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The Native Cell Population Does Not Contribute to Central-Third Graft Healing at 6, 12 or 26 Weeks in the Rabbit Patellar Tendon

Kirsten R.C. Kinneberg¹, Marc T. Galloway², David L. Butler¹, and Jason T. Shearn¹

¹School of Energy, Environmental, Biological and Medical Engineering, Biomedical Engineering Program, University of Cincinnati, Cincinnati, OH

²Cincinnati Sportsmedicine, Cincinnati, OH

Abstract

Investigators do not yet understand the role of intrinsic tendon cells in healing at the tendon-to-bone enthesis. Therefore, our first objective was to understand how the native cell population influences tendon autograft incorporation in the central-third patellar tendon defect site. To do this, we contrasted the histochemical and biomechanical properties of de-cellularized patellar tendon autograft (dcPTA) and patellar tendon autograft (PTA) repairs in the skeletally mature New Zealand white rabbit. Recognizing that soft tissues in many animal models require up to 26 weeks to incorporate into bone, our second objective was to investigate how recovery time affects enthesis formation and graft tissue biomechanical properties. Thus, we examined graft structure and mechanics at 6, 12, and 26 weeks post-surgery. Our results showed that maintaining the native cell population produced no histochemical or biomechanical benefit at 6, 12 or 26 weeks. These findings suggest that PTA healing is mediated more by extrinsic rather than intrinsic cellular mechanisms. Moreover, while repair tissue biomechanical properties generally increased from 6 to 12 weeks after surgery, no further improvements were noted up to 26 weeks.

Keywords

Patellar tendon; intrinsic healing; enthesis

Introduction

When a tendon directly inserts into bone, it transitions through regions of non-mineralized and mineralized fibrocartilage and thus forms a fibrocartilaginous enthesis.^{1, 2} This multi-component tissue organization is critical for transferring load from the relatively compliant tendon to the relatively stiff bone.^{3–6} However, it is well established that a normal, direct enthesis is not regenerated between tendon and bone after surgical re-attachment.^{7–9} Rather, fibrous scar tissue forms at the tendon-to-bone enthesis during the early phases of

Correspondence to: Jason T. Shearn.

Kirsten R.C. Kinneberg, University of Cincinnati, School of Energy, Environmental, Biological and Medical Engineering, Biomedical Engineering Program, 2901 Woodside Drive, 601 Engineering Research Center, Cincinnati, OH 45220, Phone: 513-556-4171, Fax: 513-556-3473, kirsten.kinneberg@gmail.com

Marc T. Galloway, Cincinnati SportsMedicine & Orthopaedic Center, 7423 Mason-Montgomery Rd. Mason, OH 45040-8082, Phone: 513-347-9999, Fax: 513-573-9178, mgalloway@csnoc.com

David L. Butler, University of Cincinnati, School of Energy, Environmental, Biological and Medical Engineering, Biomedical Engineering Program, 2901 Woodside Drive, 601 Engineering Research Center, ML0048, Cincinnati, OH 45220, Phone: 513-556-4167, Fax: 513-556-3473, david.butler@uc.edu

Jason T. Shearn, University of Cincinnati, School of Energy, Environmental, Biological and Medical Engineering, Biomedical Engineering Program, 2901 Woodside Drive, 601 Engineering Research Center, ML0048, Cincinnati, OH 45220, Phone: 513-556-4175, Fax: 513-556-3473, jason.shearn@uc.edu

healing.⁷⁻¹⁰ With time, the fibrous scar is remodeled toward the native enthesis.⁷⁻⁹ However, morphological progression of the enthesis does not guarantee that tendon fibers are securely anchored into the underlying bone or that the mechanical properties of the enthesis have been regenerated.⁷

Many treatment strategies have attempted to increase the degree and the rate of incorporation between tendon and bone.¹¹⁻¹³ However, few studies have examined the role of the native tendon cell population in regenerating a direct enthesis. Considering that tendon cell metaplasia may be responsible for establishing fibrocartilagenous regions during normal development of the direct enthesis^{14, 15} it is critical to understand their role in forming the direct enthesis after surgical re-attachment.

