

Enhancement of Tendon Graft Osteointegration Using Mesenchymal Stem Cells in a Rabbit Model of Anterior Cruciate Ligament Reconstruction

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Purpose: To study the effect of coating tendon grafts with mesenchymal stem cells (MSCs) on the rate and quality of graft osteointegration in anterior cruciate ligament (ACL) reconstruction. **Type of Study:** Animal model. **Methods:** Bilateral ACL reconstructions using hamstring tendon autografts were performed on 48 adult rabbits. Grafts were coated with MSCs in a fibrin glue carrier in one limb, and fibrin glue only in the other. Assessment was done at 2, 4, and 8 weeks. Histologic analysis was carried out using standard and immunohistochemical stains. Biomechanical testing of force and stiffness during loading to ultimate failure was performed. **Results:** Control reconstructions showed mature scar tissue with some Sharpey's-like fibers spanning the tendon-bone interface at 8 weeks. The MSC-enhanced reconstructions had large areas of cartilage cells at the tendon-bone junction at 2 weeks. By 8 weeks, a mature zone of cartilage was seen gradually blending from bone into the tendon grafts. This zone stained strongly for type II collagen and showed histologic characteristics similar to normal rabbit ACL insertions. Biomechanically, there was no statistical difference between limbs at 2 and 4 weeks. At 8 weeks, the MSC-enhanced grafts had significantly higher failure load and stiffness. **Conclusions:** Coating of tendon grafts with MSCs results in healing by an intervening zone of cartilage resembling the chondral entheses of normal ACL insertions rather than collagen fibers and scar tissue. MSC-enhanced ACL reconstructions perform significantly better than controls on biomechanical testing. **Clinical Relevance:** Enhancement of tendon graft osteointegration with MSCs is a novel method offering the potential for more physiologic and biomechanically stronger ligament reconstructions. **Key Words:** Graft healing—Stem cell—Mesenchymal—ACL reconstruction—Bone tunnel—Tendon graft.

Tendon grafts anchored within bony tunnels are used in a wide variety of orthopaedic procedures. Tendon pullout from bony tunnels occurs up to 12 weeks after hamstring graft anterior cruciate ligament

(ACL) reconstruction in animal models,¹⁻⁴ suggesting that mechanical strength of reconstructions before that time are dependent to a large extent on the method of fixation. For this reason, a large emphasis of recent research has been on the relative benefits and biomechanical profiles of various fixation methods for hamstring tendon autografts.⁵⁻⁷

There is increasing interest in the use of biological agents to enhance tendon-bone osteointegration within the bony tunnel. Recent studies have shown that application of bone morphogenetic protein-2¹ and a mixture of bone growth proteins⁸ to the surface of tendon autografts resulted in enhanced tendon-bone healing and improved biomechanical properties. Mesenchymal stem cells (MSCs) are pluripotential cells that are

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being investigated extensively in a wide variety of settings, including enhancement of tissue healing and tissue bioengineering.⁹⁻¹⁶ We are unaware of any study using MSCs to enhance tendon graft healing. We hypothesized that the application of MSCs at the tendon-bone junction during ACL reconstruction might result in both acceleration and enhancement of osteointegration of the tendon graft to the surrounding bone tunnel.

METHODS

Experimental Design

Bilateral ACL reconstructions with hamstring tendon autografts were performed by a single surgeon in 48 skeletally mature New Zealand White rabbits weighing 2.0 to 2.5 kg each. Autologous bone marrow-derived MSCs were harvested 3 to 4 weeks before ligament reconstruction surgery. During bilateral ACL reconstruction, the treatment limb was coated with MSCs mixed in a fibrin glue carrier and the control limb was coated with fibrin glue only. The treatment side was determined randomly, and the surgeon was blinded to the treatment limb at the time of surgery. There were 36 animals assigned for biomechanical testing and the remainder underwent histologic analysis. The animals were euthanized at 2, 4, and 8 weeks postoperatively. Biomechanical analysis was carried out by blinded observers. A further 2 rabbits were used for histologic analysis of the normal rabbit ACL. The experimental protocol, animal care, and use procedures were approved by the institutional ethics review committee.

MSC Preparation

MSCs were harvested from bone marrow of the rabbits 3 to 4 weeks before ACL reconstruction. The rabbits were anesthetized, the posterior iliac crests prepared in a sterile manner, and 5 to 10 mL of bone marrow was aspirated. The cells were separated and cultured using a protocol similar to that described by Phinney.¹⁷ The anti-coagulated bone marrow was diluted with 2 times of the volume of Hank's Balanced Salt Solution. The nucleated cell layer was obtained by Ficoll-Paque density gradient centrifugation (Sigma Chemical, St. Louis, MO) at 450g for 20 minutes. The nucleated cells were harvested and rinsed with Hank's Balanced Salt Solution using centrifugation at 250g for 10 minutes twice. The pellets were resuspended in complete Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine

serum (FBS), penicillin 100 U/mL, streptomycin 100 μ g/mL (pH 7.35), and centrifuged at 250g for 10 minutes. The pellets were resuspended with complete DMEM containing 10% FBS. The number of the nucleated cells was determined with a hemocytometer and the volume of the suspension was adjusted. The cells were plated out at a density of $1.8 \times 10^5/\text{cm}^2$ in T-75 flasks and grown at 37°C, 5% CO₂ in a humidified tissue-culture incubator. The medium was changed after 3 days, and every 3 to 4 days thereafter. When the cells became 75% to 80% confluent, they were trypsinized and transferred into 2 T-75 flasks. Having completed 1 passage, the cells were enumerated under a hemocytometer. When necessary, the cells were passaged a second time by repeating the aforementioned procedure to obtain 3 to 4 million cells for use with each graft.

