

Comparison of Achilles Tendon Repair Techniques in a Sheep Model Using a Cross-linked Acellular Porcine Dermal Patch and Platelet-rich Plasma Fibrin Matrix for Augmentation

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ABSTRACT

The primary goal of this study was to evaluate a cross-linked acellular porcine dermal patch (APD), as well as platelet-rich plasma fibrin matrix (PRPFM), for repair of acute Achilles tendon rupture in a sheep model. The 2 surgically transected tendon ends were reapproximated in groups 1 and 2, whereas a gap was left between the tendon ends in group 3. APD was used to reinforce the repair in group 2, and autologous PRPFM was used to fill the gap, which was also reinforced with APD, in group 3. All sheep were humanely euthanized at 24 weeks after the repair, and biomechanical and histological testing were performed. Tensile strength testing showed a statistically significant difference in elongation between the operated limb and the unoperated contralateral limb in groups 1 and 3, but not in group 2. All operated tendons appeared healed with no apparent fibrosis under light and polarized microscopy. In group 1, all surgical separation sites were identifiable, and healing occurred via increasing tendon thickness. In group 2, healing occurred with new tendon fibers across the separation, without increasing tendon thickness in 2 out of 6 animals. Group 3 showed complete bridging of the gap, with no change in tendon thickness in 2 out of 6 animals. In groups 2 and 3, peripheral integration of the APD to tendon fibers was observed. These findings support the use of APD, alone or with PRPFM, to augment Achilles tendon repair in a sheep model.

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Injury to the Achilles tendon commonly affects men during sporting activities, and surgical repair is considered by many to be the best way to restore strength, structural integrity, and mechanical performance in the muscle-tendon-bone unit, for lowering the risk of repeat rupture (1–3). Healing time is variable with simple surgical intervention, and identifying a means of reducing the duration of

immobilization while avoiding repeat injury can be clinically beneficial, especially for patients returning to a high level of physical activity. For challenging repairs, where the structural quality of the tendon is poor or deficient, augmentation strategies are sought to provide additional repair strength. Various biomaterials have been used to augment tendon repair; however, these can be limited by poor ingrowth potential, inflammatory response, and poor tensile strength, thereby compromising healing and functional outcome (4–6). Porcine small intestinal submucosa (SIS, Restore; DePuy, Warsaw, IN) has frequently been used as a bioscaffold for tendon repair; however, severe acute inflammatory reactions have been reported in association with this material (7, 8). Human dermal tissue (Graft Jacket; Wright Medical, Memphis, TN) has also shown promise as an augmentation material; however, this biomaterial is not chemically cross-linked, and, as such, its strength may be compromised over time. Equine pericardium (OrthADAPT Bioimplant; Pegasus Biologics, Inc., Irvine, CA) has also been used to augment tendon repair (9);

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Conflict of Interest: Hali Wang, PhD, is employed by Zimmer Orthobiologics, Inc., 9301 Amberglen Blvd., Suite 100, Austin, TX 78729.

This study was approved by the Animal Care and Use Committee at Colorado State University (CSU).

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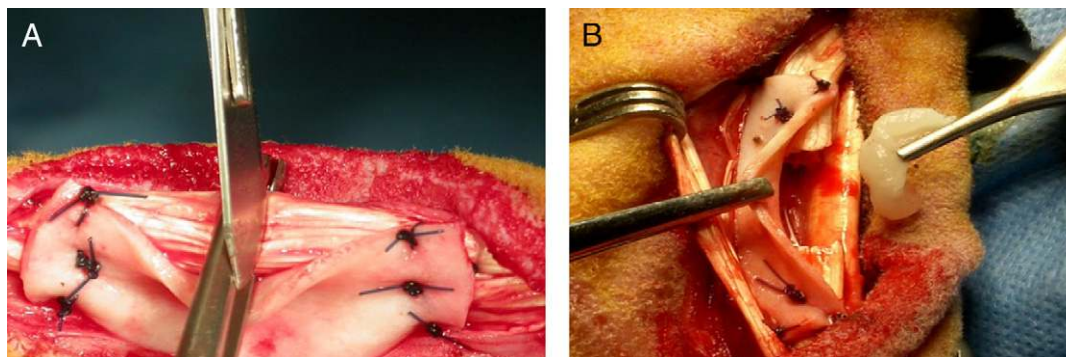


