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Biomechanical, Histologic, and Molecular Evaluation of Tendon Healing in a New Murine Model of Rotator Cuff Repair

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Abstract

Purpose: To develop a clinically relevant, robust murine model of rotator cuff tendon repair to examine cellular and molecular mechanisms of healing.

Methods: Sixty C57BL/6 male mice underwent rotator cuff transection and repair using microsurgical techniques. A modified Kessler suturing technique was used prior to tendon detachment. Sutures were passed through 2 intersecting bone tunnels that were made at the tendon attachment site. Mice were sacrificed at 2 and 4 weeks with subsequent biomechanical, histologic, micro-CT, and gene expression evaluations.

Results: Failure forces in the 2- and 4-week groups were 36% and 75% of the intact tendon, respectively. Histologic evaluation revealed complete reattachment of the tendon with no observable gap. Healing occurred by formation of fibrovascular tissue at the tendon-bone interface, similar to larger animal models. Molecular analysis revealed gene expression consistent with gradual healing of the reattached tendon over a period of 4 weeks. Comparisons were made using 1-way analysis of variance.

Conclusions: This model is distinguished by use of microsurgical suturing techniques, which provides a robust, reproducible, and economic animal model to study various aspects of rotator cuff pathology.

Clinical Relevance: Improvement of clinical outcomes of rotator cuff pathology requires in-depth understanding of the underlying cellular and molecular mechanisms of healing. This study presents a robust murine model of supraspinatus repair to serve as a standard research tool for basic and translational investigations into signaling pathways, gene expression, and the effect of biologic augmentation approaches.

It is estimated that more than 30 million tendon and ligament injuries occur annually worldwide.¹ Tendon tears affect more than 20% of the general population and up to 55% of the population older than 60 years. In the United States, approximately 250,000 rotator cuff

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tendon repairs are performed annually.² Symptomatic tears often lead to pain and disability, posing a large socioeconomic burden with use of health care resources and loss of manpower. Although rotator cuff repairs are commonly performed in an attempt to alleviate pain and restore function, results are not always satisfactory. Prior studies have shown structural failure of the repair ranging from 13% to 94%, with poorer outcomes correlated with failed healing.³⁻⁵

The enthesis (tendon-bone interface) consists of either a fibrous transition to the periosteum of cortical bone or a fibrocartilaginous transition into epiphyseal bone or a bone ridge.^{6,7} The normal enthesis microstructure is well adapted to transfer muscle force to bone. However, the native structure does not reform following injury and surgical repair. The surgically repaired rotator cuff enthesis is typically composed of a fibrovascular scar tissue interface, rather than reformation of the native fibrocartilaginous transition zone, which has inferior material properties and thus predisposes to re-rupture.^{8,9} There is currently considerable interest in improving our understanding of the biologic events in rotator cuff tendon healing to identify methods to regenerate the microstructure and composition of the native enthesis.

Prior studies have shown the involvement of a number of key molecular mediators in the differentiation of the tendon-to-bone insertion site in early development. These include Sox9, a transcription factor known to be involved in the differentiation of chondroprogenitors¹⁰⁻¹²; scleraxis (Scx), a transcription factor involved in tenogenesis^{4,13}; and the hedgehog (Hh) signaling pathway, which is known to regulate patterning of the musculoskeletal system during development.^{14,15} The use of animal models allows investigators to understand how these pathways, along with other variables such as mechanical loading, affect the healing of the repaired enthesis tissue.

The mouse is a promising model system for studying tendon-to-bone healing owing to its genetic and physiological similarity to humans, the availability of genetically engineered strains for studying molecular mechanisms, and the availability of an extensive repertoire of commercially available biological reagents for translational molecular research (Table 1). Exploiting a murine model for studying tendon-to-bone healing now allows us to take full advantage of the extensive knowledge base already existent for the murine species, the relatively low cost of research animals, and the rapid growth rate of mice, and apply these attributes to research relevant to human injury. To this end, we have developed a robust mouse model of supraspinatus tendon (SST) repair using microsurgical techniques.