Surgical Protocol

All rabbits were administered 15 mg/kg subcutaneous cephalexin and 0.3 mL/kg diazepam preoperatively. The animals were induced with Hypnorm (a mixture of fentanyl 0.315 mg/mL and fluanisone 10 mg/mL) 0.3 mL/kg (Janssen-Cilag, Saunderton, UK), followed by halothane inhalational anesthesia. Bilateral ACL reconstructions were performed under the same anesthetic. A midline incision was made, the semitendinosus tendon was harvested, and the length measured. A lateral parapatellar arthrotomy was used to expose the knee joint. The native ACL was divided and tibial and femoral tunnels were created with a 2-mm drill. The combined length of the tunnel from the anterior femoral surface, across the joint, to the anterior tibial surface was recorded. Both the treatment and control grafts (single-strand hamstring tendon) were coated with Tisseel fibrin sealant (Baxter Healthcare, Deerfield, IL) immediately before insertion into the tunnels. The fibrin sealant was supplied in 2 separate bottles, which were to be mixed just before application. On the treatment limb, the autologous MSCs were added to one of the micropipettes containing fibrin sealant solution. The grafts were advanced into the tunnels before the fibrin sealant became viscous, allowing the fibrin sealant to harden with the grafts in position in the tunnel. The grafts were sutured to the periosteum using 2-0 Ethibond (Ethicon, Somerville, NJ) in a standard fashion on the femoral side. Then the tibial side was sutured with the knee in 10° of flexion and slight tensioning of the graft. The wound was closed in layers. The

animals were allowed to move freely in their cages after the operation.

Histologic Analysis

The femur and tibia were removed, the knee joints left intact, and the specimens fixed in 10% buffered formalin. Decalcification was performed using 30% formic acid. The samples were embedded in paraffin and cut into 5- μ m thick sections longitudinal to the bony tunnels in the femur and tibia. The slides were stained using hematoxylin and eosin, Safranin-O, and immunohistochemical stains for collagens type I, II, and III. Four knees from 2 rabbits with normal ACLs were harvested and prepared in a similar manner for histologic analysis.

Biomechanical Analysis

At the time of death, the femora and tibiae were removed, and the knee joints were carefully dissected of surrounding soft tissue until the only physical connection between the 2 bones was the ACL tendon graft. The suture material used to secure the grafts during surgery was left undisturbed. The fresh samples were mounted on a materials testing machine (Instron, Canton, MA) with the line of distraction parallel to both the femoral and tibial tunnels. The specimens were cyclically loaded with 10% strain at a rate 4%/second for 20 cycles to achieve a steady state.¹⁸ The grafts were then tested to failure and the load at the point of failure recorded. Stiffness was calculated from the linear portion of the load-displacement curve. The location of failure, either by pullout of the tunnel or midsubstance graft failure, was determined by gross examination and analysis of the load-displacement curves.

Statistical Analysis

Based on load-to-failure tests obtained from a pilot study, the minimum number of animals was calculated to be 10 animals in each time frame to achieve a power of 0.8 to detect a 50% difference at the 0.05 level. The mean failure load and stiffness between treatment and control groups were compared using a paired *t* test for equality of means. The differences within groups at the different time points were analyzed with a 1-way analysis of variance. The differences between treatment groups in the frequencies of pre-sacrifice graft failure and graft pullout during

mechanical testing were cross-tabulated using the χ -square test for independence.

RESULTS

Gross Observations

There were 96 ACL reconstructions performed on 48 animals. There were 7 premature deaths, all occurring within 9 days of surgery. Of these, 4 animals had severe bilateral joint infections and 3 failed to thrive postoperatively with no evidence of joint infection on postmortem examination of the knees. There were no other joint infections observed, and the remaining 41 animals were euthanized at the planned times. At the time of death, 6 of the 82 reconstructions (in 41 remaining animals) were found to have undergone prior midsubstance graft failure, 2 in treatment and 4 in control limbs. Cross-tabulation with χ -square analysis identified no statistical difference in the incidence of early graft failure between treatment and control limbs. One specimen (treatment limb at 2 weeks) was damaged during dissection and was excluded from biomechanical testing.

The average length of the grafts was 6.0 cm and the average combined length of the tunnels was 4.0 cm. There were no statistically significant differences between treatment and control limbs for both graft and tunnel lengths.

Histologic Findings in the Control Limbs

At 2 weeks, specimens showed a distinct and wide fibrovascular interface between tendon and bone (Fig 1A). This interface consisted of irregular vascular tissue and fibroblasts. No distinct areas of organized collagen fibers or cartilage were observed. At 4 weeks, the zone between tendon and bone was seen to become more mature, with occasional oblique collagen fibers projecting from bone into the zone of fibrous tissue. By 8 weeks, the interface was still a distinct entity between tendon and bone (Fig 1B). It was significantly narrower, and occasional perpendicular collagen fibers resembling Sharpey's fibers were seen bridging the gap between tendon and bone. The fibrous interface stained moderately on collagen type I and III stains, but did not contain any type II collagen.

