Biologic Augmentation of Rotator Cuff Tendon-Healing with Use of a Mixture of Osteoinductive Growth Factors*

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Background: Clinical studies have demonstrated a high rate of incomplete healing of rotator cuff tendon repair. Since healing of such a repair is dependent on bone ingrowth into the repaired tendon, we hypothesized that osteoinductive growth factors would improve rotator cuff tendon-healing.

Methods: Seventy-two skeletally mature sheep underwent detachment of the infraspinatus tendon followed by immediate repair. The animals received one of three treatments at the tendon-bone interface: (1) an osteoinductive bone protein extract on a Type-I collagen sponge carrier, (2) the collagen sponge carrier alone, and (3) no implant. The animals were killed at six and twelve weeks, and the repaired rotator cuff was evaluated with use of magnetic resonance imaging, plain radiographs, histologic analysis, and biomechanical testing.

Results: A gap consistently formed between the end of the repaired tendon and bone in this model, with reparative scar tissue and new bone spanning the gap. Magnetic resonance imaging showed that the volume of newly formed bone (p < 0.05) and soft tissue (p < 0.05) in the tendon-bone gap were greater in the growth factor-treated animals compared with the collagen sponge control group at both time-points. Histologic analysis showed a fibrovascular tissue in the interface between tendon and bone, with a more robust fibrocartilage zone between the bone and the tendon in the growth factor-treated animals. The repairs that were treated with the osteoinductive growth factors had significantly greater failure loads at six weeks and twelve weeks (p < 0.05); however, when the data were normalized by tissue volume, there were no differences between the groups, suggesting that the treatment with growth factor results in the formation of poor-quality scar tissue rather than true tissue regeneration. The repairs that were treated with the collagen sponge carrier alone had significantly greater stiffness than the growth factor-treated group at twelve weeks (p = 0.005).

Conclusions: This model tests the effects of growth factors on scar tissue formation in a gap between tendon and bone. The administration of osteoinductive growth factors resulted in greater formation of new bone, fibrocartilage, and soft tissue, with a concomitant increase in tendon attachment strength but less stiffness than repairs treated with the collagen sponge carrier alone.

Clinical Relevance: This study is the first, as far as we know, to demonstrate the possibility of increasing tissue formation in a tendon-bone gap with use of a biologic agent. It shows the importance of the use of magnetic resonance imaging to evaluate the repaired tendon, since the findings on gross observation and even histologic examination could easily be interpreted as representing an intact repair. Further studies with use of more clinically relevant models of tendon-bone repair may support the use of growth factors to improve clinical outcomes.

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Injuries of the rotator cuff tendons in the shoulder are common in individuals who engage in overhead activities for recreation or occupation. These injuries frequently result in substantial pain and disability, and they may require surgical repair. Repair of the rotator cuff typically involves direct reattachment of the torn tendon to the bone of the proximal part of the humerus. The attachment site between the rotator cuff tendon and the bone is the so-called weak link, and because healing between the repaired tendon and the bone is a slow process, prolonged periods of relative immobility are required postoperatively. The resultant shoulder stiffness and weakness typically require extensive physical therapy for restoration of full function.

Despite careful postoperative management of rotator cuff tendon repairs, objective evaluation of the repairs has demonstrated a high rate of incomplete healing and gap formation between the tendon and the bone. Harryman et al. used ultrasonography to evaluate rotator cuff repairs at an average of five years postoperatively and found a recurrent cuff defect in 20% of isolated repairs of the supraspinatus tendon and in >50% of the repairs that had involved more than the supraspinatus. More recently, Ball et al. used ultrasound to evaluate twenty arthroscopic repairs of large chronic rotator cuff tears and found that 90% had a recurrent defect despite good clinical results. In an effort to improve the results of rotator cuff repair, Gerber et al. performed a laboratory study to determine the repair technique with the greatest initial fixation strength. The same authors later used magnetic resonance imaging to evaluate the repair site, and reported a retear rate of 34% in twenty-nine patients at an average of thirty-seven months postoperatively.

Importantly, the functional results following rotator cuff repair are superior in shoulders in which the repaired cuff is intact at the time of follow-up. Thus, it is imperative to develop methods to improve healing at the tendon-to-bone junction. Numerous studies have focused on methods to improve the initial fixation strength between the tendon and bone, with attention to the type of fixation device (suture anchors, sutures alone, or newer fixation devices), suture pattern, and type of arthroscopic knot. However, there is very little information available about biological methods to improve tendon-to-bone healing.

