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Prevention of Peritendinous Adhesions Using a Hyaluronan-Derived Hydrogel Film Following Partial-Thickness Flexor Tendon Injury

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Abstract

Purpose—Peritendinous adhesions are an important complication of flexor tendon injury. Three hyaluronan (HA)-derived biomaterials were evaluated for the reduction of peritendinous adhesions following partial-thickness tendon injury in rabbits.

Methods—Rabbits (n = 24) were divided into three groups (n = 8 per group), which were used for gross evaluation, histologic assessment, or biomechanical testing. The fourth and third toes from both hindpaws of each rabbit were randomly assigned to one of four treatments: (i) untreated control, (ii) Seprafilm®, (iii) Carbylan™-SX *in situ*-crosslinked hydrogel, and (iv) pre-formed Carbylan™-SX film.

Results—Rabbits were sacrificed at three weeks post-surgery and evaluated anatomically, histologically, and mechanically. All materials used reduced adhesions relative to untreated controls for all three evaluations. Both the gross anatomic and histologic results revealed that Carbylan™-SX film was statistically superior to Seprafilm® and Carbylan™-SX gel in preventing tendon adhesion formation. In biomechanical tests, the Carbylan™-SX film-treated hindpaws required the least force to pull the tendon from the sheath. This force was statistically indistinguishable from that required to extrude an unoperated tendon (n = 8). Carbylan™-SX gel was less effective than Carbylan™-SX film but superior to Seprafilm® for all evaluations.

Conclusions—A crosslinked HA-derived film promoted healing of a flexor tendon injury without the formation of fibrosis at 3 weeks post-operatively.

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Keywords

crosslinked hyaluronan derivative; glycosaminoglycan hydrogel; post-surgical adhesion prevention; synthetic extracellular matrix; in situ crosslinking; rabbit model

INTRODUCTION

Postoperative finger function after primary flexor tendon repair is frequently compromised by adhesions between the tendon and the surrounding tissues.[1] Such post-operative adhesions restrict the full range of motion, and repaired tendons fail to recover their original strength. Physical barriers and biological techniques have been employed to reduce tendon adhesions without adversely affecting the healing process.[2] First, mechanical barriers used to surround the tendon and physically block adhesion formation include polyethylene membranes,[3] silicone sheets,[4] chondroitin sulfate/polymer membranes,[5] and polytetrafluoroethylene membranes.[6] Materials often failed due to inflammatory responses, ingrowth of adhesions at the edges of the materials, and tendon necrosis. Second, biological approaches to limit formation of collagenous scar tissue include dextran-70,[7] fibrin,[8] 5-fluorouracil,[9] indomethacin,[10] and unmodified hyaluronic acid (hyaluronan, HA). [11, 12] Third, sliding friction can be reduced by chemically modifying the tendon surface with carbodiimide-activated gelatin and/or HA.[13]

To harness the intrinsic anti-inflammatory and adhesion-limiting effects of HA, a non-sulfated glycosaminoglycan abundant in synovial fluid, high-viscosity, high molecular weight HA was placed between the tendon and its sheath promoted tendon healing and decreased adhesion formation.[14] HA solutions diminished excursion resistance after flexor tendon repair in a canine model.[15] HA inhibited the proliferation of rabbit synovial cells *in vitro*,[16] and addition of an anti-inflammatory agent to HA reduced both inflammation and post-surgical adhesions.[11] In mammals, the half-life of HA in tissues ranging 12–72 h, and exogenously HA is diluted and eliminated within a week.[17] Slow-degrading HA materials may prolong efficacy *in vivo*.[18]

