

# Use of a Collagen-Platelet Rich Plasma Scaffold to Stimulate Healing of a Central Defect in the Canine ACL

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**ABSTRACT:** The anterior cruciate ligament (ACL) of the knee fails to heal after primary repair. Here we hypothesize that a beneficial biologic repair response can be induced by placing a collagen-platelet rich plasma (collagen-PRP) material into a central ACL defect. A collagen-PRP scaffold was used to treat a central ACL defect in vivo. In the first experiment, the histologic response in treated and untreated defects was evaluated at 3 ( $n = 5$ ) and 6 weeks ( $n = 5$ ). In the second experiment, biomechanical testing of the treated ligaments ( $n = 8$ ) was performed at 6 weeks and compared with the results of biomechanical testing of untreated defects at the same time-point ( $n = 6$ ). The percentage filling of the defects in the treated ACLs was significantly higher at both the 3- and 6-week time-points when compared with the untreated contralateral control defects ( $50 \pm 21\%$  vs.  $2 \pm 2\%$  at 3 weeks, and  $43 \pm 11\%$  vs.  $23 \pm 11$  at 6 weeks; all values mean  $\pm$  SEM. Biomechanically, the treated ACL defects had a 40% increase in strength at 6 weeks, which was significantly higher than the 14% increase in strength previously reported for untreated defects ( $p < 0.02$ ). Placement of a collagen-PRP bridging scaffold in a central ACL defect can stimulate healing of the ACL histologically and biomechanically. © 2006 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. *J Orthop Res* 24:820–830, 2006

**Keywords:** anterior cruciate ligament; wound healing; canine; platelets; collagen

## INTRODUCTION

Primary repair of the anterior cruciate ligament (ACL) was associated with a high failure rate<sup>1</sup> and has been largely abandoned in favor of ACL reconstruction. However, increasing evidence for the development of premature osteoarthritis in ACL reconstructed knees<sup>2</sup> has renewed clinical interest in revisiting primary repair. It is hoped that stimulating healing of the native structure

rather than replacing it with a single bundle construct will have a protective effect on the mechanics and subsequently the articular cartilage of the knee.

The ACL of the knee fails to heal adequately after primary repair.<sup>1</sup> The reasons for this are likely both mechanical and biologic in origin. In this article, a model with a mechanically stable defect was used in an attempt to eliminate the mechanical variables and allow focus on the biologic healing response and changes in that response with the addition of a collagen-platelet rich plasma scaffold material. If an adequate healing response could be stimulated, then future studies could be designed to address mechanical stability in a model of a complete tear.

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Many studies comparing the biologic responses of ACL and medial collateral ligament (MCL) cells have been performed showing differences between the cells from the two tissues<sup>3–8</sup>; however, while the differences are statistically significant, they do not completely explain the observed dichotomy between the ligament healing responses. A systematic evaluation of the in vivo wound healing response in the human ACL demonstrated that both cell and vessel proliferation occur within the tissue in a manner similar to that in the MCL, but that there was a complete lack of any structure spanning the gap between the ruptured ends of the ligament.<sup>9</sup> This gap grew in size as specimens were retrieved further out from the time of initial injury.

The unfilled gaps seen between the ends of the torn human ACL have also been reported within circumferential tears of menisci<sup>10</sup> and within damaged articular cartilage where they are called clefts.<sup>11–13</sup> One key strategy proposed to address these unfilled gaps and stimulate healing in the meniscus has been the use of fibrin clot. Fibrin clot, as pioneered by Arnoczky et al.,<sup>14</sup> has been used to stimulate meniscus and ligament<sup>15</sup> repair in animal studies and has been used clinically as well.<sup>16</sup> However, there is mounting evidence that there are elevated levels of plasmin circulating in joint fluid after trauma.<sup>17</sup> As the physiologic function of plasmin is to degrade fibrin clot, we elected to combine the elements of the fibrin clot with soluble collagen, which slows plasmin degradation of the fibrin,<sup>18</sup> thus extending the biologic durability of the fibrin-based implant for ACL repair. In addition, we used a simple centrifugation process to isolate platelet-rich plasma (PRP) from the whole blood. This was done in an attempt to raise the concentration of platelet-associated growth factors in the fibrin-based implant to be used in the ACL repair site.

Our hypothesis is that filling the wound site with a collagen-PRP scaffold will enhance the healing of the ACL after primary repair. To test this hypothesis, central ACL defects were treated with collagen-PRP scaffolds and the histologic and biomechanical responses for treated ACLs compared with untreated control ACLs. We hypothesized that the percentage of defect filling would be greater with use of the collagen-PRP scaffold, and that this increase in filling of the defect would be associated with an increase in mechanical strength of the ligament with time.