To investigate the role of the native cell population on graft incorporation, we designed a study to directly contrast the healing capacity of de-cellularized (i.e., without intrinsic cell population) patellar tendon autografts (dcPTAs) with patellar tendon autografts (PTAs). While intrinsic healing is associated with improved repair biomechanics and fewer complications (such as adhesions)¹⁶, extrinsic healing is associated with scar tissue formation and poor healing response¹⁷. Therefore, we hypothesized that dcPTA grafts would generate inferior biomechanical properties as compared to PTAs.

Soft tissue-to-bone healing typically requires significant time to mature (up to 26 weeks post-surgery in animal models)^{7, 9} but variations in healing can occur at earlier time points. Therefore, we examined repairs at 6, 12, and 26 weeks post-surgery. We hypothesized that osteotendinous integration and biomechanical properties of both dcPTA and PTA grafts would improve with recovery time.

Methods

Surgical Procedure

All procedures were approved by the University of Cincinnati Institutional Animal Care and Use Committee. Each rabbit was anesthetized with a cocktail of ketamine/acepromazine (40mg/kg and 5mg/kg, respectively) and isoflurane gas (as needed). Under aseptic conditions, a full-length, full-thickness, central-third defect (~3mm in width) was created in each PT to produce a soft tissue, patellar tendon autograft (PTA). PTA samples from the right limb were stored in phosphate buffered saline (PBS) soaked gauze while those from the left limb were de-cellularized (dcPTA) by three consecutive freeze-thaw cycles. Each cycle consisted of exposing the PTA to liquid nitrogen for 1 minute and sterile PBS for 3 minutes. Freeze-thaw cycling has been shown to kill 93–100% of cells.^{18, 19} Bone defects were created at the proximal and distal insertion sites (approximately 3mm wide, 5 mm long and deep enough to induce bleeding from the cortical bone) using a pneumatic sagittal saw (MicroAire Surgical Instruments, Charlottesville, VA). The excised tissues were then secured into the defect site with four sutures (Prolene 5-0, Ethicon, Somerville, NJ): two each near the patella and tibia. The sutures served to connect the autograft tissue to the adjacent native struts (NS) but not to either bone end. After recovering from anesthesia, animals were returned to their individual cages and allowed unrestricted cage activity.

Sample Collection

At 6, 12, and 26 weeks post-surgery, rabbits were anesthetized (n = 9 per time point) as described above and then euthanized by intracardiac administration of Euthasol® (1.0cc/4.54kg; Virbac Animal Health, Inc., Fort Worth, TX) followed by bilateral pneumothorax. Hind limbs were disarticulated at the hip and removed using a scalpel. For biomechanical evaluation, whole limbs were stored at –20°C until testing. For histological evaluation,

patella-PT-tibia samples were placed in 10% neutral buffered formalin and stored at room temperature (25°C) for 60–72 hours.

Biomechanical Evaluation

Limbs were thawed overnight at 4°C before testing. On the day of testing, limbs were dissected to isolate the patella, patellar tendon (PT) and tibia. Whole PT and repair tissue dimensions were obtained, as described previously.²⁰ Briefly, the length and width of both the whole PT and central-third repair tissue were measured at the medial/central/lateral and proximal/central/distal portions of the tendon, respectively, using digital calipers. Tendon length was measured on the posterior surface from tibial insertion to patellar insertion. Whole PT and repair tissue thickness were measured using a light force (<0.15 N) digital micrometer (IDCtype Mitutoyo Digimatic Indicator, MTI Corp., Aurora, IL). Due to the ellipsoid shape of the PT, whole PT width, thickness and cross-sectional area (width*thickness) are reported by region (proximal/central/distal). After recording tissue dimensions, the tibia/fibula complex was cut roughly 2.5cm distal to the tibial tuberosity to create a bone block. Patellar and tibial bone blocks were fixed into custom designed grips using polymethylmethacrylate cement (Dentsply International, York, PA).²¹ The testing grips for each sample were secured into a Plexiglas tank mounted on a materials testing system (Model 8501, Instron, Inc., Canton, MA). The tank contained phosphate buffered saline (PBS; pH 7.4) heated to 37°C. Repair tissues were preloaded (3.7 ± 1.6 N; mean $\pm$ SD), preconditioned for 50 cycles to 3% strain at 1Hz using a sinusoidal strain pattern and then failed in uniaxial tension at a constant strain rate of 20%/sec while force and displacement were continuously recorded.^{21, 22}

