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Bio-enhanced repair of the anterior cruciate ligament

Benedikt L. Proffen, MD [Research Fellow],

Sports Medicine Research Laboratory, Department of Orthopaedic Surgery, Boston Children's Hospital, Harvard Medical School

Jakob T. Sieker, MD [Research Fellow], and

Sports Medicine Research Laboratory, Department of Orthopaedic Surgery, Boston Children's Hospital, Harvard Medical School

Martha Murray, MD [Associate Professor]

Sports Medicine Research Laboratory, Department of Orthopaedic Surgery, Boston Children's Hospital, Harvard Medical School, 300 Longwood Avenue, Boston, Massachusetts 02115, USA

Abstract

Suture repair of the ACL has been widely abandoned in favor of ACL reconstruction, largely due to the high rates of failure and unreliability of the outcomes following suture repair. However, there have been recent basic science studies which suggest that combining a suture repair with a biologic adjunct may improve the results of suture repair of the ACL, with several studies in large animal models showing equivalent strength of an ACL treated with bio-enhanced repaired with that of an ACL graft at 3, 6 and 12 months after surgery. In addition, the groups treated with bio-enhanced repair had significantly less osteoarthritis when compared with the animals undergoing ACL reconstruction. These findings have led to a renewed interest in bio-enhanced primary repair as a way to make repair of the ACL a viable option for a select group of patients in the future.

HISTORY OF PRIMARY REPAIR

“Those who cannot remember the past are condemned to repeat it.” (1) This quote from George Santayana's *Life of Reason* is particularly applicable to suture repair of the ACL. Primary suture repair of the ACL has been used in clinical practice in the past, with mixed results and some successes in select groups of patients (2). However, the technique to date has been somewhat unreliable in the hands of most surgeons. Before discussing how biologic enhancement of primary repair of the ACL may make this technique more viable, a review of what has been tried in the past and the results of those trials will be reviewed here.

The first repair of the ACL in the English literature is attributed to A.W. Mayo Robson in 1895. In his report was described the care of a miner who underwent suture repair of both

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Corresponding author: Martha Murray MD, martha.murray@childrens.harvard.edu Phone: +1 617 919 2066 Fax: +1 617 730 0789.

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the ACL and PCL three years after his original injury. The case report stated that six years later, the patient was able to walk without a limp, described his knee as “perfectly strong” and he had not missed a day of work due to his knee injury – certainly an outcome of “good patient satisfaction” (3). As anesthesia and aseptic techniques became more widely available, surgery for non-life threatening knee injuries became even more prevalent. In his monograph on ligament injuries and their treatment in 1938, Ivar Palmer described the failure of spontaneous healing of a complete ACL tear and subsequent importance of repair (4). Palmer thought that early repair was critical to its success and advocated for a repair technique using silk sutures and drawing them through the opposite bone. Following on Palmer's work, Don O'Donoghue reported his technique of ACL repair in the 1950s. His technique consisted of a suture weave through the tibial stump, which then again had the ends passed through a tunnel in the femur and tied. O'Donoghue immobilized his patients for four weeks at 30 degrees of flexion (5). Both Palmer and O'Donoghue stressed the importance of early repair of the ACL.

O'Donoghue not only reported his clinical results, but he also conducted in vivo animal studies in an attempt to improve the results of ACL repair. In the 1960's, he published a study of ACL suture repair in dogs. However, in the canine model, he found that even with a suture repair of the ligament, the repaired ACL only achieved 10 percent of its normal maximum load at 4 weeks (6). H. A. Cabaud, John Feagin and William Rodkey followed up on these studies in rhesus monkeys and dogs (7), and found that while repairs reached 45% of the intact ACL strength at a year in monkeys, the repairs in dogs were less favorable, again only achieving 10% of the ACL strength, similar to that reported by O'Donoghue. Both Cabaud and O'Donoghue hypothesized that the inability to immobilize the limbs, and the subsequent early stress on the repairs, might be responsible for the low maximum loads seen in the repaired ACLs. Interestingly, animal studies of ACL reconstruction (the current gold standard of ACL treatment) have also reported high rates of failure and abnormal knee laxity in these large animal models suggesting ACL procedures may be less effective in animals that cannot undergo skilled rehabilitation (8-11).

