

Augmentation of Tendon-to-Bone Healing With a Magnesium-Based Bone Adhesive

Lawrence V. Gulotta, MD, David Kovacevic, Liang Ying, John Ehteshami, MD, Scott Montgomery, and Scott A. Rodeo,* MD
From the Laboratory for Soft Tissue Research, Hospital for Special Surgery, New York, New York

Background: Healing of an anterior cruciate ligament graft in a bone tunnel occurs by formation of fibrous scar tissue, which is weaker than the normal fibrocartilaginous insertion.

Hypothesis: We hypothesized that a magnesium-based bone adhesive would improve tendon-to-bone healing in a rabbit anterior cruciate ligament reconstruction model.

Study Design: Controlled laboratory study.

Methods: Thirty-five New Zealand White rabbits underwent bilateral anterior cruciate ligament reconstructions with semitendinosus autografts. A total of 12.5 g of bone adhesive was placed in the intraosseous tunnel around the graft in one limb, while the tunnels in the contralateral limb received no implant. Sixteen animals each were sacrificed at 3 weeks and at 6 weeks (12 biomechanical testing/4 histology). Outcomes included semiquantitative histologic analyses for new cartilage formation and fibrous tissue formation in the tendon-bone interface, microcomputed tomography to quantify new bone formation along the bone tunnel, and biomechanical testing of load-to-failure and stiffness. Three animals were sacrificed at time 0 to confirm adequate tunnel fill with the bone adhesive on microcomputed tomography.

Results: All specimens had adequate tunnel fill with the bone adhesive at time 0. Application of the bone adhesive resulted in more cartilage formation and less fibrous tissue formation at the tendon-bone interface at 6 weeks compared with controls ($P < .05$). There was significantly more bone formation in the tibia of the treated limbs at 6 weeks ($P = .01$). The load-to-failure was significantly higher in the treated group at 6 weeks (71.8 ± 31.8 N vs 43.4 ± 14.8 N; $P = .04$). There were no differences in stiffness at either time point, and there were no differences at 3 weeks in any outcome variable.

Conclusion: The magnesium-based bone adhesive improves tendon-to-bone healing based on histologic and biomechanical testing at 6 weeks in a rabbit model of anterior cruciate ligament reconstruction.

Clinical Relevance: Further studies are needed to investigate the clinical potential of this bone adhesive to enhance healing and decrease recovery time in soft tissue ligament reconstruction.

Keywords: bone adhesive; bone cement; tendon-to-bone healing; ACL

Anterior cruciate ligament (ACL) ruptures often lead to knee instability and require reconstruction. Autologous bone–patellar tendon–bone grafts are popular because they allow for bone-to-bone healing in the femoral and tibial tunnels. However, the harvest of this graft is associated with significant donor site morbidity, including persistent patellofemoral pain, quadriceps weakness, and patellar tendonitis.^{3,4} Autologous tendon grafts, such as the semitendinosus and gracilis

tendons, have minimal donor site morbidity and the highest initial tensile strength and stiffness of all graft choices.⁵ The major disadvantage with soft tissue grafts is the time required to allow secure tendon-to-bone healing for graft fixation. Several studies have shown that tendon-to-bone healing occurs more slowly and more incompletely than bone-to-bone healing.^{8,9,18,19,21,24} This raises concerns regarding the strength of fixation of the tendon within the bone tunnels and the subsequent risk for graft slippage and failure. Interventions that can accelerate and improve tendon-to-bone healing are attractive since they can potentially limit graft fixation failures, prevent slippage, and allow for early, aggressive rehabilitation.

The native ACL inserts on the tibia and femur through a direct insertion. Direct insertion sites are composed of 4 transitional zones: ligament, unmineralized fibrocartilage, mineralized fibrocartilage, and bone. This morphologic

*Address correspondence to Scott A. Rodeo, MD, Sports Medicine/Shoulder Service, Hospital for Special Surgery, 535 East 70th Street, New York, NY 10021 (e-mail: RodeoS@hss.edu).

One or more authors has declared a potential conflict of interest: Scott Rodeo, MD, received funds for research from Bone Solutions Inc.

construct is not replicated after ACL reconstruction. Instead, the tendon graft heals in the bone tunnel with an interposed layer of fibrovascular scar tissue that is biomechanically inferior to a normal tendon-to-bone insertion.^{8,9,19,21} Over time, the surrounding bone grows into this interface tissue, while collagen fibrils become oriented perpendicular to the long axis of the tendon and anchor into the bone. This arrangement is similar histologically to the Sharpey fibers seen in indirect insertion sites, such as the medial collateral ligament of the knee. Because of the formation of scar tissue between the graft and bone early in the healing process, the fixation sites of tendon grafts are seen as the “weak link” in ACL reconstruction.

Bone cements are widely available for filling osseous defects in fracture repair. Recently there has been an effort to evaluate whether these products can aid in tendon-bone healing. Most of these studies have focused on calcium phosphate-based cements and have shown encouraging preliminary results.^{16,23} Osteocrete (Bone Solutions Inc, Dallas, Tex) is a magnesium-based, injectable bone adhesive currently in experimental use. Osteocrete has been shown in preliminary studies to have a peak tensile load to failure 3 times that of calcium-based bone cements in both bone-bone and tendon-bone attachments in cadaveric models.² It has also been shown to increase the amount of callous formation in an equine metatarsal osteotomy model when compared with a calcium phosphate cement.²⁶ While these preliminary studies are encouraging, the material's ability to improve the healing of soft tissue to bone *in vivo* has not been studied.

The purpose of this study was to investigate the effect of an injectable, magnesium-based bone adhesive on tendon-to-bone healing in a rabbit ACL reconstruction model. We hypothesized that this material would act as an osteoconductive scaffold, leading to a reduction in fibrous tissue and increased bone and fibrocartilage formation at the healing tendon-bone interface, with an improvement in the biomechanical properties when compared to untreated controls at 3 and 6 weeks.

