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Increased Platelet Concentration does not Improve Functional Graft Healing in Bio-Enhanced ACL Reconstruction

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Abstract

Purpose—The use of an extra-cellular matrix scaffold (ECM) combined with platelets to enhance healing of an ACL graft (“bio-enhanced ACL reconstruction”) has shown promise in animal models. However, the effects of platelet concentration on graft healing remains unknown. The objectives of this study were to determine if increasing the platelet concentration in the ECM scaffold would; 1) improve the graft biomechanical properties, and 2) decrease cartilage damage after surgery.

Methods—Fifty-five adolescent minipigs were randomized to 5 treatment groups; untreated ACL transection (n=10), conventional ACL reconstruction (n=15), and bio-enhanced ACL reconstruction using 1X (n=10), 3X (n=10) or 5X (n=10) platelet-rich plasma. The graft biomechanical properties, anteroposterior (AP) knee laxity, graft histology and macroscopic cartilage integrity were measured at 15 weeks.

Results—The mean linear stiffness of the bio-enhanced ACL reconstruction procedure using the 1X preparation was significantly greater than traditional reconstruction while the 3X and 5X preparations were not. The failure loads of all the ACL reconstructed groups were equivalent but significantly greater than untreated ACL transection. There were no significant differences in the ligament maturity index or AP laxity between reconstructed knees. Macroscopic cartilage damage was relatively minor, though significantly less when the ECM-platelet composite was used.

Conclusions—Only the 1X platelet concentration improved healing over traditional ACL reconstruction. Increasing the platelet concentration from 1X to 5X in the ECM scaffold did not further improve the graft mechanical properties. The use of an ECM-platelet composite decreased the amount of cartilage damage seen after ACL surgery.

Keywords

Anterior cruciate ligament; collagen; platelet; reconstruction; biomechanics; osteoarthritis

Introduction

Platelet-rich plasma is used to treat patients with a myriad of orthopaedic disorders ranging from chronic tendinopathies, ligament and tendon injuries, and osteoarthritis [2]. However, the value of platelet-rich plasma remains a topic of debate [43]. The wide variation in reported outcomes may be due, in part, to the location of the tissue being treated (e.g., intra- vs extra-articular) [12], patient blood count variations [2,25], platelet-rich plasma preparations [2,12,25], whether a carrier is used to stabilize the platelets [16,31], and dosing [2]. Prior work with “bio-enhanced anterior cruciate ligament (ACL) reconstruction”, in which an ACL graft is implanted within a bio-active scaffold to enhance healing, found that the combination of the extracellular matrix (ECM) scaffold with platelets improved healing in immature pigs [11]. After 15 weeks, the grafts treated with the ECM-platelet composite made with 5X platelet-rich plasma (i.e., 5 times the systemic platelet concentration of whole blood) resulted in higher graft biomechanical properties, less graft necrosis and reduced anteroposterior (AP) knee laxity [11]. However, whether it is important to concentrate the platelets was not known. Research of other connective tissues suggests that the application of supra-physiological platelet concentrates to a wound site improves functional healing. In contrast, prior *in vitro* studies of ACL fibroblasts in an ECM scaffold suggest that physiological platelet concentrations may be more effective than supra-physiological concentrations for stimulating cell proliferation and collagen production [57]. In addition, a recent *in vivo* study suggested that reducing the platelet concentration in an ECM-platelet composite did not harm the efficacy of the implant as a stimulus for ACL healing with primary suture repair [24]. Defining the lowest effective platelet concentration would be useful, as preparations with fewer platelets typically require less blood and less expensive equipment to produce.

Patients with an ACL tear are at greater risk for post-traumatic osteoarthritis when treated conservatively or with ACL reconstruction [34,52]. Although the mechanisms are not fully realized, it is likely due to a combination of factors resulting from both the biological and mechanical insults of the acute injury [10]. It is important to note that the bio-enhanced ACL reconstruction procedure requires the use of blood products intra-articularly, and that cartilage exposure to unclotted blood can damage cartilage [19,32,39]. However, recent studies suggest that platelet-rich plasma may be chondroprotective [18,45]. In order to fully evaluate the impact of platelet concentration on graft performance, an evaluation of cartilage integrity *in vivo* is needed.

