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Augmentation of Tendon Healing in an Intraarticular Bone Tunnel with Use of a Bone Growth Factor*

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ABSTRACT

We hypothesized that an exogenous bone growth factor could augment healing of a tendon graft in a bone tunnel in a rabbit anterior cruciate ligament-reconstruction model. Seventy rabbits underwent bilateral anterior cruciate ligament reconstructions with a semitendinosus tendon graft. One limb received a collagen sponge carrier vehicle containing a mixture of bone-derived proteins while the contralateral limb was treated with either no sponge or a sponge without bone-derived proteins. The reconstruction was evaluated at 2, 4, or 8 weeks with histologic, biomechanical, and magnetic resonance imaging analysis. Histologic analysis demonstrated that specimens treated with bone-derived proteins had a more consistent, dense interface tissue and closer apposition of new bone to the graft, with occasional formation of a fibrocartilaginous interface, when compared with control specimens. The treated specimens had significantly higher load-to-failure rates than did control specimens. Treatment with bone-derived proteins resulted in an average increase in tensile strength of 65%. The treated specimens were stronger than control specimens at each time point, but the difference was greatest at 8 weeks. On the basis of signal characteristics and new bone formation, magnetic resonance imaging was useful for predicting which limb was treated, the site of failure, and the limbs with higher load-to-failure values. This study demonstrates the potential for augmenting tendon healing in an intraarticular bone tunnel using an osteoinductive growth factor.

Many ligament reconstructions and tendon transfers require healing of tendon grafts in a surgically created bone tunnel. For example, ACL reconstruction is often performed with a graft of semitendinosus or gracilis tendon or both placed into tunnels in the tibia and femur. The weak link during the early healing process is the attachment site between the tendon and bone.[9] Thus, the rate of healing and the strength of this attachment limit rehabilitation and return to activity. The intraarticular environment can add several confounding factors to the process of graft healing. Graft-tunnel motion, early or excessive loading of the graft, or synovial fluid influx may interfere with healing. Synovial fluid may impair healing by diluting the initial hematoma and preventing fibrin clot formation.

There is little information available about the mechanism of healing of a tendon graft in a bone tunnel. In an extraarticular model, Rodeo et al.[9] found that healing proceeded by formation of a fibrovascular interface tissue between the tendon and bone, followed by progressive bone ingrowth into this interface tissue. Collagen fiber continuity was gradually established between the tendon and bone, resulting in a secure attachment between the tendon and bone by an indirect type of insertion. The strength of the tendon-to-bone attachment correlated with new bone formation around the tendon and with collagen fiber continuity between the tendon and bone. Other studies have demonstrated formation of a direct type of insertion between tendon and bone, with fibrocartilage between the tendon and bone. For example, Shino et al.[14] reported establishment of a direct type of insertion between an allograft tendon and the bone tunnel in an ACL-reconstruction model in dogs. Schiavone Panni et

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al.[11,12] performed ACL reconstruction in rabbits using bone-patellar tendon-bone autografts and found formation of a direct type of insertion between the bone tunnel and the tendinous portion of the graft. Another recent study in rabbits reported formation of a direct type of insertion between a tendon graft and a bone tunnel in an intraarticular location by 12 weeks.[8] The formation of a direct or indirect type of insertion probably depends on many factors, including the specific cell types present, the local mechanical environment, and possibly the presence of specific cytokines. Further studies are required to gain understanding of the basic mechanism of healing of a tendon graft in a bone tunnel.

Because tendon-to-bone healing occurs by bony ingrowth, it is possible that healing could be improved by adding exogenous bone-growth factors. Several studies have shown the effectiveness of bone morphogenetic protein (BMP) in the induction of new bone formation.[6,15] Rodeo et al.[10] reported on the use of recombinant human BMP-2 (rhBMP-2) in tendon healing in a bone tunnel. These authors demonstrated histologic evidence of earlier bone ingrowth as well as higher attachment strength at early time points in those grafts treated with rhBMP-2 when compared with controls. This study was the first to examine the use of a growth factor in tendon-to-bone healing; its major limitation is that it was an extraarticular model.

More recently, a bone-derived extract (Bone Protein, Sulzer Biologics, Wheat Ridge, Colorado) has been reported to be effective in augmenting bone ingrowth.[1,13] For example, Bone Protein has been shown to be effective in improving fusion rates after spinal surgery.[3] The purpose of the current study was to test the hypothesis that Bone Protein, an exogenous bone-growth factor, would improve healing of a tendon graft in a bone tunnel in an intraarticular ligament-reconstruction model.

MATERIALS AND METHODS

Study Design

The study protocol was approved by the Institutional Animal Care and Use Committee. All rabbits were obtained from a United States Department of Agriculture-licensed dealer, were housed in the animal care laboratory at our institution, and were cared for according to the standards of the National Institutes of Health. Each of the 70 rabbits underwent bilateral ACL reconstruction with use of the ipsilateral semitendinosus tendon. One limb received Bone Protein on a type I collagen sponge carrier, and the contralateral limb served as an operated control. Eight animals were used for histologic evaluation and 14 for biomechanical testing at each of three time points (2, 4, or 8 weeks) (Table 1). Three animals at each time point were randomly selected for MRI of both limbs.

A preliminary pilot study was conducted on 12 rabbits using an extraarticular model.[10] Two different doses of Bone Protein were evaluated in the pilot study (35 μg or 150 μg). There was superior healing in the 35-μg group based on histologic criteria, and thus this dose was chosen for the present study.

TABLE 1
Distribution of Limbs for Histologic and Biomechanical Analysis

Sacrifice interval	Experimental limb	Control limb	
		Untreated sponge	No sponge
Histologic analysis			
2 weeks	8	4	4
4 weeks	8	4	4
8 weeks	8	4	4
Biomechanical analysis			
2 weeks	14	7	7
4 weeks	14	7	7
8 weeks	14	7	7

The proteins found in Bone Protein are purified from a noncollagenous protein extract of bovine femurs, as previously described.[1] After purification, immunoblot analysis positively identified the following proteins with known osteoinductive activity or activity that may modulate bone formation: BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7, transforming growth factor (TGF)-β1, TGF-β2, TGF-β3, and fibroblast growth factor (FGF-1).