Histological Evaluation

Tissue processing has been described previously^{20, 23} but a brief protocol is outlined below. Fixed patella-PT-tibia samples were sectioned in the transverse plane to isolate 1) the patella and proximal PT and 2) the tibia and distal PT. Patellar and tibial bone ends were decalcified in 10% formic acid (Shandon TBD2; Thermo-Scientific). Each tissue section was then dehydrated through a gradient of alcohols and xylene before being embedded in a paraffin block. The bone ends were processed by cutting eight serial sections (4µm thick) in the sagittal plane at 1 mm intervals through the sample. Serial sections were then stained with hematoxylin and eosin (H&E) and safranin O/fast green. Safranin O was used to evaluate fibrocartilage formation at the enthesis because it is a cationic dye that stoichiometrically binds to glycosaminoglycans (GAGs) and polyanionic proteoglycans (PGs) but not to collagen.²⁴

Statistical Analysis

Biomechanical and dimensional data were normal and homoscedastic. PT ruptures occurred at each time point. The ruptured samples, along with one statistical outlier, were excluded from statistical analysis (sample sizes are listed in Tables 1, 2, and 3). A two-way ANOVA was performed to determine the effect of the native cell population and recovery time on repair tissue biomechanical properties. Post-hoc testing was conducted using Fisher's protected LSD. The significance level was set at $p < 0.05$.

Results

Repair Tissue Dimensions (Table 1)

With few exceptions, whole PT and central-third graft tissue dimensions showed no significant differences among treatment groups or recovery period. At 6 weeks post-surgery, eliminating the native cell population (dcPTA graft) led to an increase in whole tissue cross-

sectional area in the proximal region relative to the PTA ($p = 0.025$). At 12 and 26 weeks post-surgery, however, dcPTA and PTA grafts showed no significant differences in tissue dimensions. Additionally, dcPTA grafts demonstrated a significant increase in whole PT width in the proximal region between 6 and 12 weeks post-surgery ($p = 0.005$) with no subsequent changes thereafter. For PTA grafts, whole PT cross-sectional area in the proximal region increased significantly between 6 and 12 weeks post surgery ($p = 0.010$). Whole PT cross-sectional area in the proximal region at 6 weeks post-surgery was less than values for both 12-week and 26-week repairs ($p = 0.010$ and 0.020 , respectively).

Graft Tissue Biomechanical Properties (Table 2)

The presence of the native cell population at the time of surgery had no effect on graft tissue biomechanical properties at 6, 12 or 26 weeks post-surgery. Additionally, graft tissue biomechanical properties typically increased from 6 to 12 weeks post-surgery but then remained constant between 12 and 26 weeks. For dcPTA grafts, maximum force, linear stiffness, maximum stress and elastic modulus were each affected by recovery time ($p = 0.03$). Each of the biomechanical properties for dcPTA graft tissues at 6 weeks post-surgery were less than the corresponding values for 12 and 26 week repairs ($p = 0.031$). However, biomechanical properties did not increase from 12 to 26 weeks post-surgery. For PTA grafts, only maximum force and maximum stress were affected by recovery time ($p = 0.015$ and 0.034 , respectively). As observed with dcPTA grafts, the maximum force and maximum stress of PTA graft tissues at 6 weeks post-surgery were less than corresponding values for 12 and 26 week grafts ($p = 0.034$). However, maximum force and maximum stress did not differ between 12 and 26 week grafts and stiffness and modulus did not change significantly with time.