Experimental studies have demonstrated that healing between the repaired rotator cuff tendon and bone is dependent on bone ingrowth. Healing begins with the formation of a fibrovascular interface tissue between the tendon and bone, followed by gradual bone ingrowth into this fibrous interface tissue and then into the tendon, resulting in eventual reestablishment of collagen fiber continuity between the tendon and bone. There is a gradual increase in attachment strength as healing progresses. Previous studies in our laboratory showed improved attachment strength of a tendon graft in a bone tunnel in specimens in which there was greater bone formation due to treatment with bone morphogenetic protein. Because of the importance of bone ingrowth in tendon-to-bone healing and the fact that rotator cuff tendon-healing is often limited by deficient tissue formation with gap formation at the repair site, we hypothesized that osteoinductive agents could improve the healing of a tendon attached to the bone surface. We had three specific hypotheses: (1) osteoinductive agents would induce greater formation of soft tissue, including fibrocartilage, at the tendon-bone interface compared with untreated repairs; (2) osteoinductive agents would induce greater bone ingrowth into the newly formed tissue in the tendon-bone interface; and (3) improved soft-tissue and bone formation in the treated specimens would result in a stronger tendon-to-bone attachment compared with untreated repairs. We used a sheep model of rotator cuff tendon repair to evaluate the effect of a combination of bone-derived growth factors on the healing process.

Materials and Methods

A total of seventy-two skeletally mature female Rambouillet X Columbian sheep were utilized for this study. This study was approved by the Colorado State University Animal Care and Use Committee. Each animal underwent unilateral detachment of the infraspinatus tendon followed by immediate repair. The experimental group consisted of twenty-four animals that received 1.0 mg of an osteoinductive bone protein extract (Growth Factor Mixture [GFm]; Sulzer Biologics, Wheat Ridge, Colorado) described in detail below) on a Type-I collagen sponge carrier applied to the tendon-bone interface. There were two control groups: twenty-four animals that received only the collagen sponge carrier with no growth factors and twenty-four animals that underwent the tendon repair with no implant. A total of thirty-six animals were killed at six weeks and thirty-six animals were killed at twelve weeks (twelve in each of the three groups at each time). The repaired rotator cuff was evaluated with use of plain radiographs, magnetic resonance imaging, histologic analysis, and biomechanical testing. The dose of the osteoinductive bone protein extract was chosen on the basis of a pilot study performed by the study sponsor (Sulzer Biologics).

Surgical Procedure

The animal model and surgical technique that we used were identical to those used in previously reported studies. After the induction of general endotracheal anesthesia, the right shoulder was prepared for sterile surgery. Anesthesia was induced with use of ketamine (4 mg/kg) and Valium (diazepam; 7.5 mg in total) and was maintained with halothane (1.5% to 3.0%) in 100% oxygen (2 L/min). A transverse incision was made over the lateral aspect of the right shoulder. The brachialis muscle was split in line with its fibers to allow exposure of the underlying acromion. The acromial head of the deltoid muscle was partially released from the humerus, exposing the infraspinatus tendon. The infraspinatus tendon was approximately 15 mm in width and very similar in size and thickness in each animal. The tendon was sharply detached from the greater tuberosity. The greater tuberosity was then prepared for repair of the tendon by removing any remaining soft tissue and fibrocartilage. The greater tuberosity was lightly decorti-
cated with use of a high-speed burr until punctate bleeding from the bone was noted. Decortication was done to a depth of only 1 mm; cortical bone still remained. Four 2.0-mm drill-holes were placed into the greater tuberosity for repair of the rotator cuff tendon to bone. These holes exited laterally over the proximal humeral cortex. Two number-5 Ethibond sutures (Ethicon, Somerville, New Jersey) were passed in a Mason-Allen configuration through the tendon, and each suture was brought through one of the four holes. The sutures were then tied over the lateral humeral cortex over a stainless-steel cortical bone augmentation plate (Synthes, Paoli, Pennsylvania). This allowed a secure repair of the tendon to the greater tuberosity. Routine wound closure was then performed. At the time of surgery, the collagen sponge implant either with or without GF_M was applied to the tendon-bone interface prior to securing the sutures (Fig. 1). In the second control group, no implant was placed at the tendon-bone interface.

All surgery was performed on the right shoulder only. Perioperative antibiotics (1 g of cefazolin) were administered. Prior to awakening the animal from anesthesia, a large softball was taped to the hoof on the operative side in order to discourage weight-bearing on the limb. This method of postoperative immobilization has been used by other investigators. The ball was kept on the hoof for five weeks, although it was evident that the animals did bear some weight on the involved limb. Postoperative analgesia was provided by phenylbutazone (1 g given orally) for three days following surgery, and a fentanyl patch (Duragesic [50 µg/h]; Janssen Pharmaceuticals, Titusville, New Jersey) was worn. The animals were returned to their pens immediately following surgery and were generally able to walk within twenty-four to forty-eight hours. A standardized scale from 0 to 17, which was based on animal alertness, movement, flock behavior, feeding behavior, and respiratory rate, was used to assess postoperative pain.