The purpose of this study was to develop a clinically relevant, reproducible murine model of rotator cuff tendon repair to examine the cellular and molecular mechanisms of healing. The hypothesis was that this technique would provide secure tendon fixation to allow superior healing, making the model suitable for biomechanical studies and investigation of therapeutic interventions.

Methods

The study protocol was approved by Institutional Animal Care and Use Committee (approval number: 04-15-03M). Sixty 12-week-old male C57BL/6 mice (Jackson laboratory, Bar Harbor, ME) (average weight 25 g) were used. This strain is the most widely used laboratory mouse and has the advantages of availability of congenic strains, easy breeding, and robustness. A recent study revealed that other commonly used mouse strains share relatively similar features with regard to overall rotator cuff anatomy.¹⁶

Animals were randomly allocated to the following experimental arms: biomechanical testing (n = 16), histology (n = 8), micro-CT analyses (n = 6), and gene expression analysis by quantitative real-time polymerase chain reaction (qRT-PCR; n = 30) (Fig 1). An additional 16 cadaveric mice were used to refine the surgical exposure and repair technique, and to measure strength and stiffness of the normal supraspinatus tendon insertion.

Baseline Measurements

Eight mouse cadavers underwent dissection and measurement of SST length, width, and thickness at the tendon midsubstance to design the surgical technique and select the appropriate suture and drill bit sizes. SST force to failure and stiffness were measured in 8 freshly euthanized mice with intact shoulders to provide baseline biomechanical data.

Surgical Technique and Postoperative Care

The surgical technique described below (Video 1, available at www.arthroscopyjournal.org) is similar to the rat SST repair model described previously in the literature.¹⁷ The feasibility of the technique was established through cadaveric dissections.

To develop this model, 3 challenging key issues were (1) access, exposure, and dissection of the supraspinatus tendon; (2) tendon-suture configuration; and (3) fixation (Video 1, available at www.arthroscopyjournal.org). Authors examined several different configurations, and found that configurations involving passage of needle in a vertical fashion were associated with a higher rate of suture pull-through. In the modified Kessler configuration, the needle and sutures are almost entirely buried in the substance of the tendon (except for the turning points where the suture emerges from the tendon). However, this model does not incorporate a compressive component, as occurs with double-row or transosseous-equivalent techniques. Such techniques would be technically difficult to achieve in the very small mouse shoulder.

The surgery was performed by a microsurgeon (a postdoctoral fellow in the lab, A.L.) assisted by a sports medicine clinical fellow (C.L.C.). These 2 surgeons initially practiced on cadaveric specimens under direct supervision of the principal investigator (S.A.R.) and the senior scientist (X-H.D.) and moved on to the live procedures after the principal investigator ascertained consistency and accuracy of their performance.

Surgery was performed on a unilateral forelimb of the mouse under ×4 magnification using a surgical microscope (Fig 2). Animals were anesthetized with isoflurane and placed on a small operating table in semilateral position. A specially designed animal holder was used to

appropriately position and immobilize the animal and to facilitate access and exposure to the mouse shoulder.

After sterile preparation of the entire forelimb and induction of anesthesia, an 8-mm incision was made on the lateral aspect of the shoulder (Fig 2A). The deltoid muscle was identified with the overlying vasculature identifying the borders of the muscle (Fig 2B). The acromioclavicular joint was identified and, using a small hook, the joint was elevated and the deltoid muscle was minimally dissected to expose the rotator cuff tendons (Fig 2 C and D). Adduction and external rotation of the humerus facilitated the exposure. Using an angled micro Adson forceps, space was developed under the SST, and this structure was separated from the infraspinatus and subscapularis tendons (Fig 2E). The Adson forceps was kept under the SST to provide the space required for suturing. A double-needled 6/0 Prolene (Ethicon, Somerville, NJ) was used to fashion a modified Kessler suture starting at the midsubstance of the SST with one needle and completing the configuration with the other needle (Fig 2F). With mild traction applied to the sutures, the SST was then detached from the humerus with a No. 15 blade (Fig 2G). Care was taken not to place the suture too close to the footprint to reduce the risk of suture pullout. Soft tissue was then carefully removed from the SST footprint without injuring adjacent tendons, and the anterior and posterior extents of the footprint were marked using a fine-tipped sterile marker to guide the drill bit. Two crossing bone tunnels were then drilled using a 30-G needle (Fig 2H). The first tunnel was drilled from the anterior extent of the footprint in a posteroinferior direction, and the second tunnel was drilled from the posterior extent of the footprint in an anteroinferior direction. These tunnels exited the humerus around the surgical neck, ensuring an adequate bone bridge between the holes. The distance between exit holes was the same as the distance between the two markings at the SST footprint. Needles were then passed through their corresponding tunnels and the suture were tightened (Fig 2I). The minimally dissected deltoid was left unrepaired. The skin was then closed with interrupted sutures. Throughout the surgery, the surgeon stabilized the shoulder by holding the humerus and elbow between his index finger and thumb. Use of excessive force was avoided to prevent inadvertent injury to the extremity.

