Supraspinatus Rotator Cuff Repair: A Mouse Model and Technique



Matthew Miller, B.A., Nikolas H. Kazmers, M.D., Peter N. Chalmers, M.D., Robert Z. Tashjian, M.D., and Michael J. Jurynec

Abstract: To improve understanding of the pathophysiology of rotator cuff repair and develop therapeutic treatments an animal model is essential. This Technical Note describes a comprehensive step-by-step description of rotator cuff repair in the mouse. This technique is particularly beneficial because the murine rotator cuff is anatomically similar to that of humans. The mouse model can also be used to test the biological role of candidate genes during repair and be used to identify drugs that accelerate the healing process.

In the United States, more than 200,000 rotator cuff repairs are performed annually, and an additional 400,000 people have surgery related to rotator cuff tendonitis and partial tears.¹ To better understand the pathology and also to evaluate for potential novel treatment approaches for rotator cuff repairs, a functioning animal model is crucial. A study by Bell et al.² confirmed that mice and humans exhibited an anatomically similar rotator cuff and supraspinatus tendon insertion. Therefore, a murine rotator cuff tear and repair model has potential utility as a research tool, and although many mouse rotator cuff repair studies have been performed, there is no comprehensive description of the step-by-step technique. Herein, we

Received February 24, 2021; accepted April 21, 2021.

Address correspondence to Matthew Miller, B.A., Department of Orthopedic Surgery, University of Utah Medical Center, 590 Wakara Way, Salt Lake City, UT 84108. E-mail: matt.miller@hsc.utah.edu

© 2021 THE AUTHORS. Published by Elsevier Inc. on behalf of the Arthroscopy Association of North America. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/ 4.0/).

2212-6287/21328 https://doi.org/10.1016/j.eats.2021.04.023 present a detailed description of a robust and reproducible surgical technique for open supraspinatus repair in mice. This technique can be used to evaluate new ways to improve rotator cuff healing both histologically and biomechanically. In addition, genetically mutant mice are relatively easy to acquire or create affording the ability to investigate the role of genetic variants in the rotator cuff repair healing.

Surgical Technique (With Video Illustration)

Drs. Kazmers, Tashjian, and Jurynec were equal contributors in the development of the technique. The use of laboratory animals was approved by the University of Utah's Institutional Animal Care and Use Committee (IACUC; Protocol number 18-09005). We apply Nair (Church and Dwight Co., Ewing, NJ) to the ventral aspect of the mouse shoulder and wipe the hair from the surgical site. The skin is rinsed well with warm water to remove any residual Nair. Next, the animals are placed in a closed chamber and anesthetized with isoflurane gas (California Pet Pharmacy [Hayward, CA], 4%-5% for induction and 2.5% for maintenance) mixed with oxygen. We place ophthalmic ointment (California Pet Pharmacy) on the animals' eyes to prevent corneal drying and trauma. The animals are positioned supine on an infrared heating pad (37°C; Kent Scientific, Torrington, CT) with the snout placed within flexible tubing, through which anesthetic is administered throughout the procedure (RWD Life Science, Shenzhen, China).

We place 2 pairs of 18-gauge needles (Becton, Dickinson, and Company, Franklin Lakes, NJ) adjacent to the bilateral axillae and chest wall to position the forelimb in abduction and to prevent movement of the

From the Department of Orthopaedics, University of Utah, Salt Lake City, Utah, U.S.A.

The authors report the following potential conflicts of interest or sources of funding: P.N.C. reports personal fees and nonfinancial support from the Journal of Shoulder and Elbow Surgery and DePuy, A Johnson e^{2} Johnson Company, outside the submitted work. R.Z.T. reports personal fees from Cayenne Medical, other from Conextions and INTRAFUSE, personal fees from the Journal of Bone and Joint Surgery – American, and other from KATOR, Mitek, Shoulder Innovations, Zimmer, and Wright, outside the submitted work; and editorial board, the Journal of Orthopaedic Trauma. Full ICMJE author disclosure forms are available for this article online, as supplementary material.



Fig 1. Positioning the mouse for rotator cuff repair surgery. Mice were anesthetized and positioned supine. The forelimbs were positioned in abduction using an 18-gauge needle (A). A-2 cm ventral incision was made to access to the rotator cuff (B).

mouse during the procedure. Next, the skin is prepared with betadine swabs 3 times followed by alcohol swabs (Medline, Northfield, IL) 3 times. The animal is draped with Press'n Seal (Glad, Oakland, CA), and a small window is made to access the surgical site. All scalpel blades and other surgical instruments are from Fine Science Tools, Vancouver Canada.

Surgery is performed under $3.5 \times$ loupe magnification. A 2-cm incision is made from the mid-clavicle to the deltoid tuberosity (Fig 1). Subcutaneous flaps are mobilized. The forelimb is next brought into extension, adduction, and external rotation (Fig 2), delivering the shoulder into the wound and placing the deltoid on tension.