**Experimental Implant**

The osteoinductive bone protein extract (Growth Factor Mixture [GF_M]) was produced by Sulzer Biologics. This material was obtained from bovine cortical bone as described previously. Briefly, the process involved removal of all soft tissue and marrow from the bone, after which the bone was pulverized and then demineralized in 1 N of hydrochloric acid for eight hours at 25°C. The demineralized bone was washed in deionized water and extracted with a 4-M guanidine hydrochloric acid solution buffered with 0.01-M Tris at pH 7.6 for forty-eight hours at 15°C. The extracted proteins were purified with a 100,000-molecular-weight cutoff ultrafilter, followed by a 10,000-molecular-weight cutoff ultrafilter. Final purification was done with use of ion-exchange chromatography and reverse-phase high-pressure liquid chromatography. Electrophoretic migration patterns of this extract demonstrate bands in the range characteristic of bone morphogenetic proteins 2 through 7 (BMP-2-7), transforming growth factor-β1-3, and fibroblast growth factor. Subcutaneous implants of this material induced bone formation in rats. Osteoinductive bone protein (1.0 mg) was added to a Type-I collagen sponge carrier (20 mm × 10 mm × 1.7 mm) and then lyophilized. The collagen sponge delivery vehicle was Type-I collagen derived from bovine tendon (ReGen, Franklin Lakes, New Jersey).

Thirty-six animals were killed at six weeks, and thirty-six animals were killed at twelve weeks. Euthanasia was performed according to the guidelines set forth by the American Veterinary Medicine Association Panel on Euthanasia. At
Each time-point there were twelve animals in each group. At the time that the animals were killed, anteroposterior and lateral radiographs were made of each limb and then the tissue was frozen and shipped to our institution for further evaluation. Of each group of twelve shoulders, six were randomly chosen for magnetic resonance imaging. After magnetic resonance imaging, three shoulders were prepared for histological analysis and nine were prepared for biomechanical testing.

**Magnetic Resonance Imaging**

We tested our hypotheses about the volume of healing tissue and new bone ingrowth using magnetic resonance imaging and histological analysis. Magnetic resonance imaging was carried out for six animals in each group at each time-point. Images were performed in a clinical 1.5-T superconducting magnet (Signa Horizon LX; General Electric Medical Systems, Milwaukee, Wisconsin) with use of a commercially available, receive-only shoulder phased-array coil (shoulder array; MedRad, Indianola, Pennsylvania). Oblique axial images were acquired through the long axis of the rotator cuff tendon with use of a fast-spin-echo pulse sequence, with a repetition time of 4000 to 5500 msec, an effective-echo time of 29.8 to 34.7 msec, and a receiver bandwidth of 31.2 to 62.5 kHz, at three excitations. The field of view ranged between 13 and 14 cm to allow for visualization of the muscle-tendon junction and tendon-to-bone attachment, and the matrix was 512 × 352, yielding an in-plane resolution of 254 µ in the frequency direction by 369 µ in the phase direction; slice thickness was 1.6 mm with no interslice gap. Pulse sequence parameters were chosen for sensitivity to fluid, to serve as an internal comparison for signal properties of the tendon. On all pulse sequences obtained, fluid in the glenohumeral joint had high signal intensity. The wider receiver bandwidth was chosen to minimize the frequency shift generated by the susceptibility artifact from the nonabsorbable sutures and surrounding plate. No frequency-selective fat suppression was utilized.

The magnetic resonance images were evaluated by a radiologist blinded to the presence or absence of the experimental implant. In order to determine precisely the complete volume of tissue in the tendon-bone gap, magnetic resonance image files were transferred onto a standard personal computer, and a manual segmentation of the interface was performed. Subsequent conversion of pixels to cubic millimeters was performed with use of conversion software and a program developed on Mat Lab 6.2 software (Applied Science Laboratory; General Electric Medical Systems). The program calculates the volume of tissue in the tendon-bone gap by the summation of the areas normally demarcated on each slice, with use of the formula: volume = Σ (Ar × ST), where Ar is the area of tissue on the slice and ST is the slice thickness. The tendon-bone gap tissue was defined as the tissue between the end of the tendon and the bone; this tissue was easily distinguished from the bone and the native tendon. We derived an estimate of the tissue material properties by dividing the biomechanical measurements (ultimate load and stiffness, described below) by the measurement of tissue volume in the tendon-bone gap.