## MATERIALS AND METHODS

### Experimental Design

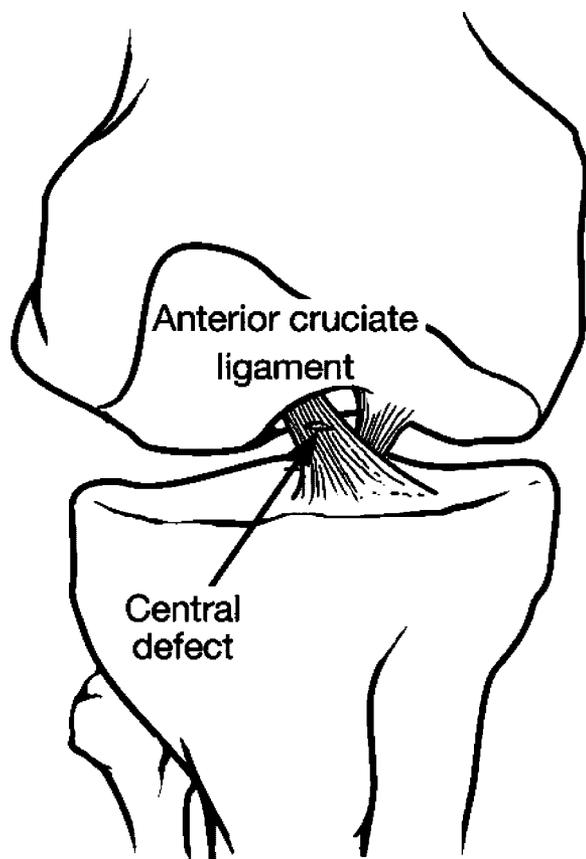
Two experiments were performed in this study. In the first experiment, bilateral defects were made and one side treated with the collagen-PRP hydrogel. A histologic comparison of treated and untreated ligaments for each animal was performed at 3 ( $n=5$ ) and 6 ( $n=5$ ) weeks. In the second experiment, unilateral ACL defects were treated with the collagen-PRP scaffold and the biomechanical properties of the treated ligaments measured at 6 weeks ( $n=8$ ). The tensile properties of the healing ligament were normalized by the intact contralateral ACL for each animal. These normalized results (TX-6) were compared with identically obtained normalized results for two previously reported control groups—a normalized untreated defect at 0 weeks (UnTX-0) and a normalized untreated defect at 6 weeks (UnTX-6).

### Collagen-PRP Provisional Scaffold

To prepare the collagen-PRP provisional scaffold, autologous whole blood was drawn from the canine jugular vein into tubes containing sodium citrate immediately prior to surgery. The blood was centrifuged to isolate the PRP fraction at 100g for 20 min. Three hundred microliters of PRP were added to 700  $\mu$ l of a mixture of acid soluble Type I collagen (Cell-a-gen, MP Biomedical, Aurora, Ohio) which had been neutralized to a pH of 7.4 with 7.5% sodium bicarbonate (Biowhittaker, Walkersville, MD). In addition, 10% antibiotic/antimycotic solution (Cell-Gro, Mediatech, Herndon, VA) and 10% 10 $\times$  Ham's F-10 culture media without bicarbonate or L-glutamine (ICN Biomedicals, Aurora, OH). The mixture was kept on ice until use. For the histologic studies, a strip of Type I collagen sponge (Cell-a-gen, MP Pharmaceuticals) was placed into the defect and the hydrogel mixture placed on top of the ligament and sponge. As the histologic results demonstrated only partial filling with this technique, in the second set of experiments for biomechanics, the Type I collagen sponge was presoaked in the gel and then the saturated sponge placed into the defect.

### Central Defect Model

The International Animal Care and Use Committee (IACUC) approval was obtained prior to starting the study. The central defect model for the canine ACL was developed by one of the authors (K. P. S.) (Fig. 1). The ACL was exposed through a medial peripatellar arthrotomy and a central defect made using a specially designed 3.5-mm blade. The incision was closed in layers. The canines allowed unrestricted weight bearing and motion of the knee.



**Figure 1.** Schematic drawing of central anterior cruciate ligament (ACL) defect model. A medial peripatellar arthrotomy was made and the ACL exposed. A central, transverse defect was made in both ACLs using a specially designed 3.5-mm blade which transected approximately 40% of the central fibers in the coronal plane. The defect went completely through the ligament in the sagittal plane.

#### Experiment A: Histologic Response to Injury in the Canine ACL

##### *Effect of Placement of a Collagen-PRP Provisional Scaffold in an Intraarticular Defect*

Ten canines, weighing between 22 and 33 kg, were used in this part of the study. Each animal had bilateral central defects created under general anesthesia. In the experimental knee, the collagen-PRP provisional scaffold was placed in the defect. In the contralateral control knee, the defect was left empty. After euthanasia, the ACLs were immediately harvested and placed in fixative. No animals experienced any postoperative complications of infection or lameness.

#### Gross Appearance

Upon retrieval of the specimens at the time of euthanasia, the healing response was graded without

knowledge of treatment received (blinded evaluation). The Wiig classification of grades 1, 2 and 3 for uncovered, partially covered and totally covered lacerations, respectively, was used.<sup>19</sup> In addition, gross observations by the surgeons as to presence of a persistent defect on either the anterior surface or posterior surface of the ligament and the presence of a vascular sheath covering the defect were made.