Histologic Findings in the MSC-Treated Limbs

Specimens at 2 weeks showed large crops of cartilage cells lining the junction between tendon and

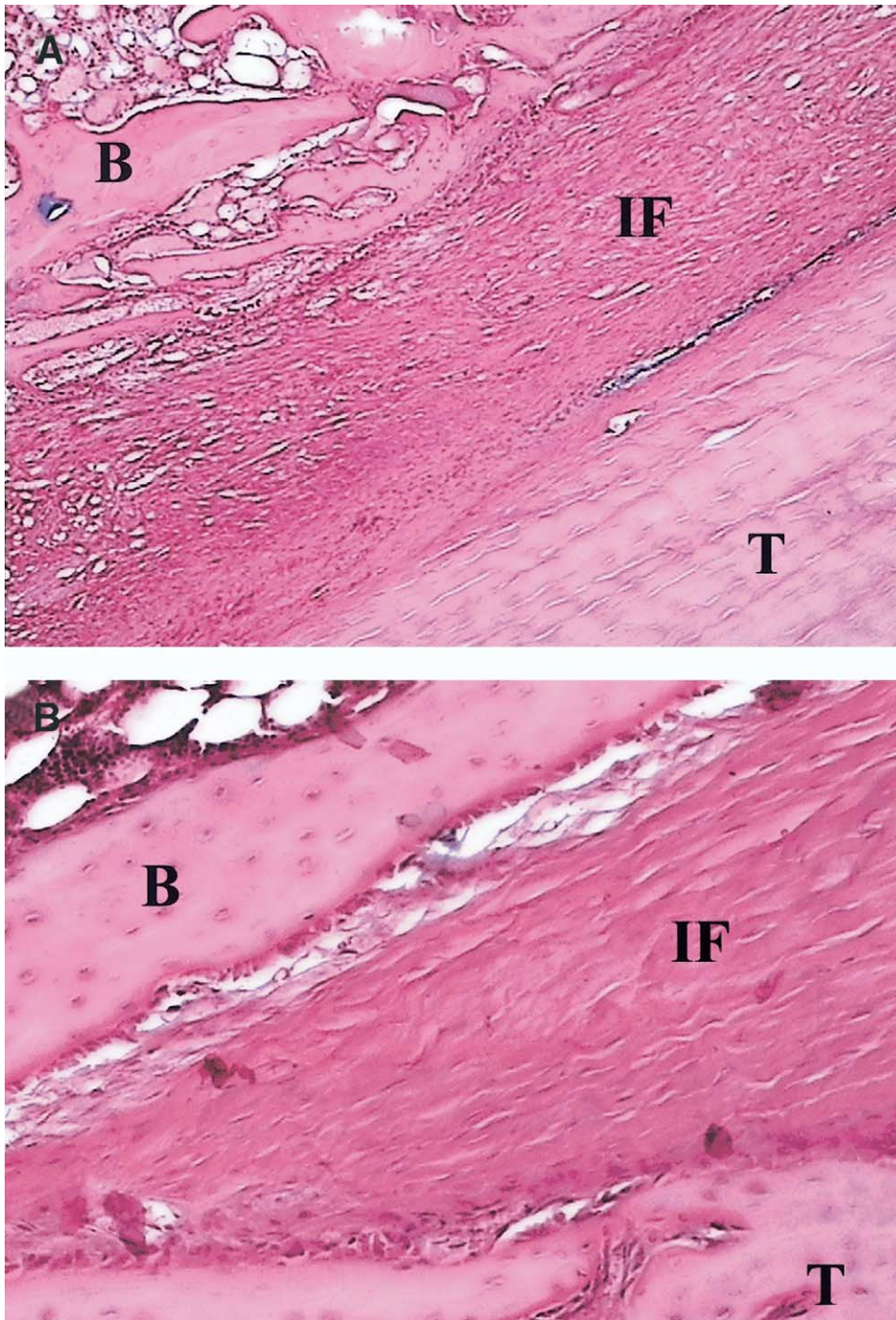


FIGURE 1. Photomicrograph of control specimens. (A) At 2 weeks, a distinct zone of disorganized fibrovascular granulation tissue is observed between tendon and bone (H&E, original magnification $\times 40$). (B) At 8 weeks, the interface is narrower, more organized, with occasional perpendicular collagen fibers crossing the junction between tendon and bone (H&E, original magnification $\times 100$). (T, tendon; B, bone; IF, interface.)

bone. The cartilage cells were immature cells arranged in large disorganized clusters around the tendon graft (Fig 2). By 4 weeks, the zone of cartilage cells appeared more organized. Cartilage matrix was being actively produced, stained strongly on Safranin-O, and contained abundant type II collagen (Fig 3). The car-

tilage cells were closely associated with surrounding bone, and a zone of cartilage was seen to gradually blend into the tendon substance. By 8 weeks, the cartilage cells had matured into a zone of organized cartilage tissue with close apposition with adjacent bone and tendon (Fig 4). A gradual transition from