Surgical Procedure

A midline skin incision was made, and a lateral parapatellar incision was used to expose the knee. The semitendinosus tendon was harvested; it averaged 3.8 cm in length. The native ACL was resected. Drill tunnels (1.7 mm) were created through the femur and tibia, entering the joint at the anatomic origin and the insertion of the native ACL. A type I collagen sponge containing 35 μg of Bone Protein was applied to the part of the graft that went into the bone tunnel (Fig. 1). The length of each tunnel was measured. The control limbs received either a sponge without Bone Protein (group 1) or no sponge (group 2). All sponges were moistened with saline solution just before fixation to the graft. The graft was advanced through the tunnels, and the ends of the graft were sutured under slight tension to the periosteum for fixation with use of 4–0 Ethibond (Ethicon, Inc., Somerville, New Jersey) suture. The retinacular incision and wound were closed in layers in standard fashion.

Histologic Analysis

At the time of sacrifice, each specimen was grossly examined for any evidence of degenerative joint changes, synovial inflammation, and effusion. The entire joint, including the proximal tibia and distal femur, was harvested and placed in 10% neutral-buffered formalin. The specimens were embedded in polymethyl methacrylate without decalcification. Five micron thick sections were cut perpendicular to the bone tunnels, yielding cross sections of the tendon graft-bone interface in both the femoral and tibial tunnels. The sections were stained with hematoxylin and eosin, Von Kossa, and Masson's trichrome and

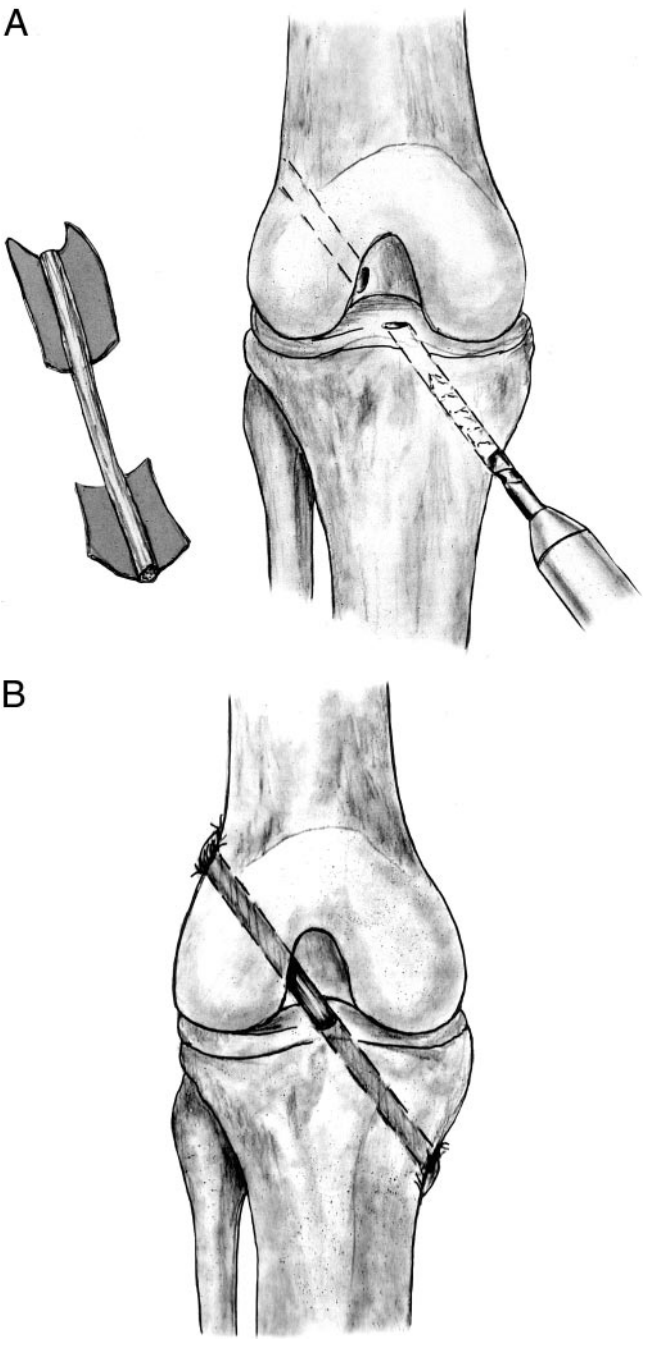

Figure 1. A rabbit ACL reconstruction with a type I collagen sponge carrier vehicle impregnated with the bone-growth factor. A, after semitendinosus tendon harvest, sponges were fixed to the portions of the graft that would be located within the bone tunnels. B, the graft was advanced through the tunnels and secured to the periosteum at the outer ends of the tunnels.

then examined with light and polarized light microscopy techniques on an Olympus BH-2 microscope (Olympus Optical Co. Ltd., Tokyo, Japan).

Healing between the tendon and the bone tunnel was assessed by determining the formation of new tissue (fibrovascular granulation tissue, fibrous tissue, cartilage, and bone) between tendon and bone. We assessed the number and type of cells as well as the density of new matrix present in the tendon-bone interface. Collagen fiber continuity between tendon and bone was assessed with the polarized light microscope. We also assessed the presence of articular cartilage degeneration, synovial hyperplasia, and foreign body giant cell response to detect any adverse effects of the Bone Protein treatment. Comparisons were made between groups (treated and control limbs), as well as between different time points.

Biomechanical Testing

The femur-ACL graft-tibia construct was harvested from each limb and frozen at −80°C until testing. Before testing, all soft tissue was removed except for the ACL graft. The bones were potted in bonding cement just above the femoral sutures and below the tibial sutures, allowing secure fixation in the testing jig. A specially designed apparatus was used that oriented the specimens such that a tensile load could be applied along the axis of the reconstructed ligament (Fig. 2). The construct was loaded on a materials testing machine (MTS Systems Corp., Eden Prairie, Minnesota) at a strain rate of 40 mm/sec (approximately 100% per second). The ultimate load to failure was recorded on a personal computer, and the site of failure was recorded. The site of failure was characterized as femoral attachment, tibial attachment, midsubstance of tendon graft, or a combination. Because the same diameter tunnel was used for all specimens and the lengths were very consistent between specimens, the absolute value for the load to failure was used for comparisons without the need to normalize the data by tunnel length for comparison.

