The purpose of this study is to determine if bipolar radiofrequency energy (bRFE) can enhance delayed surgical repair of acute supraspinatus tendon tears. Bilateral supraspinatus tendon tears were created in 42 Sprague-Dawley rats and repaired at 6 weeks either with or without bRFE augmentation. There were 8 control (sham) rats. Treatment rats were euthanized at 4, 8, and 12 weeks after repair. All specimens underwent biomechanical and histologic evaluation. Compared with standard repair, bRFE-treated repairs showed a greater average maximum stress (8.475 N/m² versus 3.95 N/m²) at 12 weeks, which was not significant (P < .11). The mode of failure was by humeral fracture in 57.14% with bRFE versus 14.29% without bRFE. Histologically, both standard and bRFE-treated repairs were indistinguishable from controls at 12 weeks. The use of bRFE showed no definitive effect on delayed repair of acute rat rotator cuff tears. (J Shoulder Elbow Surg 2007;16:640-648.)

Symptomatic rotator cuff tears significantly affect health and function and result in high costs in lost wages and treatment. Surgical repair requires 4 to 6 weeks of relative immobilization, with early restrictions on motion to minimize the chances for repair failure. Despite biologic healing, reported failure rates range between 5% and 70%.1,6,7,11,12,19,24,27 Methods to enhance rotator cuff healing have the potential benefit of earlier and more aggressive mobilization, reduced complications associated with stiffness, shorter overall rehabilitation periods, lower lost wages and disability, and possibly lower anatomic failure rates.

One potential source of rotator cuff repair augmentation is the use of bipolar radiofrequency energy (bRFE). The mechanism by which bRFE works is through a disruption of collagen molecular bonds, which triggers healing through a controlled inflammatory and angiogenic response.2,22,28 This beneficial effect of bRFE on healing has been demonstrated in several animal studies. A porcine study investigating a bRFE catheter placed percutaneously into the ventricular cavity of the heart demonstrated significant increases in vascular endothelial growth factor (VEGF) production, creation of local microvessels, and increases in local perfusion.13 A rabbit study evaluating Achilles tendons treated with a bRFE probe demonstrated an inflammatory response with significant elevations in VEGF compared with controls.3

In humans, bRFE has been used to stimulate a quicker recovery compared with laser during cosmetic surgery.9 In ear, nose, and throat surgery, a randomized, controlled, double-blind study showed that the use of bRFE during tonsillectomy resulted in quicker recovery times and improved results compared with conventional techniques.4,10

Percutaneous myocardium revascularization (PMR) in patients with congestive heart failure also has shown positive clinical outcomes.14 In a clinical pilot study of 20 patients with symptomatic areas of tendinosis of the Achilles tendon, patellar tendon, or epicondylar tissue, patients treated with bRFE demonstrated a decrease in symptomatic pain and an increase in daily function.23

To our knowledge, no current studies have evaluated the use of bRFE to enhance rotator cuff tear repair. Excluding primates, the use of the rat shoulder model most closely approximates the human shoulder.21 Previous work has determined that standard repair techniques result in inadequate recreation of the supraspinatus insertion site at 4 months after repair without biologic augmentation.25 In this study, we explored the use of bRFE as an adjunct to delayed repair of acute rat rotator cuff tears.

MATERIALS AND METHODS

The study was approved by our Independent Animal Use Committee (IACUC #02-049). Fifty Sprague-Dawley rats (weight, ≥400 grams) were used. Complete bilateral supraspinatus tendon tears were surgically created in 42 rats...
A sham group consisting of 8 rats (16 shoulders) underwent the same operative exposure of the supraspinatus tendon without tear creation. All rats were anesthetized with a combined ketamine (100 mg/kg) and xylazine (5 mg/kg) intraperitoneal injection. The upper extremities were shaved and aseptically prepared. The surgical exposure involved a 1-cm to 2-cm transverse incision centered over the lateral border of the acromion. The deltoid was split distally 8 to 10 mm to expose the greater tuberosity. The supraspinatus tendon was visualized as it passed through the bony arch created by the coracoid, acromion, and clavicle to its insertion on the greater tuberosity of the proximal humerus. The insertion of the supraspinatus tendon was further exposed by abducting and externally rotating the humerus.

