Bench-to-Bedside: Bridge-Enhanced Anterior Cruciate Ligament Repair

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ABSTRACT: Anterior cruciate ligament (ACL) injuries are one of the most well-known orthopaedic injuries and are treated with one of the most common orthopaedic procedures performed in the United States. This surgical procedure, ACL reconstruction, is successful at restoring the gross stability of the knee. However, the outcomes of ACL reconstruction can be limited by short and long-term complications, including muscle weakness, graft rupture, and premature osteoarthritis. Thus, new methods of treating this injury are being explored. This review details the pathway of how a tissue engineering strategy can be used to improve the healing of the ACL in preclinical studies and then translated to patients in an FDA-approved clinical study. This review paper will outline the clinical importance of ACL injuries, history of primary repair, the pathology behind failure of the ACL to heal, pre-clinical studies, the FDA approval process for a high risk medical device, and the preliminary results from a first-in-human study. © 2017 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 35:2606–2612, 2017.

Keywords: knee ligament; biomaterials, repair and tissue engineering; surgical repair; cell biology

CLINICAL IMPORTANCE OF ACL INJURIES

ACL reconstruction, the current gold standard of care for ACL injuries, is one of the most common orthopaedic procedures performed in United States with estimates reaching 250,000 procedures performed annually.[1,2] Adolescents and younger individuals are at increased risk for ACL injuries, and the incidence in males and females is highest between ages 15 and 34.[3] ACL reconstruction is an excellent procedure for restoring gross stability to the knee. However, there are often both short and long-term adverse sequelae. The sequelae include relatively high graft failure rates, particularly in adolescent patients,[4–6] and that the ACL reconstruction procedure does not prevent the premature onset of osteoarthritis seen in patients after an ACL injury.[7–9] Specifically, one study showed that 78% of patients developed post-traumatic osteoarthritis at 14 years after ACL injury regardless of whether or not they received ACL reconstruction.[10] Thus, novel solutions and new innovations are needed for ACL injuries.

Conflicts of interest: Dr. Murray reports grants from NIH, during the conduct of the study; In addition, Dr. Murray is an inventor listed on patents held by Boston Children's Hospital in the area of ligament repair. Drs. Murray and Fleming recently founded a company (Miach Orthopaedics Inc) in an effort to translate the scaffold to clinical use.
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HISTORY OF PRIMARY REPAIR

Primary repair, where the torn ends of the ACL were sutured back together, was previously used to treat ACL ruptures. However, this technique was met with limited success.[11] Long-term results demonstrated persistent symptoms, progressive deterioration of knee joint laxity,[12] and high failure rates at 5 and 7 years follow-up.[13,14] Primary repair of the ACL was not shown to produce better outcomes when compared to conservative treatment in two prospective randomized controlled trials.[15,16] At the same time, ACL reconstruction with a tendon graft became the accepted standard of care as it had more predictable outcomes and did not need to be performed immediately after the injury. Despite the thousands of studies that have been performed to improve ACL reconstruction outcomes, the major limitations of muscle weakness, graft rupture, and osteoarthritis persisted. This has led to investigations into alternate sources of tissue for reconstruction, including the use of allograft tissue[17] and tissue engineered graft substitutes.[18] These tissue engineered substitutes have included synthetic based materials (e.g., carbon fiber, polypropylene, Dacron, and polyester),[19,20] as well as natural materials including collagen fibers,[21] silk,[22] and chitosan.[23] In addition, cell-based strategies, where ACL cells are cultured and form a sheet that can then be used as a substitute graft[24] are also under development.

While the research into alternate tissue engineering strategies for ACL replacement or reconstruction has been plentiful, our interest is not to engineer a graft to replace the torn ACL, but instead to enhance healing of the native ACL. Approaches to ACL healing have included suture repair[12] and microfracture[25] with better success seen for proximal ACL injuries at the ligament-bone junction.[14,26] However, proximal ACL tears are only found in a small percentage of injuries and the need for a more universally applicable technique of repair would still be of interest. This led to the question

of whether there might be a way to stimulate healing of the ACL using tissue engineering techniques and thus avoid needing to replace the torn tissue with a graft.

