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Subacromial bursal preservation can enhance rotator cuff tendon regeneration: a comparative rat supraspinatus tendon defect model study

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Background: The role of subacromial bursa in rotator cuff surgery is unknown. This study aimed to assess the subacromial bursa's role in the healing of supraspinatus tendon injury in a rat model.
Methods: Twenty-three male Sprague-Dawley rats (9 weeks old; weight, approximately 296 g) were used in this study. Three rats used as biomechanical study controls were killed at 12 weeks of age. A supraspinatus tendon defect was made bilaterally in 20 rats, whereas an additional subacromial bursa sectioning was performed on the left side. Six rats were killed for biomechanical testing and 4 were killed for histologic observation at 3 and 9 weeks, respectively.
Results: The regenerated tendon in the bursal preservation group showed significantly superior biomechanical properties in maximum load to failure at 3 and 9 weeks and stiffness at 9 weeks after surgery compared with the bursal removal group. The modified Bonar scale scores showed better regenerated supraspinatus tendons in the bursal preservation group.
Conclusion: The present study found that the subacromial bursa plays an important role in rotator cuff regeneration in this rat supraspinatus injury model. Extensive bursectomy of the subacromial bursa may not be recommended in rotator cuff repair surgery, though future in vivo human studies are needed to confirm these observations.
Level of evidence: Basic Science Study; Biomechanics and Histology

Keywords: Rotator cuff tear; subacromial bursa; tendon regeneration; rotator cuff healing; bone tendon interface; extensive bursectomy

Rotator cuff tear, one of the most common chronic tendon degenerative injuries, may cause pain and disability of the shoulder, especially in the elderly population, with an incidence of 22.1%.[21] The surgical treatment such as arthroscopic or open repair, which has been well established in the past 20 years, was recommended after the failure of conservative treatment. However, this issue remains very challenging for shoulder surgeons because of the high retear rate after repair surgery.[16,26] Some authors

The animal study was performed at Asan Medical Center and was approved by the Institution of Animal Experiments Ethics Committee of Asan Medical Center. Animal care in the study was conducted in accordance with the rules and regulations of Asan Medical Center.

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attribute the issue to the avascularity of the rotator cuff insertion, which may result in poor healing and a high retear rate.[7,18,37]

The subacromial bursa, located under the coracoacromial arch and deltoid muscle covering the muscle and tendon of the rotator cuff, is the largest bursa in the human body.[14,25] Some studies have proven that the main role of the subacromial bursa is to lubricate the space between the acromion and the rotator cuff to reduce friction and subacromial impingement.[4,5,14] However, some studies also strongly suggested that the subacromial bursa, including several kinds of neurologic components and free nerve endings, is one of the most important sources of shoulder pain after rotator cuff tears, which encourages surgeons to perform excessive bursectomy as treatment.[2,9,12] Additionally, arthroscopic rotator cuff surgery is the dominant approach for treatment of rotator cuff tear because of its minimal invasiveness and lower risk of infection. To enable a better arthroscopic view and larger manipulation space, extensive bursectomy is the preferred option before rotator cuff repair surgery.

The blood supply of the rotator cuff originates from the great tuberosity, muscle, and subacromial bursa.[37] Some anatomic studies even found that compared with the rotator cuff, the subacromial bursa appears well vascularized and its blood supply at the caudal part and rotator cuff tendons was linked to the same arteries, meaning that the subacromial bursa is a very important blood supply for rotator cuff tendon healing.[28] Dyrna et al[11] recently reported that human subacromial bursal cells displayed superior engraftment and survival in murine tendon tissue and could stimulate a more robust healing response in murine tendon repair than human bone marrow stem cells.

The role of the subacromial bursa in rotator cuff healing is unknown. Because the subacromial bursa can provide a blood supply and healing stimulation for rotator cuff healing, we hypothesized that rotator cuff healing is impaired in its absence. The purpose of this study was to test this hypothesis in a rat rotator cuff tear injury model.

Materials and methods

Experimental design and sample size calculation

Twenty-five male Sprague-Dawley rats (9 weeks old; weight, 296 g) were used in this study. According to previous data[13,17] and to generate a power of 0.8 with significance at the .05 level,[34] 6 rats were estimated to be required in this study for biomechanical test at each time point, and 4 rats were planned for histologic observation at each time point. Another 3 rats with 6 shoulders killed at 12 weeks of age were used as native controls in the biomechanical evaluation. The observation time points were set at 3 and 9 weeks. Considering the unexpected death of the rat, 1 more rat was included at each time point. Finally, a total of 25 rats were used.

