Collagen-Platelet Rich Plasma Hydrogel Enhances Primary Repair of the Porcine Anterior Cruciate Ligament

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ABSTRACT: The anterior cruciate ligament (ACL) fails to heal after suture repair. One hypothesis for this failure is the premature loss of the fibrin clot, or provisional scaffolding, between the two ligament ends in the joint environment. To test this hypothesis, a substitute provisional scaffold of collagen–platelet rich plasma (PRP) hydrogel was used to fill the ACL wound site at the time of suture repair and the structural properties of the healing ACLs evaluated 4 weeks after surgery. Bilateral ACL transections were performed in five 30-kg Yorkshire pigs and treated with suture repair. In each animal, one of the repairs was augmented with placement of a collagen–PRP hydrogel at the ACL transection site, while the contralateral knee had suture repair alone. In addition, six control knees with intact ACLs from three additional animals were used as a control group. No postoperative immobilization was used. After 4 weeks the animals underwent in vivo magnetic resonance imaging to assess the size of the healing ACL, followed by biomechanical testing to determine tensile properties. The supplementation of suture repair with a collagen–PRP hydrogel resulted in significant improvements in load at yield, maximum load, and linear stiffness at 4 weeks. We conclude that use of a stabilized provisional scaffold, such as a collagen–PRP hydrogel, to supplement primary repair of the ACL can result in improved biomechanical properties at an early time point. Further studies to determine the long-term effect of primary repair enhancement are needed. © 2006 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 25:81–91, 2007

Keywords: collagen-platelet rich plasma; hydrogel anterior cruciate ligament; fibrin clot; suture repair

INTRODUCTION

The anterior cruciate ligament (ACL) has long been thought to have a limited capacity for healing. Rates of failure of healing (non-union) of the ACL, even with surgical repair, range from 40% to 100%.[1–5] This is in contrast to rates of non-union seen in the medial collateral ligament (MCL), where failure of clinical healing is the exception rather than the rule.[6–9] The lack of healing seen in the ACL and other intra-articular tissues has been attributed to their relative lack of vascularity, the hostile environment of synovial fluid,[10] to alterations in the cellular metabolism after injury,[11,12] intrinsic cell deficiencies[13–20] the complex biomechanics of these tissues, and cell loss within the tissues after injury.[21] However, recent work has reported that human ACL remnants typically contain viable cells and vasculature,[22] yet there is a gap at the rupture site which remains open.[23–25] This gap is unique to the intra-articular environment of the ACL, it is not seen in extra-articular repair sites of ligaments.[26,27] In the extra-articular MCL wound site, the gap has been filled with a provisional scaffold of fibrin and platelets that is subsequently invaded

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by surrounding intrinsic and extrinsic cells which initiate and conduct tissue healing.[26,28,29]

In the extra-articular environment of the MCL, the breakdown of this fibrin–platelet provisional scaffold occurs slowly over days to weeks in balance with new collagen formation at the injury site. However, in the intra-articular environment of the ACL, the stabilized fibrin–platelet clot is not observed to form,[30,31] even after induced bleeding into the joint. Thus, patients with an intra-articular injury form a hemarthrosis in the joint, without filling the wound site with a fibrin–platelet clot. The presence of urokinase plasminogen activator (uPA) found in the synovial fluid after injury[32] may play a key role in this failure of scaffold formation, as the presence of this enzyme results in elevated levels of circulating plasmin in the joint and accelerated fibrin dissolution. We hypothesize that the critical mechanism underlying the failed healing of the ACL is this premature loss of the fibrin–platelet provisional scaffold and that placement of an appropriate substitute for the provisional scaffold within the ACL repair site will allow functional healing to proceed.

To test this hypothesis, we transected the porcine ACL in both knees, performed suture repair alone on one side, and supplemented the suture repair on the contralateral limb by injecting a substitute provisional scaffold (the collagen–PRP hydrogel; Fig. 1). The substitute scaffold was designed to deliver platelets and plasma proteins (key components of the provisional scaffold in tissues which do heal) to the defect site, in a form that would be stable in the intra-articular environment of the ACL. Collagen is known to be resistant to degradation by plasmin[33] and was added to the fibrin and platelets to stabilize the scaffold against premature degradation by intrasynovial circulating plasmin. We then analyzed the healing response with magnetic resonance imaging to evaluate scar location and size and with tensile testing to measure biomechanical properties of the healing tissue. These experiments allowed us to test our hypothesis that the placement of a stabilized provisional scaffold within the ACL repair site would result in improved wound healing of the ACL.

