Published in final edited form as:

Vet Comp Orthop Traumatol. 2020 May ; 33(3): 212–219. doi:10.1055/s-0040-1701650.

Partial Infraspinatus Tendon Transection as a Means for the Development of a Translational Ovine Chronic Rotator Cuff Disease Model

Jeremiah Easley¹, James Johnson², Daniel Regan³, Eileen Hackett¹, Anthony A. Romeo⁴, Ted Schlegel⁵, Cecily Broomfield², Christian Puttlitz², Kirk McGilvray²

¹Preclinical Surgical Research Laboratory, Colorado State University, Fort Collins, Colorado, United States

²Orthopaedic Bioengineering Research Laboratory, Colorado State University, Fort Collins, Colorado, United States

³Flint Animal Cancer Center and Department of Microbiology, Immunology, & Pathology, Colorado State University, Fort Collins, Colorado, United States

⁴Rothman Orthopaedic Institute, New York, New York, United States

⁵Department of Orthopedics, University of Colorado School of Medicine, Aurora, Colorado, United States

Abstract

Objective—Rotator cuff tendon tears are the most common soft tissue injuries in the shoulder joint. Various animal models have been described for this condition, but all current translational animal models have inherent weaknesses in their ability to generate chronically degenerated rotator cuff tendons. The objective of this study was to evaluate a partial infraspinatus tendon transection model as a means of creating a chronically degenerated rotator cuff tendon in an ovine model and compare the injury characteristics of this model to those observed in human patients with severe chronic rotator cuff tendon injuries.

Study Design—The infraspinatus tendons of six sheep were partially detached followed by capping of the detached medial section of the tendon with Gore-Tex. Human tissue samples of the supraspinatus tendon were harvested from patients undergoing primary reverse shoulder arthroplasty and served as positive controls of chronic rotator cuff tendinopathy

Results—Transected sheep tendons were characterized predominantly by an acute reactive and reparative pathological process as compared with the chronic degenerative changes observed in the human tendons. In contrast, the non-transected portion of the ovine tendon showed histological

Address for correspondence Kirk McGilvray, PhD, 1374 Campus Delivery, Fort Collins, CO 80523, United States (kirk.mcgilvray@colostate.edu).

Authors' Contributions

Jeremiah Easley and Kirk McGilvray contributed to the conception of study, study design, acquisition of data and data analysis and interpretation. Eileen Hackett, Tony Romeo, Ted Schlegel and Christian Puttlitz contributed to conception of study and study design. Cecily Broomfield contributed to study design and acquisition of data. James Johnson contributed to acquisition of data and data analysis and interpretation. Daniel Regan contributed to study design, acquisition of data and data analysis and interpretation. All authors drafted, revised and approved the submitted manuscript.

changes, which were more chronic and degenerative in nature when compared with the transected tendon.

Conclusion—Overall, histological features of the non-transected portion of ovine tendon were more similar to those observed in the chronic degenerated human tendon.

Keywords

chronic rotator cuff degeneration; histopathology; preclinical model; sheep; supraspinatus tendon

Introduction

Rotator cuff tears represent the most common soft tissue injuries in the shoulder, resulting in debilitating pain and dysfunction.^{1–4} Around 250,000 rotator cuff tear repairs are performed annually in the United States, with a \$3.44 billion annual societal impact.^{5,6} The original insult causing these types of tears can be multifaceted, but generally stem from an acute injury resulting in chronic degeneration or emanates from a combination of over-loading and degeneration.^{7,8} Despite advanced approaches for rotator cuff repair, most surgical repairs experience a wide spectrum of failure rates (20–94%).^{9,10}

To investigate the pathogenesis of rotator cuff injuries and potential therapeutic options, it is necessary to have controlled experimental models. At this time, there is not a reproducible animal model to perform translational research on the factors that influence the healing of a chronic, degenerative rotator cuff tear. Leporine,¹¹ murine,¹² canine,¹³ ovine¹⁴ and non-human primates¹⁵ have been proposed as potential animal model candidates for investigating the aetiology and pathology of rotator cuff tears; however, none of these aforementioned models have been able to adequately emulate what is seen in chronically degenerated human tendons. Previous models have suffered from disadvantages such as incomparable healing rates,^{12,16,17} dissimilar tendon size (precluding ability to test human devices without scaling), tendon retraction and increased magnitudes of tendon and muscle degeneration as compared with human tissues.^{14,18,19}