#### Histology

Ligaments reserved for analysis with histology or immunohistochemistry were fixed at 4°C in 4% cold paraformaldehyde for 24 h, sectioned longitudinally in a sagittal plane passing through the defect, embedded in paraffin and microtomed at 7 µm. An average of 50 slides was obtained for each ligament. Hematoxylin and eosin (H&E) staining was performed on selected slides taken every 400 µm throughout the ligament (an average of six slides per ligament were stained with H&E). On slides where the central defect was noted, the percentage of filling of the defect as well as the cellularity and vascularity of the tissue within the defect and adjacent to the defect was noted. Polarized light microscopy was used to identify aligned collagen within the defect.

#### Immunohistochemistry

Immunohistochemistry to detect the presence of fibrinogen was used to verify location of the defect in all specimens at the 3- and 6-week time-points. Slides made as above were baked at 65°C for 4 h, deparaffinized in xylene and dehydrated. The presence of fibrinogen was detected by immunohistochemistry using a polyclonal rabbit anti-human antisera (Dako, Carpinteria, CA; product number A0080, dilution 1:2500). These polyclonal antibodies react to fibrinogen, fibrin, and fibrinogen fragments D, E, X, and Y. All incubations were carried out at room temperature. Endogenous peroxidase was quenched with 3% hydrogen peroxide for 5 min. Deparaffinized, hydrated slides were then pretreated with proteinase K (Dako) for 20 min. Nonspecific sites were blocked using Dako Protein Block (X 0909) for 20 min. The sections were then incubated with the primary antibody for 30 min at room temperature. Negative controls were incubated with antibody diluent, which was used to dilute the primary antibody. The sections were then incubated with a labeled polymer (Dako Envision Plus Kit) for 30 min. The labeling was developed using the Dako DAB+chromagen kit for 10 min. Counterstaining with Mayer's Hematoxylin (Sigma-Aldrich, St. Louis, MO) for 30 s was followed by a 10-min tap water wash, dehydration and coverslipping with Histomount mounting media (Thermo Electron, Waltham, MA).

### Experiment B: Biomechanical Response to Placement of a Collagen-PRP Provisional Scaffold

For the mechanical testing, 10 canines had unilateral treatment with a collagen-PRP provisional scaffold (TX-6 group), with the contralateral ACL serving as an intact internal control. One of the animals in the 6-week treatment group weighed only 16.8 kg and the 3.5-mm blade created a defect which exited the ligament medially in addition to creating a central defect. This animal was excluded from the analysis. During the mechanical testing, the ACL for one of the animals was accidentally transected during removal of the other soft tissues and this animal was also excluded from the analysis, leaving eight animals for this part of the study ( $n = 8$ ).

The data from these eight animals were compared with two previously tested groups which had undergone identical defect creation.<sup>20</sup> The first comparison group had unilateral defect placement and no treatment, and underwent immediate euthanasia (UnTX-0 group,  $n = 7$ ). The second comparison group had unilateral defect placement and no defect treatment, and underwent euthanasia after 6 weeks (UnTX-6 group,  $n = 6$ ).<sup>20</sup>

All animals had a central defect placed with an identical blade and technique. For each group, the control and defect sides were alternated sequentially during the surgical days. The surgeries were not performed in order of animal number to minimize investigator bias during results analysis. The strength of the ACL containing the defect (defect side) was normalized for each canine by the strength of the intact side. The lower limbs of all animals were frozen prior to biomechanical testing. At the time of biomechanical testing, the specimens were thawed overnight at 4°C. After thawing, the specimens were maintained at 4°C and kept moist with normal saline. One hour before testing, the specimens were brought individually to room temperature and kept at room temperature until testing was completed.

### Magnetic Resonance Imaging

The 6-week treated specimens ( $n = 9$ ) underwent magnetic resonance imaging (MRI) evaluation to non-invasively determine the percentage of defect filling and the size of the scar mass in the ligaments with a treated defect prior to mechanical testing. Magnetic resonance imaging was conducted on a 4.7 Tesla microimaging system (Biospec<sup>TM</sup>, Bruker BioSpin MRI, Inc., Karlsruhe, Germany). After the T2 localizers imaging on three orthogonal axes, spin-echo proton density weighted images (PD-WIs) were subsequently acquired in the sagittal and axial planes at the ACL site.

For quantification of filling percentage and scar size in the three planes, images were reviewed by an investigator blinded to whether the ligaments were treated or intact. In the axial, coronal, and sagittal

planes, the T2 images were reviewed to assess for the presence of fluid (bright signal) on the images. The spin-echo proton density weighted images were used to measure ligament size. Using the PD-W images, the ACL and surrounding scar on each view were identified in each plane and the area on all slices containing the ACL measured. The total number of slices for each axis and the area of each slice were recorded. Area measurements were normalized by the field of view to provide the actual ligament cross-sectional area in square centimeters. The area on each slice was recorded, and the maximum sagittal area for each ligament determined. The total area for each plane was obtained by adding the areas of the individual slices in that plane. Linear regression analysis was performed to evaluate the effect of total area in the axial, coronal, and sagittal planes, as well as the effect of the maximum sagittal scar size on the tensile strength of the treated ligaments.

