rhBMP-12 Accelerates Healing of Rotator Cuff Repairs in a Sheep Model

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**Background:** The success rate of rotator cuff repairs is variable. This study was performed to evaluate the ability of recombinant human bone morphogenetic protein-12 (rhBMP-12), administered in several carriers, to accelerate healing in a sheep model of rotator cuff repair.

**Methods:** Local retention of tracer amounts of radiolabeled rhBMP-12, added to non-radiolabeled rhBMP-12 delivered in buffer, hyaluronan paste or sponges, or Type-I or Type-I/III collagen sponges was first evaluated with use of gamma scintigraphy in a pilot study of a rat intramuscular implant model. The rhBMP-12/paste and sponge combinations were then evaluated in eight sheep each with unilateral complete detachment and subsequent double-row reattachment of the infraspinatus tendon to the proximal part of the humerus. Contralateral, normal shoulders from sixteen sheep and shoulders in which a repair had been done without administration of rhBMP-12 in fourteen sheep were also evaluated. The rhBMP-12/Type-I and Type-I/III collagen sponge combinations were each evaluated in eight additional sheep on the basis of superior efficacy. The Type-I/III collagen sponge alone was evaluated in ten sheep to examine the effect of a collagen carrier. Ultrasound imaging was performed at four and eight weeks. Radiographic evaluation, mechanical testing, and biochemical evaluation were performed at eight weeks. Histological evaluation was performed on specimens from the sites of selected repairs following mechanical testing.

**Results:** The sponge carriers had longer local retention of rhBMP-12 than did the buffer or paste carriers in the rat models. All of the sheep shoulder-repair groups demonstrated ultrasound evidence of a gap between the tendon and the humeral insertion. The gap length and the cross-sectional area of the repair tissue decreased with time. The mechanical properties of the repairs treated with rhBMP-12 and hyaluronan paste were similar to those of the untreated repairs. The maximum loads for the rhBMP-12/hyaluronan sponge and rhBMP-12/collagen sponge-treated repairs were 2.1 and 2.7 times greater, respectively, than the loads for the untreated repairs and were 33% and 42% of the value for the normal tendon at eight weeks. The maximum loads for the repairs treated with rhBMP-12 and a Type-I or Type-I/III collagen sponge were 2.1 times greater than those for the repairs treated with the Type-I/III collagen sponge alone. Changes in maximum stiffness followed a similar pattern. Histological evaluation demonstrated accelerated healing of the rhBMP-12-treated repairs compared with the untreated repairs. Bone formation was observed in all repairs, and biochemical measurements were not equivalent to those of normal tendon at eight weeks.

**Conclusions:** Delivery of rhBMP-12 in a collagen or hyaluronan sponge resulted in accelerated healing of acute full-thickness rotator cuff repairs in a sheep model.

**Clinical Relevance:** Delivery of rhBMP-12 in several sponge carriers has the potential to accelerate healing of rotator cuff repairs. Accelerated repair may allow shorter rehabilitation and an earlier return to occupational and recreational activities.

Although rotator cuff repair is one of the most commonly performed soft-tissue surgical procedures, the success of these operations varies. The majority of patients report a reduction in pain and an improvement in function after surgery. However, objective evaluation of the tendon-bone attachment sites has demonstrated a high rate of surgical failure.
despite the reported clinical success\textsuperscript{3-5}. Patients who are symptomatic following surgery are more likely to have a retear than are asymptomatic patients\textsuperscript{6}. A result, there has been a considerable amount of effort to develop surgical methods to optimize the fixation of tendon to bone in order to provide the highest possible initial strength of the repair\textsuperscript{7-9}. If it is assumed that optimal surgical repair techniques are being used, then the persistently high failure rates suggest a potential need to augment the repairs either mechanically or biologically. Mechanical augmentation has been attempted with the use of scaffold materials approved for rotator cuff repair\textsuperscript{10}, but their clinical effectiveness has not been demonstrated\textsuperscript{11-13}. Biological approaches involving gene therapy and cell-based therapy have been reported to have improved tendon healing in animal models\textsuperscript{14-17}. Recently, a mixture of extracted bone-growth factors, probably containing bone morphogenetic proteins (BMPs), delivered in a Type-I collagen sponge was reported to improve the strength of rotator cuff repairs in sheep\textsuperscript{18}. The potential involvement of BMP-12 and BMP-13 in normal tendon remodeling and repair has been inferred on the basis of the presence of these proteins in adult tenocytes\textsuperscript{19}. In addition, several studies have demonstrated induction of neotendon and ligament formation in rats\textsuperscript{20} and improved healing of tendon lacerations following gene therapy or the administration of recombinant human bone morphogenetic protein-12 (rhBMP-12), rhBMP-13 (growth and differentiation factors-7 and 6, respectively) and growth and differentiation factor-5\textsuperscript{14,17,21}. Administration of rhBMP-2 in buffer accelerated bone healing in rodents\textsuperscript{22} and rabbits\textsuperscript{23,24}. However, carrier matrices have been commonly used to achieve efficacy in higher-order animal models\textsuperscript{25-26}. The use of carriers is generally thought to increase retention of the BMPs at the repair site, localize the tissue repair, and in some instances provide a matrix for cellular ingrowth. A pilot study was performed with use of a rat intramuscular implant model to compare the retention of rhBMP-12 in several rhBMP-12/carrier formulations proposed for use in the sheep rotator cuff repair model with the retention of rhBMP-12 delivered in buffer solution. The rhBMP-12 carriers included hyaluronan paste, hyaluronan sponge, and collagen sponge. The protocols used for detecting \textsuperscript{125}I-rhBMP-12 retention have been previously reported\textsuperscript{27}. The local retention of \textsuperscript{125}I-rhBMP-12 delivered in sponges was considerably longer than that of \textsuperscript{125}I-rhBMP-12 delivered in buffer or hyaluronan paste (Fig. 1). At one, seven, and fourteen days, approximately 80\%, 45\%, and 8\%, respectively, of the initial dose of the \textsuperscript{125}I-rhBMP-12 delivered in the sponges were retained at the implant sites. At one and seven days, approximately 48\% and <1\%, respectively, of the initial dose of the \textsuperscript{125}I-rhBMP-12 delivered in the buffer or paste were retained at the injection sites.