The shortcomings in these models lead to a critical need to refine and re-develop a clinically relevant large animal model of the chronically degenerated rotator cuff injury. Although ovine shoulders lack an acromion arch, previous work has demonstrated that the infraspinatus tendon of the previous ovine models are an appropriate model for the human supraspinatus tendon, allowing devices designed for humans to be tested.^{19,20} Unfortunately, previous ovine chronic large rupture models have been unsuccessful in mimicking what is seen in the human chronic massive tear condition.¹⁹ Regardless, previous research by our group, and others, have shown that the ovine model still provides a more robust platform for evaluation of basic science questions, as well as a platform to develop novel orthopaedic devices or techniques.^{3,18,21–27} However, the current chronic ovine rotator cuff tear model has limitations, including significant tendon retraction during the degeneration cascade and increased magnitudes of tendon and muscle degeneration as compared with human tissues.^{14,18,19} The inability to anatomically appose the tendon to its original insertion increases the difficulty of repair surgeries, which when combined with the severe degenerative changes,

make it a less-than-ideal platform with which to investigate new devices such as orthobiologics designed for humans.

It has been shown that muscular and tendinous tissues will undergo degenerative changes if they are not loaded for an extended period.²⁸ It was theorized that by unloading half of the tendon and attached muscle body via the partial transection method, sheep would be able to ambulate normally while ideally still inducing mild, degenerative changes similar to those seen in humans with a chronic rotator cuff tear. Therefore, the purpose of this study was to evaluate the time-dependent changes occurring in an ovine infraspinatus partial tendon transection model (i.e. partial transection), with the ultimate goal of improving upon the translatability of current ovine chronic rotator cuff model (i.e. full transection). Specifically, the injury characteristics of a partially transected infraspinatus tendon model in sheep were compared with the injury characteristics of a chronically injured rotator cuff in humans. Additionally, it was thought that a partial rotator cuff tear of the sheep infraspinatus tendon would be more easily repairable than a full transection model following the degeneration period and that its characteristics (i.e. histopathological changes) would more closely mimic chronic rotator cuff tendinopathy characteristics noted in humans.

Materials and Methods

All surgeries were performed at the Preclinical Surgical Research Laboratory at Colorado State University (Fort Collins; Colorado, United States) under Institutional Animal Care and Use Committee (IACUC approval 15–6202A). Human tissue was collected from Rush Medical Hospital (Chicago, Illinois, United States) under Institutional Research Board approval (IRB approval 16110707-IRB01).

Human ‘Model’: Tissue Collection

Human tissue samples (20 × 10 mm, with the smaller dimension aligned with tendon fibres) of the supraspinatus tendon were harvested from 17 patients ($n = 17$ samples) undergoing primary reverse shoulder arthroplasty. Due to the infeasibility of obtaining degenerated tissue during normal rotator cuff tear repair procedures, it was determined that this tissue would serve as the most appropriate positive control of chronic rotator cuff tendinopathy in humans. This patient cohort had an average age of 68 ± 7 years, all demonstrated symptoms of chronic tendon degeneration and most had histories of previously failed rotator cuff tear repair surgeries. Tissue samples were collected intra-operatively and were placed in 10% neutral-buffered formalin for fixation at the time of collection. Due to the IRB protocol for these patients and the nature of the surgery, muscle and musculotendinous junction specimens were not collected.

Ovine Model: Surgical Procedure

Six ($n = 6$) skeletally mature *Ovis aries* ewes (3.5 years of age) were utilized in this study. Ewes were fed a grass/alfalfa mix hay and provided with tap water *ad libitum*. The sheep were divided between two sacrificial time points; three sheep were humanely euthanatized at 6 weeks and three sheep at 12-week post-surgical (with respect to the creation of the tear).

All ewes were fasted 12 hours prior to surgery and premedicated with procaine G penicillin (3 million units/kg, subcutaneous – PenOne Pro, Norbrook Laboratories Limited, Newry, Ireland), oral phenylbutazone (1 g oral – Bute Buloses, VEDCO, Inc., St. Joseph, Missouri, United States) and transdermal fentanyl patches (100 µg, 50 µg – Fentanyl Transdermal System, Watson Pharma, Inc., Corona, California, United States; one patch applied to the forelimb) 24 hours before surgery.

