Tissue-engineered augmentation of a rotator cuff tendon using a reconstituted collagen scaffold: a histological evaluation in sheep

Craig Van Kampen 1
Steven Arnoczky 2
Patrick Parks 3
Eileen Hackett 4
Dana Ruehlman 4
Anthony Turner 4
Theodore Schlegel 5

1 Rotation Medical, Inc., Plymouth, Minnesota, USA
2 Laboratory for Comparative Orthopaedic Research, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan, USA
3 Histopathometrics, LLC, Mendota Heights, Minnesota, USA
4 Small Ruminant Comparative Orthopedic Laboratory, Department of Clinical Sciences, Colorado State University, Ft. Collins, Colorado, USA
5 Steadman Hawkins Clinic Denver, Greenwood Village, Colorado, USA

Corresponding author:
Steven Arnoczky
Laboratory for Comparative Orthopaedic Research
Michigan State University
East Lansing, MI 48824, USA
E-mail: arnoczky@cvm.msu.edu

Summary

To determine if an absorbable collagen scaffold of high porosity would allow rapid tissue ingrowth and permit the functional maturation and alignment of tendon-like tissue, scaffolds were sutured to the superficial surface of the infraspinatus tendons of adult sheep. Histology demonstrated complete ingrowth with fibrovascular tissue by 6 weeks and by 12 weeks the scaffold had induced the formation of a layer of dense, regularly-oriented collagenous tissue which significantly increased the thickness of the native tendon. This new tissue was well-integrated into the host tissues at both the bone interface and along the length of the tendon. At 26 weeks the scaffold was completely absorbed leaving a stable layer of mature tendon-like tissue over the surface of the host tendon which was still present at 52 weeks. The use of a reconstituted collagen scaffold consistently increased the thickness of a rotator cuff tendon by inducing the formation of a well-integrated and mature tendon-like tissue.

KEY WORDS: tendon, collagen scaffold, rotator cuff, histology, sheep.

Introduction

Partial-thickness tears of the supraspinatus tendon comprise a complex and significant pathological process that is increasing in prevalence as the population ages 1-3. Unfortunately, there is no consensus as to the most appropriate treatment of partial-thickness, articular surface tears of the rotator cuff and a variety of treatments have been advocated ranging from strengthening exercises, to debridement, to acromioplasty, to in situ repair, to completing the tear so as to allow for a conventional surgical repair 1,4-5. In a cohort of non-operated partial-thickness tear patients, 80% of partial-thickness rotator cuff tears demonstrated progression of the tear over a two-year period, with 28% of these patients progressing to a full-thickness tear 6. Surgical repair has classically been advocated for partial-thickness tears involving 50% or more of the tendon thickness, however, these repairs are not always successful 1. Partial-thickness tears of the rotator cuff have been theorized to give rise to non-physiologic tension on the remaining tendon fibers 7. Several in vitro studies have implicated the significant increase in intra-tendinous strain following partial-thickness tears of the supraspinatus tendon as a predisposing factor for tear propagation 8-10. In addition, this increased intra-tendinous strain could further contribute to the compromised healing environment of these partial-thickness defects 1. The ability to rapidly increase the thickness and resulting cross-sectional area of an injured tendon through the natural regeneration of additional tendon-like tissue could, theoretically, decrease the stress (and resulting strain) experienced by the injured tendon. This could not only lessen the chance for future tear propagation, but may also allow for a more biomechanically conducive environment for healing.

Therefore, it was the purpose of this study to characterize the ability of a highly porous reconstituted collagen scaffold of low tensile modulus to increase the thickness of an injured tendon through the induction and remodeling of additional tendinous tissue. It was hypothesized that a highly porous, biocompatible scaffold placed on the surface of a damaged infraspinatus tendon would induce a rapid and proliferative ingrowth of host tissue to increase the thickness of the tendon. It was also hypothesized that using scaffold with a low tensile modulus relative to the ten-
barbital. During necropsy the infraspinatus muscle was detached from the scapula and the proximal one-third of the humerus was harvested. Care was taken not to disturb the deltoit muscle immediately over the implant area. The bone-tendon-muscle complex, including the scaffold and a portion of the overlying deltoit muscle, was placed in 10% buffered formalin for fixation.

Methods

Collagen Scaffold

The reconstituted collagen scaffolds were made from highly-purified, type I collagen from bovine tendons using proprietary methods (Collagen Matrix, Inc., Oakland, New Jersey)\textsuperscript{11,12}. The collagen fibers were processed to create a highly oriented and highly porous (85-90% porosity) collagen scaffold with a DNA content of less than 50 ng/mg. The scaffolds were freeze-dried after processing and rehydrated at the time of surgery. The rehydrated scaffold dimensions were approximately 15 mm wide, 30 mm long, and 2 mm thick (Fig. 1). The scaffold had a tensile modulus of approximately 6 MPa when tested in its long dimension.