Repair Tissue Failure Mechanisms (Table 3)

Of the 30 limbs tested, approximately 80% failed at the patellar and/or tibial insertion site (repair tissues either failed at the patella, the tibia or a combination of both), with almost 50% failing at the patella and less than 20% failing in the tendon mid-substance. Overall, one-third of samples experienced bone avulsion (typically, one to three small bone fragments per tendon avulsed, roughly 1mm in diameter). Due to the high rate of failures at an insertion site, it seems reasonable to associate graft biomechanical properties with the strength of the enthesis.

Patellar and Tibial Enthesis Histology

Similar to what was observed for the biomechanical properties, the presence of the native cell population at the time of surgery appears to have had a minimal effect on graft tissue morphology at the patellar and tibial entheses. Tendon-to-bone integration at the patellar and tibial entheses improved with time for both PTA and dcPTA grafts. Histologic slides were interpreted by the co-author K.R.C.K.

The patellar and tibial entheses of PTA and dcPTA grafts exhibited similar morphologies at each time point. However, at 12 weeks, there were more interdigitations between the tendon, the fibrocartilage and the tibia in dcPTA grafts relative to PTA grafts (Fig. 1, h and k). These dcPTA grafts also demonstrated enhanced fibrocartilage formation at the tibial enthesis relative to corresponding PTA grafts (as indicated by both H&E and safranin O staining; Figs. 1 and 2, h and k). A similar discrepancy in morphology was observed at the patellar enthesis at 26 weeks. However, at 26 weeks, PTA grafts appeared better integrated with the patella than corresponding dcPTA grafts.

The morphology of the tendon-to-bone enthesis improved over time for both PTA and dcPTA grafts. At 6 weeks, the tendon graft and the underlying bone were connected by

hypercellular fibrous scar tissue. The tendon graft and fibrous scar tissues were unorganized and displayed little to no interdigitation with bone (Fig. 1). There was little to no safranin O staining at the tendon-to-bone entheses, indicating little to no fibrocartilage was present (Fig. 2). At 12 weeks, fibrocartilage-like cells (rounded nuclei, residing in lacunae) were present at the tendon-to-bone entheses. There was evidence of a tidemark in each patella (Fig. 1, b and e) and some fibrocartilage-to-bone interdigitation in each tibia (Fig. 1, h and k). However, despite these signs of maturation, the fibrocartilage tissue forming at the tendon-to-bone entheses had yet to produce substantial safranin O staining of GAGs/PGs in the extracellular matrix (ECM) (Fig. 2) and the tendon graft remained poorly integrated with the underlying bone. Even at 26 weeks, when the fibrocartilage was organized in parallel rows of fibrochondrocytes, non-mineralized and mineralized layers were separated by a tidemark, and the ECM demonstrated considerable safranin O staining of GAGs/PGs, the tendon fibers still did not interdigitate with and anchor into the underlying bone. Integration was more pronounced at the fibrocartilage-to-bone interface than between tendon and fibrocartilage or tendon and bone. Despite the improvements with time, at 26 weeks the patellar and tibial entheses of both the PTA and dcPTA grafts still did not fully match the appearance of the NS (Figs. 1 and 2).

One abnormal feature which persisted over time in both PTA and dcPTA entheses was the presence of a bone growth protruding from either the proximal aspect of the patellar enthesis and/or the distal aspect of the tibial enthesis. These bone spurs are marked with an asterisk in Figures 1 and 2. Bone spurs were not evident in the native struts.

Discussion

We hypothesized incorrectly that dcPTA grafts would be inferior to PTA grafts. On the contrary, our biomechanical and histochemical data support the claim that the native cell population offers no advantage to PTA healing and incorporation at 6, 12 or 26 weeks post-surgery. Additionally, the biomechanical properties of the graft tissues did not improve with time as we expected. Significant differences were only found between the 6 week and the later time point grafts.