The objective of this study was to evaluate the ability of several rhBMP-12/carrier formulations to accelerate tendon-to-bone reattachment in a sheep model of rotator cuff repair. The specific hypotheses to be tested included (1) rhBMP-12 delivered in a collagen or hyaluronan sponge has better efficacy than an rhBMP-12/hyaluronan paste formulation and (2) use of the most efficacious rhBMP-12/carrier combination is more efficacious than use of the carrier alone.

**Materials and Methods**

**Experimental Design**

The two phases of this study included the initial screening of rhBMP-12/carrier formulations in a sheep model of rotator cuff repair and confirmation of the most efficacious rhBMP-12/carrier combinations and examination of the effect...
of the carrier alone in additional animals (Table I). The rationale for phase 1 was to screen all of the rhBMP-12/carrier combinations for efficacy and to limit the number of sheep required to evaluate the effects of the carrier alone in phase 2. The rationale for phase 2 was to expand the number of sheep used to evaluate the most efficacious rhBMP-12/carrier combinations and to evaluate the effects of the carrier alone. In phase 1, unilateral acute detachment and reattachment of the infraspinatus tendon from its insertion on the humeral head was performed in forty-six adult sheep with a body weight ranging from 60 to 90 kg and an age ranging from four to six years. In eight sheep each, the rotator cuff repair was treated with one of the four rhBMP-12/carrier formulations. In fourteen sheep, the site of the unilateral rotator cuff repair was untreated. The rhBMP-12/carrier formulations included an injectable 65% esterified untreated. The rhBMP-12/carrier formulations included an injectable 65% esterified untreated. The rhBMP-12/carrier formulations included an injectable 65% esterified untreated. The rhBMP-12/carrier formulations included an injectable 65% esterified untreated. The rhBMP-12/carrier formulations included an injectable 65% esterified untreated. The rhBMP-12/carrier formulations included an injectable 65% esterified untreated. The rhBMP-12/carrier formulations included an injectable 65% esterified untreated. The rhBMP-12/carrier formulations included an injectable 65% esterified untreated. The rhBMP-12/carrier formulations included an injectable 65% esterified untreated. The rhBMP-12/carrier formulations included an injectable 65% esterified untreated. The rhBMP-12/carrier formulations included an injectable 65% esterified untreated.
handled without tearing and that had sufficient suture-holding characteristics while limiting the amount of rhBMP-12 solution lost during surgical manipulation. The soak-load volumes for the Type-I collagen sponge, the Type-I/III collagen sponge, and the hyaluronan sponge were 33%, 50%, and 100%, respectively. Although the concentrations of rhBMP-12 were similar, the differences in the soak-load volumes of the sponge formulations resulted in differences in the total amount of rhBMP-12 delivered. Because of differences in sponge thickness, the total doses of rhBMP-12 in the Type-I collagen and hyaluronan sponges were similar. A higher rhBMP-12 concentration was chosen to adjust for the lower rhBMP-12 retention of the hyaluronan paste compared with the sponge carriers.

Surgical Procedures
The rotator cuff surgery was performed with use of aseptic technique with the sheep intubated and maintained on isoflurane anesthesia. A 6.0-cm skin incision was made over the shoulder joint, and the underlying subcutaneous colli muscle was divided. The deltoid muscle was then split along the tendinous division between the acromial and scapular heads. The insertion of the superficial head of the infraspinatus tendon was isolated, and the entire tendon was sharply detached from the humeral insertion. The thicknesses of the Type-I, Type-I/III, and hyaluronan sponges were 0.3, 0.2, and 0.1 cm, respectively. The five sutures were then tightened, pulling the tendon down onto the injected paste or the sponge against the bone. An additional 0.25 mL of 0.35 mg/mL rhBMP-12/hyaluronan paste was applied to the surface of the infraspinatus tendon in the hyaluronan-paste group. In the sponge groups, an additional 2.0 by 5.0-cm implant containing the appropriate volume and concentration of rhBMP-12 was placed over the tendon (Fig. 2-B, Table I) and sutured to the subcutaneous tissue distal to the bone tunnels on the proximal part of the humerus. The brachial fascia and subcutaneous tissues were closed with 2/0 synthetic absorbable suture, and the skin was closed with stainless-steel staples. The sheep were housed indoors without immobilization of the operatively treated limb after the surgery.

Ultrasound Evaluation
Ultrasound images were obtained for both shoulders with use of a 7.5-MHz linear transducer-equipped ultrasound imager (Aloka SSD-900V; Aloka, Wallingford, Connecticut), with the sheep manually restrained in a lateral recumbent position, at four and eight weeks after the surgery. Measurements from three longitudinal images, positioned to include the cranial, middle, and caudal aspects of the tendon, were placed in the medial aspect of the tendon insertion footprint of the humeral head. The sutures were passed through the tendon with use of a modified Mason-Allen pattern. Three sutures, passed through three bone tunnels in the greater tuberosity of the humerus, were placed in the lateral edge of the tendon with use of a modified Mason-Allen pattern. Prior to tightening of the sutures, we placed, between the tendon and the bone, a 0.25-mL volume of 0.35 mg/mL rhBMP-12/hyaluronan paste or a 2.0 by 1.5-cm sponge containing the appropriate volume and concentration of rhBMP-12 solution (Fig. 2-A and Table I). The echogenicity of the tendon and of the repair tissue approximated an ellipse. The echogenicity of the tendon and of the
tissue within the gap were subjectively compared to determine the quality of the repair tissue.

