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# Application of Bone Marrow-Derived Mesenchymal Stem Cells in a Rotator Cuff Repair Model

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**Background:** Rotator cuff tendons heal to bone with interposed scar tissue, which makes repairs prone to failure. The purpose of this study was to determine if the application of bone marrow-derived mesenchymal stem cells (MSCs) can improve rotator cuff healing after repair.

**Hypothesis:** Application of MSCs to the repair site will result in superior results compared with controls on histologic and biomechanical testing.

**Study Design:** Controlled laboratory study.

**Methods:** Ninety-eight Lewis rats underwent unilateral detachment and repair of the supraspinatus tendon; 10 rats were used for MSC harvest. Eight animals were used for cell tracking with Ad-LacZ. The remaining animals received either $10^6$ MSCs in a fibrin carrier, the carrier alone, or nothing at the repair site. Animals were sacrificed at 2 and 4 weeks for histologic analysis to determine the amount of fibrocartilage formation and the collagen organization at the insertion. Biomechanical testing was also performed.

**Results:** Specimens treated with Ad-LacZ–transduced MSCs exhibited more β-galactosidase activity at the repair site compared with controls at both 2 and 4 weeks, although activity at 4 weeks was less than that at 2 weeks. There were no differences in the amount of new cartilage formation or collagen fiber organization between groups at either time point. There were also no differences in the biomechanical strength of the repairs, the cross-sectional area, peak stress to failure, or stiffness.

**Conclusion:** The addition of MSCs to the healing rotator cuff insertion site did not improve the structure, composition, or strength of the healing tendon attachment site despite evidence that they are present and metabolically active.

**Clinical Relevance:** A biologic solution to the problem of tendon-to-bone healing in the rotator cuff remains elusive. The repair site may lack the cellular and/or molecular signals necessary to induce appropriate differentiation of transplanted cells. Further studies are needed to determine if cell-based strategies need to be combined with growth and differentiation factors to be effective.

**Keywords:** shoulder; rotator cuff; stem cells; tendon-to-bone; animal model

Rotator cuff repair surgery depends on tendon-to-bone healing. Instead of regenerating the specialized fibrocartilaginous transition zone that is seen in the native rotator cuff insertion site, an interposing layer of fibrovascular scar tissue forms between the tendon and the bone after surgical repair.[2,5-8,15,24,28] This scar tissue interface has poor material properties, which makes repairs prone to failure.[12,18] The ultimate goal of our ongoing work is to improve the biological environment around the repair to promote regeneration of the native insertion site, and to prevent the formation of scar tissue.

Mesenchymal stem cells (MSCs) are pluripotent cells that can differentiate into multiple mesenchymal tissues.[3] Numerous animal transplantation studies have found that MSCs expanded in vitro can augment the local repair and regeneration of bone, articular cartilage, intervertebral disc, and tendon.[4,11,14,21,22,25] Studies have shown that bone

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marrow-derived MSCs can improve the healing of tendon grafts in a bone tunnel.[17,20] Although these studies are encouraging, it is unclear if these findings can be extrapolated to the rotator cuff. In this study we tested the following hypotheses: (1) application of bone marrow-derived MSCs will lead to increased fibrocartilage formation and improved collagen organization at the healing tendon-to-bone insertion site at early time points, and (2) tendon attachment strength will be improved in animals treated with MSCs.

## MATERIALS AND METHODS

### Study Design

Mature, male Lewis rats weighing an average of 310.1 g were used under the guidelines of the Institutional Animal Care and Use Committee. Lewis rats were used because they are considered syngeneic. Therefore, transplantation of cells or tissue from one animal to another is analogous to an autograft and carries very little risk of graft rejection or immune response. A total of 98 animals underwent the surgical procedure, and an additional 10 animals were used for bone marrow harvest to obtain MSCs. The surgical procedure involved unilateral detachment and repair of the right supraspinatus tendon. Eight animals received $10^6$ MSCs transduced with either an adenovirus containing the reporter gene, *lacZ (*Ad-LacZ; n = 4), or an adenovirus without any gene (Ad-null) to serve as a control (n = 4). This was done to assess MSC survival. The remaining animals were randomized into 1 of 3 groups (30 animals/group). The experimental group received $10^6$ bone marrow-derived MSCs in a fibrin sealant carrier to the tendon-bone repair site, a second group received only the fibrin sealant carrier, and a control group was simply repaired. Fifteen animals in each group were sacrificed at 2 weeks, and 15 were sacrificed at 4 weeks. At each time point, 12 animals were allocated for biomechanical testing and 3 were allocated for histomorphometric analysis.

