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The effect of autologous Achilles bursal tissue implants in tendon-to-bone healing of rotator cuff tears in rats

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Background: The aim of this study was to investigate the influence of autologous bursal tissue derived from the Achilles bursa on tendon-to-bone healing after rotator cuff tear repair in a rat model.

Methods: A total of 136 Sprague-Dawley rats were randomly assigned to either an untreated or a bursal tissue application group or biomechanical testing and histologic testing after rotator cuff repair. After separating the supraspinatus tendon close to the greater tuberosity, the tendon was reattached either unaltered or with a bursal tissue interposition sewn onto the interface. Immunohistologic analysis was performed 1 and 7 weeks after supraspinatus tendon reinsertion. Biomechanical testing of the tendon occurred 6 and 7 weeks after reinsertion.

Results: Immunohistologic results demonstrated a significantly higher percentage of Type II collagen ($P = .04$) after 1 and 7 weeks in the tendon-to-bone interface using autologous bursal tissue in comparison to control specimens. The bursa group showed a significantly higher collagen I to III quotient ($P = .03$) at 1 week after surgery in comparison to the 7-week postsurgery bursa groups and controls. Biomechanical assessment showed that overall tendon stiffness ($P = .002$) and the tendon viscoelasticity in the bursa group ($P = .003$) was significantly improved after 6 and 7 weeks. There was no significant difference ($P = .55$) in force to failure between the bursa group and the control group after 6 and 7 weeks.

Conclusion: Autologous bursal tissue derived from the Achilles bursa and implanted to the tendon-to-bone interface after rotator cuff repair facilitates a faster healing response to re-establish the biologic and biomechanical integrity of the rotator cuff in rats.

Level of evidence: Basic Science Study; In Vivo Animal Model; Histology and Biomechanics

Keywords: Bursal tissue; tendon-to-bone healing; rotator cuff tear; immunohistology; biomechanical testing

The risk of rotator cuff tears and of rerupture after rotator cuff repair increases with age.[2,3] In recent years, many new arthroscopic methods for a robust reconstruction of rotator cuff tears using bone anchors and specific suture techniques have been introduced and established. These include double-row and transosseous sutures. Over a longer period of time, research has focused mainly on the biomechanical aspects of tendon-to-bone refixation,[3,9] and

Animal testing was approved by the Institutional Animal Care and Use Committee of the District Government of Upper Bavaria, and all testing regulations and guidelines were fulfilled (registration no. 55.2-1-54-1532.0-45-15).

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good short-term outcomes regarding pain reduction and functionality have been demonstrated.[2,24] However, medium- and long-term outcomes have shown a high percentage of recurrent defects. The main complications after arthroscopic repair are reruptures with occurrence of up to 50%,[12,16] although the load to failure in modern refixation techniques (500-600 N) has been shown to be significantly higher than the physiological load to failure of the native supraspinatus tendon (300 N).[4,26,38] Increased maximum failure loads with stronger anchoring techniques did not reduce the long-term rerupture rate.[11,22] Recent research has been increasingly focused on the biologic aspects of tendon-to-bone healing, improvement of the tendon quality, and rotator cuff muscle regeneration.[13,27,29-31] Mesenchymal proliferator cells, stem cells,[21] various growth factors, and translational molecules such as mitogen-activated protein kinase have been identified in the human subacromial bursa. These cells may positively influence the healing of repaired rotator cuff lesions and reduce the rerupture rate.[35] A previous study using the same conditions showed migration of mesenchymal stem cells (MSCs) originating in the Achilles bursa (also described as retrocalcaneal) into tendon-to-bone interface at 1, 3, and 6 weeks after attaching a bursa interposition onto the tendon-to-bone interface in rats.[28] This method relies on the influence of regeneration stimulation by growth factors as well as pluripotent stem cells and their ability to differentiate concerning the biologic aspects of tendon-to-bone healing.

Study purpose

The purpose of this study was to histologically and biomechanically investigate the positive healing effect of an autologous bursa patch at the tendon-to-bone interface after supraspinatus repair in an in vivo rat model. We hypothesized that the use of a bursa patch on the tendon-to-bone interface would lead to a facilitated healing and biomechanically more stable reconstruction. We aimed to investigate the immunohistologic difference of tendon-to-bone healing in specimens at 1 and 7 weeks after surgery and the biomechanical difference between a more sensitive 6-week-postsurgery model compared with the previously used 7-week postsurgery model.[7,8] The null hypothesis was that there was no significantly measurable difference between the control group and the bursal group at different stages of healing. Biomechanical testing was performed to detect if a 6-week healing period was sufficient in terms of load to failure in comparison to a healing period of 7 weeks. The primary objective of the biomechanical analysis was to determine the load needed for failure of the tendon or the tendon-to-bone area. Additionally, biomechanical parameters such as tendon stiffness and quantitative viscoelasticity were calculated. Because the subacromial bursa in the rat could not be detected macroscopically, we opted to use the Achilles bursa. The presence of MSCs and growth factors in bursal tissue have been confirmed by previous analyses as a preparative study to our study purpose.[28] Histologically, we wanted to investigate whether MSCs derived from the Achilles bursa can be used in an interposition form to improve rotator cuff healing at the tendon-to-bone interface in a rat model.

Material and methods

Study design

This is an experimental study comparing the positive healing effect of an autologous bursa patch at the tendon-to-bone interface after supraspinatus repair histologically and biomechanically in an in vivo rat model. A total of 136 female Sprague-Dawley rats (Charles River Laboratories, Sulzfeld, Germany) were used, as female rats are less aggressive when held in shared cages compared with males. The number of animals needed for this study was determined through a power analysis estimation by the Institute for Medical Information Processing, Biometry, and Epidemiology of our University.

Animal husbandry was conducted according to specific hygiene conditions (expanded FELASA criteria,[23] in type IV R cages). Food and water access was ad libitum. Tests were conducted at least 7 days after arrival of the animals to allow for regeneration from transportation and accommodation to the new environment. The rats were sacrificed for biomechanical analysis of the tendon-to-bone interface after 6 and 7 weeks of healing time to compare a shorter healing time of 6 weeks to the previously analyzed 7-week healing period.[7,8] Histologic analysis was performed on samples collected after 1 and 7 weeks (Fig. 1).