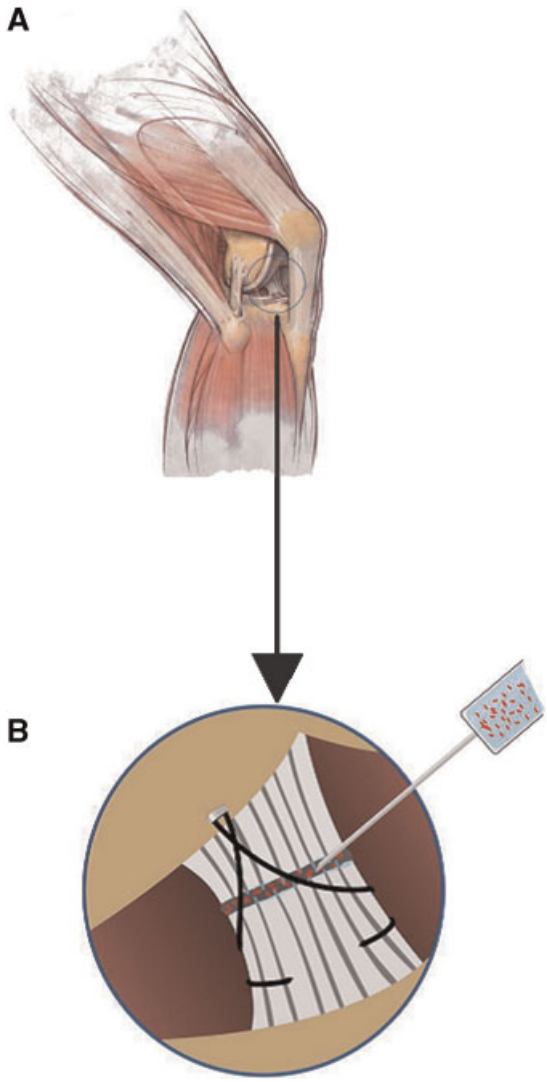

Figure 1. Schematic of the enhanced suture repair technique. (A) The location of the ACL is illustrated. (B) A schematic of a transected ACL treated with suture repair. For enhanced suture repair, the collagen–PRP hydrogel is introduced into the wound site to fill in the remaining gap.

METHODS

Experimental Design

Institutional Animal Care and Use Committee approvals were obtained prior to initiating this study. Five 30-kg female skeletally immature 4-month-old Yorkshire pigs underwent bilateral ACL transection and suture repair. Animals with uniform gender were chosen to avoid potential variability among animals as a function of gender. Each of the animals were treated on one side with suture repair alone and suture repair augmented with collagen–PRP hydrogel containing an average of $954{,}000 \pm 93{,}000$ platelets/mm^3 on the contralateral side ($n = 5$). In addition, six control knees with intact ACLs from three additional animals were used as an intact ACL control group. Sides were randomized preoperatively to suture alone and augmented repair. All animals were euthanized after 4 weeks. Just prior to euthanasia, the animals had in vivo magnetic resonance imaging (MRI) scans of both knees with gadolinium contrast to assess scar location and size as well as enhancement of the ACL wound site. Immediately after

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euthanasia, the knees were harvested and biomechanical testing of the ACL complex was performed as previously described[24,25] to measure the structural properties of the bone–ACL–bone complex for all ligaments. Intact ACLs ($n = 6$) from a separate group of age-matched, gender-matched, and weight-matched animals were used as a control group for the biomechanical studies. In addition, two additional animals were treated on one side with suture repair alone and suture repair augmented with platelet-depleted collagen–plasma hydrogel containing fewer than 20,000/mm^3 of platelets on the contralateral side. This small number of animals was included only to ascertain if removing the platelets from the hydrogel would be have a very large effect on the biomechanical properties of the healing tissue. We predicted that a sample size of two ACLs treated with platelet-depleted collagen–plasma and five treated with collagen–PRP hydrogel would provide over 80% power to detect threefold differences and 95% power to detect fourfold differences in mechanical testing parameters based on an unpaired Student's t-test.

Manufacture of Acid-Soluble Collagen Used in the Hydrogels

Rat tails were obtained from control breeder rats undergoing euthanasia for other Institutional Animal Care and Use Committee approved studies. The rat-tail tendons were sterilely harvested, minced, and solubilized in an acidified enzyme solution to obtain the acid-soluble collagen. The collagen slurry was mixed with HEPES Buffer (Cellgro, Mediatech, Inc., Herndon, VA), Ham's F-10 medium (MP Biomedicals, LCC, Aurora, OH), Antibiotic-Antimycotic solution (Cellgro, Mediatech, Inc.), sterile water, and 7.5% sodium bicarbonate (Cambrex BioScience Walkersville, Inc., Walkersville, MD).

Platelet-Rich Plasma Component Preparation

Whole blood was drawn from the femoral vein of each pig into tubes containing sodium citrate after anesthesia had been induced and immediately prior to surgery. Sixty cubic centimeters of blood were drawn for each animal for all testing (less than 3% of the total blood volume of the animal.[34] Of this amount, approximately 9 cc of blood were used to make the PRP. No physiological effects of the phlebotomy were observed for any of the animals. The blood was centrifuged to isolate the PRP fraction at 100 g for 14 min. This resulted in an approximately 2X enrichment of the platelet concentration of the blood from a range of 495,000 to 567,000/mm^3 to 780,000 to 2,300,000/mm^3. The PRP was added to the collagen slurry to keep the plasma: collagen ratio at 1:1 for the collagen–PRP gels. The mixture was kept on ice until use. The platelet-depleted collagen–plasma hydrogels in the additional two animals were prepared in the same manner, with the exception that the blood was centrifuged at 200 g for 20 min to reduce the platelet count in the plasma to below 20,000/mm^2.