THE PATHOLOGY BEHIND THE FAILURE OF THE ACL TO HEAL AND PRECLINICAL STUDIES

To identify a combination of scaffold, cells, and/or growth factors that might be useful to stimulate ACL healing, the pathology and mechanisms behind the failure of the ACL to heal had to be determined. As other ligaments around the knee joint, including the medial collateral ligament (MCL), readily heal without suture repair, comparing the response to injury of the ACL with that of the MCL might facilitate the identification of the defects in the ACL healing process that would need to be addressed by a tissue engineered construct. Work by Frank et al. demonstrated that MCL wound healing occurs via a cascade of events initiated at time of injury that continues for months after injury. These events include provisional scaffold formation, cell migration into the wound site, cell and vessel proliferation in the wound site, and production of a collagen scar mass.[27,28] Other investigators have used the MCL as a model to study the enhancement of ligament repair using growth factors.[29] While the biology was altered by adding TGF-β or other growth factors, the mechanical properties of the MCL were not affected. It was of interest to determine where this orderly and predictable process of wound healing is deficient for the ACL. It was found that three of these processes (cell/vessel proliferation, cell migration, and collagen production) occurred in the injured ACL; however, the formation of a provisional scaffold at the wound site of the ACL was not observed (Fig. 1).[30–33]

The observation that a provisional scaffold can form in the MCL wound site, but not in the ACL wound site, may be due in part to the differences in the environment in which the MCL and ACL reside. The MCL is an extra-articular ligament. When an MCL injury occurs, the ends of the ligament bleed and deliver fibrinogen to the defined wound space, which is limited by the tissue planes on each side. The fibrinogen is cleaved by thrombin to its active form, fibrin, which crosslinks to form a stable clot, resulting in a provisional scaffold which entrapes platelets, white blood cells, and red blood cells within the wound space. This provisional scaffold can then serve as a protected space into which the surrounding tissues can grow and reconnect. In comparison, the ACL is an intra-articular ligament that is surrounded by synovial fluid containing protease precursors, including plasminogen. Plasminogen is inactive in the uninjured joint; however, with injury there is an upregulation of urokinase plasminogen activator (uPa) production by the synoviocytes,[34,35] which cleaves plasminogen and forms plasmin. When the ACL tears, the ends of the ligament bleed and the fibrinogen comes into the joint. However, the presence of active plasmin in the synovial fluid results in fibrinolysis resulting in an inability to form a fibrin-platelet provisional scaffold, or "bridge," between the two torn ligament ends.[36] This lack of a "bridge" at the wound site for the ruptured ACL, in combination with the observation that many of the other biologic processes required for wound healing were already occurring in the ACL, led to the hypothesis that placement of a substitute provisional scaffold between the torn ends might provide a space for wound healing to occur.

THE DEVELOPMENT OF THE SCAFFOLD

Thinking that placement of a substitute provisional scaffold might facilitate ACL healing, the next set of studies focused on what the scaffold should contain to mimic the function of the provisional scaffold of the MCL. There are two factors that need to be considered when creating a substitute provisional scaffold; (i) the ability to stimulate cell ingrowth and proliferation, and (ii) resistance to synovial fluid degradation. Early in vitro studies showed that while individual growth factors could be helpful for stimulating cell proliferation, the effects were modest, even in vitro.[30,31,37–39] In other connective tissues, the provisional scaffold activates the entrapped platelets, stimulates additional plasma proteins and activates white blood cells.[40–43] The complex interplay of these functions results in the change of thousands of genes in the first few days after injury.[44] Thus, it remains a complex engineering task to replicate this process with the individual addition of growth factors or proteases to a scaffold.

The blood cells, including platelets, leukocytes, and erythrocytes, that are trapped in the provisional scaffold are known sources of multiple cytokines and proteases. As these cytokines and proteases play a role in soft tissue healing, they thus become attractive as biologic additives to a collagen-based scaffold for ACL healing. If it were possible to stimulate the function of