Animal Procedures

All animal procedures were approved by the Colorado State University Institutional Animal Care and Use Committee. Skeletally mature (>3.5 year-old) female sheep (\textit{Ovis aries}) were used in this study. Twenty-three sheep were randomly assigned to survival times of 6, 12, 26, and 52 weeks. Preoperative analgesics and antibiotics were provided and under general anesthesia the infraspinatus tendon of the right shoulder was surgically exposed. The infraspinatus tendon was left attached to the humerus; however, in order to simulate a tendon injury, four 2 mm-deep slits were made in the infraspinatus tendon along its entire length in the direction of the tendon fibers. Three bone tunnels were made distal to the insertion of the infraspinatus tendon for suturing the scaffold to bone. The scaffold (approximately the same width and length as the tendon) was rehydrated in physiologic saline and attached at one end to bone using 3/0 braided polyester sutures that were passed through the bone tunnels. The free end of the scaffold was then sutured under slight tension to the infraspinatus tendon with interrupted 3/0 braided polyester sutures (Fig. 2). The surgical site was lavaged with sterile saline prior to closure. Sham procedures were performed in 6 additional sheep, wherein all elements of the surgery were performed except that no scaffold was implanted. Three sham animals were evaluated at each of two time periods (6 and 12 weeks). Following recovery from anesthesia, the animals were returned to indoor pens without immobilization and allowed unrestricted activity. Postoperative analgesics were provided to control pain. The sheep were closely monitored for any adverse effects (lethargy, lack of appetite, etc.) or signs of lameness. At the allocated survival times the sheep were humanely euthanized by an intravenous injection of sodium pento-
Histological Analysis

In order to examine the entire bone-tendon-scaffold complex in situ, the formalin-fixed specimens were frozen at -80°C and the entire complex bisected along the long axis of the infraspinatus tendon using a band saw. The bisected bone-tendon specimens were photographed and the digital images used to determine the thickness of the new tissue. The thickness of the new tissue overlying the infraspinatus tendon was measured at 3 locations and the average expressed as a percentage of infraspinatus tendon thickness of each animal. The bone-tendon-scaffold specimens were then decalcified in 5% nitric acid solution, embedded in paraffin, and 5 micron-thick sections cut and stained with hematoxylin and eosin (H&E). The slides were analyzed using both transmitted and polarized light microscopy to evaluate host response, tissue ingrowth, collagen fiber alignment, and integration of new tissue with host bone and tendon.

Results

All of the sheep tolerated the surgery well and no postoperative pain or lameness was observed. One animal developed a superficial skin infection that progressed down into the shoulder joint. This animal was excluded from the study.

Collagen Scaffold Group

After 6 weeks of implantation the voids in the collagen scaffold were completely filled with proliferating fibrovascular tissue (Fig. 3). The fibers of the collagen scaffold were clearly evident and easily distinguished from the new tissue ingrowth. These observations were consistent in all specimens. There was no evidence of any inflammatory or foreign body reaction in any of the 6-week specimens. The mean thickness of the scaffold-induced tissue was 70.8% ± 11.3% (std dev) of the thickness of the underlying infraspinatus tendon.

After 12 weeks of implantation a layer of new connective tissue overlying the superior surface of the infraspinatus tendon and its bony insertion was clearly visible (Fig. 4A). The mean tissue thickness was 80.5% ± 16% (std dev) of the thickness of the underlying infraspinatus tendon. Histologically, this new tissue was composed of fibroblasts and regularly-oriented collagen fibers (Fig. 4B) and was intimately attached to the underlying infraspinatus tendon (Figs. 4C, 4D). The fibers of the collagen scaffold were occasionally visible in the 12-week sections. The scaffold-induced connective tissue demonstrated good integration into bone and there was evidence of Sharpey's fibers (Fig. 5). These observations were consistent in all specimens. There was no evidence of any inflammatory or foreign body reaction in any of the 12-week specimens.

After 26 weeks of implantation, the layer of new connective tissue overlying the infraspinatus tendon appeared more mature and regularly-oriented (Fig. 6). The mean tissue thickness was 91.5% ± 0.6% (std dev) of the thickness of the underlying infraspinatus tendon. There was no evidence of any remaining collagen scaffold in the 26-week sections. The bony insertion of the new tissue demonstrated evidence of a fibrocartilagenous component, suggestive of a more normal direct insertion (Fig. 7). There was no evidence of any inflammatory or foreign body reaction in any of the 26-week specimens.

