Does Footprint Preparation Influence Tendon-to-Bone Healing After Rotator Cuff Repair in an Animal Model?

Andreas Ficklscherer, M.D., Thomas Loitsch, Michaela Serr, Mehmet F. Gülecyüz, M.D., Thomas R. Niethammer, M.D., Hans-Helge Müller, Ph.D., Stefan Milz, M.D., Matthias F. Pietschmann, M.D., and Peter E. Müller, M.D.

Purpose: The aim of this study was to investigate the influence of footprint spongialization and radiofrequency ablation on rotator cuff repair outcomes compared with an untreated group in a rat model. Methods: We randomly assigned 189 Sprague-Dawley rats to either a spongialization, radiofrequency ablation, or untreated group. After separation of the supraspinatus tendon from the greater tubercle, the footprint was prepared by removing the cortical bone with a burr (spongialization), was prepared by ablating soft tissue with a radiofrequency ablation device, or was left unaltered (untreated). Biomechanical testing (after 7 weeks, n = 165) and histologic analysis after 1 and 7 weeks (n = 24) followed reinsertion. Results: The mean load to failure was 17.51 ± 4.46 N/mm² in the spongialization group, 15.56 ± 4.85 N/mm² in the radiofrequency ablation group, and 19.21 ± 5.19 N/mm² in the untreated group. A significant difference was found between the spongialization and radiofrequency ablation groups (P = .0409), as well as between the untreated and radiofrequency ablation groups (P = .0014). There was no significant difference between the spongialization and untreated groups (P = .2456). The mean area of fibrocartilage transition, characterized by the presence of type II collagen, was larger after 1 and 7 weeks in the spongialization group (0.57 ± 0.1 mm² and 0.58 ± 0.1 mm², respectively) and untreated group (0.51 ± 0.1 mm² and 0.51 ± 0.2 mm², respectively) than in the radiofrequency ablation group (0.11 ± 0.1 mm² and 0.4 ± 0.1 mm², respectively) with P < .05 and P < .01. Conclusions: The results of this study show that radiofrequency ablation of the footprint results in a poor biomechanical and histologic outcome in an animal model. Preparation of the footprint has the same effect as spongialization. Clinical Relevance: Different techniques of footprint preparation in rotator cuff repair may influence tendon-to-bone healing.

Open and, more recently, arthroscopic rotator cuff repair have shown good short-term results with respect to functional outcome and pain relief. Nevertheless, many clinical studies report high instances of rotator cuff tears that do not heal very well after repair. In most basic science studies, the main focus has been on improving repair techniques biomechanically, whereas recently, attention has shifted to biological aspects of tendon healing to improve tendon-to-bone healing. Several authors have reported improved rotator cuff healing using biological augmentation with stem cells, growth factors, and platelet-rich plasma, but current augmentation techniques have their limitations. An important step in rotator cuff repair is the correct reduction of the tendon to the so-called footprint, the insertion area of the tendon onto the greater tuberosity. In the literature, no recommendations are available to the surgeon on how to prepare this area for optimal reinsertion of the avulsed tendon. Clinically, radiofrequency devices are frequently used to prepare the footprint ablating soft tissue from the tendon insertion site, but their effect has never been studied. In addition, spongialization of the footprint with a burr before reinserting the tendon is also regularly performed. Since Randelli et al. proved that growth factors are released after acromioplasty with partial spongialization of the acromion, we hypothesized that spongialization of the...
footprint may improve rotator cuff healing by the release of growth factors right underneath the reduced tendon. To our knowledge, there are only 2 studies focusing on a comparable hypothesis. Levy et al. used cannulated humeral implants in rats to allow access of the bone marrow cells into the tendon insertion zone but did not find a significant influence after 4 and 8 weeks. In an earlier study, St. Pierre et al. evaluated tendon healing to a cancellous trough in a goat model and also found no significant improvement after 6 and 12 weeks.