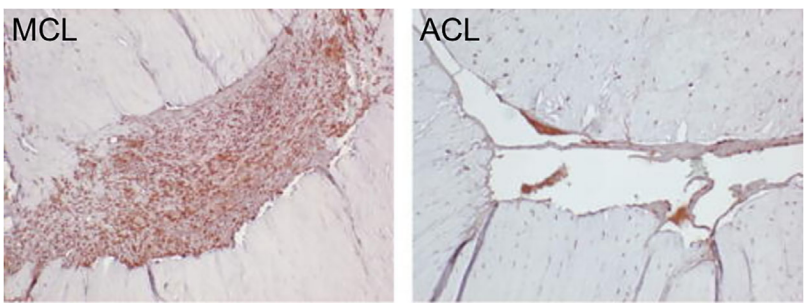

Figure 1. The primary defect for healing of intra-articular injuries. Wounds for tissues outside of the joint (like the MCL) fill with a bioactive fibrin clot after injury. In contrast, wounds inside the joint (intra-articular, like the ACL) fail to form this provisional scaffold and, therefore, are missing a key component of successful wound healing. The wound remains open, and healing cannot occur (Used with permission from Murray and Spindler[67]).

one or more of these cell types in the ACL wound site, perhaps a wound healing response similar to that in the MCL could be encouraged. Platelets in particular are carriers of multiple growth factors important in wound healing, and are known to be a critical first step in the wound healing cascade.[45] Thus platelet rich plasma seems to be a likely candidate for stimulating healing. However, there are many different forms of platelet-rich plasma (PRP), some enriching the platelet component to as high as five times the level found in whole blood; however, it was unclear what composition of PRP would be most advantageous for the ACL. In vitro studies confirmed that using higher concentrations of platelets resulted in a higher release of anabolic growth factors,[42] suggesting these enriched preparations might be of use in ACL healing as well.

To test the hypothesis that delivering a biologically active substitute scaffold (PRP) to the ACL wound site could stimulate functional ACL healing, 30 kg pigs underwent bilateral ACL transection where one side was treated with suture repair + PRP and the contralateral side was treated with suture repair alone. The outcomes, including the mechanical properties of the repaired ACL, were compared at 14 weeks after surgery. Disappointingly, the results demonstrated there was no difference between groups, that PRP was not sufficient to enhance functional healing after suture repair of the ACL.[46] One possible reason for failure of the PRP to improve the ACL repair results may have been premature dissolution of the PRP in the synovial fluid. PRP, like blood, is fibrin based, and thus the synovial joint fibrinolysis system[36] may also result in premature dissolution of the PRP clot. This may be one of the factors behind the failure of use of PRP to improve graft healing.[47,48]

This led to the next hypothesis, namely that if a carrier could be identified that could minimize the early degradation of the platelet-rich plasma clot and maintain it in the wound site long enough, that ACL healing could be possible. Prior investigators had reported that when fibrin is combined with collagen, they form a copolymer that is resistant to degradation by plasmin.[49] In vitro studies demonstrated that collagen-based scaffolds, compared to fibrin-based scaffolds, had greater resistance to degradation by synovial fluid enzymes including MMP-1, elastase, and plasmin.[50] Thus, a collagen-containing scaffold appeared to be a reasonable candidate for serving as the carrier for the biologic platelet-rich plasma. Additional work to optimize the suture construct to support the healing ligament was also performed,[51,52] and subsequently replicated by other investigators.[53,54]

To test the hypothesis that combining a collagen-based scaffold with PRP would result in improved ACL healing, bovine tissue was processed to form a porous, hydrophilic, collagen-based scaffold. This collagen-based scaffold was then loaded with PRP and placed in the gap between the ligament ends in a complete transection model of ACL repair. This "bridge-enhanced ACL repair" technique resulted in equivalent mechanical outcomes of the repaired ACL and the reconstructed ACL at 15 weeks after surgery.[55] Other groups have replicated these results using a similar extracellular matrix based material in the goat model.[56] Additional in vitro work led to the finding that both platelets and plasma proteins were important in stimulating ACL cells to heal,[40–42] red blood cells improved collagen production by fibroblasts in a simulated wound site[57,58] and white blood cells released anabolic growth factors.[43] This led to the observation that all parts of blood may play a role in wound healing. Additional in vitro and in vivo studies comparing PRP and whole blood as the biologic supplement to the collagen-based scaffold and blood combination revealed that increasing the platelet count or otherwise manipulating whole blood did not improve outcomes.[59,60] Therefore, whole blood as the biologic stimulus for ACL repair seemed reasonable and was selected for the longer-term in vivo studies for scaffold-enhanced ACL repair.

