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■ INSTRUCTIONAL REVIEW: KNEE
Basic science of anterior cruciate ligament injury and repair

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Injury to the anterior cruciate ligament (ACL) is one of the most devastating and frequent injuries of the knee. Surgical reconstruction is the current standard of care for treatment of ACL injuries in active patients. The widespread adoption of ACL reconstruction over primary repair was based on early perception of the limited healing capacity of the ACL. Although the majority of ACL reconstruction surgeries successfully restore gross joint stability, post-traumatic osteoarthritis is commonplace following these injuries, even with ACL reconstruction. The development of new techniques to limit the long-term clinical sequelae associated with ACL reconstruction has been the main focus of research over the past decades. The improved knowledge of healing, along with recent advances in tissue engineering and regenerative medicine, has resulted in the discovery of novel biologically augmented ACL-repair techniques that have satisfactory outcomes in preclinical studies. This instructional review provides a summary of the latest advances made in ACL repair.

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Introduction
Dynamic knee stability is affected by both passive (ligamentous) and active (neuromuscular) joint restraints. Among the contributors to knee joint stability, the anterior cruciate ligament (ACL) has long been considered the primary passive restraint to anterior translation of the tibia with respect to the femur.[1,2] Moreover, the ACL contributes to knee rotational stability in both frontal and transverse planes due to its specific orientation.[3,4] The ACL has been the focus of many biomechanical/anatomical studies and is among the most frequently studied structures of the human musculoskeletal system over the past decades.

Injuries to the ACL are one of the most common and devastating knee injuries mainly sustained as a result of sports participation.[5] These injuries often result in joint effusion, altered movement, muscle weakness, reduced functional performance, and may lead to the loss of an entire season or more of sports participation among young athletes.[5] ACL injuries are also associated with long-term clinical sequelae that include meniscal tears, chondral lesions and an increased risk of early onset post-traumatic osteoarthritis (OA).[3,6-10]

The ACL has long been thought to have poor healing capacity, with a substantially high rate of failure (40% to 100%), even after surgical repair using suture.[11-17] The unsatisfactory outcomes of the ACL primary repair have led to unanimous abandonment of suture repair and widespread adoption of ACL reconstruction. ACL reconstruction has remained the gold standard of care for ACL injuries, especially for young individuals and athletes who aim to return to high-level sporting activities.[5,18] However, current surgical treatment of ACL injury is costly, with variable outcomes[5] and is associated with high risk of post-traumatic OA within two decades of injury.[9,19] While few athletes are able to resume sports at the same level without surgery,[5] the surgical reconstruction is also not always successful at returning patients to their pre-injury activity level.[20] Furthermore, those athletes who successfully return to activity are at high risk of a second knee injury[21] with notably less favourable outcomes.[22]

Recent advancements in functional tissue engineering and regenerative medicine have resulted in a renewed interest in revisiting ACL repair. The promising use of novel biological/tissue engineering techniques, including growth factors, stem cells and bio-

Table I. Gender-specific rates of injury to the anterior cruciate ligament based on sports type

Authors	Sports	Level of competition	ACL injury rate (female/male)
Renstrom et al[30]	Basketball	Collegiate	3.3
Arendt et al[24,25]	Basketball	Collegiate	4.1
Messina et al[28]	Basketball	High school	3.0
Renstrom et al[30]	Soccer	Collegiate	2.5
Arendt et al[24,25]	Soccer	Collegiate	2.3
Lindenfeld et al[27]	Soccer	Youth	3.0
Stevenson et al[31]	Alpine skiing	High school	3.1
Myklebust et al[29]	Handball	-	5.0
Renstrom et al[30]	Lacrosse	Collegiate	1.4
Renstrom et al[30]	Ice hockey	Collegiate	2
Gwinn et al[26]	Military training	Collegiate	9.7

scaffolds, has been the focus of current research in ACL healing and repair. The increased number of recently published pilot clinical and basic research studies has prompted our current review of the literature, exploring the recent knowledge and indications for clinical use of these biologically enhanced techniques. In this article, we present the latest research on the biology of ACL healing and repair supplemented by a brief overview of ACL injury epidemiology, mechanism and current standard of care. Future work in this area may lead to the improvement of the current techniques along with development of novel approaches to treat this critical injury with enhanced short-term and long-term outcomes.