The surgery is performed under $3.5 \times$ loupe magnification with the use of a headlight for illumination (Designs for Vision, Bohemia, NY). With the #11-blade scalpel, a small portion of the proximal deltoid is gently peeled off the acromion to expose the underlying supraspinatus tendon (Video 1). Care is taken while



Fig 2. Positioning the shoulder for access to the proximal humerus. The forelimb is extended, adducted, and externally rotated, while a probe is used to leverage the joint. The forelimb is also pulled down and externally rotated to dislocate the shoulder joint. (A). A #11-blade scalpel is used to elevate the deltoid (B).



Fig 3. Exposing the supraspinatus tendon. The supraspinatus tendon (indicated by the arrow) is exposed and elevated with a tissue probe. The arrowhead indicates the tendon enthesis (A). The diagram highlights the anatomic relationship of the greater tuberosity and the supraspinatus attachment (B).



Fig 4. Suture attachment to the supraspinatus tendon. With the tissue probe elevating the supraspinatus tendon, a 6-0 PROLENE double-armed suture with 9.0 taper-point needles was passed through the supraspinatus tendon medial to the suture hook in a modified Kessler suture pattern with the second needle completing the pattern (A). The diagram indicates the location of the suture placement and modified Kessler suture pattern (B).



Fig 5. Generation of bone tunnels in the humeral head. Bone tunnels were manually generated with a 27-gauge needle to allow for tendon fixation. The first tunnel starts at the anterior footprint and passes out of the lateral humerus posteroinferiorly at the level of the surgical neck. The second tunnel starts at the posterior footprint and passes out of the lateral humerus anteroinferior at the level of the surgical neck. Forceps are used to stabilize the humeral head.

peeling the deltoid off the acromion as the rotator cuff is directly under the deltoid. The cephalic vein runs along the anterior border of the deltoid consistently that marks the anterior extent of the dissection. This vessel needs to be avoided during the release of the deltoid. As the deltoid muscle is released, the supraspinatus can be identified as a shiny, white, thin structure attaching to the humerus that is no more than 2 to 3 mm in width (Fig 3). A tissue hook is then used to enter the plane between the supraspinatus tendon and the articular surface of the humeral head (Video 1). The forelimb often needs to be released of some extension and adduction during this portion of the procedure to allow the tissue hook to slide under the tendon. A 6-0 PRO-LENE (eSutures, Mokena, IL) double-armed suture with 9.0 taper-point needles is passed through the supraspinatus tendon medial to the suture hook in a modified Kessler suture pattern, with the second needle completing the pattern (Fig 4). The double-armed suture is critical, as it will allow each limb to be passed independently through individual bone tunnels in the proximal humerus.

While traction on the stitch is maintained, the tendon is then fully released from the humerus with an #11blade scalpel exposing the greater tuberosity. The footprint is then debrided gently using the scalpel. We create 2 crossing bone tunnels using a 27-gauge needle (0.01625-inch diameter) in a manual fashion (Fig 5, Video 1). The first tunnel starts at the anterior footprint and passes out of the lateral humerus posteroinferiorly at the level of the surgical neck. The second tunnel starts at the posterior footprint and passes out of the lateral humerus anteroinferior at the level of the surgical neck.

Next, one limb of the previously passed 6-0 PROLENE is passed through each tunnel independently (the anterior suture limb is passed through the tunnel starting at the anterior footprint and the posterior suture limb is passed through the tunnel starting at the posterior footprint) (Fig 6). The forelimb is then placed back into abduction similar to the starting position of the forelimb to allow reduction of the tendon to the footprint. The 2 suture limbs are then tied in a square knot with 4 throws (Fig 7). The deltoid is then allowed to lay back over the acromion and no direct repair of the deltoid is performed to reattach the deltoid to



Fig 6. Fixation of the tendon to the humeral head. The supraspinatus tendon is released from the humerus and reattached by passing the suture needles through the bone tunnels. A square knot is used to maintain adequate fixation of the tendon to the greater tuberosity.



Fig 7. Securing the repair with a square knot. After the suture needles are passed through the bone tunnels (as seen in Fig 6) the 2 suture limbs are tied in a square knot with 4 throws to hold the repair to the greater tuberosity.

the acromion. The skin is closed with the remnant of the 6-0 PROLENE sutures. The superior edge of the incision is then injected with 0.1 cc of 0.25% plain lidocaine and the animals were allowed to recover from anesthesia in a recovery cage with an infrared heating pad (37°C). Nonsteroidal anti-inflammatory drugs were not used due to their potential for interfering with rotator cuff healing. Animals were monitored daily for signs of pain and infection. Total procedure time from start to finish was approximately 12 minutes.