Bilateral full-thickness supraspinatus tendon defects were created in the 42 treatment rats by incising the tendon at its insertion and excising approximately 2 mm of distal tendon. Failure to excise 2 mm of distal tendon resulted in spontaneous healing of the tendons in several prestudy trials. The deltoid muscle was then approximated using 4-0 Vicryl suture (Ethicon, Somerville, NJ) and the skin closed with absorbable 3-0 Monocryl suture (Ethicon).

Postoperatively, the rats were allowed to move freely in their cages. A pilot study (unpublished data) using the above technique resulted in nonhealed tendons at 6 weeks, allowing for subsequent repair.

After 6 weeks, the 42 rats with bilateral supraspinatus tendon tears underwent operative repair of the tear using the same anesthetic technique described previously. The surgical exposure was similar, with the exception of an additional split made in the trapezius 5 to 8 mm proximally from the acromion with division of the acromioclavicular capsule to obtain a more adequate exposure to the scarred and retracted rotator cuff tendon. The left shoulder underwent a transosseous repair using 5-0 Proline (Ethicon) placed in a modified Mason-Allen configuration through the tendon edge and then brought into approximation with the native insertion site by using a single bone tunnel (Figure 1).

The right shoulder underwent the same repair with the addition of a single application of the TOPAZ microdébrider (ArthroCare, Inc., Sunnyvale, CA). The microdébrider was connected to an Arthrocare 2000 generator using identical settings to those used in a pilot study by Tasto et al.23 with a voltage setting of 4 (175 V RMS) and a preset 0.5 second duration. The probe tip was placed by hand exactly perpendicular to the specimen 1 to 2 mm medial to the repair edge, using only the weight of the probe. The force created was measured at 15 grams. Closure was identical to the first surgical technique with the addition of the trapezius repair. The acromioclavicular joint capsule was not repaired.

The sham group (8 rats) also underwent a second surgical exposure without defect creation or repair.

Postoperatively, the rats were allowed to move freely in their cages. The 42 treatment rats (84 shoulders) were randomly euthanized using carbon dioxide gas at 4 weeks (14 rats), 8 weeks (14 rats), and 12 weeks (14 rats) postoperatively from the time of tendon repair. Within each of these groups, 8 rats (16 shoulders) were randomly assigned to biomechanical testing and 6 rats (12 shoulders) to histologic testing. The 8 sham rats were all euthanized at 8 weeks postoperatively, with 4 rats (8 shoulders) assigned to biomechanical testing and 4 rats (8 shoulders) to histologic testing (Figure 2).

All specimens were immediately dissected after euthanasia. Specimens included the intact supraspinatus muscle and tendon, which was left attached to its insertion onto the greater tuberosity of the humerus. The whole humerus was also left intact to maximize the length of bone available for potting during biomechanical analysis.

Gross pathology

The surgical site was inspected after euthanasia for tendon healing, inflammation, and character of the healed tendon. The specimens were wrapped in saline-soaked gauze, placed in 2 plastic bags, and frozen at −20°C for later analysis.

Histology

The thawed humerus was detached from the scapula, the supraspinatus tendon and muscle was kept intact, and it was fixed in 10% neutral buffered formalin. Sections (8 μm) from the entire supraspinatus attachment were obtained and placed on glass slides coated with Vectabond (Vector Laboratories, Burlingame, CA). Alternating sections were
stained with hematoxylin and eosin (H&E), safranin-O fast green (SO), or Picrosirius red (PSR) using well-established techniques. Sections were studied in detail with standard light microscopy and also with a Nikon polarization microscope (Tokyo, Japan) equipped with a lambda/4 compensator plate and interference filter (λ = 589 nm).

The relative sign of induced birefringence was determined by turning the analyzer in 2 opposite directions. The optical properties of the extracellular matrix (ie, the presence or absence of birefringence indicating orientation of the collagen fibers) was observed. The most representative histologic sections in each specimen were examined and scored in a fashion similar to that described by Carpenter et al.3

Our scoring system accounted for (1) transition zone architecture in H&E sections, (2) cellularity in H&E sections, (3) proteoglycan staining in SO-stained sections, and (4) collagen fiber orientation in PSR-stained sections. For each category, the specimen was graded on the following scale: 0 = normal, + = mild changes, ++ = moderate changes, and +++ = marked changes. A number of other histologic features were also noted, including articular cartilage, inflammation, foreign body reaction, and vascularity. The sections were blindly read by 2 different observers, with the final score being an average of each of the two readings.