Fig. 1. Application of a cross-linked acellular porcine dermal patch (APD) with preparation for transection of Achilles (A) and placement of platelet rich plasma fibrin matrix (PRPFM) into gap (B).

however, in an animal study, it revealed little host incorporation and induced a foreign-body reaction (10). A cross-linked acellular porcine dermal patch (APD) (Zimmer Collagen Repair Patch, Zimmer, Inc.) has been shown to incorporate well into host tissues in an ovine model (11) and has been used to augment rotator cuff repair and to improve clinical outcomes in humans (12). Platelet-rich plasma fibrin matrix (PRPFM, Cascade Autologous Platelet System-4; Musculoskeletal Transplant Foundation, Edison, NJ) recently has also been shown to enhance soft tissue healing in a variety of applications (13–15) through release of growth factors, cytokines, and hemostatic factors. Cytokines also play an important role in initiating the early healing response (15). The primary aim of this investigation was to evaluate healing characteristics, in terms of histological evidence of tendon repair and graft incorporation, as well as inflammation, and the mechanical characteristics of APD augmentation of primary suture repair of acutely ruptured Achilles tendon, in comparison with primary suture repair alone, and gap (bridging model) repair augmented with ADP and PRPFM, in a sheep model.

Materials and Methods

This pilot study was undertaken at Colorado State University, and the university's Animal Care and Use Committee approved all of the procedures. Six skeletally mature (> 3.5 years of age) Columbian × Rambouillet ewes, each weighing approximately 70 to 80 kg, were randomly assigned (random number generator list) to each of the 3 intervention groups. An acute model of Achilles tendon rupture was simulated by means of transection, after which surgical repair was undertaken in accordance with the method predetermined by the random allocation to a specific treatment group. Group 1 consisted of primary reapproximation of the tendon ends with suture repair only. Group 2 consisted of primary reapproximation with suture augmented with APD wrapped around the repair and sutured to the tendon (Figure 1). Group 3, the bridging model, consisted of repair with APD wrapped around the proximal and distal margins of the tendon, bridging a 1.5-cm gap between the tendon ends, with PRPFM sutured in place within the gap. The PRPFM (Cascade Autologous Platelet System-4, Musculoskeletal Transplant Foundation) was prepared from approximately 9 cc of centrifuged autologous blood, the resultant matrix of which contained approximately 5 times more platelets and growth factors than did the noncentrifuged blood, and was durable enough to be sutured within the gap between the tendon ends. The unoperated, contralateral limb was used for comparison, allowing each animal to act as its own control.

In all cases, the operative procedure entailed first collecting blood for platelet counts and, for sheep allocated to group 3, preparation of the PRPFM. All sheep had 5-mm transdermal analgesic patches (Duragesic; Janssen Pharmaceutical, Titusville, NJ) applied preoperatively to one front limb, proximal to the carpus, and all received 3×10^6 IU of procaine penicillin G (PenOnePro; MWI Veterinary Supply, Meridian, ID) infused intravenously, and 1 g phenylbutazone infused intravenously preoperatively, immediately postsurgery, and for 3 consecutive days postsurgery. General anesthesia was induced with intravenous diazepam (0.2–0.5 mg/kg) and ketamine hydrochloride (3.3 mg/kg) and maintained with isoflurane via inhalation. The surgical area was aseptically prepared with alternating scrubs of povidone iodine and alcohol, and the limb was sterilely draped with an adhesive barrier (Ioban; 3M, St. Paul, MN) placed directly on the limb. With the left limb uppermost, a lateral approach to the left gastrocnemius tendon was made, and the skin and subcutaneous tissues were reflected to expose the medial and lateral portions of the gastrocnemius tendon. The larger medial branch was isolated, and the APD positioned about the tendon for reinforcement (group 2), or bridging (group 3) and anchored with

simple, interrupted 2-0 sutures composed of polyglycolide and polylactide (Polysorb; Covidien, Manchester, MA) (Figure 1A). The tendon was then sharply transected with a scalpel and the tendon ends reapproximated with 2-0 synthetic absorbable monofilament glycomer sutures (Biosyn, Covidien) in a Kessler locking loop pattern (groups 1 and 2). For sheep in group 3, a gap was left between the tendon ends, and this was filled with autogenous PRPFM (Figure 1B) that was sutured in place with simple, interrupted sutures of 2-0 sutures composed of polyglycolide and polylactide (Polysorb). The adventitia and subcutaneous tissues were then closed with the 2-0 polyglycolide and polylactide sutures, and the skin was closed with 2-0 nylon (Dermalon, Covidien) in an interrupted cruciate pattern. A nonadherent bandage was then applied to protect and pad the operated extremity.