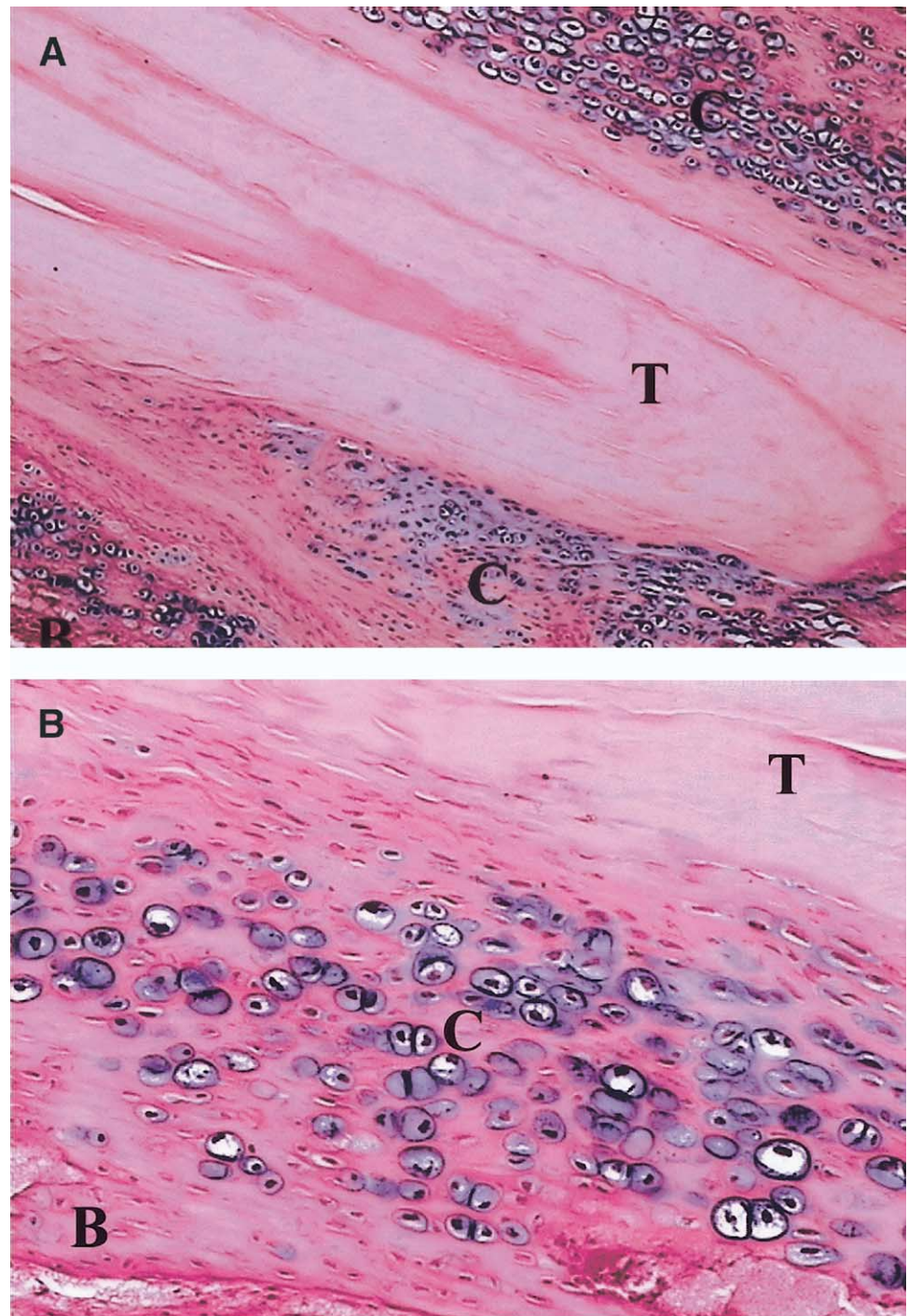


FIGURE 2. Photomicrographs of a 2-week MSC-treated specimen. (A) Large collections of cartilage cells are observed lining the junction between tendon and bone (H&E, original magnification $\times 40$). (B) At higher magnification, these cells are noted to be immature, and arranged in disorganized clusters (H&E, original magnification $\times 100$). (T, tendon; B, bone; C, cartilage.)

bone, through a distinct zone of fibrocartilage, into the tendon substance was seen.

Histology of Normal ACL Insertions

The normal ACL insertion was seen to be a chondral enthesis with 4 distinct zones (Fig 5). Tendon

substance blended gradually into a zone of fibrocartilage with cartilaginous cells organized in columns. This fibrocartilage then became progressively mineralized before blending into the bony substance of the tibia and femur. The fibrocartilage zone contained matrix proteoglycan that stained strongly on Safr-

