Bone Marrow–Derived Mesenchymal Stem Cells Transduced With Scleraxis Improve Rotator Cuff Healing in a Rat Model

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Background: Rotator cuffs heal through a scar tissue interface after repair that makes them prone to failure. Scleraxis (Sex) is a basic helix-loop-helix transcription factor that is thought to direct tendon development during embryogenesis. The purpose of this study was to determine if the application of mesenchymal stem cells (MSCs) transduced with adenoviral-mediated scleraxis (Ad-Sex) could improve regeneration of the tendon-bone insertion site in a rat rotator cuff repair model.

Hypothesis: Bone marrow–derived cells transduced with Sex would improve the structure of the healing tendon-bone interface and result in increased tendon attachment strength.

Study Design: Controlled laboratory study.

Methods: Sixty Lewis rats underwent unilateral detachment and repair of the supraspinatus tendon. Thirty animals received MSCs in a fibrin glue carrier, and 30 received Ad-Scx-transduced MSCs. Animals were sacrificed at 2 weeks and 4 weeks and evaluated for the presence of fibrocartilage and collagen fiber organization at the insertion. Biomechanical testing was performed to determine the structural and material properties of the repaired tissue. Statistical analysis was performed with a Wilcoxon rank sum test with significance set at \( P = .05 \).

Results: There were no differences between the Sex and MSC groups in terms of histologic appearance at 2 weeks. However, the Sex group had higher ultimate stress-to-failure (2.6 ± 0.9 vs 1.7 ± 0.3 MPa; \( P = .03 \)) and stiffness (8.4 ± 2.9 vs 5.0 ± 1.9 N/mm; \( P = .01 \)) compared with the MSC group. At 4 weeks, the Sex group had more fibrocartilage (728.7 ± 50.4 vs 342.6 ± 217.0 mm\(^2\); \( P = .04 \)), higher ultimate load to failure (26.7 ± 4.6 vs 20.8 ± 4.4 N; \( P = .01 \)), higher ultimate stress to failure (4.7 ± 1.3 vs 3.5 ± 1.0 MPa; \( P < .04 \)), and higher stiffness values (15.3 ± 3.4 vs 9.3 ± 2.2 N/mm; \( P < .001 \)) as compared with the MSC group.

Conclusion: Mesenchymal stem cells genetically modified with Sex can augment rotator cuff healing at early time points.

Clinical Relevance: Biologic augmentation of acutely injured rotator cuffs with Sex-transduced MSCs may improve rotator cuff tendon healing and reduce the incidence of re-tears. However, further studies are needed to determine if this remains safe and effective in larger models.

Keywords: tendon-to-bone healing; rotator cuff; mesenchymal stem cells; scleraxis; Sex; gene therapy

Although rotator cuff repair surgery remains one of the most common and successful procedures in orthopaedic surgery, there is concern over the tendon’s ability to heal to bone in the postoperative period. Studies that have used imaging have shown that repaired tendons have a persistent defect at the repair site in a surprisingly high percentage of cases. \( 7,8,13,14,15,20,25 \) Patients who have persistent rotator cuff defects have worse functional results when compared with patients without a defect. A possible reason for this is that the normal histologic properties of the rotator cuff insertion are not re-created after repair. \( 8,10,11,24 \) The rotator cuff tendons normally insert into the bone through a highly specialized, fibrocartilaginous transition zone that dissipates force. After repair, a layer of fibrovascular scar tissue forms between the tendon and the bone. This scar tissue is weaker than normal tissue and may make repairs prone to failure. \( 6,24 \) Tendon-to-bone healing in the rotator cuff likely depends on a combination of the mechanical strength of the repair as well as biologic factors that influence healing.
This phenomenon is also similar in the tibial side. However, we could not identify a constant posterior and central position landmark. For these reasons, the posterior end of the Blumensaat line was chosen as a femoral landmark, and the connecting point between the anterior cortex of the proximal tibia and tibial plateau was selected as a tibial landmark.

This BAT method showed superior reproducibility in ACL-injured patients. On the other hand, although the BAT method showed the best results, all 5 methods are suitable for PCL-injured patients. This suggests that slight changes in rotation or flexion are permitted with this BAT method, although the problem of relaxation and large changes in rotation or flexion were not solved.