The sheep were prepared for unilateral surgery and placed in left lateral recumbency under general anaesthesia. The skin over the right shoulder joint was prepared for aseptic surgery using alternating scrubs of povidone-iodine (Betadine) and alcohol. The shoulder joint was draped for aseptic surgery. A skin incision of approximately 12 cm in length was made over the point of the shoulder. The *musculus subcutaneous coli* was divided in line with the incision and the *musculus deltoideus* was split along the tendinous division between the heads of the acromion and scapula. The superficial head and insertion of the infraspinatus tendon was isolated, followed by sharp transection/detachment of the cranial 50% of the infraspinatus tendon at its insertion to the greater tuberosity of the humerus (►Fig. 1A). This portion of the tendon was chosen as the transection region at this location as it was deemed to be repeatable during surgery. The released portion of infraspinatus tendon was then capped/wrapped with an approximately 3 cm × 3 cm patch of Gore-Tex using metric 3 nylon in a mattress pattern (►Fig. 1B). The purpose of the Gore-Tex was to inhibit scarring of the tendon to the surrounding soft tissue, while still allowing some nutrition to diffuse to the covered aspect of the tendon.^{3,29}

The capped end of the tendon was not re-attached to the original site of insertion on the humerus (►Fig. 1B). Two 1.2-mm Kirschner wire markers were inserted along the lateral edge of the humeral insertion site to serve as anatomical landmarks indicating the site of partial transection. After Kirschner wire insertion, the brachial fascia and subcutaneous tissues were closed as separate layer using 2–0 absorbable suture material (Polysorb), followed by skin closure with stainless steel staples. Following complete recovery, the sheep were allowed to eat and ambulate *ad libitum*. The sheep were monitored daily throughout the study period for any signs of adverse events or complications and to evaluate pain, lameness/ambulatory function and incisional site healing.

Surgical Assessment after 6 or 12 Weeks of Healing

Prior to euthanatization of both the 6- and 12-week time point animals, surgical assessment of scar tissue formation of the capped portion (i.e. was the scar tissue removable or affixed) and tendon retraction measurement were performed under general anaesthesia. After the *musculus deltoideus* was split along the tendinous division between its acromial and scapular head, the acromial head of the *musculus deltoideus* was reflected with malleable retractors. Overlying fibrous scar tissue was dissected away, and the Gore-Tex patch capping the transected portion of the infraspinatus tendon was localized and removed. The capped portion of tendon was assessed, and the retraction distance from the proximal end of the tendon to the anatomical insertion on the humeral head was measured (utilizing a sterile surgical ruler). Surgical reattachment of the transected portion was simulated by two board-certified veterinary surgeons and a medical doctor familiar with the acute repair technique.

This ‘simulation’ served as a means to determine the relative level of tension required to achieve normal tendon apposition to the anatomical insertion of the humeral head, thus providing insight into the potential feasibility of repair surgery (a necessity for utilizing this animal model to test orthopaedic devices in a pre-clinical setting). Following the surgical assessment, all animals were humanely euthanatized and the infraspinatus tendon samples were collected within 1 hour of euthanatization. Samples were then placed in 10% neutral-buffered formalin for fixation.

Histological Analysis

Tendon biopsies from both the transected and non-transected sides were taken from the proximal end of the tendon (~1 cm in length) (►Fig. 1C). Muscle and myotendinous junction specimens were obtained from locations outlined in ►Fig. 1C. Following the tissue collection and fixation for both the human and ovine samples, standard paraffin-embedding techniques were used, and one section was cut through the mid-portion in the sagittal plane from each formalin-fixed sample and stained with haematoxylin and eosin. Haematoxylin and eosin stain was used to highlight and quantify the amount of muscle and fat within the samples. Sections were evaluated using a semi-quantitative scoring system based on an adaptation of the scheme put forth by Longo and colleagues.³⁰ As samples were collected in close proximity to the enthesis of the tendon, care was taken to analyse both proximal and distal aspects of the tendon to ensure that any variations in structure and constituents were not structural or cellular changes associated with the insertional region of the tendon. Tenocyte reactivity, stromal cell proliferation, inflammation, neo-vascularization, discreteness of muscle-tendon junction and myocyte degeneration/atrophy were evaluated. Slides were scored on a five-point scale where zero corresponds to normal, 1 with minimal change, 2 with mild change, 3 with moderate change and 4 with severe change as compared with healthy controls. A board-certified veterinary pathologist unblinded to species but blinded to tendon/muscle section (medial vs. lateral) performed the histopathological analysis on all samples. The pathologist who performed the analysis of the specimens in this study utilized samples from previous studies^{3,18,21,26,27,31} to generate and calibrate to a baseline of uninjured ovine tendons before performing the analysis on the specimens in this study.

