Surface Modification with Chemically Modified Synovial Fluid for Flexor Tendon Reconstruction in a Canine Model In Vivo

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


Introduction: Flexor tendon injuries in the digits are common. Flexor tendon reconstruction is performed if the direct repair fails due to severe adhesions, re-ruptures or a large tendon defect. Clinical and experimental studies have shown restricted tendon gliding and reduced digit function, which is related to the inferior surface durability of the extrasynovial tendons compared to that of intrasynovial flexor tendons. Studies using a compound, carbodiimide derivatized hyaluronic acid combined with lubricin (cd-HA-Lubricin) in a canine model, have showed its ability to greatly reduce tendon adhesion and improve digit function, while having an adverse effect on tendon healing (1, 2). Tendon surface modification using a compound of carbodiimide derivatized SF with gelatin (cd-SF-G) showed significant improvement on gliding ability in vitro (3). Moreover, SF was found to have no inhibition on human osteoblasts in vitro, indicating SF might not affect tendon-to-bone healing (4). The purpose of this study was to investigate the effects of the surface modification of autograft extrasynovial tendon with cd-SF-G for flexor tendon reconstruction using a clinically relevant canine model.

Methods: Eleven purpose-bred dogs weighing 20 to 25 kg were used for this study after approval by our Institutional Animal Care and Use Committee. This study included a repair failure phase followed by a reconstruction phase. In the first phase, flexor digitorum profundus (FDP) tendons in the 2nd and 5th digits were transected at the proximal interphalangeal (PIP) joint level and repaired with a modified Kessler technique. Free cage activity caused all tendons to rupture and a scar to form on the digital sheath by post-operative week six. Flexor tendon reconstruction was performed using the peroneus longus (PL) tendons as grafts. Synovial fluid was first aspirated from both knees intraoperatively. One PL tendon was randomly selected as a control treated with saline, while the other was coated with cd-SF-G comprised of 46% native SF, 10% gelatin (from porcine skin; Sigma Chemical, St. Louis, MO, USA), 1% 1-ethyl-3-[(3-dimethylaminopropyl) carbodiimide hydrochloride] (EDC) (Sigma), 1% N-hydroxysuccinimide (NHS) (Sigma) in 0.1 M 2-[N-morpholino]ethanesulfonic acid (MES) buffer (Sigma), pH 6.0. A high radial neurectomy was performed to avoid weight-bearing through a lateral humeral approach. The operated limb was immobilized with a custom-made canine jacket and a modified passive synergistic rehabilitation therapy was performed daily for six weeks until sacrifice. After sacrifice, 2nd and 5th digits of each dog were tested for normalized digit work of flexion (nWOF) according to a well-established protocol. Following nWOF testing, the digits were exposed for adhesion score evaluation, on a scale ranging from 0 (no adhesion) to 8 (severe adhesion), and proximal adhesion breaking force. The FDP tendon was then isolated to test gliding resistance as previously described (5). The distal pullout strength and stiffness were measured to assess the healing strength following an established protocol (2). Indentation testing was performed to evaluate the mechanical response of a 5-
mm long tendinous segment to compression. Two tendons each in normal, control and cd-SF-G groups were histologically evaluated at the proximal and distal repair sites. Hematoxylin and eosin staining was used to assess the proximal tendon-to-tendon healing and the distal tendon-to-bone healing. Four additional FDP tendons were harvested from dogs for other IACUC approved studies and divided into two groups. The tendons of cd-SF-G group were treated with 1 ml cd-SF-G compound for 10 seconds and then incubated in 10 ml minimum essential medium (MEM) for 7 days. The tendons of control group were cultured for 7 days without other treatments. Cell viability of the tendons was assessed by calcein-Am (cal Am) and ethidium homodimer (EthD-1).

All the data were presented as a mean ± standard deviation. One-way analysis of variance was used to analyze differences in biomechanical properties. Statistical significance was set at P<0.05 in all cases. **Results:** No graft ruptures were found either at the proximal or distal repair sites. The cd-SF-G group showed significantly lower nWOF compared to the control group (P < 0.001) (Fig. 1 A). Tendons coated with cd-SF-G compound showed lower adhesion score, proximal adhesion breaking force and stiffness compared to the tendons treated with saline (P < 0.001, P =0.029, P =0.035, respectively) (Fig. 1 B & C). Gliding resistance in the cd-SF-G group was significantly lower than that of the control group (P < 0.05) (Fig. 2 A). There was no significant difference between the cd-SF-G group and control group for distal pullout strength (P = 0.226) and stiffness (P = 0.343) (Fig. 2 B). The compressive stiffness of cd-SF-G group was not significantly different compared to the control group (P=0.390) (Fig. 2 C).

Graft tendons coated with cd-SF-G presented a smooth layer of paratenons covering the tendon surfaces, without evident adhesions (Fig. 3 A). Abundant adhesions were found on the surfaces of tendons treated with saline. Graft tendons and native recipient tendons formed secure healing at the interface without obvious gaps, as did the distal tendon-to-bone insertions (Fig. 3 B).

Viable cells were found on the surfaces of both two groups, which were round in shape and randomly distributed (Fig. 3 C).

**Discussion:** No rupture was found in either the cd-SF-G or saline groups from macroscopic inspection of the proximal repair site. H&E staining showed solid union and cells infiltration into both host and graft tendons. These findings indicated that the surface coating of cd-SF-G might not affect tendon-to-tendon healing in this short-term observation. For the distal attachment, mechanical testing revealed that tendons coated with cd-SF-G could achieve similar failure strength and stiffness compared to the tendons coated with saline. Histological images also showed solid union between graft tendon and bone tissue in the two groups, without visual gaps. Hence, the surface modification of cd-SF-G might not interfere with tendon-to-bone healing of the flexor tendon reconstruction using an autograft.

**Significance:** Flexor tendon surface modification with cd-SF-G significantly improved digit function and reduced adhesion formation without effecting tendon healing and stiffness in this canine in vivo model.

![Figure 1](image_url)
Figure 2. The comparison of gliding resistance (A), distal attachment failure strength and stiffness (B), indentation stiffness (C). An asterisk indicates that the difference was significant.

Figure 3. The H&E staining of surfaces of normal FDP tendon, cd-SF-G or saline treated graft tendons (A). Secure healing was achieved at proximal and distal repair sites for both cd-SF-G and saline treated tendons (B). Viable cells were found on the surfaces of both groups (C).

ORS 2015 Annual Meeting  
**Paper No: 0107**