### Bone Marrow-Derived Mesenchymal Stem Cell Harvest and Culture

A total of 10 Lewis rats were euthanized by carbon dioxide inhalation, and bilateral femurs, tibias, and humeri were harvested under sterile conditions. The MSCs were harvested by lavaging the intramedullary canals of the long bones with Hanks' Balanced Salt Solution (Gibco, Gaithersburg, Maryland) into a conical tube. Harvested cells were washed with Red Blood Cell Lysis Solution (Sigma, St. Louis, Missouri) 3 times and centrifuged. The cell pellet was resuspended in 20 mL of complete medium consisting of Dulbecco's Modified Eagle Medium, 1% antibiotic-antimycotic, and 10% fetal bovine serum (all from Gibco), and then plated onto T-175 culture flasks. Cells were grown in an incubator at 37°C, humidified with 5% $CO_2$. After 2 days, the contents of the flask were removed and washed with complete medium, leaving behind MSCs that adhered to the bottom of the flask. Once confluent, the MSCs were detached with trypsin (Gibco) and serially subcultured. Third-passage cells were used for implantation. This protocol has been used for bone marrow-derived MSC harvest.[4,17,20] Previous studies have shown that these cells maintain their pluripotency up to the third passage.[16,23,30]

### Surgical Technique

Animals were anesthetized with isoflurane in high-flow oxygen. All animals underwent unilateral detachment and repair of the right supraspinatus tendon. A longitudinal incision was made on the anterolateral aspect of the shoulder and the deltoid was split to reveal the supraspinatus tendon. The supraspinatus tendon was then sharply detached from its insertion on the greater tuberosity, and the underlying greater tuberosity was decorticated. A Mason-Allen stitch was placed into the supraspinatus tendon with 3-0 Ethibond (Ethicon, Somerville, New Jersey). Bone tunnels were created with a 22-gauge needle at the anterior and posterior margins of the insertion site, 2 mm from the articular surface, in a crossed fashion. The sutures in the tendon were then passed through the tunnels.

At this point the animals were randomized to receive either MSCs in a fibrin carrier, the fibrin carrier alone, or to receive no implant and serve as a control. For animals randomized to receive MSCs, $10^6$ cells were pelleted down and mixed with fibrin sealant for implantation. Briefly, the fibrin sealant (Tisseel, Baxter AG, Vienna, Austria) was prepared from a 2-component mixture according to the manufacturer's protocol. Thrombin was dissolved in calcium chloride solution (40 nmol/L) to yield solution A. Tisseel was then dissolved in aprotinin solution (3000 KIU/mL) with use of a heated stirring device (Fibrinotherm, Baxter AG) to yield solution B. For the MSC group, 25 µL of solution A was mixed with $10^6$ MSCs before use. A Duploject System (Baxter AG) was used to simultaneously apply equal amounts (25 µL each, for a total of 50 µL) of the 2 solutions between the tendon and the bone. This protocol has been used in previous studies to deliver cells to a healing site.[4,17,20] Before our study, we tested the ability of the cell to survive in the fibrin sealant solution. We suspended $10^6$ MSCs in solution A of the fibrin sealant for 12 hours and then placed the cells back into culture with complete medium. These cells survived and were capable of proliferation in culture.

The same procedure was done for animals randomized to receive the fibrin sealant alone, except the MSCs were omitted. Animals randomized to serve as the control group received neither the fibrin sealant nor the cells. The suture through the detached tendon was then tied over a bone bridge on the lateral aspect of the humerus. Skin closure was performed with 4-0 Vicryl sutures (Ethicon). Buprenorphine (0.05 mg/kg) was administered subcutaneously for analgesia during the postoperative period. Weightbearing activities were ad libitum postoperatively.