Healing at the tendon-to-bone enthesis followed the expected progression of inflammation, proliferation and remodeling for both dcPTA and PTA grafts. At 6 weeks, the enthesis for both dcPTA and PTA grafts were in a state of late inflammation/early proliferation. Cellular infiltration during early graft healing/inflammation elicits an increase in cross-sectional area and a deterioration of biomechanical properties.¹⁹ Such infiltration may account for both the increased tissue dimensions in the proximal PT and the reduced biomechanical properties of dcPTA and PTA grafts observed at 6 weeks (Tables 1 and 2, respectively). The dcPTA grafts likely experienced a greater amount of cellular infiltration than PTA grafts due to liquid nitrogen-induced necrosis, which triggers an inflammatory response²⁵. This inflammatory response could explain the discrepancy in whole PT cross-sectional area observed in the proximal region between dcPTA and PTA repairs at 6 weeks. Additionally, early remodeling during the revascularization and proliferation phase of graft healing reduces graft strength until roughly 6–8 weeks.²⁶ This corresponds to our biomechanical data where grafts were generally weakest at 6 weeks. At 12 weeks, the enthesis for both dcPTA and PTA grafts had entered the remodeling phase of healing. During the remodeling phase of healing, the graft is continually adapting towards the morphology and strength of the native structure.²⁶ Morphological progressions at the tendon-to-bone entheses were observed for both grafts from 6 to 12 weeks (as evidenced by the interdigitation of tendon, fibrocartilage and bone and the fibrocartilage organization observed at the enthesis; Figs. 1 and 2). Additionally, both grafts experienced increases in their biomechanical properties from 6 to 12 weeks. Since a clear endpoint of the remodeling phase cannot be defined²⁶, it is

likely that both dcPTA and PTA grafts were still in the remodeling phase at 26 weeks. While both grafts continued to show signs of improved integration at the enthesis, there was no corresponding increase in biomechanics.

The plateau in biomechanical properties at 26 weeks may be due to limited loading at the entheses. While the native struts served to protect the graft during the early phases of healing and incorporation, they may truncate enthesis formation later in the healing process by limiting the amount of load transmitted from healing tendon to bone. There is significant evidence pointing to the importance of mechanical loading on the formation of the tendon-to-bone enthesis.^{27, 28} Specifically, compressive and shear forces at the enthesis may be essential for directing metaplasia of fibroblasts into fibrocartilage cells.^{14, 15}

While others have proposed that the native tendon cell population does not contribute to enthesis formation¹⁰, there are no studies to our knowledge that directly compare tendon-to-bone healing of cellular and de-cellularized grafts. Although autologous and de-cellularized allogeneic bone-PT-bone grafts have been compared for anterior cruciate ligament reconstruction^{29, 30} the discrepancies in their respective healing responses may be due to an increased and prolonged immune response within the autograft^{29, 30}. Thus, studies of auto- vs. allograft ACL reconstruction do not provide a direct comparison and cannot be used to elucidate the role of the native cell population. The results of this study confirm that the native tendon cell population does not contribute to biomechanical integrity or morphological healing and incorporation at the tendon-to-bone enthesis.¹⁰

This study is not without limitations. 1) Although sample sizes for our groups were modest ($n=4-6$), the coefficient of variation for each response measure was typically less than 20%, exceeding 30% in only three cases. Doubling our sample size ($n = 9-13$) might have allowed us to detect a difference in stiffness and modulus between 6 and 12 week PTA repairs. However, more than 100 samples at each time point would be needed to detect differences in most biomechanical response measures for dcPTA and PTA repairs. Therefore, our primary conclusions from this study would remain the same even with modest increases in sample size. 2) Our model does not replicate graft replacement or surgical reconstruction of a tendon or ligament tear. However, we have created a reproducible and accessible model to test the healing response of autografts and tissue-engineered strategies. In addition, our model can examine methods to promote tendon healing and integration into bone. 3) We did not determine cell viability in our PTA autografts. Cellularity could have been adversely affected by preparing the grafts but these changes were likely small.³¹ 4) Although we did not verify that dcPTAs were fully de-cellularized, our laboratory and others have previously shown that repeated freeze-thaw cycles kill 93–100% of all cells in tissues.^{18, 19}