In the 1970s, John Feagin continued the clinical studies of primary repair of the ACL. Feagin was the first active duty Army officer to attend medical school. During his four year tour at Keller Army Hospital at West Point, he became team physician for the Army football and basketball teams and noted that the natural history of an untreated ACL tear was not usually conducive to continuation of the active lifestyle of a cadet or officer. He began operating on acute ACL tears and repairing them. Feagin noted that these acute tears were often Z shaped, with the posterolateral bundle proximally torn and the anteromedial bundle often torn more distally (JAF, personal communication). Along with Walter Curl, Feagin reported on their experience with primary suture repair for ACL injuries in West Point cadets. Feagin advocated use of a Dexon suture woven through the tibial stump and passed up through bone tunnels in the femur (Figure 1A). He immobilized his patients for 6 weeks after surgery at 30 degrees of flexion. For these active patients, 25 of 30 were doing well at 2 years out from surgery (12); however, the five-year follow-up results had 71% of patients with pain and 94% with knee instability (13). Feagin felt that shortening the immobilization time after surgery led to an increased rate of re-injury (14). He did however feel that 1/3 of patients who had a primary repair had good outcomes (14) and that the patients who had

good outcomes at five years had good outcomes at 30 years (15). In follow-up animal and clinical studies, Feagin, Cabaud, and Rodkey reported that post-operative immobilization was important for the knee, augmenting the repair with a strip of patellar tendon was useful, and adding a Vicryl braid to the repair all helped improve the knee laxity for the repaired ACL (16).

John Marshall reported a suture repair technique using multiple loop-varying depth sutures in both the femoral and tibial cruciate stump, using polyester sutures and individually tensioning each suture (Figure 1B). Marshall felt the repair should happen urgently. The meticulously placed sutures were then passed through drill holes in the opposite bone (17, 18). Marshall's studies appeared more promising, with a 2.5 year follow up study where no patients with repair had giving way symptoms or had needed subsequent meniscal surgery (even though 93% of them were active in sports) (17). The reasons for the improved results in Marshall's compared to Feagin's studies may have been due to the different patient populations for each (the injury mechanism for the West Point cadets was typically contact sports or combat while in the Marshall studies, was often a slow, twisting fall as in skiing). They may also have been due to the activity level post-operatively, where the West Point cadets were typically highly active. In addition, Feagin noted that the West Point cadets who could choose more sedentary activities had better outcomes (15).

After these initial reports of suture repair, two randomized clinical trials were reported in the 1980s. In 1985, Odensten et al published the first prospective, randomized trial of suture repair vs. iliotibial band reconstruction or non-operative therapy for ACL injuries (19). The technique utilized for suture repair involved using non-absorbable sutures to reattach the tibial stump to the ACL insertion point on the lateral femoral condyle. This was done by passing the sutures exiting the tibial stump through two drill holes in the lateral femoral condyle and tying them over a bone bridge. No sutures were placed in the femoral stump. The knees which had primary repair were immobilized for six weeks at 30 degrees of flexion. At 18 months out from surgery, the authors reported no significant difference between operated and non-operated knees in terms of overall outcome scores; however, 95% patients in the repaired group had a stable knee at follow up (with a negative pivot shift or Slocum test), while only 11% in the non-operated group had a stable knee. In addition, the patients who did not have a repair needed meniscal surgery twice as often as patients undergoing suture repair in the first 18 months after injury (19).

The second prospective randomized trial comparing the results of primary suture repair and non-operative treatment was published 1987 by Sandberg et al (20). Similarly to the results published by Odensten, the printed conclusion was that suture repair was no better than conservative treatment. However, it is interesting to note that in this second study, the rate of a positive pivot shift test also decreased with surgery (from 62% to 28%) as did the risk of presenting with a subsequent meniscal tear (24% vs. 8%). Thus, while both of these studies concluded that functional performance was comparable between the two groups, other important characteristics (stable knee, preservation of the menisci) appeared to be better in the group undergoing primary repair.

