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COLLAGEN-PLATELET COMPOSITES IMPROVE THE BIOMECHANICAL PROPERTIES OF HEALING ACL GRAFTS IN A PORCINE MODEL

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Abstract

Background—The outcome of ACL reconstruction is variable and many patients have increased joint laxity postoperatively.

Hypothesis—We hypothesized that placement of a collagen-platelet composite (CPC) around the graft at the time of ACL reconstruction would decrease postoperative knee laxity and improve the structural properties of the graft when compared to standard ACL reconstruction.

Study Design—Controlled laboratory study.

Methods—Thirteen immature pigs underwent unilateral ACL reconstruction with bone-patellar tendon-bone allograft. In 6 pigs, a standard allograft was used to reconstruct the ACL. In 7 pigs, a CPC was placed around the allograft. After 15 weeks of healing, the animals were euthanized, and the anterior-posterior (AP) knee laxity and structural properties of the graft were measured. Qualitative histology of the grafts was also performed.

Results—The AP laxity values of the reconstructed knees, normalized to the contralateral control, were significantly reduced by 28% and 57% at 60° and 90° of knee flexion, respectively, with the addition of CPC ($p < 0.001$). Significant improvements in the graft structural properties were also found; the normalized yield ($p = 0.044$) and maximum failure loads ($p = 0.025$) of the CPC group were 60% higher than the standard ACL reconstructed group. Although cellular and vessel infiltration were observed in the grafts of both groups, regions of necrosis were present only in the standard ACL reconstructed group.

Conclusions—These data demonstrate that the application of CPC at the time of ACL reconstruction improves the structural properties of the graft and reduces early AP knee laxity in the porcine model after 15 weeks of healing.

Clinical Relevance—Application of a CPC to an ACL graft at the time of surgery decreased knee laxity and increased the structural properties of the graft after 15 weeks of healing.

Key Terms

ACL; reconstruction; platelets; laxity; mechanical properties; healing; collagen-platelet composite; allograft

INTRODUCTION

The current “standard of care” for an ACL tear is reconstruction with either autograft or allograft tendon to provide gross stabilization of the knee and improve post-injury function. Despite the popularity of these procedures, clinical studies report temporal increases in knee joint laxity during the first few months of healing that persist for the life of the graft.^{3, 9, 30} Increases in knee laxity have been shown to correlate with decreased mechanical graft properties in animal models,^{2, 11} suggesting that the grafts are inferior. Strategies that could improve the graft structural properties (yield load, maximum failure load, linear stiffness) and reduce the initial increase in anterior-posterior knee laxity (AP laxity) could potentially improve ACL reconstruction outcome in patients.

Collagen-platelet composites (CPCs) have been shown to stimulate histological and biomechanical healing of ACL injuries.^{20–22, 29} ACL injuries treated with CPC demonstrated a similar growth factor expression profile as the extra-articular medial collateral ligament.²¹ This was in contrast to no healing and severely limited growth factor expression without CPC. In addition, two factors normally concentrated in platelets (PDGF and TGF) have been tested in large animal models of ACL reconstruction.^{31, 34, 36} Exogenous application of these growth factors improved the graft failure load and linear stiffness values 12 weeks post-operatively.^{31, 34} In contrast, VEGF, a growth factor to stimulate revascularization, resulted in weaker grafts at 12 weeks.³⁶ Recently, it was shown that application of CPC around an ACL autograft improved AP knee laxity after 6 weeks of healing even when the platelet level was not concentrated in the caprine model.²⁸ These studies suggest that the use of a CPC, particularly one with concentrated platelets to release a myriad of growth factors to stimulate healing, may have potential clinical application.

The objective of this study was to evaluate if a CPC can improve the structural and material properties of the ACL and reduce post-operative knee laxity after 15 weeks of healing. The porcine model was selected because of its anatomic similarity to the human knee,^{4, 20, 33} and similar hematologic characteristics.¹⁷ We hypothesized that placement of a CPC around an ACL allograft at the time of surgery would decrease postoperative AP laxity and increase the early structural properties (i.e. yield load, maximum failure load, linear stiffness) and material properties (i.e. yield stress, tangent modulus) fifteen weeks after surgery (Fig. 1).