Surgical procedure

Animals were anesthetized with ketamine hydrochloride (5-15 mg/kg) (Pfizer Inc., Berlin, Germany) and general anesthesia maintained by isoflurane (1 mL/mL) (CP Pharma, Burgdorf, Germany). All surgical procedures were performed under sterile conditions and in a lateral position. The lower right paw of the animals receiving the autologous Achilles bursa tissue was shaved and a 0.5-cm-long incision of the skin and subdermal tissue was carried out. The right Achilles tendon was exposed and the Achilles bursa dissected. The wound was then sutured closed. A 1-cm-long skin incision was made cranially to the glenohumeral joint in both the bursa and control groups, and the deltoid muscle was split along the fiber lines. The clavicle was divided in the proximity of the acromioclavicular joint for better access to the supraspinatus tendon. Once the tendon had been exposed, the distal end was detached from the greater tuberosity using a scalpel. A Mason-Allen suture was prepared (Ethicon Prolene, Norderstedt, Germany). For transosseous refixation, 2 channels of 0.5 mm width were drilled into the greater tuberosity (Micromot 50/E; Proxxon, Wecker, Luxembourg). In the bursa group, the extracted bursal tissue was strung onto the suture attached to the supraspinatus tendon and both suture legs were pulled through the drill channels. By tying both ends of the suture, the bursal interposition glided between the end of the supraspinatus tendon and

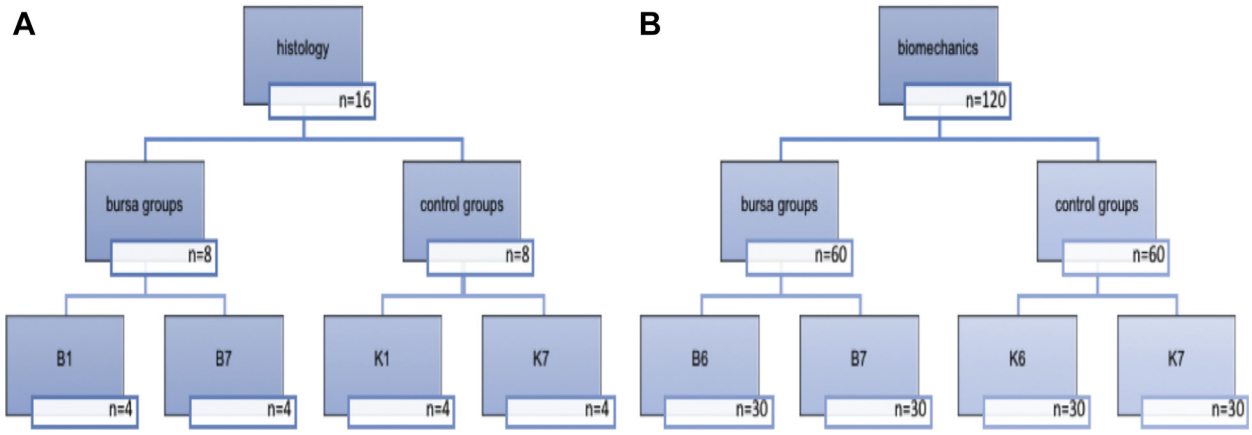

Figure 1 (**A**) Histologic and (**B**) biomechanical experimental setup. Animals were assigned to groups by randomized pattern. For histologic assessment (**A**), the control group consisted of an SSP suture without a bursal interposition (K) with a 1-week healing period (K1) or a 7-week healing period (K7), and the bursa group consisted of an SSP suture with a bursal interposition (**B**) with a 1-week healing period (B1) or a 7-week healing period (B7). For the biomechanical setup (B), the control group 1 consisted of an SSP suture without a bursal interposition (K) with a 6-week healing period (K6) and a 7-week healing period (K7), and the bursa group consisted of an SSP suture with a bursal interposition (B) with a 6-week healing period (B6) and a 7-week healing period (B7). *SSP*, supraspinatus.

the prepared anatomic footprint on the greater tuberosity. The suture legs were then tightly bound. The deltoid muscle and the skin were then closed, using muscle and skin single-stitch sutures. In the control group, surgery was performed in an identical manner except that bursal tissue was not interpositioned at the tendon-to-bone interface. After surgery, the animals were separated from those who had not undergone surgery to receive postoperative care and to allow for a proper recovery. They were placed in a heated box until they achieved full consciousness and mobility. The animals received a single shot of 0.5 mg/kg enrofloxacin (Baytril; Bayer AG, Leverkusen, Germany) as a prophylactic antibiosis.

Histology and immunohistomorphometry

The 16 animals allocated to undergo histologic and immunohistomorphometric analysis (Fig. 1) were euthanized by a lethal overdose of isoflurane at weeks 1 and 7. The right shoulder joints were harvested and fixed in 4% neutral-buffered formalin (Merck, Darmstadt, Germany) for 48 hours. Afterward, the shoulders were decalcified in ethylenediaminetetraacetic acid tetrasodium (EDTA-4Na) 20% citric acid (Merck) for 14 days. Then the specimens were processed in multiple ascending grades of ethanol in an automatic tissue processor into paraffin (Hypercenter XP, Thermo Scientific Fisher). From each specimen, 20 slices were cut sequentially at 3 µm, in the coronal plane. The 10th section of each specimen was stained with hematoxylin-eosin, with the adjacent sections being immunohistomorphometrically assessed for collagen type I (Novus Biologicals, Nordenstadt, Germany), collagen type II (CIIC2, Development Studies Hybrid), and collagen type III (Novus Biologicals) using image processing and analysis (Image J Java 1.8.0_172; National Institute of Health, Bethesda, MD, USA). Images of histologic and immunohistochemically stained sections were captured and digitized using a light microscope (Axioskop 40; Zeiss, Jena, Germany) interfaced to a video camera (Axio Cam MRc5; Zeiss). Images and/or slides of each of the histologic specimens were evaluated by the authors (A.Z., A.F., R.K.) using the Bonar score for histopathology of tendinopathy. The Bonar score describes 4 stages of degeneration in tendinopathy by the parameters of increasing collagen disorganization, mucoid ground substance, prominence of vascular spaces with or without neovascularization, and focal necrosis or calcification.[17] Each investigator was blinded with respect to specimen groups. The vascularity of the tendon-to-bone interface, fibrocartilage at the tendon-to-bone interface, organization of collagen tissue, inflammation, and collagen fiber continuity between tendon and bone tissue were also evaluated.