### Mesenchymal Stem Cell Tracking

To track implanted MSCs and confirm survival, 4 animals received MSCs transduced with Ad-LacZ to the repair site

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and 4 received MSCs transduced with Ad-null (both viruses were gifts from Chisa Hidaka, MD). Ad-LacZ is an adenovirus that contains the reporter gene *lacZ*, which codes for the protein β-galactosidase. When this protein interacts with its substrate, X-Gal, a blue color is emitted. The presence of blue stain indicates that transduced MSCs are present and metabolically active. Ad-LacZ or Ad-null was used to transduce MSCs in vitro at $10^5$ particle units per cell 24 hours before the surgical procedure. The morning of surgery, the transduced MSCs were harvested, suspended in the fibrin sealant, and implanted at the repair site as previously described. Four animals were sacrificed at 2 weeks and the remaining 4 were sacrificed at 4 weeks. At the time of necropsy, the supraspinatus-humerus complex was harvested en bloc and the tissue was analyzed for β-galactosidase activity. The specimens were fixed with Tissue Fixative buffer for 1.5 hours on ice, rinsed with solution A once, and maintained in solution A for 30 minutes at room temperature, then rinsed with solution B once and washed for 5 minutes in solution B (all from Specialty Media, Millipore, Billerica, Massachusetts). The washed tissues were incubated in prewarmed (37°C) 1% X-Gal (American Bioanalytical, Natick, Massachusetts) in solution C for 4 hours, and the blue stain was monitored at regular intervals. The amount of β-galactosidase activity was assessed by the amount of blue seen at the tendon-bone insertion site. This was subjectively determined by 2 independent, blinded observers (L.V.G. and D.K.) on the following scale: none (–), minimal (+), moderate (++), or intense staining (+++).

## Histomorphometric Analysis

Animals were sacrificed at 2 and 4 weeks for analysis. After euthanasia with inhaled carbon dioxide, all animals underwent dissection such that the specimen consisted of the humerus and the attached supraspinatus tendon and muscle. Specimens designated for biomechanical testing were wrapped in gauze soaked in phosphate-buffered saline (Gibco) and stored at –80°C until the time of analysis. For specimens undergoing histologic examination, the dissected humerus-supraspinatus sample was fixed in 10% neutral-buffered formalin, decalcified with Immunocal (Decal, Congers, New York), and embedded in paraffin. Five-micron thick sections were cut in the coronal plane. These slides were then alternately stained with hematoxylin and eosin, safranin O/fast green, and picrosirius red. The greater tuberosity, repaired tendon-bone insertion site, and the midsubstance of the supraspinatus tendon were examined under light and polarized microscopy using an Olympus BH-2 light microscope (Olympus Optical, Lake Succuss, New York). Digital images were taken using a SPOT RT camera (Diagnostic Instruments, Sterling Heights, Michigan).

To evaluate the organization of collagenous tissue in the repaired supraspinatus tendon, sections were stained with picrosirius red and illuminated with monochromatic polarized light. By quantifying the birefringence of collagen under polarized light (based on brightness), differences in collagen deposition and maturation in the healing tendon could be detected.[13,15] Images of the slides underwent 8-bit digitization with ImageJ software (National Institutes of Health, Bethesda, Maryland). The slides were then analyzed as follows: noncollagenous material was dark (gray level 0) and collagenous material was depicted by gray scales from 1 to 255. Higher gray scales signify more organized and mature collagen. Ten rectangular areas (2500 $\mu m^2$ each) were randomly selected at the insertion site, and gray scales were measured (mean ± standard deviation) with ImageJ. Three sequential coronal sections of each specimen were examined to reduce sampling error. The light intensities were measured under exactly the same conditions of illumination, and during the same sitting, for all specimens.