Search strategy and selection criteria
For the purpose of this literature review, peer-reviewed journals were consulted and the findings summarised to provide an understanding of the information gained from the current literature. Studies were identified by searching the MEDLINE, CINAHL and SPORTDiscus electronic databases. The last search was undertaken on September 15 2013. The following search terms were used: "Anterior Cruciate Ligament AND Injury", "ACL AND Injury", "Anterior Cruciate Ligament AND Healing", "ACL and Healing", "Anterior Cruciate Ligament AND Repair" and "ACL AND Repair". Searches were repeated using the keywords as MeSH terms as well.

The search algorithm was intentionally general to maximise return. In addition to the online searches, the bibliographies of the included studies were reviewed to identify additional publications. No date limits were considered for the publications on ACL healing and repair. However, literature covering the injury epidemiology, mechanism and surgical reconstruction were deemed either seminal published works or publications after 2010. The citations identified from the searches were combined and duplicates excluded.

All *in vivo* and *in vitro* studies that focused on ACL repair following injury, not reconstruction, were considered. All titles resulting from the search criteria were reviewed and those that clearly referred to a topic other than the focus of current review were excluded. All case reports and expert opinions were excluded. Abstracts were also reviewed to confirm inclusion eligibility. Finally, full texts were obtained for the eligible studies for final review.

ACL injury epidemiology
The ACL is one of the most frequently injured ligaments of the knee, with a prevalence estimated to be 1 in 3000 in the United States (greater than 120 000 cases annually).[23] Despite trivial injury incidences in the general population, ACL injury frequently affects young, active individuals, and females are at a reported two- to ten-fold greater risk than males playing the same sport (Table I).[24-31] High risk of injury along with the high rate of sports participation among girls and young women over the last three decades has led to a rapid rise in ACL injuries in females. ACL injuries are mainly associated with other concomitant articular injuries, and may result in an increased risk of early onset post-traumatic OA at ten to 15 years post-injury (as high as 80%), especially in the presence of concomitant meniscal damage.[6,7,9,32]

In addition to pain, instability and associated long-term sequelae, ACL injury may affect the athletes' quality of life economically as well as socially.[7,32] Using a conservative cost estimate of between USD $17 000 and $25 000 per patient for surgery and rehabilitation, the estimated cost for treatment in ACL injured patients in the United States is over $1.7 billion annually. This estimate does not consider the resources necessary for non-surgical treatment, or to treat the long-term complication of post-traumatic OA associated with both the ACL-injured and ACL-reconstructed knee.[33] Moreover, patients who have suffered an ACL injury face long-term consequences that include lowered activity levels, high risk of re-injury and long-term disability due to post-traumatic OA.[5-7,9,21,32]

Injury mechanism
More than 70% of ACL injuries occur as non-contact (without a direct blow to the knee joint).[2-4] They occur as a result of landing from a jump and lateral cutting manoeuvres that may occur in different athletic activities such
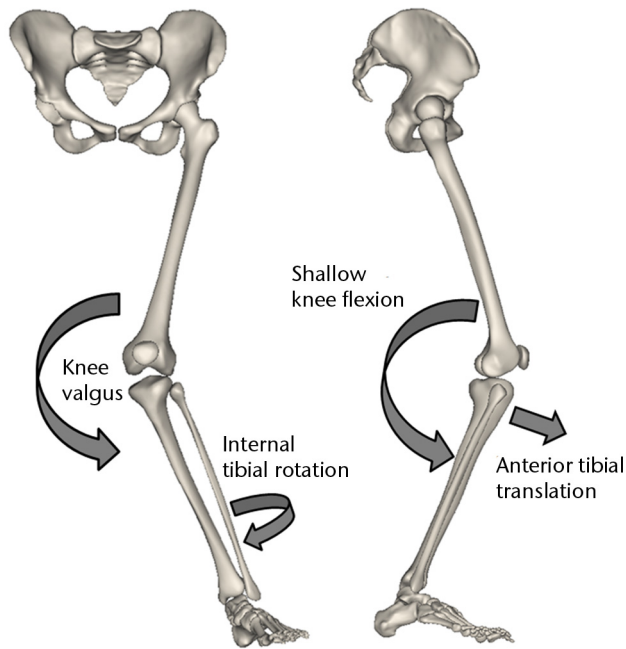
Fig. 1