We previously described the use of a crosslinked thiol-modified HA that reduced adhesion formation in a rat uterine horn model,[19] comparing the use of an *in situ*-crosslinkable hydrogel with a pre-formed film. In addition, an HA hydrogel containing a bioconjugate of mitomycin C (MMC) acted as a bioresorbable barrier that locally released an antiproliferative agent and reduced the formation of postoperative intraperitoneal adhesions. [19] More recently, we developed a new carboxylated and thiol-modified derivative of HA.[20] This material, known as CMHA-S or Carbylan™-S, could be crosslinked to give Carbylan™-SX biomaterials. Both the Carbylan™-SX sprayable gel and a pre-formed Carbylan™-SX film were statistically more effective than Seprafilm® in reducing intraabdominal post-operative adhesion formation in both rat cecum-abdominal wall and rat uterine horn models. [21] Herein we describe the use of these new HA-derived biomaterials to reduce adhesions in partial-thickness flexor tendon tears in rabbits. We compare the Carbylan™-SX sprayable gel and film formulations with Seprafilm®, an approved antiadhesive barrier that contains chemically-modified HA.[22]

MATERIALS AND METHODS

Preparation of Carbylan™-SX films and crosslinkable gels

Carbylan™-S (Carbylan BioSurgery, Palo Alto, CA), was synthesized[20] by modifications of methods used for HA-DTPH [23]. A 2.5% (w/v) solution of Carbylan™-S in Dulbecco's phosphate-buffered saline (DPBS, GIBCO, Rockville, MD) was adjusted to pH 7.4. A

solution of 4% poly (ethyleneglycol) diacrylate (PEGDA; 3,400 kDa, Nektar Therapeutics, Huntsville, AL) was prepared by dissolving PEGDA in DPBS. All solutions were sterilized by filtration (pore size 0.45 μm), aliquoted, and stored at −80 °C. Carbylan™-SX films were prepared one week before surgery. Thus, the 2.5% Carbylan™-S and 4% PEGDA solutions were mixed in a volume ratio of 4:1, vortex-mixed for 15 sec, and then poured into a plastic base mold (0.8 ml per mold) (Fisher Scientific, 2 cm × 2 cm × 0.5 cm). Each mold with its crosslinking solution was held at 37 °C for 48 h to obtain the films. Sprayable Carbylan™-SX hydrogels were prepared on the same day and employed for *in situ* crosslinking at the site of surgery as described below. Seprafilm® was purchased from Genzyme Corporation (Cambridge, MA).

Experimental Design and Animal Model

A total of twenty-four 4-month-old New Zealand White rabbits (Western Oregon Rabbit Company, Philomath, OR), each weighing 2.6–3.0 kg were used. Procedures followed protocols approved by Institutional Animal Care and Use Committee at the University of Utah. The rabbits were randomly divided into three groups with eight rabbits per group: Group 1 rabbits were used for gross evaluation, Group 2 for histologic assessment, and Group 3 for biomechanical testing.

Each rabbit received one control and each of the three experimental treatments. The fourth and third toes from both hindpaws of eight rabbits of each group were randomly assigned to one untreated control and three treatments: Seprafilm®, Carbylan™-SX gel, and Carbylan™-SX film. Rabbits were anesthetized with intramuscular xylazine, 5 mg/kg (Akorn, Inc., Decatur, IL) and ketamine, 35 mg/kg (Fort Dodge Labs, Fort Dodge, IA). The depth of anesthesia was maintained with inhalation of 2% isofluorane. The hindpaws were shaved, scrubbed, disinfected with iodine and 70% alcohol, draped, after which a tourniquet was applied above the os calcis. Under a 4× loop of magnification, separate longitudinal midline skin incisions were made on the volar aspect of the proximal phalanx of each toe and carefully dissected to expose the synovial sheath. The sheath was incised transversely between the A2 and A3 pulleys to access the flexor digitorum profundus (FDP) distal to the flexor digitorum superficialis (FDS) bifurcation. Standard tendon injuries were then made as described [24] by lifting the FDP with the aid of curved microforceps just distal to its emergence from the FDS bifurcation and cutting transversely halfway through and, from this point, longitudinally 5 mm both proximally and distally. This procedure created two flaps, which were sutured back in position with interrupted 5-0 nylon sutures. The pulleys were preserved. The sheath incision was not repaired.