We also assessed the amount of new cartilage formed at the insertion site as native rotator cuff insertions contain a fibrocartilaginous transition zone. Safranin O/fast green stains proteoglycans reddish-purple, a phenomenon referred to as *metachromasia*.[15] ImageJ software was used to manually outline the areas of metachromasia on safranin O slides at ×40 magnification on 3 sequential coronal sections. The total area for each specimen was then recorded as squared micrometers and the mean plus or minus standard deviation for each group was determined. All histomorphometric analyses were performed in a blinded fashion by 2 independent reviewers (L.V.G. and D.K.).

## Biomechanical Testing

At the time of testing, the specimens were thawed at room temperature overnight and all excess supraspinatus muscle and the sutures were carefully removed. The cross-sectional area of the supraspinatus tendon at the level of its insertion was measured with digital calipers. The specimen was then placed into a custom-made uniaxial testing system. The tendon was secured in a screw grip using sandpaper and ethyl cyanoacrylate glue. The humerus was secured in a custom-designed vice grip that prevented fracture through the humeral physis. The supraspinatus tendon was secured to a 45-N load cell attached to a linear bearing that allowed alignment of the tendon in the direction of its pull. The humeral jig was secured to a linear stage. The specimen was preloaded to 0.10 N and then loaded to failure at a rate of 14 microns/sec, corresponding to approximately 0.4% strain. Displacement was measured using a 1-micron resolution micrometer system attached to the linear stage. The maximum load at failure and the failure site was recorded. Peak stress was calculated by dividing the maximal load at the time of failure by the cross-sectional area. Stiffness was also calculated by determining the slope of the linear portion of the load-displacement curve (Excel, Microsoft, Redmond, Washington). This testing protocol has been used in previous studies from our laboratory.[5]

## Statistical Analysis

A power analysis was performed before this study. The primary outcome was biomechanical testing of tendon attachment strength. We based our power study on a prior study that evaluated rotator cuff tendon healing in rats.[5]

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TABLE 1
β-Galactosidase Activity in Ad-LacZ–Transduced Mesenchymal Stem Cells as Compared With Ad-Null–Transduced Controls[a]

| Group | 2 Weeks (Specimen 1/ Specimen 2) | 4 Weeks (Specimen 1/ Specimen 2) |
|---|---|---|
| Ad-LacZ | +++/+++ | ++/++ |
| Ad-Null | +/+ | +/+ |

[a]Scoring system: +, minimal; ++, moderate; +++, intense staining.

In this study, the average ultimate load to failure was 8.4 ± 3.0 N at 2 weeks and 20.0 ± 4.7 N at 4 weeks. For the current study, an increase in strength of 20% would be considered clinically significant. Using these estimations, a power of 0.80 is achieved using 12 specimens per group with $\alpha = .05$ for biomechanical testing. The power calculation was performed using the SigmaStat program (Jandel Scientific, San Rafael, California).

Nonparametric statistical methods were used for all analyses because of the nonnormality of the data in the groups being compared. Kruskal-Wallis tests were used because the distributions of 3 groups were compared. A $P < .05$ value indicated a statistically significant difference. SAS statistical software (version 9.1, SAS Institute, Cary, North Carolina) was used to perform the analyses.

## RESULTS

Three rats suffered proximal humerus fractures intraoperatively and were thus euthanized. Two animals died intraoperatively as a complication of anesthesia. These complications were a reflection of the technical and anesthetic adjustments required to operate on the smaller Lewis rats as compared with the larger Sprague Dawley rats. These animals were replaced. There were no postoperative complications, and all animals resumed a normal gait by 5 days postoperatively.

### Cell Tracking

There was significantly more β-galactosidase activity in the specimens treated with Ad-LacZ–transduced MSCs as compared with the controls treated with Ad-null–transduced MSCs. However, specimens sacrificed at 4 weeks showed lower activity than those sacrificed at 2 weeks (Table 1 and Figure 1).

### Gross Observations

There was continuity between the repaired tendon and the bone in all animals at the time of necropsy. There were no signs of infections in any specimen. At 2 weeks, there was poorly organized, thin tissue connecting the tendon to the bone, with no detectable differences between groups. By 4 weeks, the tendon and the tendon-bone interface tissue was markedly more robust than the 2-week group animals, but again there were no appreciable differences noted between groups.