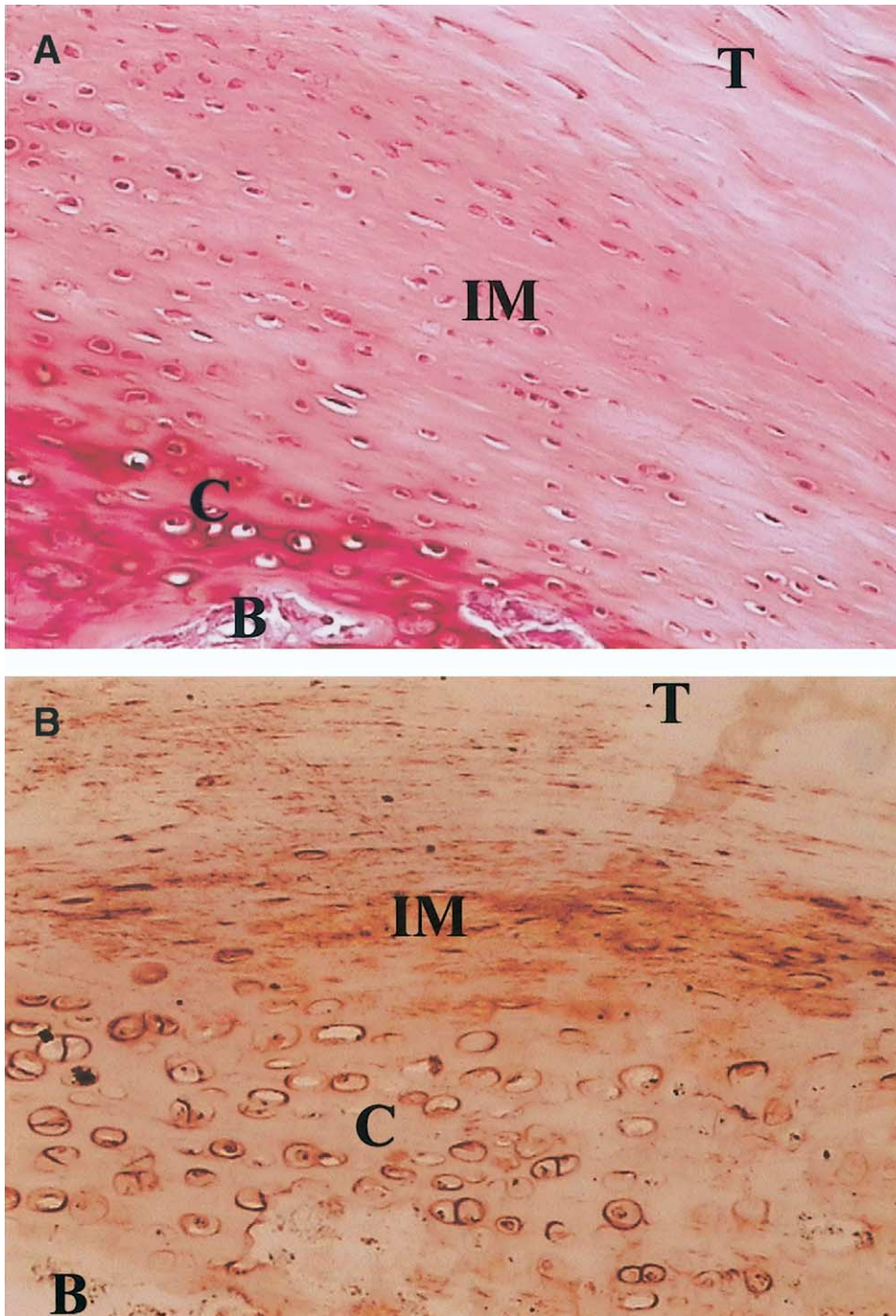


FIGURE 3. Photomicrograph of a 4-week MSC-treated specimen. (A) Increasing organization of the cartilage interface is observed with close apposition to surrounding bone, and a distinct intermediate zone of cartilage blending into the tendon graft (Safranin-O, original magnification $\times 100$). (B) The interface between tendon and bone stains for collagen type II (immunohistochemistry collagen type II, original magnification $\times 40$). (T, tendon; B, bone; C, cartilage; IM, intermediate zone.)

nin-O, and contained abundant type II collagen on immunohistochemical analysis.

Biomechanical Analysis

Thirty-one pairs of bilateral ACL reconstructions were analyzed (Table 1). The overall mean failure

load was 66% greater in the MSC-treated reconstructions compared with controls. The overall mean stiffness during load-to-failure was 51% greater in the MSC-treated reconstructions compared with controls. There was no difference in the mean failure load or stiffness between treatment limbs at 2 weeks. At 4

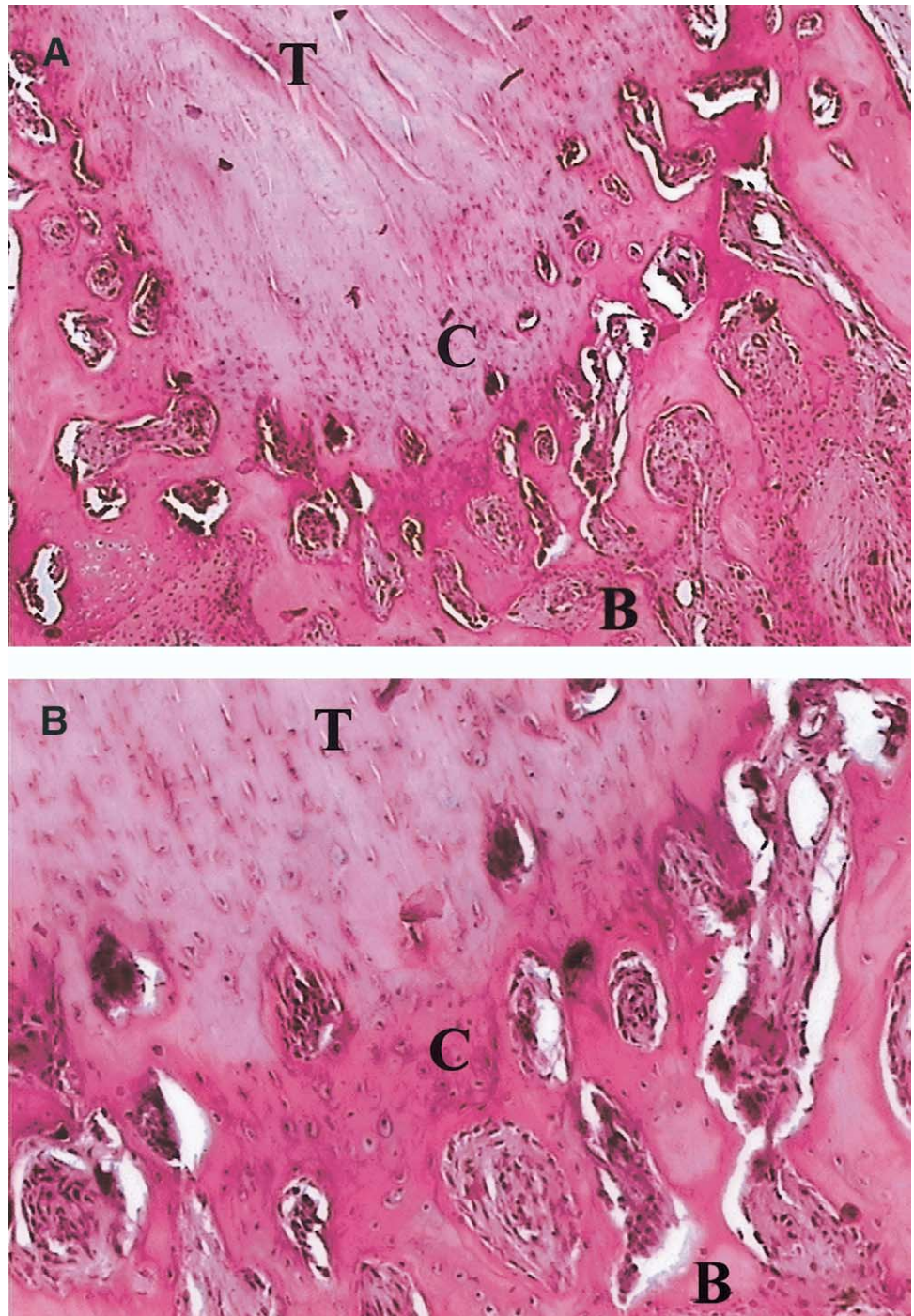


FIGURE 4. Photomicrographs of an 8-week MSC-treated specimen. (A) A mature zone of cartilage is observed gradually blending into the adjacent bone and tendon substance (H&E, original magnification $\times 40$). (B) At higher magnification, the fibrocartilage cells are seen in organized columns blending into tendon substance (H&E, original magnification $\times 100$). (T, tendon; B, bone; C, cartilage.)