Biomechanical testing

The aim of the biomechanical testing was to analyze parameters such as the viscoelasticity, tendon stiffness, and load to failure. The following experimental setting was based on the setup protocols described by Galatz et al[10] and Ficklscherer et al.[7,8] To avoid drying out, the samples remained wrapped in damp gauzes with 0.9% saline solution. All measurements were done using a tensile testing machine (Z010/TN2A; Zwick/Roell, Ulm, Germany). For detection of the strength applied, a transducer (Z6FD1; Zwick/Roell) within a measuring range of 0.4 and 100 N was used as a sensor. The samples were embedded in aluminum cylinders that were sealed at the bottom and filled with a combination of RenCast (OBO Werke GmbH, Stadthagen, Germany) FC 52/53 isocyanate and RenCast FC 53 polyol, which produces polyurethane with a mass ratio of 1:1. The humerus bone of each sample shoulder was put vertically into the polyurethane up to the surgical column. After hardening of the polyurethane, the muscle belly of the supraspinatus muscle was stripped from the tendon. The tendon was fixed near the insertion site into the clamps of the transducer. The cylinder and transducer were attached at a perpendicular angle. Traction to the tendon was simulated to physiological conditions as closely as possible, in a 90° angle to the vertical axis of the humerus bone. Management of the tensile testing machine was logged according to the testing requirements into the software testXpert V12.1 (Zwick/ Roell). To define the same initial tension value for all samples, a preload of 0.2 N with

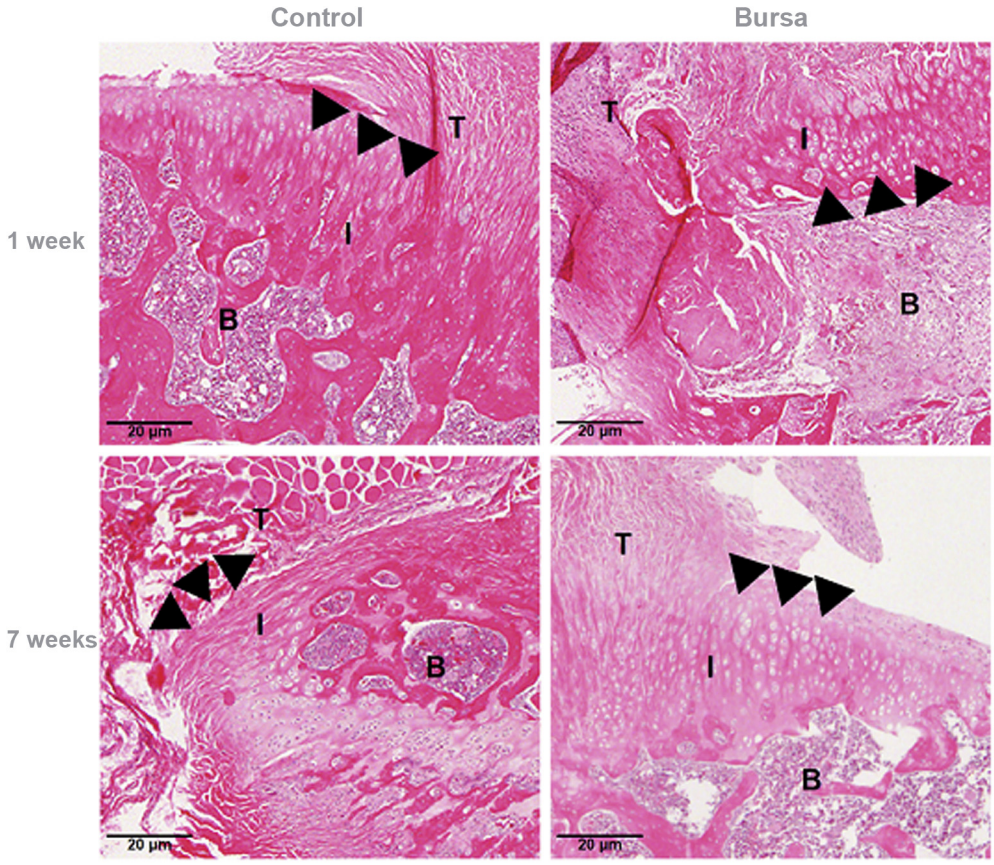

Figure 2 Hematoxylin-eosin staining of rat shoulders shows less cellularity and vascularization and more interface organization tissue bursa groups after 1 and 7 weeks. The areas of interest in the interface tissue regions are marked with ▶. *T*, tendon; *I*, tendon-to bone interface; *B*, bone.

0.1 mm/s was applied for every measurement. Tendon viscoelasticity (Nmm) was calculated with the area of the increasing and decreasing value points of the hysteresis loop using integral calculus. The calculated value of viscoelasticity is contrary to the value of elasticity. To determine load-to-failure (N), it was preloaded with 0.2 N and with speed at 0.1 mm/s. Subsequently, the sample was strained with an incrementally increasing speed of 0.1 mm/s until failure of the tendon suture or the tendon-to-bone interface.

Statistical analysis

Statistical analysis for biomechanical testing was done using Mann-Whitney *U* test (Graph Pad Prism 7.03 for Windows; Graph Pad Software, San Diego, CA, USA). Statistical analysis for histomorphometry was performed using 1-way analysis of variance with univariate General Linear Model (Graph Pad Prism 7.03). The confidence interval was defined at 95% and the significance level at $P < .05$.