Animals were allowed free, unrestrained cage activity postoperatively and were examined daily for general signs of well-being, which included normal activity, grooming, and hydration and nutritional status.

Repair Site Evaluation

For the biomechanics, histology, and micro-CT experimental arms, one-half of the animals were euthanized at 2 weeks and the remaining animals were sacrificed at 4 weeks postoperatively. For the qRT-PCR experimental arm, animals were divided equally among 1-, 2-, and 4-week sacrifice time points.

Biomechanical Testing

For biomechanical testing, shoulder specimens were carefully dissected under $\times 2.5$ loupe magnification to isolate the supraspinatus muscle, tendon, and the humerus from all surrounding tissue. The humerus was potted in Bondo Lightweight Filler 265 (3M, St. Paul,

MN) in 2.0-mL cryogenic tubes (VWR). Dimensional measurements of the tendon were performed at midway between footprint and tendon-muscle junction. At the time of conduct of this experiment, noncontact laser measurement was not available and, therefore, a digital micrometer with fine tips and a resolution of 0.01 mm was used to measure the dimensions. Muscle tissue was then scraped from the supraspinatus tendon with a scalpel and suture material used for tendon fixation was removed. The tendon was flattened and placed in a custom serrated clamp and immobilized using sandpaper and cyanoacrylate glue (Krazy glue, Elmer's Products, Columbus, OH). A custom-designed materials testing system (MTS) was used to measure tendon failure force. The specimen was placed into the MTS machine to allow uniaxial tensile testing at a 60° abduction angle to approximate the anatomic positioning of the supraspinatus tendon (Fig 3). The specimen was loaded to failure at a rate of 1 mm/min. Load-to-failure data were recorded, and stiffness was calculated from the load-deformation curves using Microsoft Excel (Microsoft, Redmond, WA). The site of tendon failure was also recorded.

Histology

Specimens from 8 animals were used for histologic evaluation. After removal of the skin and overlying deltoid muscle, the specimens were fixed briefly in 10% neutral buffered formalin, decalcified in Immunocal (StatLab, McKinney, TX), embedded in paraffin, and sectioned in the coronal plane. Standard hematoxylin and eosin (H&E) and Safranin O preparations were then examined for tendon-to-bone interface characteristics. New tissue formation at the healing tendon-bone interface was evaluated, noting the amount, type, and location of new tissue formation. Safranin O-stained sections were used to identify glycosaminoglycan. The phenotype of cells in the healing area was evaluated on the H & E-stained sections. Qualitative descriptions of the histologic findings are reported based on consensus grading of 3 reviewers. Reviewers were the principal investigator (S.A.R.), the senior scientist (X-H.D.), and a post-doc fellow (A.L.). All of these 3 individuals had extensive experience with small animal musculoskeletal histology.