Model setup

The bilateral shoulders of each rat were included in the experiment. Anesthesia was induced through the intramuscular injection of 50 mg/kg zolazepam and tiletamine (Zoletil 50; Virbac, Carros, France) and 10 mg/kg xylazine (Rompun; Bayer HealthCare, Leverkusen, Germany). A skin incision was made over the deltoid muscle, and the septum of the anterior and lateral parts of the muscle was split to expose the proximal part of humerus, which was clamped using a mosquito clamp. The acromioclavicular joint was dislocated and retracted to expose the subacromial bursa and the insertion of the supraspinatus tendon on the greater tuberosity. The supraspinatus tendon around 4 mm from the insertion to the muscle tendon junction was transected, and the bursa was preserved on the right side. The bursa tissue was moved to fill the defect area of the supraspinatus tendon on the right side as the bursa preservation (BP) group; on the other hand, the subacromial bursa was resected and the same tendon defect was created on the left side as the bursa removal (BR) group (Fig. 1). A mattress stitch was used to repair the acromioclavicular joint, and the muscle incision was closed with no. 4-0 Vicryl sutures. A no. 4-0 black silk suture was used to close the incision after the procedure. After the surgery, all animals were housed individually at 21°C under a 12-hour light-dark cycle. The animals were provided food and water ad libitum and allowed to move freely within their cages until being killed at 3 or 9 weeks postoperatively.

Biomechanical testing

Following the killing, the supraspinatus muscle and tendon of each shoulder along with the humeral head were harvested. The proximal two-thirds of the supraspinatus muscle was removed and the native muscle tendon junction with a small amount of muscle was preserved for fixation. The prepared samples were then preserved at –20°C and thawed at room temperature on the day of the biomechanical testing. Subsequently, the native muscle tendon junction was secured with running 5-0 sutures knot over the distal end of the muscle to prevent slippage and firmly clamped using a large needle holder. The tip of the needle holder just clamped the distal end of the muscle tendon junction, and no muscle was exposed. The humerus and needle holder were fixed using a custom fixture-clamping system (Instron, Norwood, MA, USA). Based on a similar previous study, the uniaxial testing condition was set using an Instron 3344 material testing machine.[8] The tendon was loaded until it pulled apart from the its midsubstance. Data from the tensile load-to-failure test were automatically collected using a data acquisition system on a personal computer.

Tendon histologic analysis

Following the killing, the humeral head–attached supraspinatus tendon with a small amount of muscle was fixed in 10% neutral buffered formalin for 24 hours and subsequently decalcified for 24 hours (Formical-2000; Decal Chemical Corporation, Tallman, NY, USA), processed, and embedded in paraffin.[27] Coronal sections (3 μm thick) of tendon-bone at the middle of the tendon insertion of each shoulder were placed on glass slides and stained using hematoxylin and eosin (HE) and immunohistochemical staining for type I and type III collagen according to routine protocols.[30]