The histological response remained stable out to 52 weeks of implantation. The scaffold-induced tissue was less cellular and histologically resembled tendon-like (dense, regularly-oriented) connective tissue and was well-integrated with the underlying host tendon (Fig. 8). The mean tissue thickness was 86.1% ± 13.6% (std dev) of the thickness of the underlying infraspinatus tendon. There was no evidence of any inflammatory or foreign body reaction in any of the 52-week specimens.
Sham Control Group

The sham procedures, wherein all elements of the surgical procedure were performed except that no scaffold was implanted, demonstrated that, in the absence of the scaffold, there was no discernible induc-
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Discussion

Collagen-based extracellular matrices have previously been advocated as an augmentation to the surgical re-attachment of large tears of the rotator cuff tendons\textsuperscript{13,14}. The primary indication of these matrices is to provide supplemental support, protection, and reinforcement (i.e., augmentation) of repaired rotator cuff tendons\textsuperscript{13}. However, variations in degradation rates, biocompatibility, and tissue-induction properties of currently available collagen-based scaffolds may affect the predictability of their biologic behavior\textsuperscript{14,15}. The results of the current study support the hypothesis that a highly porous, biocompatible, reconstituted collagen scaffold of low tensile modulus could consistently support the rapid ingrowth of host tissue and swiftly induce its progressive maturation into a thick layer of regularly-oriented connective tissue. The new tissue became integrated with the underlying infraspinatus tendon and was able to increase the thickness of the tendon by 80% at 12 weeks. This increase in mass was maintained over the next 9 months as the new tissue matured and became more tendon-like in its histological appearance. It is likely that the low (6 MPa) modulus of the scaffold permitted sufficient deformation of the new tissue to allow its modulation from fibrovascular repair tissue to dense, regularly-oriented connective tissue. The initial mechanical properties of the collagen scaffold were an order of magnitude below that reported for the rotator cuff tendons of animals\textsuperscript{13} and humans\textsuperscript{16}. The lower tensile modulus could be an important factor in the rapid orientation of the collagen fibers seen in the current study. Increased strain has been shown to induce collagen fiber alignment\textsuperscript{17} which relates to improved material properties (over less-aligned collagen fibers)\textsuperscript{17}. While mechanical loading is necessary for the functional remodeling of the newly synthesized tissue\textsuperscript{18}, it is also required for the creation of a normal tendon-

Figure 6. At 26 weeks after implantation the new tissue consisted of well-organized connective tissue. The collagen scaffold was completely absorbed by 26 weeks. No inflammatory or foreign body reaction. H&E, original magnification=50x.

Figure 7. (A) At 26 weeks after implantation the new tissue (NT) was well-integrated with the native bone (NB) with a layer of fibrocartilage (FC) at the bone interface. No inflammatory or foreign body reaction. H&E, original magnification=50x. (B) Polarized light image of Panel A.

Figure 8. At 52 weeks after implantation the new tissue consisted of well-organized connective tissue that was well-integrated with the underlying tendon. No inflammatory or foreign body reaction. H&E, original magnification=100x.
bone insertion\textsuperscript{19}. In the current study, the scaffold-induced tissue appeared well-integrated into cortical bone by 12 weeks with evidence of Sharpey's fibers spanning the osseous attachment of the new tissue. By 26 weeks the new tissue demonstrated a fibrocartilagenous transition zone at its insertion site into bone. This is similar to the direct insertion site described for the bony attachment of rotator cuff tendons. The creation of a direct type of insertion site that allows gradations in structure, composition, and material properties does not always occur in surgical reattachment of the rotator cuff and this is thought to be responsible for the high incidence of failure of these repairs\textsuperscript{19,20}.

The primary limitation of this research is that it was performed in healthy sheep with intact tendons. While sheep are considered appropriate animal models for studying the repair of full-thickness tears of rotator cuff tendons, partial-thickness tendon lesions in healthy animals can heal spontaneously\textsuperscript{21}. Therefore, the premise that adding new regularly-oriented, connective tissue to a rotator cuff tendon with a partial-thickness tear could decrease the incidence of tear propagation or allow for a more biomechanically optimal healing environment for these partial-thickness tears cannot be accurately tested in healthy animals. However, the lack of any new tissue accretion in sham animals supports the inductive and conductive capabilities of the reconstituted collagen scaffold in generating new tendon-like tissue. Additionally, the current study did not examine the material or biochemical characteristics of the new tissue augmenting the infraspinatus tendon. While this limits our ability to fully characterize the new tissue as being a tendon, the histologic characteristics of the maturing tissue observed in this study (i.e. dense, regularly-oriented connective tissue) is similar to that of tendons\textsuperscript{22}. However, the ultimate test of any regenerated tissue will be its ability to integrate and function within the host environment. Finally, although increasing the thickness of the infraspinatus tendon by 80\% through the addition of collagenous tissue highly oriented along the axis of loading would likely decrease any applied stress (and ultimately strain) experienced by the tendon, additional studies are needed to confirm this supposition.

The results of this study demonstrate that a highly porous, low modulus scaffold made of highly-purified reconstituted collagen has the ability to induce the formation of new tendon-like tissue consisting of well-organized collagen with fibers oriented in the direction of the load. The scaffold did not elicit an inflammatory response or foreign body reaction and was completely absorbed within 6 months after implantation. The new tissue induced by the scaffold was well-integrated with the underlying rotator cuff tendon and demonstrated a fibrocartilagenous transition zone at its insertion into bone. The increased thickness of the infraspinatus tendon appeared stable throughout the 12-month follow-up of this study. These results suggest that the reconstituted collagen scaffold may have a role in preventing tear propagation in partial-thickness tears of the supraspinatus tendon by increasing tendon thickness and decreasing intra-tendinous strain.

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References

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