The objective of this study was to determine the effects of platelet concentration on the graft structural properties, graft histology, AP knee laxity, and cartilage integrity 15 weeks following a “bio-enhanced ACL reconstruction” procedure [11]. The first hypothesis was that the linear stiffness, AP knee laxity, Ligament Maturity Index and macroscopic cartilage damage would improve when an ACL allograft was treated with an ECM-platelet composite

as compared to traditional ACL reconstruction or untreated ACL transection. The contralateral knee served as the uninjured negative control, while the ACL transection group served as a positive control to show how the treatments compare to no treatment. The second hypothesis was that the ECM-platelet composite made with the lowest platelet concentration (1X) would result in higher linear graft stiffness, lower AP knee laxity, and reduce macroscopic cartilage damage scores when compared to the higher concentration preparations (3X or 5X). To test these hypotheses, a validated pre-clinical model of ACL injury was used [11,24,29,30].

Material and Methods

Fifty-five Yucatan mini-pigs in late adolescence (with closed tibial and femoral physes) [age (mean $\pm$ SD): 15.4 $\pm$ 1.3 months; weight: 48 $\pm$ 9 kg] underwent unilateral ACL transection and were randomized to one of five experimental groups: no treatment (positive control, n=10), conventional ACL reconstruction with bone-patellar tendon-bone (BPTB) allograft (n=15) [46], or bio-enhanced ACL reconstruction with BPTB allograft [11] using an extracellular matrix (ECM) scaffold bio-activated with 1X (n=10), 3X (n=10) or 5X (n=10) platelet-rich plasma (Fig. 1). The surgical knee was randomly selected and the contralateral ACL intact knee served as the negative control.

Extra-cellular matrix scaffold

The ECM scaffolds (MIACH, Boston Children's Hospital, Boston MA) were manufactured by producing a slurry of ECM proteins from bovine connective tissue as previously reported [30]. The ECM scaffold was molded into a porous hollow cylinder with an outer diameter of 22 mm, inner diameter of 10 mm, and length of 30 mm [11]. Once the scaffold was implanted in the knee, it was activated by the addition of platelet-rich plasma at one of three concentrations; 1, 3 and 5 times the pre-operative systematic platelet count (1X, 3X and 5X, respectively). The platelet-rich plasma was created by centrifuging autologous blood as previously described [11]. The systemic platelet count and final platelet concentrations of platelet-rich plasma were determined using a VetScan HM2 Analyzer (Abaxis, Union City, California).

Surgical Procedures

All animals underwent an ACL transection procedure through a medial arthrotomy. The ACL was cut with a scalpel between the proximal and middle thirds of the ligament. After confirming transection with a positive Lachman, the knee was irrigated with 500 cc of normal saline. For the animals assigned to receive no treatment, the incisions were closed, and the untreated ACL injury was allowed to heal naturally [30,46].

Fifteen of the pigs underwent conventional ACL reconstruction immediately following ACL transection [11,29,46]. Fresh-frozen BPTB allografts were harvested from age, weight, and gender-matched donors. The entire patellar tendon (~10 mm in width) was used for the soft tissue portion of the graft and tunnels were placed in the native ACL insertion sites. Grafts were fixed in the femur and tibia with 6 $\times$ 20 mm bio-absorbable interference screws (Biosure; Smith & Nephew, Andover, MA) with the knee in maximal extension.

For the animals assigned to the bio-enhanced ACL reconstruction groups, the same reconstruction procedure was performed; however, the ECM scaffold was threaded over the graft and positioned around its intra-articular portion just after femoral graft fixation (Fig. 1) [11,29]. Three cubic centimeters of the platelet concentrate (1X, 3X or 5X) were used to saturate the ECM scaffold *in situ* and the graft then fixed in the tibial tunnel. The knee was closed in layers and the animal kept under anesthesia for one hour after graft placement.

