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The Biology of Anterior Cruciate Ligament Injury and Repair: Kappa Delta Ann Doner Vaughn Award Paper 2013

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Abstract

Anterior cruciate ligament (ACL) injuries are currently treated by removing the injured ligament and replacing it with a tendon graft. Recent studies have examined alternative treatment methods, including repair and regeneration of the injured ligament. In order to make such an approach feasible, a basic understanding of ACL biology and its response to injury is needed. Identification of obstacles to native ACL healing can then be identified and potentially resolved using tissue engineering strategies - first, with in vitro screening assays, and then with in vivo models of efficacy and safety. This Perspectives paper outlines this path of discovery for optimizing ACL healing using a bio-enhanced repair technique. This journey has required constructing indices of functional tissue response, pioneering physiologically-based methods of biomechanical testing, developing and validating clinically relevant animal models, and creating and optimizing translationally feasible scaffolds, surgical techniques and biologic additives. Using this systematic translational approach, "bio-enhanced" ACL repair has been advanced to the point where it may become an option for future treatment of acute ACL injuries and the prevention of subsequent post-traumatic osteoarthritis associated with this injury.

CLINICAL SIGNIFICANCE OF ACL INJURY

The annual incidence of ACL injury in the US is estimated at 1 per 1000 people¹. ACL injuries have immediate and long-term effects on the quality of life, and are known risk factors for post-traumatic osteoarthritis.³ In the past, surgeons tried to repair the ACL; however, it failed to heal in over 90% of patients.⁴ The reason for this was unknown. Thus, the current gold standard of treatment, ACL reconstruction, which involves removal and replacement of the ligament with a tendon graft, has become popular (Fig. 1). However, patients treated with ACL reconstruction continue to exhibit progressive articular cartilage and joint damage in the injured knee. A recent prospective cohort study suggests that 62% of ACL reconstructed patients with an isolated ACL injury presented with radiographic evidence of post-traumatic osteoarthritis 10 to 15 years post-surgery.⁵ Considering that many patients sustain ACL injuries before the age of 16, these injuries may place young patients at risk for premature post-traumatic osteoarthritis before age 30 even with our current best treatment methods.

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THE BIOLOGIC MECHANISM BEHIND THE FAILURE OF THE ACL TO HEAL

Based upon the observations that ACL reconstruction does not preserve the long-term integrity and function of the knee, researchers set out to understand the mechanisms that underlie the inability of an injured ACL to heal so that novel, clinically relevant ways to repair the ACL could be designed. By developing a means to biologically enhance the repair of the torn ACL, the need to remove and replace it would be obviated and post-traumatic osteoarthritis might be prevented. First, a series of experiments were designed to improve our understanding of why a torn ACL does not heal. These experiments would differentiate between intrinsic cellular deficiency of ACL fibroblasts, vascular deficiency, and local environmental factors, such as the bathing of the damaged ends of the ligament by synovial fluid or an inability to bring apposing ends of the torn ligament sufficiently close together using suturing or other stabilizing techniques. Studies compared ACL fibroblasts⁶ with those obtained from the medial collateral ligament (MCL),^{7,8} an extra-articular ligament that has no difficulty healing with conservative “non-surgical” treatment. By combining *in vitro* cell culture assays with *in vivo* histological and immunohistochemical techniques, it was found that MCL and ACL cells within injured ligaments have comparable rates of proliferation, and that each ligament is able to revascularize after rupture.^{6,7} Comparable collagen production within the ligaments was observed at time points up to one year after injury.⁹ Importantly, however, the germinal observation was made that the provisional scaffold found in the wound site of the MCL and other extra-articular ligaments was missing in the ACL (Fig. 2).

Among the potential explanations for poor healing response of the ACL was the difference in mechanical stabilization between the ACL and the altered environments surrounding these torn ligaments (Fig. 2). The ACL is surrounded by synovial fluid, whereas the MCL and all other extraarticular ligaments are not. Thus, while the cells and vascularity of the ACL were capable of mounting the same functional healing response, there was no structure in place to rejoin the two ends of the ligament for the cells to invade and remodel (Fig. 3). These findings^{6,7,10,11} led to the hypothesis that the lack of provisional scaffold between the two ends of the torn ACL was the key mechanism behind the failure of this tissue to heal (Fig. 3).