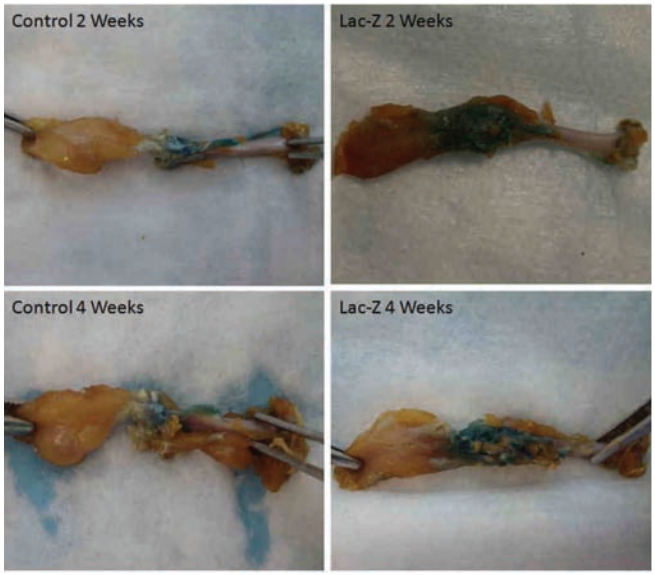


**Figure 1.** Gross specimens depicting increased β-galactosidase activity (blue stain) at the repair site of specimens treated with Ad-LacZ.

### Histomorphometric Analysis

At 2 weeks, there was a poorly organized, highly cellular, fibrovascular granulation tissue at the tendon-to-bone interface. The tendon proper was hypercellular, containing mostly fibroblasts and inflammatory cells. Fibrocartilage was seen starting to form at the interface even at this early time point; however, the chondrocytes were immature and disorganized. The interface tissue became progressively more organized with time. At 4 weeks, the interface was less cellular and began to show matrix organization in line with the tensile pull of the tendon. There was more fibrocartilage, and this fibrocartilage was more organized. Although the tendon and the tendon-bone insertion tissue became more organized and contained more fibrocartilage at 4 weeks as compared with 2 weeks, there were no differences between groups.

Semiquantitative histologic analysis confirmed our qualitative assessment of the specimens. At 2 weeks, the control group had a brightness of 16.5 ± 5.5 gray scale, the fibrin group had 20.3 ± 7.0 gray scale, and the MSC group had 20.3 ± 5.9 gray scale ($P = .73$). At 4 weeks, the control group had 24.8 ± 2.5 gray scale, the fibrin group had 25.5 ± 4.4 gray scale, and the MSC group had 26.5 ± 5.5 gray scale ($P = .96$) (Figures 2 and 3).

The amount of new cartilage formation as measured by the area of metachromasia showed no statistical improvement within each group from 2 to 4 weeks, nor did it show a difference between groups at either time point. At 2

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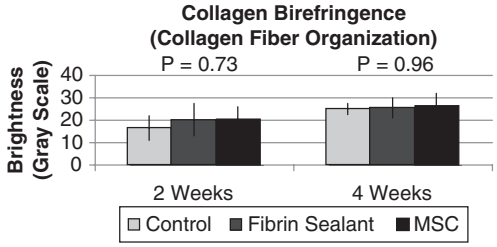


**Figure 2.** Collagen fiber organization was measured by calculating the brightness in gray scales of slides stained with picrosirius red under polarized light microscopy. There were no differences seen between groups at either time point.