Following all surgical procedures, the incisions were closed [46]. Pigs were individually housed for the duration of the 15 week follow-up period. They were then euthanized, the limbs were harvested and immediately frozen at -20°C until mechanical testing.

Biomechanical Testing

Knees were prepared for biomechanical testing as previously described [11]. Measurements of AP knee laxity and the structural properties were performed using a servohydraulic load frame (MTS 810; MTS Systems Corporation, Eden Prairie, MN) and custom fixtures [11]. The resolutions for the load and displacement measures were 0.1 N and 0.1 mm. AP knee laxity was measured with the joint capsule intact at 30° , 60° , and 90° of knee flexion with anterior-posterior shear loads of $\pm 40\text{N}$ [9]. The structural properties of the ligaments and grafts were determined by tensile failure testing after the capsule and other ligaments were removed [16]. The linear stiffness, yield and failure loads were calculated from the load displacement data [17]. The cross-sectional areas of the graft mid-substance were estimated assuming an elliptical cross section by measuring the depth and width of the midsubstance with Vernier calipers (measurement resolution of 0.5 mm) [29].

Graft Histology

After biomechanical testing, the graft tissue was dissected free and fixed in formalin. The grafts were embedded in paraffin, sectioned, and stained with hematoxylin and eosin [36]. A central sagittal slice through each graft was scored using the Ligament Maturity Index [36] by independent blinded examiners (Intraclass Correlation Coefficient = 0.92) and their scores were averaged. The Ligament Maturity Index is the sum of three subscores evaluating the cellular, collagen, and vascular organization [36]. The cellular subscore evaluated the presence of inflammatory cells, number of fibroblasts, fibroblast nuclear aspect ratio and the orientation of the fibroblast nuclei relative to the collagen fascicles. The collagen subscore considered bundle orientation and crimp appearance. The vascularity subscore assessed blood vessel density, orientation and maturity. The maximum Ligament Maturity Index achievable was 22 and indicative of a normal ligament.

Macroscopic Cartilage Assessment

After tissue harvest, cartilage damage of each knee was scored using a common macroscopic scoring method (ICC = 0.96) [42]. A five point scale ranging from 0 (no damage) to 4 (lesions with exposed bone greater than 10% of the lesion area) was used to grade six regions of interest; the anterior medial femoral condyle, posterior medial femoral condyle, anterior lateral femoral condyle, posterior lateral femoral condyle, medial tibial plateau, and lateral tibial plateau. The total score was the sum of the scores from each region with a maximum possible score of 24. A larger score correlated with more cartilage damage.

Institutional Animal Care and Use Committee approvals for this study were obtained from Brown University (#0806004) and Rhode Island Hospital (#0176-08).

Statistical Analysis

Generalized estimating equations were used to model the surgical and intact limb values as a function of treatment group for all outcome measures. All models employed a lognormal distribution. The intact limbs were first compared between groups to determine if there were any contralateral limb effects. In the event of treatment differences between contra-lateral limbs, primary hypotheses compared surgical limbs and intact limbs individually. When there were no differences between the treatment groups' intact limbs, surgical limbs were compared after normalizing to their intact limb values. All comparisons were carried out as orthogonal linear estimates and adjusted for multiplicity using the Holm test to maintain alpha at 0.05. The geometric means and confidence intervals were reported.

Results

Blood and PRP Cell Counts

There were no significant differences between groups with respect to the mean platelet, red blood cell, or white blood cell counts in the whole blood (Table 1). For the 1X, 3X and 5X platelet preparations, the mean $\pm$ 95% confidence limits of the platelet multipliers were 1.0 $\pm$ 0.0, 3.1 $\pm$ 0.2, and 5.2 $\pm$ 0.3 times the whole blood levels, respectively (Table 1).