Other magnetic resonance imaging parameters that were graded included (1) the signal characteristics of the cancellous bone at the humeral head repair site (normal or hyperintense); (2) the signal characteristics of the tendon-bone gap tissue, i.e., low signal intensity (isosignal intense to “normal” tendon), intermediate signal intensity (isosignal intense to skeletal muscle), and high signal intensity (isosignal intense to fluid in the glenohumeral joint); (3) the thickness of the end of the tendon (anteroposterior dimension); and (4) the width of the gap measured from the greater tuberosity repair site to the lateral edge of the tendon. Gap measurements were made on five or six serial images and then were averaged. Relative signal intensity measurements were performed on an Advantage Windows workstation (General Electric Medical Systems), with use of a relative scale of signal intensity acquired directly from the magnetic resonance images. A standardized 1-mm² region of interest was sampled from the tendon remote from the site of repair, as well as from three 1-mm² samples from the tissue in the tendon-bone gap. These three values were then averaged. The signal intensity was measured relative to other tissues in the joint on the same image, thus providing an internal control.

**Histological Analysis**

The tissues for histological analysis were fixed in 10% neutral buffered formalin for approximately two weeks and then were decalcified in 5% nitric acid. After the tissue was fully decalcified, the tissue was further trimmed and embedded in paraffin. Five-micrometer-thick sections were made and were stained with hematoxylin and eosin and safranin O. Coronal sections were made in line with the tendon, which allowed evaluation of the bone, the tendon-bone gap tissue, and the end of the repaired tendon. The histological sections were viewed with use of light and polarized light microscopy on an Olympus BH-2 microscope (Olympus Optical, Lake Success, New York). The sections were evaluated in a blinded fashion with no knowledge of experimental or control group. We assessed new-bone formation at the greater tuberosity, cellularity and vascularity in the tendon-bone gap tissue, new matrix deposition in the tendon-bone gap, the presence of cartilage in the tendon-bone gap, and collagen fiber continuity and so-called organization from the bone surface into the gap tissue.

**Biomechanical Testing**

We tested our third hypothesis about the tendon attachment strength, using standard biomechanical testing protocols. At the time of biomechanical testing, the specimens were thoroughly thawed. All muscle was removed from the infraspinatus, but the tendon itself was left intact. A specially designed jig was made for mounting the proximal part of the humerus. Testing was carried out on a load frame (MTS, Eden Prairie, Minnesota) coupled to a controller (Instron, Canton, Massachusetts). The specimens were mounted in the load frame for uniaxial tensile loading in line with the pull of the infraspinatus tendon. The proximal part of the humerus was mounted and secured with clamps, and then the tendon was gripped in
a specially fabricated gripping device with serrated edges that allowed secure gripping of the soft tissue and prevented slippage. The tendon was gripped at a constant distance (25 mm) from the bone in each specimen. Data were collected on a personal computer with use of data acquisition software (Lab-Tech Notebook, Andover, Massachusetts). The load-frame data were collected at a rate of 20 Hz. All testing was carried out at room temperature, and the specimens were kept moist with normal saline solution during testing.

Each specimen was cycled between 10 N and 60 N (approximately 1% strain) at a rate of 1 Hz for a total of ten cycles to precondition the specimens prior to loading. Each specimen was then loaded to failure at a cross-head displacement rate of 20 mm per minute. The ultimate load-to-failure and the site of failure were recorded for each specimen. Grip-to-grip displacements were used to compute stiffness.

Data Analysis
Our primary purpose was to compare the growth factor-treated group with the control groups. As a secondary analysis, we made comparisons within each group over time. The volume of new bone and new soft-tissue formation at the tendon-bone attachment site and the signal intensity measurements in the tendon and the interface (as measured by magnetic resonance imaging) were compared with use of Kruskal-Wallis rank analysis of variance (version 11.0; SPSS for Windows, Chicago, Illinois). For the biomechanical data, the ultimate failure load and stiffness in the control and experimental groups were compared at each time-point with use of analysis of variance for normally distributed data and a Kruskal-Wallis rank analysis of variance for non-normally distributed data. Post hoc tests were done with use of the Mann-Whitney rank-sum test and Bonferroni corrected comparisons. The same statistical tests were used to compare the six-week and twelve-week data within each group. Correlations between the biomechanical data and the magnetic resonance imaging measurements were performed with use of linear regression analysis. Significance was set at p < 0.05.