We checked both sides, but only the injured side was used for the analysis for a number of reasons. First, measurement differences are made at both sides, and the rotation and flexion could be different between sides. Second, the difference itself is smaller than is absolute measurement in case of mild instability, and the results could be magnified with small measurement error.

It is very important to define how much time occurred before and after examination for these radiographs. We repeated the same measurement after 3 weeks for the evaluation of ICCs. However, we checked stress radiographs twice (before and after the examination at the outpatient clinic) on the same day for the evaluation of test-retest reproducibility. It is possible to have some inherent bias in this type of radiograph, as the patient would have memory within 1 to 2 hours of their experiences in this circumstance. However, we intended this effect. In other words, this can minimize patient factor among variables because the patients would have memory of their experiences. Furthermore, there is little possibility of fundamental change of ligament characteristics.

CONCLUSION

Different methods for measuring stress radiographs resulted in different levels of reproducibility for both anterior and posterior instability using the Telos device. In the anterior drawer test, the BAT method showed the best measurement reliability and test-retest reproducibility. In the posterior drawer test, the BAT method showed favorable measurement reliability and reproducibility, but the superiority could not be demonstrated.

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Mesenchymal stem cells (MSCs) have shown promise in their ability to augment tendon graft healing in a bone tunnel. However, previous work in our laboratory has failed to show improvement in rotator cuff healing when MSCs are added to the repair site in a rat model. On the basis of these findings, we believe that bone marrow–derived stem cells alone are insufficient to augment rotator cuff healing where the contact area between the tendon and the bone is limited and the shear stresses on the repair are great. We hypothesized that growth and differentiation factors are necessary to guide the differentiation of the MSCs throughout the healing process to optimize their effectiveness. In a recent study, we found that MT1-MMP, a developmental gene present during the formation of the tendon-bone insertion site during embryogenesis, was able to improve rotator cuff healing at 4 weeks but not at 2 weeks after repair. Although these results were encouraging, they still left significant room for improvement in the development of a biologic strategy to augment rotator cuff repairs.

Scleraxis (Sex) is a basic helix-loop-helix transcription factor that has been implicated in tendon development and regeneration. Endogenous expression of Sex is detected early in mesoderm formation, defines a zone between sclerotomal and myotomal cells within the developing somite, and persists throughout formation of the tendon proper, as well as the tendon-bone insertion sites, in the mouse embryo. Specifically, Sex is believed to direct the tendinous attachments to bone. Studies have shown that overexpression of Sex in osteoblastic cell lines induces chondrocyte-specific gene expression. Furthermore, it is expressed in a close temporal and spatial association with Sox9, a transcription factor associated with cartilage differentiation. These findings of a simultaneous role for Sex in both tendon and cartilage development suggest its potential to improve regeneration of the rotator cuff insertion site consisting of the tendon and its cartilaginous transition zone into the bone.

The purpose of this study was to determine if bone marrow–derived MSCs transduced with adenoviral-mediated scleraxis (Ad-Sex) could improve healing when applied to the tendon-to-bone insertion site after rotator cuff repair. We hypothesized that the application of these cells would result in an insertion site that more resembled the native insertion site in terms of composition (regeneration of the fibrocartilaginous transition zone) and structure (more organized collagen fibers). Furthermore, it was hypothesized that these histologic improvements would correspond to stronger tendon-to-bone fixation as evidenced by biomechanical testing.

**MATERIALS AND METHODS**

**Study Design**

A total of 60 Lewis rats underwent unilateral detachment and acute repair of the supraspinatus tendon. Ten rats were used for MSC harvest, for a total of 70 rats that were used in this study. Approval for this study was obtained from our Institutional Animal Care and Use Committee. Lewis rats were chosen because they are inbred to the point they are considered syngeneic. Therefore, transplantation of cells from one rat to another is analogous to an autograft transplantation and limits the risk of graft rejection. Animals were randomized into 1 of 2 groups with 30 rats per group: MSCs in a fibrin glue carrier (TissueGel, Baxter AG, Vienna, Austria) (10^6 cells) or Ad-Sex-transduced MSCs in a fibrin glue carrier (10^6 cells). Fifteen animals in each group were sacrificed at 2 weeks, and 15 were sacrificed at 4 weeks. At each time point, 12 specimens were used for biomechanical testing, and 3 animals were used for histologic examination. Outcomes included semi-quantitative histologic analysis to determine the amount of fibrocartilage and collagen fiber organization. Biomechanical testing was performed to determine the structural and material properties of the repaired tendon.