MATERIALS AND METHODS

Animal Model

Fourteen immature female 30kg Yorkshire pigs underwent unilateral ACL reconstruction using a bone-patellar tendon-bone allograft. ACL reconstruction with standard allograft (ACLR; control) was performed in 7 animals while the procedure was performed using the same allograft enhanced with CPC in 7 animals (E-ACLR; experimental). One animal in the control group was euthanized at the time of surgery due to a condylar fracture and was not included in the study. The study was 80% powered to detect an effect size of 2.

Following the induction of anesthesia and clinical examination of both knees, the knee was shaved, prepared with a surgical iodine solution and alcohol, and draped. An 8 cm incision was made at the medial border of the patellar tendon. The patella was retracted laterally without

dislocating it from the trochlear groove. The fat pad was partially resected to expose the ACL, which was completely transected at the junction of the proximal and middle thirds of the ligament. ACL transection was verified by a positive Lachman exam.

The fresh frozen allografts were harvested from fourteen age, weight and gender matched donor knees. The entire patellar tendon, which was approximately 10 mm in width, was used. The bone blocks were trimmed to 7 mm diameter to allow for smooth graft passage through 8 mm osseous tunnels in the femur and tibia. The osseous tunnels were drilled to the insertion sites of the native ACL using a commercial drill guide system (Acufex Elbow Aimer, Smith & Nephew, Inc, Andover MA). Once the tunnels were completed, the graft was introduced intra-articularly through the arthrotomy. One bone block was passed into the femoral tunnel and rigidly fixed using a 6 mm bioabsorbable interference screw (CALAXO, Smith-Nephew, Andover, MA). In the standard ACLR group, the graft was then passed retrograde into the tibial tunnel, preconditioned using 20 cycles of firm manual tension, held in maximum manual tension, and secured in the tibia using another 6 mm interference screw. The tibial screw was inserted into the tibial tunnel at the distal end of the tunnel, and the screw was countersunk 3 mm below the cortical surface of the tibia (Fig. 1). In the E-ACLR group, a tubular collagen sponge (details below) was threaded onto the graft after femoral fixation. The graft was then pulled retrograde into the tibial tunnel, preconditioned, tensioned, and secured with another interference screw as described for the ACLR group. The concentrated platelets were added to the sponge to create the CPC.

The retinacula and subcutaneous tissues were closed with interrupted 2-0 Vicryl, and a 3-0 Vicryl subcuticular stitch was used to close the skin incision. The animals were allowed unrestricted weight bearing. Postoperative pain was controlled with narcotics. All animals were monitored closely after surgery for any signs of discomfort, lameness or weight loss. The pigs were euthanized after 15 weeks of healing. The knees were immediately harvested and frozen until mechanical testing.

Collagen-Platelet Composite (CPC)

Whole blood was drawn from each animal at the time of surgery using a large bore needle (≤ 18 g) and placed into tubes containing sodium citrate. Tubes were centrifuged at 150g and the supernatant collected. The supernatant was then centrifuged at 500g to form a platelet pellet. The second supernatant was collected as platelet-poor plasma. The platelet pellet was then resuspended in the specified amount of platelet-poor plasma to create a platelet concentrate approximately 5 times the systemic platelet level. Initial and final platelet concentrations were determined using a VetScan HM5 Analyzer (Abaxis, Union City, CA). The platelet concentrate was kept at room temperature until use (less than 30 minutes).

The collagen sponge was made by solubilizing bovine fascia. Fresh bovine fascia was harvested from the hindlimbs, minced, and solubilized in a pepsin solution to create a bovine atelocollagen solution. The resulting solution was then frozen, lyophilized, and rehydrated with a specified amount of water to create a solution with a collagen content >10 mg/ml. The resulting collagen slurry was neutralized using HEPES buffer (Mediatech Inc, Herndon, VA), sodium hydroxide (Fisher Scientific, Fair Lawn, NJ), PBS (HyClone, Logan UT), and calcium chloride (Sigma-Aldrich, St. Louis, MO). The neutralized atelocollagen solution was placed into cylindrical molds with an inner diameter of 9.5 mm, and an outer diameter of 22.5 mm and lyophilized. The resulting sponges were stored frozen and under vacuum until use.