MRI Analysis

Nine rabbits (three from each time point) were randomly selected to undergo MRI before biomechanical testing. All specimens were imaged in a 1.5-T clinical MR unit (Signa Horizon LX, GE Medical Systems, Milwaukee, Wisconsin). Specimens were imaged in either a commercially available quadrature-receive wrist coil (Medical Advances, Milwaukee, Wisconsin) or a prototype phased-array wrist coil (MRI Devices Corp., Waukesha, Wisconsin). Knees were placed in the coil in full extension and were surrounded by a pliable nonferrous putty to increase regional signal-to-noise ratio. A water phantom was placed to localize the center of the bore.

Pulse sequences included sagittal and coronal fast spin echo techniques, with use of a proton-density weighted sequence (repetition time, 3500 to 5000 msec; echo time [effective], 18 to 42 msec), a bandwidth of 31.2 kHz, a field of view of 8 cm with an imaging matrix of 512 × 288 to 320, four excitations, and a slice thickness of 1.5 mm with no interslice gap. Images were obtained tangential to the coronal and sagittal orientation of the ACL graft from an axial localizer image.

The MR images were reviewed by a musculoskeletal radiologist (HGP) who was blinded to the treatment

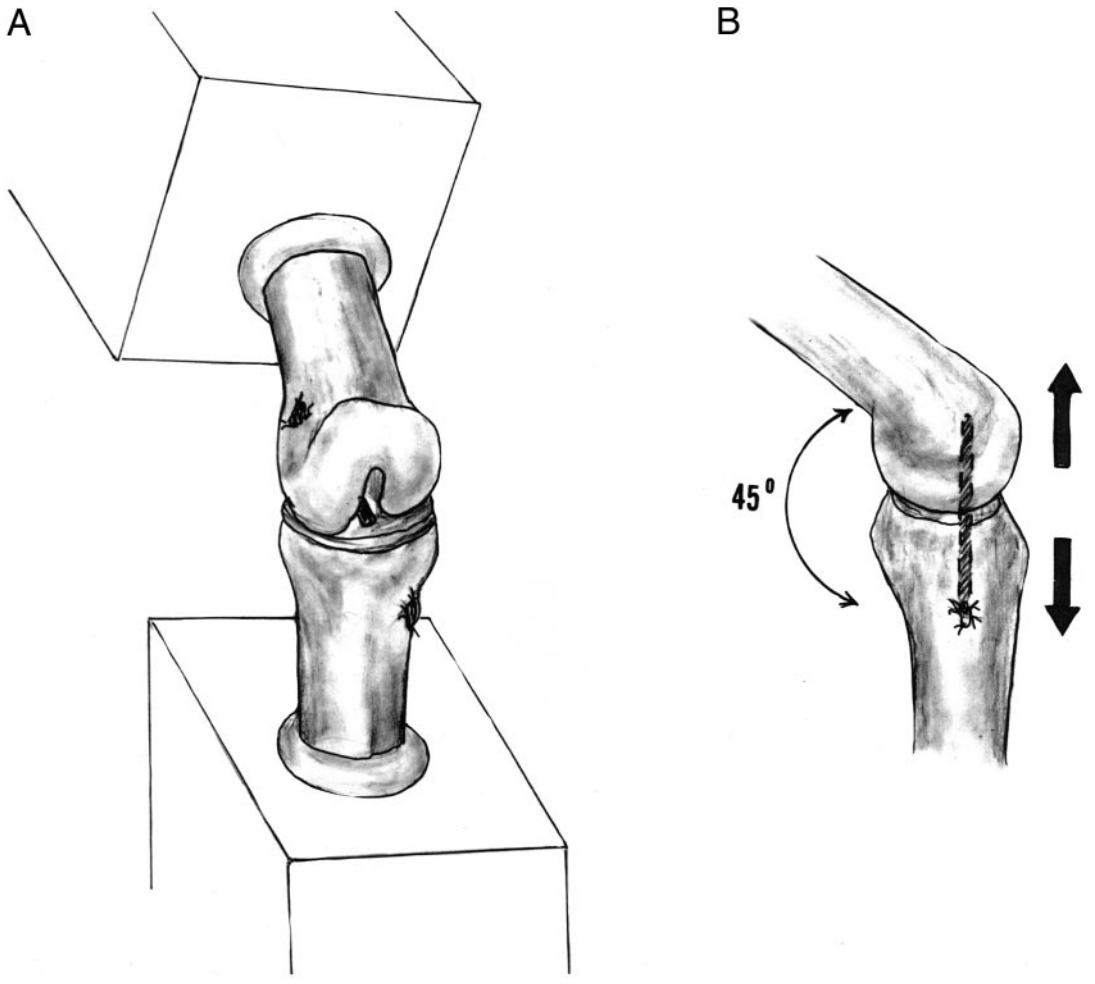

Figure 2. A, rabbit knee specimen mounted in the biomechanical testing apparatus. B, load was applied along the axis of the graft with the knee at 45° of flexion.

group. The graft position relative to the parallel axis of Blumensaat's line was assessed, and any site of gross graft discontinuity was noted. The signal characteristics were also qualitatively assessed and denoted as low signal intensity (isosignal intense to the adjacent PCL), moderate signal hyperintensity (isosignal intense to skeletal muscle), and severe signal hyperintensity (isosignal intense to joint fluid). Quantitative T2-weighted relaxation times were not measured. We looked for the presence of new bone formation, either within the tunnel or at the periosteal surface, the presence of fluid signal in the tunnel, and tunnel widening and morphologic characteristics. These data were used to make the following predictions: 1) the limb that received Bone Protein, 2) the limb with the higher ultimate load to failure, and 3) the site of failure for each specimen. It was postulated that the limb that had more bone formation in the tunnel had received Bone Protein, and that the more homogeneous grafts with good orientation and no gross disruption would have the higher load to failure. These predictions, based on the qualitative MR data, were then compared with the results of the subsequent biomechanical testing.