For untreated control toes, the skin incision was closed with 5-0 silk interrupted stitches. A firm expand-over bandage was then applied to the hindpaw to immobilize the toes and ensure the comfort and ambulation of the animals after recovery from anesthesia. The bandage was then removed 3 days post-surgery to permit normal digit motion during the healing process. For the Carbylan™-SX film and Seprafilm® treated toes, a 5 × 8 mm dry film was trimmed and wrapped around the injured tendon. After saturating the film with DPBS buffer, the films swelled slightly and completely encircled the tendon with an overlay covering a 1-cm segment of the repair zone. Then the skin incision was closed as above. For the Carbylan™-SX gel treated toes, the 2.5% Carbylan™-S and 4% PEGDA solutions were mixed in a volume ratio of 4:1 in a 1 ml syringe. Within 5 min, as the mixture was becoming viscous, 0.2 ml was sprayed around the injured and sutured tendon. Spraying of the gelling mixture was accomplished under CO_2 pressure using the applicator as described.[21] Within 5–10 minutes after spraying, a thin layer of crosslinked hydrogel formed on the surface of the tendon. The skin incision was then closed.

Gross Evaluation—Eight rabbits were euthanized three weeks after surgery using pentobarbital sodium. The toes were transected at the metatarsophalangeal level. The skin sutures were removed and the skin incision was opened along the original incision. A semiquantitative grading scale was used to evaluate the extent and severity of adhesion formation within the intrasheath region: grade 1, no adhesions; grade 2, filmy (separable from surrounding tissue); grade 3, mild (not separable from surrounding tissue); grade 4, moderate (35–60% of injured area); grade 5, severe (> 60% of injured area).[25] All gross evaluations were made by an investigator blinded to the treatments.

Histologic Assessment—Eight rabbits were euthanized three weeks after surgery, the toes were transected as above, and then fixed in 10% buffered formalin. After fixation, the distal femora were decalcified with 10% formic acid. The toes were then trimmed longitudinally to expose the repaired tendon. Samples were then dehydrated by a graded series of alcohol and xylene washes and paraffin embedded. Sections were cut at 5 μm thickness and stained with Masson's trichrome. Stained sections were examined for the degree of adhesion and quality of tendon healing. Histologic evaluations were blinded and were made by two independent investigators. The area of tendon surface with adhesion was estimated by observing ten slides prepared at 1.0-mm intervals for each specimen. Adhesions were quantified into 4 grades as follows: Severe, >66% of the tendon surface; moderate, 33% to 66% of the tendon surface; mild, <33% of the tendon surface; and no adhesions.[5] Each slide was graded, and the average grade of ten slides was calculated.

Histologic quality of tendon healing was graded as described.[26] Well-established tendon continuity and a smooth epitenon were graded as *excellent*. Samples in which the intratendinous collagen bundles had healed well, but the epitenon was interrupted by adhesions in some locations were *good*. Irregularly-arranged and partly interrupted intratendinous collagen bundles were *fair*. Samples that exhibited repair failure, e.g., separation of the sutured parts, or with massive overgrowth of granulation tissue on the repair were *poor*.

Biomechanical tests—The force necessary to remove the tendon from the toe was employed as a measure of adhesion strength, and was accomplished by modification of an Instron tensile-strength testing protocol.[27] Eight rabbits were euthanized three weeks after surgery as above. The toe was amputated through the metatarsophanlangeal joint, leaving a 2-cm length of profundus tendon attached, which was secured in the bottom plate clamp in a buckled, unloaded fashion. The distal toe was attached to the hook via a hole drilled through distal phalange and secured in the top crosshead. The sample was then brought to a tension of 10 g, and preconditioned [28] using a cyclic triangular displacement profile of 10 cycles at an amplitude that produced 5 g of additional tension. The movement of the crosshead ultimately pulled the tendon from the sheath. The force in newtons (N) needed to initiate separate the tendon and surrounding adhesion was represented by the highest peak of the curve.[24, 29]