MRI

Just prior to euthanasia, the animals were anesthetized and the morphometry of the grafts were measured using volumetric sequences on MRI at 1.5 Tesla (GE Medical Systems, Milwaukee,

WI) using an eight-channel phased array coil. The ligament cross-sectional area, and length were measured using a gapless PD sagittal series through the knee using a 3D Fiesta acquisition protocol (TR= 4.9, TF = 1.6, 0.8 mm slice thickness with 0 mm gap, FOV=16, BW = 62.5 and flip angle of 65°). The length and cross-sectional area measurements were used to assess the material properties of the graft.

Clinical Examination

Following the induction of anesthesia, the range of motion (ROM) was assessed using a goniometer. The minimum extension angle, maximum flexion angle, and the total ROM were recorded. A clinical Lachman test was performed by the operating surgeon (MMM) pre-operatively. The measurements were performed in conjunction with the MR imaging protocol.

Mechanical Testing

The knees were thawed and immediately prepared for AP laxity and tensile failure testing. The soft tissues surrounding the tibia and femur were dissected free leaving the joint capsule intact. The distal tibia and proximal femur were then potted in 15 cm lengths of PVC piping using an epoxy (SmoothCast 300; Smooth-On Inc., Easton, PA) to facilitate mounting on the material testing platform¹⁹. The joints were wrapped in towels saturated with physiologic saline to prevent dehydration. All testing was done at room temperature.

AP laxity values for the ACL reconstructed and contralateral intact joints of both the ACLR (control) and E-ACLR (experimental) animals were measured using a custom fixture with the knee locked at 30°, 60° and 90° flexion (Fig. 2a).^{8, 10, 19} Anterior and posterior directed loads of ± 40 N were applied to the femur with respect to the tibia by an MTS 810 Materials Testing System (MTS, Prairie Eden, MN) while the AP displacements were measured.¹³ AP laxity was reported as motion of the tibia with respect to the femur between the load limits of ± 30 N.¹⁰

After the AP laxity tests, the tibia and femur were positioned in a tensile testing fixture so that the mechanical axis of the ACL was collinear with the load axis of the material test system (Fig. 2b).^{19, 28, 32} All soft tissues were dissected from the joint leaving the graft and scar mass intact. The femur-graft-tibia complexes were then loaded in tension to failure at 20 mm/min,^{20, 26} while the load-displacement data were recorded. Identical protocols were performed on the contralateral ACL-intact knees. From the load-displacement tracing, the yield and maximum failure loads, and the linear stiffness were determined. The load-displacement data were then normalized by the minimum graft cross-sectional area and the initial resting graft length as determined by MRI to calculate the material properties (yield stress and tangent modulus).

Histology

After biomechanical testing, the graft tissue was dissected from the femoral and tibial insertion sites and fixed in formalin for 7 days. After fixation, the grafts were cut in cross-section and embedded in paraffin, and microtomed into 7 micron sections. These sections were placed onto glass slides, and stained with hematoxylin and eosin for qualitative assessment by one unblinded examiner (MMM).

Statistical Analysis

Statistical comparisons of platelet counts, clinical examination data, AP laxity and tensile failure testing were made between the ACLR and E-ACLR groups. Differences between systemic platelet counts at the time of surgery were compared using an unpaired t-test. Clinical examination data (ROM, maximum flexion angle, minimum extension angle, and Lachman test) were analyzed after subtracting the difference between the experimental knee and the

contralateral ACL-intact control knee (reconstructed minus control). Comparisons between the two treatment groups (ACLR vs E-ACLR) were then performed using the Mann-Whitney U-test and presented in terms of the median and range. AP laxity data were analyzed after subtracting the difference between the experimental and control knees using multivariate analyses of variance. To compare the structural properties, the value of the treated knee was normalized by that of the contralateral ACL-intact knee (reconstructed/control). The ratios were then transformed by the natural logarithms before the statistical analyses to account for nonlinearity and non-normality of the raw ratios. Comparisons of the structural properties (yield load, maximum failure load, linear stiffness) were then based on multivariate analyses of variance. Comparisons of the material properties (yield stress, tangent modulus) between the ACLR and E-ACLR grafts were made using non-paired t-tests. A two-tailed value of $p < 0.05$ was used as the criterion for statistical significance.

RESULTS

Surgical Outcomes

There were no significant post-operative complications in any of the animals. All animals were able to weight-bear within 12 hours of surgery, and were walking normally within one week. There was no evidence of infection in any knees.