Results
Gross Observations
All animals tolerated the surgical procedure well with no intraoperative or perioperative complications. There was no evidence of infection or immunological reaction in any specimen. By postoperative day 2, the pain scores were 0 to 1, and by postoperative day 3, the scores were 0 in all animals.

Direct observation of the specimens showed reparative scar tissue spanning a gap between tendon and bone in all specimens. It was difficult to discern scar tissue from normal tendon by gross observation. Gross observation demonstrated a greater volume of new tissue formation at the tendon repair site at the greater tuberosity.

Comparison of Experimental and Control Animals
Radiographs
Plain radiographs demonstrated new-bone formation at the greater tuberosity as well as over the cortical bone augmenta-
tion plate in the growth factor-treated specimens. This new bone appeared to be remodeled over time, such that less new bone was present at twelve weeks compared with six weeks. New bone was not present in either of the control groups. There was no evidence of substantial bone resorption at the tendon repair site on plain radiographs.

Magnetic Resonance Imaging
Magnetic resonance imaging allowed clear distinction between the end of the repaired tendon, the newly formed tissue in the tendon-bone gap, and the greater tuberosity (Figs. 2-A, 2-B, and 2-C). The tendon had normal low-signal intensity, while the newly formed tissue between the tendon and bone had intermediate to high-signal intensity. There was extensive periosteal new-bone formation and increased signal intensity in the humeral head in the growth factor-treated group compared with controls. In the six-week group, the average gap (and standard deviation) between the repaired tendon and bone was 23.0 ± 5.6 mm in the controls with no implant, 25.9 ± 5.6 mm in the collagen-carrier control, and 19.3 ± 4.6 mm in the growth factor-treated group; the differences were not significant. In the twelve-week group, the average gap between the repaired tendon and bone was 22.9 ± 10.4 mm in the controls with no implant, 24.0 ± 12.1 mm in the collagen carrier control, and 19.1 ± 11.9 mm in the growth factor-treated group; the differences were not significant.

Fig. 2-B
Axial magnetic resonance image acquired from a twelve-week specimen from a control with no implant demonstrates the interface tissue between tendon and bone (arrows) and very little new-bone formation at the bone surface.

Fig. 2-C
Axial magnetic resonance image acquired from a twelve-week specimen from the growth factor-treated group demonstrates extensive new-bone formation at the bone surface (arrows). Note the signal heterogeneity of the tendon-bone interface.
Magnetic resonance imaging demonstrated a significantly greater volume of new-bone formation in the tendon-bone gap in the growth factor-treated group at both six weeks and twelve weeks compared with the collagen carrier control group (p < 0.05). Similarly, there was a significantly greater volume of newly formed soft-tissue in the tendon-bone gap in the growth factor-treated group compared with the collagen carrier control at both six weeks (p < 0.05) and twelve weeks (p < 0.05) (Fig. 3).

There was significantly greater magnetic resonance imaging signal intensity in the interface tissue in the collagen carrier control at six weeks (p < 0.05), but this was no longer significant at twelve weeks. There were no significant differences in signal intensity in the tendon itself between the controls and the growth factor-treated specimens at either six or twelve weeks.

**Histological Analysis of the Repaired Tendon**

**Six-Week Specimens**

The normal infraspinatus tendon in the sheep inserts to the bone by means of a direct insertion, with a zone of fibrocartilage between the tendon and bone. In this animal model, the gap between the tendon and bone fills in with fibrovascular granulation tissue in both the controls and growth factor-treated specimens. A completely normal-appearing insertion site was not reformed in any group. The infraspinatus tendon itself appeared essentially normal, with viable cells and well-organized collagen fibrils in both groups. The native tendon could be easily distinguished from this interface scar tissue. In the control specimens, the newly formed tissue in the tendon-bone interface gap was highly cellular, containing a mix of spindle-shaped fibroblastic cells, mononuclear cells, and occasional chondrocytes (Figs. 4-A through 4-D). The collagen fibers in the interface tissue between the tendon and bone were moderately well organized. At the bone surface, there was a thin seam of newly formed woven bone, and in some specimens there were small areas of cartilage in the interface. In contrast, the growth factor-treated specimens demonstrated extensive new-bone and cartilage formation in the tendon-bone gap by six weeks. There was a greater volume of interface tissue in the growth factor-treated specimens than in both of the control groups. This tissue was heterogeneous, with some areas moderately well organized (collagen fibers aligned in the same direction) and other areas poorly organized. No remnants of the collagen sponge could be identified histologically in either sponge group at six weeks.