Surgical Outcomes

All of the animals recovered well and survived the 15 week follow-up period. One animal in the traditional ACL reconstructed group was excluded as the ACL from its contralateral "ACL-intact" knee was absent at harvest.

Knee Biomechanics

The mean normalized linear stiffness (Fig. 2) of the 1X ECM-platelet composite bio-enhanced ACL reconstructed group was significantly greater than that of traditional ACL reconstruction ($p=0.03$). There were no significant differences in linear stiffness between the 3X or 5X ECM-platelet composite groups and traditional ACL reconstruction. All three bio-enhanced ACL reconstructed groups resulted in significantly higher mean linear stiffness values compared to untreated ACL transection ($p<0.03$) while the traditional ACL reconstructed group tended to be greater than untreated ACL transected ($p=0.07$).

The mean normalized maximum failure loads (Fig. 3) and yield loads of the three bio-enhanced ACL reconstructed groups and the traditional ACL reconstructed group were significantly greater than the untreated ACL transected group ($p<0.01$). However, there were no significant differences between the bio-enhanced ACL reconstruction groups and the traditional ACL reconstructed group.

In the operated knees, the mean cross-sectional areas of the grafts were significantly greater ($p<0.01$) than the healing ACL transected ligaments except for the 5X ECM-platelet composite group (Table 2). There were no significant differences between the normalized

mean AP laxity values between treatment groups when measured at 30°, 60° and 90° of knee flexion (Table 2).

Ligament Histology

No significant differences in the total Ligament Maturity Index were found between the grafts following traditional or bio-enhanced ACL reconstruction (Fig. 5). Similar findings were observed when evaluating the ligament subscores across treatment groups (Table 3). There was no evidence of inflammatory cells within any grafts.

Cartilage Assessment

The mean macroscopic cartilage grades of the untreated ACL transected and traditional ACL reconstructed groups were significantly greater (i.e., exhibited greater cartilage damage) than the three bio-enhanced ACL reconstructed groups (Fig. 6; $p < 0.03$). There were no differences between the ACL transected and traditional ACL reconstructed groups or between the three bio-enhanced ACL reconstruction groups.

Discussion

The most important findings of this study were that the bio-enhanced ACL reconstructed animals treated with the physiologic concentration (1X) of platelets had a significantly higher linear stiffness than those undergoing traditional (i.e., non-bio-enhanced) ACL reconstruction while the bio-enhanced ACL reconstructions treated with higher concentrations (3X and 5X) of platelets did not and that cartilage damage was less when all three ECM-platelets composite were used. The bio-enhancement did not significantly influence the mean yield and maximum failure loads as none of the ACL reconstructed treatment groups were significantly different from each other (bio-enhanced or traditional). The linear stiffness results are encouraging as this is the parameter that tissue engineers attempt to replicate as ligaments and tendons routinely carry loads that are less than 10% of the failure load when patients perform activities of daily living [3].

The results of the present study are in contrast to a previous study of bio-enhanced ACL reconstruction where there were significant improvements in all of the structural properties with bio-enhancement when using a 5X ECM-platelet composite [11]. The differences between studies are likely due to the mean ages of the animals. Juvenile farm pigs (approximately 3 months old) were used in the previous study as compared to adolescent minipigs (approximately 15 months old) in the current study. The growth plates of the adolescent pigs, while not fused, were closed, and representative of a child in mid-teens. It has been previously shown that the healing capacity of adolescent and adult pig ACLs was significantly less than in juvenile pigs [30]. The reasons may be due to differences in the regenerative potential of fibroblasts [23], the number of growth factor receptors on the fibroblasts [48], cell density [22], or because adolescent fibroblasts migrate and proliferate less than juvenile fibroblasts [21].