Systemic Platelet Counts

No significant differences in the systemic platelet counts (mean $\pm$ standard deviation) were found between ACLR and E-ACLR groups at the time of surgery (379 ± 90 K/ μ l vs. 292 ± 82 K/ μ l, $p = 0.358$). The average platelet count in the platelet rich plasma used to create the CPC for the E-ACLR group was 1141 ± 527 K/ μ l, with an average enrichment factor of $4.8X \pm 1.0X$. At the time of retrieval, the average systemic platelet counts in the ACLR and E-ACLR groups were 335 ± 102 K/ μ l and 335 ± 43 K/ μ l. The changes in white blood cell concentrations, over the time course of the experiment, were also similar in both groups (-1.6 ± 3.1 K/ml for ACLR and -2.5 ± 3.0 K/ml for E-ACLR).

Clinical Assessment/Gross Appearance

The clinical assessment between treatments at the time of harvest demonstrated that there were no differences in the normalized values of ROM, maximum flexion, minimum extension limits, or manual Lachman tests between the ACLR and E-ACLR groups (Table 1). There was none to minimal cartilage wear in both groups per visual inspection.

There were no differences between the ACLR and E-ACLR groups on gross appearance in terms of rate of reformation of the ligamentum mucosum, rate of scar or adhesion formation from the notch scar mass to the harvest defect of the patellar tendon or amount of joint adhesions observed. Both the ACLR and the E-ACLR appeared to be similar.

Biomechanical Testing

The AP laxity values at all knee flexion angles (30° , 60° , and 90°) were greater in the ACL reconstructed knees as compared to the ACL intact knees. The mean difference of the AP laxity of the knees treated with the CPC was significantly less than that of the knees treated with standard ACL reconstruction when tested at 60° ($p < 0.001$) and 90° ($p < 0.001$) of flexion (Table 1), however, there was no significant difference when the knees were tested at 30° ($p = 0.45$); full extension in the pig.

The tensile load-displacement curve for the E-ACLR group was closer to that of the intact ACL when compared to the standard ACLR group (Fig. 3). The structural properties were generally improved in the E-ACLR animals compared to the ACLR animals across animals (Table 2).

Significant improvements (approximately 60%) in both the normalized yield load ($p=0.025$) and the normalized failure load ($p=0.044$) resulted from the E-ACLR treatment when compared to ACLR alone (Fig. 4). There was no difference ($p=0.33$) in linear stiffness between treatment groups (Fig. 4).

The MRI measurements of graft length were similar in both groups (ACLR: 23.1 ± 3.55 mm; E-ACLR: 20.2 ± 3.77 mm, $p=0.20$). However, the cross-sectional area in the ACLR group was significantly larger than that of the E-ACLR group (37.3 ± 20.89 mm² vs 16.1 ± 3.43 mm², $p=0.022$). The tangent modulus of the E-ACLR group was three times higher than the ACLR group (103 ± 31.6 N/mm² vs 40.4 ± 32.0 N/mm², $p<0.005$). The yield stress was also significantly higher in the E-ACLR group (27.4 ± 7.06 N/mm² vs 11.4 ± 13.79 N/mm², $p=0.021$).

Histology

There was reasonable cellular and vessel infiltration in both the E-ACLR and ACLR grafts (Fig. 5A,B). However, areas of necrosis were noted within the ACLR midsubstance (Fig. 5C), which were not seen in the tissue from the E-ACLR group. In addition, there was hypercellular and hypervascular tissue circumferentially surrounding the E-ACLR graft (Fig. 5D). The degree of vascularization was similar to that seen in the epiligamentous tissue of the ruptured ACL.¹⁸ The E-ACLR graft tissue was more vascular than the ACLR grafts, where vessels were noted to extend from the hypercellular and hypervascular epiligamentous tissue into the graft substance (Fig. 5D).

DISCUSSION

The addition of CPC resulted in a significant reduction in the post-operative AP knee laxity at 60° and 90° of flexion (by approximately 50%), and significant improvements in the yield load and maximum failure load of the graft (by approximately 60%) fifteen weeks after ACL reconstruction using an allograft patellar tendon graft in the porcine model. The 15 week time period was selected for study since it falls well after the nadir where graft strength and stiffness is increasing^{5, 7, 13, 23}. Likewise improvements of approximately 250% were seen in the yield stress and tangent modulus. Application of a CPC to a healing graft may therefore provide a clinically relevant means to improve early graft healing following ACL reconstruction.