weeks, the treated limbs showed a trend toward higher failure loads and stiffness than controls. At 8 weeks, the MSC-treated reconstructions had significantly higher failure loads and stiffness than control reconstructions.

Analysis of variance between time frames revealed

no significant changes in failure load in the control limbs over time. The control limbs showed a significant increase in stiffness between 2 and 8 weeks. The MSC-treated reconstructions had a significant increase in both failure load and stiffness from 2 to 8 weeks (Fig 6).

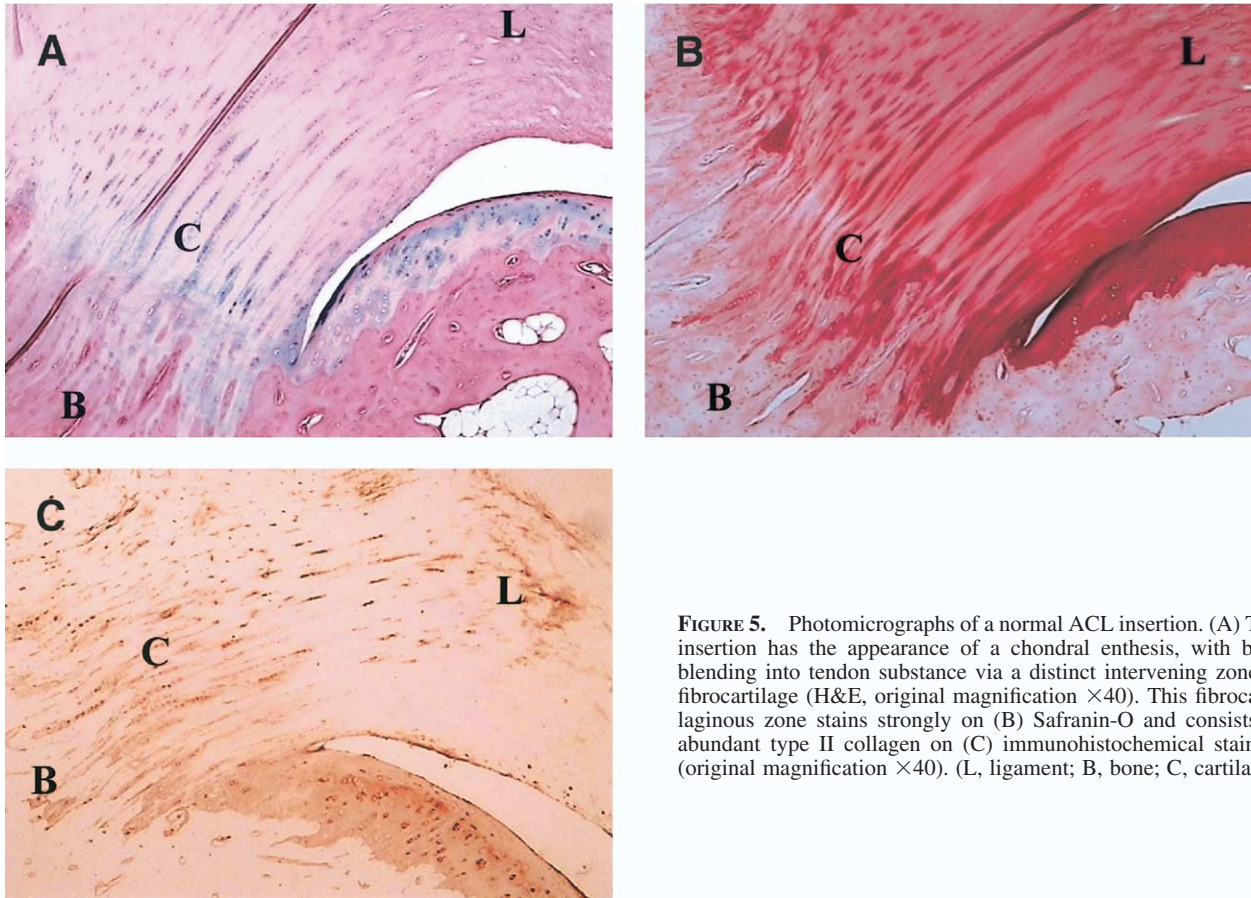


FIGURE 5. Photomicrographs of a normal ACL insertion. (A) This insertion has the appearance of a chondral enthesis, with bone blending into tendon substance via a distinct intervening zone of fibrocartilage (H&E, original magnification $\times 40$). This fibrocartilaginous zone stains strongly on (B) Safranin-O and consists of abundant type II collagen on (C) immunohistochemical staining (original magnification $\times 40$). (L, ligament; B, bone; C, cartilage.)

Fifteen of 34 MSC-treated limbs (44%) failed by pullout, whereas the remainder failed by midsubstance graft rupture. Twelve of 32 control limbs (38%) failed by

pullout. χ -square analysis showed no significant difference between the rates of pullout in the treatment and control reconstructions overall and in each time frame.