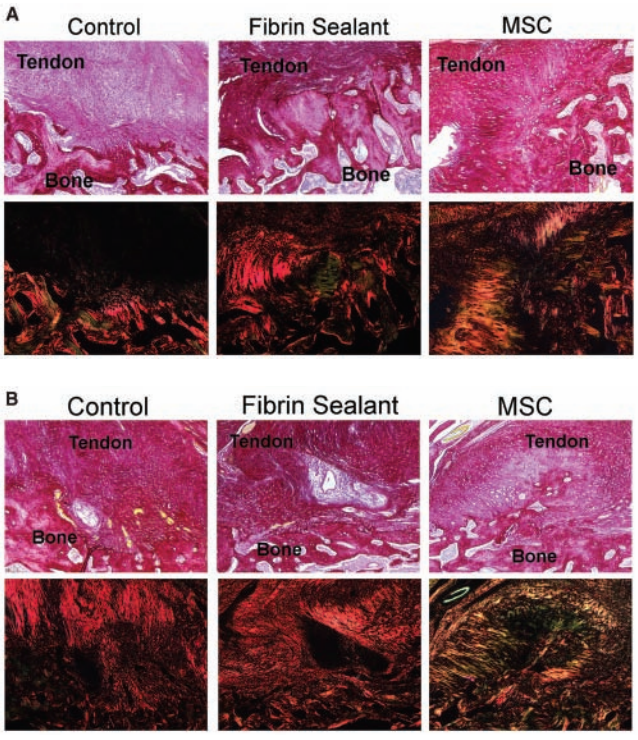


**Figure 3.** Representative histology photographs of picrosirius-stained slides (top) and the image created under polarized light (bottom) at 2 weeks (A) and 4 weeks (B). Collagen organization is proportional to brightness under polarized light. There is no appreciable difference between groups.

weeks, the control group had an area of metachromasia of 341 652.3 ± 201 279.3 $\mu m^2$, the fibrin group had 340 298.0 ± 803 38.1 $\mu m^2$, and the MSC group had 596 500.7 ± 192 408.5 $\mu m^2$ ($P$ = .06). At 4 weeks, the control group had an area of metachromasia of 437 693.0 ± 115 267.4 $\mu m^2$, the fibrin group had 340 214.7 ± 161 318.9 $\mu m^2$, and the MSC group had 342 606.7 ± 216 980.6 $\mu m^2$ ($P$ = .88) (Figures 4 and 5).

## Biomechanical Testing

Although there were significant increases within each group in terms of ultimate load to failure from 2 weeks to

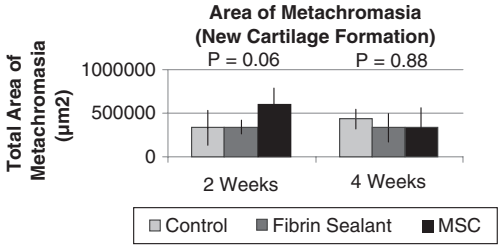


**Figure 4.** The amount of new cartilage formation was determined by measuring the area of metachromasia with safranin O staining. There were no differences between groups at either time point, although there was a trend toward significance at 2 weeks.

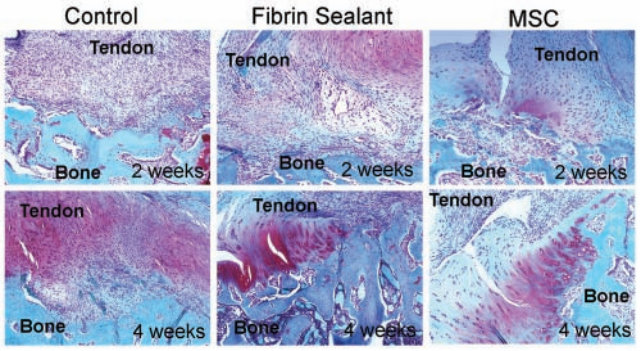


**Figure 5.** Representative histology photographs of safranin O-stained slides at 2 (top) and 4 (bottom) weeks. Safranin O stains bone and tendon blue, and stains the proteoglycans in the fibrocartilage reddish-purple. There were no appreciable differences between groups.

4 weeks, there were no differences seen between groups at either time point. At 2 weeks, the mean load to failure for the control group was 10.5 ± 2.4 N, the fibrin group was 11.6 ± 3.5 N, and the MSC group was 11.2 ± 2.3 N ($P$ = .63). At 4 weeks, the mean load to failure for the control group was 22.1 ± 3.5 N, the fibrin group was 20.4 ± 3.4 N, and the MSC group was 20.8 ± 4.4 N ($P$ = .57) (Table 2). There were no differences between groups at either time point in the cross-sectional area of tendons at the insertion site, the peak stress at failure, and the stiffness of the repair construct (Table 2). All specimens failed at the tendon-bone attachment site during biomechanical testing.