Results

A total of 136 animals were used in the study. Of these, 120 specimens were allocated for biomechanical testing, and 16 specimens were used for histologic analysis using the Bonar score for assessing histopathology of tendinopathy. The immunohistomorphometric analysis of the Bonar score rates the specimens by the parameters tenocytes, ground substance, collagen, and vascularity.

All 136 animals survived the surgical implantations, with no deaths occurring postoperatively. An average body mass gain of 15% was observed across all experimental and control groups. HE staining showed less cellularity and vascularization and more interface organization tissue in bursa groups using the Bonar score for histopathology of tendinopathy (Fig. 2).

Immunohistomorphometry

Collagen II expression

The mean area of collagen II expression in the interface region was significantly higher ($P = .04$) using the bursa interposition (0.3 ± 0.1 mm^2) after 1 week in comparison to the control group (0.2 mm^2) (Fig. 3). Similarly, significant results ($P = .04$) could be found in the bursa interposition group (0.3 ± 0.1 mm^2) after 7 weeks compared with the control group (0.2 ± 0.1 mm^2).

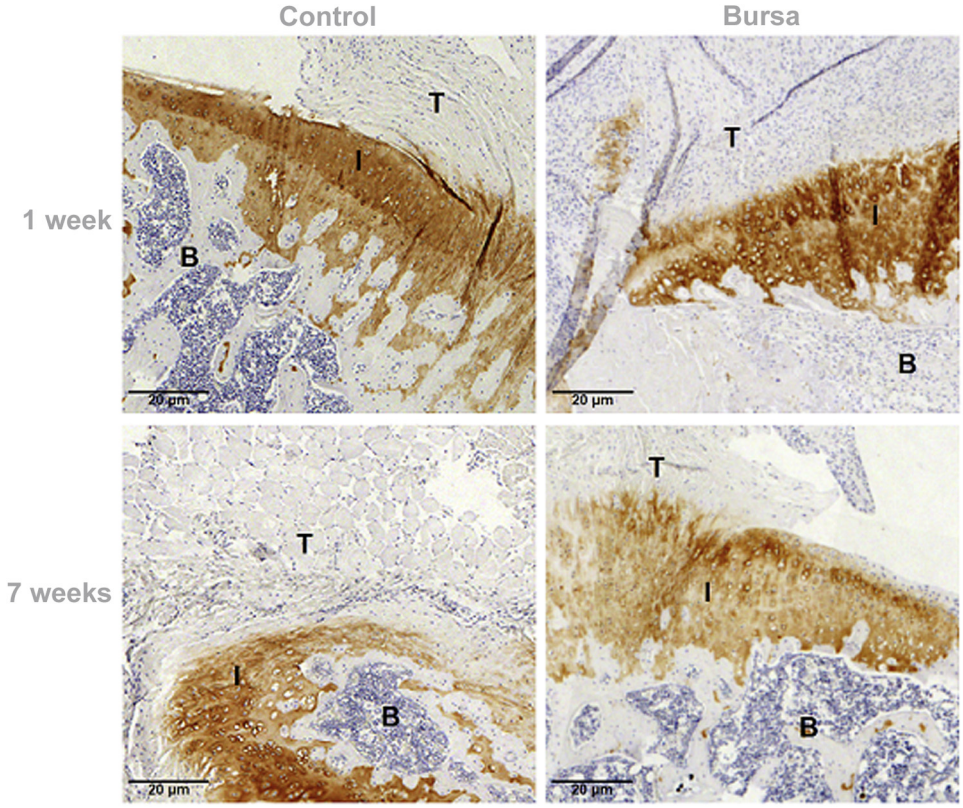

Figure 3 Collagen II immunohistologic staining of rat shoulders shows less cellularity and vascularization and more interface organization tissue bursa groups after 1 and 7 weeks. The mean area of collagen II expression in the interface region was significantly higher ($P = .04$) in the bursa interposition group after 1 week in comparison to the control group. Similarly, significant results ($P = .04$) could be found in the bursa interposition group after 7 weeks compared with the control group. *T*, tendon; *I*, tendon-to bone interface; *B*, bone.

Collagen I and III quotient

Overall, collagen I and III staining showed significantly higher presence of collagen I to III quotient ($P = .03$) in the bursa interposition group at 1 week after surgery (5.1 ± 1.4), (Fig. 4). There was a higher presence of collagen I in the first week after surgery, whereas collagen III was present in significantly greater proportion 7 weeks after inserting the bursa interposition in all groups.

Biomechanical testing

Viscoelasticity

The evaluation of viscoelasticity (Nmm) demonstrated significant differences in the investigated groups. Lower values of viscoelasticity were shown in the bursa group than in the control group after 6 weeks. Therefore, greater elastic properties ($P = .002$) could be demonstrated in the bursa group. The difference was highly significant comparing the 7-week measurements of the bursa group vs. those of the control group. In analogy to the previous paired comparisons, significantly higher elasticity was demonstrated in the bursa groups ($P = .003$) (Fig. 5).

Tendon stiffness

A comparison of the bursa group 6 weeks after surgery to the control group detected a significantly increased stiffness in the bursa group ($P = .02$). No significant difference was detected in the comparison of the 7-week bursa group vs. the 7-week control group. Within the bursa group, the stiffness was almost identical after 6 and 7 weeks (Fig. 6).

Force at failure

Ninety specimens were used for biomechanical testing. Eleven specimens failed as a result of humeral head fractures during testing, and in 79 cases, the specimens failed at the tendon at the tendon-to bone interface. When evaluating the tensile strength, comparable yield values were found in all groups. We found no significant differences in maximum failure load (N) between the experimental groups (Fig. 7).