Animals were observed daily for signs of poor health and their general response to the surgery. All exceptions to normal were recorded and documented according to Colorado State University animal care and husbandry protocols. Sheep remained inside, housed in 12 × 12-foot stalls, for the entire convalescence period. After 3 weeks, the padded bandages were removed. At 24 weeks postoperative, all of the sheep were euthanized with an intravenous overdose of barbiturate. Immediately after euthanasia, both left and right gastrocnemius musculotendinous units, including the calcaneal attachment, were harvested from each animal. All of the specimens were then wrapped in saline solution-soaked gauze and frozen to -20°C in preparation for subsequent histological and biomechanical evaluations.

Before biomechanical testing, each specimen was thawed in a bath of normal saline solution at 37°C . The tendon was separated from the calcaneus with a scalpel, and each end of the tendon was potted in an acrylic-based resin that polymerized quickly, allowing the tendon ends to be placed into pneumatic grips so that biomechanical testing could be performed to evaluate uniaxial longitudinal tensile strength with an Instron 4500 model (Instron Corporation, Norwood, MA), retrofit to 5500 capabilities, mechanical testing device. The specimens were loaded at a constant displacement rate of 6 mm/min, recording the load (newtons) at the yield point (when the load curve reached its peak and then sharply dropped). Tendons were not taken to ultimate failure, so that they could subsequently be used for histological inspection. The force displacement curve was recorded and the area under the curve used to calculate the energy (millijoule), and the slope of the linear portion of the curve used to determine stiffness (newton/millimeter) of the healed tendinous unit. Load at the yield point was reported as group mean and standard deviation. Total elongation (millimeter), and the ultimate change in length (stretch) of the tendon when under the constant pulling rate at 6 mm/min, was reported as mean percent difference.

Immediately after the biomechanical testing, all of the specimens were placed into 10% neutral buffered formalin and attached to cardboard to prevent contraction before histological evaluation. The specimens were then separated longitudinally into 3 slabs, approximately equal in thickness, and processed through gradient dehydration. Sections of 5 to 8 μm were then embedded in paraffin with the use of a large (4×6 cm) cassette. The sections were stained with hematoxylin and eosin and placed on 1 large (5×7.5 cm) slide to view tissue reactions over the entire thickness of the specimen. All sections were examined under transmission and polarized light microscopy to identify cell and tissue morphology, vasculature, incorporation of the scaffold into the operated tendon, and the presence of any inflammatory or untoward immunologic response to the APD. The data were stored in a personal computer and the statistical analyses performed with one-way analysis of variance to compare differences between the groups. The Tukey post-hoc test was also used for between-group comparisons, and statistical significance was defined at the 5% ($P \leq .05$) level.

Results

No sheep developed outward signs of systemic infection, inflammation, or swelling at the surgical site at any time postoperatively. Some sheep exhibited knuckling of the metatarsophalangeal joint due

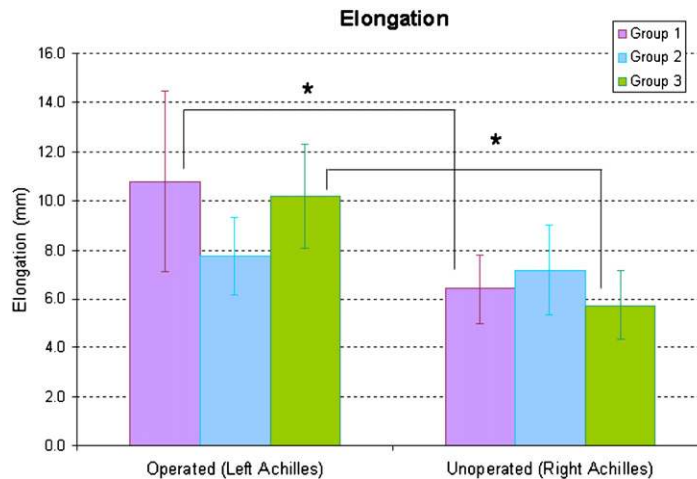


Fig. 2. Mean elongation (millimeter) for operated and unoperated Achilles tendons. There is a statistically significant difference in elongation between operated and its contralateral limbs in groups 1 and 3 ($P < .05$), but not between operated limbs with APD augmentation and its contralateral limbs in group 2.

to postoperative functional lameness from surgically injured nerves that innervate the digital extensors postoperatively, and required the use of a fiberglass splint on the extremity. Most sheep improved over 2 to 3 days, with 1 sheep remaining splinted for 11 days. All of the animals were minimally weight bearing on the affected limb thereafter and regained full weight-bearing capacity by 8 weeks postoperation.