The use of platelets to stimulate graft healing was selected for this study as they are known to release a variety of growth factors known to stimulate healing in ligament and tendon tissues [2,26,28]. Animal models have been used to evaluate exogenous application of

specific growth factors including transforming growth factor beta (TGF- β , epidermal growth factor (EGF), platelet derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) [53,54,56,59,60]. PDGF and the combination of TGF- β and EGF have been shown to improve the structural properties of the healing graft in the early stages of healing [54,56]. As with the current study, these improvements were particularly evident in the measures of graft stiffness. No changes in AP knee laxity were found when compared to traditional ACL reconstruction in all three studies. Application of VEGF has been shown to improve the vascularity of healing grafts, though this came at the expense of increased AP knee laxity and decreased structural properties [59,60]. However, the combination of TGF- β and VEGF improved the structural properties of the graft compared to either treatment alone [53], emphasizing the importance of including multiple growth factors to potentially enhance graft healing.

There have been several clinical trials to determine the effects of platelets, platelet concentrates or platelet-derived growth factors on graft healing in human patients [4,5,8,33,35,38,40,44,50,51]. A recent systematic review identified eight trials for biologically improving ACL reconstruction, seven of which focused on graft maturation (via histology and/or MRI assessments) [49]. Four of these studies reported better “maturation” outcomes when grafts were treated with platelets, though none of them were able to detect differences in clinical outcomes. For the present porcine study, the 1X ECM-platelet concentration improved graft healing. These findings suggest that the use of platelets to enhance graft healing could be translated into clinical practice once the preparation and dosing have been optimized.

The amount of macroscopic cartilage damage noted in the present study was relatively minor across treatment groups within the short 15 week follow-up period. Nonetheless, the extent of cartilage damage in knees treated with the 1X, 3X or 5X ECM-platelet composites was significantly less than that of traditional ACL reconstruction. Additional research is required to determine the long-term response of the cartilage following bio-enhancement with platelet concentrates. In addition to the lower rates of cartilage damage seen with the use of an ECM-platelet composite in this study (Fig. 6), and a prior study at 6 and 12 months [29], are the significant differences in cartilage damage on the non-operated knee. The etiology of this effect remains unknown.

This study utilized a commonly used semi-quantitative macroscopic cartilage scoring system [6,13,15,29,42] while the examiners were blinded to specimen number, treatment group and limb to ensure validity. Macroscopic grading was selected over semi-quantitative histological grading because it considers the entire cartilage bearing surface and not just representative slices within each compartment. Nonetheless a formal histological assessment could provide additional insight into cartilage health and should be considered for future studies.

For the structural property measurements, the 1X ECM-platelet composite produced the highest linear stiffness values and was the only treatment group significantly different from traditional ACL reconstruction. It is possible that the study was underpowered to detect the differences between bio-enhanced treatments of different platelet concentrations; however,

the study was 92% powered to detect a change in stiffness of 20 N/mm with 10 animals per group.

The pig model has some limitations that are also common to all animal models of ACL reconstruction. The pig is a quadruped and post-operative rehabilitation is difficult to control. Thus, it does not fully represent the human condition. Nonetheless, similar anatomical and biomechanical features between the pig and human have been noted [1,37,55]. For this study, the minipig strain has some advantages over other commonly used ACL reconstruction models (i.e. sheep and goat) in that the blood of the pig closely matches the hematology profile of the humans [27]. This is an important consideration for studies involving blood derivatives for bio-enhancement.

Another limitation was that the ACL reconstruction procedures utilized fresh frozen allografts instead of autografts. In the porcine model, harvesting a patellar tendon autograft would compromise the extensor mechanism while the hamstring autograft is not of sufficient length. It is possible that autografts would have provided different results. This is unlikely a major concern in that all treatment groups utilized the same allograft and the structural properties of the allografts in this study were similar to those reported for autografts in other quadruped models [7,14,41]. It is also possible that the allografts and/or ECM scaffolds (i.e., xenografts) could have elicited an immune response. However, the allografts were fresh frozen and obtained from animals of the same strain under sterile conditions. There was no evidence of an inflammatory response in the current study or in previous studies of bio-enhanced ACL reconstruction [11,29]. Likewise, the ECM scaffolds were manufactured from bovine tissue under sterile conditions using a method to remove cellular debris. The biocompatibility of the treated scaffolds has been extensively studied with no evidence of an immune response [20,47,58]. Finally, the cellular subscore of the Ligament Maturity Index considered the presence of inflammatory cells. None were noted in any of the allografts treated with or without the ECM scaffold (Table 3).