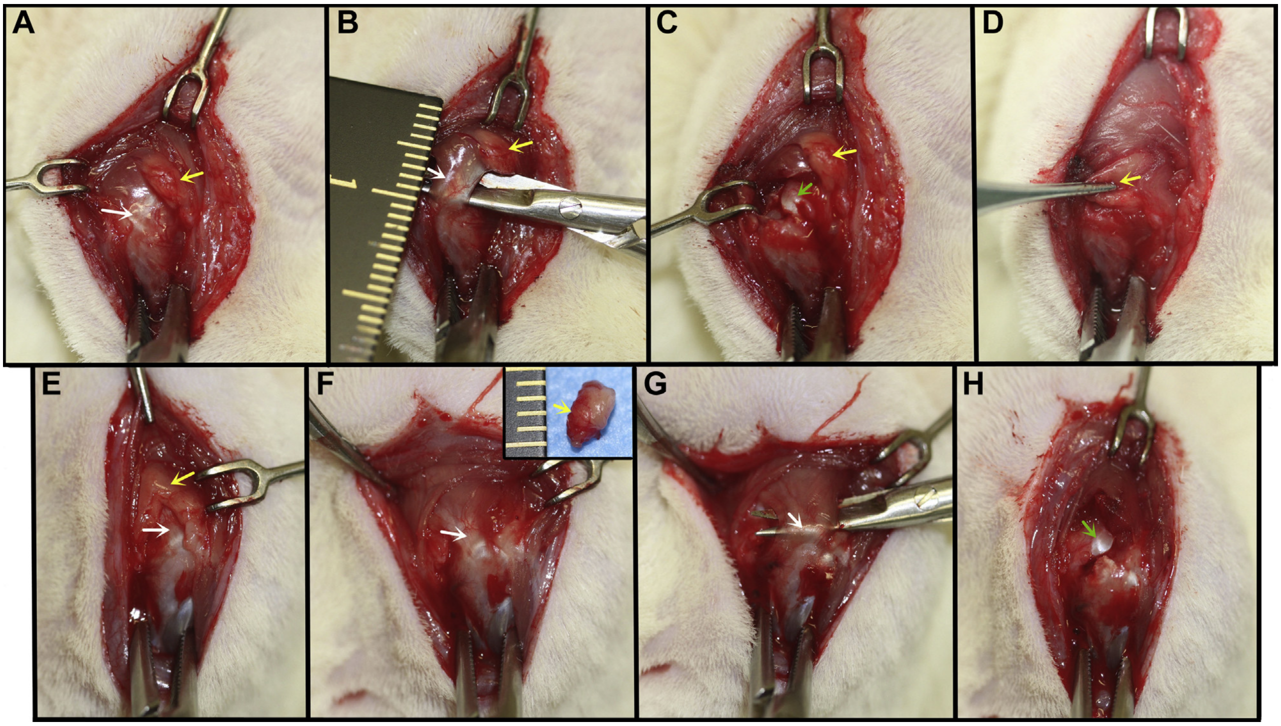

Figure 1 Surgical procedure in the bursal preservation and bursal removal groups. (**A-D**) Surgical procedure in the bursal preservation group. (**A**) Supraspinatus tendon insertion exposure. (**B**, **C**) A tendon defect was induced from the insertion to the muscle tendon junction at around 4 mm. (**D**) Bursa tissue was placed into the tendon defect to repair the acromioclavicular joint. (**E-H**) Surgical procedure in the bursal removal group. (**E**) Supraspinatus tendon insertion exposure. (**F**) Subacromial bursal removal. (**G**, **H**) A tendon defect was induced from the insertion to the muscle tendon junction around 4 mm. (*White arrow*, insertion of the supraspinatus; ←, subacromial bursa tissue; ←, long head of the biceps.)

HE staining was used for the pathologic analysis of the tendon regeneration as a whole. Anti–collagen I and anti–collagen III staining were used to analyze the different components in the regenerated tendon. All slides were assessed by 2 investigators (Y.C.S. and J.M.K.). Histologic images were obtained using an inverted microscope (Nikon TS100; Nikon, Amstelveen, Netherlands).

The modified Bonar score, which evaluates tenocytes, ground substance, collagen fibers, and vascularity, was used to evaluate each sample, whereas tenocytes were judged for spindle shape, increased roundness, increased size, and cytoplasm amount as described previously.[19,22,23]

Data analysis

The statistical analysis was performed using Student *t* test for intergroup comparisons of biomechanical properties. The significance level was set at $P \leq .05$.

Results

Gross observation

One rat in the 3-week group planned for histologic analysis died right after the dosage injection of anesthesia. Twenty-four rats survived for the postdeath analysis. After careful dissection, the humerus-supraspinatus junction was exposed. The regenerated tendon from the BP side usually showed thicker tendon regeneration (Fig. 2).

Biomechanical testing

The failure sites of the BR group were at the regenerated tendon at 3 and 9 weeks. All samples in BP groups failed at the regenerated tendon at 3 weeks. However, 3 samples ruptured at tendon insertion and 3 samples ruptured at regenerated tendon at 9 weeks in the BP group. The tendon from the BP side showed significantly better biomechanical properties in terms of maximum load at 3 (12.1 ± 4.7 N vs. 6.7 ± 1.3 N, $P = .049$) and 9 weeks (37.0 ± 5.5 N vs. 26.8 ± 3.9 N, $P = .017$) and stiffness at 9 weeks postoperation (18.1 ± 5.1 N/mm vs. 11.5 ± 2.3 N/mm, $P = .047$) (Fig. 3). No significant difference was found in terms of stiffness at 3 weeks (12.0 ± 5.1 N/mm vs. 7.5 ± 1.6 N/mm, $P = .107$) between the 2 groups.