Surgical Procedure

The pigs were premedicated with telazol 4.4 to 6.6 mg/kg IM, xylazine 1.1 to 2.2 mg/kg IM, and atropine 0.04 mg/kg. They were intubated and placed on isoflurane 1% to 3% for anesthesia maintenance. After anesthesia had been obtained, the pigs were weighed and placed in the supine position on the operating room table. Both hind limbs were shaved, prepared with chlorhexidine followed by betadyne paint, and sterilely draped. No tourniquet was used. To expose the ACL, a 4-cm incision was made over the medial border of the patellar tendon. The incision was carried down sharply through the synovium using electrocautery. The fat pad was released from its proximal attachment and partially resected to expose the intermeniscal ligament. The intermeniscal ligament was released to expose the tibial insertion of the ACL. A Lachman maneuver was performed prior to releasing the ACL to verify knee stability. Two #1 Vicryl sutures were secured in the distal ACL using a variable depth technique. The ACL was transected completely at the junction of the middle and proximal thirds using a no. 12 blade. Complete transection was verified visually and with a repeat Lachman maneuver that became positive in all knees with no significant end point detected after complete transection. An absorbable suture anchor [TwinFix AB 5.0 Suture Anchor with DuraBraid Suture (USP#2); Smith & Nephew, Inc., Andover MA] was placed at the back of the femoral notch. The knee was irrigated with 500 cc of sterile normal saline to remove all synovial fluid. Hemostasis was carefully achieved using pressure and a solution of 1:10,000 of epinephrine as needed. Once hemostasis had been achieved, a strip of Gelfoam (Surgifoam Absorbable Gelatin Sponge, Ethicon, Inc., Somerville, NJ; Ferrosan, Soeborg Denmark) was presoaked in 1 cc of the collagen–PRP mixture, and threaded onto sutures and up into the region of the proximal ACL stump in the notch. The Durabraid sutures were tied individually to the Vicryl sutures in the distal ACL with the knees in resting flexion (approximately 70° of flexion) and 2 additional cc of the collagen–PRP mixture were placed on top of the Gelfoam in the experimental knees. The additional collagen–PRP hydrogel filled the intercondylar notch. The knee was closed after the gel reached a soft set (approximately 10 min). The knee was left in resting flexion while the identical technique of suture repair was performed without the collagen–PRP hydrogel or Gelfoam placement on the contralateral knee (approximately 1 h). The procedure was identical in the suture repair alone knees; however, the Gelfoam sponge and collagen–PRP hydrogel were not used. In these knees treated with suture repair alone, blood clot was seen to fill the notch after suture placement. The incisions were closed in multiple layers with absorbable sutures.

The animals were not restrained postoperatively, and were allowed ad libitum activity. Once the animals recovered from anesthesia, they were permitted to resume normal cage activity and nutrition ad libitum.

Buprenex 0.01 mg/kg IM once and a Fentanyl patch 1 to 4 μg/kg transdermal were provided for postoperative analgesia. All animals were bearing weight on their hind limbs by 24 h after surgery. After 4 weeks in vivo, the animals were again anesthetized and underwent in vivo MRI using the protocol detailed below.

After the MRIs had been obtained, the animals were euthanized using Fatal Plus at 1 cc/10 lbs. No animals had any surgical complications of difficulty walking normally, redness, warmth and swelling of the knee, fever, or other signs of infection that would have necessitated early euthanasia. The knees were retrieved and taken for same-day biomechanical testing. The knees were kept at 4°C until biomechanical testing and kept moist using a saline spray and moist wraps.

The six intact control knees were obtained from age-, gender-, and weight-matched animals after euthanasia following surgical procedures to the chest. The hind limbs were frozen at −20°C for 3 months and thawed overnight at 4°C before mechanical testing. All other testing conditions for these knees were identical to those in the experimental groups.

Magnetic Resonance Imaging

In vivo magnetic resonance imaging was performed at 1.5 T (GE Medical Systems, Milwaukee, WI) with an eight-channel phased array coil at the specified time points. Scanning was performed with the knees placed in maximum extension (between 30°–45° of flexion). Conventional MRI included multiplane T1, FSE PD, and T2 weighted images. Field of view (FOV): 16–18 cm; matrix: 256 × 256, (repetition time/echo time); TR/TE: 400/16, 2500/32, 3000/66 ms; echo train length (ETL): 8; bandwidth (BW): 15 kHz; slice thickness: 3 mm; interslice gap: 1 mm). Enhancement was evaluated by using spoiled gradient echo sequence (TR/TE = 200/2 ms; flip angle = 60° 3-mm slice thickness; 0.625 mm plane resolution) with an intravenous contrast agent (Magnevist; Berlex, Wayne, NJ) 0.2 mL/kg injected 10 s after the start of scan. Five images were obtained per slice, 78 s apart. Post contrast T1-weighted images were obtained (FOV:16 cm; matrix: 256 × 256; TR/TE: 400/9 ms; slice thickness: 3 mm; interslice gap: 1 mm) in the coronal and sagittal planes.