Micro-CT Analysis

Micro-CT was performed to evaluate the effect of drilling and creation of bone tunnels on basic bone parameters at 2- and 4-week time points. Specimens from 6 animals underwent micro-CT analysis using a Scanco μ CT 35 (Scanco Medical, Brüttisellen, Switzerland) system. All soft tissues except for the glenohumeral joint capsule were removed following sacrifice and forequarter amputation of the right upper limbs. Micro-CT was performed on the day of sacrifice with the specimens kept in normal saline at room temperature. The scans included a phantom containing air, saline, and an intact upper limb serving as the bone reference material for calibration of Hounsfield units to tissue mineral density. Imaging was performed with 15- μ m voxel size, at 55 KVp, 0.36° rotation step (180° angular range) and a 400-ms exposure per view.

The images were thresholded to distinguish bone voxels by use of a global threshold for each specimen, with the threshold set between 240 and 1,000. To analyze the bone surrounding the tendon attachment site, 3D images were reoriented to position the long axis

of each suture tunnel in the vertical axis using the accompanying Scanco μ CT software (HP, DECwindows Motif 1.6).

The following parameters were measured for trabecular bone surrounding the tendon attachment site: total volume (TV), bone volume (BV), bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp) as outlined by Bouxsein et al.¹⁸ To prevent inclusion of adjacent cortical bone in the analysis, the volume of interest was defined as a hemicylinder incorporating half of each suture tunnel starting from the bone surface and extending distally with a height and diameter of 1 mm. To obtain baseline data, identical micro-CT analysis was performed on intact right humeri from 3 freshly sacrificed mice of the same age and strain subsequent to drilling the same bone tunnels. The measurements reported for each mouse are the average of the measurements for each repair site.

Gene Expression Analysis

All qRT-PCR tissue specimens were collected following a careful dissection protocol. Following CO₂ euthanasia, skin over the shoulder was incised, the acromioclavicular joint was disarticulated, and both bony components were bluntly reflected, revealing the healing tendon enthesis. The supraspinatus tendon was transected at the midsubstance, and the enthesis tissue was thoroughly detached from its bony attachment. The tissue was immediately immersed in RNeasy Lysis Buffer (Qiagen, Crawley, UK). Contralateral control tendon was collected in the same manner. Tissues were then minced using microsurgical scissors under RNeasy Lysis Buffer immersion and flash-frozen in air-phase liquid nitrogen. Specimens were stored at -80°C until RNA isolation.

For RNA isolation, specimens were suspended in TRIzol (Thermo Fisher Scientific). Tissue was homogenized using microtube pestles (Argos Technologies, Elgin, IL) and a cordless motor (VWR, Bridgeport, NJ) for 2 minutes or until visibly disrupted. RNA isolation was performed using the column-elution method (RNeasy Mini Kit; Qiagen, Hilden, Germany). All purified RNA was tested for integrity using an RNA Integrity Number (RIN) cutoff of >7.0 (2100 Bioanalyzer; Agilent Technologies, Santa Clara, CA).

For qRT-PCR, cDNA was generated by reverse transcription (QuantiTect Reverse Transcription Kit; Qiagen) from a total of 50 ng RNA. qRT-PCR amplification (CFX96 Touch real-time PCR detection system, Bio-Rad, Hercules, CA) was performed using custom primers and SYBR green (SsoAdvanced Universal Supermix; Bio-Rad) for 7 genes of interest (Table 2). GAPDH was used as housekeeping reference. The data were normalized to the housekeeping gene, and the relative expression of mRNA was calculated by delta-delta Ct ($\Delta\Delta\text{Ct}$) method, where the fold differences of gene expression was analyzed by $2^{-\Delta\Delta\text{Ct}}$.

Statistical Analysis

The number of animals per time point (30) was based on authors' previous experiments with murine shoulder specimens. It was found that at least 6 specimens were needed for biomechanical testing, 4 for histologic evaluations, and 5 to 8 for gene expression analysis. In addition, based on cadaveric testing, authors expected an effect size of at least 70% at 4

weeks. Defining α and β at 0.05 and 0.2, respectively, the sample size (if authors were to compare failure forces at 2- and 4-week time points with those of intact tendon) would be 28 to 30 mice per time point.

Statistical analysis was performed using IBM SPSS version 21 and plotted using GraphPad Prism 6. Comparison of means for micro-CT and gene expression analysis data were made by 1-way analysis of variance with Tukey post hoc test for significance. Significance level was set at .05.