Bovine atelocollagen was used to make the CPC. It was made by digesting the collagen with pepsin, which cleaves the telopeptides from the central collagen region. Removal of these antigenic components is responsible for the low rate of cross-species reaction when it is clinically used as a soft tissue filler. A bovine source was selected because it was readily available, commonly used in humans, and has been FDA approved for other uses.^{15, 25, 27}

The use of platelets to stimulate healing of the ACL graft is a plausible means to enhance healing and improve outcome. Platelets release a myriad of growth factors that are essential to wound healing. Some of these growth factors are known to stimulate the healing responses of the ACL and the ACL graft. In vitro, ACL cell migration has been stimulated by TGF- β 1, while PDGF-AB and FGF-2 can stimulate ACL cell proliferation in a 3-D collagen scaffold.¹⁶ CPCs have been found to release PDGF-AB in relevant quantities, suggesting platelet activation and cytokine release.¹⁴ In vivo, high levels of FGF-2, PDGF-AB and TGF- β 1 are found in the area of CPC implantation for up to 3 weeks after gel implantation, suggesting sustained presence of these platelet-related growth factors in the wound site after platelet activation.²¹ When combined with collagen, platelets have been shown to help heal partial and complete transections of the ACL.^{20–22, 29}

Recent studies have demonstrated the feasibility of enhancing the healing response of an ACL graft using platelets or platelet associated growth factors.^{28, 31, 34, 36} Previous translational

models of ACL graft healing with TGF- β 1/EGF³⁴ demonstrated a significant increase in strength at 12 weeks, while PDGF-BB³¹ demonstrated a significant increase in linear stiffness at 24 weeks. Neither study reported significant differences in knee laxity. In the goat model, a CPC was made to augment the healing response of a patellar tendon autograft by adding whole blood to the collagen scaffold.²⁸ A 30% reduction in AP laxity was observed in autograft ACL reconstruction when treated with a CPC when compared to autograft treatment with the collagen scaffold only (no platelets) after 6 weeks of healing at 60° of flexion. Although no significant difference was found with regards to the structural properties in the goat, there was a significant correlation between the systemic platelet count, graft strength and linear stiffness suggesting that increasing the platelet count could improve the structural properties. This goat study differed from the present study in that the platelets were not concentrated, an autograft was used, and the time of healing was only 6 weeks. Thus, the current study was performed to determine if concentrating the platelets would affect both the structural properties and AP laxity over a 15-week period.

The increased hypercellular and hypervascular tissue seen around the ACLR grafts and the elimination of the central necrotic regions of the ACL grafts with CPC enhancement (E-ACLR) suggests that placement of a biologic scaffolding at the time of surgery may enhance biologic incorporation of the graft tissue. The CPC may provide a conduit for vascular ingrowth and subsequently encourage cellular migration and proliferation within the graft substance as a result of an improved nutritional environment. In this study, the presence of a hypertrophic epiligamentous layer was seen in the E-ACLR group, the group which also had improved structural and material properties. Additional quantitative studies evaluating the changes in the cellular and vascular density on the functional graft properties will be necessary in the future.

To our knowledge, this is the first report of an improvement in AP laxity and the structural and material properties for an ACL graft using a device with a biologic stimulus. Previous attempts to accelerate the revascularization phase with VEGF in a sheep model found increased vessels but increased laxity.³⁵ By applying platelets in a stabilized CPC we have simulated a more “natural” environment with the natural occurrence of platelets deposited in a fibrin clot as occurs in extra-articular healing. The collagen matrix stabilizes the platelets and protects the clot from premature dissolution by the synovial fluid. The platelets contain a multitude of growth factors in addition to TGF- β 1 and PDGF that should be in the appropriate concentration for extra-articular healing, though this remains to be proven. Highly relevant to clinical application is that the cost to apply autologous platelets from peripheral blood would be much less than recombinant TGF- β 1, PDGF or EGF.