As this study was intended to be a pilot study, the low sample size of each experimental group precluded meaningful statistical analysis of the results. Therefore, all results are reported as mean $\pm$ standard deviation.

Results

All six animals survived to the 6- and 12-week time points with three shoulders being used from each time point. No intra- or postoperative complications were noted. At both time points, the capped portion of the tendon was covered with a moderate amount of scar tissue that was easily removed via surgical dissection to expose the Gore-Tex capped tendon.

Reattachment of Medial Half of Tendon

At the 6-week time point, the detached portion of the infraspinatus tendon was able to be re-attached to the humeral footprint to create an anatomically correct repair (3 out of 3 samples); the average retraction of the detached portion at the 6-week time point was 1.6 ± 0.2 cm (mean $\pm$ standard deviation). It was not possible to re-attach the infraspinatus tendon to the humeral footprint at the 12-week time point due to significant retraction (2.2 ± 0.6 cm) and fibrosis of the detached tendon section (2 out of 3 samples).

Histology Results

Tendon Region

As compared to uninjured ovine tendon (►Fig. 2A), the medial infraspinatus tendon section within the ovine model that underwent transection (►Fig. 2B) was primarily composed of stromal cell proliferation and tenocyte reactivity, the latter was characterized by tenocytes containing large nuclei with open chromatin and increased numbers of plump, reactive, mesenchymal/stromal cells, often times arranged in dense streaming bundles which separated and replaced normal tendon collagen (►Fig. 2C, 2D). The ovine 12-week transected and human samples had mild-moderate changes in the tenocyte reactivity and stromal cell proliferation category, demonstrating a decrease from the 6-week time point which had moderate-severe changes (►Table 1). Multi-focally, ovine tendon collagen also frequently appeared degenerate, characterized by loss of the tightly packed parallel fibre arrangement, collagen fibre pallor, with increased waviness, separation and disorganization of collagen bundles (►Fig. 2C, 2D). Both the 6- and 12-week samples showed similarity to what was seen in the human condition regarding levels of inflammation (mild level), although they were still graded as slightly more inflamed than the human condition (minimal) (►Table 1). All ovine tendon samples demonstrated evidence of neo-vascularization, characterized by the presence of variably sized bloodvessels lined by plump endothelium, often located between parallel bundles of collagen (►Fig. 2D). Overall, ovine samples scored higher than the human samples in the tendon neo-vascularization category (►Table 1). In the ovine samples, infiltrates of mononuclear inflammatory cells, primarily composed of lymphocytes, with fewer plasma cells and rare macrophages, were often present between collagen bundles and surrounding neo-vessels (►Fig. 2C, 2D).

In contrast, histological changes within the lateral (non-transected) ovine infraspinatus tendon (►Fig. 2E, 2F) were typified by a less inflamed and reactive process, but instead more chronic degenerative histological changes. These changes were characterized by diffuse pallor, marked separation and haphazard arrangement of collagen bundles, with loss of collagen fibre structure. All of these lateral tendon sections also contained multi-focal regions of mucinous degeneration of collagen, and multi-focally, tenocytes and their nuclei resembled chondrocytes and were forming clusters of chondrocytes with multiple nuclei per lacunae, consistent with chondroid metaplasia.

In brief, the most consistent histological feature present within the human tendons was the loss of the tight, parallel organization of collagen bundles, with haphazard arrangement, fibrillation and separation of collagen fibres. Additionally, multi-focal regions of increased

tenocyte reactivity and stromal cell proliferation were also present, again characterized by increased overall tendon cellularity, enhanced prominence of tenocytes between collagen bundles and occasional streaming bundles of plump, reactive mesenchymal cells. In addition, the overall tinctorial properties of the tendon collagen was markedly different from normal tendons, characterized by diffuse pallor (pale pink staining) which coincided with marked loss of normal collagen bundle architecture and the presence of variably sized regions of chondroid metaplasia and mucinous degeneration, and less frequently, smaller foci of collagen hyalinization. Multi-focal, mild neovascularization was present in the majority of samples, while inflammation was minimal to often times essentially non-existent (►Fig. 2A). Similar gross histological findings were noted across all animals at both 6 and 12 weeks and severity of tendon changes did not seem to progress beyond the 6-week healing period (►Fig. 2C–F).