The results of this study demonstrate no histochemical or biomechanical benefit of the native cell population for PTA repair of the patellar tendon central-third at 6, 12 or 26 weeks. Therefore, we propose that PTA healing is mediated by extrinsic cellular mechanisms. However, further study is needed to understand if a phase of necrosis and repopulation occurs similar to that of the ACL graft. The second objective was to understand how osteotendinous integration progresses with time and if changes at the insertion site correlate to changes in biomechanical properties. While changes at the enthesis between 6 and 12 weeks post-surgery did correspond to increases in biomechanical properties for dcPTA and PTA grafts, the grafts reached a threshold at 12 weeks. Our future studies will use tissue engineering strategies to augment the tendon-to-bone entheses with the goal of improving osteotendinous integration and graft tissue biomechanical properties.

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Figure 1.

Patellar and tibial insertion sites for PTA and de-cellularized PTA (dcPTA) grafts at 6, 12 and 26 weeks post-surgery stained with H&E. Fibrocartilage at the tendon-to-bone interface matured with time for both PTA and dcPTA repairs. However, the tendon-to-bone insertion site never fully matched that of the patellar and tibial native strut. Images are labeled to reflect: B, bone; T, tendon; * bone spur. All images are at 4X magnification.

Figure 2.

Patellar and tibial insertion sites for PTA and de-cellularized PTA (dcPTA) grafts at 6, 12 and 26 weeks post-surgery stained with safranin O. Fibrocartilage at the tendon-to-bone entheses matured with time for both PTA and dcPTA repairs. However, the tendon-to-bone insertion site never fully matched that of the patellar and tibial native strut. Images are labeled to reflect: B, bone; T, tendon; * bone spur. All images are at 4X magnification.

Table 1

Tissue Dimensions for Whole and Central-Third PT Graft Tissues, Mean $\pm$ SD

		dcPTA				PTA			
Variable	Region /	6 week n=6	12 week n =6	26 week n=4	6 week n=4	12 week n=6	26 week n=4	26 week n=4	
Whole Tissue									
CSA (mm ²)	Proximal	35.5 ± 4.7	41.8 ± 10.1	39.2 ± 7.2	27.0 ± 2.6 ^{a,b,c}	39.7 ± 6.8		38.9 ± 4.8	
	Central	39.5 ± 6.4	43.9 ± 5.1	37.3 ± 2.8	31.8 ± 8.7	47.1 ± 16.0		35.7 ± 7.4	
	Distal	32.2 ± 8.4	30.9 ± 5.5	27.2 ± 3.3	25.4 ± 8.0	32.3 ± 12.9		28.7 ± 5.4	
Width (mm)	Proximal	9.5 ± 0.6 ^a	11.7 ± 1.6	10.6 ± 0.8	9.0 ± 1.0	11.9±2.6		11.5 ± 2.0	
	Central	11.7± 1.7	15.2 ± 4.0	13.1 ± 1.7	11.0± 1.4	15.6±3.8		13.7 ± 1.7	
Thickness (mm)	Distal	10.0 ± 1.3	10.9 ± 3.0	10.2 ± 0.9	9.2 ± 1.0	11.2 ± 2.4		11.5 ± 1.3	
	Proximal	3.7±0.3	3.6 ± 0.7	3.7±0.4	3.1 ± 0.6	3.4±0.7		3.4 ± 0.5	
	Central	3.4 ±0.4	3.0 ± 0.6	2.9 ± 0.3	2.9 ± 0.7	3.1 ± 1.0		2.6 ± 0.4	
Length (mm)	Distal	3.2 ± 0.8	2.9 ± 0.6	2.7 ± 0.3	2.7 ± 0.7	3.0 ± 1.3		2.5 ± 0.3	
		23.3 ± 3.2	23.9 ± 3.9	24.0 ± 3.0	23.1±3.2	24.9±3.7		24.0± 4.1	
Central-Third Repair									
CSA (mm ²)		7.7 ± 0.8	8.0 ± 0.0	7.0 ± 1.3	7.9 ± 1.4	8.4 ± 2.7		7.2± 0.9	
Width (mm)		2.8 ± 0.3	3.0 ± 0.2	2.9±0.3	2.9 ± 0.3	3.0 ± 0.2		3.0 ± 0.1	
Thickness (mm)		2.7 ± 0.4	2.7 ± 0.5	2.4 ± 0.3	2.7 ± 0.6	2.8 ± 0.8		2.4 ± 0.2	
Length (mm)		23.6 ± 2.9	24.7 ± 5.0	23.9 ± 4.0	24.6 ± 4.3	26.3 ± 6.6		24.6 ± 5.6	