Discussion

In recent years, the biomechanical quality of modern anchorage and suture techniques for the surgical care of rotator

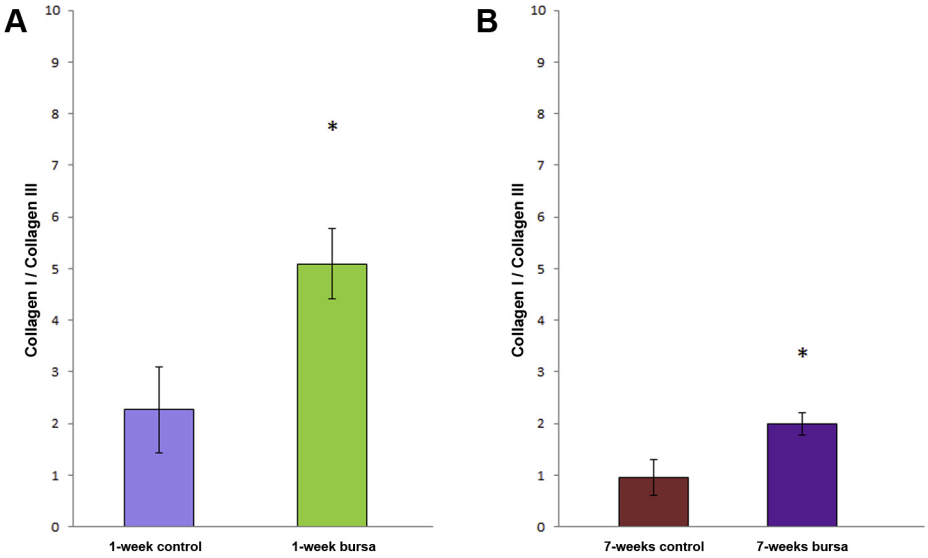

Figure 4 Immunohistomorphometric assessment of collagen type I and III formation. (**A**) 1-week control and bursa groups; (**B**) 7-week control and bursa groups. The *x*-axis shows the surgery groups whereas the *y*-axis demonstrates the collagen I to III quotient. The bursa interposition group 1 week after surgery showed a significantly higher presence of collagen I to III quotient ($P = .03$, *green bar* with *) in comparison to 1 week (*blue bar*). The bursa interposition group 7 weeks after surgery (*brown bar*) showed a significantly higher presence of collagen I to III quotient compared with postoperative controls and 7 weeks postsurgery (*purple bar*).

cuff tears has been increasingly improved and optimized. However, the problem of high rerupture rate after surgical tendon refixation is still unresolved, and the biology of tendon healing is increasingly becoming the focus of research.[37] In previous studies, it could be shown that the restoration of the complex visual tendon-to-bone insertion (enthesis) is not successful and that the biomechanical properties of the scar tissue are insufficient in comparison to the original tendon to bone complex.[1] Many studies have investigated the influence of growth factors, cytokines, platelet concentrates, and pharmaceutical therapeutics on the modulation and support of tendon-to-bone healing.[1,14] The investigation of platelet concentrates (platelet-rich plasma) and the growth factors contained therein (including transforming growth factor beta 1-3, vascular endothelial growth factor, platelet-derived growth factor, insulinlike growth factor 1c, immunoglobulin A 1a, bone morphogenetic protein 12, epidermal growth factor) has been in particular focus with regard to clinical application. Significantly positive results have been achieved in vitro in terms of biomechanical and histologic tendon quality. However, as of this writing, no reliable proof of efficacy could be provided for clinical application.[18] Additionally, there is considerable uncertainty concerning the application process, the timing, and the correct dosage of the more than 1500 cytokines involved in the healing process.[37]

We therefore developed the main hypothesis that the stem cells and growth factors of bursa tissue used as an interposition can lead to a higher quality of biomechanical healing of the tendon compared with the suturing alone. We confirmed that a reduced healing time of 6 weeks is biomechanically sufficient for tendon healing at the tendon-to-bone interface when a bursal interposition is used. We were also able to demonstrate that bursal tissue containing MSCs might improve rotator cuff healing. MSCs from an autologous Achilles bursa interposition increases connective tissue, that is, collagen II production and a higher collagen I to III quotient, at the tendon-to-bone interface in a rat model. Only a few studies currently have shown the presence of MSCs in bursal tissue.[14,19,32] No study has yet demonstrated signs of better histologic tissue healing in rotator cuff tears in an animal model with the use of this tissue. Even though the presence of bursal stem cells was investigated by Utsunomiya in 2013,[36] the clinically relevant question remains of whether a partial and sparing bursectomy can be recommended rather than subtotal bursectomy during arthroscopic surgeries. In their in vivo study on the differentiation potential of MSCs from the subacromial bursa, Song et al[32] were able to demonstrate that the growth factor bone morphogenetic protein–12 can support MSCs in their differentiation into tenocytes and thus the formation of tendon tissue. It is possible that one of the induced mediators in combination with a longer healing time is responsible for our results; it must be considered whether there is a role of inflammatory factors in healing of tendon-to-bone. Further immunohistologic analysis in the context of follow-up studies could contribute to a better understanding. Currently, there is no study that has investigated the influence of MSCs on the viscoelasticity that could be used for comparison. In the bursa and control groups, we could not find any tendency or significant difference between a 6- and 7-week healing period.