Musculotendinous Junction Region

For human patients, only tendon tissue samples were obtainable due to the nature of the primary reverse shoulder arthroplasty; thus, comparisons of ovine muscle and musculotendinous regions to human tissues could not be performed. Importantly, however, the infraspinatus musculotendinous junction in this sheep model was characterized by varying degrees of infiltration and separation of myocyte bundles by adipocytes. Also present were multi-focal areas of myofiber atrophy and fibrosis. These pathological changes observed within the proximal infraspinatus muscle and musculotendinous junction, are somewhat similar to those described for ‘fatty atrophy’ of human rotator cuff muscles³² (►Fig. 2G). The data demonstrated that the level of neovascularization decreased slightly on the transected side of the musculotendinous junction during the final 6 weeks of the study. Similarly, tenocyte reactivity and stromal cell proliferation decreased in going from the 6- to 12-week time point on the transected and non-transected portion (►Table 2).

Infraspinatus Muscle Region

Evaluation of the histological scoring of the infraspinatus muscle biopsies demonstrated a clear difference in the degree of inflammation between the transected and non-transected sides at both 6 and 12 weeks, with the non-transected side showing minimal to no evidence of inflammation. The level of atrophy seen on the transected side was much greater than the non-transected side at the 6-week time point (►Table 2). The muscle tissue from the transected half had a noticeably increased degree of neo-vascularization as compared with the non-transected portion.

Discussion

This translational animal model development study described an ovine model of chronic rotator cuff degeneration. To our knowledge, this is the first study to include serial histological analysis of the pathological effects on the tendon originating muscle at multiple time points.^{33,34} As compared with the degenerated human condition, sheep tendons were characterized by a more acute to sub-acute reactive and reparative pathological process, as evidenced by increased inflammation, neo-vascularization and markedly increased tenocyte reactivity and stromal cell proliferation. These changes were most evident within the

transected infraspinatus tendon. In contrast, histological changes within the human tendons evaluated in this study were characterized by more diffuse, chronic, degenerative changes, which contrasts with the acute, reactive and reparative changes more frequently observed in the transected portions of the sheep tendons. Unexpectedly, we observed that these histological changes in our human rotator cuff tendon samples appeared more similar to those observed in the lateral non-transected sheep infraspinatus tendons, which were also predominantly characterized by chronic degeneration of tendon collagen, with pale staining, separation and haphazard arrangement of collagen bundles. As compared with the transected portion of the tendon, the non-transected infraspinatus tendon showed more mild changes, which appeared more chronic and degenerative in nature as opposed to the reactive/reparative changes observed for the transected tendons.

Other studies have noted similar pathological findings in partial tendon transection ovine models. Smith and colleagues performed a partial tendon transection ovine model and analysed histological results at the 4-week time point.³³ While this was a shorter time point than what we had analysed, increased vascularity and decreased collagen fibre alignment were likewise noted. Another large animal partial tendon transection model was tested by Derwin and colleagues, which found comparable levels of tendon retraction in their canine model.³⁴ Increased cellularity was also a key finding of a rat model of partial transection.³⁵ Jacobson and colleagues utilized a partial equine flexor tendon injury to investigate changes that occur in both the injured and non-injured halves of the tendon. They found that there were no significant differences between pathological parameter between the transected and non-transected sides of the tendon. This is in contrast to what was observed in this ovine model. Specifically, pathological changes (e.g. inflammation and cellularity) were observed in both sides of the ovine tendon, with these changes being more severe in the transected.³⁶

As with all translational animal models, there are limitations correlating the results from this study to clinical human cases. The main limitation of this study is that our low sample size ($n = 3$ tendons at each time points) resulted in low statistical power (0.22), which precludes statistical conclusions and analysis of further time points. This study compared the transected and non-transected sides of the muscle, musculotendinous junction and tendon; however, only half of the tendon was transected. Since the distal portion of the tendon was not split in half (and therefore was still attached), the degenerative effects may be seen in both sides of the muscle and musculotendinous junction. As previously mentioned, human tissue samples were limited to only tendon, therefore a direct comparison between human and ovine muscle and musculotendinous sections was not possible. One of the primary purposes of pre-clinical models is to analyse the effects of different treatments in repair scenarios. Due to the level of tendon retraction seen, reattachment of these ovine tendons at delayed time points during any subsequent repair surgeries would most likely be infeasible. As such, the testing of musculoskeletal implants in a delayed setting utilizing this model would be intractable, thus significantly impairing the usefulness of this model.