Postmortem Evaluation
Harvested specimens were qualitatively evaluated for the continuity of the repair between the tendon and bone, the quality of the repair tissue, evidence of residual carrier material, and the presence of mineralized nodules. High-resolution radiographs (MX-20; Faxitron X-Ray, Wheeling, Illinois) were used to confirm the presence of mineralization in the repairs. Mineralized nodules were dissected from the repairs and weighed following mechanical testing.

Tensile Biomechanical Testing
The infraspinatus muscle, tendon insertion, and proximal part of the humerus were dissected from the scapula and associated soft tissues by two of the authors (H.J.S. and S.A.R.), who were blinded to the type of treatment. The muscle was stripped away from the fascial attachment of the tendon. The humeral shaft was potted in polymethylmethacrylate. A dry-ice-cooled serrated grip secured the tendon fascia at a distance of 6.0 cm from the bone. specimens were tested in uniaxial tension at room temperature with use of a materials testing system (model 8500; Instron, Canton, Massachusetts). After preloading of the specimen to 10.0 N, ten 2.0%-strain sine-wave preconditioning cycles were performed. Specimens were loaded to failure at a crosshead displacement rate of 2.0 cm/min (0.5% strain/sec). The maximum load and stiffness as well as the location of the failure were determined. The maximum stiffness was determined from the linear portion of the load-displacement curve, which generally occurred between 7.0 and 11.0 mm. Tendon displacement was determined on the basis of grip displacement, not optical displacement of tendon markers. Nonuniform displacement of the tendon could not be distinguished from displacement of the fascia with this technique. Lack of tendon slippage was confirmed by maintenance of the regular crimp pattern of the fascia in the serrated grip after testing. Average load-displacement curves were derived for each group by averaging the loads at 0.5-mm increments in crosshead displacement for each animal. A wide range of mechanical testing crosshead displacement rates have been reported in studies of sheep rotator cuff repairs. The 2.0-cm/min crosshead displacement rate used in this study was chosen to allow direct comparison with data in a report on extracted bone-growth factors that were likely to contain BMPs delivered in a Type-I collagen sponge. In order to determine the relationships among crosshead displacement rate, mechanical measurements, and failure location, similar measurements were made on nine normal tendons each, tested at 20.0 cm/min (5.5% strain/sec) or 72 cm/min (20% strain/sec) and were compared with the measurements made at 2.0 cm/min (0.5% strain/sec). The results of this comparison indicated that the maximum tensile load was independent of the crosshead loading rate (see Appendix). The stiffness measurements were lowest at the highest loading rate (p < 0.05). The prevalence of humeral shaft failure was highest at the slowest loading rate.

Biochemical Analysis
After biomechanical testing was completed, three, four, or five pieces of the repair tissue between the tendon and the bone of the operatively treated shoulder and corresponding pieces of normal tendon from the contralateral shoulder were removed near the insertion onto the bone. The harvested specimens did not include bone but included the fibrocartilaginous insertion. The glycosaminoglycan content (a measure of the proteoglycan content), the deoxyribonucleic acid content (a measure of cellularity), and the hydroxyproline content (a measure of collagen content) were evaluated separately for each piece and were normalized to dry weight. The data for the individual pieces were averaged for each shoulder. The pieces were lyophilized to determine the water content and were digested overnight with proteinase K (Sigma-Aldrich, St. Louis, Missouri). An aliquot of the digestate was used to determine the glycosaminoglycan content with use of a dimethylmethylen blue colorimetric assay. The deoxyribonucleic acid content was determined with use of a fluorescent nucleic acid dye (PicoGreen; Invitrogen, Carlsbad, California). The remainder of the digestate was hydrolyzed overnight with 6.0-N hydrochloric acid and was assayed for hydroxyproline content with Ehrlich reagent.

Water content was expressed as a percentage of wet weight. Hydroxyproline content was expressed as a percentage of dry weight. Glycosaminoglycan and deoxyribonucleic acid content was expressed as micrograms per milligram of dry weight.

Histological Evaluation
Selected repairs with sufficient tissue remaining attached to the proximal part of the humerus following mechanical testing were subjected to histological evaluation. Four rhBMP-12-treated repairs, two untreated repairs, and two normal tendons were evaluated. Prior to fixation, the majority of the proximal part of the humerus was removed with a band saw, leaving a thin layer of bone at the repair insertion. The specimens were divided sagittally into 2.0-mm sections with a low-speed diamond band saw for histological processing. Sections were decalcified in 20% EDTA for five weeks and then embedded in paraffin after ten hours of paraffin infiltration. Six-micrometer sections were cut, and two slides were made: one stained with hematoxylin and eosin and one stained with Weigert safranin O.

Data Analysis
The data for the shoulders treated with the rhBMP-12/collagen carriers in phases 1 and 2 were combined. Analysis of variance was used to compare ultrasound measurements, maximum tensile load, maximum stiffness, and biochemical measurements among normal tendons, rhBMP-12-treated tendons, and untreated repairs. When significant group effects were observed, comparisons of all pairs of group means were performed with the Tukey-Kramer post-hoc test. Repeated-measures analysis of variance was used to compare changes in the ultrasound measurements between four and eight weeks. The Student t test with use of the pooled variance estimate was employed for within-group comparisons between four and eight weeks. The Pearson coefficient was used to evaluate the correlation of ultrasound and biochemical
measurements with maximum load. All tests were two-tailed, and differences were considered significant at \( p < 0.05 \). When important comparisons did reveal a significant difference, the difference between population means required to detect a significant difference at a power of 0.80 and \( \alpha = 0.05 \) was calculated.