The aim of this study was to investigate the influence of footprint spongialization and radiofrequency ablation on rotator cuff repair outcomes compared with an untreated group in a rat model. Our hypothesis was that with spongialization of the footprint, functionally competent tendon-to-bone regeneration can be achieved that is superior to radiofrequency ablation.

Methods

Study Design and Experimental Groups

A rat model was used because of the similarities to the human anatomy and based on previous recommendations. After approval was obtained from the Institutional Animal Care and Use Committee, 189 twelve-week-old Sprague-Dawley rats, with a mean weight of 250 g, were obtained (Charles River Laboratories, Sulzfeld, Germany). There was no difference in weight at the time of the operations among the groups. All animals were randomly assigned to either the spongialization, radiofrequency ablation, or untreated group. Furthermore, they were divided into a biomechanical arm and a histologic arm of testing, with 55 rats and 8 rats in each group, respectively. Biomechanical testing took place 7 weeks after surgery, whereas 4 animals from each subgroup were analyzed histologically after 1 week and 7 weeks. Figure 1 shows the classification of all groups and subgroups.

Surgical Technique

All rats were anesthetized with isoflurane followed by an intramuscular injection of 50-mg/kg ketamine and subcutaneous injection of enrofloxacin, 2.5 mg/kg of body weight, for antibiosis. During surgery, isoflurane and oxygen were administered by nose cone. All operations were performed under sterile conditions by a single surgeon (A.F.). The right shoulder region was shaved; the animals were put in the lateral position and kept warm with a heating pad. Only the right arm was operated on, allowing the rats to ambulate and feed. The skin incision was followed by a deltoïd-splitting cut. To visualize the rotator cuff, the acromioclavicular joint was divided. The supraspinatus tendon was carefully identified and cut off at the insertion to the greater tuberosity. The tendon was dissected transverse; no dog-bone configuration was achieved. The footprint was prepared in 3 different ways, depending on the group assignment. In 1 group (n = 63) the cortical bone of the greater tuberosity was removed by a fine burr (Proxxon Micromot 50/EF; Proxxon, Föhren, Germany). In another group (n = 63) the soft tissue was completely ablated from the insertion area with the help of a bipolar radiofrequency ablation device (CoolCut 45 SJ; Arthrex, Munich, Germany). Finally, in the untreated group (n = 63), the tendon was reattached without any preparation of the footprint. Tendon repair was carried out with a single Mason-Allen stitch using a No. 5-0 double-armed Prolene suture (Ethicon, Somerville, NJ) through a single supraspinatus tendon. On the greater tuberosity, 2 mm from the articular surface, two 0.5-mm tunnels were drilled. Both suture ends were passed through the tunnels and tied together on the humeral cortex, reconnecting the supraspinatus tendon and the differently prepared footprints. The deltoid split was closed with No. 3-0 Ethibond (Ethicon), and the skin was closed with No. 3-0 Vicryl subcutaneous suture (Ethicon).

For analgesia, the rats were subcutaneously given buprenorphine, 0.05 mg/kg of body weight, directly at 6 and 12 hours postoperatively. All animals were monitored according to the guidelines provided by the Institutional Animal Care and Use Committee for discomfort, distress, and pain.

Biomechanical Testing

The humerus, with its attached supraspinatus tendon and muscle, was carefully dissected from the scapula

---

Fig 1. Study setup and number of animals in each group.
and surrounding tissue. The supraspinatus muscle was then stripped from the supraspinatus tendon, whose midsubstance diameter was measured with a micrometer, and the cross-sectional area was calculated. Specimens were stored in a −30°C freezer and thawed at room temperature before biomechanical testing, which was performed in a blinded manner. According to preceding investigations by Galatz et al. and Ficklscherer et al., the humerus was embedded in a custom-made aluminum cylinder, by use of polymethyl methacrylate. The probe was then placed into a Zwick Universal Testing Machine (model Z010/1N2A; Zwick, Ulm, Germany). The proximal end of the tendon was placed between 2 aluminum clamps lined with sandpaper and also secured with cyanoacrylate (Pattex Ultra Gel; Henkel, Düsseldorf, Germany). The clamps were screwed together securely. A transducer (model HBM Z6FD1; Zwick) with a measurement range of up to 100 N and a measurement uncertainty of 0.2% registered force to the shoulder at 90° of shoulder abduction. We used TestXpert software (version 5.0; Zwick) to record measurements and perform data evaluation. To define a standard “zero load,” a load state from which all samples begin, a preload to 0.2 N was applied. After the preload, 5 cycles of preconditioning followed at 5% grip-to-grip strain at a rate of 0.1%/s to define a consistent load history for each sample. For testing force at failure, a constant strain rate test to failure was performed afterward.