Biomechanical testing

On the day of biomechanical analysis, specimens were thawed and moistened using a phosphate-buffered solution bath and kept at precisely 39°C until immediately before testing. All remaining muscle was stripped from the tendon. An established technique by Carpenter et al5 was used to fashion the tendon precisely into a dumbbell shape (5-mm gauge length, 3.5-mm width). Specimen thickness was measured manually using a digital caliper (Precision Graphic Instruments, Inc, Spokane, WA) by observing when the caliper arms came into contact with the specimen. This measurement was recorded and used to calculate the cross-sectional area of the tendon.

The humerus was then potted using poly-isocryl bone cement (Isocryl, Lang Dental, Chicago, IL). The free tendon side was fixed to a custom-made cryoclamp. A material testing system (Instron Model 8871, Canton, MA) was used to apply a uniaxial load to each specimen at a constant strain rate of 14 μm/s (approximately 0.4%/s) and at an angle of 110° from the humerus.3,26 Specimens were pretensioned to 5 N. The force and displacement were recorded using Instron com-
puter software (Instron Corporation, Canton, MA), and failure mechanism was observed and recorded.

Statistical analysis
Data were entered and evaluated using SPSS 11.5 software (SPSS Inc, Chicago, IL). Descriptive statistics were calculated, including frequencies, mean, standard deviation, and minimum and maximum values. Analysis with the Kruskal-Wallis test was used for comparison of between groups due to the nonnormal nature of the data. Analysis by \( \chi^2 \) was used for nominal data. Results were considered statistically significant at \( P < .05 \).

RESULTS
Gross and histologic analysis
The gross appearance of the supraspinatus tendon during the initial surgery was similar in all rats. No preexisting tears, tendinosis, or inflammation were observed in any specimen. During the second surgery (after creation of the iatrogenic tear), gross analysis showed scar formation and tendon retraction in all specimens, with a dull and thickened appearance. Sham rats at the identical interval had tendons that were normal in appearance. The gross appearance of repaired tendons showed a grossly intact repair in all specimens at 4, 8, and 12 weeks. No difference in gross appearance could be identified between treatment groups (standard versus bRFE) at any of the treatment periods.

In all sham control specimens, the insertion site transition zone of the supraspinatus into the humerus consisted of collagen fibers interwoven into the uncalcified zone of cartilage, which blended directly into the calcified layer and subsequently gave rise to the bone of the humeral head. Normal proteoglycan staining could be demonstrated around the cells in the transition zone. Cells in the sample were evenly distributed. Fibroblasts were oriented along the longitudinal axis of the collagen fibers without any signs of hypercellularity or hypocellularity. Collagen was highly organized and oriented longitudinally. There were no signs of inflammation. Articular cartilage appeared histologically normal. No tears in the tendon were encountered (Figure 3).

At 4 weeks after repair in the treatment groups, no qualitative difference between the standard and bRFE group was observed. Histologic analysis revealed a smaller zone of calcification and an overall thinner transition zone. There was grossly more fibrous tissue and minimal proteoglycan staining. Cellularity was increased without any signs of inflammation. Fibroblast clustering was observed in several specimens. Collagen was moderately disorganized (Figure 4).

At 8 weeks after repair, the transition zone of the bRFE group appeared slightly thicker than the standard group. Proteoglycan staining remained minimal in both groups. Cellularity was decreased when compared to the 4-week specimens, but even spacing was once again observed for both groups. Neither group showed signs of inflammation. Minimal fibroblast clustering was seen. Collagen was minimally disorganized (Figure 5).

At 12 weeks after repair, there was a qualitatively thicker area of cells in the transition zone in both groups. In 1 sample in the standard repair group, a longitudinal intrasubstance tear was observed adjacent to the insertion site, with clusters of cells with intense pericellular proteoglycan staining. No signs of inflammation were seen. Cellularity was nearly normal. Collagen organization was essentially normal (Figure 6).