Statistical Analysis—Gross evaluation and histologic assessment of tendon adhesion and healing were compared across groups with Kruskal-Wallis One Way Analysis of Variance on Ranks, and a value of $p < 0.05$ was considered significant. Biomechanical results were also analyzed using One Way Analysis of Variance and a p value < 0.05 was considered significant. All statistical analyses were performed with SigmaStatt (Version 3.5, Systat Software, Inc., San Jose, CA).

RESULTS

All control and experimental animals survived the surgical procedures. At three weeks post-surgery, all animals were euthanized and the three groups of rabbits were separately used for macroscopic gross evaluation of tendon adhesions, histologic assessment of adhesion formation, and mechanical testing of the force required to remove the tendon from the hindpaw. The results for the four different treatments were then analyzed and compared statistically.

Gross evaluation

Figure 1 shows the gross view of representative tendons receiving different treatments. The untreated tendons exhibited dense adhesion formation with the surrounding tissues. These adhesions were characterized by a large bundle of fibrous tissue bridging between tendon and surrounding tissue (Figure 1a). For the tendons wrapped with Seprafilm®, there were small bundles of fibrous tissue loosely bridging between the tendon and surrounding tissue (Figure 1b). No adhesions were evident between tendon and surrounding tissues in either the Carbylan™-SX gel or the Carbylan™-SX film treatments, but the surface of tendons in the Carbylan™-SX film treatments was smoother than the surfaces of tendons with the Carbylan™-SX gel treatment (Figure 1c and d). The results from the semi-quantitative gross evaluation of each treatment in the eight rabbits of Group 1 are summarized in Table 1 and the comparisons among groups are summarized in Table 2.

Histologic assessment

Representative histologic sections of the tendons receiving each of the four treatments are shown in Figure 2. In untreated tendons, severe adhesions were observed between the tendon and surrounding vascular granulation tissue. The fibrous adhesion tissue invaded the epitenon and superficial layers of the repaired tendons and exhibited poor collagen maturation (Figure 2a). In tendons treated with Seprafilm® (Figure 2b), loose bundles of fibrous tissue bridged the repaired tendon and surrounding tissue. The surfaces of the repaired tendons were very rough, indicating only partial healing of the injury. In the Carbylan™-SX gel treatments (Figure 2c), no adhesions formed between the repaired tendons and the surrounding tissues; an interface at the repaired sites was quite noticeable. Moreover, residual fragments of undegraded gel remained between the repaired tendon and surrounding tissue. In the Carbylan™-SX film treatments (Figure 2d), no adhesions formed between the repaired tendons and surrounding tissues. The surfaced of repaired sites on the tendon were smooth and thus better healed. The collagen in these tendons was well organized at the repaired site. The scored histologic assessments for the degree of tendon adhesion and tendon healing are presented in Table 1 and the comparisons among groups are summarized in Table 2.

Biomechanical measurements

Each tendon was first placed under low tension and preconditioned with ten cycles of a low level of force insufficient to extrude the tendon, a technique which yields more consistent results in mechanical testing of biological tissues.[28] Then, the peak force required to remove the tendon from the tendon sheath was determined. When this point was reached, the force decreased back to baseline as the tendon was extruded. The area under this curve represents the total energy used to remove the tendon from the tendon sheath. The resulting energies and forces generated the same rank order of efficacy for adhesions strength. Figure 3 illustrates the force required to completely remove the tendons from the tendon sheaths. The untreated, injured tendons required the largest force to remove the tendon from the hindpaw, while unoperated tendons required the least force. Among the three treatments, tendons treated with the Carbylan™-SX film required the least force, the Carbylan™-SX gel

treatment needed an intermediate force, and the Seprafilm® treatment required the greatest force to remove the tendon from the tendon sheath. Comparisons among groups are summarized in Table 2.