Histologic analysis of regenerated supraspinatus tendon

HE staining revealed slightly delayed tendon healing on the BP side. Slight inflammation and disordered alignment of immature tenocytes was observed at 3 weeks; however,

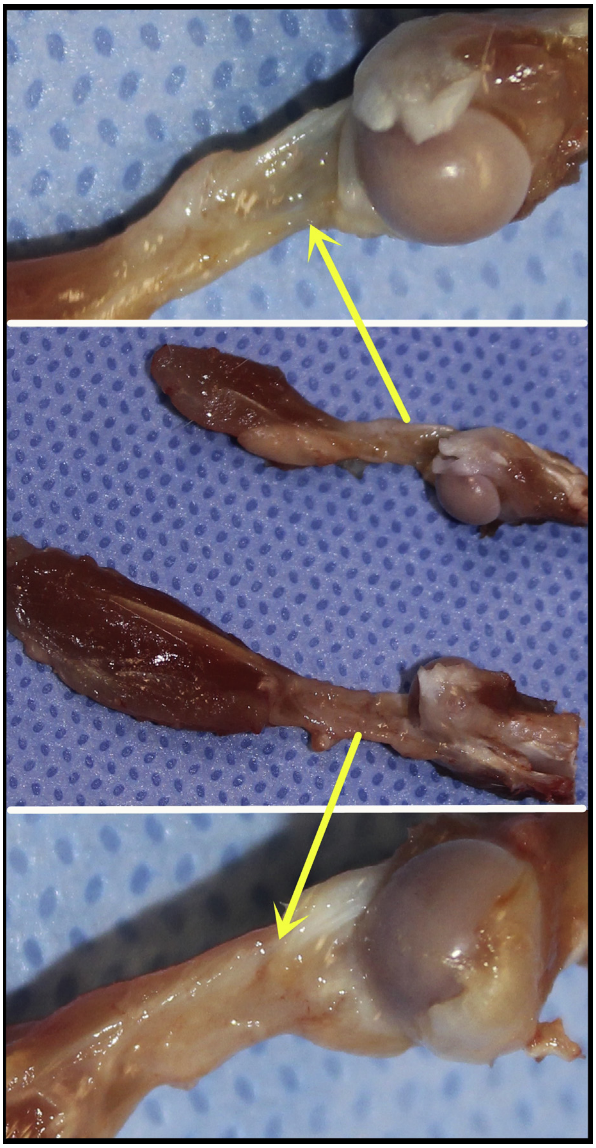

Figure 2 Gross observation of a paired sample showing supraspinatus tendon regeneration at 3 weeks after surgery. This upper shoulder sample shows inferior tendon regeneration on the bursal removal side. However, the low shoulder sample shows thicker tendon regeneration on the bursal preservation side (*yellow arrows show magnification*).

tenocytes were better aligned along the long axis of the tendon in the BP group. After 9 weeks, mature tenocytes were well organized like the native tendons in the BP group, which showed superior tendon quality compared with the BR side (Fig. 4).

The modified Bonar score grading tenocytes, ground substance, collagen fibers, and vascularity from normal (0) to markedly abnormal (3) showed comparable improvement in healing for both groups between 3 and 9 weeks (BP, 1.8 and 0.5, respectively; BR, 2.5 and 1.5, respectively) (Fig. 5).

Discussion

The key finding of the present study was that the subacromial bursa plays an important role in rotator cuff regeneration in this rat supraspinatus injury model. First, the regenerated tendon in the BP side showed significant superior biomechanical properties regarding maximum load to failure at 3 and 9 weeks and stiffness at 9 weeks after surgery compared with the BR side. Second, the modified Bonar scale shows better scores of regenerated supraspinatus tendon in the BP side. Therefore, this study suggested that surgeons try to preserve the subacromial bursa whenever possible in rotator cuff repair arthroscopically or via open surgery. Extensive bursectomy of subacromial bursa may not be recommended.