### Biomechanical Testing

The bone-ligament-bone ACL complex from both knees for each dog was tested in uniaxial tension. After euthanasia, the hind limb of each dog was amputated through the midshaft of the femur and tibia. Taking care to preserve the medial (MCL) and lateral (LCL) collateral ligaments and ACL, all other soft tissues including muscle, tendon, joint capsule, and the posterior cruciate ligament were removed from the knee by sharp dissection prior to embedding. The MCL and LCL were preserved until just prior to mechanical testing to facilitate correct spacing and alignment of the femur and tibia during mounting of the knee in the mechanical test apparatus. The femur and tibia were embedded in cylindrical molds using polymethylmethacrylate (PMMA) resin. The knee was embedded in 30 degrees of flexion with the tibia oriented in parallel with the testing axis and the femur oriented 30 degrees to the testing axis, as previously reported.<sup>20</sup> The remaining soft tissues, except the ACL, were transected after the knee was loaded into the tensile grips at 30 degrees of flexion.

All mechanical testing was conducted using an Interlaken Series 3300 Load frame controlled by an MTS TestStar IIm Digital Controller (Eden Prairie, MN). For the 6-week treated and intact knees, close-range digital images of the bone-ligament-bone ACL complex 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 identify the failure location (i.e., midsubstance at repair site, femoral or tibial attachment sites). Before conducting the tensile test, the bone-ligament-bone ACL complex was preconditioned with 10 cycles of loading and unloading at a strain amplitude of approximately 3%, at a rate of 5 mm/min.<sup>21</sup> Immediately after preconditioning, each specimen was tested to failure in uniaxial tension at 30 degrees of flexion at 20 mm/min.<sup>21,22</sup>

The tangent modulus (maximum slope of force-displacement curve), maximum load 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 tested. Data was analyzed using MATLAB (The Math Works, Natick, MA). Each measured parameter for the knee that had placement of a central defect was normalized (divided) by that same parameter measured for the intact contralateral ACL. The yield force represents the point along the normalized force-displacement curve where the mechanical behavior of the ACL complex departed from “linear” behavior, and for the purposes of this analysis, was defined as the point where the tangent modulus declines by at least 2% from its maximum value. The ultimate force was deduced from the maximal normalized force sustained by the ACL complex prior to failure. The work to failure was derived by integrating the total area under the force-displacement curve.

Sequential digital images of the ACL complex were used to determine the site of failure of the bone-ligament-bone complex.

#### **Statistical Methods and Power Calculations**

To determine the number of animals required for the biomechanical study, a power analysis was performed. For the untreated ACL defects, the average maximum tensile load experimental/control ratio at time zero was  $0.57 \pm 0.12$  (mean  $\pm$  SD).<sup>20</sup> To detect a clinically significant increase from 0.60 to 0.80 for maximum load, a power analysis (nQuery Advisor software version 6.0, Statistical Solutions, Saugus, MA) indicated that a sample size of seven ligaments in each group would provide 80% power to detect an effect size of  $\delta = 1.7$  using an unpaired *t*-test (two-tailed  $\alpha = 0.05$ ,  $\beta = 0.20$ ). To detect a clinically significant increase from 0.60 to 0.80 between the treated and untreated ligaments for stiffness (assuming an SD of 0.13), a power analysis (nQuery Advisor software) indicated that a sample size of eight ligaments in each group would provide 80% power to detect an effect size of  $\delta = 1.5$  using an unpaired *t*-test (two-tailed  $\alpha = 0.05$ ,  $\beta = 0.20$ ). Therefore, a group of 10 animals was used to allow for any losses of animals due to illness, infection, or delivery problems. Continuous data were correlated with the Pearson product-moment coefficient (*r*). One-way analysis of variance (ANOVA) was performed to evaluate whether the independent variable treatment method (treated, untreated, control) had a significant effect on each of the four dependent (response) biomechanical variables: structural stiffness, yield strength, ultimate strength, and work to failure. An overall F-test was followed by Fisher’s least significant difference (LSD) procedure for multiple group comparisons to protect against Type I errors (false positives). For assessing differences in filling of defects, a two-factor ANOVA

model was chosen to test for the effects of treatment and time. Linear regression analysis was applied to model the relationship between healing ligament strength and maximal area recorded on the sagittal magnetic resonance images with *R*-squared utilized as the criterion for judging goodness-of-fit of the model. Data analysis was performed using the SPSS statistical package (version 12.0, SPSS Inc., Chicago, IL).