Statistical Analysis

A power analysis was performed to determine the minimum number of animals necessary to draw statistically valid conclusions. We used data from previous work at this institution and from a pilot study for this project. This analysis revealed that 14 specimens (7 of each control group) would be sufficient at each time point for biomechanical analysis to achieve a power of 0.80 with $\alpha = 0.05$. This number would allow detection of a 50% increase in attachment strength, which we thought would be clinically significant. We compared the ultimate load to failure between the experimental and control limbs using paired Student's *t*-tests. Comparisons of groups at different time points and between control groups were performed with analysis of variance. Frequency of failure at given sites (femoral, midsubstance, tibial) was compared with use of chi-square analysis. The applicability of these tests was confirmed by a statistician and calculated using the statistical software package Sigmastat (SPSS, Inc., Chicago, Illinois).

RESULTS

Gross Observation

Eight animals died prematurely, three because of failure to thrive (two at 9 days and one at 10 days postoperatively), three were sacrificed early because of postoperative infections (one experimental limb, one control limb, one bilateral), and two died from perioperative anesthetic complications. All grafts were intact (Fig. 3), although two appeared grossly atrophic. Two grafts were partially lacerated during dissection and therefore had to be excluded.

Gross observation demonstrated no evidence of a significant adverse effect of the Bone Protein treatment on the joint tissue. There was minimal chondral degeneration and mild synovial inflammation. These findings were thought to be due to the surgical intervention itself because the changes were mild and similar between treated and control specimens. Occasional heterotopic bone formation was observed at the extraarticular tunnel entrance in both the treated and the control specimens, with more extensive new bone formation in the Bone Protein-treated specimens. Ossification was not seen at the intraarticular aperture of the drill tunnel. In three knees, there was abundant scar formation at the periosteal end of the drill tunnel. This scar formation was seen in two knees that received Bone Protein and in one control knee. Abundant scar tissue was not seen at the intraarticular aperture of the drill tunnel. No meniscal specimens had evidence of ossification.

Histologic Analysis

Two-Week Specimens. In the control specimens, healing began with formation of a fibrovascular interface tissue between the tendon and bone. There was occasional cartilage formation in the interface zone. Cellular proliferation seemed to be derived from bone and bone marrow cells, as there was no evidence of consistent cell proliferation within the tendon. There was considerable variability in the amount of cartilage and the density of the interface tissue between different specimens as well as within a given specimen. In some specimens there was dense interface tissue containing cartilage at one end of the tunnel, whereas the other end of the tunnel was much less organized and did not have cartilage in the tendon-bone interface. There appeared to be widening of the bone tunnel in some specimens, with a large gap between the tendon and bone filled only with loose fibrous tissue, whereas in other specimens the tendon-bone interface was denser and contained cartilage. There was very little new bone formation along the edge of the tunnel. Remnants of the collagen sponge were evident in the tendon-bone interface. There was no significant difference in the overall histologic appearance of the interface tissue between the tibial and femoral tunnels at this time point, although the cancellous bone surrounding the tunnel on the femoral side was generally denser than in the tibia.

In the Bone Protein-treated specimens, there was ex-

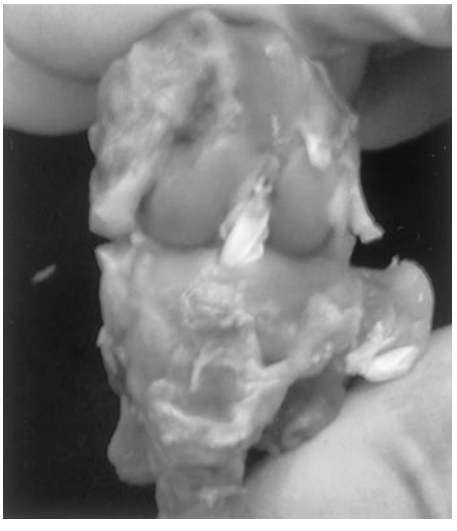

Figure 3. Necropsy photograph demonstrating an intact ACL graft.

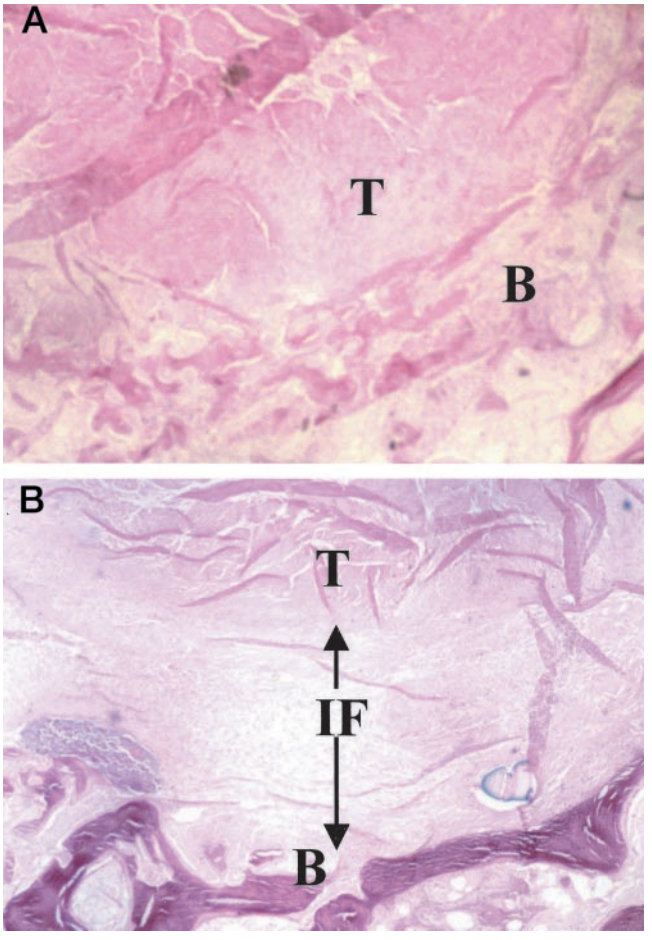

Figure 4. Photomicrographs of a 2-week specimen. A, in the limb treated with Bone Protein there is a relatively narrow interface zone between the tendon and bone. B, the control specimen had a wider interface between tendon and bone. T, tendon; IF, interface; B, bone. (hematoxylin and eosin stain, ×40)