Schematic showing the multi-planar loading mechanism of non-contact injury to the anterior cruciate ligament (Adapted and modified with permission from Levine et al[3]).

as basketball and soccer.[24-31] Over the past twenty years, a myriad of research has examined potential mechanisms and associated risk factors for ACL injury using *in vivo*, *ex vivo* and *in silico* techniques.[1-4,20,21,24-31,34-36]

Neuromuscular control deficit during dynamic movements has been hypothesised to be the primary cause for both primary and secondary ACL injury risk (re-injury following ACL reconstruction).[5] The deficit in dynamic active neuromuscular control manifests as excessive joint loads and leads to detrimental ACL stress/strains and ultimate failure. Non-contact ACL injury mechanisms are multi-planar in nature, involving the tibiofemoral joint articulation in all three anatomical planes.[3,4,36] Previous studies have identified combined multi-planar loading including anterior tibial shear, knee valgus and internal tibial rotation to be the worst case scenario and primary mechanism of non-contact ACL injury (Fig. 1).[3,4]

Treatment options

ACL repair by re-approximating the two ends of the ruptured ligament using suture was one of the earliest suggested methods described for treatment of ACL injuries. It was in early 1900s that Robson[37] described the primary repair of ACL, a technique that was later studied and documented in detail by O'Donoghue et al.[14,38] Feagin[11] and Cabaud et al[39,40] were the first to report the long-term outcomes of primary ACL repair in the 1970s. Feagin[11] showed

that the primary repair of ACL failed in over 90% of patients in a five year follow-up study. These findings were further backed up by the observations of Sandberg et al[15] showing no difference in outcomes after primary repair *versus* conservative treatment in a randomised controlled trial. The high rates (40% to 100%) of the ACL failure to heal, even with surgical repair,[11,12,14-17] have led to abandonment of suture repair and almost universal adoption of ACL reconstruction for treatment of ACL injuries.

In ACL reconstruction, the torn ACL tissue is removed from the knee surgically and replaced with an allo- or autograft tendon taken either from the medial hamstrings or the middle third of the patellar tendon. Although ACL reconstruction has become the current gold standard for restoring the gross stability of a symptomatic ACL-deficient knee, significant problems persist. In the short term, conventional ACL reconstruction fails to restore the normal joint kinematics and kinetics.[41,42] This alteration in joint mechanics has been mainly associated with non-anatomic ligament insertion (location and geometry) and alignment, loss of tissue neurosensory function (proprioception), graft-tissue degeneration and neuromuscular deficit.[43-45] Many studies have shown significantly greater translational and rotational laxity of the reconstructed knees relative to the contralateral uninjured sides, regardless of the graft type.[46-49] Additionally, reconstruction requires tissue harvest from the knee (autograft), which is associated with tissue morbidity. Alternatively, using allografts is associated with high risk of biologic incorporation failure and disease transmission in addition to financial and tissue availability complications. Most importantly, patients remain at high risk for development of early onset OA even after surgical reconstruction. This risk has been reported to be between 66% and 100%.[6,7,9,19] A meta-analysis of 33 clinical follow-up studies reported that ACL reconstruction was unable to slow the premature onset of OA following ACL tear.[50]

Over the last decade, substantial effort has been made to make the surgical reconstruction more anatomical by altering tunnel position and introducing the concept of a double-bundle reconstruction.[17,51,52] This evolution in ACL reconstruction has resulted in an improved joint translational and rotational stability closer to the intact knee, compared with conventional, non-anatomic single-bundle reconstruction.[53-56] However, no consensus has been reached on the improved clinical outcomes of anatomic double-bundle reconstruction over the traditional single-bundle technique.[53,57-62] A recent randomised trial of 130 patients with a minimum four-year follow-up have reported that although anatomic double-bundle reconstruction results in improved IKDC score, it was not superior to the conventional single-bundle technique in preventing post-traumatic OA.[61]