Some authors suggested that the subacromial bursa is the major cause of shoulder pain after rotator cuff tear. Soifer et al[32] and Tomita et al[35] also reported that the subacromial bursa was well innervated and demonstrated an extensive neural network, including free nerve endings and mechanoreceptors. Several other studies proposed that excessive inflammation and nerve-related growth factors, which have been proven to be associated with pain, were found in the subacromial bursa tissue in rotator tear cases.[6,15,24,31] This kind of result demonstrates that the nerve components in the subacromial bursa worked as pain afferent neural input in the proprioceptive reflex arcs and that inflammation of the subacromial bursa occurs in patients with rotator cuff tears; therefore, bursectomy during rotator cuff repair surgery may be an effective treatment for pain relief. However, some studies found that the inflammation and density of the neural elements of the subacromial bursa that are directly associated with pain-related reaction are also very important for tissue healing.[1,9,35] Matthews et al[20] found that small-sized tears have greater amounts of inflammatory reactions than massive rotator cuff tears, which are less likely to heal. Tomita et al[35] also found that the population density of neural elements of the subacromial bursa associated with massive cuff tears, which have lower healing potential and high retear rates after repair, was significantly lower than that observed in control shoulders. Based on the above 2 studies, we may find that the healing potential of the rotator cuff has a positive relationship with inflammation and density of the neural elements. In addition, in a randomized controlled study, Nam et al[25] found that patients did not benefit from extensive bursectomy in terms of pain relief compared with limited bursectomy during rotator cuff repair. Therefore, extensive bursectomy for pain relief also depresses the healing ability of the rotator cuff, especially in elderly people and in large to massively sized lesions.[9,10,36]

However, another group of authors have advocated that the bursa tissue actually plays a very important role in cuff healing because of the avascularity of tendon insertion of rotator cuff compared to subacromial bursa.[3,18,29] Most of

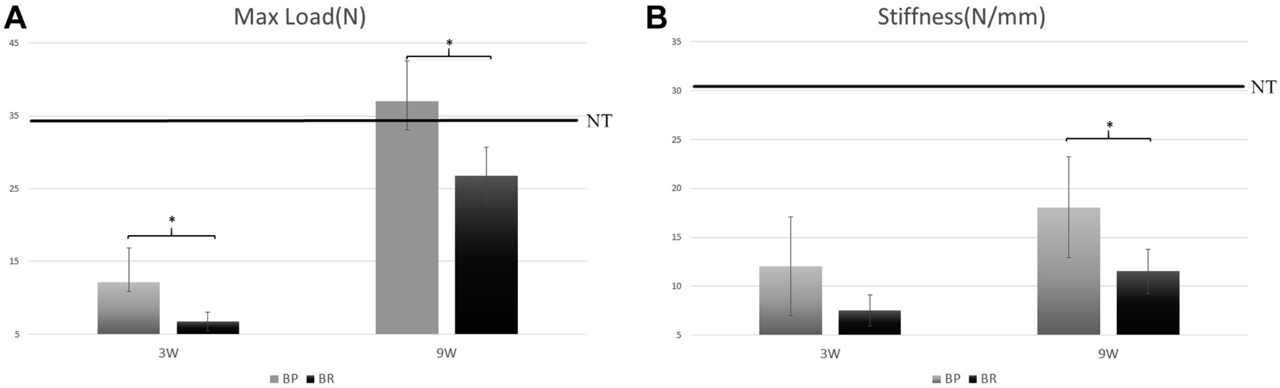

Figure 3 Biomechanical results. (**A**) Maximum load to failure. (**B**) Stiffness. *NT*, native tendon; *, significant difference.