TABLE 1. Failure Load and Stiffness

	Failure Load (N)		Stiffness (N/mm)	
	Mean (95% CI)	P	Mean (95% CI)	P
2 weeks (n = 11)				
Control	30.9 (18-42)		9.8 (6-14)	
MSC-treated	27.0 (14-39)	.60	9.5 (4-15)	.85
4 weeks (n = 10)				
Control	30.6 (18-43)		11.5 (8-15)	
MSC-treated	55.7 (21-90)	.09	17.5 (11-24)	.10
8 weeks (n = 10)				
Control	38.1 (26-50)		17.6 (14-21)	
MSC-treated	84.7 (46-124)	.02	32.6 (18-47)	.05
Overall (n = 31)				
Control	33.1 (27-39)		12.9 (11-15)	
MSC-treated	54.9 (37-72)	.01	19.5 (14-26)	.02

NOTE. P values indicated the degree of statistical difference between control and treatment limbs on paired t testing.

DISCUSSION

This study shows that MSCs may be used to biologically alter the normal healing process of hamstring tendon grafts to their surrounding bony tunnels, resulting in biomechanically better ACL reconstructions. Healing of the control reconstructions occurred in the manner previously reported by numerous authors^{2-4,19,20} with the formation of a dense layer of fibrous scar tissue containing perpendicular collagen fibers (resembling Sharpey's fibers). The MSC-enhanced grafts healed with a zone of intervening fibrocartilage containing type II collagen. This zone of fibrocartilage gradually transitioned from the tendon substance to the surrounding bone. This mode of healing has been shown to occur in bone-patellar tendon-bone grafts^{3,4,20-23} and has been suggested to be more

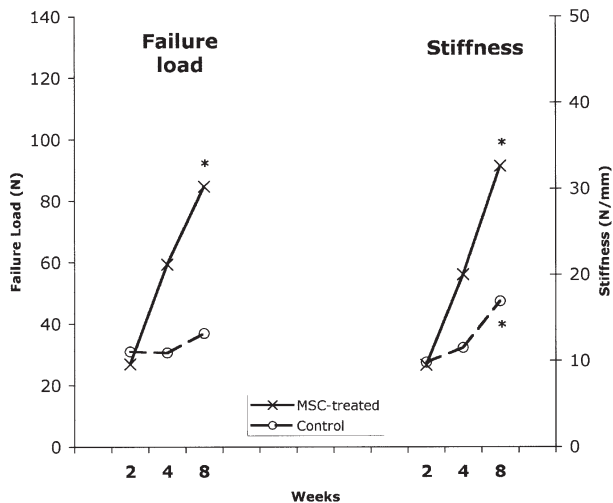


FIGURE 6. Mean failure load and stiffness in control and MSC-treated reconstructions at each time frame. *A significant change from 2-week mean values on analysis of variance testing.

physiologic because it resembles the chondral entheses of the native ACL.²⁰ To our knowledge, this is the first report of the use of MSCs to enhance tendon graft healing. Furthermore, this is the first time that hamstring tendon grafts have been observed to heal with a distinct intervening zone of fibrocartilage.

There are few reports on the use of biological substances to augment tendon graft healing in bony tunnels. Recently, 2 reports described enhancement of bone formation and healing at the tendon-bone fibrovascular interface using bone morphogenic proteins. Rodeo et al.¹ analyzed the effect of bone morphogenic protein-2 in a canine model using hind limb extensor tendons transplanted into drill holes in the proximal tibia. They reported healing with a fibrovascular interface, which had more extensive bone formation with closer apposition of new bone to the tendon in the treated limbs. The treatment grafts had higher pullout strengths at all time frames, the differences of which achieved statistical significance in the 2-week group, but not the 4- and 8-week groups. Most control limbs failed by tendon pullout through the bony tunnel, whereas a significant number of treated grafts failed by midsubstance failure. Anderson et al.⁸ performed ACL reconstructions in rabbits and applied a heterogeneous bone growth factor containing BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7, TGF- β 1, TGF- β 2, TGF- β 3, and FGF-1. They reported that treated specimens healed by a fibrovascular interface that showed more bony and cartilaginous formation at 8 weeks. In the present study, the treated reconstruc-

tions had higher failure loads and stiffness than controls (66% and 51%, respectively), and these differences were significant both overall and at 8 weeks. The biomechanical findings of this present study show improvements in graft performance comparable to that of these 2 reports. This suggests that MSC-enhanced osteointegration by a fibrocartilaginous interface results in biomechanical properties at least matching that of bone protein-enhanced reconstructions.

Both Rodeo et al.¹ and Anderson et al.⁸ used a collagen sponge carrier to apply the bone proteins to the surfaces of the tendon grafts. While Rodeo et al. detected no biomechanical differences between controls with and without collagen sponge carriers, they raised the possibility that the collagen sponge might somehow interfere with tendon-to-bone healing. In view of the uncertainties regarding the contribution of collagen sponge carriers to tendon-to-bone healing, we chose instead to use a fibrin glue carrier. Shoemaker et al.²⁴ examined the effects of fibrin sealant on the healing of tendons to bony tunnels in the proximal tibia in dogs. The fibrin sealant appeared to accelerate the organization of the fibrovascular interface in the first 1 to 2 weeks after surgery, but no visible histologic differences between treatment and control (no fibrin sealant) were visible at 28 days. There were no infections or reoperations in the 12 animals studied. While the authors did not perform biomechanical analysis in that experiment, we believe that, at least histologically, using a fibrin glue carrier both in treatment and control limbs would not appreciably influence the outcome of our results.