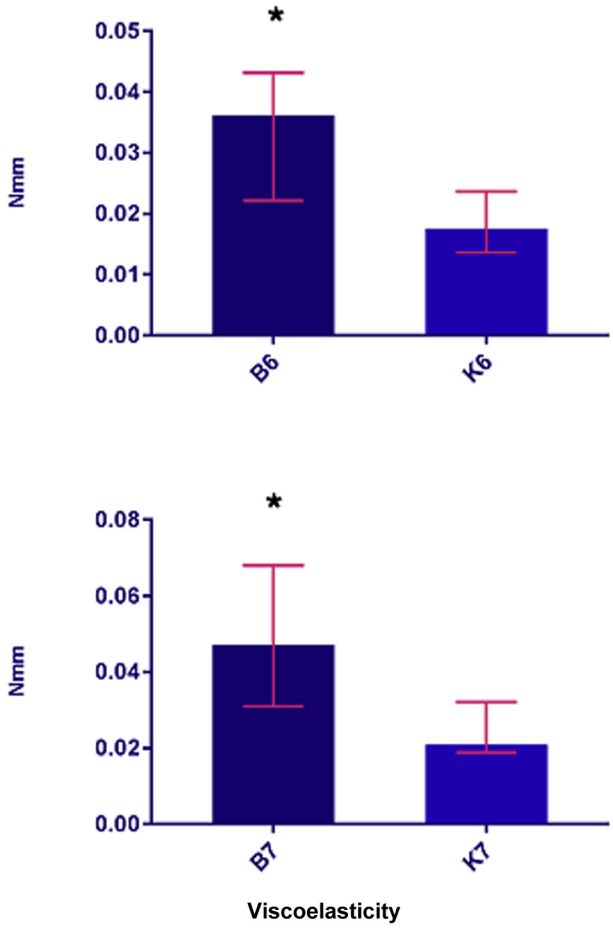

Figure 5 Biomechanical viscoelasticity measured in newton-millimeters in controls after 6 (K6) and 7 (K7) weeks vs. the bursa group after 6 (B6) and 7 (B7) weeks postsurgery. Significant results were noted at $P < .05$ (shown with *) in the bursa groups after 6 and 7 weeks (B6 and B7).

In biomechanical aspects, based on the experiments of Galatz et al,[10,11] we have developed a standardized test procedure for measuring the tensile strength and viscoelastic behavior of the supraspinatus tendon in a rat model. The control groups after 6 and 7 weeks' healing time vs. the experimental groups after 6 and 7 weeks demonstrated a significantly better elasticity. From a different angle, the loss of potential energy may help protect the tendon-bone suture. This would be a desirable condition in the immediate postoperative setting but is disadvantageous in the medium and long term. The comparison of viscoelasticity between the sixth and seventh postoperative week showed inconsistent values in this respect.

In terms of stiffness, we were able to confirm our hypotheses. The bursa group showed significantly higher results compared with the control group. We interpret higher tendon stiffness as a positive result, albeit with limitations. Stiff tendons provide tensile forces with greater resistance to deformation and transfer muscle strength to the bone with little energy loss. However, the tendon is less extensible, and the possibility of length variation is limited. Muscle force impulses are thus transmitted to the bone with less attenuation, and high strain of the tendon suture is possible after surgical treatment of a rotator cuff tear. Degen et al[5] investigated the effect of MSCs from bone marrow on rotator cuff tendon healing in their work. In 26 animals of their experimental group, the supraspinatus tendon was released and refixed. MSCs dissolved in fibrin glue were then applied to the tendon-bone interface. In the control group, the fibrin glue was applied without MSCs. Biomechanical measurements were taken 4 weeks after surgery. In contrast to our results, Degen et al found no significant difference between their experimental and control groups. Only the comparison with intact tendons showed significantly lower results in the experimental group. This was also noted by Ficklscherer et al,[7,8] who in their rat models showed 10 times higher stiffness values in shoulders without surgery in comparison to shoulders after rotator cuff repair. Notwithstanding limitations of viscoelasticity inconsistency, we were able to demonstrate that the interposition of bursa tissue showed a higher quality of biomechanical healing as well as histologic tissue stability in a rotator cuff tear rather than suturing alone.

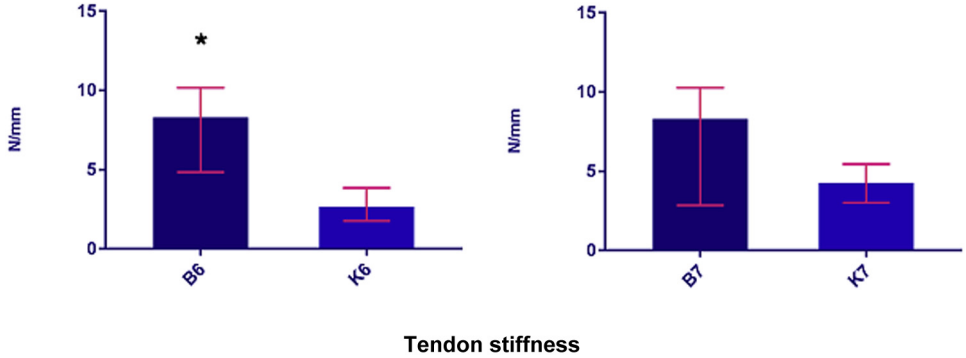

Figure 6 Tendon stiffness measured in newton-millimeters in controls after 6 (K6) and 7 (K7) weeks vs. the bursa group after 6 (B6) and 7 (B7) weeks postsurgery. Significant results were noted at $P < .05$ (shown with *) in the bursa groups after 6 weeks (B6).

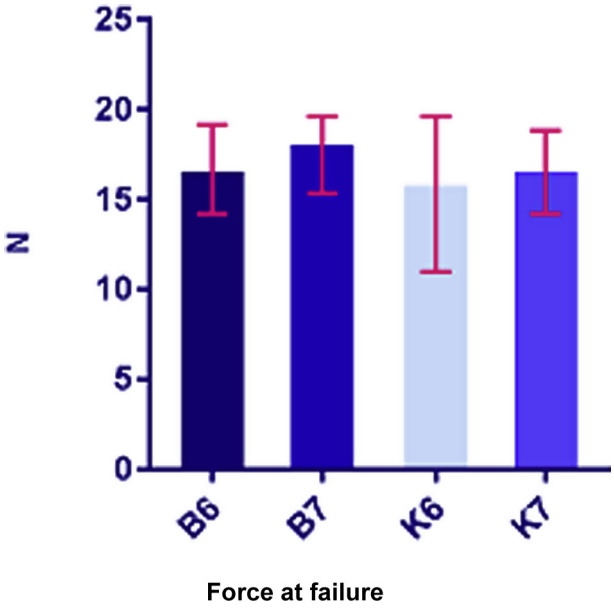

Figure 7 Overview of force at failure results (N) of all groups. The bursa group 6 weeks (B6) and 7 (B7) weeks postsurgery and the control groups 6 (K6) and 7 (K7) weeks postsurgery are shown in a tabular overview.