METHODS

Study Design

A total of 35 New Zealand White rabbits underwent bilateral ACL reconstruction surgery with semitendinosus autografts. The right limb received Osteocrete (Bone Solutions), a magnesium-based, injectable bone adhesive, while the left limb received no bone adhesive and served as control. Sixteen rabbits were sacrificed at 3 weeks, and the remaining 16 were sacrificed at 6 weeks. At each time point, 12 animals were used for biomechanical testing, and 4 were used for micro-computed tomography (μ CT), scanning electron microscopy, and histology. Three animals were used for time 0 μ CT analysis to evaluate tunnel fill with the bone adhesive.

Animal Model

This study used an established model of ACL reconstruction in the knee of skeletally mature male New Zealand White rabbits.¹³ The rabbits were obtained from a licensed

United States Department of Agriculture dealer and were housed in the Facility for the Care of Laboratory Animals at our institution, in accordance with the standards established by the National Institutes of Health for the care and use of laboratory animals. Upon arrival, the animals were housed in individual cages and allowed free cage activity for 1 week before surgery. This study was approved by the Institutional Animal Care and Use Committee.

Surgical Procedure

Rabbits received no food or water 12 hours before surgery. Anesthesia was induced with ketamine (40 mg/kg) and acetylpromazine (0.5 mg/kg) delivered in a single syringe subcutaneously. Anesthesia was then maintained throughout the surgery via isoflurane inhalation through an endotracheal tube. Animals received ampicillin (25 mg/kg) 30 minutes before surgery for antibiotic prophylaxis.

A midline incision was made, and a lateral parapatellar arthrotomy was used to expose the ACL. A lateral arthrotomy was used to preserve the medial patellar retinaculum in an effort to minimize the risk of postoperative patellar dislocation. The semitendinosus tendon was then harvested and placed in saline-soaked gauze until preparation. Tunnels were drilled with a 2.78-mm-diameter drill bit through the femur and tibia at the insertion of the native ACL. In contrast to the established model of ACL reconstruction in New Zealand White rabbits, a slightly larger drill bit was used in this study to ensure adequate space for the bone adhesive.¹³ The graft was then passed through the bone tunnels to replace the ACL. The tunnels were irrigated with saline before application of the experimental agent.

The Osteocrete (Bone Solutions) bone adhesive was then prepared using a strict sterile technique. Twelve grams of the bone adhesive was mixed with 3 mL modified phosphate buffered saline in a 50-mL syringe as per the manufacturer's protocol. The mixture was manually stirred for 2 minutes. When the material became viscous and began to harden, which took about 5 minutes, it was transferred into a 12-mL curved-tip syringe (MonoJect; Tyco Healthcare Group LP, Mansfield, Mass) with approximately half of the tip cut off to form an opening measuring an eighth of an inch (no needle was used) and injected into the bone tunnels of the femur and tibia of the right knee. The bone tunnels were roughly 2 cm long, and about 1 mL of cement was injected into each tunnel from the extra-articular end. There were no problems with the viscosity of the cement during injection. The graft was passed back and forth to ensure even coating of the agent along the entire tunnel. Extravasated bone adhesive was cleaned using a saline-moistened sponge. The grafts were then secured to the periosteum and the surrounding soft tissues outside the femoral and tibial tunnels using 3-0 Ethibond sutures (Ethicon Inc, Somerville, NJ). After the graft was fixed, the remaining bone adhesive was used to “caulk” the exits of the bone tunnels. The wounds were then closed in layers, paying special care to ensure adequate closure of the lateral patellar retinaculum with 3-0 Ethibond.

Postoperatively, all animals were allowed free cage activity. They were given buprenorphine (0.05 mg/kg) subcutaneously for 3 days for pain control. The rabbits typically

had a mild limp for up to 2 weeks and regular pain-free activities after 2 weeks. Three animals were sacrificed immediately after the surgical procedure and served as time 0 controls to verify adequate tunnel fill with the bone adhesive. Sixteen animals were sacrificed at 3 weeks, and 16 animals were sacrificed at 6 weeks. These animals were tranquilized with acetylpromazine (0.4 mg/kg) 30 minutes before euthanasia to reduce stress. The animals were then euthanized with sodium pentobarbital 26% ("Sleepaway"; Fort Dodge Animal Health, Fort Dodge, Iowa) administered intravenously through the auricular vein.

Microcomputed Tomography Analysis

Upon necropsy at their respective time points, limbs were carefully dissected, and axial sections were obtained from the tendon-to-bone interfaces at the tibial and femoral tunnels. These sections were fixed in 10% neutral buffered formalin. Microcomputed tomography (μ CT) analysis at 19 μ m of isotropic resolution was subsequently performed using an MS-8 In Vitro Specimen Scanner (GE Medical System, London, Ontario, Canada). Each scan included a phantom containing air, saline, and an SB-3 bone analog (1.18 g/cc) for calibration of image Hounsfield units to tissue mineral density.⁷ Individual CT slices were reconstructed using a modified Parker algorithm with a resolution of 24 μ m.²⁰ Images were thresholded using 25% of the mineral attenuation of the cortical bone for each specimen. Regional analyses of the thresholded scans were performed using the system software (MicroView; GE Healthcare Technologies, Waukesha, Wis). A 4.5 \times 6.0-mm cylindrical volume of interest (VOI) was centered along the longitudinal axis of the bone tunnel in the middle portion of the tunnel. The VOI contained the graft and the bone surrounding the tunnel, as all tunnels were drilled with a 2.78-mm drill bit. Total bone volume (TBV, mm³) and bone volume fraction (BV/TV) were calculated based on the number of bone voxels compared with the total number of voxels in the VOI. Tissue mineral content (TMC, mg) and bone mineral content (BMC) were also determined based on the known standard. Trabecular architecture was characterized by the direct trabecular thickness (TbTh, μ m) measurements. For the 3 time 0 specimens, qualitative assessments were made to determine the extent of the canal filled with the bone adhesive.