Results

Baseline Measurements

The length, width, and thickness of SST in intact mice were 1.54 ± 0.08 mm, 0.48 ± 0.04 mm, and 0.31 ± 0.06 mm, respectively (Table 3).

Operative Time

The mean operative time was 20 minutes in the initial cadaveric procedures, which was reduced to 7 minutes in final cadaveric procedures. The mean skin-to-skin operative time in live procedures was 6 minutes.

Biomechanical Testing

Table 3 summarizes the biomechanical findings in the 2- and 4-week repair groups. The mean failure force in the 2- and 4-week groups was 1.98 ± 0.33 N and 4.12 ± 0.94 N, which were 34% and 75% of the intact SST failure force, respectively. All failures in intact SSTs occurred at the tendon midsubstance and all failures in the 2- and 4-week repair groups occurred at the tendon-to-bone attachment site.

Histology

Histologic evaluations showed reattachment of the SST at the tendon-to-bone insertion without a gap at both time points. Healing occurred by formation of fibrovascular scar tissue at the tendon-bone interface. Although Safranin O staining showed some glycosaminoglycan deposition at the healing attachment site, an organized transition zone with calcified and noncalcified fibrocartilage was not consistently reformed. Figure 4 depicts a histologic view of a specimen at 4 weeks.

Micro-CT Analysis

Although the mean bone volume/total volume (BV/TV) and trabecular number (Tb.N) were higher in the 4-week group, there were no significant differences in mean total volume, bone volume, trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp) between the 2 groups (Fig 5). Figure 5B shows a 3D reconstruction of a humeral head. Table 4 summarizes the measurements of the trabecular bone surrounding the tunnels.

Gene Expression Analysis

qRT-PCR analysis showed upregulation of all genes of interest at the repaired enthesis (Fig 6). Collagen I, scleraxis, and MMP-14 expression were upregulated at 1 week, followed by a

statistically significant decrease toward baseline by 4 weeks. SOX-9 and MMP-13 expression followed a similar, statistically nonsignificant trend. Aggrecan expression at the repaired enthesis revealed a nonsignificant peak at 2 weeks. MMP-3 expression exhibited an increase from 2 to 4 weeks, suggesting ongoing tissue matrix turnover and remodeling during the 4-week period.

Discussion

The data presented in this study show that this model is capable of restoring 75% of the failure force of the intact SST by the fourth postoperative week. Histologic evaluations in our study corroborate the biomechanical data by showing healing at the tendon-to-bone attachment site, with no appreciable gapping at the repair site.

The overall purpose of this study was to design and test a robust and reproducible supraspinatus tendon repair technique that could be used to study the cellular and molecular aspects of tendon-to-bone healing, with the longer-term goal to use genetically modified strains to study the role of specific molecular factors in healing.

The technique used in this study is clinically relevant, as both the tendon-suture configuration and the tendon-to-bone attachment resemble actual surgical techniques used in clinical settings. Both of these features could contribute to the strength of repair achieved in our model. Moreover, the relatively small standard deviations in the biomechanical data (the mean values for failure force and stiffness) is indicative of the reliability and reproducibility of this model. Meticulous care was taken while performing biomechanical evaluations as mouse structures are very delicate and need to be handled carefully during the tissue dissection and preparation for biomechanical testing.

Evaluation of therapeutic interventions to improve rotator cuff repair outcome, either surgical or nonsurgical, require reliable animal models. The murine model can now be used with defined transgenic mouse strains to allow studies of the cellular and molecular mechanisms of healing. Another important application of this model would be induction of tendinopathy in the rotator cuff tendons using a variety of methods, with subsequent tendon repair to allow in-depth investigation of cellular and molecular features of rotator cuff repair in a more clinically relevant fashion.

We reported the average operative time (6 minutes, almost 10 specimens per hour) to help highlight the economic feature of this model. This rate will allow the investigators to economize on costs of procedure rooms and animal care. Also, a large sample size can be procured in 1 day, with an overall shorter study period and superior study power. The latter is especially important in quantitative histologic and gene expression analyses where a larger sample size is usually required to address data variability, an inherent feature of tissue sectioning and preparation methods.

