SKELETAL MATURITY SIGNIFICANTLY AFFECTS FUNCTIONAL ANTERIOR CRUCIATE LIGAMENT HEALING

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INTRODUCTION
The influence of age and skeletal maturity on the ability of the ACL to heal has not been thoroughly defined. In a study comparing ACL healing in immature and mature rabbits, after one year, the partially torn ligaments had regained a higher percentage of ligament strength in the mature group when compared to the immature group. Building upon the previous rabbit study, the current study was designed to evaluate the effects of skeletal maturity on the functional healing of the torn ACL in a large animal model when left untreated or treated with enhanced suture repair using a collagen-platelet composite. We hypothesized that the structural properties of the torn ACL of juvenile animals (open physes) would be greater than those of adolescent (open physes nearing closure) and adult animals (physes closed) after 15 weeks of healing, and that those ligaments treated with enhanced repair would be greater than those not treated.

METHODS

Experimental Design
Institutional Animal Care and Use Committee approvals were obtained prior to initiating this study. Twenty one Yucatan mini-pigs representing three different age groups; JUVENILE (6 to 9 months of age, n=8), ADOLESCENT (12 to 13 months of age, n=8) and ADULT (36 to 60 months of age, n=5) were utilized. Each animal underwent bilateral surgical ACL transection. One side was left unrepaired while the other side was immediately repaired with suture-repair enhanced with the collagen-platelet composite (Figure 1). The knees were allowed to heal for fifteen weeks. A separate group of knees with intact ACLs from age-matched controls were also evaluated.

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Figure 1: Schematic diagram depicting the primary suture repair with the collagen-platelet composite scaffold in place. Sutures were fixed proximally with an Endobutton device. The sponge was threaded onto four of the trailing suture ends (RED) which were then passed through the tibial tunnel and tied over a button to provide initial knee stability. The remaining two suture ends (GREEN) were tied to the sutures in the tibial stump of the ACL. All sutures were resorbable, and there was no sign of suture material remaining at the time of post-mortem testing (Reprinted with permission of John Wiley & Sons, Inc.).

Collagen-Platelet Composite: At the time of surgery, 60ml of whole blood was drawn from each animal using a large bore needle (≤18g) and serially centrifuged to obtain 5X platelet-rich plasma. Initial and final platelet concentrations were determined using a VetScan HM5 Analyzer (Abaxis, Union City, CA). The collagen sponge was made by solubilizing bovine fascia in an acidic pepsin solution and lyophilizing. The collagen sponge was made by solubilizing bovine fascia in an acidic pepsin solution and lyophilizing.

Mechanical Testing: AP laxity values for the JUVENILE, ADOLESCENT and ADULT groups were measured with the knee positioned at 30°, 60° and 90° of flexion using a custom designed fixture. After completing the AP laxity tests, the joint capsule, menisci, collateral ligaments, and the PCL were dissected from the joint leaving the ACL graft and integrated scar mass intact. The femur-graft-tibia complexes were loaded in tension to failure at 20mm/min. Identical testing was performed for the knees with intact ACLs in each age group.

Statistical Analysis
Anterior-posterior knee laxity results, and the structural properties after 15 weeks of healing were compared between the three maturity groups (JUVENILE, ADOLESCENT, vs. ADULT) by analysis of variance (ANOVA) while treatment (not treated vs. enhanced repair) was considered as a within subject variable.

RESULTS

Untreated ACL Transection (Intrinsic Healing Potential of the ACL)

While the JUVENILE ACLs reached 25% of the intact load at three months after transection, the ADULT only reached 7% at the same time point (p<0.01). Similar results were found for the maximum load to failure (Figure 2). In addition, the linear stiffness of the healing ACL in the JUVENILE knees was double that found for the ADULT knees, although the difference only approached statistical significance (p=0.09).

Interestingly, the AP laxity at 60 degrees was 25% lower in the ADULT knees than in either the ADOLESCENT or JUVENILE knees (p<0.01 for both comparisons).

Figure 3. The maximum load in each age group as normalized by the maximum load of the intact ACL for that age group. The JUVENILE animals had a 300% higher normalized maximum load than the ADULT animals in the untreated group (p<0.01). For ligaments treated with collagen-platelet composite (CPC), both the JUVENILE and ADOLESCENT animals had higher normalized maximum loads that the ADULT group (p<0.01 for both comparisons). §The addition of the collagen-platelet composite resulted in an 85% increase in maximum load in the ADOLESCENT group (p<0.01).

ACL Transection treated with Enhanced ACL Suture Repair

The repaired ligaments also had biomechanical properties that were dependent on animal age. The yield load in the ADOLESCENT animals was 50% greater than that of the ADULT animals (p<0.01). The maximum load of both the ADOLESCENT and JUVENILE animals after repair was 40% greater than that of the ADULT animals (p<0.01 for both comparisons). The AP laxity at 90 degrees of flexion was 30% lower in the ADOLESCENT and JUVENILE knees than in the ADULT knees (p<0.01). Finally, the stiffness of the repaired ADOLESCENT and JUVENILE animals was 50% greater than the stiffness of the repair tissue in the ADULT knees (p<0.01).

DISCUSSION

The results of this study support the hypothesis that the capacity for functional healing of a ligament after injury and enhanced repair is dependent on the level of skeletal maturity of the animal. JUVENILE animals had a more productive repair response to ACL transection than the older animals, since the repair tissue had a higher maximum load and stiffness. The ADOLESCENT animals derived the most benefit from suture repair using a collagen-platelet composite, with a doubling of the yield load in this group with treatment. The ADULT animals had the least functional repair response, both in terms of intrinsic healing (no treatment of the transected ACL) and enhanced suture repair. This work demonstrates partial healing of the ACL with an enhanced suture repair technique may provide a new treatment alternative – to stimulate healing of this important ligament rather than developing new ways to replace or reconstruct it. Further investigation into the biology of the ligament and the surrounding tissues, including evaluation of the presence of stem cells adjacent to the injury site, the molecular signals, and potential molecular manipulations of the healing process, are all clinically relevant avenues of study that could potentially lead to a revolution in the treatment of ACL injuries, particularly for young patients.

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REFERENCES


Figure 2. The yield load and normalized yield load as a percentage of the intact ACL is shown for each age group. The JUVENILE knees was double that found for the ADULT knees, although the difference only approached statistical significance (p=0.09).