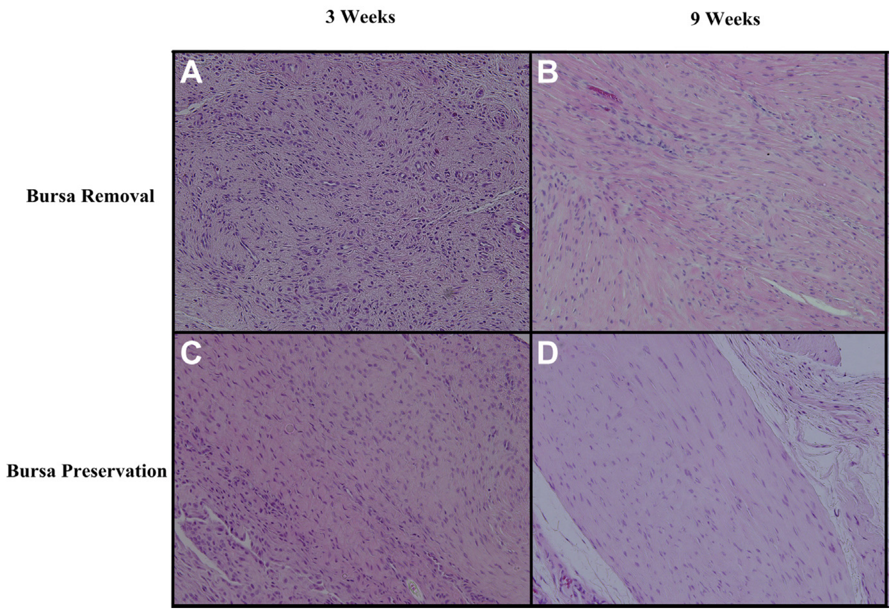

Figure 4 Representative hematoxylin and eosin staining of tendon regeneration in the 2 groups. (**A**) Representative tendon regeneration on the bursal removal side at 3 weeks. Immature tenocytes were in chaos arranged with some inflammation reaction. (**B**) Representative tendon regeneration on the bursal removal side at 9 weeks. Tenocytes become mature compared with their status at 3 weeks. Obvious collagen fibers are visible and aligned together with tenocytes in a row. (**C**) Representative tendon regeneration on the bursal preservation side at 3 weeks. Compared with tenocytes on the bursal removal side, tenocytes are slightly mature and were arranged slightly in a row. (**D**) Representative tendon regeneration on the bursal preservation side at 9 weeks. Spindle-like mature tenocytes are well organized in a row. Magnification, 100×.

them suggested that the blood supply from the subacromial bursa is the major blood supply used for rotator cuff healing.[9,28] On the other hand, some authors found that the cells from human bursa tissue have better engraftment and survival potential in tendon tissue healing than those of bone marrow stromal cells from the humerus.[11,33] Other authors even suggested that rotator cuff healing occurs mostly from the bursa side rather than the articular side.[10]

Arthroscopic shoulder surgery is becoming popular worldwide because of its lower invasiveness, lower infection rates, and early rehabilitation. An increasing number of surgeons are switching from open surgery to arthroscopic surgery, especially rotator cuff surgery. However, the view, cuff mobilization, and working space with arthroscope use are always disturbed by subacromial bursa tissue during rotator cuff repair surgery compared with open surgery. Together with the concept of bursectomy for pain relief from some surgeons and researchers, extensive bursectomy during rotator cuff surgery is gaining support. However, in this study, we found that the bursa tissue is

Figure 5 Modified Bonar scores of the 2 groups. Bonar scores for bursal preservation (BP) and bursal removal (BR) groups evaluate tenocytes, ground substances, collagen fibers, and vascularity, with 0 indicating normal, 1 indicating slightly abnormal, 2 indicating abnormal, and 3 indicating a markedly abnormal appearance.

helpful for cuff healing, and limited bursectomy for visualization and cuff mobilization and manipulation are recommended during arthroscopic rotator cuff repair.

To date, no study has provided direct evidence that the subacromial bursa plays an important role in rotator cuff healing. Some of the studies supporting the concept that rotator cuff healing benefits from the subacromial bursa only found indirect evidence using cadaveric and clinical studies.[5,9,10,28] In this controlled laboratory study, we provide sound histologic and biomechanical evidence that the bursa tissue is very important for rotator cuff regeneration by comparing BP and BR in cuff tendon defect scenarios.

Some limitations of the current study should be acknowledged. First, this rat model had its own limitations, especially the superior healing potential compared with humans. However, animal study has its own advantages. In this study, we could better evaluate the bursa tissue's role in rotator cuff tendon regeneration because the same condition and intervention were applied for the rats after surgery. However, in humans, rotator cuff healing is affected by a variety of factors of human life such as smoking, tear size, age, and diet. It is very difficult for authors to control so many such factors in a single study. Hence, we infer that one study did not demonstrate significantly different healing after rotator cuff repair between its extensive and limited bursectomy groups.[25] Second, a tendon defect model was used in the study rather than a rotator cuff tear model as in humans. Considering the higher self-healing potential after rotator cuff tears in rats compared with humans, a single tear healing may not demonstrate the differences because of the subacromial bursa. A similar strategy was used to evaluate the role of tissues adjacent to the tendon in Achilles tendon regeneration.[23] Third, functional evaluations were not included. Fourth, the results of the current study cannot be directly translated to clinical practice, but the findings may prompt more clinical studies on the topic.

Conclusion

The present study found that the subacromial bursa plays an important role in rotator cuff regeneration in a rat supraspinatus injury model. According to our findings, extensive bursectomy of the subacromial bursa may not be recommended in rotator cuff repair surgery, though future in vivo human studies are needed to confirm these observations.

Disclaimer

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