The associated complications with the surgical reconstruction, despite its undeniably large success, in addition

to the advent of functional tissue engineering, precipitated increased interest in bio-enhanced ACL repair as an alternative to reconstruction.[63-65] However, development of a regenerative method for repair of the torn ACL begs an enhanced understanding of why the earlier primary ACL repair was largely unsuccessful. Over the past decade, researchers set out to understand the mechanisms that underlie the inability of an injured ACL to heal, a finding which is in direct contrast to the high healing capacity of extra-articular connective tissues like the medial collateral ligament (MCL).[63-72] Several factors have been reported to be responsible for this discrepancy in tissue healing ability including, but not limited to, the 'hostile' environment of synovial fluid,[66,73-75] alterations in the post-injury inflammatory response and cell metabolism,[67-70,72,76-84] intrinsic cell deficiencies,[71,85-92] different vascular environment,[93,94] and load bearing characteristics.[4,95]

Biologically augmented ACL repair

The improved knowledge of ACL healing characteristics has helped researchers and clinicians to introduce novel biologic ACL repair approaches. These alternatives to the current surgical reconstruction have the potential to preserve the native insertion site and proprioceptive function, which may in turn lead to more normal joint mechanics and decreased risk of post-traumatic OA. One such approach was the 'healing response technique' pioneered by Steadman et al.[96] In this technique, micro-holes within the femur near the ACL insertion site are created, leading to blot clot and subsequent haematoma formation. Ligament healing is then thought to be induced by the high concentration of the reparative cells near the torn ends of the ACL as a result of the created haematoma.[96-99] This technique has been reported to be successful in middle-age patients with very proximal ACL tears.[96] However, a recent study by Wasmaier et al[100] showed no differences between patients treated by healing response technique and patients treated conservatively with regard to Lysholm and Tegner scores, normalised joint laxity, and rate of required revision surgery. Recent integration of advanced functional tissue engineering in the area of ACL repair has left researchers with multiple novel approaches to treat ACL injuries with improved outcomes. A brief overview of these methods follows.

Cell therapy. Cell therapy using mesenchymal progenitor cells (MPCs) or mesenchymal stem cells (MSCs) has been widely studied *in vitro* and in preclinical studies within the area of sports medicine research.[101-103] MSCs harvested from mesenchymal tissues (i.e., bone marrow) can differentiate into various cell types (i.e., fibroblasts) required to regenerate different tissues such as bone, cartilage, tendon, ligament and fat.[104-111] In a rat model of partial ACL tear, Kanaya et al[112] showed that intra-articular injection of MSCs resulted in a healed ligament with superior histological scores and greater failure load compared with non-treated control knees. Lim et al[113] and Soon et al[114] have

shown similar improved biomechanics in rabbit models of ACL reconstruction using autografts and allografts, respectively, all enhanced by the application of MSCs.

In a recent study, Oe et al[115] used intra-articular injection of either fresh bone marrow cells (BMC) or cultured MSCs at one week after ACL transection in a rat model. They showed that the donor cells were located within the wound site and ACL exhibited almost normal histology, with more mature spindle cells with higher levels of transforming growth factor (TGF-β) in the BMC group. They concluded that the direct intra-articular BMC injection is an effective solution for the treatment of partial ACL tears,[115] which was in line with previous findings of Kanaya et al.[112] These findings are encouraging considering the potential of MSCs to carry and deliver therapeutic molecules in addition to the positive role of MSCs in the healing of ligaments. Despite the advantages of stem cell-based therapies, unresolved challenges exist in optimising the MSC applications in ACL repair. One such challenge is the development of proper methods to effectively differentiate these multi-pluripotent cells into specific cell types required to enhance tissue repair. Another concern is the delivery and maintenance of the stem cells within the wound site, which underscores the need for further research in this field.

Gene transfer and gene therapy. Gene transfer is a recent promising strategy to modulate durably the application of various therapeutic factors essential to the healing of injured tissues such as ligaments. Gene transfer in ligaments mainly occurs using nonviral gene delivery vectors or vectors derived from viruses with natural entry pathways in the cell (adenoviruses, lentiviruses/retroviruses) in order to alter tissue endogenous protein synthesis by mediating certain gene expression.[116-124] Such gene-based approaches may have the potential to modulate the biochemical changes following an ACL injury such as variations in collagen expression, the wound contractile α–smooth muscle actin (α-SMA) markers, and nuclear factor–kB (NF-kB) markers.[119,125,126] Hildebrand et al[127] tested the possibility of gene transfer to normal and ACL ruptured knees in a rabbit model. They concluded that adenoviral vectors are able to express more efficiently than retroviral vectors in ACL cells and can lead to a considerably long period of gene expression *in vivo* (six weeks).