**Results**

**Surgical Repair and Clinical Evaluation**

The rhBMP-12 was easily delivered with both the paste and the sponge carriers. All of the sponges had acceptable handling and suture-holding properties. With the exception of one sheep with substantial neuromuscular atrophy of the shoulder, none of the animals exhibited lameness at eight weeks.

**Postmortem Evaluation**

Surgical reattachment of the infraspinatus tendon onto the proximal part of the humerus resulted in the induction of scar tissue of variable length and thickness between the retracted tendon edge and the insertion on the bone in all of the repair groups at eight weeks (Fig. 3). Residual carrier was detected only in the rhBMP-12/hyaluronan sponge-treated repairs. Palpable mineralized nodules were present in some of the specimens in each group. Adhesions to the surrounding soft tissues were the greatest in the rhBMP-12-treated repairs. There was evidence of fatty infiltration and fibrosis of the infraspinatus muscle in all of the repairs compared with the findings in the contralateral, normal shoulders. Two specimens from the rhBMP-12/Type-I/III collagen sponge-treated group were excluded because of a positive Q-fever (Coxiella burnetii) titer in one animal and severe shoulder neuromuscular atrophy in the second animal. Two additional specimens from this group were not included in any of the analyses because of mechanical testing errors. One specimen each in the untreated and the rhBMP-12/hyaluronan paste-treated groups were not included in any of the analyses because of dissection errors.

**Radiography**

Ectopic bone was present at the sites of nine of the thirteen untreated repairs and three of the ten repairs treated with the Type-I/III collagen sponge alone (see Appendix). In the rhBMP-12-treated groups, ectopic bone formation was present in two of the seven shoulders treated with hyaluronan paste, eleven of the sixteen treated with Type-I collagen sponge, seven of the twelve treated with Type-I/III collagen sponge, and one of the eight treated with hyaluronan sponge. The bone nodules in the rhBMP-12-treated groups were larger than those in the untreated-repair group.

**Ultrasound Evaluation**

There was a variably hypoechoic region between the distal end of the transected infraspinatus tendon and the insertion on the proximal part of the humerus in all of the repaired groups compared with the contralateral, normal shoulders (Figs. 4-A, 4-B, and 4-C). The presence of the hypoechoic region is consistent with retraction of the repaired tendon from the humeral insertion as noted on gross examination. Transverse ultrasound images of the normal infraspinatus tendons demonstrated a uniform echogenicity and parallel fiber orientation. The repaired tendons had mixed ultrasound density and less uniform parallel fiber orientation. Hyperechoic regions with hypoechoic shadowing, consistent with mineralization,
were observed at several repair sites in all of the groups. Hyperechoic regions, consistent with fatty degeneration, were also observed in the infraspinatus muscle of the repaired shoulders compared with more uniform echogenicity observed in the contralateral, normal infraspinatus muscle (Figs. 4-A, 4-B, and 4-C).

There was a significant group effect on the cross-sectional area at four weeks \( (p = 0.008) \) and a significant group-by-time effect between four and eight weeks \( (p < 0.0001) \) (Table II). The cross-sectional area of the repaired tendons was greater than that of the normal infraspinatus tendons at both four and eight weeks \( (p < 0.0001) \). The cross-sectional area in the untreated-repair group was significantly greater than that in the treated-repair groups at four weeks \( (p < 0.01) \); it was also greater at eight weeks, but that difference was not significant \( (p > 0.05) \). With the exception of the value in the Type-I/III-collagen sponge group, the cross-sectional area decreased by approximately 19% to 28% between four and eight weeks \( (p < 0.01) \). There was no significant difference between the treated and untreated-repair groups with regard to the percentage decrease in the cross-sectional area between four and eight weeks \( (p > 0.9) \). There was also no significant difference in cross-sectional area between the group treated with the rhBMP-12 and Type-I/III collagen sponge and the group
treated with the Type-I/III collagen sponge only (p = 0.08; a mean difference in cross-sectional area of 23.1% is required to show significance at a power of 0.80).

There was no group effect on gap length at either four or eight weeks (p > 0.55 and 0.60, respectively) (Table II). However, there was a significant group-by-time effect between four and eight weeks (p = 0.005). The gap length decreased by approximately 13% to 20% between four and eight weeks (p < 0.05) in all groups except for the one treated with the Type-I/III-collagen sponge alone; in that group, the gap length increased between four and eight weeks (p < 0.04). There was no difference in the percentage decrease, compared with the four-week gap length, at eight weeks between the rhBMP-12 treated and untreated-repair groups (p > 0.9). There was a significant difference in the percentage change in the gap length at eight weeks in the group treated with the Type-I/III collagen sponge alone compared with the untreated group (p = 0.03) and the group treated with the rhBMP-12 and Type-I/III collagen sponge (p < 0.002).

### TABLE II Ultrasound Measurements of the Cross-Sectional Area of Normal Tendon and Repair Tissue and Gap Length at Four and Eight Weeks After Surgery*

<table>
<thead>
<tr>
<th>Group</th>
<th>Cross-Sectional Area † (cm²)</th>
<th>Gap Length ‡ (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 Wk</td>
<td>8 Wk</td>
</tr>
<tr>
<td>Normal (n = 16)</td>
<td>0.58 ± 0.19</td>
<td>0.60 ± 0.23</td>
</tr>
<tr>
<td>Untreated (n = 13)</td>
<td>1.69b ± 0.32</td>
<td>1.23b ± 0.30</td>
</tr>
<tr>
<td>rhBMP-12/hyaluronan paste (n = 7)</td>
<td></td>
<td>Not done</td>
</tr>
<tr>
<td>rhBMP-12/hyaluronan sponge (n = 8)</td>
<td>1.26c ± 0.27</td>
<td>0.86b ± 0.22</td>
</tr>
<tr>
<td>rhBMP-12/Type-I collagen sponge (n = 16)</td>
<td>1.30d ± 0.34</td>
<td>1.01b ± 0.33</td>
</tr>
<tr>
<td>rhBMP-12/Type-I/III collagen sponge (n = 12)</td>
<td>1.25c ± 0.16</td>
<td>1.00b ± 0.24</td>
</tr>
<tr>
<td>Type-I/III collagen sponge (n = 10)</td>
<td>1.22c ± 0.13</td>
<td>1.17b ± 0.24</td>
</tr>
</tbody>
</table>

