this study after Institutional Animal Care and Use Committee approval. The FDP allograft tendons were obtained after animal sacrifice from other IACUC approved projects and prepared with a standard freeze-thaw and lyophilization procedure (2). In order to mimic a clinically relevant condition for flexor tendon reconstruction, a chronic FDP tendon rupture with scar model was used, based on a previously published protocol (3). Six weeks after primary repair failure, the 2nd and 5th digits underwent tendon reconstruction using allograft FDP tendons. The ruptured FDP tendons along with surrounding scar were excised, after which a tendon graft was performed, either with an untreated or CHL treated graft. Postoperative rehabilitation was performed once per day, 7 days per week, for 6 weeks after tendon grafting, after which the animals were sacrificed and the digits were harvested for assessment.

CHL: 1% sodium hyaluronate (HA) (95%, 1.5x106MW, Acros), 10% gelatin (Sigma), 1% 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (Sigma), 1% N-hydroxysuccinimide (NHS) (Pierce), 0.1 M Mes pH 6.0, and 260 μg/ml lubricin (bovine synovial lubricin provided by Gregory Jay, MD)

Biomechanical Evaluation: After sacrifice, the graft digits were dissected and digit work of flexion normalized by digit joint motion (nWOF) was evaluated based on a well-established protocol (3). Following nWOF testing, the digit was carefully exposed in the zone II area and adhesions were scored from 0 (no adhesion) to 8 (Severe adhesion) based on previous grading criteria (3). Following adhesion evaluation, the graft tendon was isolated, and friction was measured between the graft tendon and proximal pulley as previously described (3). Finally, the mechanical strength of the distal phalanx and the distal tendon/bone attachment was tested to evaluate distal tendon/bone repair healing.

Histology: Calcein-AM and ethidium homodimer stain was used in two graft tendons from each group immediately following sacrifice to assess cell viability. The graft tendons from the same samples were also prepared for histologic evaluation with routine hematoxylin and eosin (H&E) staining of paraffin embedded sections.

Statistical Analysis: Mean and standard deviations of outcomes were reported. One-way ANOVA was used to compare means between groups with significance set at p<0.05.

Results: The adhesion score in grafts treated with CHL was 0.75 ± 1.5, which was significantly lower than the grafts that were treated with saline (3.1 ± 2.1) (p < 0.05). The nWOF and graft frictional force in the CHL group were all significantly lower than that in the saline group, but still higher than in the normal contralateral digit group (p < 0.05) (Figure 1A & B). The force to failure of the distal tendon to bone attachment in the CHL group was significantly lower than that in saline control group and in the time-0 repair group (p < 0.05) (Figure 2). There was no significant difference in maximal failure force between the six week grafts treated with saline and the grafts repaired to the distal phalanx at time 0. However, the stiffness of the saline-treated grafts was significantly higher than both the grafts treated with cd-HA-Lub and the grafts in the time-0 groups (p < 0.05) (Figure 2).

Discussion: Although allograft has been commonly used for ACL reconstruction, it is rarely used for flexor tendon applications due to problems with adhesion formation and function. In the current study, we describe a potentially useful and clinically applicable technique to improve allograft tendon quality, thereby decreasing adhesions and improving function for flexor tendon reconstruction. Our preliminary results are encouraging, though further work is needed to accelerate allograft recellularization and improve allograft-to-host healing.

Significance: Allograft tendon treated with two carbodiimide derivatized bio-lubricating molecules, hyaluronic acid and lubricin,
improves the results of flexor tendon reconstruction in a canine model in vivo.

Figure Legends:
Fig 1. nWOF (A) and graft frictional force (B). Asterisks indicate a significant difference.
Fig 2. Mechanical strength at distal graft/bone attachment. Asterisks indicate a significant difference.
Fig 3. Viable cells presented on the surface and the tendon midsubstance in normal FDP tendon (A.D.G). Viable cells were found in both graft groups but were limited to the tendon surface (B.E.H: CHL graft and C.F.I: control).

Acknowledgments: This study was funded by a grant from NIH/NIAMS (AR057745).