With the limited success of primary suture repair of the ACL, additional procedures were added to try to improve the results of ACL surgery. Suture repair was augmented with other tissue (the iliotibial band or a bone-patellar tendon-bone graft) (21), and eventually, the tissue augmentation or the implantation of a graft was performed without the suture repair yielding good results.

The history of primary repair has shown us that suture techniques alone may be useful for ACL injuries where the ends of the torn tissue can be re-approximated under compression. Studies of this technique in lower demand patients reported better results than studies in high demand patients; however, the procedure has been abandoned in favor of ACL reconstruction largely due to the unpredictable results of the procedure. This may be due to the fact that many ACL tears cannot be re-approximated or sutured without a gap between the torn ends, and as we will discuss in the next section. The presence of a gap, even one that is less than a millimeter wide, can result in failure of the suture repair due to the fact that the ACL exists in the synovial fluid of the knee joint. Biologics and scaffolds may be useful in filling the gap and enhancing ACL healing.

WHY DOESN'T PRIMARY ACL REPAIR WORK?

If repair of the ACL could be performed successfully, this might provide several advantages over reconstruction, including the preservation of the natural anatomy of the ligament, in particular its insertion sites and multiple bundle morphology. It is also believed that repair of the ACL would preserve the native physiology, nerves and intrinsic cell populations, as well as some of the complex biomechanical properties of the ligament. Preserving any proprioceptive nerve function of the ACL, in particular, might confer protection to the knee. However, the clinical practice of ACL repair has been limited by the unreliability of this procedure. The question remains, why the ACL doesn't heal with suture repair.

In contrast to the ACL, the medial collateral ligament (MCL) heals uneventfully (22). To begin to understand the biologic reasons for the differences between these two tissues, researchers have studied several of the basic biological responses essential to successful healing in both ligaments and compared the two sets of responses. In both ligaments, an elevated growth factor synthesis is observed after injury (22, 23). Cells in both the torn MCL and ACL are capable of migration into an adjacent wound site, and are able to proliferate and produce essential matrix components, including collagens (23-28). The major difference observed between the ACL and MCL after injury is the lack of a scaffold in the wound site of the injured ligaments (FIGURE 2). When the MCL is ruptured, the ends bleed and a blood clot forms in the wound site. This clot serves as a provisional scaffold, or structure for the surrounding cells to migrate into and remodel into a fibrovascular scar, thus restoring function to the torn ligament (22). However, while the torn ends an injured ACL bleed as well, enzymes present in the synovial fluid prevent a clot or a bridging provisional scaffold from forming. Harrold et al. demonstrated that a fibrin clot does not form in the posttraumatic intra-articular milieu (29). Rather, rapid clot breakdown is observed due to the abundance of plasmin in the synovial fluid, which is a very efficient cleaver of fibrin (30). Also, an increased PLAU gene expression (encoding urokinase plasminogen activator) is seen in the injured ACL, suggesting a local activation of plasmin at the site of clot formation

(23). One could hypothesize that this early loss of a provisional scaffold or bridge between the two torn tissue ends might play a significant role in the failure of primary repair of the ACL. Subsequent research was focused on the development of a biologically active scaffold that is placed between the torn tissue ends to stabilize the blood clot, prevent premature fibrinolysis and thereby facilitate healing repaired ACL. Those studies will be detailed in the next section.

BIOLOGIC ENHANCEMENT OF PRIMARY ACL REPAIR

Multiple growth factors have been evaluated in vitro and in vivo for ACL healing. Transforming Growth Factor Beta 1 (31), Fibroblast Growth Factor 2 (FGF-2) (32, 33), Growth and Differentiation Factor (GDF) 5 (33) and GDF-7 (33) have been shown to stimulate type I collagen production in ACL-derived cells in vitro, whereas Insulin-like Growth Factor I (34) did not result in substantial increases. In vivo studies by Kobayashi et al. showed that FGF-2 improved healing and neovascularization of partially lacerated ACL in canines (35). In addition, localized injection of recombinant human hepatocyte growth factor yielded improved biomechanical results in a rabbit model for ACL reconstruction (36).