The observation that there was essentially no provisional scaffold formation or wound site filling between the ends of the ruptured human ACL led to the additional hypothesis that there would be a quantitative difference in the amount of wound site filling in the ACL and MCL even in a mechanically stable and contained defect.¹¹ It was additionally hypothesized that any molecular deposition within the wound site would differ between the ACL and the MCL including differences in fibrinogen, fibronectin, PDGF-A, TGF- β 1, FGF-2 and vWF.^{7,12} To test this hypothesis, central defects (Fig 3) were created in extra-articular ligaments (MCL and patellar ligament) and an intra-articular ligament (ACL) in canine knees and the histologic response to the injury was evaluated at 3 days, 7 days, 21 days and 42 days. The findings were that the MCL and patellar ligament defects exhibited far greater filling of the wound site with increased presence of fibrinogen, fibronectin, PDGF-A, TGF- β 1, FGF-2 and vWF when compared to ACL defects at all time points (Fig. 3).⁷ Thus, these data supported the hypothesis that there was a lack of a provisional scaffold within the intra-articular wound site of the ACL, and that this loss is associated with a decreased presence of important extracellular matrix proteins and cytokines within the ACL wound site.

ENGINEERING A SUBSTITUTE PROVISIONAL SCAFFOLD

After finding that the premature provisional scaffold loss was a major mechanism behind the failure of ACL healing, the next step was to develop a substitute provisional scaffold that

would be easily implanted and capable of providing the growth factors and enzymes required to optimize fibroblast and neurovascular ingrowth. Such a substitute scaffold would need to withstand the physical conditions of the intra-articular environment. Additional experiments validated the hypothesis that multiple cytokines could stimulate cell proliferation, migration and collagen production *in vitro*; however, the improvements were modest.¹³ This led to a search for a more physiologically relevant cytokine and growth factor delivery system. Platelets were one candidate target as the classic studies of wound healing proved that platelet coagulation and granule release are incipient events. Strategies for delivering platelets to an ACL wound using a stable carrier (scaffold) were evaluated to see whether this would promote ACL healing in a manner similar to that of the MCL. In a series of *in vitro* experiments, it was determined that the concentration of growth factors released from platelet rich plasma was dependent on the platelet concentration¹⁴ and that the timing of that release was dependent on the activation method for the platelets.¹⁴⁻¹⁶ Thrombin resulted in an immediate release of the platelet-derived growth factors, while the use of collagen as an activator resulted in a more sustained release of multiple platelet-derived growth factors.¹⁷ It was also found that keeping the platelets in their physiologic plasma resulted in an improved ability for the platelets to stimulate collagen synthesis by ACL fibroblasts.¹⁸

IN VIVO VALIDATION STUDIES

In vivo animal models of ACL injury were then required to translate what was learned in the petri dish to a living organism. ACL transection models (i.e., the Pong-Nuki model) have been historically used to promote joint failure in the study of osteoarthritis. In this application, the ligament is not repaired so that joint damage can develop post-operatively. To study the ACL repair process, an ACL injury model that could undergo surgical repair was needed. Mouse and rodent models were not suitable, since their joint size, structure, morphology and life expectancy are very different from humans. Thus, attention was turned to large animal models, where clinically relevant outcome measures that were translatable to the human condition could be identified and measured.^{7,11,12,19-21} The initial large animal experiments focused on a central defect model in which a cut was created within the ACL midsubstance and then left to heal on its own or after treatment with a tissue engineered scaffold or biologic agent. This model eliminated the confounding factor of joint instability on the wound physiology. In the canine model, it was found that these partial ACL injuries would not heal spontaneously.^{11,21} A histological scoring system was developed to grade the ligament healing response,⁷ and this set of histological criteria were used to test the hypothesis that placement of a substitute scaffold could stimulate histological healing of the ACL. Mechanical testing was used to determine if the histological healing resulted in improved mechanical properties as well. It was reported that the use of an extracellular matrix (ECM)-based scaffold loaded with autologous platelets encouraged both biological and mechanical healing of the central ACL defect.^{7,12}

The finding that the use of a tissue engineered composite stimulated healing of a central defect led to combining this scaffold with a suture repair (a “bio-enhanced repair”) for the treatment of a complete ACL tear. A model for the complete tear was developed in the porcine knee, and in that model, it was determined that the use of a extracellular matrix-platelet scaffold could stimulate functional ACL healing,²⁰ while use of platelets alone,²² or the ECM scaffold alone,²³ did not improve repair healing. It was also found that the suture technique used during ACL repair significantly influenced the outcomes,^{24,25} but that increasing the platelet concentration above three times the normal level did not further improve results.²⁶

Recent ACL repair studies in three large animal models have demonstrated that placement of the extracellular matrix device in the injury site of the ACL can stimulate biological and mechanical healing of the ligament.^{7,11,12,20,24,27-30} Studies in canine,^{7,12} porcine,^{20,24,27,29-31} and ovine²⁸ models have shown that the use of a platelets with a scaffold, which immobilizes and activates the platelets in the injury site, releases growth factors with spatial and temporal sequences that matches their release in healing extra-articular tissues such as the MCL.⁷ The use of the collagen device significantly improved both the yield load and stiffness of the repair tissue over the case where no scaffold was placed.^{20,30} When used in bio-enhanced ACL repair, the yield load increased almost 100% from 200 ± 145 N to 395 ± 110 N (mean $\pm$ SD, $p < 0.03$), and the stiffness of the repair improved 60% from 50 ± 32 N/m to 83 ± 15 N/m (mean $\pm$ SD, $p < 0.02$), resulting in values that are nearly equivalent to that of a graft after ACL reconstruction (the gold standard).²⁰ In a recent randomized trial in a large animal model,³¹ the biomechanical outcome of bio-enhanced ACL repair was equivalent to that of ACL reconstruction (Fig. 4).