The large increases in AP laxity seen in the ACLR group (Table 1) are consistent with those of previous studies of ACL reconstruction with autogenous and allogeneous patellar tendon grafts using large animal models.^{1, 6, 13, 24} After 16 weeks of healing, Cummings et al reported an increase in laxity of approximately 8 mm in the ACL reconstructed knee when compared to the contralateral control with the most of the increase occurring in the first two weeks.⁶ Similar increases in AP laxity of an ACL allograft have also been noted after 6 months of healing.¹³ Although baseline (Time 0) AP laxity values were not measured in the injured knee in the current study, similar increases in AP laxity occurred when comparing the values of the contralateral intact knees to the ACLR knees after 15 weeks. Further work is needed to dissect out the mechanism behind the AP laxity improvement seen in the E-ACLR group, which may be due to direct action of the CPC on graft healing, other interactions with the secondary restraints of the joint, or adhesions. TGF- β 1 has been associated with capsular contractures in other animal models.¹² However, in our study there was no difference in the amount of small adhesions formed between the graft site and arthrotomy site between the two groups, and no animals in either group had any significant thickening of the joint capsule suggestive of an arthrofibrotic process. Furthermore, there were no measurable differences in the range of

flexion-extension motion, maximum flexion angle, or minimum extension angle between the two experimental groups (Table 1).

The load-displacement responses of the femur-graft-tibia complexes were recorded to permit a comparison of the graft structural properties between groups. The structural properties were evaluated because surgeons are typically interested in the response of the entire graft construct (i.e. strength and stiffness). However, measurements of the mechanical properties (yield stress and tangent modulus) provide information regarding the quality of the tissue. Whether examining the structural or mechanical properties of the graft, improvements were noted for the E-ACLR when compared to the ACLR grafts.

The porcine model was chosen for this study for several reasons including its anatomic similarity to the human knee, size, and anatomic equivalence to other large animal models of ACL reconstruction.³³ The pig knee is dependent on its ACL for joint stability.⁴ The pig has hematologic characteristics that are similar to human which is particularly important for wound healing studies involving platelets.¹⁷ Finally, the animals are relatively easy to work with and care for, and age, weight and gender matched animals are readily available.

A limitation of the Yorkshire pig model is its rapid rate of growth. Immature animals weighing 30 Kg were used. The animals doubled in size during the 15 week duration of the study. In a prior study of intact ACLs from 3 month old (30 Kg) pigs averaged 398 ± 75 N (mean $\pm$ SD),²⁰ while in this study, the strength of the intact ACLs (6 month old, 60 Kg pigs) averaged 992 ± 137 N; suggesting a doubling in strength of the intact ACL during this three months of growth. Therefore, the contralateral limb was used as a control to ameliorate this concern. We selected the immature pig since a mature Yorkshire pig is too large to work with. An alternative for future studies may be the minipig, but the model has yet to be fully characterized. adult minipigs are also expensive and difficult to obtain.

The porcine model has other weaknesses common to large animal studies in quadrupeds; predominantly that these animals weight bear on four limbs. There may also be subtle differences in anatomy, gait, and rehabilitation. Furthermore, there may be small differences in the wound healing cascade that are not yet appreciated. These are limitations to consider when interpreting the results of this study and other studies utilizing large animal models.

The application of CPC to fresh frozen allografts was evaluated in this study. No steps were taken to sterilize the allografts. It was our intent to see if we could alter the healing response of a graft with CPC treatment. It should be noted that no negative effects, such as synovitis or cartilage degradation, were observed in any of the allograft reconstructed knees. However, it is possible that the beneficial results of the CPC treatment could be different for sterilized allografts. It seems reasonable to assume that the beneficial effect seen in the CPC treated frozen allografts would also translate to other allograft preparations and autografts. Future studies are required to address these questions.

Another limitation of this study is that the animals were not randomized to a particular treatment group. The animals in the standard ACLR group underwent surgery before those of the E-ACLR group. All surgeries were performed by one experienced surgeon (MMM) within a 6-month period using the same instrumentation and protocol, other than the application of the CPC. The animals were received from the same source and were the same age and size. For these reasons, and given the large differences in AP laxity and structural properties that were detected, this should not be a major concern. The lack of randomization, however, makes the results susceptible to bias, such as learning effects.

Finally, there was no difference between treatment groups in regards to AP laxity at 30° of flexion (full extension in the pig) or linear stiffness. The relatively small difference in AP laxity

in full extension was expected since the posterior capsule and other ligaments become taut as the knee reaches full extension and contribute to stability. We did not measure varus-valgus laxity or internal-external rotational laxity in this study. These outcome measures could provide additional insight into the graft healing using CPC.