The width of the scar tissue was measured in both the sagittal and coronal planes using the oblique sagittal proton density images taken in the plane of the ACL and the coronal T1 images also taken in the plane of the ACL. These two values were multiplied to obtain an estimate of the cross-sectional area of the scar mass. The length of the ACL was measured from the tibial attachment to the femoral ACL attachment on the sagittal images and from the tibial insertion to the top of the lateral notch on the coronal images and the two values averaged to obtain an estimate of ligament length. These measurements were made on the digital images using a calibrated digital ruler. On one of the pairs of knees, a complete set of quantitative MRI data could not be obtained and this animal was excluded from the dimensional analysis and material properties analysis, and the paired *t*-testing was performed on the remaining four animals.

Biomechanical Testing

The bone–ligament–bone ACL complex from both knees for each pig was tested in uniaxial tension as previously described.[24] In brief, all soft tissues except the ACL scar mass were transected at the joint line and the menisci removed from each knee. Biomechanical testing was performed with the knee at 30° of flexion and at room temperature. The bone–ligament–bone construct was preconditioned to compensate for the effects of viscoelasticity by subjecting each specimen to 10 sinusoidal cycles at a rate of 0.833 mm/s and an amplitude of 0.6 mm displacement. After preconditioning, each specimen was tested to failure in uniaxial tension at 20 mm/min.[35,36] Close-range digital images were acquired at 3 Hz using a high-resolution digital camera with a macro lens (PixeLINK PLA662 Megapixel Firewire camera, PixeLINK, Ottawa, ON, Canada) to determine failure mode. The yield load, displacement at yield, linear stiffness (maximum slope of force–displacement curve), maximum load at failure, displacement at failure, and total work to failure (area under force–displacement curve) were determined from the force–displacement curve measured for each bone–ligament–bone ACL complex. The yield load represented the point along the normalized force–displacement curve where the structural behavior of the ACL complex departed from linear behavior and for the purposes of this analysis was defined as the point where the linear stiffness declines by at least 2% from its maximum value. The displacement at yield was the displacement recorded at this same point. The maximum load is the highest load sustained by the ACL complex prior to failure and the displacement at failure the displacement recorded at the maximum load. The energy to failure was derived by integrating the total area under the force–displacement curve. The yield stress, yield strain, linear modulus (maximum slope of stress–strain curve), maximum stress at failure, strain at failure, and total work to failure (area under stress–strain curve) were determined from the force–displacement information using the MRI measurements of ligament cross-sectional area and length of each ACL complex. The engineering stress was calculated by dividing the force by the cross-sectional area of the ACL as measured on MRI, while the engineering strain was calculated by dividing the displacement of the ACL by its original length. The yield stress was assigned to the point along the stress–strain curve where the structural behavior of the ACL complex departed from linear behavior. The yield strain was the strain value at this same point. The maximum stress was the maximal load normalized by the ACL cross-sectional area prior to failure and the maximum strain at failure was calculated from the displacement recorded at the maximum failure load.

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Histology

Histology was performed after mechanical testing to determine if the scar tissue were cellular and vascular. The knees were fixed in neutral buffered formalin for 1 week, decalcified carefully over 24 to 72 h (DELTA-Cal, Delta Products Group, Aurora, IL) just until softening of the bone was observed, and the entire knee cut longitudinally in a sagittal plane passing through the ACL. Each half of the knees were dehydrated and embedded in paraffin. Large sections, 7 microns in thickness, containing the ACL scar mass were microtomed, placed onto pretreated large section glass slides, and stored at 4°C. Sections were stained with hematoxylin and eosin.

Statistical Analysis

Measurements of scar size, bone–ligament–bone structural properties and the material properties of the repair tissue were compared for three groups of ACLs: transected ACLs treated with suture repair alone, transected ACLs treated with suture repair enhanced with collagen–PRP hydrogel, and a historical age- and gender-matched group of intact ACLs. Comparisons used *F*-tests from multivariate analysis of variance (MANOVA) with 95% confidence intervals (95% CI).[37] A *F*-test exceeding the critical value of 3.84 would be regarded as evidence for statistical significance. Paired *t*-tests were used to evaluate differences in scar size between the ligaments repaired with suture alone compared to the contralateral side treated with suture and collagen–PRP hydrogel. For the structural properties of the bone–ligament–bone preparations, each of the six variables (load at yield, maximum load, displacement at yield, displacement at failure, linear stiffness, and energy to failure) followed a normal (Gaussian-shaped) distribution and therefore data are presented in terms of the mean and standard deviation (SD). Paired *t*-tests were used to evaluate differences in ACLs treated with suture repair alone compared to the contralateral side receiving sutures with PRP for both structural properties of the bone–ligament–bone complex and the material properties of the repair tissue. Statistical analysis was performed using SPSS version 14.0 (SPSS Inc., Chicago, IL). All values of $p < 0.05$ were considered statistically significant.

RESULTS

Magnetic Resonance Imaging: Changes in Scar Size and Enhancement with Collagen–PRP Hydrogel

The cross-sectional area of the transected ligaments treated with collagen–PRP hydrogel was not significantly different than the cross-sectional area of the ligaments treated with suture repair alone (41 ± 9 mm^2 vs. 33 ± 17 mm^2, respectively; mean $\pm$ SD; $p > 0.50$). Ligament length was also similar in the two groups (17.6 ± 1.5 mm for suture alone vs. 17.1 ± 2.5 mm for suture plus collagen–PRP hydrogel; $p > 0.70$).