The anatomy and morphology of the mouse SST was found to be similar to the rat model, which has been used extensively. A prior study evaluated rotator cuff anatomy and shoulder function in 33 different animals to determine their utility for modeling the human rotator cuff, but the list of the animals did not include mouse.¹⁹ Only the rat shoulder satisfactorily

fulfilled all anatomic criteria for modeling the human supraspinatus tendon, with a prominent supraspinatus tendon passing under an enclosed arch.¹⁹ A more recent study confirmed that the mouse rotator cuff is similar to that of human, including the presence of a coracoacromial arch.¹⁶

Prior studies have directly tested the mechanical properties of the native mouse SST and have evaluated how mechanical loading affects collagen fibrillogenesis in neonatal mice.^{20–24} These authors quantified transition stress (stress at the intersection of toe and linear regions of the load-displacement curve), transition strain (strain measured at the transition point), local linear-moduli, cross-sectional area of the SST, and the spread of the collagen fibers at several spatial and temporal points throughout mechanical testing.²⁰ These authors reported that collagen fibril development and alignment depended on the developmental age and that the timing of collagen fibrillogenesis may impact the SST's ability to appropriately respond to load.²⁰

Only 1 prior study has reported supraspinatus tendon repair in the mouse.¹⁶ The investigators used a figure-of-8 suture pattern in the SST and used the needle of the same suture to create a transverse, anteroposterior tunnel.¹⁶ They reported a maximum load for the contralateral (intact) shoulder of 1.22 ± 0.52 N, whereas we found higher load-to-failure of the native tendon (5.43 ± 0.79 N). This discrepancy could be due to different MTS setups, including the grip mechanism. Furthermore, the failure strength of the repaired tendon at 2 weeks following repair in the prior study was 0.45 ± 0.14 N, versus 1.98 ± 0.33 N in our study. No biomechanical evaluation was performed beyond 2 weeks postoperatively in the previous study.

In this study, gene expression at the repaired enthesis was examined using qRT-PCR for gene markers of tissue turnover and healing to begin to understand basic molecular mechanism(s) of healing. The repaired tissue revealed a peak in expression of tissue matrix turnover markers (MMPs 3, 13, and 14) and matrix genes (collagen I, aggrecan) at 1 to 2 weeks postrepair, with a trend toward baseline by 4 weeks postrepair (Fig 6). These trends are consistent with a previous study showing gene expression in a rat tendon midsubstance healing model.²⁵

The MMPs and their endogenous inhibitors play a critical role in maintaining the dynamic homeostasis and integrity of the extracellular matrix. Imbalance between MMPs and their inhibitors resulting in elevated MMP activity has been associated with a number of pathologic conditions of connective tissue, including degenerative tendinopathy and rotator cuff tears.^{26–35} Biologic modulation of endogenous MMP activity to basal levels may reduce pathologic tissue degradation and favorably influence tendon-to-bone healing.^{35–38} MMP-13 has been previously identified as playing a role in diseases characterized by excessive degradation of the ECM, including osteoarthritis, rheumatoid arthritis, and cutaneous ulcers.^{39–41} Doxycycline-mediated inhibition of MMP-13 favorably influenced early healing after rotator cuff repair in a rat model.⁴²

Expression of genes involved in embryologic development of the enthesis was also evaluated. MMP-14, also known as matrix type 1-MMP (MT1-MMP), plays a fundamental