Development of a more clinically translatable chronic rotator cuff injury model is critical to advancing the surgical and medical treatment of human rotator cuff tears. Currently, there remains no reproducible large animal model to perform translational research investigations on factors that influence healing of a chronic, degenerative rotator cuff tear. While this

model does not completely emulate all aspects of chronic tendon degeneration seen in humans, it has the advantage of having a tendon of similar structural characteristics as the human supraspinatus tendon, allowing for the study of safety and efficacy of controlled variables including: implantable devices, cellular therapies, prevention of degenerative changes, rehabilitation options or novel surgical techniques.

Acknowledgments

All work was internally funded by the Orthopaedic Bioengineering Research Laboratory and the Preclinical Surgical Research Laboratory. One of the authors has received personal fees from Arthrex, Inc., Atreon Orthopaedics and Paragen. D.P.R. is supported by National Institutes of Health Grant K01OD022982.

Conflict of Interest

Dr. Romeo receives royalties from devices/products from Arthrex, Inc. and Atreon Orthopaedics. He also has been paid to present in the last 12 months by Arthrex, Inc. He is a paid consultant for Arthrex, Inc. and Atreon Orthopaedics. He has stock/stock options in Paragen. Dr. Schlegel reports grants from Arthrex Education Grant, grants from Gemini Mountain Education Grant, outside the submitted work; and stock/stock options in the following companies: Kaleo, PRIVIT, Avenu and NASH.

References

1. Lorbach O, Baums MH, Kostuj T, et al. Advances in biology and mechanics of rotator cuff repair. *Knee Surg Sports Traumatol Arthrosc* 2015;23(02):530–541 [PubMed: 25573661]
2. Apreleva M, Özbaydar M, Fitzgibbons PG, Warner JJP. Rotator cuff tears: the effect of the reconstruction method on three-dimensional repair site area. *Arthroscopy* 2002;18(05):519–526 [PubMed: 11987064]
3. Santoni BG, McGilvray KC, Lyons AS, et al. Biomechanical analysis of an ovine rotator cuff repair via porous patch augmentation in a chronic rupture model. *Am J Sports Med* 2010;38(04): 679–686 [PubMed: 20357402]
4. Chakravarty K, Webley M. Shoulder joint movement and its relationship to disability in the elderly. *J Rheumatol* 1993;20 (08):1359–1361 [PubMed: 8230019]
5. Colvin AC, Egorova N, Harrison AK, Moskowitz A, Flatow EL. National trends in rotator cuff repair. *J Bone Joint Surg Am* 2012;94(03):227–233 [PubMed: 22298054]
6. Mather RC III, Koenig L, Acevedo D, et al. The societal and economic value of rotator cuff repair. *J Bone Joint Surg Am* 2013;95(22):1993–2000 [PubMed: 24257656]
7. Savin D, Meadows M, Verma N. Rotator cuff healing: improving biology. *Oper Tech Sports Med* 2017;25:34–40
8. Oh LS, Wolf BR, Hall MP, Levy BA, Marx RG. Indications for rotator cuff repair: a systematic review. *Clin Orthop Relat Res* 2007;455 (455):52–63 [PubMed: 17179786]
9. Edelstein L, Thomas SJ, Soslowsky LJ. Rotator cuff tears: what have we learned from animal models? *J Musculoskelet Neuronal Interact* 2011;11(02):150–162 [PubMed: 21625052]
10. Galatz LM, Ball CM, Teefey SA, Middleton WD, Yamaguchi K. The outcome and repair integrity of completely arthroscopically repaired large and massive rotator cuff tears. *J Bone Joint Surg Am* 2004;86(02):219–224 [PubMed: 14960664]
11. Grumet RC, Hadley S, Diltz MV, Lee TQ, Gupta R. Development of a new model for rotator cuff pathology: the rabbit subscapularis muscle. *Acta Orthop* 2009;80(01):97–103 [PubMed: 19234889]
12. Barton ER, Gimbel JA, Williams GR, Soslowsky LJ. Rat supra-spinatus muscle atrophy after tendon detachment. *J Orthop Res* 2005;23(02):259–265 [PubMed: 15734235]
13. Derwin KA, Baker AR, Codsi MJ, Iannotti JP. Assessment of the canine model of rotator cuff injury and repair. *J Shoulder Elbow Surg* 2007;16(5, Suppl):S140–S148 [PubMed: 17560802]
14. Gerber C, Schneeberger AG, Perren SM, Nyffeler RW. Experimental rotator cuff repair. A preliminary study. *J Bone Joint Surg Am* 1999;81(09):1281–1290 [PubMed: 10505524]