Histology

After removal of the humerus with the attached supraspinatus muscle, specimens were fixed in 4% neutral-buffered formalin (Micros GmbH, Garching, Germany) for 48 hours. Afterward, the decalcification process was performed in EDTA-4Na 20% citric acid for 14 days. Dehydration and paraffin embedding were fully automated by a tissue processor (Hypercenter XP; Thermo Scientific Fisher, Schwerte, Germany). Three-micrometer-thick sections were cut parallel to the long axis of the tendon in the coronal plane. The sections were stained with hematoxylin-eosin and immunohistochemically labeled for the presence of type II collagen (CIIC2; Development Studies Hybridoma Bank, Iowa City, IA). From each specimen, 3 different sections were obtained and evaluated by 3 investigators (S.M., A.F., and M.S.) who were not informed about and were blinded to the specimen group assignment. The organization of collagen tissue, vascularity, and fibrocartilage at the tendon-bone interface; inflammation; cross-sectional area; and collagen fiber continuity between tendon and bone tissue were evaluated. Furthermore, the type II collagen—positive area at the tendon-bone interface was measured and quantified (in square millimeters) (Zeiss Visio Release 4.5; Zeiss, Göttingen, Germany). Pictures were recorded with an Axio Cam MRC5 camera (Zeiss), attached to a Zeiss Axioskop 40 light microscope. All images were obtained at identical illumination and magnification.

Statistical Analysis

Load to failure (primary endpoint) and cross-sectional area were compared with the nonparametric Mann-Whitney U test (GraphPad Prism software, version 5.02 for Windows; GraphPad Software, San Diego, CA). Strong control for multiple comparisons of groups regarding the primary endpoint at the 2-sided .05 level of significance for 2-sided testing differences was pre-planned by closed testing (principle of Marcus et al.); this enhanced nonparametric Dunnett-type testing of the footprint preparation groups versus the control group (Steel test) for all pair-wise comparisons (subordinately including the comparison of the footprint preparation groups). Experience regarding the load of failure in the control group from a former experiment yielded estimated means (15.7 N/mm² for operative specimens [SD, 4.0 N/mm²] and 20.3 N/mm² for nonoperative specimens) but with suspicion of relevant deviations from the normal distribution. Originally, 3 footprint preparations with calculated 60 rats per group were fixed in the protocol. Feasibility forced us to reduce the number of preparations to 2, and the number of rats was recalculated to be 55 per group. The sample size aimed to detect a relevant difference of 2.5 N/mm² between at least 1 of the footprint preparations and the control group with a power of 80%, assuming normal distributions with an SD of 4 N/mm². The calculations were based on the closed testing procedure using the correlated Wilcoxon–Mann-Whitney tests but with slightly conservative Bonferroni correction for 2 tests instead of laborious calculations of the Steel critical values. For histologic analysis, the type II collagen—positive areas were statistically compared by use of the t test; all other parameters were qualitative in nature. The number of animals per histologic group (4 per group) was chosen in accordance with similar histologic investigations in several other publications.

Results

No animal was lost during the experiment or excluded from analysis. No anesthesia-related, surgery-related, or postoperative complications of any kind occurred during the study. Food intake and gait did not change as a result of pain or restricted shoulder function from surgery. There was no significant difference in body weight among the groups when the animals were killed.