Histomorphometric Analysis

The same specimens that underwent μ CT analysis were then decalcified in Immunocal (Decal, Congers, NY) and embedded in paraffin. Five-micrometer-thick sections were cut perpendicular to the bone tunnel and stained with hematoxylin and eosin and safranin-O/fast green for routine histologic evaluation using light microscopy (Eclipse E800; Nikon, Melville, NY). Digital images were taken using a SPOT RT camera (Diagnostic Instruments, Sterling Heights, Mich). Computerized image analysis (Image J, NIH) was then used to measure the width of the tendon-bone interface and the area of new cartilage formation. To determine the interface width, the tendon-to-bone interface was divided into 4 quadrants. Each histologic section was

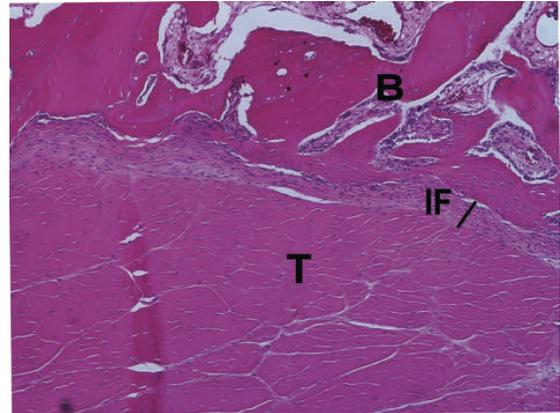


Figure 1. Interface width (IF) was measured as the distance between the tendon graft (T) and the bone tunnel (B). Four measurements were taken in each of the 4 quadrants and then averaged.

taken at the midportion of the tunnel, approximately 1 cm from the articular surface. In each of the 4 quadrants, the interface width was measured as the distance between the edge of the bone tunnel and the outer tendon as determined under 100 \times magnification.¹³ Four separate measurements were made in each of the quadrants for a total of 16 measurements for each specimen. The interface width was then determined by averaging these numbers for each specimen (Figure 1). The area of new cartilage formation was determined by outlining the area of metachromasia on the safranin-O slides at 40 \times magnification. The total area of metachromasia was recorded for each specimen.¹⁰ Three observers performed the histomorphometric measurements together as a group and arrived at a consensus.

Biomechanical Analysis

The limbs that were used for biomechanical analysis were frozen at -80°C until testing. At the time of testing, the limbs were thawed, and all soft tissue was removed except the grafted tendon. All scar tissue and sutures at the tunnel exits were carefully removed so as to determine the effects of the bone adhesive on the strength of healing and eliminate any confounding factors such as suture material. The femur-ACL graft-tibia complexes were fixed in specially designed clamps allowing tensile loading along the axis of the graft in a materials testing machine. A preload of 1 N was applied. After cyclic preconditioning of the constructs between elongation limits of 0 and 0.75 mm, a load-to-failure test was performed at an elongation rate of 10 mm/min. The failure load was recorded, and stiffness (N/mm) was calculated from the slope of the linear region of the load-displacement curve between 1.5 and 2 mm of elongation. The site of graft failure (femoral tunnel, mid-substance, or tibial tunnel) was recorded. This testing protocol has been used in previous studies from our laboratory.^{1,13}

Statistical Analysis

Before this study, a power analysis was performed. In our previous work with this rabbit ACL reconstruction model,

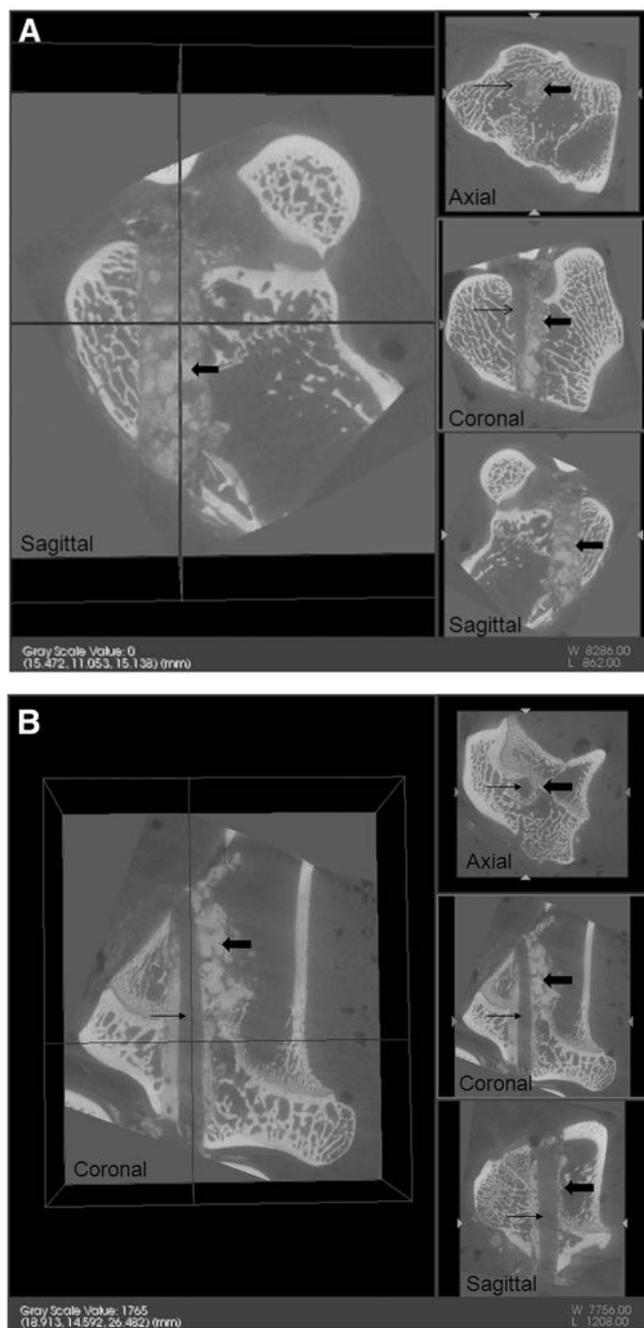


Figure 2. Microcomputed tomography at time 0 of the femur (A) and tibia (B) confirms adequate tunnel fill with the bone adhesive at the time of surgery. Line arrow marks the tendon graft, and block arrows denote the bone adhesive.