Using the objective scoring system described by Carpenter et al,\(^3\) the overall histologic grade was 0.30 for
the sham group. The overall histologic grade of the standard treatment group and bRFE group was 2.2, 2.1, and 1.7 and 2.4, 1.9, and 1.3 at 4, 8, and 12 weeks, respectively (Figure 7). There was no statistically significant difference between bRFE and standard treatment groups at any of the time intervals. Comparison of the overall histologic grade between the sham and both treatment groups (standard and bRFE) was statistically different at 4 and 8 weeks (P < .05) but not at 12 weeks.

**Biomechanical results**

During biomechanical testing, the tendon cryoclamp failed in 5 specimens (1 standard repair and one bRFE at week 4, 1 standard at week 8, and 1 standard and one bRFE at week 12), and their data could not be collected. All other specimens were tested successfully, producing the expected load-deformation curves seen in tendinous tissue. The toe region of the curve was minimal and was likely the result of pretensioning. The sham group showed an average max stress of 7.89 N/mm² and an average tissue modulus of 28.89.

Biomechanical testing of the standard repair group showed an average maximum stress of 4.99 N/mm² at 4 weeks, 3.80 N/mm² at 8 weeks, and 3.95 N/mm² at 12 weeks. All of these values were inferior to the sham control group, showing statistical significance, even 12 weeks after surgical repair (Table I). The tissue modulus was calculated to be 18.03, 20.89, and 33.37, at 4, 8, and 12 weeks, respectively. The standard repair group showed no statistically significant difference by 8 weeks.

The bRFE treated repair group had an average max stress of 7.05 N/mm², 6.28 N/mm², and 8.48 N/mm² at 4, 8, and 12 weeks, respectively (Figure 8). When compared with the control group, there was no difference by week 8. The tissue modulus was significantly different than the sham group at week 4, but was statistically similar at weeks 8 and 12 (Table I).

Comparison between the standard and bRFE enhanced repair groups showed an increasing disparity in maximum stress between the 2 groups at 8 and 12 weeks after repair, which did not reach statistical significance (P = .17 and P = .11, respectively; Table I). Comparison of modulus also revealed no statistically significant difference.

During biomechanical testing, failure of the specimen occurred either through rupture of the tendinous repair or fracture through the humeral neck in all specimens. All sham control specimens failed through humeral neck fracture. In both treatment groups, fracture was seen in 14% to 28% in the 4-week and 8-week groups. At 12 weeks, the incidence of fracture in the bRFE repair group was 4 times higher.
than the standard repair group (57.14% versus 14.29%). However, this did not reach statistical significance ($P = .11$; Table II).

**DISCUSSION**

This study assessed the effect of bRFE on the healing of rotator cuff tendons. Previous animal studies have looked at various aspects of supraspinatus tendon healing and the resulting biomechanical and histologic changes.3,8,21,25,26 None of these studies duplicates the current scenario of delayed repair of complete supraspinatus tendon tear used in this study. Thomopoulos et al.25 studied the histologic and local expression of extracellular matrix components in immediately repaired rotator cuff tears during a 16-week period. Gimbel et al.8 studied the histologic and biomechanical properties of unrepaired, complete supraspinatus tendons. In both of these studies, they found poor quality repair tissue during initial time intervals, which approached normal architecture by 16 weeks.

In our experiment, both treatment groups showed a minimal increase in cellularity by 4 weeks and histologically similar tendons by 12 weeks. Although these results cannot help us delineate the relative

![Table I: Biomechanical properties of experimental groups](image)