Once it was discovered that this “bio-enhanced ACL repair” technique resulted in a repair with equal strength to ACL reconstruction in young animals, the effect of age on functional ligament healing was evaluated where it was found that the ACL of skeletally immature animals heal more quickly and more effectively than adult animals.²⁷ Skeletally immature animals had significantly improved ACL structural properties over those of adult animals after three months of healing in both the untreated and repair groups (Fig. 5).²⁷ It was subsequently determined that the increased cell infiltration of the wound site in vivo³² was likely a result of the improved migration and proliferation abilities of the younger cells,^{33,34} which in turn was likely due to the increased number of growth factor receptors on the younger cells.³⁵

It has also been recently reported that the stimulation of ligament repair and regeneration using the “bio-enhanced repair” technique prevents the development of post-traumatic osteoarthritis after an ACL injury.³⁶ In the most recent study, 80% of the knees that had a conventional ACL reconstruction developed post-traumatic osteoarthritis while the knees in the bio-enhanced ACL repair group did not one year after surgery (Fig 6). The power of the animal model is that a specific injury that strongly predisposes the injured joint to post-traumatic osteoarthritis can now be used to compare changes in the joints of animals where bio-enhanced ACL repair prevents post-traumatic osteoarthritis from those in which conventional therapy (i.e., ACL reconstruction) does not.

SUMMARY AND CONCLUSIONS

The investigations of ACL repair and regeneration summarized here have focused on a new potential treatment for ACL injury – namely, bio-enhanced ACL repair. To date, researchers have proven the hypotheses that 1) early loss of a provisional scaffold (i.e. scar tissue) inhibits ACL healing, 2) that placement of a substitute provisional scaffold can restore functional healing, and 3) that growth factor delivery systems can be specifically designed for use in the joint. In addition, it has been demonstrated that the healed ligament following bio-enhanced ACL repair is equivalent in strength to the graft after ACL reconstruction, and that it also minimizes post-traumatic osteoarthritis following ACL injury and reconstruction in preclinical models. However, preclinical models are not identical to the human condition and further studies of the clinical safety and efficacy of this method are a required next step in the translation of this technology.

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Fig. 1. Schematic demonstrating an ACL tear (left panel), our current method of treatment with removal of the ACL and replacement with a tendon graft (ACL reconstruction, middle panel) and the novel treatment of repair and regeneration we have developed for this injury (“Bio-enhanced ACL repair”, right panel) (Adapted with permission from Murray & Fleming³⁶).

Fig. 2.

Differences in the intrinsic healing response of the ACL (top row) and medial collateral ligament of the knee (MCL, bottom row). The ACL is injured, but no blood clot forms in the injury site, likely due to the synovial fluid which bathes the ligament washing the clot out. In contrast, in the MCL, blood clot forms at the site of the tear and stabilizes the two ligament ends. The MCL tissue can then grow into this provisional scaffold and the defect can be healed. The loss of the provisional scaffold in the ACL is likely the key mechanism behind its failure to heal.

Fig. 3.

Representative photomicrographs of the patellar ligament wounds (extra-articular (EA; first row)) untreated ACL wounds (intra-articular (IA; second row)) and ACL wounds treated with collagen-platelet scaffold (intra-articular (IA TX; third row)) 21 days after wounding (10x). Similar distributions of protein presence were noted in the treated ACL wounds and the healing patellar ligament wounds. The untreated ACL wounds remain relatively empty of any substratum (Used with permission from Murray et al⁷).

Fig. 4. Tensile structural properties of the bio-enhanced stress-protected ACL repair (labeled as “Enhanced ACL Repair”) were identical to those of ACL reconstruction with a graft in a randomized large animal study ($n=8/\text{group}$; $p>0.60$ for all parameters). Both treatment groups had a more functional repair than that of the untreated ACL transection.

Fig. 5.

The maximum load in three age groups 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 than 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 ($p < 0.01$) (Used with permission from Murray et al²⁷).

Fig. 6.

The distal femur cartilage 1-year after (A) an untreated ACL rupture, (B) after conventional ACL reconstruction and (C) after bioenhanced ACL repair. Note the damage to the medial femoral condyle in the untreated and ACL reconstructed knees (white arrows) and the lack of damage in the medial femoral condyle in the bio-enhanced ACL repaired knee (black arrow) (Adapted with permission from Murray & Fleming³⁶).