The enhancement of the scar mass as evidenced on postgadolinium in vivo imaging was qualitatively more intense in the transected ligaments treated with collagen–PRP hydrogel than the ligaments treated with suture repair alone. Both groups had some enhancement of the fat pad anterior to the scar mass (likely due to postoperative changes); however, in the knees treated with suture and collagen–PRP hydrogel the entire scar mass enhanced strongly immediately on perfusion of the knee with the IV gadolinium contrast (Fig. 2). Greater enhancement was seen in the knees treated with suture and collagen–PRP hydrogel on the postgadolinium coronal images as well (Fig. 3).

Biomechanics: Mechanical Response of the Repair with and without Collagen–PRP Hydrogel

Failure Mode

Use of a collagen–PRP hydrogel to enhance suture repair resulted in a change in failure mode for two of the five treated ligaments. In the intact ligaments, failure was at the bone–ligament junction in six out of six cases. In the ligaments treated with absorbable suture repair alone ($n = 5$), the mode of failure was intrasubstance in five out of five ligaments, while in those treated with suture repair + collagen–PRPhydrogel, the repaired ligaments failed at the bone–ligament junction in two out of five cases.

Structural Properties of the Bone–ACL–Bone Complex

The use of a collagen–PRP hydrogel to enhance suture repair resulted in significantly increased stiffness, yield load, and maximum load of the healing ligaments compared to suture repair alone; however, both groups remained significantly inferior to the intact ligament group. When suture repairs was supplemented with a collagen–PRP hydrogel, the yield load was approximately twofold greater than suture repair alone (Table 1; $t = 5.76$, $p = 0.004$). Similar improvements were seen in the maximum load ($t = 4.15, p = 0.014$), and linear stiffness ($t = 3.44, p = 0.026$). While mechanical performance was improved for the enhanced repair group, displacement at yield ($t = 0.16$, $p = 0.80$), displacement at failure ($t = 0.11$, $p = 0.33$), and energy at failure ($t = 2.29$, $p = 0.08$) did not attain statistical significance.

Material Properties of the Scar Mass

The yield stress of the enhanced ACL repairs was significantly higher than the repairs performed