^f If a region is not indicated, the dimension listed reflects either the tissue as a whole or the central-third repair tissue (tissue length is reported as an average of the medial, central and lateral lengths).

^a Less than 12 week repair for same graft material, $p < 0.05$

^b Less than 26 week repair for same graft material, $p < 0.05$

^c Less than dcPTA repair at same time point, $p < 0.05$

Table 2
Biomechanical Properties of Patellar Tendon Autograft (PTA) and De-Cellularized Patellar Tend on Autograft (dcPTA), Mean $\pm$ SD

Response Measure	dcPTA				PTA			
	6 wk n =6	12 wk n =6	26 wk n =6	6 wk n =4	12 wk n =6	26 wk n =4	6 wk n =4	26 wk n =4
Maximum Force (N)	100.6 $\pm$ 38.3 ^{a,b}	176.5 $\pm$ 32.3	152.8 $\pm$ 14.8	94.3 $\pm$ 8.6 ^{a,b}	164.8 $\pm$ 33.2	147.9 $\pm$ 41.0	62.2 $\pm$ 18.7	59.4 $\pm$ 17.7
Stiffness (N/mm)	46.6 $\pm$ 13.6 ^{a,b}	66.7 $\pm$ 16.5	72.0 $\pm$ 11.8	43.5 $\pm$ 2.7	20.8 $\pm$ 5.9	20.2 $\pm$ 3.6	136.6 $\pm$ 13.0	192.5 $\pm$ 21.5
Maximum Stress (MPa)	12.8 $\pm$ 4.3 ^{a,b}	22.1 $\pm$ 3.9	22.5 $\pm$ 5.0	12.3 $\pm$ 2.5 ^{a,b}	20.8 $\pm$ 5.9	20.2 $\pm$ 3.6	136.6 $\pm$ 13.0	192.5 $\pm$ 21.5
Modulus (MPa)	138.3 $\pm$ 21.9 ^{a,b}	201.5 $\pm$ 35.5	250.2 $\pm$ 60.2	136.6 $\pm$ 13.0	199.1 $\pm$ 66.8	192.5 $\pm$ 21.5	136.6 $\pm$ 13.0	192.5 $\pm$ 21.5

^aLess than 12 week repair for same graft material, $p < 0.05$

^bLess than 26 week repair for same graft material, $p < 0.05$

Table 3

Failure and Bone Avulsion Locations

Failure Location			
	Patella	Tibia	Combination of Patella and Tibia
6 Week	5	1	2
12 Week	6	3	1
26 Week	3	3	1
Tendon Mid-Substance			
6 Week			2
12 Week			2
26 Week			1

Bone Avulsion Location			
	Patella	Tibia	Combination of Patella and Tibia
6 Week	3	1	1
12 Week	4	0	0
26 Week	0	1	0