*The values are given as the mean and the standard deviation. The values within each column that share the same letter are not significantly different. †The group effect on cross-sectional area was p = 0.008 at four weeks and p > 0.09 at eight weeks. The group-by-time effect between four and eight weeks was p < 0.0001. ‡The group effect on gap length was p > 0.55 at four weeks and p > 0.60 at eight weeks. The group-by-time effect between four and eight weeks was p = 0.005. §There was a significant difference between the four and eight-week values in the same row.

### TABLE III Biomechanical Measurements of Maximum Load, Maximum Stiffness, and Failure Location During Testing Under Tension at Eight Weeks After Surgery*

<table>
<thead>
<tr>
<th>Group</th>
<th>Maximum Load † (kN)</th>
<th>Maximum Stiffness ‡ (kN/mm)</th>
<th>Failure Location (no. of repairs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bone Insertion</td>
</tr>
<tr>
<td>Normal (n = 16)</td>
<td>3.68a ± 0.48</td>
<td>0.273a ± 0.054</td>
<td>See Appendix§</td>
</tr>
<tr>
<td>Untreated (n = 13)</td>
<td>0.58b ± 0.24</td>
<td>0.074b ± 0.024</td>
<td>10</td>
</tr>
<tr>
<td>rhBMP-12/hyaluronan paste (n = 7)</td>
<td>0.68b ± 0.19</td>
<td>0.082b ± 0.020</td>
<td>7</td>
</tr>
<tr>
<td>rhBMP-12/hyaluronan sponge (n = 8)</td>
<td>1.21c ± 0.47</td>
<td>0.126c ± 0.048</td>
<td>8</td>
</tr>
<tr>
<td>rhBMP-12/Type-I collagen sponge (n = 16)</td>
<td>1.56c ± 0.64</td>
<td>0.171c ± 0.064</td>
<td>5</td>
</tr>
<tr>
<td>rhBMP-12/Type-I/III collagen sponge (n = 12)</td>
<td>1.57c ± 0.50</td>
<td>0.178c ± 0.038</td>
<td>2</td>
</tr>
<tr>
<td>Type-I/III collagen sponge (n = 10)</td>
<td>0.75a ± 0.37</td>
<td>0.099a ± 0.037</td>
<td>5</td>
</tr>
</tbody>
</table>

*The values are given as the mean and the standard deviation. The values within each column that share the same letter are not significantly different. †The group effect on maximum load was p < 0.0001. ‡The group effect on maximum stiffness was p < 0.0001. §Thirteen of the sixteen normal tendons failed at the proximal part of the humeral shaft; one, at the tendon-to-bone insertion on the humerus; one, in the midpart of the body of the tendon; and one, in the fascia at the upper clamp.
Tensile Biomechanical Evaluation

In the majority of the normal shoulders, failure occurred in the humeral shaft, at the interface between the embedded bone and the exposed proximal humeral metaphysis (see Appendix). The similarity between the maximum load and stiffness in the shoulders in which failure occurred in the tendon midsubstance and those variables in the shoulders in which the failure occurred in the bone indicates that the values for tendon may not differ substantially from those for bone under these testing conditions. The untreated repairs failed by avulsion of the repair tissue from the insertion or in the midsubstance of the repair tissue (Table III). All of the rhBMP-12/hyaluronan paste-treated repairs failed by avulsion of the repair tissue from the insertion. The rhBMP-12/hyaluronan sponge-treated repairs failed in the midsubstance of the repair tissue. The repairs treated with the rhBMP-12/collagen sponge combination failed by avulsion of the bone at the insertion site or by avulsion of the repair tissue from the insertion or they failed in the midsubstance of the repair tissue. The repairs treated with the Type-I/III collagen sponge alone failed by avulsion of the repair tissue from the insertion or in the midsubstance of the repair tissue.