we found that the average width of the tendon-bone scar interface was approximately $130 \mu\text{m} \pm 20 \mu\text{m}$.^{1,6,13} Using these estimations, 4 specimens per group provided a power of .80 to detect a 30% difference in tendon-bone interface width with $\alpha = .05$. For biomechanical testing, our prior testing with this model found an average tensile strength of 20 N at 28 days after repair, with a standard deviation (SD) of 5.0 N.^{1,6,13} For the current study, an increase in

strength of 40% would be considered clinically significant. Using these estimations, a power of .80 is achieved using 12 specimens per group with $\alpha = .05$ for biomechanical testing. The power calculation was performed using SigmaStat (Jandel Scientific, San Rafael, Calif). The histomorphometry, μCT , and biomechanical data were all compared between the experimental and control limbs using a paired Student *t* test (Excel; Microsoft, Redmond, Wash). Statistical significance was set at $P < .05$.

RESULTS

Gross Observations

Upon necropsy, the knee joints of all limbs contained clear serous fluid, but there were no signs of gross infection. Synovitis was seen in all limbs at 3 weeks (no noticeable difference between control and experimental), but by 6 weeks, the synovitis had subsided. There were no obvious chondral injuries noted. Although the bone cement is exothermic, there was no evidence of graft or tissue necrosis on histologic testing. Moreover, no cement residue was found on the cartilage or in the intra-articular space. Five grafts failed to heal: 1 in the control limb of an animal sacrificed at 3 weeks; 1 from the experimental limb at 3 weeks; 1 from an experimental limb at 6 weeks; and 2 from control limbs at 6 weeks. The location of graft failure was intra-articular (midsubstance) in all limbs. The specimens with failed grafts were deducted from the biomechanical testing groups. Therefore, there were 11 specimens available for biomechanical testing in the control group at 3 weeks, the bone adhesive group at 3 weeks, and the bone adhesive group at 6 weeks; and there were 10 specimens available in the control group at 6 weeks. Four specimens in each group remained available for histomorphometric analysis. In the 3 animals that were sacrificed at time 0, the bone adhesive had not completely hardened by the time of necropsy. The time from surgery to necropsy was approximately 30 minutes for all animals in the time 0 group. In vitro, the bone adhesive had completely hardened after 20 minutes, suggesting that perhaps the blood and synovial fluid in vivo may delay hardening.

Microcomputed Tomography

Three specimens underwent μCT at time 0 to confirm adequate tunnel fill with the bone adhesive. Qualitative analysis of these scans confirmed that the bone adhesive had circumferentially coated the graft throughout the length of the tunnel (Figure 2). By 6 weeks, the TBV was significantly greater in the tibial tunnels of the experimental limbs when compared with the control limbs ($27.0 \pm 8.1 \text{ mm}^3$ for the experimental tibia, $12.0 \pm 3.6 \text{ mm}^3$ for the controls; $P = .03$). There were no differences in TBV between the experimental and control femurs at 6 weeks, nor were there any differences in either bone at 3 weeks (Figures 3 and 4). Furthermore, there were no differences between any groups for TMC, BMC, BV/TV, or TbTh (data not shown).

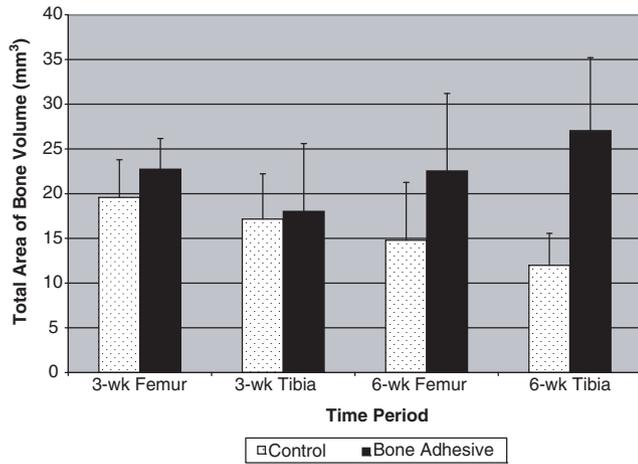


Figure 3. Total bone volume ($\text{mm}^3 \pm \text{SD}$) in the femoral and tibial tunnels as determined by microcomputed tomography. The tibial tunnels that received the bone adhesive had more new bone formation at 6 weeks than controls. *Represents a significant difference compared with the control group at the same time point ($P < .05$).

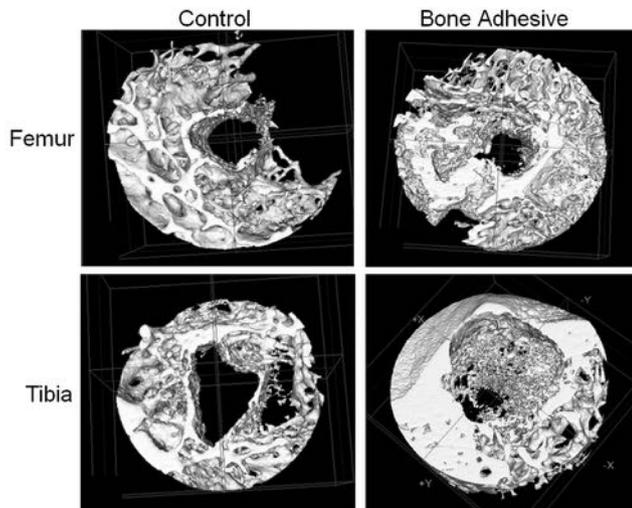


Figure 4. Three-dimensional reconstruction of microcomputed tomography images at 6 weeks.