Construction of Ad-Sex for Gene Therapy

The Ad-Sex virus for gene therapy was constructed in our laboratory using the AdEasy Adenoviral Vector System Kit (Stratagene, La Jolla, California) according to the manufacturer's protocol. Briefly, this adenoviral vector is based on the human adenoviral serotype 5 with deleted E1 and E3 genes and a multiple cloning site between a cytomegalovirus (CMV) promoter and a SV40 polyadenylation signal. The entire Sex gene was polymerase chain reaction (PCR)–subcloned from mouse Sex cDNA (gift of Ronen Schweitzer, PhD, Oregon Health and Sciences University) into the pShuttle-CMV vector (Stratagene) with the following primers: 5'-TAAGCGGCGGATGTCGCTGAGC CATGCTCCTTCGCTATGAGG-3' (forward, NotI site underlined, ATG start codon italicized) and 5'-TATTACGAGCTCT CACCTCGAATGCCGCTCTGCTG-3' (reverse, XhoI site underlined, TGA stop codon italicized). Successful subcloning was confirmed with DNA sequencing (Cornell DNA Sequencing Core Facility, Cornell University, Ithaca, New York). The pShuttle vector was then linearized with Pme I and transformed by electroporation into BJ5183 Escherichia coli cells that had been previously transformed with pAdEasy-1 (Stratagene) that encodes for the adenoviral backbone. Homologous recombination between the Sex cloned pShuttle vector and the pAdEasy plasmid occurred in vivo within the BJ5183 cells. Recombinants were subsequently identified by restriction digestion and again confirmed with DNA sequencing. The recombined adenovirus plasmid was transformed into XLI10-Gold cells (Stratagene) for amplification. The plasmid was linearized with Pme I and the linearized plasmid was used to transfect AD206 (Stratagene) cells with Polyfect Transfection Reagent (Qiagen, Valencia, California) according to the manufacturer's protocol. The transfected AD206 cells were harvested when approximately 90% of them exhibited cytopathic effects (cpe) and placed into 50-mL conical tubes. They underwent 4 freeze-thaw cycles followed by centrifugation at 12,000 g for 10 minutes to pellet the cellular debris. The supernatant was collected as a primary viral stock. The primary stock was used to transduce BJ5183 cells. Transduction of BJ5183 cells was accomplished by using the protocol.
viral stock was diluted in a minimal amount of infection medium (Dulbecco's modified Eagle's medium (DMEM), 1% antibiotic-antimycotic, and 2% fetal bovine serum (all from GIBCO, Gaithersburg, Maryland) and placed in 10 T-175 plates that were approximately 70% confluent with AD293 cells and incubated at 37°C on a rocker for 2 hours. Complete medium (DMEM, 1% antibiotic-antimycotic, and 10% fetal bovine serum) was added and the cells were cultured at 37°C until they reached 100% cpe. At this point, the medium and cells were harvested and the virus was purified with the Adeno-X Maxi Purification System (Clontech, Mountain View, California) using the manufacturer's protocol. Purified Ad-Sex virus was quantified using spectrometry and stored at −80°C until it was used.

Bone Marrow-Derived Mesenchymal Stem Cell Harvest and Culture

The bilateral femurs, tibias, and humeri of 10 Lewis rats euthanized by carbon dioxide inhalation were harvested under sterile conditions. Hank's balanced salt solution (GIBCO) was used to harvest MSCs from the intramedullary canals of the long bones. The cells were then washed with Red Blood Cell Lysis Solution (Sigma, St Louis, Missouri) 3 times and centrifuged at 2000 rpm for 5 minutes. The supernatant was discarded and the cell pellet was resuspended in 20 mL complete medium consisting of DMEM, 1% antibiotic-antimycotic, and 10% fetal bovine serum and then plated onto T-175 culture flasks. The cells were grown at 37°C for 2 days and were subsequently washed with complete medium, yielding bone marrow-derived MSCs adherent to the bottom of the flask. The cells were then isolated from the bottom of the flask with trypsin (GIBCO) and then subcultured. This process was repeated twice before the cells were either transduced with the Ad-Sex virus or suspended in the fibrin sealant for implantation.