## **RESULTS**

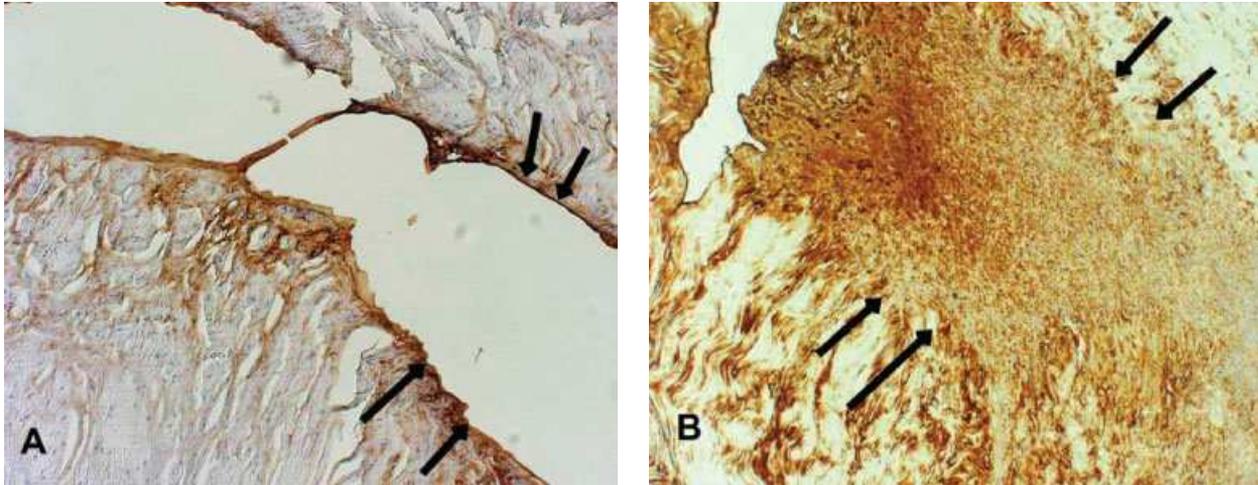
### **Experiment A: Histologic Response to Injury in the Canine ACL**

#### **Gross Observations**

Neither the Wiig classification nor the other gross observation measures were predictive of treated versus untreated defect on the 20 ACLs used for the histologic arm of the study. For the 6-week specimens, all five defects on the experimental knees were covered (Wiig class 3), but two of the control knees were also completely covered (Wiig class 3), and the remaining three knees were partially covered (Wiig class 2). A matched pairs analysis with *t*-testing indicated a trend ( $p = 0.10$ ) suggesting increased coverage as a result of treatment which did not reach statistical significance, due at least in part to the small number of pairs analyzed ( $n = 5$ ).

#### **Microscopic Evaluation**

On microscopic evaluation, the defects in the untreated controls were easily visible on the microscopic sections. However, for three of the five treated ACL defects, the lesions were more difficult to discern definitively with standard light microscopy at the 6-week time-point. Therefore, immunohistochemistry for the presence of fibrinogen and polarized light microscopy were used to verify the location of the defect for all specimens. The percentage filling of the defects in the treated ACLs was significantly higher at both the 3- and 6-week time-points when compared with the untreated controls ( $50 \pm 21\%$  vs.  $2 \pm 2\%$  at 3 weeks, and  $43 \pm 11\%$  vs.  $23 \pm 11\%$  at 6 weeks, all values mean  $\pm$  SEM; Fig. 2) but no significant change was seen with time (two-factor ANOVA,  $p = 0.03$  for treatment group,  $p = 0.60$  for time effect). After 6 weeks in vivo, longitudinal collagen bundles with crimp lengths smaller than that in the normal tissue were observed (Fig. 3).



**Figure 2.** Photomicrographs showing the appearance of the gap in the untreated ACL defect (A, arrows) and a cellular repair tissue with positive staining for fibrinogen within the treated ACL defect (B, arrows) at 3 weeks. (Immunohistochemistry for fibrinogen where brown represents a positive stain, original magnification,  $\times 400$ ).

### Experiment B: Biomechanical Response to Placement of a Collagen-PRP Provisional Scaffold

#### Results, MRI

In the magnetic resonance imaging of the specimens prior to biomechanical testing, fluid was noted to persist in the defect in two of eight of the treated ligaments at 6 weeks. An inverse relationship was found between the total area of the scar and scar strength for the area measured on the axial slices ( $R^2=0.54$ ), the coronal slices ( $R^2=0.48$ ), and the sagittal slices ( $R^2=0.58$ ). The highest correlation coefficient was found with the maximal sagittal area parameter (Fig. 4;  $r = -0.77$ ,  $p < 0.05$ ).