Histomorphometric Analysis

All tendon grafts healed by the formation of fibrovascular interface tissue at the tendon-bone interface. There was progressive new matrix formation and bone in-growth by 6 weeks. This resulted in the establishment of collagen fiber continuity between tendon and bone in all samples (Figure 5). An enthesis with fibrocartilage between tendon and bone circumferentially was not observed in any sample. However, there were several samples in which a portion of the tendon-to-bone interface replicated the fibrocartilaginous transition zone of the native insertion site at 6 weeks. Three of the 4 femoral specimens (75%) and all 4 of the tibial specimens (100%) treated with the bone adhesive exhibited fibrocartilage formation. In the control group, fibrocartilage was found

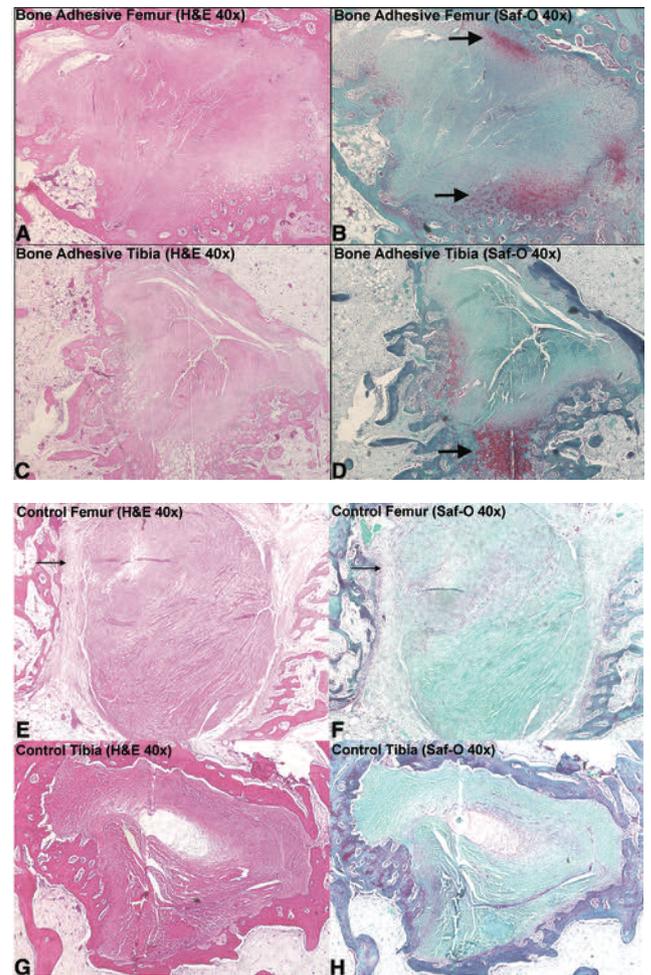


Figure 5. Histology images at 6 weeks. The bone adhesive induced more new cartilage formation at the interface as seen on the safranin-O–stained slides (B and D, block arrows indicate areas of metachromasia). Application of the bone adhesive also resulted in less fibrous tissue formation between the tendon graft and the bone tunnel (E and G, line arrows indicate interface tissue in a control specimen).

in only 1 of the 4 specimens in both the tibia (25%) and femur (25%). In 1 sample that received the bone adhesive in the 6-week group, a granuloma was observed in the tendon-bone interface. This was the only example of the adhesive potentially inciting an inflammatory response. The adhesive does not have any known side effects or toxicity.

Treatment with the bone adhesive resulted in less apparent scar tissue formation at the tendon-bone interface as evidenced by interface widths at 6 weeks (Figure 6). There was a reduction in the interface width of 44% for the femur and 50% for the tibia in the limbs that received Osteocrete (Bone Solutions) when compared with controls. The mean interface width for the femurs in the experimental group at 6 weeks was $69.5 \pm 36.2 \mu\text{m}$, while the mean width for the control femurs at 6 weeks was $156.9 \pm 57.3 \mu\text{m}$ ($P = .04$). The mean interface width for the tibia in the experimental group at 6 weeks was $75.8 \pm 25.1 \mu\text{m}$, while the mean width for the 6-week control tibia was $150.5 \pm 42.2 \mu\text{m}$ ($P = .04$). There were no differences between groups at 3 weeks.

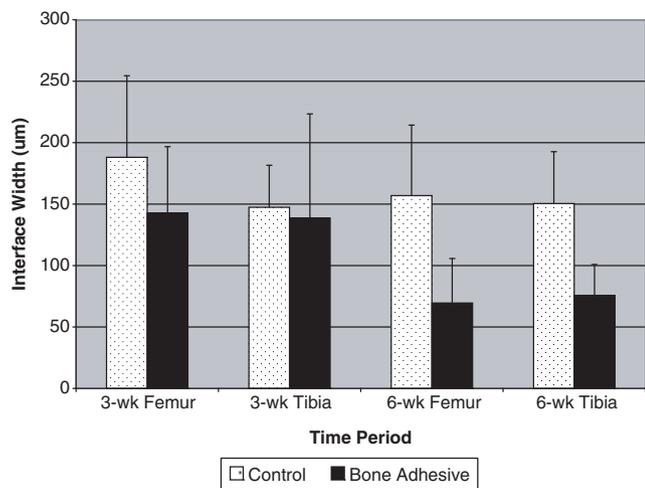


Figure 6. Interface width. Femoral and tibial specimens that received the bone adhesive had narrower interface widths ($\mu\text{m} \pm \text{SD}$), indicating less fibrous tissue formation at the interface compared with controls at 6 weeks ($P < .05$). *Represents a significant difference compared with the control group at the same time point ($P < .05$).