Limitations

Among the small animals, the rat is most similar to human anatomy and biomechanics (including the anatomy of the acromial arch and the compression of the supraspinatus tendon during arm elevation and overhead activities).[33] The cost-effectiveness of the rat model is better in comparison to a large animal model. The tendon defects created by us correspond more to the pathologic mechanism of an acute rupture and not to the degenerative lesions usually found in humans. The refixation was carried out in a single operation. Tendon retraction and degeneration as well as muscle belly fattening, which are treatment-limiting factors in humans, were therefore excluded. This restriction may have been compensated for by Thangarajah et al,[34] who used a 2-stage surgical procedure. In a first operation, the supraspinatus tendon was detached from the bone and marked with a Prolene thread. In a second surgery after 3 weeks, the tendon was refixed to the footprint of the bone. Furthermore, the self-healing power of rats are far superior to those of humans, so tendon tears heal much faster. Thus, reruptures do not occur that often.[6] The rat tendons were stored at –20°C between collection and biomechanical testing. Studies in 2017 by Oswald et al[25] and Lee et al[20] conclude that freezing at –20°C has no effect on the biomechanical and structural properties of tendons, and this is therefore currently recommended as a method of storage choice. Hirpara et al[15] investigated the effects of cryopreservation of varying duration (24 hours, 3 months, and 6 months) on the stability of tendon sutures and found no significant differences. We also found no significant differences between our groups. Despite these limitations, the rat is seen as an adequate model for the study of repair mechanisms and new treatment approaches in rotator cuff pathologies.[6,18]

Conclusion

This study demonstrates the influence of bursal tissue interposition during rotator cuff surgery to the tendon-to-bone interface and histologically and biomechanically better tendon-to-bone healing. Stem cells and growth factors from bursal tissue might aid in faster healing processes of tendon-cartilage-bone interfaces in rat models and may be an indication for applying bursal tissue or bursal cells by injection in clinical use. Future recommendations may include using the already present bursa as an interposition into the tendon-to bone interface during arthroscopic or minimal-open surgeries and development of intraoperative bursal MSC extraction kits with processing and application of bursal stem cells as in autologous conditioned or platelet-rich plasma kits.

Disclaimer

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References

1. Berebichez-Fridman R, Gomez-Garcia R, Granados-Montiel J, Berebichez-Fastlicht E, Olivos-Meza A, Granados J, et al. The holy grail of orthopedic surgery: mesenchymal stem cells—their current uses and potential applications. Stem Cells Int 2017;2017:14-5. https://doi.org/10.1155/2017/2638305
2. Boszotta H, Prunner K. Arthroscopically assisted rotator cuff repair. Arthroscopy 2004;20:620-6. https://doi.org/10.1016/j.arthro.2004.04.058
3. Burkhart SS, Danaceau SM, Pearce CE Jr. Arthroscopic treatment of massive rotator cuff tears. Clin Orthop Relat Res 2001:107-18.
4. Burkhart SS, Denard PJ, Konicek J, Hanypsiak BT. Biomechanical validation of load-sharing rip-stop fixation for the repair of tissue-deficient rotator cuff tears. Am J Sports Med 2014;42:457-62. https://doi.org/10.1177/0363546513516602
5. Degen RM, Carbone A, Carballo C, Zong J, Chen T, Lebaschi A, et al. The effect of purified human bone marrow-derived mesenchymal stem cells on rotator cuff tendon healing in an athymic rat. Arthroscopy 2016;32:2435-43. https://doi.org/10.1016/j.arthro.2016.04.019
6. Depres-Tremblay G, Chevrier A, Snow M, Hurtig MB, Rodeo S, Buschmann MD. Rotator cuff repair: a review of surgical techniques, animal models, and new technologies under development. J Shoulder Elbow Surg 2016;25:2078-85. https://doi.org/10.1016/j.jse.2016.06.009
7. Ficklscherer A, Hartl TK, Scharf M, Sievers B, Schroder C, Milz S, et al. Effects of selective paralysis of the supraspinatus muscle using