Statistical analysis

The statistical analysis of the data from the gross evaluation, the histologic degree of adhesion formation, the histologic degree of tendon healing, and the biomechanical force measurements (Figure 3 and Table 1) are summarized in Table 2. When compared with the untreated controls, all treatments showed statistically significant efficacy in reducing adhesion formation by all four measurements. The Carbylan™-SX film treatment was the most effective, compared to the Seprafilm® treatment ($p = 0.018$) or to the Carbylan™-SX gel treatment ($p = 0.043$). The Carbylan-SX gel treatment showed intermediate effectiveness while the Seprafilm® treatment was the least effective in the gross evaluation. Importantly, this rank order of efficacy was identical for the histologic degree of tendon adhesion, the histologic degree of healing, and the force required for extrusion of the tendon from the tendon sheath.

DISCUSSION

During surgical repair of tendon injuries, the integrity of the protective sheath should be maintained to recover full function.[30] A number of surgical approaches have been used to to reconstruct the defect in the flexor sheath when primary sheath repair is not feasible.[6, 8, 31] Clinically, proper sheath repair and reconstruction can reduce adhesions.[26] However, surgical closure of the sheath may mar the diameter of the gliding tunnel (e.g., by scar formation).[6, 26]. In other cases, surgical repair of the sheath is not possible because of major defects after injury.[6]

HA is an important constituent of synovial fluid and flexor tendon sheath fluid,[32] and the use of HA in tendon healing decreased adhesion formation *in vivo*.[12, 17, 33] However, even high molecular weight HA (> 1 MDa) was rapidly eliminated.[17]

Seprafilm®, a bioresorbable membrane composed of chemically-modified HA and carboxymethylcellulose (CMC), is commonly-used intervention for the reduction of postsurgical adhesions.[22] Seprafilm® equal in efficacys to HA in the prevention of peritendinous adhesion in primary flexor tendon repair in rabbits.[34] However, Seprafilm® film suffers from several drawbacks that limit its efficacy in abdominopelvic and orthopedic surgeries. Seprafilm® can be difficult to handle during the surgery, particularly when wrapping the tendon, because the material loses its integrity and strength during hydration. Seprafilm® has a short *in vivo* residence time, often too short to bridge the critical 5–7 day period necessary to permit adhesion-free healing to occur. In many off-label surgeries, Seprafilm® is frequently less effective than newer anti-adhesive products.[21, 35]

In contrast, the Carbylan™-SX films (i) are elastic, robust, and easy to handle, (ii) maintain integrity during rehydration and repositioning, (iii) have a 2–3 week half-life *in vivo*[36] (iv) can also be deployed as an *in situ* crosslinkable hydrogel, and (v) show increased efficacy for a variety of animal abdominal and orthopedic adhesion models.[20] The Carbylan™ HA-based biomaterials have been found to promote wound healing and to reduce scarring in sinus mucosal injuries,[37, 38] biopsied vocal folds,[39] and airway injuries.[40]

In addition to reducing adhesion formation, Carbylan™-SX materials also showed improved effectiveness relative to Seprafilm® in enhancing tendon healing. In this orthopedic model, as opposed to the rat abdominal wall-cecal abrasion model, the Carbyan™-SX film was found to be superior to sprayable gel. In this orthopedic model, in contrast with the rat

abdominal wall-cecal abrasion model,[21] the Carbyan™-SX film was found to be superior to sprayable gel. The abdominal environment benefits from a soft gel that conforms to the irregular tissue surfaces that are not experiencing frequent gliding motion. In contrast, the Carbylan™-SX film has greater biomechanical strength and concomitant improved ability to isolate the moving tendon from surrounding tissue during healing.