Transduction of MSCs With Ad-Sex and Confirmation

Twenty-four hours before implantation, MSCs at 90% confluence in T-175 flasks were transduced with the Ad-Sex virus at a ratio of 10^8 physical particle counts/cell. This multiplicity of infection (MOI) was determined based on preliminary work with this adenoviral delivery system with an Ad-LacZ virus. The appropriate amount of viral solution was added to enough infection media to barely cover the cells for 2 hours at 37°C while the flasks were placed on a rocker. Complete medium was then added and the cells were incubated overnight. The following morning, the medium was aspirated and the cells were washed twice with phosphate-buffered saline (PBS). The cells were then trypsinized and harvested for implantation (described below).

An aliquot of transduced and untransduced MSCs from 2 plates each was saved to determine the Sex gene expression using reverse transcription followed by real-time polymerase chain reaction (RT-PCR). Total RNA was isolated from the cell samples using the TRizol Reagent (Invitrogen, Carlsbad, California) extraction method. Equal concentrations of mRNA were then reverse transcribed using the Bio-Rad iScript cDNA synthesis kit (Bio-Rad, Hercules, California) following the manufacturer's suggested protocol. The cDNA product was amplified and quantified through RT-PCR using iQ SYBR Green Supermix on a MyiQ Single-Color Real Time PCR Detection System machine (both Bio-Rad). All reactions were run for 40 cycles. Relative expression levels were calculated based on ACT values (the difference in the threshold cycle between Sex and the housekeeping gene GAPDH). The following primers were used for this analysis: mouse Sex forward 5'-TGGGCTCCAGCTACAGTTCT-3', mouse Sex reverse 5'-TGTCACGGTCCTTGCTGAAC-3', rat GAPDH forward 5'-TGCCACTCAGAAAGACTTGG-3', and rat GAPDH reverse 5'-GGATGCAGGGATGATGTTCT-3'.

Surgical Procedure

The surgical procedure has been previously described.

Briefly, the deltoid was split and the supraspinatus was detached from its footprint. A #15 blade knife was used to decorticate the area of the greater tuberosity directly underlying the original insertion of the supraspinatus tendon. Care was taken to ensure that all existing fibrocartilage was removed from the footprint and that only bleeding bone was seen. A Mason-Allen stitch was placed into the supraspinatus tendon with 3-0 Ethibond (Ethicon, Somerville, New Jersey). A 22-gauge needle was used to create 2 bone tunnels at the anterior and posterior margins of the insertion site. The sutures in the tendon were then passed through the tunnels.

The Lewis rats were randomly assigned to receive 1 of 2 different treatments: 30 animals received MSCs in a fibrin glue carrier, and 30 animals received Ad-Sex-transduced MSCs in a fibrin glue carrier. The fibrin sealant (Baxter AG) was composed of 2 separate solutions. Solution A was composed of thrombin dissolved in 40 mmol/L calcium chloride, whereas solution B was prepared by dissolving Tissec in 3000 KIU/mL aprotinin using a heated stirring rod (Fibrinotherm; Baxter AG). In both groups, 10^6 MSCs were harvested and mixed with 25 μL solution A on the morning of surgery, which was subsequently added to solution B using a Duploject System (Baxter AG). This system allowed the simultaneous application of equal amounts (25 μL) of solution A and solution B at the interface between the tendon and the bone for a total of 50 μL. This protocol has been used in previous studies to deliver cells to a healing site.11,16,20 The amount of MSCs used, 10^6 cells, was based on the technical limitations of the surgical procedure. Previous studies on the effects of MSCs to improve healing in a rabbit ACL model used 3 to 4 × 10^6 cells.5,17,26 However, the overall surface area of a rat supraspinatus footprint is much less than 26% of the total surface area in a rabbit ACL model when one considers both the tibial and femoral tunnels. Therefore, we thought that the number of cells was proportionate to the surface area we were working with in this model based on previous studies.
The techniques for MSC harvest, transduction, and transplantation have been validated in a previous study. In that study, MSCs transduced with an adenovirus containing the reporter gene for beta-galactosidase, LacZ, were implanted at the rotator cuff repair site using the same protocol. Specimens were sacrificed at 2 and 4 weeks, and the presence of LacZ was confirmed by blue staining at the repair site when the samples were treated with X-Gal, beta-galactosidase's substrate. These data demonstrate that these cells are able to survive the transplantation process and remain metabolically active up to 4 weeks after transplantation. The exact number of cells that survive the implantation process was not quantified.