**Twelve-Week Specimens**

By twelve weeks, the interface tissue in both controls and growth factor-treated specimens was denser because of an increased matrix deposition and was less vascular and less cellular than at six weeks. The collagenous matrix was more oriented in line with the tendon in all three groups. In both control groups, there were occasional small areas of cartilage in the tendon-bone gap, but the gap was mostly filled with fibrous tissue (Figs. 5-A and 5-B). Polarized light microscopy showed collagen fiber continuity between the tendon and bone. Although some specimens in both control groups had a small zone of fibrocartilage between the tendon and bone, a normal-appearing direct tendon insertion site did not consistently reform by twelve weeks. The mineralized fibrocartilage zone and the tidemark (mineralization front) that are found in the normal direct tendon insertion site were not reformed in the specimens in either control group.

In the growth factor-treated specimens at twelve weeks, the gap tissue demonstrated well-organized collagen fibers that were oriented in line with the tensile pull of the tendon and this tissue was less cellular and more organized than that
in the six-week specimens. The large areas of new bone and cartilage that were present at six weeks had been remodeled by twelve weeks and were more organized. The cells in the gap tissue also became aligned with the tensile load on the tendon. Polarized light microscopy demonstrated collagen fiber continuity from the bone surface into the fibrocartilage gap tissue. The cartilage in the tendon-bone gap had remodeled into a more normal-appearing fibrocartilage zone (Figs. 5-C and 5-D). There was more cartilage formation in the tendon-bone gap in the growth factor-treated specimens compared with both control groups. However, the columnar arrangement of chondrocytes in a normal direct insertion was not consistently reestablished, and the resulting insertion site still did not consistently demonstrate the histological criteria of a normal direct insertion. The collagen sponge had completely resorbed by twelve weeks in all animals.

**Biomechanical Testing**

All of the specimens failed at the soft tissue-to-bone attachment site. In several specimens, small bone spicules were found on the end of the soft tissue after failure. The ultimate load-to-failure was significantly higher in the growth factor-
treated group compared with the collagen carrier control at six weeks (p < 0.05) and was significantly higher in the growth factor-treated group compared with both control groups at twelve weeks (p < 0.05). When the failure load data were normalized by dividing by the volume of new tissue at the attachment site, no significant difference was found among the groups at either six weeks or twelve weeks (Figs. 6-A and 6-B).

The collagen control group was significantly stiffer than the growth factor group (p = 0.005) at twelve weeks but did not differ from the controls with no implant (p = 0.07); no significant difference was found between the growth factor group and the controls with no implant (Fig. 7). At the six-week time-point, there was no significant difference among the groups.

We found a significant correlation between the normalized load to failure and the volume of new soft-tissue in the tendon-bone gap at both six weeks ($r^2 = 0.65$, p = 0.009) and twelve weeks ($r^2 = 0.37$, p = 0.02). There was a trend for a cor-
relation between load to failure and the tendon-bone gap distance ($r^2 = 0.42$, $p = 0.06$).

Changes Over Time within Each Group
There was gradual maturation of the healing tissue from six weeks to twelve weeks in all three groups, with more marked changes occurring in the growth factor-treated specimens. This reflected the fact that there was extensive formation of immature reparative scar tissue in the growth factor-treated specimens at the early time-point. As described above, the scar tissue in the tendon-bone gap became less cellular, with increased matrix deposition and improved organization. There was a decreased volume of new-bone formation in the tendon-bone gap in the growth factor-treated group at twelve weeks.
compared with six weeks, while there was no significant change in the volume of new soft-tissue formation in the tendon-bone gap between six weeks and twelve weeks as measured by magnetic resonance imaging (Fig. 3). There was no significant difference in the signal intensity in the gap tissue or native tendon between six and twelve weeks in any group.

There were significant increases in ultimate load-to-failure between six weeks and twelve weeks in the growth factor-treated group (p < 0.05) and the controls with no implant (p < 0.05), but there was no change in the collagen carrier control group (Figs. 6-A and 6-B). Within the collagen carrier control group, the twelve-week specimens were significantly stiffer than the six-week group (p = 0.04); no differences were found between the six-week and twelve-week time-points within either the growth factor-treated group or the controls with no implant (Fig. 7).