The site of biomechanical weakness in the first 6 to 12 weeks after ACL reconstruction has been shown to be the graft-tunnel interface in both hamstring tendon^{2,3} and bone-patellar tendon-bone³ grafts. The control reconstructions in this present study remained the same from 2 to 4 weeks, before displaying a trend toward improvement in failure load at 8 weeks. In contrast, the MSC-enhanced reconstructions showed progressively increased failure loads between time frames. Assuming the MSCs were influencing primarily the tendon-bone junction, we hoped to observe a lower rate of pullout failures in the treatment group. Rodeo et al.¹ reported significantly lower rates of pullout failures in the treatment groups at all time frames. Andersen et al.⁸ did not observe any differences between treatment or control groups in relation to type of failure. The results of the present study also show no difference in the incidence of pullout failure between treatment and control reconstructions, while showing significantly higher failure load and stiffness

in the MSC-treated limbs. These findings may suggest that, aside from the obvious histologic enhancement of the tendon-bone junction healing, the MSCs may in fact be influencing the biomechanical properties of the tendon substance itself.

Goradia et al.²⁵ reported that hamstring tendon autografts progress through a period of relative hypocellularity at 4 weeks before gradually being repopulated by fibroblast-like cells from 8 to 12 weeks. This is accompanied by collagen fiber disorganization that slowly begins to show a crimp pattern from 12 weeks onward. MSCs have been successfully used to enhance tendon defect healing.²⁶ Acceleration of fibroblast/tenocyte repopulation of the tendon graft in the present study may explain the enhanced early biomechanical properties of reconstructions. The MSCs applied to the surface of tendon grafts may have served as a source of additional recruitable fibroblast-like cells for tendon repopulation, or they may have been involved in activation and recruitment of local fibroblast precursors.

Hamstring grafts have been associated with a higher incidence of bony tunnel widening²⁷⁻²⁹ and the development of graft laxity in the medium-term in some reports.^{30,31} Tunnel widening in hamstring ACL reconstruction has been postulated to result from graft fixation points being further away from the joint surface.²⁷ However, recent reports suggest that femoral tunnel widening still occurs in hamstring tendon grafts with anatomic fixation,³² whereas bone-patellar tendon-bone grafts have less tunnel widening, even when femoral suspensory fixation is used.³¹ The findings of these reports suggest that tunnel widening may in fact be related to the differences in healing of hamstring and bone-patellar tendon-bone grafts to their surrounding bony tunnels. By modifying the healing process and creating a more physiologic tendon-bone interface, MSC-enhanced ACL grafts may be less likely to develop tunnel widening.

We chose to use a rabbit model of ACL reconstruction. This is a model that has been validated in previous reports in the literature.^{8,22,33-39} The mortality (14%) and infection rates (8%) were comparable to those reported in previous studies (0 to 17% and 0 to 9%, respectively).^{8,33,35,36,38} Six of 82 reconstructions (7%) were found to have failed at the time of death. Sakai et al.³⁸ reported that 2 of 27 reconstructions (7%) had unrecordable force measurements. Similarly, Anderson et al.⁸ reported that 2 of 84 reconstructions (2%) were grossly atrophic and resulted in unrecordable force measurements on biomechanical testing. We did not perform control reconstructions

without fibrin glue, and cannot comment whether the use of fibrin as a carrier might have contributed to these findings. Although there were statistically significant differences between treatment and control limbs in biomechanical testing, the range of test results was wide, with standard errors of between 8% and 27% of the mean failure load and stiffness in each time frame. We believe this is an inherent problem with the use of small animal models of ACL reconstruction and previous studies^{4,8,38} have also reported a wide range in their biomechanical results. More accurate and reliable testing may have been achieved with the use of a larger animal model, such as sheep or dog. Nonetheless, the range of values for force and stiffness in our study are similar to those obtained by other investigators performing ACL reconstructions on rabbits^{4,8,33,35,38} and, thus, useful comparisons and conclusions may still be drawn.

There are several other limitations of this study. First, we did not perform the experiment with labeled MSCs. We are thus unable to comment on whether the fibrocartilage interface formed originated from the MSCs themselves, or whether this cartilage originated from cells recruited locally. Analysis of the fate of the MSCs with green fluorescent protein labeling is the subject of a subsequent experiment by our group.

Second, we chose to analyze our results at and before 8 weeks because the original intention of the experiment was to detect acceleration of early healing when tendon grafts are known to fail at the tendon-graft junction. However, with the discovery that the MSC-enhanced reconstructions healed by fibrocartilage rather than granulation tissue, it would have been desirable to observe the histologic appearances of these fibrocartilaginous tendon-bone junctions at intervals even up to a year after surgery. Longer time frames would have also allowed us to document whether the histologic and biomechanical differences between treatment and control limbs remain the same or diminish over time.

Last, the mean failure loads of the control reconstructions in this present study did not increase significantly over the 8 weeks of the experiment. Some have reported an increase in failure loads in control reconstructions from 2 to 8 weeks.^{1,3,4} The fact that the control reconstructions did not change in strength may have accentuated the differences in results between treatment and control limbs at 4 and 8 weeks. Nonetheless, the biomechanical behavior of the control limbs in this present study is similar to that of several other reports^{2,8,33} that also found no increase in failure loads in the initial 8 to 12 weeks.

CONCLUSIONS

Applying MSCs to tendon grafts results in a zone of fibrocartilage at the graft-tendon junction that more closely resembles that of the normal ACL chondral entheses. These enhanced grafts have improved biomechanical properties compared with controls and exhibit a rapid and significant increase in failure load and stiffness in the first 8 weeks following ACL reconstruction. Further research is required to evaluate this novel method of enhancing tendon-graft osteointegration, which offers more physiologic healing, stronger reconstructions, and an earlier return to activity.

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