Conclusions

Bio-enhanced ACL reconstruction with the 1X ECM-platelet composite significantly improved the linear stiffness of the healing graft as compared to traditional ACL reconstruction after 15 weeks of healing in the adolescent porcine model. This improvement, however, was not seen at the higher platelet concentrations (3X or 5X). There were significant reductions in cartilage damage in all of the bio-enhanced ACL reconstructed groups when compared to traditional ACL reconstruction or untreated ACL transection. The biomechanical similarities and improved cartilage outcomes for bio-enhanced ACL reconstruction using the 1X ECM-platelet composite suggest that bio-enhancement of graft healing is possible and may justify the translation of this technique to clinical trials.

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Fig. 1.

Schematic of standard ACL reconstruction (ACLR) and bio-enhanced ACL reconstruction (BE-ACLR) with the ECM-platelet composite. The ECM sleeve was threaded over the graft and then loaded with autologous platelets of different concentrations (1X, 3X or 5X the systemic concentration) to form the ECM-platelet composite (Adapted with permission from Fleming et al[11]).

Fig. 2.

The linear stiffness values normalized to the contralateral ACL-intact (control) knee for the five experimental groups (ACLT = untreated ACL transection, ACLR = traditional ACL reconstruction, 1X-ACLR = BE-ACLR with 1X platelets, 3X-ACLR = BE-ACLR with 3X platelets, 5X-ACLR = BE-ACLR with 5X platelets). The mean data are plotted with the 95% confidence intervals. Means that do not differ between groups after Holm adjustment within each time point have the same upper case letter (A, B or C).

Fig. 3.

The maximum failure loads normalized to the contralateral control knee for the five experimental groups. The mean data are plotted with the 95% confidence intervals. Means that do not differ between groups after Holm adjustment within each time point have the same upper case letter (A or B).

Fig. 4.

The yield loads normalized to the contralateral control knee for the five experimental groups. The mean values are plotted with the 95% confidence intervals. Means that do not differ between groups after Holm adjustment within each time point have the same upper case letter (A or B).

Fig. 5.

The Ligament Maturity Index for the five experimental groups. The mean values are plotted with the 95% confidence intervals. Means that do not differ between groups after Holm adjustment within each time point have the same upper case letter (A or B). The subscores making up the Ligament Maturity Index can be found in Table 3.

Fig. 6.

The macroscopic cartilage scores for the five experimental groups for the surgical (Sx; left panel) and contralateral control (Ctl; right panel) limbs. The maximum score (denoting the worst cartilage damage) is 24. The mean values are plotted with the 95% confidence intervals. Means that do not differ between groups after Holm adjustment within each time point have the same upper case letter (A or B). It should be noted that it was not appropriate to normalize the data to the contralateral knee as there were significant treatment effects, albeit minor, in the control knee.

Table 1

The mean (95% Confidence Limits) platelet, white cell and red blood cell counts for the whole blood of each group and the platelet preparation for the 1X, 3X and 5X groups.