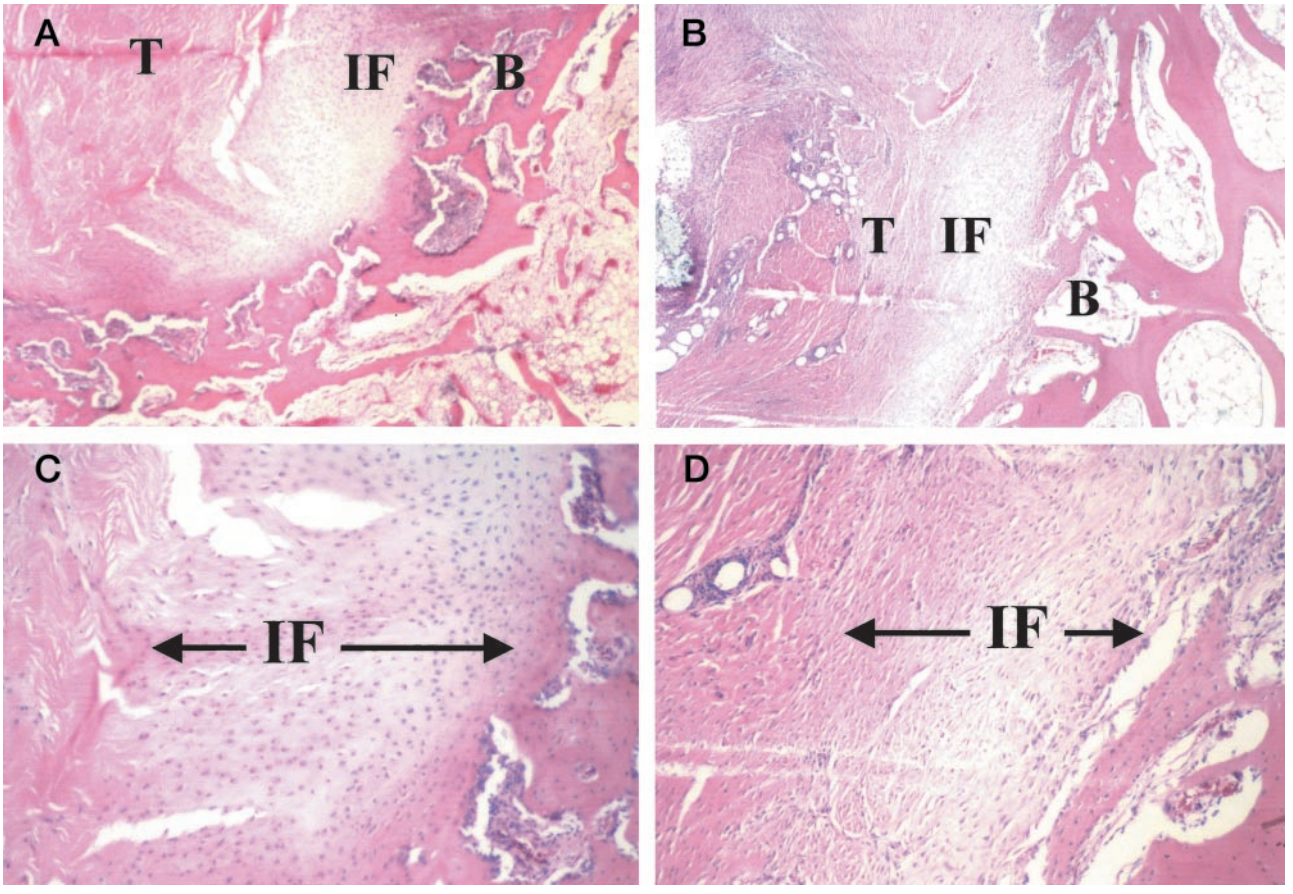

Figure 5. Photomicrographs of a 4-week specimen. There is a distinct tendon-bone interface in both the treated (A) and control (B) specimens. The newly forming bone trabeculae lining the bone tunnel are more dense in the Bone Protein-treated limb (A) compared with the paired control limb (B). At higher magnification, the interface tissue in the treated limb (C) appears cartilaginous, whereas there is a fibrous tissue interface in the control limb (D). T, tendon; IF, interface; B, bone. (hematoxylin and eosin stain; A and B, ×40; C and D, ×100)

tensive formation of new bone trabeculae and cartilage in the tendon-bone interface. There was more new bone formation than in the contralateral control specimen, and this new bone was in closer apposition to the tendon (Fig. 4). Overall, the amount of cartilage in the tendon-bone interface was similar between treated and control limbs; however, there was less variability in healing in the Bone Protein-treated limbs than in the control limbs. There was no evidence of foreign body giant cell response to the Bone Protein-containing collagen sponge. There was no evidence of an adverse effect on the articular cartilage on the tibia or femur. There was a similar degree of mild hyperplasia of the synovial lining in both treated and control limbs.

Four-Week Specimens. In both the control and treated specimens, there was progressive maturation of the interface tissue between tendon and bone (Fig. 5, A and B). There was generally more cartilage in the tendon-bone interface in the treated specimens compared with the controls, and the cartilage and fibrovascular tissue in the tendon-bone interface was more mature in the treated specimens (Fig. 5, C and D). Incorporation of the tendon with the establishment of collagen-fiber continuity between tendon and bone was often more advanced in the treated specimen, as seen with polarized light microscopy. Proliferation of cells along the edge of the tendon was apparent in some of the control and treated specimens, with no obvious difference between groups. There was generally more vigorous healing in the femoral tunnel than in the tibial tunnel.

Eight-Week Specimens. At 8 weeks, there was further matrix deposition in the tendon-bone interface. There continued to be variability in the degree of healing between the tendon and bone in the control specimens, with some specimens demonstrating a persistent wide interface zone between tendon and bone with only loose fibrous tissue formation (Fig. 6). The treated specimens demonstrated more cartilage and new bone formation around the tendon graft than did the control specimens.