At postmortem examination, all sheep had gross evidence of primary healing of skin incisions and no infection was macroscopically visible. There were no seromas, hematomas, or any evidence of inflammatory reactions at the operative sites. One animal in group 3 was found dead 11 weeks postoperatively, and full necropsy revealed internal hemorrhage of gastrointestinal illness despite apparently normal healing of the tendon surgical site. Microscopic inspection revealed no evidence of an inflammatory reaction to the APD. The tendon from this sheep was not subjected to biomechanical testing, and the data from this sheep were not included in the final analyses.

Biomechanical Testing

As previously stated, load was applied only until the yield point was achieved for all groups undergoing biomechanical evaluation, keeping specimens intact for histological evaluation. The results showed no statistically significant differences between the groups. Load at the yield point was 35.93 ± 34.17 N for group 1, 36.56 ± 11.59 N for group 2, and 38.55 ± 22.63 N for group 3. Data from each individual animal were expressed as a percentage of the unoperated contralateral limb to obtain group means and standard deviations for comparisons within groups. Among 6 animals in group 1, 5 animals in group 2, and 5 animals in group 3, there were large inter-group variations in the recorded values, indicative of variation in the healing process among individuals. Group 2, however, demonstrated elongation at the yield point of the healing tendon that was similar to the elongation at the yield point of the tendon of the contralateral limbs, indicative of the fact that tendon augmented with APD displayed stiffness similar to that of the unoperated limb. Contrary to this, however, both the primary suture repair and bridging repair groups displayed statistically significantly higher elongation values (Figure 2), indicating that these repairs resulted in lower stiffness than that of the unoperated limbs.

Histopathology

As previously noted, 1 sheep in group 3 died of gastrointestinal bleeding at 11 weeks postoperatively, and this was deemed unrelated

to the tendon surgery. Hematoxylin and eosin staining and polarized light microscope examinations showed that the surgical sites displayed increased tendon thickness in all of the animals at 24 weeks postsurgery (Table 1). All of the operated tendons appeared healed with no apparent fibrosis under light microscopy. Visual approximation and physical superimposition of slides of operated tendons over the unoperated contralateral side (Figure 3) were used to estimate changes in the thicknesses of the tendons. In group 1 (suture repair only), all 6 specimens had identifiable surgical separation sites in at least 1 of the 3 slabs, visible either by light or polarized microscopy (Figure 4). Healing occurred by increasing tendon thickness and the defect was bridged with horizontally arranged fibers, and some larger fibers appeared in spiral fashion along the outer surfaces of the transected tendon. In group 2 (suture repair augmented by APD), healing occurred with new tendon fiber bundles directly across the separation, without increasing total tendon thickness in 2 animals (Figure 5). In the other 4 sheep, the thickness of the tendon was moderately increased, and the surgical separation site was also still visible. One animal in group 2 had mild and localized inflammation at the junction of the patch and surrounding tendon fibers that extended to deeper portions of the APD in some locations. Integration of the APD was found at the periphery in most parts, and occasionally ingrowth of new fibers was seen in deeper portions of the APD. Group 3 (APD bridging the gap with PRPFM interposed) showed complete bridging of the gap in all specimens. Three of the 5 specimens had identifiable surgical separation sites. Healing was assumed to occur with insertion of new tendon fiber bundles directly across the gap and around the implanted patch (Figure 6). Total thickness was moderately increased in 3 animals but unchanged in 2 others. Axial orientation of new fiber bundles around those preexisting tendon fibers was similar to those in group 2, and the degree of ingrowth of fibers into the scaffold was slightly deeper than that seen in group 2. In

Table 1

Histological evaluation at 24 weeks postoperative (N = 17 tendons, 6 in group 1, 6 in group 2, and 5 in group 3 [1 sheep in group 3 died])

Groups	Identifiable surgical site	Increased tendon thickness
Suture only (group 1)	6/6	6/6
Suture + APD (group 2)	4/6	4/6
APD bridging + PRPFM (group 3)	3/5	3/5

Abbreviations: APD, acellular porcine dermal patch; PRPFM, platelet-rich plasma fibrin matrix.