Long-term results, at 6 and 12 months after surgery, of in vivo testing of the collagen-based scaffold combined with whole blood in a large animal model showed that the repaired ACLs using the scaffold had similar mechanical properties to ACLs treated with ACL reconstruction at both time points.[61,62] In addition, the use of the whole blood with a collagen-based scaffold resulted in significantly less development of osteoarthritis of the porcine knee at 1 year following surgery[61] (Fig. 2). The mechanisms of the chondroprotection are currently under investigation.

TRANSLATION FROM BENCH-TO-BEDSIDE

With the results of the long-term studies demonstrating similar mechanical properties for the scaffold-enhanced ACL repair and ACL reconstruction with a graft, the next step was to complete the testing required by the FDA to translate it to clinical trials. The collagen-based scaffold was given a device designation and guidance for the studies required for an Investigational Device Exemption (IDE) for a first in human study was provided by the Center for Devices and Radiologic Health (CDRH) of the FDA. Details of this process for an academic laboratory has been previously described in detail.[63]

PRELIMINARY RESULTS FROM A FIRST IN HUMAN SAFETY STUDY

Pre-clinical studies validating sterility and efficacy in large animal models were completed,[63–65] and an IDE approval for a first-in-human study of 20 patients was granted. Institutional approval and registration of the study on Clinical Trials.gov (NCT 02292004) were completed before opening the study to enrollment.

The first-in-human study was designed to primarily assess the safety of the collagen-based scaffold. As all previous studies had been done in animals and we

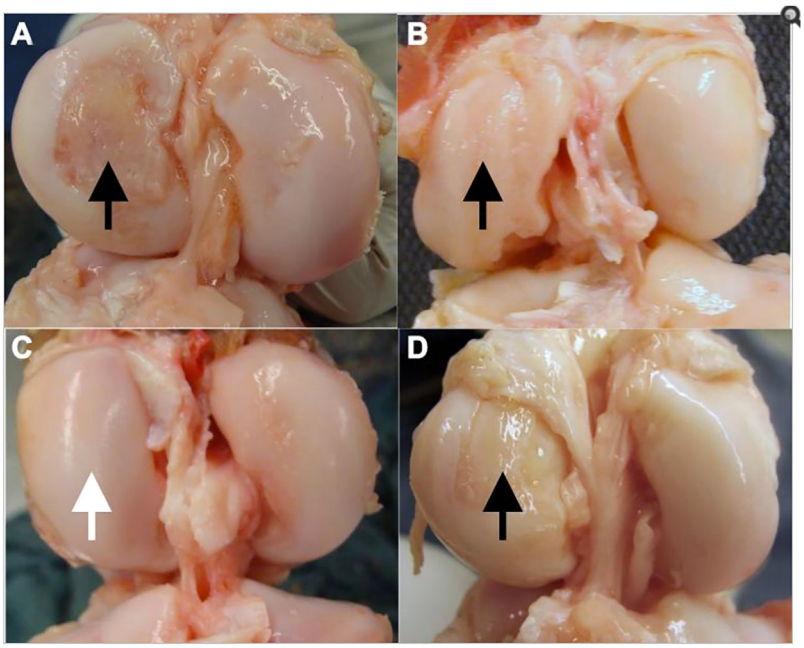

Figure 2. The distal femur cartilage 1-year after (A) an untreated ACL rupture, (B) after conventional ACL reconstruction, (C) after bridge-enhanced ACL repair, and (D) after bridge-enhanced ACL reconstruction. Note the damage to the medial femoral condyle in the untreated, ACL reconstructed knees, and bridge-enhanced ACL reconstructed knees (black arrows), and the lack of damage in the medial femoral condyle in the bridge-enhanced ACL repair and bridge-enhanced ACL reconstructed knees (white arrow)[61] (Used with permission from Murray and Fleming).

were aware of the fact that the intra-articular environment is complex with regard to its response to implanted materials, this study was designed to determine the rate of significant adverse reactions to the implanted scaffold. As safety was the primary study metric, the primary hypothesis was that the implanted scaffold would not result in deep joint infection or significant inflammation. In order to generate additional data that would help tailor future study designs, additional secondary outcomes aimed at efficacy were also included, including anterior-posterior (AP) laxity of the knee, patient reported outcomes, and muscle strength. Twenty patients were enrolled in the non-randomized, first-in-human safety study where ten patients received the collagen-based scaffold and ten patients received a hamstring autograft ACL reconstruction. The collagen-based scaffold was used in conjunction with primary suture repair of the ACL. The scaffold was activated using 10 cc of autologous blood placed on it at the time of repair (Fig. 3).