In a series of *ex vivo* and *in vitro* studies, Pascher et al[122] confirmed the ability of vector-laden hydrogels in *in situ* gene delivery to the injury site for potential biological repair of the ACL. They showed increased cellularisation and collagen (I and III) deposition by *in situ* transfer of TGF-β1 using an adenoviral vector in a collagen hydrogel placed between the torn ends of the ACL.[122] The same authors further demonstrated increased deposition of collagen (I and III), elastin, tenascin, and vimentin through *in situ* transfer of insulin-like growth factor-1 (IGF-1) cDNA by an adenovirus vector in the same

model.[123] Most recently, Madry et al[119] tested the enhanced healing of the human ACL by over expression of fibroblast growth factor-2 (FGF-2) via direct recombinant adeno-associated virus (rAAV) vector–mediated gene transfer. They showed that stable FGF-2 expression using rAAV resulted in remarkable decrease in ACL lesions mainly due to increased expression of α-SMA, ligament-specific transcription factor scleraxis, and NF-kB for collagen proliferation and deposition.[119]

Despite these advantages, there are several issues that need to be considered during gene therapy. The loss or decrease of expression of the transferred gene after several weeks, especially in adenoviral vectors, is one of the major and most frequent challenges in gene therapy.[128] Safety is also a major concern using this technique, which can lead to high risks of side effects including mutagenesis.[129] Moreover, abnormal cell growth, toxicity under chronic over-expression of growth factors, and development of any malignancy are other possible side effects associated with gene-modified cell therapy. As a result this technique is a current topic of research to identify the ideal gene vectors and further optimise the current methods in an effort to overcome the difficulties associated with viral gene therapy.

Application of growth factors. The use of growth factors has gained a lot of traction in treatment of soft-tissue injuries since the late 1990s. A wide range of growth factors, including insulin-like growth factor (IGF), TGF-β, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and nerve growth factor (NGF) have been used previously to improve ligamentous and tendon tissue repair.[78,130-140] They have shown to be able to regulate and improve the cellular activities and proliferation, extra-cellular matrix (ECM) deposition, and influence the differentiation of MSCs into fibroblasts to repair the torn ligaments. In particular, these growth factors have exhibited positive effects on various biological processes needed to improve ACL healing.

Early in vitro studies by Marui et al[141] demonstrated that the application of TGF-β1 resulted in increased collagen synthesis up to 1.5 times greater than controls in both MCL and ACL fibroblasts. Kobayashi et al[142] reported the positive effect of basic-FGF (bFGF) in improved ACL tissue healing with increased vascularity compared with the control in a canine model. More recently, Kondo et al[132] studied the effect of TGF-β1 in an in vivo model of ACL injury in rabbits. They showed significant improvement of biomechanical and histological healing properties of injured ACLs treated with TGF-β1 compared with controls.

In addition to the mentioned growth factors, the use of platelet-rich-plasma (PRP), which contains a multitude of growth factors, has been the centre of attention as a novel, non-invasive treatment for sports related injuries.[143-149] PRP is a simple, efficient method of obtaining a high concentration of growth factors through separation of platelets from autologous blood. Platelets are the first cells reaching the injury site and are a substantial reservoir of critical growth factors and signaling molecules, including leukocyte-derived catabolic cytokines and fibrinogen.[150,151] This combination of bio-active agents can mediate the tissue healing process, following an injury through both the inflammatory and remodeling phases.[146,150,151] Platelets are involved in homeostasis, aggregation and clot formation steps, which finally lead to enhanced tissue healing.[150] This is done by the release of PDGF, TGF-β1, VEGF, bFGF and epidermal growth factor (EGF) through degranulation of alpha granules.[144,145] Among these growth factors, PDGF and TGF-β1 have been reported to be the most critical modulators in the healing process by contributing to increased fibroblast proliferation and collagen production.[144]