**Discussion**

Failure of secure healing between tendon and bone is a major problem in rotator cuff repair. Various factors are thought to account for poor healing of the rotator cuff tendon, including intrinsic tendon degeneration, fatty infiltration of the muscle and tendon, muscle atrophy, poor bone quality, and weak tendon-to-bone fixation. Our goal in this study was to examine the hypothesis that osteoinductive growth factors would improve healing of the rotator cuff tendon to bone. Despite the use of the repair technique that has been found to have the greatest biomechanical strength in vitro models, the repaired tendon consistently detached from the repair site in this animal model. Thus, in this study, we modeled tissue formation in a gap between tendon and bone. The gap was evident only by magnetic resonance imaging. Notably, gross inspection of the specimens demonstrated stout tissue connecting the infraspinatus muscle to the bone, with no evidence of a gap. Furthermore, histological analysis showed a well-organized, tendon-like fibrous tissue connecting to bone; the histological findings could easily be interpreted as representing an intact repair. If magnetic resonance imaging were not performed, it may have been concluded that the tendon remains firmly attached to the bone. We believe that this is an important finding, as other investigators are using this same model to evaluate healing of the rotator cuff tendon. On the basis of our results, we urge caution in interpreting the results of other studies that have used a sheep model for rotator cuff repair.

It is likely that the tendon detached from the repair site in the early postoperative period because of an inability to control the loads on the repaired tendon immediately postoperatively. The resulting gap subsequently filled in with a fibrovascular scar tissue. This scar tissue gradually matured and acquired substantial load-bearing capacity. Other investigators have also reported gap formation at a tendon-to-bone repair site. Gerber et al. used the same animal model with an identical suture pattern and a cortical bone augmentation plate, and they also found that the gap between the end of the repaired tendon and the bone filled in with scar tissue that was often indistinguishable from normal tendon. They termed this a “failure in continuity.” Similar to our results, they reported that the scar tissue in the defect was composed of well-organized, dense collagen bundles by six months and was difficult to distinguish from the end of the tendon.

Although we were not able to study healing of an intact rotator cuff tendon repair, the resultant model of healing in a tendon-bone gap has clinical relevance. Clinical studies of rotator cuff repair have demonstrated a high rate of incomplete healing and gap formation between the tendon and the bone. There are important concerns among shoulder surgeons about the strength of rotator cuff tendon-to-bone fixation and the potential for formation of a tendon-bone gap and failure of healing. Furthermore, various materials have been developed to use as a scaffold to bridge a gap in rotator cuff tendon repair. The ability to improve tissue formation in a tendon-bone gap may allow improved clinical results.

Our results support our hypotheses about the induction of new soft-tissue and bone formation. To our knowledge, this is the first report of the use of growth factors to augment tissue formation between the rotator cuff tendon and bone. Examination of the group with no implant showed that healing occurs in this animal model by formation of a fibrovascular scar-tissue interface between tendon and bone. Although there was partial reformation of a fibrocartilage interface in the growth factor-treated animals, we did not observe consistent formation of a normal insertion site. The growth factors appeared to induce a greater reactive scar-tissue response rather than regeneration of a morphologically normal insertion site. Although there was abundant new tissue formation, the quality of this tissue was relatively poor, as evidenced by the finding that at twelve weeks the failure loads were only approximately 31% of the normal strength of a sheep infraspinatus tendon insertion, on the basis of previ-
ously reported data. Magnetic resonance imaging demonstrated persistently high signal in the reparative tissue in the tendon-bone interface, reflecting increased mobile water and indicative of a relatively poorly organized matrix. The growth factor-treated group had significantly greater failure loads compared with both control groups; however, when the data were normalized by tissue volume to provide an estimation of material properties, there were no differences between groups. Furthermore, the stiffness of the tendon-bone construct was not improved in the growth factor-treated group compared with the controls. These results all support the conclusion that the growth factor treatment results in the formation of poor-quality scar tissue rather than true tissue regeneration. Previous studies of the effect of growth factors on tendon and ligament-healing have also described the formation of new tissue with inferior material properties. The reparative tissue in our model underwent remodeling and became more mature over time, as demonstrated histologically by improved matrix organization and biomechanically by increased attachment strength in the growth factor-treated group between six and twelve weeks.

There were important differences between the groups that received the collagen sponge implant (the collagen carrier control group and the growth factor group). The stiffness was higher in the collagen carrier control group compared with the growth factor group at twelve weeks. At the same time, we found a lower volume of new tissue formation in animals receiving the collagen sponge alone. The collagen sponge appeared to inhibit excessive tissue formation. The presence of the sponge directly between tendon and bone may have acted as a physical block to tissue formation. We also found increased magnetic resonance imaging signal intensity in the tendon-bone interface in the collagen sponge group at six weeks. It is possible that a subtle immune response against the bovine-derived collagen sponge contributed to the apparent inhibition of tissue formation and the increased magnetic resonance imaging signal intensity, although histological evaluation did not support that. The diminished new tissue formation in the collagen sponge group makes the finding of new tissue formation in the growth factor-treated animals even more notable.