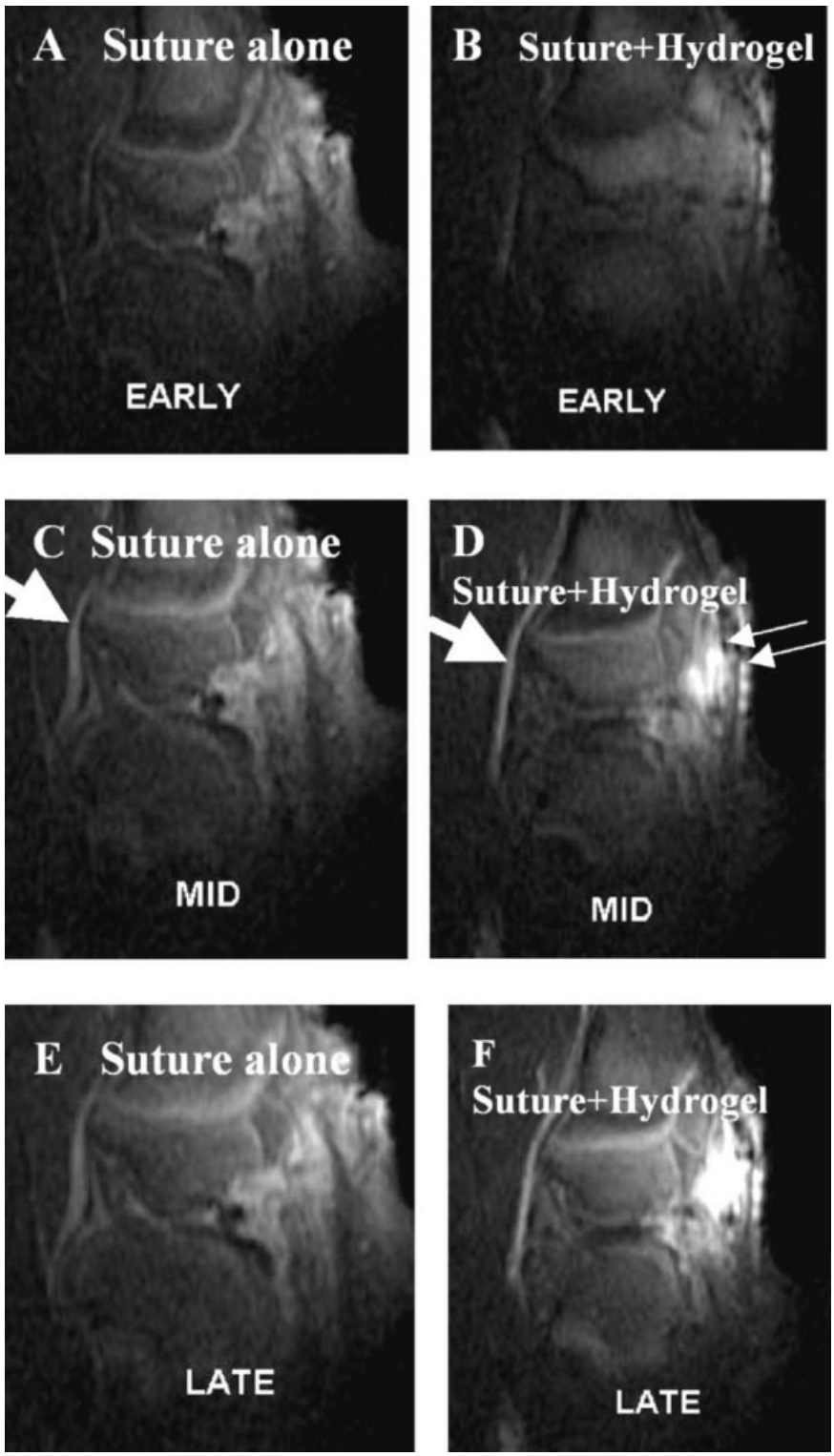

Figure 2. In vivo perfusion images during IV contrast administration. The left column (A, C, and E) shows the images obtained during contrast administration where the ACL transection was treated with suture repair alone, while the right column (B, D, and F) shows the images for treatment with enhanced repair. In the top row, no contrast has yet reached the knee. In the second row, contrast has perfused the popliteal artery (white arrow) and greater enhancement is noted in the enhanced repair (D). The difference is noted to increase with additional perfusion of the limb (E, F).

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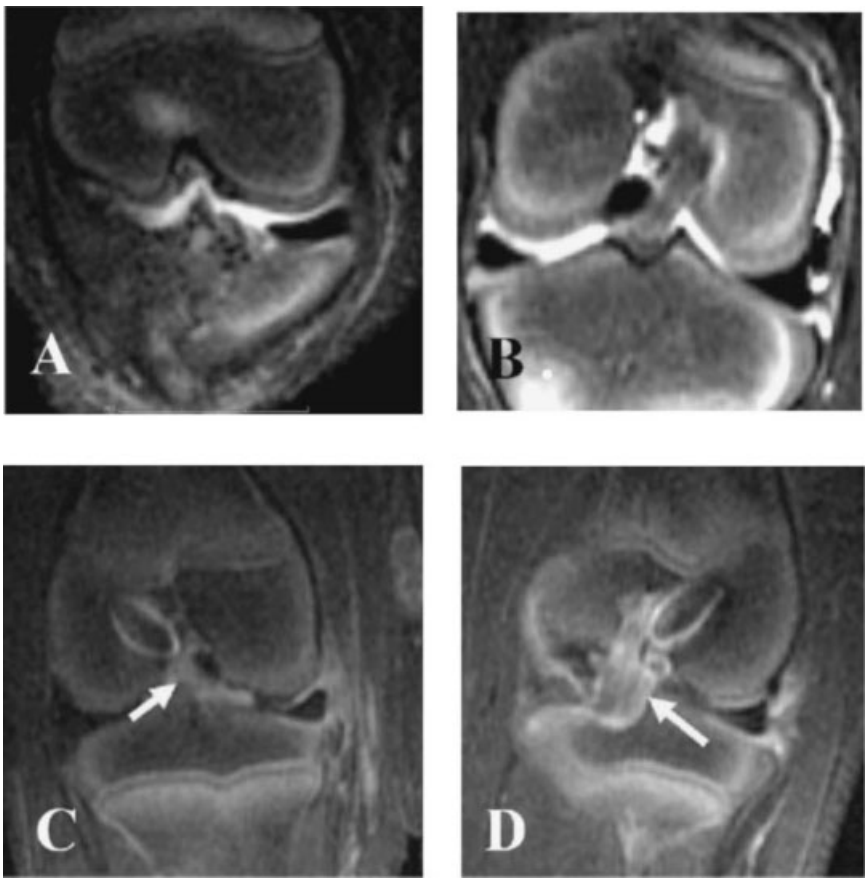

Figure 3. Coronal magnetic resonance images of two knees of the same animal in which the knee in the left column was treated with suture repair alone and the contralateral knee in the right column was treated with suture repair enhanced with collagen–PRP hydrogel. In A and B, precontrast coronal images show a gap in the knee on the left column (A), while the contralateral side has a continuous scar mass from tibia to femur (B). In C and D, postcontrast images in the coronal plane show an area of bright signal corresponding to a perfused scar mass is larger on the side treated with a collagen–PRP hydrogel (white arrow, D) than the side treated with suture alone (C).

with suture alone (2.42 ± 0.8 MPa vs 1.0 ± 0.3 MPa, respectively; mean $\pm$ SD; $p < 0.02$). The ultimate tensile stress of the scar mass was also almost twice as high in the ligaments treated with collagen–PRP hydrogel than those repaired with suture alone, a difference which approached, but did not reach statistical significance (2.7 ± 0.7 MPa vs. 1.4 ± 1.0 MPa, respectively; $p > 0.08$). The yield strain at yield and failure strain were not significantly different between the two groups ($p > 0.35$ for both comparisons).