The effects of the Carbylan™-SX film and gel in reducing peritendinous adhesion formation can be attributed to their inhibition of fibroblast proliferation and attachment. Crosslinked HA derivatives such as thiol-modified HA or Carbylan™-S do not support cell attachment, spreading, or proliferation, because the crosslinked HA-derived films are negatively charged, hydrophilic, and lack peptidic epitopes that activate integrins.[20, 36, 41] Carbylan™ materials have longer residence times (ca. 2 weeks) *in vivo*, allowing them act as physical barriers for a longer time. Carbylan™ materials are non-immunogenic and completely biodegradable, and the time for complete resorption varies with the site of insertion.[19–21, 36]

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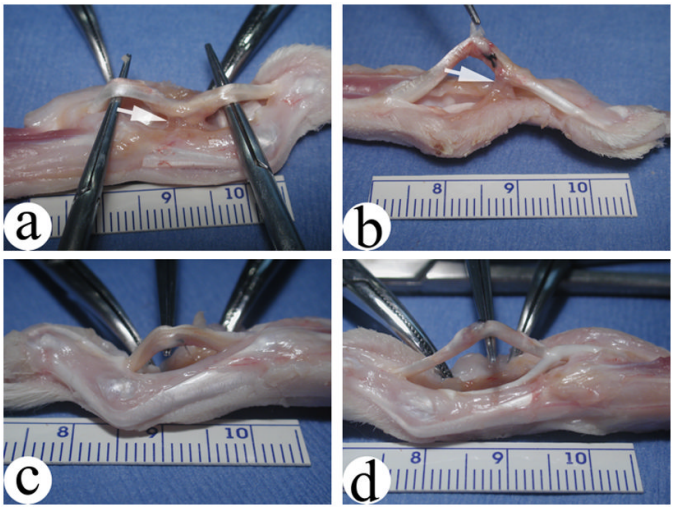

Figure 1.

Gross evaluation of rabbit toes after the skin was removed and the flexor digitorum profundus tendon was lifted with a hemostat. Each target tendon was 50% severed and sutured prior to closure of the skin incision. Treatments: **panel a,** no treatment; **panel b**, wrapped with Seprafilm®; **panel c**, sprayed with Carbylan™-SX gel; **panel d**, wrapped with Carbylan™-SX film.

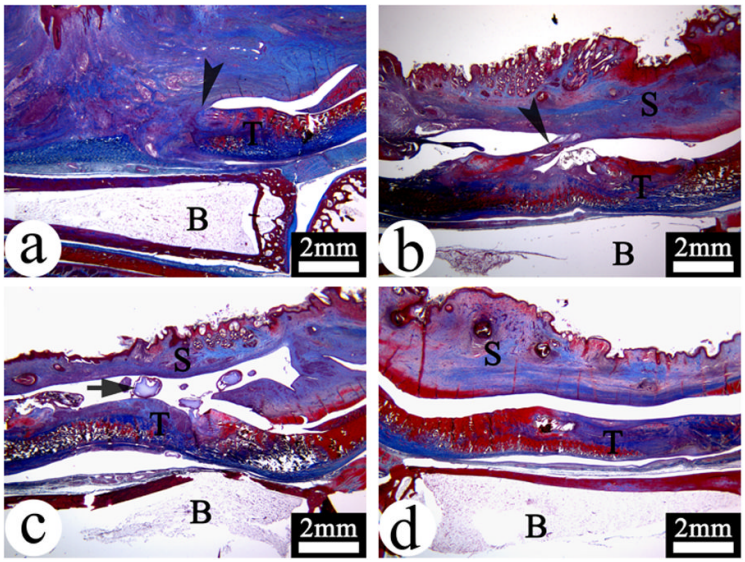

Figure 2.