Biomechanical Testing

The average load to failure in the treated specimens was significantly greater than in the control specimens for the

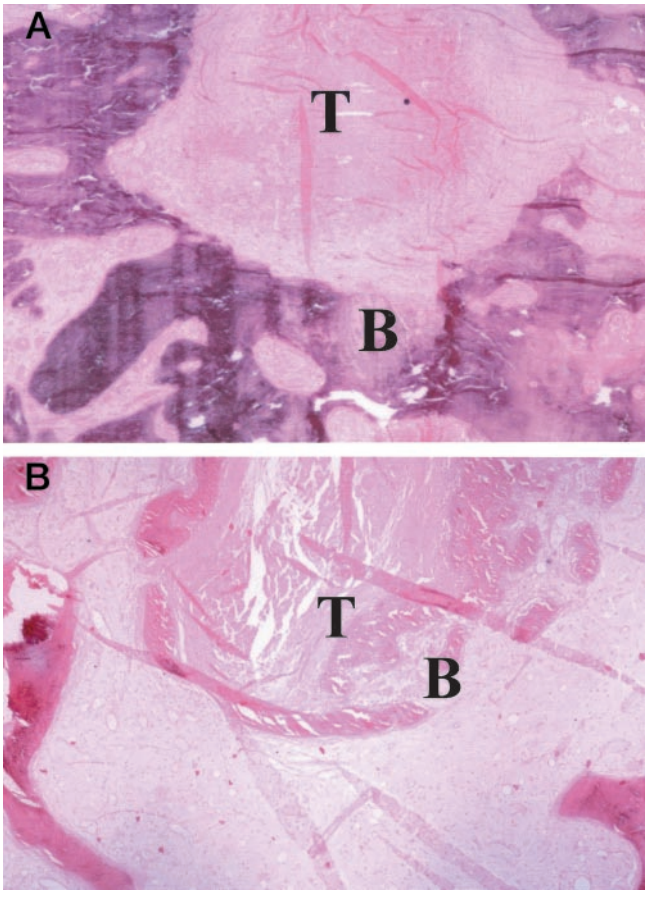

Figure 6. Photomicrographs of 8-week specimens. In the treated limb (A), the tendon graft is surrounded by bone, whereas there is little bone formation around the tendon graft in the control limb (B). T, tendon, B, bone. (hematoxylin and eosin stain, ×40)

entire study population ($P < 0.001$) as well as at the individual 2-, 4-, and 8-week time points ($P = 0.04$, 0.01, <0.001, respectively) (Fig. 7). The treated side was significantly stronger ($P = 0.001$) compared with the group 1 control specimens (untreated collagen sponge carrier) for the entire population. However, at individual time points, the treated limbs were significantly stronger than the group 1 control limbs only at 8 weeks ($P = 0.0003$). In addition, the Bone Protein-treated limbs were significantly stronger ($P = 0.02$) than the group 2 control limbs (no collagen sponge carrier) for the entire population. Again, when compared at each individual time point, the treated limbs were significantly stronger than group 2 control limbs only at 8 weeks ($P = 0.05$). Overall, the ultimate load to failure in the treated limbs was 65% greater than that of the control limbs. At the 2-, 4-, and 8-week time points, the treated limbs had, on average, 47%, 69%, and 80% greater ultimate tensile strength than did the control limbs, respectively. This increase was similar when compared with either the untreated sponge (group 1) or no sponge (group 2) control groups. There were no significant differences in ultimate load to failure

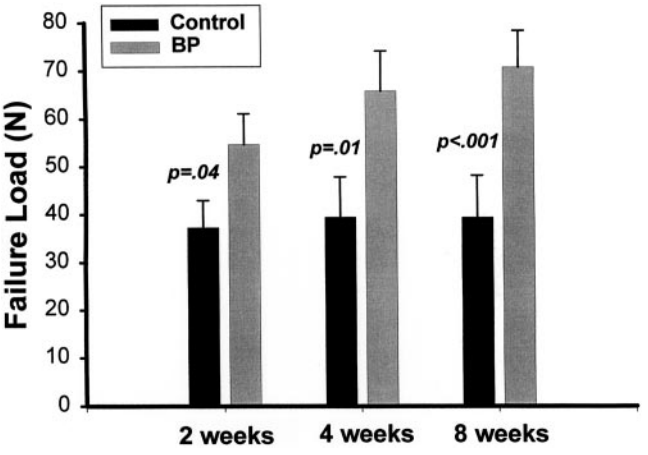

Figure 7. The average load to failure in the Bone Protein (BP)-treated specimens and control specimens at each time point.

between control group 1 and control group 2 ($P = 0.90$). A summary of load-to-failure data and the site of failure is presented in Table 2.

The site of failure changed as healing time increased. Failure was more common in the femoral tunnel at 2 weeks and in the tibial tunnel by 8 weeks ($P = 0.0006$, chi-square). Midsubstance failure was uncommon at 2 weeks and more common at 8 weeks. The experimental and control groups did not differ significantly in the distribution of failure sites. Also, there were no significant differences in failure sites between group 1 and 2 control specimens. The ultimate load to failure did not differ significantly between different failure sites.

Magnetic Resonance Imaging

Magnetic resonance imaging demonstrated homogeneous low signal intensity in the graft substance in 3 femoral and 11 tibial tunnels, moderate hyperintensity in 4 femoral and 6 tibial tunnels, and severe hyperintensity in 11 femoral tunnels and no tibial tunnels (Fig. 8). Signal hyperintensity was thought to reflect newly forming tissue in the bone tunnel. One of the tibial grafts was obscured because of suture artifact. New bone formation was seen at the extraarticular entrance to six femoral and no tibial tunnels.

From the MRI findings, we were able to accurately predict the side receiving Bone Protein in 8 of 9 animals. Periosteal or intratunnel new bone formation was generally the strongest indicator of the presence of Bone Protein. In 7 of 9 cases, the higher load to failure of the two limbs was predicted with use of the MRI appearance of graft position, signal, and morphologic characteristics. In 14 of 18 limbs, new bone formation within the tunnels was correctly correlated with the treated side.