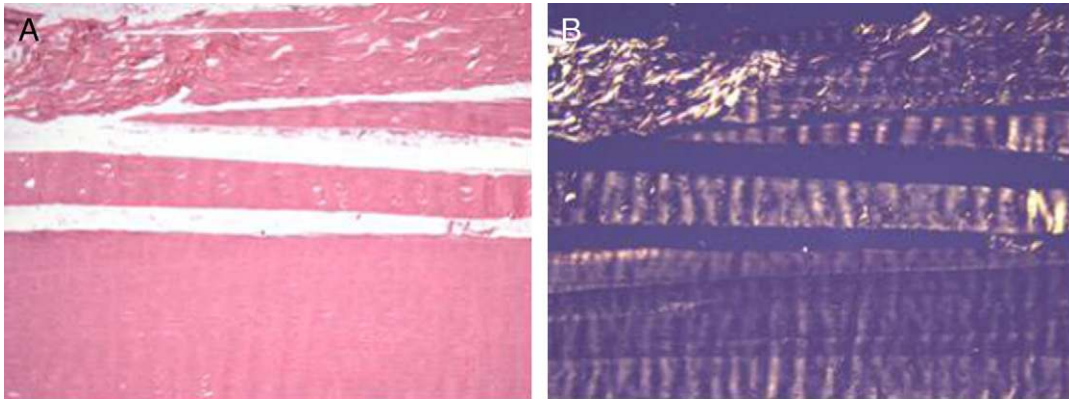


Fig. 3. Unoperated contralateral tendon tissue. Hematoxylin and eosin stain (A) and polarized light (B) images taken at 4× magnification.

groups 2 and 3, integration of the patch to new and existing tendons was found in peripheral zones (Figure 7) with vascular ingrowth. Group 3 also demonstrated occasional blending of new fibers within the marginal portions of the APD.

Discussion

None of the sheep euthanized at 24 weeks postoperative had profound inflammatory reactions as viewed on microscopy at this time point. Evaluating all specimens at this time would not account for those reactions that might be seen in the immediate postoperative period. However, the APD has been evaluated at multiple time points after rotator cuff repair, and no inflammatory response at any time could be observed (11). In previous accounts of cell reaction noted with the use of scaffold products like SIS, tissue reaction was noted beyond the initial postoperative period, not subsiding until absorption of the implant at 8 weeks in a rabbit model (8). Severe inflammatory reaction in several human patients, mice, and rabbits has been noted with the use of SIS (7, 8, 16), and this may be related to the fact that SIS has been shown to contain porcine DNA and cellular material (8), which could potentially increase the likelihood of an adverse inflammatory reaction. Aseptic inflammatory reaction has also been reported with the use of APD in patients undergoing trapeziectomy (17). However, in that report, the reaction was attributed to where particles generated from articulation when the scaffold was applied as an interpositional spacer. Furthermore, it is known that cross-linking may mask antigenicity, and the decreased inflammatory response noted with the APD may be attributed to cross-linking as well as the decellularization process through which porcine DNA is completely

removed from APD scaffold material. The APD is a scaffold material cross-linked with hexamethylene diisocyanate. The chemical cross-linking of the porcine dermal tissue serves to aid repair by providing additional mechanical stability to the healing tendon throughout all phases of repair. Without the stability afforded by cross-linking, premature degradation of the augmentation scaffold is likely to occur, typically within 9 to 12 weeks after implantation. Should the scaffold degrade at this time, ongoing healing would have to rely solely on the integrity of the native tendon. Non-cross-linked scaffolds are also more susceptible to collagenase activity and hence prone to more rapid degradation, less tensile strength, and ultimately less favorable clinical outcomes (16, 18, 19). It is also interesting to note that the histological evidence of tendon healing in this study confirmed the presence of cellular infiltration into the APD scaffold at 24 weeks postoperatively, and this finding was consistent with those previously observed in association with rotator cuff repair (11). These findings indicated fibroblast infiltration into the scaffold, thereby incorporating the implanted material with native tendon and surrounding tissues before its eventual degradation, which may serve to inhibit fibrosis (scar formation) and clinical tendon contracture. Surgeons should keep in mind, too, that an augmentation scaffold should act as a load-sharing device during the course of tendon healing, thereby minimizing the risk of recurrent tendon injury during the healing phase and allowing the tendon to heal under conditions of protected load (20, 21).