Histologic examination (Masson's trichrome staining) of rabbit toe after flexor digitorum profundus tendon injury. Treatments: **panel a,** no treatment; **panel b**, wrapped with Seprafilm®; **panel c**, sprayed with Carbylan™-SX gel; **panel d**, wrapped with Carbylan™-SX film. Key: **B** = bone; **T** = tendon; **S** = skin; arrow heads indicate adhesions between the target tendon and surrounding tissue; arrows indicate the residual of Carbylan™-SX gel. Scale: 2 mm.

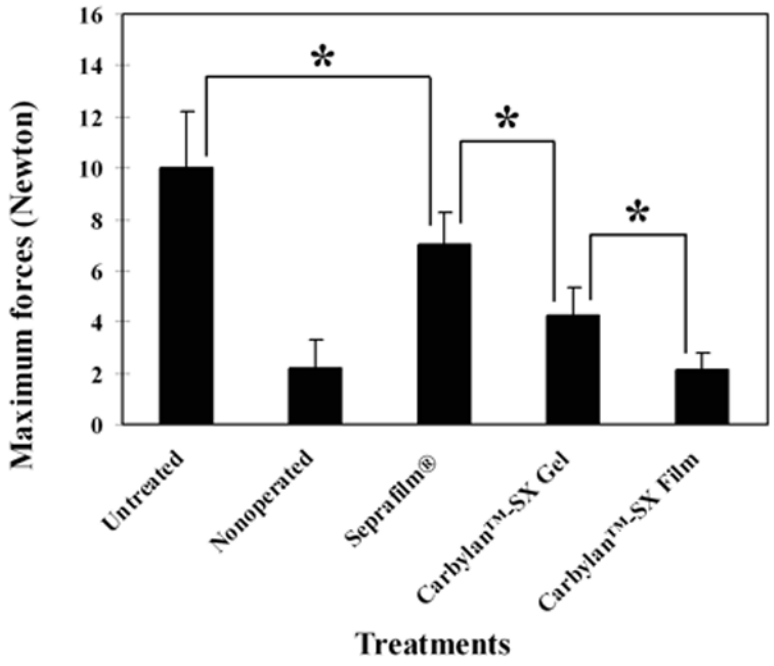

Figure 3.

Biomechanical test which shows the maximum force (N) applied to pull the flexor digitorum profundus tendon out of the hindpaw tendon sheath. Statistically different ($p < 0.05$) treatments are indicated with asterisks, with p values reported in Table 2. The difference between non-operated controls and the Carbylan™-SX film group was not statistically different.

Table 1

Gross and Histological Evaluation of Tendon Adhesion and Healing

	Gross Evaluation of Tendon Adhesion					Histologic Degree of Tendon Adhesion				Histologic Degree of Tendon Healing			
	Severe	**Moderate**	**Mild**	**Filmy**	**None**	**Severe**	**Moderate**	**Mild**	**None**	**Excellent**	**Good**	**Fair**	**Poor**
Untreated control	6	1	1	0	0	6	1	1	0	0	1	2	5
Seprafilm	1	2	2	2	1	3	1	3	1	1	2	2	3
Carbylan-SX gel	0	1	2	2	3	0	2	4	2	2	3	2	1
Carbylan-SX film	0	0	1	2	5	0	1	1	6	4	2	2	0

Table 2

The Comparisons among the groups

Comparisons	*P* values			
	Gross Evaluation of Tendon Adhesion	Histologic Degree of Tendon Adhesion	Histologic Degree of Tendon Healing	Biomechanical test-maximum forces
Seprafilm vs. Untreated control	0.018	0.0431	0.0431	0.0057
Carbylan-SX gel vs. Untreated control	0.0117	0.0117	0.018	< 0.0001
Carbylan-SX film vs. Untreated control	0.0117	0.0117	0.0117	< 0.0001
Carbylan-SX gel vs.Seprafilm	0.0118	0.0431	0.0431	0.0084
Carbylan-SX film vs. Seprafilm	0.018	0.018	0.018	< 0.0001
Carbylan-SX film vs. Carbylan-SX gel	0.0431	0.0431	0.0679	0.0450