## DISCUSSION

In this study, we hypothesized that the application of bone marrow-derived MSCs would increase the amount of fibrocartilage formation and improve the collagen fiber organization at the insertion site. We further hypothesized that the ultimate strength of the repair would be greatest in specimens that received the MSCs. However, our data do not support these hypotheses.

The goal of improving the biology of rotator cuff healing remains elusive. The highly specialized structure of the native fibrocartilaginous insertion site is not regenerated

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TABLE 2
Biomechanical Testing Results[a]

| Group | Time Point (Weeks) | Ultimate Load to Failure (N) | Cross-Sectional Area ($mm^2$) | Ultimate Stress to Failure (MPa) | Stiffness (N/mm) |
|---|---|---|---|---|---|
| Control | 2 | 10.5 ± 2.4 | 5.8 ± 1.1 | 1.9 ± 0.6 | 5.7 ± 2.3 |
| Fibrin | 2 | 11.6 ± 3.5 | 6.3 ± 1.2 | 2.0 ± 0.8 | 6.3 ± 3.0 |
| MSC | 2 | 11.2 ± 2.3 | 7.1 ± 2.4 | 1.7 ± 0.7 | 4.9 ± 1.8 |
| *P* value | 2 | .63 | .28 | .50 | .16 |
| Control | 4 | 22.1 ± 3.5 | 6.5 ± 1.3 | 3.5 ± 1.0 | 9.8 ± 4.7 |
| Fibrin | 4 | 20.4 ± 3.4 | 5.7 ± 1.0 | 3.7 ± 1.0 | 9.1 ± 3.8 |
| MSC | 4 | 20.8 ± 4.4 | 6.1 ± 1.2 | 3.5 ± 1.0 | 9.3 ± 3.3 |
| *P* value | 4 | .57 | .37 | .66 | .89 |

[a]MSC, mesenchymal stem cell. All values are mean ± 1 SD.

after rotator cuff repair surgery. Instead, the fibrovascular scar tissue that forms between the tendon and the bone makes repairs prone to failure.[5,6,15] Although pluripotent stem cells are present in adults, there is concern that they may not be present in numbers sufficient to promote regeneration of the insertion site after repair. Several studies have demonstrated the ability of MSCs to improve healing in various orthopaedic models.[4,14,17,20,21] However, this study failed to show that the application of bone marrow-derived MSCs to a rotator cuff repair can improve healing at early time points in a rat model, despite evidence that they are present and metabolically active as evidenced by the β-galactosidase assay.

Mesenchymal stem cells have been shown to improve healing in other tendon-to-bone models. Ouyang et al[20] evaluated the effect of rabbit-derived bone marrow stromal cells in a fibrin glue carrier on the healing of hallucis longus tendons in a calcaneal bone tunnel. They found that the application of the stem cells improved healing by formation of a fibrocartilaginous attachment between the tendon graft and the bone. These results were seen as early as 2 weeks after the surgery. Biomechanical testing was not included in this study. Lim et al[17] used an ACL reconstruction model in rabbits to test the effect of rabbit-derived MSCs on healing of the semitendinosus tendon in a bone tunnel. They also reported healing by the formation of fibrocartilage in the stem cell–treated animals at early time points when compared with controls. In contrast, the controls healed through a scar tissue interface with the presence of some Sharpey-like fibers that became more organized at later time points. By 8 weeks, the stem cell–treated animals had higher failure loads and stiffness, whereas no differences were seen at earlier time points. We found that these results of graft healing in a bone tunnel could not be extrapolated to our rotator cuff model.