#### Results, Mechanical Testing

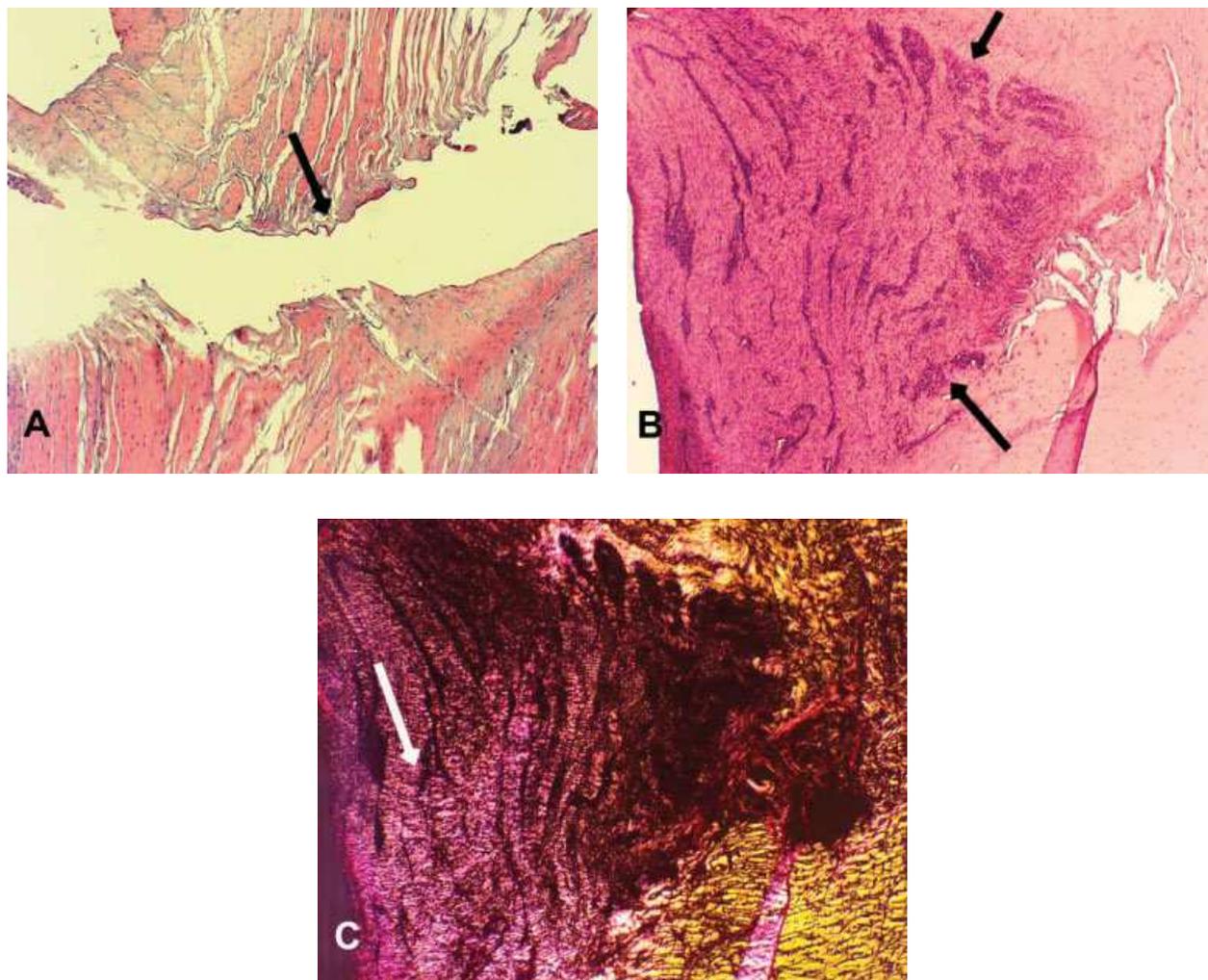
In the intact ligaments, four of eight ligaments failed in the midsubstance, while the remaining four failed at the bone–ligament interface. In the ligaments with a treated defect, seven of eight ligaments failed initially in the midsubstance with failure of the remaining intact fascicles occurring in some combination of midsubstance and bony interface failure. Only one ligament in the experimental group failed at the femoral insertion site.

Treatment (UnTX-0, UnTX-6, TX-6) of the central defect improved the normalized failure load ( $F$ -test = 6.0,  $p < 0.01$ ). There was a 40% increase in failure strength between 0 and 6 weeks

in the treatment group (0.795–0.568/0.568), a difference which was statistically significant ( $p = 0.003$ ). During the same time period, the untreated control group had only a 14% increase in failure strength (0.648–0.568/0.568), a difference which was not statistically significant ( $p > 0.25$ ) (Fig. 5). Treatment also affected the normalized work to failure ( $F$ -test = 3.5,  $p < 0.05$ ) (Fig. 5). For the treated group, the work to failure was 51% greater (0.777–0.513/0.513) than that for the dogs at time zero (UnTX-0) ( $p = 0.02$ ), but the 35% difference between the treated (TX-6) and untreated dogs (UnTX-6) at 6 weeks was not statistically significant ( $p = 0.08$ ), perhaps reflecting the small number of animals in each of these groups. The normalized maximum tangent modulus appeared to be less influenced by treatment ( $F$ -test = 2.1,  $p = 0.15$ ) (Fig. 5). The maximum tangent modulus of the ACL complex tended to be greater for the dogs treated for 6 weeks (79% of the intact contralateral knee) than the dogs in the treatment control group (56% of the intact contralateral knee), and this mean difference nearly reached significance ( $p = 0.06$ ).