In addition to porcine dermal matrix, a non-cross-linked acellular human dermal matrix graft also has been used in augmentation models of rotator cuff repair (22–25) and in human patients with Achilles tendon rupture (26, 27). Valentin et al (4) found that fibrous

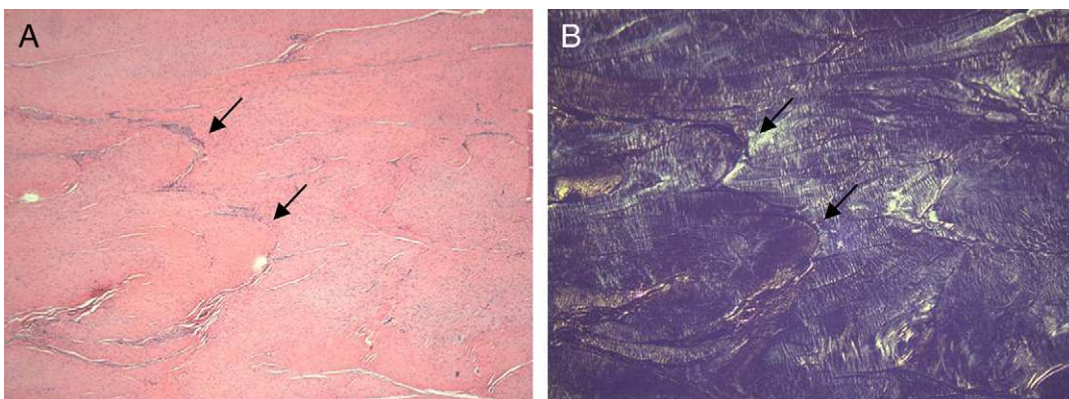


Fig. 4. Group 1, suture repair only. Healed surgical site (arrows) are visible in all 6 tendons. Hematoxylin and eosin stain (A) and polarized light (B) images taken at 4× magnification.

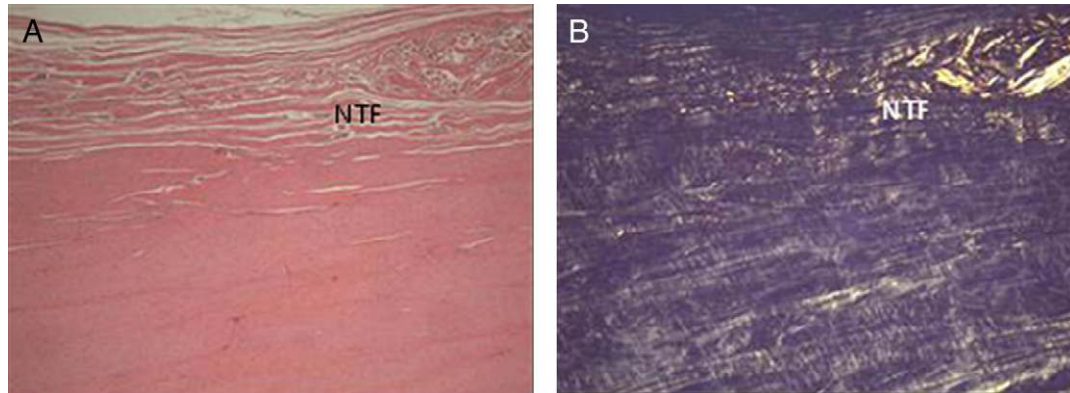


Fig. 5. Group 2, suture + APD. New tendon fibers (NTF) healed across the surgical site. Hematoxylin and eosin stain (A) and polarized light (B) images taken at 4× magnification.

connective tissue in rats had replaced the human dermal matrix in 16 weeks. In contrast, the porcine dermal matrix (Permacol, Covidien), which is a thinner version of APD, has been shown to be encapsulated by fibrous tissue with minimal scaffold degeneration and minimal host cellular response within 16 weeks. Although fibrous encapsulation is considered to be a process of persistent chronic inflammation, we observed no histological evidence of this reaction by 24 weeks postoperatively in the current investigation. A possible explanation for this is that inflammation may have subsided from week 16 to 24, as a result of the aforementioned features of the cross-linked ADP scaffold, including masked antigenicity due to the masking of surface proteins that would normally initiate a cellular hypersensitivity response (6, 18).