There are several possible explanations for our findings in light of the previous studies on MSCs in tendon-to-bone healing. The rotator cuff insertion of a rat offers less tendon-to-bone surface area for healing than a tendon graft in a bone tunnel. Studies have shown that the overall contact area between soft tissue and bone is a major determinant of healing.[10] There are also considerable shear forces distributed over this relatively small surface area from the pull of the supraspinatus muscle. This is particularly true as the animals were not immobilized postoperatively for practical reasons. This may have resulted in stresses that hindered the ability of the tendon to adequately heal regardless of the stimulus used. However, the rat has been used in a number of rotator cuff studies and is a generally accepted model by which to test healing.[9,27] We also used fewer MSCs than in previous studies because of the anatomic constraints of the rat rotator cuff. We used roughly 1 million cells as compared with the 3 to 4 million used in the previous rabbit studies.[4,17,20] Because the tendon-bone surface area in the rat rotator cuff model is roughly 25% of the overall surface area in a rabbit ACL model when one considers both tibial and femoral tunnels, we believe that the number of cells to surface area in our study was proportionate to that used in previous studies.

The application of a fibrin sealant also had no beneficial effects on healing when compared with the untreated controls in this study. Fibrin sealant has been investigated as an adjunct to improve the initial fixation strength and healing potential in tendon-to-bone healing models.[26,29] It has also been used as a carrier for growth factors and cells.[17,31] Most studies have shown that fibrin sealants alone provide minimal, if any, improvements in healing. Shoemaker et al[26] placed xenograft and autograft anterior and posterior tibialis tendons into bone tunnels created in the distal tibial metaphysis of dogs and evaluated whether the application of a fibrin sealant system could improve healing. They found that the fibrin sealant promoted organization and maturation of fibrous connective tissues at early time points, but failed to see differences by 28 days. Yamazaki et al[31] performed ACL reconstruction surgeries with flexor tendon autografts in dogs. They placed either fibrin sealant alone, fibrin sealant with transforming growth factor-β1, or nothing in the bone tunnels. They found that the fibrin sealant alone failed to improve the histology or pull-out strength as compared with controls, but that the application of transforming growth factor-β1 improved both. Our findings are similar to these in that the fibrin sealant alone provided no beneficial effects on healing.

There are several limitations to our study. First, we chose to analyze the animals at only 2 and 4 weeks. It is possible that we could have seen beneficial effects from the stem cells at later time points. We chose these early time points because the initial goal of the study was to detect an

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acceleration of healing. Furthermore, previous work in our laboratory with rat rotator cuff repair models has shown that most control tendons heal by 8 weeks. This makes it difficult to detect differences between control and experimental animals because it is hard to improve on solid healing. Lim et al[17] did not show biomechanical improvements until 8 weeks; however, they were using a rabbit model of ACL reconstruction that, in our experience, heals at a slower rate than a rat rotator cuff.[1,19]

Second, it is possible that there has been a type II statistical error (false-negative) for the biomechanical testing. The study was powered only for biomechanical testing based on previous data in Sprague Dawley rats. The Lewis rats used in this study are considerably smaller than Sprague Dawley rats. However, the mean values for the ultimate loads to failure were so similar across groups in each time point that it is unlikely that a clinically significant difference would be detected with more animal testing. Statistical error may be present in our histologic analysis. We attempted to quantify our histologic findings based on the area of cartilage formation and collagen birefringence, instead of relying simply on qualitative assessments. Given the inherent variations in animal models, 3 specimens in each group were not sufficient to show anything less than a profound difference in histologic appearance. However, our goal was to provide at least semiquantitative data to help alleviate the subjectivity inherent in histologic analyses. Both of these techniques have been used in previous studies for the same purpose.[5,15] In this study, the semiquantitative histologic data correlated with our qualitative assessments that there were no appreciable differences between groups at both time points.

This study highlights the difficulty of developing a biologic solution to the problem of tendon-to-bone healing in the rotator cuff. Although the application of MSCs has shown modest improvements in the healing of tendon grafts in bone tunnels, it appears that these results cannot be extrapolated to the complex biomechanical environment of the rotator cuff. It is possible that the application of stem cells alone is insufficient, and that a signal must also be provided that can induce regeneration. The repair site may lack the cellular and/or molecular signals necessary to induce appropriate differentiation of the transplanted cells, suggesting that cell-based strategies may need to be combined with appropriate growth and differentiation factors to be effective. In that scenario, the stem cells serve as the raw materials for regeneration, while the signal serves as the impetus toward that goal. Further studies are proceeding in our laboratory to determine if the application of stem cells concomitantly with either a growth or differentiation factor can improve healing.

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