However, delivery of individual growth factors to the ACL wound site in a localized and sustained fashion has been difficult in vivo. This has been a relatively large barrier for translation into clinical use. Autologous platelets contain multiple growth factors and, when delivered as platelet-rich-plasma (PRP) or whole blood, are capable of forming a blood clot. This has resulted in interest in platelets as a potential growth factor delivery system. However, the use of PRP to stimulate ACL graft healing has had mixed results (37-41). In the porcine model using clotted PRP in the gap of a transected ACL, no beneficial effect of adding PRP was observed (42). The mixed results of PRP alone may be related to the fact that the main protein to provide structure in the clotted PRP is fibrin. In the prior section, it was noted that the synovial fluid contains a large amount of fibrin-degrading enzymes after injury. Therefore the fibrin clot containing the platelets may be prematurely dissolved in the post-traumatic or post-surgical environment, leading to the failure of isolated PRP as a system for localized growth factor delivery.

These observations led to the exploration of carriers to hold the PRP in the wound site of the ACL and protect it from early degradation. Collagen, when mixed with fibrin, formed a copolymer which is more resistant to degradation by plasmin (30). Further, collagen has been found to activate platelets in a sustained fashion, allowing the release of the platelet-associated factors over 10 to 14 days (43). In contrast the physiological platelet activator, thrombin, results in a bolus release of all factors within the first few hours (43). Thus, collagen has been explored as a carrier for platelets for ACL repair.

Supplementation of a primary repair of ACL in pigs with collagen-PRP hydrogel improved the biomechanical properties of the healing ACL after four weeks in vivo, when compared with suture repair alone (44). The use of a collagen scaffold (instead of a hydrogel) soaked with whole blood to deliver platelets in combination with a novel bio-enhanced primary repair technique using a suture stent (Figure 3) resulted in biomechanical properties of the

repaired ACL equivalent to an ACL reconstruction after 3, 6 and 12 months of healing (45-49). Neither the collagen scaffold itself nor PRP alone were found to be effective in promoting the ACL healing or repair (42, 50). Further, this novel technique of bio-enhanced repair prevented the development of the cartilage lesions, which were seen 12 months after untreated ACL transection and ACL reconstruction (46) (Figure 4). Those findings appear promising and suggest that bio-enhanced repair of the ACL may help to minimize the premature development of osteoarthritis which is currently observed after ACL injury and reconstruction in human patients (51-54). The complete effects of collagen-platelet composites on the intraarticular tissues and their interaction are unknown and further investigations are ongoing to define the underlying mechanism of cartilage protection.

While a collagen-platelet composite is sufficient as provisional scaffold to allow healing of an immediately repaired ACL, this technique was less effective when the repair was performed with a delay of 2 or 6 weeks (55). This might be due to undamped apoptosis, inflammation and/or matrix degradation within the ACL after injury. To further define the biologic mechanism behind this finding, researchers have characterized the biological response of the anterior cruciate ligament to injury, as well as the intraarticular milieu in which the healing would need to take place. Within the ligament, fibroblasts undergo nitric oxide induced and Caspase-9 mediated apoptosis early after injury (56-59). Further, an increase in protein fragments indicating type I collagen breakdown is detected (60) and those fragments were localized in the injured ACL in subsequent animal experiments (24). Those findings suggest a degradative environment with high activity of matrix metalloproteinases (24). These are enzymes that break down collagen proteins and were shown to be produced by the synovial membrane, as well as the injured ligament after an ACL injury (23-26, 61, 62). In addition, several studies have demonstrated elevated levels of proinflammatory cytokines in the synovial fluid after ACL injury (63-68) and experimental evidence suggest that inflammation is a driver of apoptosis (57) and matrix degradation (69). Among others, these processes are thought to be contributing to the development of posttraumatic osteoarthritis as well (70, 71), which is not within the scope of this review.