Discussion

Other animal rotator cuff repair models have been described including sheep, rabbit, and rat.³⁻⁵ A larger animal model, as opposed to the mouse, eases the ability for performing the repair and the testing of the repair. In contrast, mouse models provide researchers control of the genetic and pharmacologic environment while still allowing findings to translate to the human condition and are thus critical tools in scientific progress. We have successfully used the model described here to examine the effect of an estrogen-like compound on rotator cuff tendon healing.⁶ While there have been several previous studies of rotator cuff repair in mice, there are no previous detailed

Table 1. Key Points

- The murine rotator cuff is anatomically similar that of the human, allowing this animal model to provide translational knowledge relevant to rotator cuff repair in humans.
- Supine positioning, anterior approach, adduction/flexion/external rotation of the forelimb to expose the humerus, and elevation of the deltoid allow excellent exposure of the supraspinatus.
- Tendon fixation with a modified Kessler pattern and bone fixation with 2 crossed bone tunnels exiting at the level of the surgical neck laterally provides excellent initial repair strength.
- This approach avoids the need for difficult to obtain instrumentation or implants.

descriptions of surgical technique and our technique offers numerous advantages (Table 1).^{2,7} For instance, other studies discuss using a lateral decubitus position to perform rotator cuff repairs in mice.² The supine method presented here is simple and provides excellent exposure. In addition, our technique using a 6-0 suture is easy to perform under loupe magnification commonly used in hand or microsurgery.⁶ Other studies have used 7-0 PROLENE suture or a 7-0 polydioxanone suture that is smaller and more difficult to manipulate and potentially not as strong.^{2,7,8} Furthermore, previous studies have suggested a single tunnel transversely from posterior to anterior, which may not provide as good of footprint coverage as crossing tunnels which replicates the open bone tunnel repairs performed in humans.^{2,7-9} Finally, previous studies have not described how to create the tunnels. While our initial attempts were with a power drill, the small bone makes this method very unreliable as any wobble creates critical bone loss. Our technique avoids this problem by presenting a simple method to drill the tunnels by hand using a 27-guage needle

Table 2. Pearls and Pitfalls

For adequate exposure of the deltoid and supraspinatus, the forelimb needs to be brought into extension, adduction, and external rotation A right-angle probe or suture hook can be helpful to find the plane between the supraspinatus and the articular surface to allow the tendon to be released in a controlled fashion. During this maneuver, slightly reduce the extension and abduction to allow more space under the tendon for the plane here the supraspinatus and the first supraspinatus and the articular surface to allow the tendon to be released in a controlled fashion. During this maneuver, slightly reduce the extension and abduction to allow more space under the tendon for the back.	The mouse humerus is so small that attempts to create tunnels with a powered instrument can quickly lead to significant bone loss and loss of fixation. It is very easy to damage the humerus or the cuff if you are overly aggressive with the mouse limb and all manipulation of the limb must be gentle.
A double-armed suture is helpful to create a modified Kessler stitch and allow independent passage of the 2 limbs of the stitch through independent tunnels.	Because of the size of the supraspinatus and humerus in a mouse, biomechanical testing of the repair requires customized equipment

available in any clinical setting. The technique presented herein is a reproducible, robust, and simple technique for the repair of the supraspinatus in mice using materials readily available and hopefully assisting other researchers in performing these types of animal studies (Table 2).

References

- 1. Burns KA, Robbins L, LeMarr AR, Childress AL, Morton DJ, Wilson ML. Rotator cuff repair with knotless technique is quicker and more cost-effective than knotted technique. *Arthrosc Sports Med Rehabil* 2019;1:e123-e130.
- **2.** Bell R, Taub P, Cagle P, Flatow EL, Andarawis-Puri N. Development of a mouse model of supraspinatus tendon insertion site healing. *J Orthop Res* 2015;33:25-32.
- **3.** Gerber C, Meyer DC, Flück M, Valdivieso P, von Rechenberg B, Benn MC, et al. Muscle degeneration associated with rotator cuff tendon release and/or denervation in sheep. *Am J Sports Med* 2017;45:651-658.
- **4.** 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;80:97-103.

- Archambault JM, Jelinsky SA, Lake SP, Hill AA, Glaser DL, Soslowsky LJ. Rat supraspinatus tendon expresses cartilage markers with overuse. *J Orthop Res* 2007;25:617-624.
- 6. Tashjian RZ, Kazmers NH, Epperson RT, et al. The effect of estrogen-like compound on rotator cuff tendon healing in a murine model [published online February 2, 2021]. *J Orthop Res.* https://doi.org/10.1002/jor.25000.
- 7. Lebaschi AH, Deng XH, Camp CL, et al. Biomechanical, histologic, and molecular evaluation of tendon healing in a new murine model of rotator cuff repair. *Arthroscopy* 2018;34:1173-1183.
- **8.** Wang Z, Liu X, Davies MR, Horne D, Kim H, Feeley BT. A mouse model of delayed rotator cuff repair results in persistent muscle atrophy and fatty infiltration. *Am J Sports Med* 2018;46:2981-2989.
- **9.** Wang Z, Liu X, Jiang K, Kim H, Kajimura S, Feeley BT. Intramuscular brown fat activation decreases muscle atrophy and fatty infiltration and improves gait after delayed rotator cuff repair in mice. *Am J Sports Med* 2020;48:1590-1600.