botulinum neurotoxin A in rotator cuff healing in rats. J Orthop Res 2013;31:716-23. https://doi.org/10.1002/jor.22260
8. Fickscherer A, Loitsch T, Serr M, Gulecyuz MF, Niethammer TR, Müller HH, et al. Does footprint preparation influence tendon-to-bone healing after rotator cuff repair in an animal model? Arthroscopy 2014;30:188-94. https://doi.org/10.1016/j.arthro.2013.11.016
9. Flury M. Patch augmentation of the rotator cuff. A reasonable choice or a waste of money? Orthopade 2016;45:136-42. https://doi.org/10.1007/s00132-015-3211-z
10. Galatz LM, Charlton N, Das R, Kim HM, Havlioglu N, Thormopoulos S. Complete removal of load is detrimental to rotator cuff healing. J Shoulder Elbow Surg 2009;18:669-75. https://doi.org/10.1016/j.jse.2009.02.016
11. Galatz LM, Rothermich SY, Zaegel M, Silva MJ, Havlioglu N, Thomopoulos S. Delayed repair of tendon to bone injuries leads to decreased biomechanical properties and bone loss. J Orthop Res 2005;23:1441-7. https://doi.org/10.1016/j.orthres.2005.05.005.1100230629
12. Gulecyuz M, Bortolotti H, Pietschmann M, Ficklscherer A, Niethammer T, Roßbach B, et al. Primary stability of rotator cuff repair: can more suture materials yield more strength? Int Orthop 2016;40:989-97. https://doi.org/10.1007/s00264-015-3012-3
13. Gulecyuz MF, Macha K, Pietschmann MF, Ficklscherer A, Sievers B, Roßbach BP, et al. Allogenic myocytes and mesenchymal stem cells partially improve fatty rotator cuff degeneration in a rat model. Stem Cell Rev 2018;14:847-59. https://doi.org/10.1007/s12015-018-9829-6
14. Gulotta LV, Kovacevic D, Packer JD, Deng XH, Rodeo SA. Bone marrow–derived mesenchymal stem cells transduced with scleraxis improve rotator cuff healing in a rat model. Am J Sports Med 2011;39:1282-9. https://doi.org/10.1177/0363546510395485
15. Hirpara KM, Sullivan PJ, O'Sullivan ME. The effects of freezing on the tensile properties of repaired porcine flexor tendon. J Hand Surg Am 2008;33:353-8. https://doi.org/10.1016/j.jhsa.2007.12.011
16. Kamath G, Galatz LM, Keener JD, Teefey S, Middleton W, Yamaguchi K. Tendon integrity and functional outcome after arthroscopic repair of high-grade partial-thickness supraspinatus tears. J Bone Joint Surg Am 2009;91:1055-62. https://doi.org/10.2106/JBJS.G.00118
17. Khan KM, Cook JL, Bonar F, Harcourt P, Astrom M. Histopathology of common tendinopathies. Update and implications for clinical management. Sports Med 1999;27:393-408.
18. Kim YS, Lee HJ, Ok JH, Park JS, Kim DW. Survivorship of implanted bone marrow-derived mesenchymal stem cells in acute rotator cuff tear. J Shoulder Elbow Surg 2013;22:1037-45. https://doi.org/10.1016/j.jse.2012.11.005
19. Lebaschi A, Deng XH, Zong J, Cong GT, Carballo CB, Album ZM, et al. Animal models for rotator cuff repair. Ann N Y Acad Sci 2016;1383:45-57. https://doi.org/10.1111/nyas.13203
20. Lee AH, Elliott DM. Freezing does not alter multiscale tendon mechanics and damage mechanisms in tension. Ann N Y Acad Sci 2017;1409:85-94. https://doi.org/10.1111/nyas.13460
21. Lhee SH, Jo YH, Kim BY, Nam BM, Nemeno JG, Lee S, et al. Novel supplier of mesenchymal stem cell: subacromial bursa. Transplant Proc 2013;45:3118-21. https://doi.org/10.1016/j.transproceed.2013.07.052
22. Lorbach O, Baums MH, Kostuij T, Pauly S, Scheibel M, Carr A, et al. Advances in biology and mechanics of rotator cuff repair. Knee Surg Sports Traumatol Arthrosc 2015;23:530-41. https://doi.org/10.1007/s00167-014-3487-2
23. Mahler Convenor M, Berard M, Feinstein R, Gallagher A, Ilgen-Wilcke B, Pritchett-Corning K, et al. FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. Lab Anim 2014;48:178-92. https://doi.org/10.1177/0023677213516312
24. Nho SJ, Delos D, Yadav H, Pensak M, Romeo AA, Warren AF, et al. Biomechanical and biologic augmentation for the treatment of massive rotator cuff tears. Am J Sports Med 2010;38:619-29. https://doi.org/10.1177/0363546509343199
25. Oswald I, Rickert M, Bruggemann GP, Niehoff A, Fonseca Ulloa CA, Jahnke A. The influence of cryopreservation and quick-freezing on the mechanical properties of tendons. J Biomech 2017;64:226-30. https://doi.org/10.1016/j.jbiomech.2017.08.018
26. Quigley RJ, Gupta A, Oh JH, Chung KC, McGarry MH, Gupta R, et al. Biomechanical comparison of single-row, double-row, and transosseous-equivalent repair techniques after healing in an animal rotator cuff tear model. J Orthop Res 2013;31:1254-60. https://doi.org/10.1002/jor.22363
27. Rothrauff BB, Tuan RS. Cellular therapy in bone-tendon interface regeneration. Organogenesis 2014;10:13-28. https://doi.org/10.4161/org.27404
28. Safi E, Ficklscherer A, Bondarava M, Betz O, Zhang A, Jansson V, et al. Migration of mesenchymal stem cells of bursal tissue after rotator cuff repair in rats. Joints 2018;6:4-9. https://doi.org/10.1055/s-0038-1636948
29. Salata MJ, Sherman SL, Lin EC, Sershon RA, Gupta A, Shewman E, et al. Biomechanical evaluation of transosseous rotator cuff repair: do anchors really matter? Am J Sports Med 2013;41:283-90. https://doi.org/10.1177/0363546512469092
30. Savin D, Meadows M, Nikhil V, Cole B. Rotator cuff healing: improving biology. Oper Tech Sports Med 2017;25:34-40. https://doi.org/10.1053/j.otsm.2016.12.006
31. Scheibel M. Recurrent defects of the rotary cuff: causes and therapeutic strategies. Oper Orthop Traumatol 2012;24:458-67. https://doi.org/10.1007/s00064-012-0179-2
32. Song N, Armstrong AD, Li F, Ouyang H, Niyibizi C. Multipotent mesenchymal stem cells from human subacromial bursa: potential for cell based tendon tissue engineering. Tissue Eng Part A 2014;20:239-49. https://doi.org/10.1089/ten.TEA.2013.0197
33. Soslowsky LJ, Carpenter JE, DeBano CM, Banerji I, Moalli MR. Development and use of an animal model for investigations on rotator cuff disease. J Shoulder Elbow Surg 1996;5:383-92.
34. Thangarajah T, Sanghani-Kerai A, Henshaw F, Lambert SM, Pendegrass CJ, Blunn GW. Application of a demineralized cortical bone matrix and bone marrow-derived mesenchymal stem cells in a model of chronic rotator cuff degeneration. Am J Sports Med 2018;46:98-108. https://doi.org/10.1177/0363546517727512
35. Uhthoff HK, Sarkar K. Surgical repair of rotator cuff ruptures. The importance of the subacromial bursa. J Bone Joint Surg Br 1991;73:399-401.
36. Utsunomiya H, Uchida S, Sekiya I, Sakai A, Moridera K, Nakamura T. Isolation and characterization of human mesenchymal stem cells derived from shoulder tissues involved in rotator cuff tears. Am J Sports Med 2013;41:657-68. https://doi.org/10.1177/0363546512473269
37. Zumstein MA, Ladermann A, Raniga S, Schar MO. The biology of rotator cuff healing. Orthop Traumatol Surg Res 2017;103:1-10. https://doi.org/10.1016/j.otsr.2016.11.003
38. Zumstein MA, Jost B, Hempel J, Hodler J, Gerber C. The clinical and structural long-term results of open repair of massive tears of the rotator cuff. J Bone Joint Surg Am 2008;90:2423-31. https://doi.org/10.2106/JBJS.G.00677