Histomorphometric Analysis

Three animals in each group were euthanized at 2 weeks and at 4 weeks for histologic examination. The shoulder of each animal was dissected to yield a specimen consisting only of the humerus with the attached supraspinatus tendon and muscle. Each specimen was fixed in 10% neutral buffered formalin, decalcified with Immunocal (Decal, Congers, New York), and embedded in paraffin with the tendon at approximately 45° from the bone. Multiple sections were cut in the coronal plane through the repaired supraspinatus tendon and the greater tuberosity. The sections were 5 microns thick and were subsequently used to make slides stained with hematoxylin and eosin (H&E), safranin-O/fast green, and picrosirius red. An Olympus BH-2 light microscope (Olympus Opticals, Lake Success, New York) was used to examine the greater tuberosity, repaired supraspinatus tendon, and the midsubstance of the supraspinatus tendon under light and polarized light microscopy. Digital images were taken using a SPOT RT camera (Diagnostic Instruments, Sterling Heights, Michigan), and semi-quantitative analyses were performed using computerized image analysis (Image J, National Institutes of Health) to determine the amount of fibrocartilage based on the area of metachromasia with safranin-O staining and collagen fiber organization based on birefringence under polarized light microscopy on the picrosirius red-stained slides. Semi-quantitative histomorphometric analyses were performed by 3 independent observers (LVG, DK, SAR) who were blinded as to the group and time point of the specimens. Specific details of the methods for both of these analyses have been previously described.

Biomechanical Testing

Twelve animals per group were euthanized at 2 weeks and at 4 weeks for biomechanical testing. All testing was performed in a blinded fashion. After thawing at room temperature overnight, the supraspinatus tendon was isolated by carefully dissecting the supraspinatus muscle belly away from the tendon. The sutures were also removed. Digital calipers were used to measure the cross-sectional area of the supraspinatus tendon at the point of insertion into the humerus, and a rectangular shape was assumed. The specimen was then transferred to a custom-made uniaxial testing system and secured in a screw grip using sandpaper and ethyl cyanoacrylate (Krazy Glue, Elmer's Products, Inc, Columbus, Ohio). A 45-N load cell was 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. Each specimen was initially preloaded to 0.10 N. The tendon was then loaded at a rate of 14 microns/s until the tendon repair failed, at which point the maximum load at failure and failure site were recorded. The rate of loading created a tendon strain of roughly 0.4%. A 1-micron resolution micrometer was used to measure displacement. Stiffness was calculated by determining the slope of the linear portion of the load displacement curve. Ultimate stress at failure was calculated by dividing the ultimate force at failure by the cross-sectional area. This testing protocol has been used in previous studies from our laboratory.

Statistical Analysis

At the onset of the study, a power analysis was performed with the primary outcome being biomechanical testing of tendon attachment strength, whereas histomorphometric analysis was a secondary outcome. Our power analysis was based on a previous study that evaluated rotator cuff tendon healing in Lewis rats. In that study, the average ultimate tensile load to failure was 11.2 ± 2.3 N at 2 weeks and 20.8 ± 4.4 N at 4 weeks. A strength increase of 20% between 2 groups at the same time point was considered clinically significant for this study. Using these estimates, a power of 0.80 is achieved using 12 specimens per group with α = 0.05 for biomechanical testing. The SigmaStat program (Jandel Scientific, San Rafael, California) was used to perform the power calculation. All semi-quantitative histomorphometric and biomechanical testing outcomes were expressed as mean ± standard deviation. Nonparametric statistical methods were used for all analyses because of the nonnormality of the data in the groups being compared. Wilcoxon rank sum tests were used, and a P value ≤ 0.05 was used to indicate a statistically significant difference between groups. SAS statistical software (Version 9.1; SAS, Cary, North Carolina) was used to perform the analyses.