There was a significant group effect on maximum load and stiffness (p < 0.0001) (Table III). The maximum load and stiffness in the normal shoulders were greater than those in all of the repaired shoulders at eight weeks (p < 0.0001). The maximum load in the group treated with the rhBMP-12 and hyaluronan paste was similar to that in the untreated-repair group (p = 0.97; a mean difference of 0.4 kN is required to show significance at a power of 0.80). The maximum load in these two groups was 18% and 16%, respectively, of the value for the normal tendon. The maximum load in the rhBMP-12/hyaluronan paste-treated group was also significantly less than that in the rhBMP-12/collagen sponge-treated groups (p < 0.001). The difference in maximum load between the rhBMP-12/hyaluronan paste and rhBMP-12/hyaluronan sponge-treated groups was not significant (p = 0.08). The maximum load of the repairs treated with the rhBMP-12 and hyaluronan sponge was 2.1 times greater than that of the untreated repairs (p = 0.01) and 33% of the value for the normal tendon (p < 0.0001). The maximum load of the repairs treated with the rhBMP-12 and collagen sponge was 2.7 times greater than that of the untreated repairs (p < 0.0001) and 42% of the value for the normal tendon (p < 0.0001). The difference between the maximum loads in the rhBMP-12/hyaluronan sponge and rhBMP-12/collagen sponge-treated groups was not significant (p = 0.1). The maximum load in the group treated with the rhBMP-12 and Type-I/III collagen sponge was 2.1 times greater than that in the group treated with the Type-I/III collagen sponge alone (p =0.0004). The maximum load in the group treated with the rhBMP-12 and Type-I collagen sponge was 3.6 times greater than the reported twelve-week value in a similar sheep model treated with Type-I collagen sponge alone (0.43 ± 0.16 kN)\(^{18}\). There was a moderate negative correlation between the cross-sectional area of the repairs at eight weeks and the maximum failure load (r = −0.27, p < 0.05).
The same pattern of treatment effect was evident in the analysis of the maximum stiffness of the repaired tendons (Table III). The stiffness of the rhBMP-12/hyaluronan paste-treated repairs was similar to that of the untreated repairs (p > 0.45; a mean difference of 0.04 kN/mm is required to show significance at a power of 0.80). The stiffness in these two groups was 30% and 27%, respectively, of the value for the normal tendon. The stiffness of the repairs treated with the rhBMP-12 and hyaluronan sponge was 1.7 times greater than that of the untreated repairs (p = 0.025) and 46% of the value for the normal tendon (p < 0.0001). The stiffness of the repairs treated with the rhBMP-12 and collagen sponge was approximately 2.3 times greater than that of the untreated repairs (p < 0.0003) and 64% of the value for the normal tendon (p < 0.0001). The stiffness of the repairs treated with the rhBMP-12 and Type-I/III collagen sponge was approximately 1.8 times greater than that of the repairs treated with the Type-I/III collagen sponge alone (p = 0.005). The stiffness of the repairs treated with the rhBMP-12 and Type-I collagen sponge was approximately 2.9 times greater than the value reported for repairs treated with Type-I collagen sponges alone at twelve weeks in a similar sheep model (0.06 ± 0.12 kN/mm)\textsuperscript{9,19}.

The average stiffness/displacement curve for the untreated repairs was lower, throughout the range of crosshead displacements, than the curve for the normal shoulders (Fig. 5). At crosshead displacements of <6.0 mm, measurements for the rhBMP-12/collagen sponge-treated repairs and the repairs treated with the Type-I/III collagen sponge alone were similar to those for the untreated repairs. At crosshead displacements of >6.0 mm, the average stiffness/displacement curves for the rhBMP-12/collagen sponge-treated repairs were higher than the curve for the repairs treated with the Type-I/III collagen sponge alone and the curve for the untreated repairs. However, the curves for all treatment groups remained lower than the curve for the normal shoulders. Similar results were obtained for the other treatment groups.

**Biochemical Analysis**

There was a significant group effect on water, hydroxyproline, glycosaminoglycan, and deoxyribonucleic acid contents (p < 0.001, Table IV). The water, glycosaminoglycan, and deoxyribonucleic acid contents were lower and the hydroxyproline content was higher in the normal tendons compared with the repair groups (p < 0.0001). The water and glycosaminoglycan contents were similar in all of the repair groups. The hydroxyproline content of the repairs treated with the rhBMP-12 and collagen sponge and of the repairs treated with the Type-I/III collagen sponge alone were significantly higher than that of the untreated repairs (p < 0.01). The deoxyribonucleic acid content in the repairs treated with the rhBMP-12 and Type-I collagen sponge and the repairs treated with the Type-I/III collagen sponge alone were significantly lower than that of the untreated repairs (p < 0.05). All measurements in the group treated with the rhBMP-12 and Type-I/III collagen sponge were similar to those in the group treated with the Type-I/III collagen sponge alone. A moderate positive correlation was observed between the glycosaminoglycan content of the repairs and the maximum failure load (r = 0.39, p = 0.002), and a moderate negative correlation was found between the deoxyribonucleic acid content of the repairs and the maximum failure load (r = −0.42, p < 0.001).

**Histological Evaluation**

Histological evaluation of specimens from the sites of the limited number of repairs with sufficient tissue remaining demonstrated fibrocartilaginous attachment of the repair tissue to bone in the rhBMP-12-treated groups and a primarily cartilaginous attachment in the untreated-repair group. Compared with the untreated repairs, the rhBMP-12-treated repairs showed a decrease in the density of the cells and the number of blood vessels and an increase in the alignment of the collagen fibers in the midbody of the repair tissue. Taken together, these histological findings suggest that the rhBMP-12-treated repairs demon-

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**TABLE IV Biochemical Measurements of Water, Hydroxyproline, Glycosaminoglycan, and Deoxyribonucleic Acid Contents (Normalized to Milligrams of Dry Weight) at Eight Weeks After Surgery**

<table>
<thead>
<tr>
<th>Group</th>
<th>Water Content ( \pm ) (%)</th>
<th>Hydroxyproline Content ( \pm ) (%)</th>
<th>Glycosaminoglycan Content ( \pm ) (( \mu g ))</th>
<th>Deoxyribonucleic Acid Content ( \pm ) (( \mu g ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 16)</td>
<td>65.7( \pm ) 2.6</td>
<td>76.7( \pm ) 4.4</td>
<td>4.9( \pm ) 2.4</td>
<td>0.44( \pm ) 0.02</td>
</tr>
<tr>
<td>Untreated (n = 13)</td>
<td>78.0( \pm ) 2.0</td>
<td>52.2( \pm ) 3.8</td>
<td>16.3( \pm ) 4.6</td>
<td>2.73( \pm ) 0.87</td>
</tr>
<tr>
<td>rhBMP-12/hyaluronan paste (n = 7)</td>
<td>76.3( \pm ) 3.0</td>
<td>61.6( \pm ) 6.1</td>
<td>16.5( \pm ) 3.6</td>
<td>3.28( \pm ) 0.91</td>
</tr>
<tr>
<td>rhBMP-12/hyaluronan sponge (n = 8)</td>
<td>77.3( \pm ) 2.3</td>
<td>58.3( \pm ) 9.8</td>
<td>17.6( \pm ) 4.6</td>
<td>1.96( \pm ) 0.39</td>
</tr>
<tr>
<td>rhBMP-12/Type-I collagen sponge (n = 16)</td>
<td>79.6( \pm ) 1.9</td>
<td>63.9( \pm ) 5.9</td>
<td>16.3( \pm ) 6.2</td>
<td>1.70( \pm ) 0.36</td>
</tr>
<tr>
<td>rhBMP-12/Type-I/III collagen sponge (n = 12)</td>
<td>80.3( \pm ) 2.0</td>
<td>63.9( \pm ) 9.8</td>
<td>19.8( \pm ) 9.3</td>
<td>2.42( \pm ) 1.0</td>
</tr>
<tr>
<td>Type-I/III collagen sponge (n = 10)</td>
<td>78.8( \pm ) 2.9</td>
<td>67.8( \pm ) 9.9</td>
<td>13.0( \pm ) 3.2</td>
<td>1.72( \pm ) 0.53</td>
</tr>
</tbody>
</table>