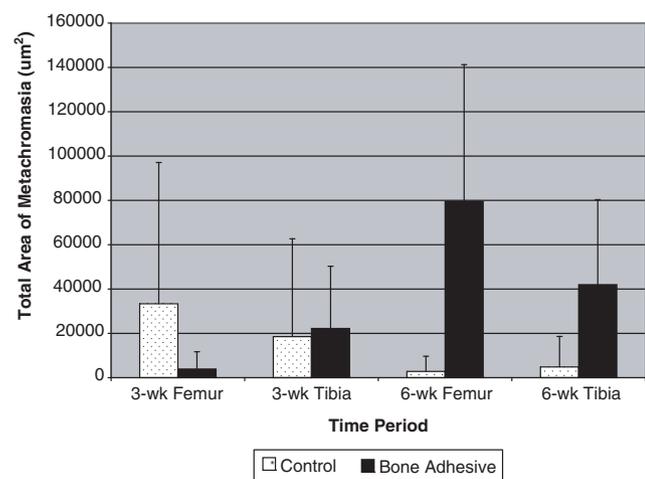


Figure 7. Metachromasia. Addition of the bone adhesive induced more new cartilage formation at the interface as evidenced by an increased area of metachromasia ($\mu\text{m}^2 \pm \text{SD}$; $P < .05$). *Represents a significant difference compared with the control group at the same time point ($P < .05$).

The bone adhesive also resulted in more cartilage formation as evidenced by the area of metachromasia at 6 weeks (Figure 7). The mean area of metachromasia in the experimental 6-week femurs was $79\,556.2 \pm 61\,664.0 \mu\text{m}^2$ compared with $2806.2 \pm 6\,873.7 \mu\text{m}^2$ for the control femurs at 6 weeks ($P = .01$). The mean area in the experimental 6-week tibia was $41\,979.2 \pm 38\,345.7 \mu\text{m}^2$, while the control 6-week tibia contained $2806.2 \pm 6\,873.7 \mu\text{m}^2$ ($P = .04$). No differences were seen between the groups at 3 weeks.

Biomechanical Testing

The mean load-to-failure for the experimental limbs at 6 weeks was twice that seen for the control limbs at the

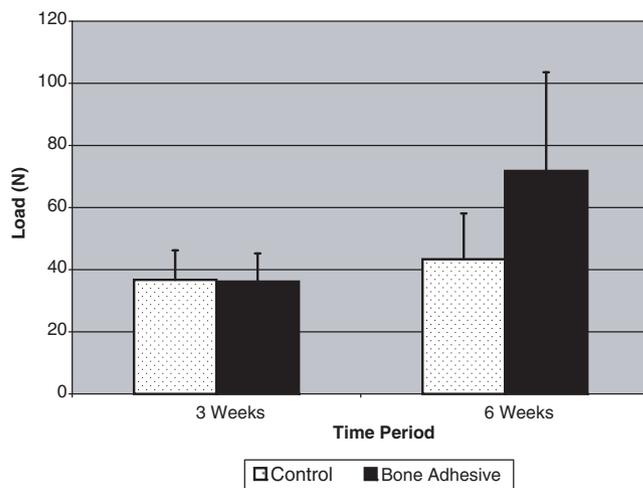


Figure 8. Ultimate tensile load to failure. Addition of the bone adhesive resulted in higher ultimate loads to failure at 6 weeks. *Represents a significant difference compared with the control group at the same time point ($P < .05$).

same time point ($71.8 \pm 31.8 \text{ N}$ for the experimental limbs compared with $43.4 \pm 14.8 \text{ N}$ for controls; $P = .04$). There were no differences between the groups at 3 weeks ($36.1 \pm 9.1 \text{ N}$ for the 3-week experimental limbs, $36.7 \pm 9.5 \text{ N}$ for controls; $P = .90$). There were no differences between groups at either time point in stiffness of the bone–tendon graft–bone construct (Figure 8). All specimens failed at the graft–tunnel junction, with an equal number of failures occurring from the tibial and femoral sides.

DISCUSSION

It is well established that tendon grafts heal in a bone tunnel with an intervening layer of fibrovascular scar tissue. This scar tissue is mechanically inferior to normal tissue and represents a “weak link” after surgical reconstruction. As these tissues remodel with time, bone from the tunnel grows into the fibrous interface tissue and confers mechanical strength. For these reasons, there is much interest in developing interventions that can accelerate and improve bone in-growth. Several experimental agents have been studied, including the application of bone morphogenetic protein-2 (BMP-2),^{1,13,14} transforming growth factor- β 1 (TGF- β 1),²⁷ the antiosteoclastogenic protein osteoprotegerin,⁶ stem cells,^{12,17} periosteum,²⁸ transcutaneous ultrasound,²⁵ and calcium-based cements.^{13,16,23}

In this study, we evaluated the ability of a magnesium-based bone adhesive to improve tendon-to-bone healing in a rabbit ACL reconstruction model. We found that this material had a positive effect in terms of limiting scar formation, promoting the regeneration of a fibrocartilage insertion site, and increasing the amount of bone formed in the tunnels at 6 weeks. These histologic findings correlated with improved biomechanical properties in terms of load-to-failure.

The use of bone cements to augment bone in-growth into the scar interface is an attractive option because these materials are readily available and relatively inexpensive

when compared with other biologic therapies. Using a similar model, Tien et al²³ evaluated the ability of a calcium-phosphate bone cement to augment tendon-to-bone healing. They found the cement caused diffuse bone in-growth into the interface tissue at early time points when compared with untreated controls. Furthermore, they showed that ultimate loads to failure were 3 times that of the control group at 1 week and approximately twice that of the control group at 2 weeks. Biomechanical testing at later time points was not conducted. Matsuzaki et al¹⁶ hybridized semitendinosus tendon grafts with a calcium phosphate-containing solution *in vitro* before using them to perform ACL reconstructions in rabbits. They noted an abundance of osteoclasts and osteoblasts around the tendon-bone interface by 2 weeks, with newly formed bone and cartilage around the tendon grafts by 3 weeks. However, biomechanical testing was not conducted.