### DISCUSSION

The ACL, meniscus, and articular cartilage are intraarticular structures which all have limited healing capacity. When the injury sites of meniscus, articular cartilage, and the ACL are

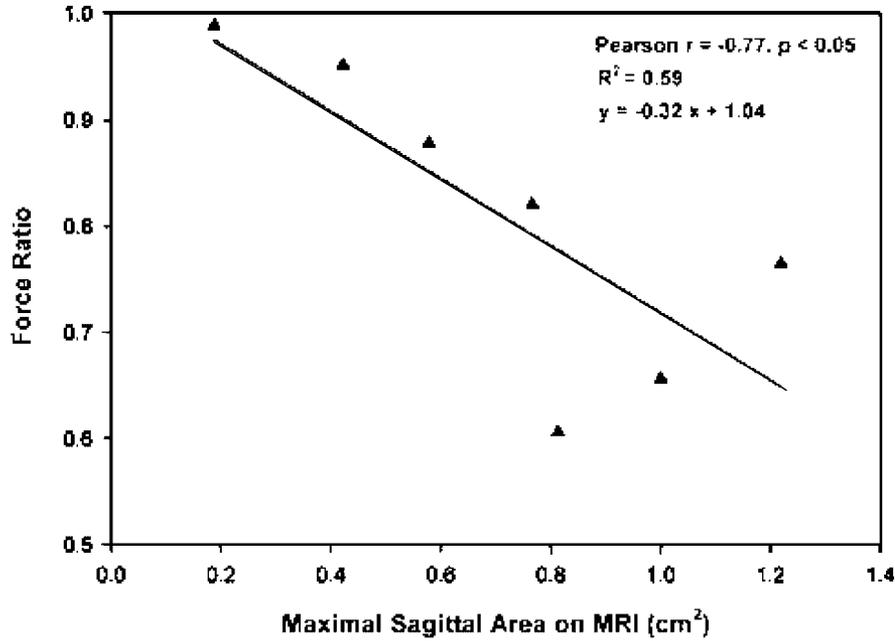


**Figure 3.** Photomicrographs showing the appearance of the empty defect after 6 weeks in vivo (A, arrow), the appearance of the treated defect after 6 weeks (B, arrows), and the appearance of the same area in (B) with polarized light (C). Note the presence of collagen bundles with small crimp lengths (arrow) within the defect consistent with scar formation.

examined histologically, the remaining tissue typically contains viable cells while there is a gap at the rupture site which remains open.<sup>23</sup> Even with suture repair and gross reapproximation of the tissue edges, the gap persists.<sup>10,24</sup> This gap, often called a cleft in the articular cartilage literature, also persists after mosaicplasty or implantation of tissue engineered substrates designed to induce cartilage healing.<sup>11-13</sup> This gap is unique to the intraarticular environment, it is not seen in extraarticular repair sites of bone, tendon,<sup>25,26</sup> or ligament,<sup>27,28</sup> even in cases of experimentally induced healing failure in these tissues.<sup>29</sup> In those extraarticular sites, the gap

has been filled with a provisional scaffold of fibrin and platelets that is subsequently invaded by surrounding intrinsic and extrinsic cells which initiate and conduct tissue healing.<sup>30,31</sup>

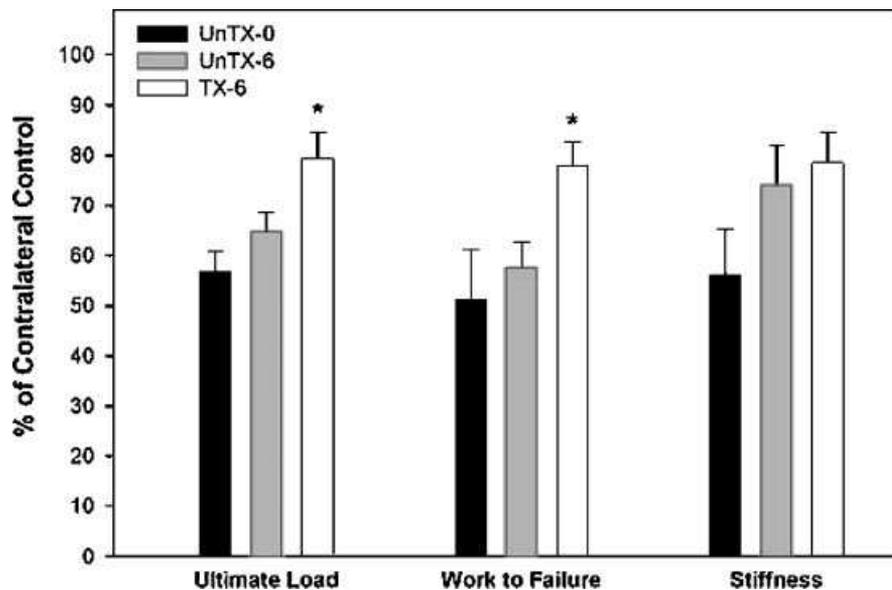
In the extraarticular environment, the breakdown of the provisional scaffold is a well balanced process, with clot degradation occurring slowly over days to weeks as the reparative cells initiate new collagen formation at the injury site. However, in the intraarticular environment, the stabilized fibrin clot is not observed to form,<sup>32,33</sup> even after induced bleeding into the joint. Thus, patients with an intraarticular injury form a hemarthrosis (rust colored bloody fluid) in the