*The values are given as the mean and the standard deviation. The values within each column that share the same letter are not significantly different. †The group effect on water content was p < 0.0001. ‡The group effect on hydroxyproline content was p < 0.001. §The group effect on glycosaminoglycan content was p < 0.001. #The group effect on deoxyribonucleic acid content was p < 0.001.
Discussion

In this study, rhBMP-12 administered in collagen sponges resulted in greater maximum tensile load and stiffness at eight weeks compared with that seen in repairs treated with rhBMP-12 in hyaluronan paste, repairs treated with Type-I/III collagen sponge alone, or untreated repairs in a sheep model of a complete rotator cuff tear. However, there was less maximum stiffness, maximum load, and load as a function of displacement in all of the repair groups at eight weeks than in the normal tendon. All of the repairs healed with a gap between the infraspinatus tendon and the insertion on the proximal part of the humerus despite reattachment of the tendon to the bone with the use of a double-row suture technique. The results of the mechanical testing and biochemical analysis indicated the presence of immature repair tissue at eight weeks in all of the repair groups compared with the normal tendon. However, the ultrasound evaluation demonstrated that the cross-sectional area of the repair tissue and the gap length decreased between four and eight weeks in all repair groups, a finding consistent with ongoing remodeling of the repair tissue.

Rotator cuff repairs have a high rate of incomplete healing and gap formation between the tendon and bone. Recurrent defects have been found in a large proportion of repaired large rotator cuff tears\(^{36,37}\). Better functional results have been reported when the repaired rotator cuff is intact at the time of the follow-up examination\(^{38,39}\). Although gap tissue was still present at eight weeks in our study, the maximum tensile load for the repairs treated with rhBMP-12 administered in collagen sponges was 2.7 times greater than that for the untreated repairs. The maximum tensile load for the repairs treated with the rhBMP-12 and Type-I/III collagen sponge was 2.1 times greater than that for the repairs treated with the Type-I/III collagen sponge alone. The maximum tensile load and stiffness for the rhBMP-12/collagen sponge-treated repairs were approximately 42% and 64%, respectively, of the values for the normal tendon; in comparison, the load and stiffness values for the repairs treated with the Type-I/III collagen sponge alone and those for the untreated repairs were approximately 30% and 17%, respectively, of the values for the normal tendon at eight weeks. The use of extracted bone-growth factors delivered in a Type-I collagen sponge resulted in a maximum tensile load of the repairs, at twelve weeks, that was 1.3 times that of repairs treated with Type-I collagen sponge alone or untreated in a similar sheep model\(^{40}\). The fact that the rhBMP-12/collagen sponge-treated repairs had a higher maximum tensile load and stiffness but a similar ultrasound-measured cross-sectional area compared with the untreated and collagen alone-treated groups suggests that the rhBMP-12/collagen sponge-treated repairs had increased material properties of the repair tissue compared with the other groups. However, the tissue in all of the repair groups had decreased material properties compared with those of the normal tendon.

The clinical relevance of the increased maximum tensile load and stiffness in response to the rhBMP-12/collagen sponge treatment is difficult to determine since none of the sheep in any of the repair groups demonstrated any functional deficits. The maximum tensile load in all of the repair groups in our study exceeded the highest recorded in vivo tensile loads measured for the infraspinatus tendon in actively struggling suspended sheep (0.31 kN)\(^{40}\). However, to our knowledge, maximal tensile loads in the shoulder have not been reported during normal weight-bearing activity in sheep. The average load-displacement curves were also similar among all of the repair groups below the reported 0.31-kN load (Fig. 3). At higher applied loads, the rhBMP-12-treated repairs were stiffer than those treated with carrier alone or the untreated repairs, but they were still less stiff than normal tendon. Ideally, the shape of the load-displacement curves (tangent stiffness) for repairs should mimic the values for normal tendon up to and beyond peak loads experienced during activity\(^{41}\). This is particularly important since the eightfold or greater safety factor measured for most tendons suggests that tendons are designed to maintain adequate stiffness for increased control of limb position and muscle length rather than sufficient strength\(^{42}\). The tangent stiffness of mechanically stimulated, cell-impregnated collagen sponge implants placed in a middle-third patellar tendon defect matched values for normal tendon up to 125% of peak loads measured during activity in rabbits\(^{43}\). However, these results were observed after two weeks of in vitro mechanical stimulation and twelve weeks after implantation compared with eight weeks in the current sheep study. The biomechanical, biochemical, and histological measurements in our sheep study showed that the repair tissue was far from normal at eight weeks after surgery. Because treatment of rotator cuff tears with rhBMP-12 on a collagen sponge carrier improves the structural and material properties of the repair compared with those of untreated repairs, such treatment may allow patients to begin active rehabilitation and return to full function sooner as a result of accelerated healing. Previously reported results\(^{44}\) indicate that untreated repairs would require approximately twenty-two weeks to attain maximum tensile strength and stiffness similar to those of the rhBMP-12/collagen sponge-treated repairs in our series at eight weeks. Since the target parameters for a functionally superior rotator cuff repair in sheep have not been fully determined, the true benefit of the proposed treatment can be determined only in human clinical trials.