The differences between the collagen carrier control group and the growth factor group suggest distinctly different biologic responses to these implants. We hypothesize that the collagen sponge by itself acts as a scaffold to orient newly forming fibrous tissue. Because a gap forms in this model, the sponge by itself may play a positive role in healing (as evidenced by improved stiffness). The collagen sponge with the growth factor induces a vigorous biologic response, leading to excessive new tissue formation. However, this newly forming tissue has poor material properties at twelve weeks. We further hypothesize that any beneficial effect on healing due to the collagen sponge alone may not have been evident in the group that received a collagen sponge with growth factor because of more rapid sponge resorption in this group. The intense biologic response to the growth factors (including an early inflammatory response) would likely accelerate sponge resorption. Analysis of earlier time-points would be required to examine the kinetics of sponge resorption. These findings have clinical relevance since there are currently available collagen-containing extracellular matrix materials that are used as a scaffold to improve rotator cuff tendon-healing. Similar to the findings in our study, Schlegel et al. recently reported improved stiffness but no change in failure loads with use of a swine small intestine submucosa patch to augment rotator cuff tendon-healing in the same sheep model.

The potential for osteoinductive growth factors to improve rotator cuff tendon repair is supported by previous studies that have examined healing of tendon to bone. Although the basic cellular and molecular mechanism of rotator cuff tendon-to-bone healing is poorly understood, previous animal studies have shown that healing proceeds by bone ingrowth into the interface between tendon and bone. Gerber et al. used a sheep model of infraspinatus tendon repair and reported gross and histological findings very similar to our results, with an osteoblastic response at the tendon-to-bone attachment site. Uhthoff et al. used a rabbit model of supraspinatus tendon repair and found that, while there was little cellular proliferation in the tendinous stump, there was cellular and vascular proliferation within the underlying bone and subacromial bursa at two weeks following repair. Further support for the use of osteoinductive growth factors is provided by studies that have demonstrated bone resorption and potentially impaired bone formation at tendon and ligament insertion sites. For example, Kansus et al. reported regional osteoporosis in the proximal part of the humerus in patients with a rotator cuff tendon tear. Regional osteoporosis in the proximal part of the humerus not only diminishes the pull-out strength of sutures or bone anchors but also may result in impaired bone formation at the healing tendon-bone junction. The use of an osteoinductive agent at the insertion site may ameliorate these deleterious bone changes.

There are limitations to the animal model used in this study. As discussed above, the repaired tendon consistently detached from the repair site. In humans, when a rotator cuff tendon repair fails, there is a persistent gap in the tendon, whereas in the sheep model the repair site is extrasynovial and the gap fills with scar tissue. It appears that the sheep is able to produce abundant new bone at the greater tuberosity, which is unlikely to occur in the typical patient undergoing rotator cuff repair who may be elderly and have poorer bone-forming potential. Another limitation of this model is that in this study we examined healing of an acute rotator cuff repair, which does not mimic the typical clinical situation in which there is retraction and atrophy of the torn tendon. There are typically intrinsic degenerative changes in the torn rotator cuff tendon (tendinosis), tendon retraction, and osteoporosis of the greater tuberosity in shoulders that have a long-standing rotator cuff tear. The healing capacity of the tissue in these clinical situations is likely diminished. Gerber et al. performed delayed repair of a retracted tendon in the sheep model and reported an exceedingly high failure rate after repair, which they
attributed to the high tension on the repairs’. Soslowsky et al. developed a rat model of overuse tendinosis, which may be useful to evaluate the effect of growth factors onrotator cuff tendon-healing. Although the rat model has been used to study tendon repair, it is not known whether repair failure and scar formation also occur in the rat.

Our results should be considered preliminary and only a first step toward the development of growth factors for clinical application in rotator cuff repair. We had a relatively small sample size, resulting in low power for some comparisons. These findings would currently have limited application to rotator cuff repair in patients, as a hypertrophic tissue response in the subacromial space could lead to subacromial impingement. The abundant new tissue likely forms in this model to fill the tendon-bone gap, resulting in a stronger attach-

In conclusion, we found that a mixture of osteoinductive growth factors leads to increased formation of new bone and soft tissue in a tendon-bone gap, resulting in a stronger attachment between the tendon and bone at six and twelve weeks after repair. We also found improved stiffness of the repairs treated with a collagen scaffold alone. Because clinical studies of rotator cuff repair have demonstrated a relatively high prevalence of failure of complete healing of rotator cuff repairs, the use of extracellular matrix scaffolds and growth factors to augment healing may provide a clinically important improvement in rotator cuff repair. The use of biologic agents to improve healing is likely to be especially valuable in patients with diminished biologic healing potential due to rotator cuff tendinosis and associated osteoporosis of the greater tuberosity.

References