**Figure 4.** Linear regression scatterplot showing the inverse relationship between healing ligament strength and maximal area recorded on the sagittal magnetic resonance images (MRI). Thus, ligaments with larger scars had lower recovery of tensile strength.

joint, without forming a stabilized fibrin-platelet scaffold (blood clot) at the site of injury. The presence of urokinase plasminogen activator (uPA) found in the synovial fluid after injury<sup>17</sup>

may play a key role in scaffold failure, as the presence of this enzyme results in circulating plasmin in the joint and accelerated fibrin dissolution. Thus, one possible hypothesis for the failure



**Figure 5.** Comparison of normalized biomechanical variables according to method of treatment. Based on ANOVA, asterisks denote significant improvement in ultimate load for TX-6 weeks group compared to UnTX-0 ( $p < 0.05$ ) and UnTx-6 ( $p = 0.003$ ), as well as work to failure for dogs in TX-6 group compared to UnTx-0 control ( $p = 0.02$ ).

of intraarticular tissues, such as the ACL, to heal is the premature loss of the fibrin-platelet provisional scaffold.

This study demonstrates that placement of a substitute collagen-PRP provisional scaffold in the defect will stimulate histologic filling and biomechanical healing of the defect. Thus, healing of these types of defects may be possible using an appropriate substitute scaffold. This is a critical finding as prior research into stimulation of healing in articular tissue defects has focused on overcoming cellular deficiencies rather than scaffolding deficiencies. In this study, no cells (except the platelets and white blood cells contained in the platelet-rich plasma) were transplanted, yet a highly cellular repair tissue was seen within the defect by 3 weeks. This suggests that at least in the ACL, there is a sufficient intrinsic and/or extrinsic cellular response from the environment around the ACL defect to stimulate histologic healing of the defect if an appropriate scaffold is provided. This is an important distinction, as stimulation of a local cell response could eliminate the need for cellular transplantation in this application. 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,<sup>34,35</sup> or stem cells, or implanted recombinant growth factors, or even viral vectors for gene therapy.<sup>34,36,37</sup>

However, even with treatment using the collagen-PRP provisional scaffold, incomplete defect filling was seen histologically. Only an average of 50% filling was seen in the treated defects when examined at 3 weeks, and only 42% filling at 6 weeks. Possible explanations for this incomplete filling include insufficient implantation of material into the defect, partial premature dissolution of the scaffold, and scaffold contraction within the defect. Thus, there is room for improvement in defect filling and scaffold retention. However, even with incomplete defect filling, a significant increase in failure load was seen in the treated group, suggesting that even incomplete filling can significantly alter the return of mechanical strength.

In addition, while this is the first time healing of the ACL defect has been demonstrated biomechanically, the recovery of biomechanical strength in the defect remained incomplete at 6 weeks. While the percent recovery in the defect was similar to that previously reported for the healing MCL at 6 weeks,<sup>38</sup> there remains room for improvement

for stimulating an earlier return of biomechanical strength, perhaps using additional implanted growth factors and extracellular matrix proteins. In addition, while a significant return of biomechanical strength was seen at 6 weeks, it is not clear that this return would continue with time; further studies examining results after longer periods of recovery are needed.

One of the limitations of this study was that the sponge and gel components of the scaffold were administered differently in the two arms of the study. In the histology study, the insoluble collagen sponge was placed first into the defect and the soluble collagen-PRP gel placed on top of the sponge. Based on the histology work, which showed incomplete filling of the defect using this technique, in the mechanical studies, the sponge was first saturated in the collagen-PRP gel and then placed in the defect in an attempt to improve the defect filling. Thus, the two arms of the study were not completely equivalent.

In addition, this model differs from the clinical situation in three important ways. First, the scaffold was introduced immediately after the defect was created, whereas in the clinical situation the defect is treated days or weeks after injury.<sup>39</sup> Second, the defect that was created was a knife cut, with little or no damage to the cut fascicles proximal and distal to the cut. This is clearly different from a clinical rupture where many of the fascicles are thought to sustain tensile injury along their length prior to rupture. Third, this model was a partial tear model, and clearly the more relevant clinical situation is a complete tear. Additional work defining the effects of each of these components (lag time between injury and treatment, mechanism of injury, and partial versus complete tear) is required to begin to develop clinically applicable methods of ACL primary repair.

In summary, use of a collagen-PRP scaffold can stimulate healing in a mechanically stable small defect as seen with optimal primary ACL repair. Future studies focusing on the longer term effects of such a treatment, the effect of gap size on the biologic healing capacity of the ACL, and extension of scaffold use to avascular meniscus and cartilage lesions are warranted.

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