Effect of Platelet Depletion on Biomechanical Parameters

The results of platelet depletion can only be considered a trend as only two animals were treated with a platelet-depleted hydrogel. The removal of platelets from the hydrogel implanted in the ACL wound site resulted in a lower load at yield (19.3 ± 4.5 N vs. 92.7 ± 22.7 N; $p < 0.01$), maximum failure load (24.8 ± 12.3 N vs. 102.9 ± 27.1 N; $p = 0.01$), and linear stiffness (4.5 ± 2.2 N/mm vs. 24.2 ± 4.9 N/mm; $p < 0.01$) as compared to the collagen–PRP hydrogel treatment group. The platelet-depleted repaired ligaments had similar displacement at failure than the ligaments treated with a collagen–PRP hydrogel (7.6 ± 3.3 mm vs. 6.7 ± 1.3 mm; $p > 0.43$). Platelet depletion also had no apparent effect on the ligament displacement at yield (4.7 ± 0.7 vs. 5.4 ± 1.0 mm; $p = 0.43$) or energy at failure (336.8 ± 121.8 N $\times$ mm vs. 145.1 ± 111.6 N $\times$ mm; $p = 0.11$).

Table 1. Results of Mechanical Testing

Variable	Suture Repair ($n = 5$)	Collagen-Suture/ PRP ($n = 5$)	Percentage Collagen-Change with PRP Supplementation	Intact ACL ($n = 6$)
Load at yield (N)	35.1 ± 21.3	92.7 ± 22.7^a	164%	141.7 ± 38.1^b
Maximum load (N)	46.1 ± 27.7	102.9 ± 27.1^a	123%	178.8 ± 36.6^b
Displacement at yield (mm)	5.3 ± 2.1	5.4 ± 1.0	−2%	3.7 ± 0.69
Displacement at failure (mm)	7.7 ± 0.8	6.7 ± 1.3	−13%	5.3 ± 1.2^c
Tangent modulus (N/mm)	11.5 ± 8.5	24.2 ± 4.9^a	110%	48.7 ± 7.8^b
Energy to failure (N × mm)	176.4 ± 82.8	336.8 ± 121.8	91%	492.2 ± 203.8^c

Abbreviations: PRP, collagen-platelet rich plasma; ACL, anterior cruciate ligament.
Data are represented as mean ± SD.
[a] $p < 0.05$ vs. suture.
[b] $p < 0.05$ vs. suture and vs. suture/PRP.
[c] $p < 0.05$ vs. suture.

Post-Testing Histology: Characteristics of the Scar Tissue after Treatment with a Collagen–PRP Hydrogel

Sagittal large sections of the entire knee through the ACL scar mass were made and representative sections stained with hematoxylin and eosin. Histological examination of the sections suggests that the scar mass between the ends of the transected ligaments in the collagen–PRP hydrogel group is hypercellular and hypervascular at 4 weeks. The vast majority of the cells seen within the scar mass were fibroblastic, with only rare inflammatory cells seen within the repair tissue. The distal tibial remnant of the transected ACL appeared to be covered anteriorly with a hypertrophic tissue that was continuous with the anterior fat pad and the ACL wound site. In addition, the ACL remnants themselves (both proximal and distal) contained areas of hypertrophic epiligamentous tissue between fascicles.

DISCUSSION

This study demonstrates that biomechanical properties measured early after primary repair of the ACL transection can be enhanced with use of a collagen–PRP hydrogel placed within the repair site. These results support our hypothesis that an important mechanism for failure of the ACL to heal is a lack of appropriate provisional scaffold.[24,25,38] This is a critical finding as prior research into stimulation of healing in intra-articular tissue defects has focused on overcoming cellular deficiencies,[14–16,18,19,26,27,39–44] rather than scaffolding deficiencies.[26] In this study, no cells (except the platelets and white blood cells contained in the collagen–PRP hydrogel) were transplanted, yet a highly cellular repair tissue was seen within the defect after only 4 weeks. This suggests that at least in the acutely repaired ACL, there is a sufficient intrinsic and/or extrinsic cellular response from the environment around the ACL to stimulate histological and functional healing if an appropriate scaffold is provided.

Proposed mechanisms for the lack of functional healing seen in the ACL after suture repair have previously focused on alterations in the cellular metabolism after injury,[11,12] intrinsic cell deficiencies,[13–20] the complex biomechanics of these tissues, and cell loss within the tissues after injury.[21] However, cell and vessel proliferation,[38] cells with migratory potential,[45] and collagen production[22] have recently been reported within the ACL tissue after rupture. Thus, the contributions of intrinsic cellular and vascular mechanisms of healing failure appear less likely to completely explain the observed dichotomy between healing ligaments, including the medial collateral ligament, and nonhealing ligaments, such as the ACL. One additional histological observation recently noted is that the provisional scaffold found in the wound site of other ligaments has been noted to be missing in the ACL.[25,38] This finding is possibly due to the upregulation of urokinase plasminogen activator (and subsequent increased conversion of plasminogen to plasmin) in the synovial fluid after injury.[32] As plasmin is the principal enzyme responsible for the dissolution of fibrin clot, it is possible that one additional mechanism for failure of provisional scaffold function in an ACL wound site is premature fibrinolysis of the naturally occurring fibrin–platelet provisional scaffold. The addition of collagen to the fibrin–platelet scaffold confers resistance to plasmin degradation to the construct, as the collagen–fibrin copolymer requires an additional matrix metalloproteinase for dissolution[33] and this enzyme is not typically present in the synovial fluid. In this study, filling of the wound

site with the collagen–PRP hydrogel resulted in successful fibrovascular scar formation, a finding that lends support to intrinsic scaffolding deficiency as a possible mechanism of failure of healing of the untreated ACL.