Future biological enhancement could address the synovial fluid milieu in order to counteract these processes. Potential strategies could be derived from studies aiming at lowering cartilage breakdown in the knee joint through the injection of therapeutics. These strategies may include Caspase inhibition to decrease fibroblast apoptosis (72), the application of antagonists to inflammatory cytokines (e.g. Interleukin 1 receptor antagonist) (73) or reducing metalloproteinase activity (74, 75). In the context of ACL repair, a short-term intraarticular treatment with these therapeutics might impede the above mentioned detrimental biological processes that initiate ligament and cartilage degradation after ACL injury.

SUMMARY

In summary, repair of the ACL with sutures is a procedure that has been largely supplanted by ACL reconstruction, primarily due to the unreliability of the outcomes of the original procedure in the hands of most surgeons. ACL repair in these patients most likely failed due to an insufficient approximation of the torn ends, resulting in a gap that prevented the

ligament from healing. In the MCL, that heals successfully, the gap is filled with a blood clot, or provisional scaffold, that allows surrounding cells to invade and produce a functional fibrovascular scar. In contrast, the formation of a bridging clot is not observed in the ACL, likely due to premature dissolution of that scaffold by enzymes in the synovial fluid. Without healing of the tissue itself, the sutures of a primary repair eventually fatigue and fail. Surgical placement of a scaffold which can stabilize a provisional clot in the gap between the torn ACL ends for several weeks has recently been found to result in a healing ACL with similar mechanical properties to an ACL reconstruction and less development of post-traumatic osteoarthritis in preclinical models. While this bio-enhanced ACL repair has thus shown promise in preclinical studies, clinical data is not yet available, since the technique has only recently been FDA approved for a first-in-human clinical safety study. Therefore it remains to be seen if biologically active scaffolds that fill the gap between the torn tissue ends may one day make bio-enhanced repair of the ACL a viable option for patients with ACL injuries.

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Figure 1.

(A) Method of surgical ACL repair by Feagin et al. (Published with permission from Feagin et al. 1976). A figure eight suture is made in the ligament, the suture is then passed through drill holes in the lateral condyle and secured. (B) Method of surgical ACL repair by Marshall et al. (Published with permission from McCulloch et al. 2007). Individual suture loops are threaded each ACL stump. The tibial suture is then passed through a femoral, while the femoral suture is passed through a tibial drill-tunnel and secured.

Figure 2.

FGF-2 expression within the ligament defect at day 7 after defect creation. (A) untreated ACL defect remains empty. (B) MCL defect has filled with tissue, which is releasing FGF-2. Immunohistochemistry for FGF-2 where a red stain is positive. (x100). (Published with permission Murray et al. 2005)

Figure 3.

Scheme of bio-enhanced ACL repair. (A) Defect model. (B) Femoral and tibial tunnels (dashed lines) and EndoButton pulled through femoral tunnel and placed on femoral cortex. The EndoButton is loaded with 3 sutures, resulting in 6 free-ending strands (4 red and 2 green). (C) A Kessler suture is placed in the tibial ACL stump, and a collagen scaffold is threaded onto 4 strands (red), pushed into the notch, and saturated with 3 mL of autologous blood. (D) The 4 suture strands running through the scaffold (red) are passed through the tibial tunnel, while the remaining suture (green) is tied to the tibial Kessler suture, using it as a pulley to reduce and stabilize the tibial ACL stump. (E) The transtibial sutures (red) are tightened and tied over an extracortical button. The free ends of the ACL suture pulley (green) are knotted to secure the reduced ACL in the collagen-platelet composite. (Published with permission from Vavken et al. 2012)

Figure 4.

The distal femoral cartilage 1-year after an untreated ACL transection (ACLT), conventional ACL reconstruction (ACLR), and bio-enhanced ACL repair (BE-Repair). Note the damage to the medial femoral condyle in the untreated and ACL reconstructed knees and the lack of damage in the medial femoral condyle in the bio-enhanced ACL repair (black arrows).

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