RESULTS

In Vitro Confirmation of MSC Transduction

Quantitative real-time PCR confirmed that the in vitro Sox gene expression was 115-fold greater in the transduced MSCs when compared with the untransduced MSCs (Figure 1).

Histomorphometric Analysis

Fibrovascular scar tissue was found at the tendon-bone interface in all specimens. At 2 weeks, there were no differences in the area of metachromasia between the Ad-Sox
Biomechanical Testing

All specimens failed at the tendon-bone interface during biomechanical testing. The data for 1 specimen in the Ad-Sex group at 2 weeks and 1 specimen from the MSC group at 2 weeks were discarded because of slippage of the tendon in the testing grips, resulting in an irregular load displacement curve.

At 2 weeks, there was an increase in the ultimate stress to failure in the Ad-Sex group (2.6 ± 0.9 MPa) compared to the MSC group (1.7 ± 0.3 MPa; \( P = .03 \)) (Figure 3). There was also an increase in stiffness in the Ad-Sex group (8.4 ± 2.9 N/mm) compared with the MSC group (5.0 ± 1.9 N/mm; \( P = .01 \)). There were no differences at 2 weeks between the groups in terms of the ultimate load to failure or the cross-sectional area.

At 4 weeks, the ultimate load to failure was greater in the Ad-Sex group (26.7 ± 4.6 N) as compared with the MSC group (20.8 ± 4.4 N; \( P = .01 \)) (Figure 3). The Ad-Sex group had a higher stiffness (15.3 ± 3.4 vs 9.3 ± 2.2 N/mm; \( P < .001 \)) and a higher ultimate stress to failure (4.7 ± 1.3 vs 3.5 ± 1.0 MPa; \( P < .04 \)). There were no differences at 4 weeks in terms of the cross-sectional area of the insertion site.

There were statistically significant increases in the ultimate load to failure, the stiffness, and the ultimate stress to failure within each group from 2 weeks to 4 weeks. This corresponded to an increase in the amount of metachromasia from 2 to 4 weeks within the Sex group, but there was no difference in the amount of metachromasia from 2 to 4 weeks in the MSC group.

**DISCUSSION**

In this study, we demonstrated a significant improvement in tendon-to-bone healing when specimens were treated...
MSCs Transduced With Scleraxis to Improve Rotator Cuff Healing

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Figure 3. The results of the biomechanical testing. A, There were higher ultimate loads to failure in the scleraxis (Scx) group compared with the mesenchymal stem cell (MSC) group at 4 weeks. B, There were higher values for stiffness in the Scx group at 2 and at 4 weeks. C, There were no differences between groups at either time point in terms of cross-sectional area of the tendon at the insertion site. D, There were higher ultimate stresses to failure at 2 and 4 weeks in the Scx group. (Bar graphs represent the mean for each group, and error bars represent ±1 standard deviation. *P = .05.)

with Ad-Scx-transduced MSCs when compared with the use of MSCs alone. Scx overexpression resulted in greater stiffness and stress at failure at 2 and 4 weeks, as well as an increased load to failure at 4 weeks. These findings corresponded to more fibrocartilage at the insertion site of the Ad-Scx-treated specimens at 4 weeks, which more closely resembles the native fibrocartilaginous insertion site of the rotator cuff. The amount of fibrocartilage seen in the healing insertion site at 4 weeks was still much less than that seen in the normal insertion site, but it is relatively early in the healing process. Although the numbers in this study were insufficient to determine a statistical correlation between fibrocartilage formation and mechanical strength of the repair, it is logical to deduce as an association.

The development of biologic therapies to augment rotator cuff healing is a challenging but worthwhile goal. 