<table>
<thead>
<tr>
<th>Sample week</th>
<th>Max stress (n/mm²)</th>
<th>Tissue Modulus</th>
<th>P value* vs Std</th>
<th>vs bRFE</th>
<th>vs Sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>4.99</td>
<td>18.03</td>
<td>—</td>
<td>.39</td>
<td>.014</td>
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<tr>
<td>bRFE</td>
<td>7.05</td>
<td>15.39</td>
<td>.39</td>
<td>.35</td>
<td>.002</td>
</tr>
<tr>
<td>Sham</td>
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<td>28.89</td>
<td>.014</td>
<td>.002</td>
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</tr>
<tr>
<td>8 week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>3.80</td>
<td>20.89</td>
<td>—</td>
<td>—</td>
<td>.23</td>
</tr>
<tr>
<td>Sham</td>
<td>7.89</td>
<td>28.89</td>
<td>—</td>
<td>—</td>
<td>.23</td>
</tr>
<tr>
<td>12 week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>3.95</td>
<td>33.37</td>
<td>—</td>
<td>.35</td>
<td>.20</td>
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<tr>
<td>bRFE</td>
<td>8.48</td>
<td>35.31</td>
<td>.004</td>
<td>.004</td>
<td>.161</td>
</tr>
<tr>
<td>Sham</td>
<td>7.89</td>
<td>28.89</td>
<td>—</td>
<td>—</td>
<td>.23</td>
</tr>
</tbody>
</table>

Std, Standard repair; bRFE, bipolar radiofrequency energy.

*Statistically significant values in bold ($P < .05$).

![Figure 8: Comparison of maximum stress at 4, 8, and 12 week after repair in sham (patterned bar), standard (solid bar), and bipolar radiofrequency energy (bRFE, clear bar) treatment groups. Error bars indicate 1 standard deviation.](image)

![Table II: Mode of failure of experimental groups](image)

<table>
<thead>
<tr>
<th>Sample week</th>
<th>Repair site rupture (%)</th>
<th>Humeral neck fracture (%)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>85.71</td>
<td>14.29</td>
<td>.52</td>
</tr>
<tr>
<td>bRFE</td>
<td>71.43</td>
<td>28.57</td>
<td></td>
</tr>
<tr>
<td>8 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>85.71</td>
<td>14.29</td>
<td>.55</td>
</tr>
<tr>
<td>bRFE</td>
<td>75.00</td>
<td>25.00</td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>12 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>85.71</td>
<td>14.29</td>
<td>.11</td>
</tr>
<tr>
<td>bRFE</td>
<td>42.86</td>
<td>57.14</td>
<td></td>
</tr>
</tbody>
</table>

bRFE, Bipolar radiofrequency energy.

*Standard vs bRFE; statistical significance at $P < .5$. 
contribution of the use of bRFE in the accelerated healing response, they do demonstrate the return of tendon strength, stiffness, and histologic grade occurs at a much earlier time than with the techniques used in the 2 previous studies.

Using gross anatomic observation, objective histologic grading, and biomechanical evaluation, our data showed that during the 3 time intervals studied (4, 8, and 12 weeks), there was no statistically significant difference between standard and bRFE-treated repairs \( (P \leq .05) \). One possible explanation for the lack of differences seen at the gross and microscopic level is the lack of sensitivity of our current methods. Semiquantitative methods of analysis such as immunohistochemical staining or scanning electron microscopy, or quantitative methods such as molecular biologic assays for growth factors and cytokines would have increased the sensitivity of detecting more subtle differences in collagen organization and biochemical composition. Such changes have been observed in previous studies that used bRFE.\(^{15,23}\)

A possible explanation for our inability to demonstrate significance in both maximum stress and tissue modulus is the confounding effect of the humeral neck. The high incidence of fractures in our study indicates a significant amount of stress was being applied to this region of our tested material. The tissue modulus measured in our study would have been a composite of the tendon as well as the bone. The relative contribution of each to the measured modulus cannot be determined. Failure at the humeral neck implies that the strength of the tendon exceeds the bone, and therefore, our measurements may have underestimated the tendon’s true maximum stress. If one examines the mode of failure in the 2 groups, humeral fracture was observed in 4 times as many cases in the bRFE group than in the standard group at 12 weeks \((14.29\% \text{ versus } 57.14\%)\). One of 2 conclusions can be drawn from this finding. If the bone was of equal strength, the bRFE tendons had a higher maximum stress than the standard repair tendons, although the difference is unknown. If the tendons were of equal strength, the bone strength may have been less in the standard repair group.