The increased efficacy of the sponge carriers may be due in part to the increase in local retention of rhBMP-12 at the repair site compared with that in the paste carrier. The sponge carriers may also provide a scaffold for cellular and vascular ingrowth. However, histological evaluation of rhBMP-2/Type-I collagen sponge-treated rabbit ulnar osteotomy sites indicated that the majority of the repair tissue occurs in the soft tissue surrounding the implant rather than within the matrix\(^{45}\).

The histological appearance of the repair tissue and the higher collagen content suggest that repairs treated with rhBMP-12/collagen sponge were more mature than untreated repairs.
The similarity between the collagen content and cellularity of the rhBMP-12/collagen sponge repairs and the repairs treated with the Type-I/III collagen sponge alone suggests that the increased mechanical properties of the rhBMP-12/collagen sponge-treated repairs may be due to collagen alignment and/or collagen cross-linking rather than collagen content. The amount of collagen in the repairs was only weakly correlated to the maximum load. The moderate positive correlation between the glycosaminoglycan content and the maximum load may indicate accelerated reestablishment of the fibrocartilaginous insertion site.

Ultrasound imaging has been used clinically to determine if a repair is intact after surgery. However, the relationship between the state of the repair and the clinical outcome is not well established. Ultrasound images of the repairs in this study clearly indicated the presence of poorly aligned repair tissue compared with normal tendon between the resected end of the infraspinatus tendon and the insertion on the proximal part of the humerus. Ultrasound quantification indicated that the cross-sectional area of the repair tissue decreased in all of the repair groups between four and eight weeks. In all of the repair groups except for the one treated with the Type-I/III collagen sponge alone, the gap length decreased between four and eight weeks. Ultrasound imaging of manually restrained sheep may not be indicative of the predictive power of this imaging modality for people with rotator cuff repairs because of the difficulty in obtaining images from the same location within the repair on subsequent evaluations of the sheep.

A limitation of the sheep model used in this study is that the surgical repair procedure was performed on normal tendon rather than on degenerated and retracted tendon, which would have been more clinically relevant. A sheep model of delayed repair of a retracted tendon has been used previously; however, the high failure rate and extensive initial tendon retraction associated with this model limit its suitability for evaluation of therapeutic interventions. The large amount of connective tissue in sheep infraspinatus tendon repairs is also not indicative of failed rotator cuff repairs in people, in whom minimal connective tissue is often observed. Induction of repair tissue with improved material properties by rhBMP-12 may reduce the number of surgical failures in people if the repair tissue forms before retearing occurs. Unfortunately, there is little information indicating when retears occur following repair. Remodeling of the rhBMP-12-induced repair tissue between the retracted tendon and the bone may improve the outcome of failed repairs in people, in whom minimal repair tissue is observed following surgical repair. Another limitation of the sheep model is the inability to immobilize the repair site after surgery, as is routinely done in people. Despite use of a surgical repair method that has been shown to provide the best initial fixation strength, all of the repairs in this study would be classified as surgical failures on the basis of the ultrasound appearance of a gap between the normal tendon end and the attachment on the bone and on the basis of the scar tissue observed in the repair on gross pathological evaluation. Immobilization of the operatively treated limb, although difficult to achieve, may reduce the gapping at the repair site. However, the long-term strength of the repair appears not to be influenced by postoperative immobilization in this model. Despite the presence of gapping in all of the repairs, improved healing was observed in response to treatment with the rhBMP-12/sponge matrices. Since surgical failures are often associated with massive rotator cuff repairs, this model appears to parallel the clinical scenario.

Bone formation has been reported after the use of high doses of rhBMP-12 and related protein family members in rodent models of tendon healing. This sheep model could not be used to assess the potential for bone formation in response to rhBMP-12 treatment because ectopic bone formation was observed in many of the untreated repairs as well as in the repairs treated with the rhBMP-12/carrier combinations and with the Type-I/III collagen sponge alone. Partial decortication of the proximal part of the humerus prior to the repair may also have contributed to bone formation. Bone formation is not commonly observed in rotator cuff repairs in people and would not be desirable. The potential for rhBMP-12 treatment to induce excessive fibrous tissue leading to impingement cannot be evaluated in this animal model because of the absence of an acromial process in sheep.

Despite these limitations, the current study demonstrates that rhBMP-12 delivered in either a collagen or a hyaluronan sponge accelerates healing of the infraspinatus tendon to the proximal part of the humerus in sheep at eight weeks. In particular, rhBMP-12 delivered in a collagen sponge improved the repairs, as compared with the untreated repairs, by increasing peak failure load, peak stiffness, and collagen content; decreasing cellularity and cross-sectional area; and accelerating progression of healing as seen histologically. The combination of rhBMP-12 and a collagen sponge may have the potential to improve rotator cuff repairs in people. However, mechanical fixation of the repair must be achieved by other means since the rhBMP-12/collagen sponge implants provide no additional mechanical strength.

Appendix

A table describing the effects of various crosshead displacement rates on mechanical testing measurements and figures illustrating bone nodule formation in the tendon repairs and selected histological sections are available with the electronic versions of this article, on our web site at jbjs.org (go to the article citation and click on “Supplementary Material”) and on our quarterly CD/DVD (call our subscription department, at 781-449-9780, to order the CD or DVD).
References