As the collagen-based scaffold was previously found to be reabsorbed by 8 weeks after in vivo implantation,[65] the primary safety outcomes for all patients were assessed specifically for the first 3 months postoperatively as specified in the pre-study protocol (NCT 02292004). The 3 month results found there were no joint infections or significant inflammation in either group, no differences between groups in effusion or pain, and no failures as determined by Lachman exam criteria. Magnetic resonance images (MRIs) from all patients in both groups demonstrated a continuous ACL or intact graft. The only statistically significant

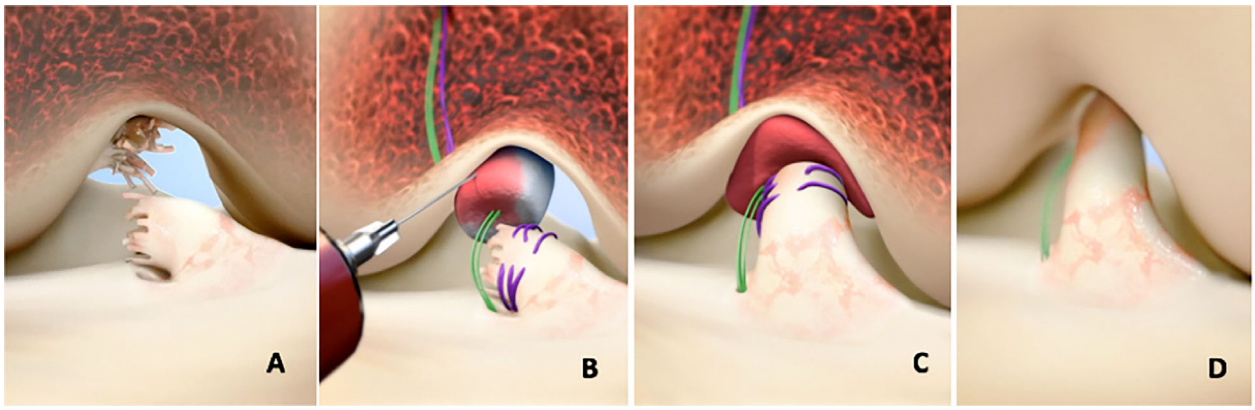

Figure 3. Stepwise demonstration of the "Bridge-Enhanced ACL repair" technique using the collagen-based scaffold. In this technique, the torn ACL tissue is preserved (A). A whip stitch of #2 absorbable suture (purple suture) is placed into the tibial stump of the ACL. Small tunnels (4 mm) are drilled in the femur and tibia and an extracortical button with two #2 nonabsorbable sutures (green sutures) and the #2 absorbable ACL sutures attached to it is passed through the femoral tunnel and engaged on the proximal femoral cortex. The nonabsorbable sutures (green) are threaded through the scaffold, tibial tunnel and secured in place with a second extracortical button. The collagen-based scaffold is then saturated with 5 ml of the patient's blood (B), and the tibial stump pulled up into the saturated scaffold (C). The ends of the torn ACL then grow into the collagen-based scaffold and the ligament reunites (D) (Used with permission from Murray et al.[66]).

difference between the groups was that the hamstring strength at three months was significantly better in the group treated with the collagen-scaffold and suture repair than in the hamstring autograft group (mean ± SD: 77.9% ± 14.6% vs. 55.9% ± 7.8% of the contralateral side, $p < 0.001$).[66] The results of this first study demonstrated that the rate of adverse reactions may be low enough to warrant additional efficacy studies in a larger cohort of patients. A 100-patient randomized control trial is currently enrolling patients at Boston Children's Hospital (NCT 0264545).

CONCLUSION

Injuries to the ACL are a common problem and often result in adverse long-term health problems with currently available treatment. While previous human studies have shown limited utility in primary repair of the ACL, a new procedure that augments suture repair of the ACL with the collagen-based scaffold has shown success in vivo studies and is currently being evaluated in clinical trials. This review serves as a summary of how tissue engineering technologies can be translated from the laboratory to clinical trial by an academic laboratory.

AUTHORS' CONTRIBUTIONS

GSP, BLP, AMK, JTS, BCF, and MMM contributed to literature review and manuscript preparation. All authors have read and approved the final submitted manuscript.

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