Biomechanical Testing

After 7 weeks, a total of 165 specimens were available for biomechanical testing. The load to failure was
17.51 ± 4.46 N/mm² in the spongialization group, 15.56 ± 4.85 N/mm² in the radiofrequency ablation group, and 19.21 ± 5.19 N/mm² in the untreated group. A significant difference was found between the spongialization and radiofrequency ablation groups (P = .0409), as well as between the untreated and radiofrequency ablation groups (P = .0014). There was no significant difference between the spongialization and untreated groups (P = .2456). The results are shown in Fig 2.

Tendon cross-sectional area was significantly smaller in the radiofrequency ablation group (0.07 ± 0.03 mm²) compared with the spongialization group (0.14 ± 0.08 mm², P < .0001) or untreated group (0.12 ± 0.08 mm², P < .0001). We found no significant difference in cross-sectional area when comparing the spongialization and untreated groups (P = .1178). The tendons of 3 untreated specimens ruptured because in order to perform biomechanical testing of the tendon one has to separate the muscle; therefore, a piece of yarn was looped around the tendon and slowly pulled, which was then stripped from the attached supraspinatus muscle. The tendons of 2 samples ruptured in the decorticated and radiofrequency ablation groups during the same procedure. These specimens were lost to biomechanical testing. Ultimately, 158 specimens were biomechanically tested. Furthermore, 9 specimens in the spongialization group, 2 specimens in the radiofrequency ablation group, and 8 specimens in the untreated group failed during load-to-failure testing because of proximal humeral fractures. All other failures occurred at the bone-tendon transition zone.

Histologic Analysis

After 1 week, specimens from the untreated group showed reduced cellularity and vascularity compared with the 2 other groups. There were fewer cells and less vascularity in the spongialization group than in the radiofrequency ablation group. The transition tissue in the untreated and spongialization groups was better organized and more interdigitated compared with the radiofrequency ablation group. In the spongialization group the decorticated area was filled with connective tissue and showed greater vascularity. The humeral epiphysis was intact in all samples. There were still more cells detectable after 7 weeks in the radiofrequency ablation group. The area of interdigitation was smaller when compared with the control and spongialization groups. Fiber orientation was disorganized and heterogeneous. The spongialization and untreated groups both showed a tidemark indicating better-organized

191
transition tissue. Representative histologic images are shown in Figs 3 and 4.

The area of type II collagen was both significantly larger after 1 and 7 weeks in the spongialization (1 week: $0.57 \pm 0.1 \text{ mm}^2$; 7 weeks: $0.58 \pm 0.1 \text{ mm}^2$) and untreated groups (1 week: $0.51 \pm 0.1 \text{ mm}^2$; 7 weeks: $0.51 \pm 0.2 \text{ mm}^2$) than in the radiofrequency ablation group (1 week: $0.11 \pm 0.1 \text{ mm}^2$; 7 weeks: $0.4 \pm 0.1 \text{ mm}^2$). Details are given in Figs 5 and 6.

**Discussion**

The results presented in this study support our hypothesis that a functionally competent tendon-to-bone regeneration that is superior to radiofrequency ablation can be achieved using footprint spongialization in rotator cuff reconstruction. We were also able to show the restoration of the tidemark as an indicator for better-organized and functionally competent fibrocartilaginous transition tissue with superior attachment strength. We were not able to show a biomechanically superior load-to-failure rate in the spongialization group when compared with the untreated group.

In our opinion the most probable explanation for the previously mentioned finding is the high intrinsic healing potential in rats. Thus positive effects of locally present growth factors and mesenchymal stem cells due to spongialization might be outweighed. Remarkably, although the footprint was likely weakened by decortication, the loads to failure in the 2 groups were comparable. Possibly, these positive effects may be more pronounced in humans, in whom the intrinsic healing potential is marginal.