Radiologic studies have shown that the repair site has a persistent defect in up to 94% of cases.7,8,13,14,16,18,25 Although many of these patients experience symptomatic relief, patients with these defects have poorer shoulder function when compared with patients without defects. Attempts to improve the biomechanical properties of the repair, through various suture techniques such as double row or transosseous equivalent techniques, have had little effect on rates of postoperative radiographic defects at the repair site. This has led investigators to pursue biologic therapies that can augment healing.

Stem cell–based therapies to augment rotator cuff healing are attractive because they provide a renewable source of pluripotent cells that can contribute to the healing environment. Although several studies have shown improvements in soft tissue graft healing in a bone tunnel,6,17,20 previous work in our laboratory has shown that MSCs alone were insufficient to improve healing in the same rat rotator cuff repair model used in this study.11 This led us to investigate potential factors that can signal the differentiating cells to augment regeneration of the native tendon-to-bone
insertion site. We have previously reported our results with the developmental gene MT1-MMP. The Ad-MT1-MMP-transduced MSCs were able to improve healing based on histologic and biomechanical properties at 4 weeks when compared with specimens treated only with MSCs. However, there were no differences in any outcome variable at 2 weeks. In this study, the use of Ad-Scx-transduced MSCs resulted in improvements in histologic and biomechanical properties at 2 weeks as well as 4 weeks.

The mechanism by which Scx exerts its healing effects is unknown and beyond the scope of this study. Theoretically, Scx may act through several mechanisms to promote tendon-to-bone healing. Because it is a transcription factor, it is possible that gene transfer of Scx into MSCs causes differentiation of these cells into tenocytes, which results in more collagen production. However, because there was also an increase in the amount of fibrocartilage as well as the material properties of the repaired tendons, we believe that Scx acts through a more complex signaling mechanism that directs the transition of tendon to bone through a cartilaginous transition zone. The mechanisms by which this occurs are just starting to be elucidated. Blitz et al. showed that Scx regulates BMP-4 expression in tendon cells and directs the formation of the deltid bone ridge that ultimately serves as its tendinous insertion site. Furumatsu et al. have also shown that Scx, together with E47, cooperatively regulates Sox9 transcription. Sox9 is thought to play a significant role in the formation of cartilage. Future studies should investigate the ability of Scx to induce tenoblastogenesis in vitro, as well as its ability to coordinate the formation of the fibrocartilaginous transition zone. Other future areas of interest include the role of scleraxis in the formation of mineralized fibrocartilage, which may be more important than the amount of unmineralized fibrocartilage in the overall strength of the insertion site.

There are several limitations to this study. Semi-quantitative histologic analysis failed to show any difference in collagen fiber organization between the Ad-Scx group and the MSC group at either time point, nor were there any differences between groups in the amount of fibrocartilage formation at 2 weeks. Only 3 animals per treatment group were used for histologic analysis, and this facet of the study may have been statistically underpowered. Thus, it is possible that a difference between the Ad-Scx group and the MSC group did exist at 2 weeks but was not observed because of a type II error. Other possible explanations for the observed biomechanical improvements at 2 weeks in the absence of histological improvement include increased collagen cross-linking, improvements in the collagen ultrastructure, or an increase in the production of other noncollagenous matrix proteins. However, these factors were not directly tested for in this study, and these possibilities remain only speculative. Regardless of the biochemical changes at the insertion site, the primary outcome in this study was ultimate load to failure because that is the outcome most clinically applicable to rotator cuff repair surgery.

There are limitations to the single-plane polarized light microscopy used in our methods. A more accurate means would be to perform circularly polarized light microscopy or computerized image analysis algorithms. However, care was taken to ensure that the orientation of our slides and the intensity of the light source were consistent. Also, we used a contact method to determine the cross-sectional area of the unloaded insertion site, and this may have introduced variability into those measurements, as well as the stress measurements. However, the same person performed these, so the error that was introduced was a systematic one that should affect both groups in the same manner.

Another limitation is that we did not track Scx expression in vivo and thus cannot be entirely certain that the MSCs were present at the tendon-bone insertion site and that these cells were overexpressing Scx. However, expression of Scx by transduced MSCs was confirmed with RT-PCR in vitro. We have proven in a previous study that our model is able to deliver and overexpress the LacZ gene at the repair site as late as 4 weeks after application.