	Whole Blood Levels			Platelet Preparation Levels		
	Platelets	RBC	WBC	Platelets	RBC	WBC
ACLT	435 (377.1–492.7)	5.7 (5.3–6.1)	13.5 (10.9–16.2)	-	-	-
ACLR	368 (301.2–434.7)	5.7 (5.4–6.1)	11.3 (9.2–13.3)	-	-	-
1X-ACLR	466 (387.3–544.7)	6.0 (5.6–6.3)	11.7 (9.8–13.6)	466 (387.3–544.7)	6.0 (5.6–6.3)	11.7 (9.8–13.6)
3X-ACLR	432 (367.7–496.5)	5.9 (5.5–6.3)	12.4 (10.1–14.7)	1386 (1153–1618)	0.1 (0.0–0.1)	2.7 (1.4–4.1)
5X-ACLR	466 (383.7–548.5)	5.5 (5.1–5.8)	10.1 (8.0–12.3)	2473 (2011–2937)	0.1 (0.1–0.2)	6.0 (2.3–9.6)

Table 2

The mean (95% Confidence Limits) for the graft cross sectional areas and AP laxity at 30° (AP30), 60° (AP60), and 90° (AP90) of flexion for the five treatment groups; ACL transection (ACLT), ACL reconstruction (ACLR), IX bio-enhanced ACL reconstruction (1X-ACLR), 3X bio-enhanced ACL reconstruction and 5X bio-enhanced ACL reconstruction (5X-ACLR) 15 weeks after surgery.

Surgical Limb	ACLT	ACLR	1X-ACLR	3X-ACLR	5X-ACLR
CSA (mm ²)	34.7 (22.0–47.4)	63.3 (41.2–85.4)	65.8 (49.4–82.2)	62.5 (38.4–86.6)	54.9 (31.6–78.2)
AP30 (mm)	5.5 (4.2–7.2)	3.2 (2.6–4.0)	6.1 (5.2–7.0)	4.1 (3.0–5.6)	4.3 (3.0–6.1)
AP60 (mm)	9.9 (7.8–10.8)	8.7 (7.9–9.7)	8.9 (7.5–10.8)	8.0 (6.7–9.7)	7.9 (5.6–11.0)
AP90 (mm)	6.3 (5.2–7.7)	7.6 (6.8–8.6)	7.1 (5.8–8.7)	6.7 (5.2–8.6)	6.4 (4.6–8.9)
Control Limb					
CSA (mm ²)	30.2 (21.0–39.4)	27.5 (21.5–33.5)	33.7 (32.4–35.0)	27.8 (20.6–35.0)	34.3 (30.1–38.5)
AP30 (mm)	2.3 (1.9–2.8)	2.1 (1.8–2.4)	2.3 (1.9–2.9)	2.0 (1.7–2.3)	2.0 (1.5–2.6)
AP60 (mm)	2.5 (2.1–2.9)	2.2 (1.9–2.6)	2.5 (2.0–3.1)	2.1 (1.9–2.5)	2.6 (2.1–2.9)
AP90 (mm)	1.8 (1.5–2.1)	1.7 (1.5–1.9)	1.9 (1.6–2.3)	1.5 (1.4–1.6)	1.6 (1.3–1.8)

Table 3

The mean (95% Confidence Limits) for the Ligament Maturity Index Subscores. The cell subscore includes the presence of inflammatory cells, number of fibroblasts, fibroblast nuclear aspect ratio and orientation of the fibroblast nuclei with the collagen fascicles. The collagen subscore evaluates bundle orientation with long axis of the ligament and crimp. The vascularity subscore assesses blood vessel density, orientation of vessels with long axis of the ligament and vessel maturity. The maximum score for the the cell, collagen and vascular subscores are 8, 8, and 6, respectively. A higher score is indicative a more normal ligament.

Group	Cellularity	Collagen	Vascularity
ACLT	4.4 (4.1–4.8)	2.3 (1.6–3.1)	2.9 (2.1–3.7)
ACLR	5.6 (4.8–6.4)	5.0 (4.1–5.9)	4.3 (3.9–4.7)
1X-ACLR	6.0 (5.2–6.8)	5.5 (4.4–6.6)	4.0 (3.2–4.8)
3X-ACLR	6.4 (5.4–7.4)	4.8 (3.7–5.9)	3.6 (2.6–4.6)
5X-ACLR	5.7 (5.0–6.4)	4.2 (3.1–5.3)	3.8 (3.5–4.0)