We have previously studied the ability of recombinant human bone morphogenetic protein-2 (rhBMP-2) to improve bone in-growth in the same rabbit ACL model. In our first study, we found that rhBMP-2 on a collagen sponge resulted in early osteoclastic resorption around the tunnel edges, followed by extensive formation of new bone that resulted in a stronger tendon-bone interface. In a subsequent study, calcium phosphate cement was used as a carrier for the application of rhBMP-2. The calcium phosphate cement was effective in limiting the early resorptive phase seen with the collagen sponge.¹³ The controls treated with the calcium phosphate cement alone showed superior healing compared to the historical controls using a sponge carrier alone. These findings suggested that perhaps an osteoconductive scaffold alone might prove sufficient to augment tendon-to-bone healing. However, calcium phosphate acts more like a grout than a glue. These properties make it an ideal choice to resist compressive forces that are seen in bone defect models but might not be the ideal material to resist the tensile forces seen at the tendon-bone interface.

The bone adhesive used in this study, Osteocrete (Bone Solutions), is composed of calcium, phosphate, and magnesium. Magnesium oxide comprises 41% of the weight of the powder form of Osteocrete and 32% by weight with the addition of the modified phosphate-buffered saline. The calcium phosphate component provides an osteoconductive biologic scaffold onto which new bone can form. The magnesium gives the cement an adhesive quality that theoretically allows it to resist tensile forces at time 0, thereby potentially limiting graft-tunnel motion. In preliminary *in vitro* testing of the material's ability to adhere tendon-to-bone at time 0, the magnesium-based bone adhesive was more than 3 times stronger than a commercially available calcium phosphate cement. *In vivo* testing in an equine metatarsal osteotomy model has shown that application of the magnesium-based bone adhesive results in more callus formation and a quicker time to union when compared with a calcium phosphate cement.²⁶ However, no differences were seen in biomechanical testing at 7 weeks.

While these characteristics offer theoretical advantages, further study is required to evaluate the clinical applicability of this material for tendon-to-bone repair. We observed that the bone adhesive had not completely hardened by the

time of necropsy on the 3 time 0 animals. Our finding of significantly improved attachment strength at 6 weeks is likely due to the osteoconductive properties of the material. Earlier time points should be studied to determine if the adhesive properties alone improve graft fixation strength. However, it is also possible that the adhesive properties of the material alone improved eventual graft healing by minimizing detrimental graft-tunnel motion. At any rate, the adhesive did appear to have a positive biological effect on tendon-to-bone healing as evidenced by superior histologic, radiographic, and biomechanical results at 6 weeks. The average ultimate load-to-failure for the limbs receiving the bone adhesive at 6 weeks was 71.8 ± 31.8 N, while the average load seen in our previous study with calcium phosphate cement alone at 8 weeks was 38.6 ± 18.0 N.¹³ The average ultimate load-to-failure of the native rabbit ACL has been shown in a previous study to be 351.8 ± 41.6 N.¹¹ Conclusive comparisons cannot be made since these represent historical controls; however, it appears that any augmented healing is still far from re-creating the biomechanics of the native ACL insertion at these time points.

While there was more fibrocartilage at the interface in limbs treated with the bone adhesive, no specimens had fibrocartilage circumferentially around the graft as seen in normal direct-ligament insertion sites. This was seen despite μ CT analyses at time 0 that showed circumferential fill of the tunnel with the bone adhesive. This finding is most likely due to the varying mechanical stresses that are placed on different portions of the tendon-bone interface. We have recently shown that graft-tunnel motion can affect graft healing in a bone tunnel in the same rabbit model.²² Further studies are needed to define the role that graft-tunnel motion, or lack thereof, plays in the healing process.

We used μ CT to objectively determine the amount of new bone formed in the tibial and femoral tunnels. While μ CT has been used to assess bone formation in a rotator cuff model of tendon-to-bone healing, we believe this is the first to use it to determine the amount of bone formed in a tunnel.¹⁵ We found the tibias of the 6-week animals that received the bone adhesive had significantly greater bone volume compared to controls. We did not find a significant difference between experimental and control femurs at this time point. This is most likely due to the high variability seen in this group that limited statistical power. In past studies, we have measured new bone formation using semi-quantitative histology, which has the potential to introduce errors because of interobserver variability. We found that μ CT allowed accurate, precise quantification of osseointegration of the tendon graft in a bone tunnel in this model.

We acknowledge several limitations to our study. The use of a rabbit model makes extrapolation into human patients difficult. While the technique of ACL reconstruction in this model has been well established, rabbits place unusual stresses on their grafts because they sit with their knees in hyperflexion. As discussed earlier, the effect these stresses have on healing is not completely understood. This may have contributed to the 5 graft failures seen on necropsy and the inconsistent presence of fibrocartilage at the interface. Additionally, it is unclear what the long-term effects on hardening would be if this were done in a fluid-filled

arthroscopic environment. Also, there were high variances in the semiquantitative histology and μ CT data as evidenced by large standard deviations that limited the statistical power and raised the possibility of a type II error. This could explain why no differences were seen between groups at 3 weeks. However, for most of the outcome variables at 3 weeks, the mean values were so similar that it is difficult to imagine significance emerging with more samples. Finally, the study groups had been powered according to prior biomechanical testing data. However, 2 specimens at 3 weeks were lost due to graft failure. This resulted in suboptimal power and raises the possibility of a type II error.

In summary, the results of this study indicate that a magnesium-based bone adhesive can improve tendon-to-bone healing in a rabbit model. We found that this material can induce fibrocartilage formation, limit fibrous tissue formation at the healing tendon-bone interface, and increase osteointegration at 6 weeks. It may eventually be possible to use this bone adhesive clinically to augment tendon-to-bone healing, potentially leading to increased attachment strength and a diminished risk of graft failure or slippage.