In summary, this study showed that MSCs genetically modified to overexpress Scx can aid in the regeneration of the tendon-bone insertion site based on histologic and biomechanical outcomes at early time points. The mechanism by which this works is unknown, but given the encouraging results in this small animal model, it is worthy to investigate these mechanisms further. Further studies are needed to determine whether this remains safe and effective in larger models.

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Outcomes of Revision Arthroscopic Type II Superior Labral Anterior Posterior Repairs

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Investigation performed at the Kerlan-Jobe Orthopaedic Clinic, Los Angeles, California

Background: Outcomes of arthroscopic type II superior labral anterior posterior (SLAP) repairs have been reported with success. However, published data regarding outcomes of revision arthroscopic type II SLAP repairs are lacking.

Hypothesis: Outcomes of revision arthroscopic type II SLAP repairs are inferior to those of primary repairs.

Study Design: Case series; Level of evidence, 4.

Methods: A retrospective chart review was performed to identify patients who had undergone revision arthroscopic type II SLAP repairs at our institution. Patients who underwent concomitant rotator cuff repairs or labral repairs for instability were excluded. Twelve patients were contacted, and the following outcome data were prospectively gathered: American Shoulder and Elbow Surgeons (ASES) score, patient satisfaction level, return to work, return to sports, and physical examination. Demographics and intraoperative report data were also collected from the charts.

Results: The mean age at the time of revision arthroscopic type II SLAP repairs was 32.6 years (range, 19-67 years) with a mean follow-up of 50.5 months (range, 8-81 months). There were 5 workers’ compensation patients and 6 overhead athletes. Pain was the chief complaint at the time of initial and revision SLAP repairs. The mean ASES score was 72.5, patient satisfaction level was 6.4 (scale of 0-10), mean return to work was at 57.8% of the previous level, and mean return to sports was at 42.2% of the previous level. In overhead athletes, mean return to sports was at 41.3% of the previous level, and none of the 4 baseball players returned to preinjury level. The mean values for all outcome data and range of motion values were lower in workers’ compensation patients. There were no reported complications, but 2 patients required additional arthroscopic surgeries.

Conclusion: Arthroscopic revision type II SLAP repairs yield worse results than primary repairs as reported in the literature, with workers’ compensation patients and overhead athletes doing especially worse. A larger prospective study of this relatively rare procedure is needed to better determine which patients may benefit from this procedure.

Keywords: SLAP; revision; outcomes; superior labrum anterior posterior

Superior labral anterior posterior (SLAP) lesions were first described by Andrews et al2 and classified into 4 types by Snyder et al.23 Subsequently, several additional types were described and added to the classification.16,20 Of these, the type II lesions are the most common23 and are characterized by detachment of the superior labrum/biceps anchor from the glenoid with resulting instability of this complex.

The first line of treatment for type II SLAP lesions is generally nonoperative, and surgical intervention is reserved for those who are refractory to nonoperative treatment. Although level I and II studies are lacking, the outcomes of arthroscopic type II SLAP repairs have been generally favorable.4,15,18,19,51,26 However, there are patients who remain dissatisfied after arthroscopic type II SLAP repair and necessitate further surgical treatment.11 In those patients who sustain a retear or lack of healing of the original lesion, revision arthroscopic type II SLAP repair can be performed and may be a reasonable option. But currently, there are no data regarding the outcome of this treatment.

The purpose of this study was to examine the subjective and objective outcomes of revision arthroscopic type II SLAP repairs performed at our institution. We hypothesized that the outcomes would be inferior to those of primary arthroscopic type II SLAP repairs reported in the literature.

MATERIALS AND METHODS

Institutional review board approval was obtained before this study. A retrospective chart review was performed to identify patients from January 1, 2003 to July 1, 2009 who had undergone arthroscopic revision type II SLAP repairs at our institution. Inclusion criteria were as follows: history of arthroscopic type II SLAP repairs with suture anchors and patients who are able to be contacted for collection of outcomes data. To best isolate the effect of...