15. Sonnabend DH, Howlett CR, Young AA. Histological evaluation of repair of the rotator cuff in a primate model. *J Bone Joint Surg Br* 2010;92(04):586–594 [PubMed: 20357340]
16. Galatz LM, Sandell LJ, Rothermich SY, et al. Characteristics of the rat supraspinatus tendon during tendon-to-bone healing after acute injury. *J Orthop Res* 2006;24(03):541–550 [PubMed: 16456829]
17. Liu X, Manzano G, Kim HT, Feeley BT. A rat model of massive rotator cuff tears. *J Orthop Res* 2011;29(04):588–595 [PubMed: 20949443]
18. Coleman SH, Fealy S, Ehteshami JR, et al. Chronic rotator cuff injury and repair model in sheep. *J Bone Joint Surg Am* 2003;85 (12):2391–2402 [PubMed: 14668510]
19. Turner AS. Experiences with sheep as an animal model for shoulder surgery: strengths and shortcomings. *J Shoulder Elbow Surg* 2007;16(5, Suppl):S158–S163 [PubMed: 17507248]
20. Gerber C, Schneeberger AG, Beck M, Schlegel U. Mechanical strength of repairs of the rotator cuff. *J Bone Joint Surg Br* 1994;76(03):371–380 [PubMed: 8175836]
21. Hee CK, Dines JS, Dines DM, et al. Augmentation of a rotator cuff suture repair using rhPDGF-BB and a type I bovine collagen matrix in an ovine model. *Am J Sports Med* 2011;39(08):1630–1639 [PubMed: 21555508]
22. Rodeo SA, Potter HG, Kawamura S, Turner AS, Kim HJ, Atkinson BL. Biologic augmentation of rotator cuff tendon-healing with use of a mixture of osteoinductive growth factors. *J Bone Joint Surg Am* 2007;89(11):2485–2497 [PubMed: 17974893]
23. Lewis CW, Schlegel TF, Hawkins RJ, James SP, Turner AS. Comparison of tunnel suture and suture anchor methods as a function of time in a sheep model. *Biomed Sci Instrum* 1999;35:403–408 [PubMed: 11143386]
24. Lewis CW, Schlegel TF, Hawkins RJ, James SP, Turner AS. The effect of immobilization on rotator cuff healing using modified Mason-Allen stitches—a biomechanical study in sheep. *Biomed Sci Instrum* 2001;263–268 [PubMed: 11347400]
25. Schlegel TF, Hawkins RJ, Lewis CW, Turner AS. An in vivo comparison of the modified Mason-Allen suture technique versus an inclined horizontal mattress suture technique with regard to tendon-to-bone healing: a biomechanical and histologic study in sheep. *J Shoulder Elbow Surg* 2007;16(01):115–121 [PubMed: 17113318]
26. McGilvray KC, Lyons AS, Turner AS, MacGillivray JD, Coleman SH, Puttlitz CM. Shoulder tendon repair biomechanics using a polyurethane patch in a chronic ovine defect model. *Am Soc Mech Eng* 2007; (47985):853–854
27. Luan T, Liu X, Easley JT, Ravishankar B, Puttlitz C, Feeley BT. Muscle atrophy and fatty infiltration after an acute rotator cuff repair in a sheep model. *Muscles Ligaments Tendons J* 2015;5(02):106–112 [PubMed: 26261789]
28. Kang JR, Gupta R. Mechanisms of fatty degeneration in massive rotator cuff tears. *J Shoulder Elbow Surg* 2012;21(02):175–180 [PubMed: 22244060]
29. Barbolt TA, Odin M, Léger M, Kangas L, Hoiste J, Liu SH. Biocompatibility evaluation of dura mater substitutes in an animal model. *Neurol Res* 2001;23(08):813–820 [PubMed: 11760872]
30. Longo UG, Franceschi F, Ruzzini L, et al. Histopathology of the supraspinatus tendon in rotator cuff tears. *Am J Sports Med* 2008; 36(03):533–538 [PubMed: 18006676]
31. Easley J, Puttlitz C, Hackett E, et al. A prospective study comparing tendon-to-bone interface healing using an interposition bioresorbable scaffold with a vented anchor for primary rotator cuff repair in sheep. *J Shoulder Elbow Surg* 2020;29(01):157–166 [PubMed: 31401128]
32. Gibbons MC, Singh A, Anakwenze O, et al. Histological evidence of muscle degeneration in advanced human rotator cuff disease. *J Bone Joint Surg Am* 2017;99(03):190–199 [PubMed: 28145949]
33. Smith MM, Sakurai G, Smith SM, et al. Modulation of aggrecan and ADAMTS expression in ovine tendinopathy induced by altered strain. *Arthritis Rheum* 2008;58(04):1055–1066 [PubMed: 18383380]
34. Derwin KA, Codsi MJ, Milks RA, Baker AR, McCarron JA, Iannotti JP. Rotator cuff repair augmentation in a canine model with use of a woven poly-L-lactide device. *J Bone Joint Surg Am* 2009;91(05): 1159–1171 [PubMed: 19411465]