In summary, the potential use of a CPC to improve the graft structural properties and reduce AP knee laxity after ACL allograft reconstruction was demonstrated in the porcine model. Further work investigating the mechanism behind these actions will surely provide additional insight into how we can improve the healing response. Studies that characterize the potential role of a CPC on improving ACL reconstruction outcomes in the near and long term are also warranted.

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Figure 1. Schematic of standard ACL reconstruction (ACLR; Top) and enhanced ACL reconstruction with the CPC (E-ACLR; Bottom) is shown. A collagen sleeve was threaded over the graft and infiltrated with autologous 5X platelet-rich plasma to form the CPC.

Figure 2.

Schematics of the test fixtures used for AP laxity and tensile failure testing. For AP laxity testing (A), the knee flexion angle was prescribed, axial tibial rotation was constrained in the neutral position, and the translations in the coronal plane were unconstrained while the AP loads were prescribed. Figure 2A used with permission.¹⁹ For tensile testing (B), the knee flexion angle was initially set at 30°. The tibia was mounted to the base of the MTS via a sliding X-Y platform while the femur was unconstrained to rotations so that the specimen could seek its own position to ensure that the load was distributed over the entire graft cross section.

Figure 3. Load-displacement behavior of ACL-intact, ACL reconstruction enhanced with a CPC (E-ACLR) and standard ACL reconstruction (ACLR) groups demonstrating the improvement of the E-ACL graft, particularly in the functional loading region of the load-displacement curve. The error bars represent one standard error of the mean.

Figure 4.

E-ACLR produced yield and maximum failure loads that were closer to that of the ACL. The structural properties are expressed as a percent of the ACL-intact contralateral control. A value of 100% indicates that the property is equal to that of the control. The error bars represent one standard deviation.

Figure 5.

Histology of the ACLR (A, C) and E-ACLR (B, D) groups. Cellular repopulation of the allografts is seen in both the (5A) ACLR and (5B) E-ACLR groups, with vessels seen in both groups of grafts (black arrow, 5B). Central necrosis was found in the ACLR group (5C) but not in the E-ACLR grafts. A thick, vascular epiligamentous tissue was seen around the E-ACLR grafts, and vessels were actually noted to be extending from the hypercellular and hypervascular epiligamentous tissue into the graft substance (white arrow, 5D).

Table 1

Physical examination and AP laxity results (ACL reconstructed ACL intact knee). ROM = range of motion, Flexion = maximum flexion angle, and Extension = minimum extension angle. Values reported are median (minimum, maximum).

Variable	Differences (Tx – Intact)		p-values
	E-ACL	ACL	
ROM	5 (–15, 10)	–7 (–10, 10)	0.30
Flexion	0 (–5, 5)	–2 (–5, 10)	0.53
Extension	–5 (–5,10)	2 (–15, 20)	0.45
Lachman	2 (–4,6)	2 (–4, 4)	0.99
AP laxity 30°	3.8 (0.0, 7.9)	4.9 (3.7, 7.9)	0.45
AP laxity 60°	7.6 (0.8, 8.2)	10.6 (8.4, 14.2)	<0.001
AP laxity 90°	3.7 (1.5, 6.1)	8.7 (6.9, 13.1)	<0.001

Table 2

The structural properties (yield load, maximum failure load, linear stiffness) for each animal.

Animal #	Group	Yield load (N)		Maximum load (N)		Stiffness (N/mm)	
		Tx	Intact	Tx	Intact	Tx	Intact
876	E-ACLR	429	865	439	935	206	60.4
878	E-ACLR	405	857	495	874	176	101
882	E-ACLR	528	729	560	729	102	176
880	E-ACLR	580	1000	661	1000	121	181
881	E-ACLR	191	850	196	896	44	184
883	E-ACLR	420	811	420	835	64	187
879	E-ACLR	543	1031	619	1031	87	230
715	ACLR	143	910	153	1106	34	146
716	ACLR	320	840	324	1093	51	138
717	ACLR	170	840	269	1186	48	121
718	ACLR	617	1080	617	1194	76	124
724	ACLR	340	928	402	1003	56	159
725	ACLR	89	903	95	1019	25	159