The current settings and technique of application of the bRFE probe may also have affected our results. The settings used in our study were modeled after the previous study by Tasto et al.\(^{23}\) Using these settings, they were able to demonstrate an angiogenic response by showing an increase in VEGF mRNA levels. It is possible that these settings are insufficient for rotator cuff tears in rats. Furthermore, we did not tightly control the amount of pressure applied to the probe during application. This may have caused variability in the amount of energy transferred to the repaired tendon ends.

Another possible explanation of why we did not find statistical significance in our study was lack of power. Although the exact percent difference that is clinically relevant is not known, a 30% difference was used as a frame of reference. Using post hoc power analysis with the values obtained in our study, we would need 14 samples in each treatment group to have an 80% chance of detecting a 30% difference. In our study, we had only a 60% chance of detecting a 30% difference.

A final possible explanation of our inability to demonstrate statistical significance between the groups was our use of delayed repair of an acute tear. Our current technique may have resulted in a ceiling effect where there was already enough inherent ability of the tendon to repair itself when it was repaired at 6 weeks. As a result, any observable beneficial effect of the bRFE may have been dampened or diminished.

Currently, there is no universally accepted chronic rotator cuff model in rats. Gimbel et al.\(^{8}\) studied the properties of un repaired tears at intervals of 1, 2, 4, 8, and 16 weeks. They did not determine which time interval was the threshold for a chronic tear. Furthermore, even at 16 weeks, the characteristics of the tendon tear were not completely identical to those found in chronic human tears. Perhaps waiting 16 weeks to repair the tendon would have amplified the effects of bRFE on the repair.

Our study had methodologic weaknesses that have not yet been discussed. Several factors during biomechanical testing may have confounded our results. Creating dumbbell-shaped specimens resulted in samples that were very difficult to test owing to the resulting small size. Even with the use of a cryoclamp, slippage of the specimen could not be prevented in 5 tendons, leading to inadequate data for those specimens. Maintaining as much of the tendon as possible would have made testing more reliable and easier.

Second, the contribution of the humeral neck to measured values of both tissue modulus and maximum stress may have been diminished by potting the specimens more extensively to support this location within the humerus.

Third, the use of contact methods to measure our tendons without controlling the amount of force placed on the specimen during measurement may have introduced another source of error to our calculations.

Finally, the use of position data to calculate stress and strain is flawed by lack of uniform stress throughout the specimen.\(^{21}\) However, in this study, we were more interested in the comparison between treatment groups rather than the true tendon mechanical and structural properties, and therefore, it is unlikely that our conclusions would have changed even under ideal testing conditions.
The strength of our study was that it was a controlled study examining the effect of a single variable: the use of bRFE on the repaired tendon. Inconsistencies in technique were also minimized by having only a single surgeon for all procedures and a single biomechanical tester.

The safety of the use of bRFE in rotator cuff tears was verified in our study. No gross or histologically detrimental effect to the surrounding soft tissue, bone, or cartilage was observed, paralleling previous studies using this modality.13,23 Macroscopic observation revealed a successful repair in all specimens, with no qualitative difference between the 2 treatment groups.

CONCLUSION

Based upon a PubMed search at the time of publication of this article, this is the first article, to our knowledge, to report the use of bRFE to enhance rotator cuff repairs in rat tendons. It was found to be safe when used for this specific procedure. Compared with standard repair, the advantage of adding bRFE in our current model has yet to be proven. However, many questions remain to be answered, such as the optimal voltage setting, application duration, application location, and number of applications. Changes in any of these parameters with precise control of the pressure during application may increase the size of the effect and further separate the standard and the enhanced repair groups.

Quantitative methods of analysis, such as use of molecular biologic assays, and semiquantitative methods, such as immunohistochemical staining and scanning electron microscopy, may also help shed light on the biologic effects of bRFE on rotator cuff tendons and help refine the optimal usage parameters. Finally, effects may also become more apparent in a chronic model, where the healing process is at a greater disadvantage.4,29

We thank the animal research facility staff for their work in maintaining the rats and creating a suitable operating environment, Dr Nadim Hallab and his lab for sharing use of the Instron machine, Jennifer Hayden for her invaluable work on the statistics, Tammy Pylawka for her help with the data calculation, and James Williams for his guidance and help with the histology. Special thanks also to Stephen Lee, MS, and Amit Goyal, MS, for their assistance in the project.

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