In this study, the use of a variable-depth suture repair technique alone resulted in a 4-week maximum load of 26.9% of the intact control group. This failure load is comparable with prior studies of ACL suture repair, where reports of 10% strength at 4 weeks and 43% at 13 months have been reported,[46] as well as reports of partial ACL defects where 14% of control strength is recovered at 6 weeks[23,25] and 26% of the control ACLs maximum load at 3 months.[23] In addition, the ACL treated with suture repair alone had favorable strength at failure compared with ACL reconstruction where 26% of intact ACL strength is recorded at 3 weeks.[47] The supplementation of the suture repair with collagen–PRP hydrogel resulted in a further increase in maximum load to 58% of the intact group—a value more than twice as high as the reconstruction strength at a similar time point.[47]

The advantages of this large animal model of complete ACL transection and suture repair are multiple. The suture repair provides initial structural stability, and the use of absorbable suture that has minimal strength at the end points of interest prevents the need for searching through (and possibly destroying) the scar mass to release suture and allow for testing of the scar mass itself. Use of a large animal model allows for easy identification of the structures of interest, both at the time of ligament transection and retrieval, and relative ease of structural repair for surgeons versed in repair of human ligaments.

The collagen–PRP hydrogel used here also has several major advantages over prior tissue-engineered implants. There is no required cell or tissue harvest prior to implantation (other than phlebotomy at the time of surgery). The collagen form used to mix with the PRP is similar to that used currently in plastic surgery procedures,[48,49] where it is obtained either autologously or as a xenograft.[49,50] Thus, future clinical application is likely to be relatively low risk, as opposed to treatment methods which require an additional procedure to procure cells for expansion,[51,52] or stem cells, or implanted recombinant growth factors or viral vectors for gene therapy.[51,53,54]

After 4 weeks in vivo, the distal tibial remnant of the transected ACL appeared to be covered anteriorly with a hypertrophic tissue that was continuous with the anterior fat pad and the ACL wound site. This finding suggests that some of the vascularity seen within the ACL wound site may arise from the anterior fat pad. In addition, the ACL remnants themselves (both proximal and distal) contained areas of hypertrophic epiligamentous tissue between fascicles, suggesting intrinsic epiligamentous and perivascular ACL cells may also be playing a role in repopulation of the wound site. One of the limitations of the histological analysis was that all specimens were taken at 4 weeks, when the wound site had completely filled with cells, which precluded any ability to accurately determine the sources of wound repopulation; histological studies at earlier time points would be extremely valuable in determining early sources of cells and vasculature for the wound site.

In addition, while this is the first time healing of a complete transection of the ACL has been demonstrated biomechanically, the recovery of biomechanical strength in the defect remained incomplete at 4 weeks. While the percentage recovery in the defect was similar to that previously reported for the healing MCL at 6 weeks,[55] there remains room for improvement for stimulating an earlier return of biomechanical strength, perhaps using additional implanted growth factors and extracellular matrix proteins.

Another feature of this study is that it was performed in pigs that were skeletally immature. Conventional orthopedic wisdom states that "kids heal faster than adults" for fractures, and basic science studies in animals appear to support this clinical wisdom.[3] However, basic science studies of partial ACL transection and patellar tendon healing suggest that the scar formed in skeletally mature animals is actually stronger that that formed in skeletally immature animals.[23,56] As patients with open physes are vulnerable to ACL injury, and stand to have the longest period of disability if premature osteoarthritis occurs, it is clinically important to begin to examine ACL healing in this population; however, the effects of skeletal maturity on ligament healing have yet to be defined and the results obtained here may be less applicable to adult models.

In addition, the intact ligaments were stored at -20°C for 3 months before undergoing testing while the experimental group was tested immediately after harvest. While prior work has shown that storage at -20°C has no detrimental effect on the mechanical properties of ligaments,[57] it is important to note that the experimental and intact knees were treated differently.

Finally, only two animals were treated with a platelet-depleted hydrogel. The small number of animals was studied only to detect if very large

effects of platelet depletion on healing tissue mechanical properties were present. Interestingly enough, even with s sample of only two animals, the differences in load at yield, linear stiffness, and maximum load between the groups treated with and without platelets were so large as to achieve statistical significance. However, given the small number of animals treated with a platelet-depleted hydrogel, these results must be considered only a trend, and future studies are required to adequately investigate the effects of platelets on the healing properties of the ACL.

A final limitation is that these results were obtained only for an early 4-week time point. While a significant return of biomechanical strength was seen at 4 weeks, it is not clear that this return would continue with time. Additional work evaluating this technique at longer time points, as well as studies of the articular cartilage and synovium to document safety are clearly needed before considering translation to clinical use.

In summary, use of a collagen–PRP hydrogel can stimulate healing resulting in improved biomechanical properties after suture repair of the porcine ACL at an early time point. Future studies focusing on the longer-term effects are warranted. Pressing forward with this line of inquiry is likely to result in significant changes in our clinical approach to ACL rupture, from resection and replacement towards repair and regeneration.

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