REFERENCES

- Anderson K, Seneviratne AM, Izawa K, Atkinson BL, Potter HG, Rodeo SA. Augmentation of tendon healing in an intra-articular bone tunnel with use of a bone growth factor. *Am J Sports Med.* 2001;29(6):689-698.
- Bertone AL, Hackett B, Litsky AS, Johnson AL, Kaeding CC, Lally T. A magnesium injectable formulation adheres bone to bone and tendon to bone. *Trans Orthop Res Soc.* 2005;30:1007.
- Bonamo JJ, Krinick RM, Sporn AA. Rupture of the patellar ligament after use of its central third for anterior cruciate reconstruction. A report of 2 cases. *J Bone Joint Surg Am.* 1984;66(8):1294-1297.
- Christen B, Jakob RP. Fractures associated with patellar ligament grafts in cruciate ligament surgery. *J Bone Joint Surg Br.* 1992;74(4):617-619.
- Cooper DE, Deng XH, Burstein AL, Warren RF. The strength of the central third patellar tendon graft. A biomechanical study. *Am J Sports Med.* 1993;21(6):818-823, discussion 823-824.
- Dynybil C, Kawamura S, Kim HJ, Ying L, Perka C, Rodeo SA. The effect of osteoprotegerin on tendon-bone healing after reconstruction of the anterior cruciate ligament: a histomorphological and radiographical study in the rabbit [in German]. *Z Orthop Ihre Grenzgeb.* 2006;144(2):179-186.
- Gardner MJ, van der Meulen MC, Carson J, et al. Role of parathyroid hormone in the mechanosensitivity of fracture healing. *J Orthop Res.* 2007;25(11):1474-1480.
- Goradia VK, Rochat MC, Grana WA, Rohrer MD, Prasad HS. Tendon-to-bone healing of a semitendinosus tendon autograft used for ACL reconstruction in a sheep model. *Am J Knee Surg.* 2000;13(3):143-151.
- Grana WA, Egle DM, Mahnken R, Goodhart CW. An analysis of autograft fixation after anterior cruciate ligament reconstruction in a rabbit model. *Am J Sports Med.* 1994;22(3):344-351.
- Koike Y, Trudel G, Curran D, Uthoff HK. Delay of supraspinatus repair by up to 12 weeks does not impair enthesis formation: a quantitative histologic study in rabbits. *J Orthop Res.* 2006;24(2):202-210.
- Labs K, Perka C, Schneider F. The biological and biomechanical effect of different graft tensioning in anterior cruciate ligament reconstruction: an experimental study. *Arch Orthop Trauma Surg.* 2002;122(4):193-199.
- Lim JK, Hui J, Li L, Thambyah A, Goh J, Lee EH. Enhancement of tendon graft osteointegration using mesenchymal stem cells in a rabbit model of anterior cruciate ligament reconstruction. *Arthroscopy.* 2004;20(9):899-910.
- Ma CB, Kawamura S, Deng XH, et al. Bone morphogenetic protein signaling plays a role in tendon-to-bone healing: a study of rhBMP-2 and noggin. *Am J Sports Med.* 2007;35(4):597-604.
- Martinek V, Latterman C, Usas A, et al. Enhancement of tendon-bone integration of anterior cruciate ligament grafts with bone morphogenetic protein-2 gene transfer: a histological and biomechanical study. *J Bone Joint Surg Am.* 2002;84(7):1123-1131.
- Meyer DC, Jacob HA, Pistoia W, von Roll A, Gerber D. The use of acrylic bone cement for suture anchoring. *Clin Orthop Relat Res.* 2003;410:295-302.
- Mutsuzaki H, Sakane M, Nakajima H, et al. Calcium phosphate-hybridized tendon directly promotes regeneration of tendon-bone insertion. *J Biomed Mater Res A.* 2004;70(2):319-327.
- Ouyang HW, Goh JC, Lee EH. Use of bone marrow stromal cells for tendon graft-to-bone healing: histological and immunohistochemical studies in a rabbit model. *Am J Sports Med.* 2004;32(2):321-327.
- Panni AS, Milano G, Lucania L, Fabbriani C. Graft healing after anterior cruciate ligament reconstruction in rabbits. *Clin Orthop Relat Res.* 1997;343:203-212.
- Papageorgiou CD, Ma CB, Abramowitch SD, Clineff TD, Woo SL. A multidisciplinary study of the healing of an intra-articular anterior cruciate ligament graft in a goat model. *Am J Sports Med.* 2001;29(5):620-626.
- Parker DL. Optimal short scan convolution reconstruction for fan-beam CT. *Med Phys.* 1982;9(2):254-257.
- Rodeo SA, Arnoczky SP, Torzilli PA, Hidaka C, Warren RF. Tendon healing in a bone tunnel. A biomechanical and histological study in the dog. *J Bone Joint Surg Am.* 1993;75(12):1795-1803.
- Rodeo SA, Kawamura S, Kim HJ, Dynybil C, Ying L. Tendon healing in a bone tunnel differs at the tunnel entrance versus the tunnel exit: an effect of graft-tunnel motion? *Am J Sports Med.* 2006;34(11):1790-1800.
- Tien YC, Chih TT, Lin JH, Ju CP, Lin SD. Augmentation of tendon-bone healing by the use of calcium-phosphate cement. *J Bone Joint Surg Br.* 2004;86(7):1072-1076.
- Tomita F, Yasuda K, Mikami S, Sakai T, Yamazaki S, Tohyama H. Comparisons of intraosseous graft healing between the doubled flexor tendon graft and the bone-patellar tendon-bone graft in anterior cruciate ligament reconstruction. *Arthroscopy.* 2001;17(5):461-476.
- Walsh WR, Stephens P, Vizesi F, Bruce W, Huckle J, Yu Y. Effects of low-intensity pulsed ultrasound on tendon-bone healing in an intra-articular sheep knee model. *Arthroscopy.* 2007;23(2):197-204.
- Waselau M, Samii VF, Weisbrode SE, Litsky AS, Bertone AL. Effects of a magnesium adhesive cement on bone stability and healing following a metatarsal osteotomy in horses. *Am J Vet Res.* 2007;68(4): 370-378.
- Yamazaki S, Yasuda K, Tomita F, Tohyama H, Minami A. The effect of transforming growth factor-beta 1 on intraosseous healing of flexor tendon autograft replacement of anterior cruciate ligament in dogs. *Arthroscopy.* 2005;21(9):1034-1041.
- Youn I, Jones DG, Andrews PJ, Cook MP, Suh JK. Periosteal augmentation of a tendon graft improves tendon healing in the bone tunnel. *Clin Orthop Relat Res.* 2004;419:223-231.