DISCUSSION

The primary weakness of a tendon graft reconstruction during the early postoperative period is at the interface

TABLE 2
Biomechanical Data and Site of Failure

Group	Average load to failure (N)	Failure site (No.)		
	Mean (range)	Tibial tunnel	Midsubstance	Femoral tunnel
2 weeks				
Treated (N = 13)	54.69 (13–92)	2	1	9
Control (N = 13)	37.27 (7–72)	4	1	8
No sponge (N = 7)	33.13 (12–59)	1	1	5
Sponge (N = 6)	42.11 (7–72)	3	0	3
4 weeks				
Treated (N = 13)	65.82 (7–109)	6	4	3
Control (N = 13)	39.44 (0–96)	5	6	2
No sponge (N = 7)	40.12 (0–96)	2	4	1
Sponge (N = 6)	38.65 (5–74)	3	2	1
8 weeks				
Treated (N = 11)	70.74 (27–112)	5	5	1
Control (N = 11)	39.37 (0–116)	3	7	1
No sponge (N = 6)	41.46 (0–116)	2	4	0
Sponge (N = 5)	36.86 (24–52)	1	3	1

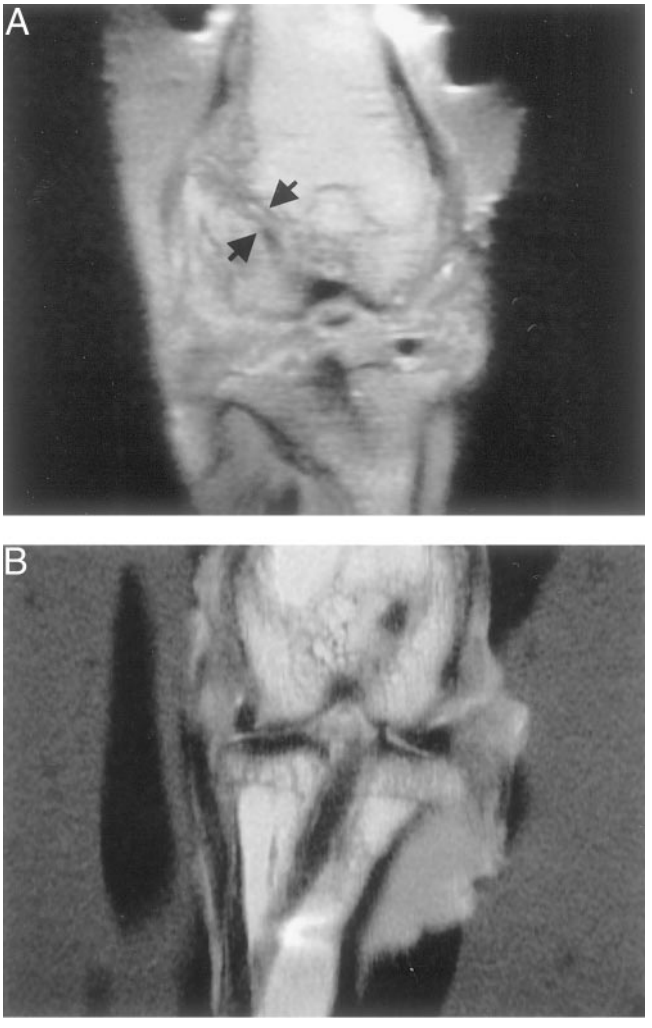

Figure 8. A, coronal MR image of an 8-week treated specimen demonstrating obliteration of the femoral tunnel by new bone formation (arrows). B, coronal MRI of a 4-week control specimen demonstrating less new tissue formation in the tibial and femoral tunnels compared with the treated specimen.

between the tendon graft and the bone, particularly when tendon must heal to bone within an intraarticular environment. The results of this study demonstrate that the tendon-to-bone healing process can be enhanced by the delivery of a bone growth factor to the healing interface. Overall, the limbs that received Bone Protein had more advanced healing and had greater ultimate load to failure. Addition of the collagen sponge carrier alone did not have any effect on healing when the result was compared with that of limbs that received no implant.

These results are similar to those of a previous study of an extraarticular bone tunnel model in a dog, in which BMP-2 was used to enhance healing.[10] Healing of a tendon graft in an intraarticular environment may be slower and more variable because of the effect of synovial fluid influx into the tunnel. The findings of these two studies suggest the possibility of accelerating healing in procedures in which a tendon graft is implanted into bone tunnels, such as cruciate ligament reconstruction in the knee, ankle ligament reconstruction, and medial collateral ligament reconstruction in the elbow.

Although we have demonstrated augmentation of healing, the ultimate failure load of the Bone Protein-enhanced reconstructions averaged less than 20% of the intact ligament-bone complex (when compared with previously published data for strength of the normal insertion site).[2,5,7] In the case of the control limbs, the strength was only 10% of that of the intact knee. It is not known how strong the attachment needs to be during the early healing period because the loads on the healing tendon-bone interface during early postoperative rehabilitation in patients are unknown. However, it is likely that some degree of micromotion and "slippage" of the tendon graft occurs during early activity and may compromise healing and affect ultimate graft laxity.

There was considerable variability in the histologic degree of healing between animals. Differences in graft appearance, density of fibrous interface tissue, width of tunnels, and bone apposition were observed between animals. It is likely that similar variability exists in patients and

may partly account for the variability in the rate of radiographic tunnel widening, instrumented knee laxity, and eventual graft function that has been reported after ACL reconstruction in patients.[4] It is notable that healing was more consistent and there was less variability in the overall histologic appearance in the Bone Protein-treated specimens.

It is not known why there was a shift in failure site from the femoral tunnel to the tibial tunnel as the time of healing increased. There is a relative paucity of cancellous bone in the tibial metaphysis in rabbits, with a correspondingly less vigorous healing response. There was often high signal intensity in the femoral tunnel on MRI, which is a nonspecific finding but suggestive of reactive tissue formation, either as a result of osteolysis or immature bone formation at the interface of the soft tissue graft. It is possible (but not proven) that such vigorous healing and remodeling along the femoral tunnel initially weakened the attachment but eventually resulted in a stronger attachment as healing progressed. It is also possible that the axis of tension in the testing jig did not match precisely with the bone tunnels. This may have affected the site of failure by altering the magnitude and type of load occurring at the healing interface.