A study performed by Kida et al. on bone marrow–chimeric rats promotes that aspect of cell migration, showing the migration of bone marrow–derived cells through holes drilled in the humerus footprint. Kida et al. were also able to show that these cells infiltrated the repaired rotator cuff, contributed to postsurgical rotator cuff healing with quicker tissue remodeling, and led to a significantly higher load-to-failure force (the release of growth factors was not evaluated in that study).

St. Pierre et al. performed a study comparing the outcome of tendon healing after reinserting the infraspinatus tendon either into a cancellous canal or onto the cortical bone in a goat model. They used 16 goats for biomechanical analysis and 4 goats for histologic analysis (each bilateral), measuring the outcome (load to failure, energy to failure, and stiffness) at 6 and 12 weeks after surgery. They found a mean difference in load to failure of 3.9% in favor of cancellous repair, which was not statistically significant, and stated that there was “no significant benefit from the creation of a trough,” with almost identical biomechanical properties in both groups after 6 and 12 weeks. Contrary to
St Pierre et al., we did find significant differences between the groups. One possibility may be the relatively small group size, with only 16 animals.

A recently published study by Levy et al.16 focusing on the same hypothesis used cannulated humeral implants in rats to deliver local bone marrow to the healing tendon. Biomechanical testing was performed on 10 animal shoulders after 4 and 8 weeks, with histologic analysis in 4 specimens per group. Although biomechanical and histologic parameters improved over time, Levy et al. were not able to show a significant difference between groups. They supposed that the diminutive size of the implants might have been the reason for an insufficient amount of bone marrow migration.

In our study the footprint underwent complete spongialization and the supraspinatus tendon was in direct contact with the humeral bone marrow. We were able to show that both the spongialization group and the untreated group had a significantly higher mean load to failure than the radiofrequency ablation group. Histologically, we were able to show the restoration of the footprint with interdigitating fibers in the untreated and spongialization groups.

Data about the effects of radiofrequency ablation on tendons are sparse and diverse. Some studies suggest a faster return to mechanical integrity allowing earlier rehabilitation of the treated extremity.24 Others describe thermal complications after the use of a radiofrequency ablation device.25,26 There are, however, scarce data on heat distribution on bone. Groetz et al.27 detected a mean temperature of 52.2°C after radiofrequency ablation of vertebral metastases in a cadaveric study. Changes in tissue quality were not evaluated. To date, no study has evaluated heat distribution in cortical bone at the shoulder girdle. On the basis of our interpretation, our study results for the radiofrequency ablation group are in accordance with these studies and do not favor footprint preparation with this method.

We did not find significant differences in our primary endpoint—load to failure—between the spongialization and untreated groups. In our opinion the most probable explanation for this is the high intrinsic healing potential in rats. Thus positive effects of locally present growth factors and mesenchymal stem cells due to spongialization might be outweighed. Nevertheless remarkable is the fact that although we attenuated the footprint by spongialization, we did find nearly equal values in load to failure. In our opinion possible positive effects may be more pronounced in humans, in whom the intrinsic healing potential is marginal.

Limitations
This study has limitations, including differences between rat and human rotator cuff healing and between chronic and acute tears. Rotator cuff healing in the rat is faster and more likely than that in humans. Furthermore, tears treated immediately are more likely to heal than chronic tears, making it harder to observe a difference between the groups.28,29 To our astonishment, not a single retear was found during this study, which one might have expected, especially in the radiofrequency ablation group. This, again, is probably because of the high intrinsic healing potential in this animal model. Therefore an animal model with nearly equal healing potential to humans with an adequate group size should be favored.

We did not distinguish between monopolar and bipolar devices. Another limitation relates to the size of the holes drilled for re-adaptation of the supraspinatus tendon. Because 0.5-mm holes are relatively large for a rat’s humerus, it could be that these holes were large enough for mesenchymal cells to migrate, having the same effect as spongialization.23

Conclusions
The results of this study show that radiofrequency ablation of the footprint results in a poor biomechanical and histologic outcome in an animal model. No preparation of the footprint has the same effect as spongialization.

References