role in embryonic development of the junction between calcified and uncalcified cartilage.⁴³ Scleraxis (Scx) is a well-known specific marker for tendons and ligaments with high expression in tendon progenitors.⁴ The transcription factor Sox9 is essential for the initiation of organogenesis and the determination of cell lineage in a variety of tissues, including ligaments.^{11,44,45} It has been shown that the tendon cells originate from Sox9-expressing chondrogenic mesenchymal cells in the cartilage primordia.⁴⁶ These cells become committed to tendon cells at and just after mesenchymal condensation. Once the tendon forms and attaches to cartilage, Sox9-expressing cells in the cartilage primordia no longer contribute.⁴⁵ The coordinated expression of Sox9 and Scx affects the phenotype of the developing tissue. Sox9+/Scx+ progenitor pool constitutes a multipotent cell population that gives rise to tenocytes, ligamentocytes, and chondrocytes and contribute to establishment of the enthesis.^{46,47} Scleraxis and SOX-9 are also highly expressed in the acute phase following supraspinatus tendon repair. Despite the expression of these genes, consistent regeneration of the organized fibrocartilage transition zone at the healing enthesis was not found. This may be due to lack of translation to protein, absence or deficiency of appropriate cell populations and/or organized extracellular scaffolds to aid in the response to these genes and proteins, or inadequate healing time. This preliminary analysis of gene expression following supraspinatus tendon repair can provide the basis for future studies to examine molecular mechanisms of healing.

Healing between tendon and bone depends upon new bone ingrowth into the interface between tendon and bone, with eventual re-establishment of collagen fibril continuity.^{7,17} Micro-CT was used to examine micro-structural changes in bone at the healing attachment site. These data can serve as a baseline for further studies.

Limitations

The main disadvantage of mice as a surgical model is their small size, which can make identification of structures difficult and render operative procedures tedious. Basic microsurgical skills combined with meticulous surgical practice are required to address the small size of the murine structures and achieve consistent, reliable results. It is necessary that surgeons interested in development or application of murine surgical models dedicate significant time and effort to cadaveric dissection and surgical practice to successfully overcome the steep learning curve associated with microsurgical procedures. Intermittent practice sessions will also help to maintain this skill for research purposes.

The main limitation of this model is the small size of the murine shoulder. As a corollary, it is not possible to replicate all steps of an authentic human procedure in a murine model. The technique presented here mimics the commonly used transosseous rotator cuff repair procedure. Other limitations include the inability to control postoperative load (weight bearing, shoulder motion) on the healing tendon, and the acute repair of a normal tendon, which differs from repair of a degenerative tendon that occurs in humans.

Conclusions

This model is distinguished by use of microsurgical suturing techniques, which provides a robust, reproducible, and economic animal model to study various aspects of rotator cuff pathology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig 1.
Animal allocation.

Fig 2.

Surgical technique of mouse supraspinatus tendon (SST) detachment and repair. (A) An 8-mm incision is made on the lateral aspect of the shoulder. (B) The deltoid muscle is identified with the overlying vasculature. (C and D) The acromioclavicular joint is elevated and the deltoid muscle is minimally dissected to expose the rotator cuff tendons. (E) Using an angled micro Adson forceps, space is developed under the SST. The Adson forceps is kept under the tendon to provide the space required for suturing. (F) A double-needled 6/0 Prolene is used to fashion a modified Kessler suture starting at the midsubstance of the tendon with one needle and completing the configuration with the other needle. (G) With mild traction applied to the sutures, the tendon is then detached from the humerus with a No. 15 blade. (H) Two crossing bone tunnels are drilled using a 30-G needle. The first tunnel is drilled from the anterior extent of the footprint in a posteroinferior direction, and the second tunnel is drilled from the posterior extent of the footprint in an anteroinferior direction. These tunnels exit the humerus around the surgical neck. (I) Needles are then passed through their corresponding tunnels and the sutures are tightened. (SST, supraspinatus tendon.)

Fig 3. Setup for biomechanical testing in the materials testing system (MTS). The immobilized humerus and the insertion site of the supraspinatus tendon (arrow) are visualized at the center of this image. The load cell is to the right. The computerized image display is off-screen.

Fig 4. Coronal sections of mouse shoulder specimens at 2 weeks (A and B) and 4 weeks (C and D) following supraspinatus tendon repair, revealed by H&E (A and C) and Safranin O (B and D) histologic stains. The black arrow points to the healing supraspinatus tendon enthesis. Black arrowheads refer to the location of the intersecting transosseous sutures used to achieve tenodesis. Safranin O staining suggests the presence of proteoglycan at the insertion site (B; inset). Magnification is as shown.