Regarding mechanical properties, we found no statistically significant differences between the intervention groups in this pilot study. This was not surprising, because variations in animal anatomy and behavior could have caused the large variations observed within each group. When we compared stiffness and load, we noted that both of these outcome variables, though not statistically significant between groups, were much lower in the operated limbs when compared with the unoperated contralateral controls. When evaluating soft tissue healing in a musculotendinous unit, it is probably more relevant to examine stiffness as a material property of the tendon. Load measurement reflects the ability of a repaired tendon to resist a single increasingly applied force at one time point. Load to failure has, in fact, been compared between many available products, and dermal-based grafts demonstrate higher failure loads than those manufactured from subintestinal mucosa (5). However, stiffness data

may be more clinically relevant in terms of the chronic cyclical nature of “subdestructive” loads seen during the rehabilitation process in the postoperative period. In this study, both groups 1 and 3 had significantly lower stiffness measurements when compared with the control limbs. However, it would be unrealistic to expect a healing tendon at 24 weeks postsurgery to have equal strength to the contralateral tendon in animal models (28, 29), even though patients can often return to strenuous physical activities, including sports, after 24 weeks postsurgery (30). When comparing stiffness results between augmented tendon and control populations, others have reported a decrease in the strength of the repair when compared with that in control populations (28, 29). Based on these observations, we suspect that scarring tissues, manifested as an increase in stiffness, can alter the material properties of a healing tendon. Tendon hypertrophy via increasing numbers and sizes of fiber bundles also elevates stiffness, therefore compromising elasticity and normal stretching and retracting.

Platelet-rich plasma appears to be advantageous relative to tendon healing when a gap is present between the tendon ends, as depicted in our investigation. The combined use of APD and PRPFM may be efficacious for the repair of a ruptured tendon with the scaffold providing mechanical augmentation and PRPFM providing biological enhancement. In addition to secretion of a variety of key growth factors like vascular endothelial growth factor, platelet-derived growth factor, and transforming growth factor- β 1, PRPFM also has been shown to upregulate matrix metalloproteinases (1 and 3) and to enhance angiogenesis and collagen production (31, 32). Whether these actions are beneficial in the presence of degenerative or

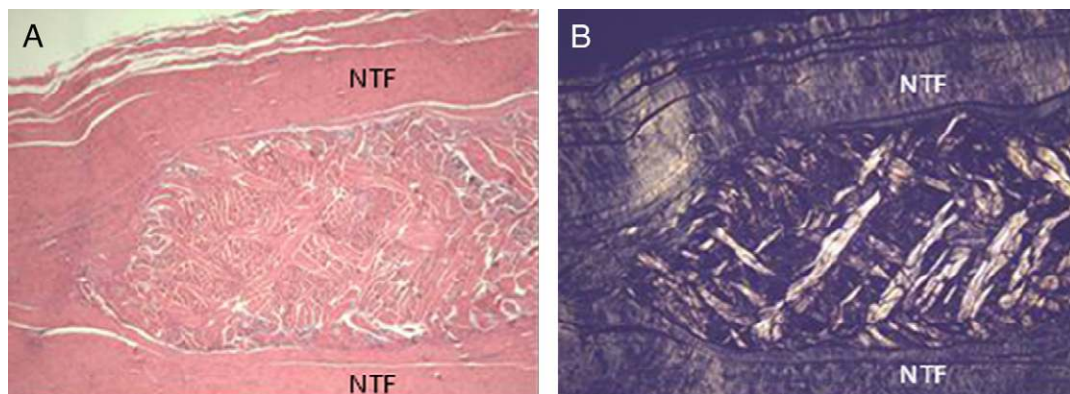


Fig. 6. Group 3, suture (1.5-cm gap) + APD + PRPFM. New tendon fibers (NTF) healed across the gap and around APD. Hematoxylin and eosin stain (A) and polarized light (B) images taken at 4× magnification.