35. Lemmon EA, Locke RC, Szostek AK, Ganji E, Killian ML. Partial-width injuries of the rat rotator cuff heal with fibrosis. *Connect Tissue Res* 2018;59(05):437–446 [PubMed: 29874950]
36. Jacobson E, Dart AJ, Mondori T, et al. Focal experimental injury leads to widespread gene expression and histologic changes in equine flexor tendons. *PLoS One* 2015;10(04):e0122220 [PubMed: 25837713]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 1.

Surgical photos. (A) Transected infraspinatus tendon capped with Gore-Tex. (B) Uncapped transected infraspinatus tendon. (C) Histological biopsy locations. (1) Proximo-lateral muscle, (2) proximo-medial muscle, (3) disto-medial musculotendinous junction, (4) distolateral musculotendinous junction, (5) transected medial tendon (shown capped with Gore-Tex) and (6) non-transected lateral infraspinatus tendon.

Fig. 2.

Photomicrographs of haematoxylin and eosin-stained human and ovine tendons. (A) Normal infraspinatus tendon, 20 × . (B) Human rotator cuff supraspinatus tendon, 10 × . Asterisk = cartilaginous metaplasia. (C and D) Transected portions of the sheep infraspinatus tendon, 10 × . Asterisk = proliferations of stromal cells. Arrowhead = pale staining collagen fibres. Arrows = neo-vascularization. (E and F) Non-transection portions of the sheep infraspinatus tendon, 10 × . Asterisk = collagen fibre structure, arrows = nuclei per lacunae. (G)

Proximomedial aspect of the sheep infraspinatus musculotendinous junction, $10\times$. Asterisk = myocyte bundles, arrows = adipocytes. (H) Mucinous degeneration, $20\times$.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Histological scoring of tendon biopsies

		Human	Ovine: 6-wk	Ovine: 12-wk	
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
Tendon histological scoring	Inflammation	0.59 $\pm$ 0.87	1.83 $\pm$ 0.85	1.00 $\pm$ 0.00	Transected
			1.50 $\pm$ 0.71	1.33 $\pm$ 0.47	Non-transected
	Neo-vascularization	2.24 $\pm$ 1.03	3.67 $\pm$ 0.47	3.33 $\pm$ 0.94	Transected
			3.00 $\pm$ 0.00	3.00 $\pm$ 1.41	Non-transected
	Tenocyte reactivity and Stromal cell proliferation	2.44 $\pm$ 0.79	3.67 $\pm$ 0.47	2.67 $\pm$ 0.94	Transected
			3.33 $\pm$ 0.47	2.00 $\pm$ 0.82	Non-transected

Abbreviation: SD, standard deviation.

Note: Human samples $N=10$. Ovine 6- and 12-week, transected and non-transected samples $N=3$.

Table 2

Histological scoring of musculotendinous junction and muscle biopsies

		Ovine: 6-wk		Ovine: 12-wk	
		Mean	SD	Mean	SD
Musculotendinous junction	Discreteness of muscle-tendon junction	2.50	± 0.87	1.67	± 0.47
		1.00	± 0.82	1.50	± 0.50
	Neo-vascularization	1.25	± 0.43	1.00	± 0.00
		0.33	± 0.47	0.50	± 0.50
Muscle	Tenocyte reactivity and Stromal cell proliferation	2.75	± 0.83	1.00	± 0.00
		1.00	± 0.82	0.50	± 0.50
	Inflammation and Cellular composition	1.08	± 0.84	0.83	± 0.69
		0.20	± 0.40	0.17	± 0.37
	Neo-vascularization	0.92	± 0.45	0.50	± 0.50
		0.00	± 0.00	0.17	± 0.37
	Myocyte and Degeneration/atrophy	2.17	± 0.69	1.83	± 0.69
		0.80	± 0.75	1.42	± 0.61

Abbreviation: SD, standard deviation.

Note: Ovine 6- and 12-week, transected and non-transected samples $N = 3$.