Fig 5.

(A) Volume of interest was defined as a hemicylinder incorporating half of the suture tunnel to avoid cortical bone as much as possible. (B) 3D reconstruction of the humeral head in a mouse from 2-week group, with the posterior tunnel in vertical position. Arrows show the position of the bone tunnels.

Fig 6.

Gene expression analysis of enthesis tissue following rotator cuff repair surgery versus native tendon control for 7 genes of interest. Gene expression is reported relative to GAPDH reference. (* $P < .05$; ** $P < .01$).

Table 1.**Advantages of Mice as a Model for Supraspinatus Tendon Repair**

High level of anatomic resemblance to human anatomic structures
High level of genetic similarity to human
Low purchase, housing, and care costs
Easy transportation within and between facilities
Fast and safe anesthesia and recovery profile
Fast surgical procedures, reducing operation room costs and overall time and costs of the projects
Imaging studies performed faster and less expensively compared with larger animals
Ability to perform in vivo imaging, permitting repeat measurements over time in the same animal
Fast growth rate
Wide availability of transgenic technology and knockout strains

Table 2.

Oligonucleotides Used in qRT-PCR

Gene	Forward	Reverse	Accession No.
Aggrecan	CCGCTTGCCAGGGGGAGTTG	CCTGCAGCCAGCCAGCATCA	NM_007424.2
Collagen1a1	AATGGCACGGCTGTGTGCGA	AACGGGTCCCCCTTGGGCCTT	NM_007742.3
Mmp3	TGTGTGCTCATCTTACCCATTGC	CCCTGTCTATCTTCCAACCCGAGGA	NM_010809.2
Mmp13	ATGGTCCAGCGCATGAAGACCCC	GTGCAGGCGCCAGAAAGAAATCTGT	NM_008607.2
Mmp14	AACTTCAGCCCCCGAAGCCTGG	ACAGCGAGGGCGGCTCATGG	NM_008608.3
Sclerajds	CCTCAGCAACCAAGAGAAAGTTGAGCA	GCCATCACCCGCGCTGTCCATC	NM_198885.3
Sox9	AAGCTCTGGAGGCTGCTGAACGAG	CGGCCTCCGCTTGTCCGTCT	NM_011448.4
GAPDH	GGGCTCATGACCACAGTCCATGC	CCTTGCCCAACAGCCTTGGCA	NM_001259726.1

qRT-PCR, quantitative real-time polymerase chain reaction.

Table 3.

Two- and 4-Week Biomechanical Data for Mouse Supraspinatus Tendon Repair Model

	Failure Force, N	Stiffness, N/mm
Intact SST	5.43 ± .79	5.28 ± 0.68
2 weeks postrepair	1.98 ± 0.33	1.21 ± 0.33
4 weeks postrepair	4.12 ± 0.94	2.94 ± 0.93

Values are mean ± standard deviation.

SST, supraspinatus tendon.

Table 4.

Basic Micro-CT Measurements for Trabecular Bone Surrounding Each Suture Tunnel

	Group			P Value
	2-Week	4-Week	Baseline*	
TV, mm ³	0.2137 ± 0.0924	0.1208 ± 0.0561	0.2113 ± 0.0146	.277
BV, mm ³	0.0701 ± 0.0260	0.0402 ± 0.0203	0.08625 ± 0.0403	.254
BV/TV	0.3348 ± 0.0252	0.3499 ± 0.1081	0.4156 ± 0.2197	.762
Tb.N, 1/mm	7.2407 ± 0.9948	9.2209 ± 1.4033	9.3112 ± 2.6914	.319
Tb.Th, mm	0.0524 ± 0.0002	0.043367 ± 0.0018	0.0716 ± 0.0278	.134
Tb.Sp, mm	0.1492 ± 0.0177	0.1226 ± 0.0309	0.1258 ± 0.0425	.541

Data are presented as mean ± standard deviation.

BV, bone volume; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; TV, total volume.

* Baseline refers to data from humeri of fresh murine cadavers with bony tunnels in place.