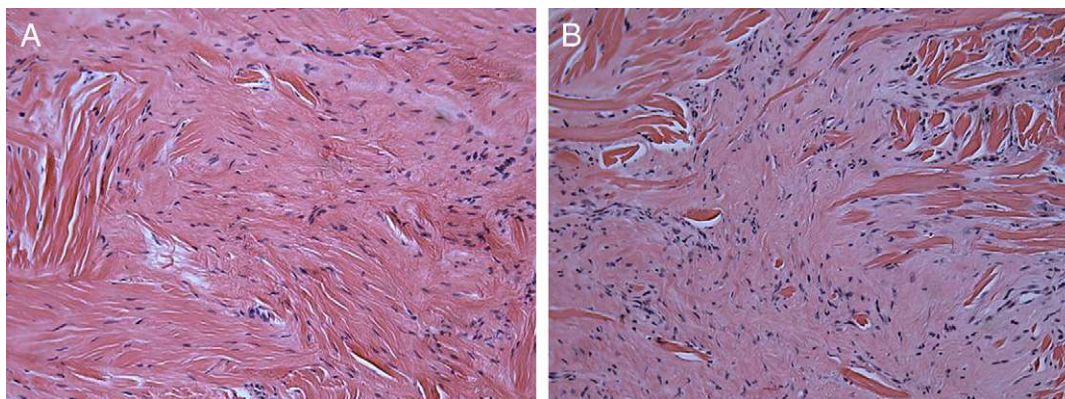


Fig. 7. Integration of APD with host tissue. Hematoxylin and eosin stain at 20 \times magnification. (A) Group 2. (B) Group 3.

ruptured tendons remains to be elucidated, but specific matrix metalloproteinases certainly have a role in remodeling and possible prevention of tendinopathic changes (33). PRPFM has also been previously shown to enhance healing of human Achilles tendon repairs (34) and likely was a factor contributing to increased healing noted in our investigation.

The procedure used in this study for preparation of autologous plasma results in the formation of a fibrin matrix with embedded platelets. The procedure does not require the use of exogenous bovine thrombin, and thus, there is no risk of immunogenic reaction or disease transmission. The end product is also a material that can be sutured and made for ease of surgical application. Additionally, the preparation of PRPFM allows the slow release of growth factors and proteins from platelets responsible for initiating and modulating wound healing. The fibrin matrix is more representative of natural conditions of platelet release and provides a scaffold that could assist with quality and time of ultimate repair (34). We believe that the use of PRPFM provides the opportunity to enhance the clinical outcome and to accelerate repair of tendon lesions.

It is also interesting to consider that fibrin glue has been shown to be as effective as suture repair for Achilles tendon rupture (35). The findings from this study support the use of PRPFM and APD in combination to bridge the Achilles tendon in the sheep model. The histological data showed that healing occurred in all specimens, even in group 3, with the presence of a 1.5-cm gap. Group 2 also showed promising results with regard to elongation at the yield point in longitudinal biomechanical testing. There were also noticeable differences in healing between groups 1 and 2. For example, 2 out of 6 tendons did not show the surgical sites with APD application. Future studies to evaluate how the APD would perform when used solely to bridge a remnant gap are warranted, and the results of this pilot investigation could be used in the design of subsequent studies. The combination of APD and PRPFM could also serve as a therapeutic regimen when resection of a torn tendon is necessary to enhance the quality and strength of healing.

Because this was a pilot study to evaluate the operational characteristics of APD for Achilles tendon repair in an acute injury sheep model, some limitations of the study need to be mentioned. First, the number of animals in each intervention group ($n = 6$) was low, and this could have led to a type 2 statistical error, wherein the null hypothesis was not rejected when, in fact, a statistical difference may have actually existed. One animal in group 3 actually died 11 weeks postoperatively, and full necropsy revealed internal hemorrhage secondary to gastrointestinal illness despite normal healing at the surgical site. This could have biased toward the null, because we excluded this particular sheep's data from the analyses. Even further, 1 animal's contralateral limb in group 2 was damaged and no mechanical data could be

obtained. Therefore, the data from the same animal's operated limb were eliminated for biomechanical analyses. Second, there was no control group with PRPFM to enhance the primary suture repair, which could provide direct information on the efficacy of PRPFM. We believe that this specific association should be investigated further. Still further, our study period was limited to 24 weeks, and a longer follow-up may allow the operated tendon to become stronger and more likely to match the strength of the unoperated contralateral tendon. Statistically significant differences, moreover, in regard to the mechanical properties of the tendon among the groups may be detected if a longer observation period was used.

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