To the best of our knowledge, this study is the first of its kind to compare MRI findings with results from mechanical testing in a tendon-to-bone healing model. These preliminary data suggest that MRI may be used to provide accurate information about graft healing and that this information may eventually be useful in the comparison of healing between different treatments. Further study with a larger sample size is needed to confirm and expand these preliminary findings. Such evaluation requires moderately high-resolution techniques if clinical MR units (1.5 T) are used because the conspicuity of trabeculae surrounding and within the tunnels depends on a small pixel size. Further information about the relationship between MR appearance and graft mechanical function may allow the use of MRI in future animal and, eventually, human studies.

There are several limitations to consider when interpreting the results of this study. The time points evaluated were relatively early in the healing process. The goal of augmentation was to improve the rate of healing and subsequent rehabilitation; therefore, we thought that longer intervals from surgery would be less relevant to the clinical question. However, the differences in this study were greater at the 8-week point, raising the question of whether they would be even greater at longer time points. It is also unknown whether the differences in attachment strength will persist over time or whether the graft attachment sites will remodel to a similar end point. Mortality from the bilateral procedure was slightly higher than projected when the study was designed. Thus, the statistical power of some of the comparisons at individual time points and between time points was limited. The data presented do not conclusively demonstrate the time at which Bone Protein treatment has its greatest effect.

Although dose-response studies have been performed with use of this growth factor in spine fusion models, only two doses were evaluated in our pilot study for this project. The optimal dose for augmentation of tendon-to-bone healing remains uncertain. The dose used in this study appears to achieve a balance between enhancing the healing of tendon to bone without causing adverse effects on intraarticular structures. Further studies in large animals are required to identify the optimum dose and carrier vehicle.

We chose to measure ultimate load to failure rather than mechanical behavior during repetitive cyclic loading. Graft reconstructions in patients are exposed to repeated loads, and failure is more likely to occur because of gradual, microscopic slippage of the tendon in the tunnel. However, ultimate load-to-failure testing is used in animal studies because, after sacrifice, the tissue cannot repair the microscopic damage that likely occurs with in vivo repetitive cyclic loading. The activity level of these animals postoperatively was not strictly limited. Each animal was allowed to walk as tolerated. It is likely that the reconstructed ligaments were exposed to some level of repeated loading before sacrifice. Thus, we sought to answer the simple question of whether the ultimate strength of the attachment of tendon to bone was improved by the addition of the bone growth factor.

In conclusion, the addition of an osteoinductive factor improved healing of a tendon graft in an intraarticular bone tunnel, as measured by histologic, biomechanical, and MRI criteria. Delivery of a growth factor may prove especially useful in situations in which impaired healing may be expected, such as during revision ACL reconstruction with enlarged bone tunnels or in the presence of graft-tunnel motion.

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REFERENCES

1. Atkinson BL, Fantle KS, Benedict JJ, et al: Combination of osteoinductive bone proteins differentiates mesenchymal C3H/10T1/2 cells specifically to the cartilage lineage. *J Cell Biochem 65:* 325–339, 1997
2. Blickenstaff KR, Grana WA, Egle D: Analysis of a semitendinosus autograft in a rabbit model. *Am J Sports Med 25:* 554–559, 1997
3. Boden SD, Schimandle JH, Hutton WC: The use of an osteoinductive growth factor for lumbar spinal fusion. Part II: Study of dose, carrier, and species. *Spine 20:* 2633–2644, 1995
4. Daniel DM, Stone ML, Dobson BE, et al: Fate of the ACL-injured patient: A prospective outcome study. *Am J Sports Med 22:* 632–644, 1994
5. Danto MI, Woo SL-Y: The mechanical properties of skeletally mature rabbit anterior cruciate ligament and patellar tendon over a range of strain rates. *J Orthop Res 11:* 58–67, 1993
6. David SM, Gruber HE, Meyer RA Jr, et al: Lumbar spinal fusion using recombinant human bone morphogenetic protein in a canine model. A comparison of three dosages and two carriers. *Spine 24:* 1975–1979, 1999
7. Grana WA, Egle DM, Mahnken R, et al: An analysis of autograft fixation after anterior cruciate ligament reconstruction in a rabbit model. *Am J Sports Med 22:* 344–351, 1994

8. Park MJ, Lee MC, Seong SC: A comparative study of the healing of tendon autograft and tendon-bone autograft using patellar tendon in rabbits. *Int Orthop 25:* 35–39, 2001
9. Rodeo SA, Arnoczky SP, Torzilli PA, et al: Tendon-healing in a bone tunnel: A biomechanical and histologic study in the dog. *J Bone Joint Surg 75A:* 1795–1803, 1993
10. Rodeo SA, Suzuki K, Deng X-H, et al: Use of recombinant human bone morphogenetic protein-2 to enhance tendon healing in a bone tunnel. *Am J Sports Med 27:* 476–488, 1999
11. Schiavone Panni A, Fabbriciani C, Delcogliano A, et al: Bone-ligament interaction in patellar tendon reconstruction of the ACL. *Knee Surg Sports Traumatol Arthrosc 1:* 4–8, 1993
12. Schiavone Panni A, Milano G, Lucania L, et al: Graft healing after anterior cruciate ligament reconstruction in rabbits. *Clin Orthop 343:* 203–212, 1997
13. Sciadini MF, Dawson JM, Johnson KD: Evaluation of bovine-derived bone protein with a natural coral carrier as a bone-graft substitute in a canine segmental defect model. *J Orthop Res 15:* 844–857, 1997
14. Shino K, Kawasaki T, Hirose H, et al: Replacement of the anterior cruciate ligament by an allogeneic tendon graft: An experimental study in the dog. *J Bone Joint Surg 66B:* 672–681, 1984
15. Yasko AW, Lane JM, Fellinger EJ, et al: The healing of segmental bone defects, induced by recombinant human bone morphogenetic protein (rhBMP-2): A radiographic, histological, and biomechanical study in rats. *J Bone Joint Surg 74A:* 659–670, 1992