Despite the large number of research studies conducted on the role of PRP treatment on ACL reconstruction,[148,152-156] the use of PRP in ACL healing and repair is not as well considered. In a series of in vivo large animal studies, Murray et al[157-160] have reported improved ACL healing using a collagen-platelet hydrogel in an ACL central defect model. They demonstrated that the presence of collagen-platelet hydrogel in the wound site can result in release of growth factors with similar spatial and temporal sequence as healing extra-articular tissue. They further reported significant increases in tissue formation and mechanical properties following biologically augmented primary ACL repair.[157-160]

Despite these advantages, there are concerns regarding the optimised use of growth factors. One of the major concerns is the short life span of these bio-active agents, which have limited their efficacy. Delivery and maintenance of the growth factors within the wound site is another challenge using this technique for treatment of soft-tissue injuries. Therefore, safe and reproducible systems that allow sustained delivery of growth factors to the injury site are essential.

Use of bio-scaffolds. A wide range of synthetic and biologic-based scaffolds made from alginate, chitosan, collagen or hyaluronic acid have been used in functional tissue engineering and regenerative medicine.[161,162] ACL tears have been previously treated with synthetic scaffolds loaded with growth factors[142] and also with hyaluronic acid.[163,164] Wiig et al[164] reported improved healing of a ACL central defect using intra-articular injection of hyaluronic acid in a rabbit model. They showed that the group treated with hyaluronic acid showed greater angiogenic response with increased amount of reproduced type III collagen. However, these techniques are associated with critical challenges such as problems with implant–host integration, cell survival after transplantation, and short-time degradation. Alternatively, the use of collagen-based scaffolds has shown to be more effective. ACL fibroblasts have been previously shown to effectively attach, proliferate and express collagen on collage-based scaffolds.[165]
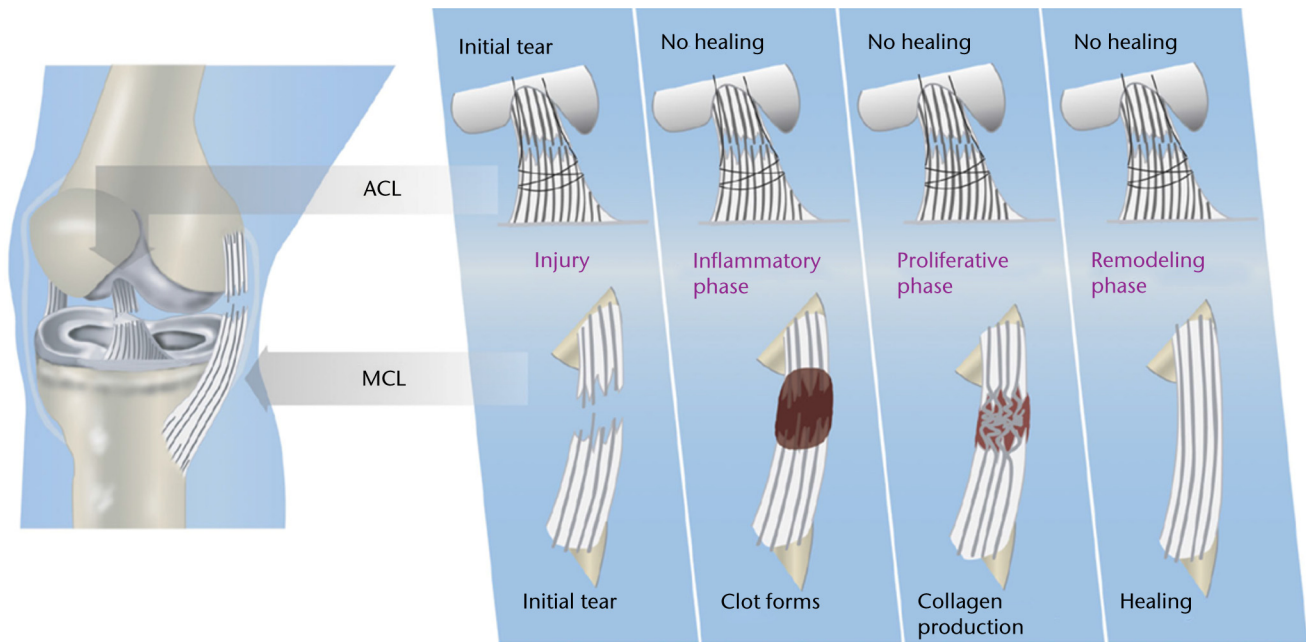
Fig. 2

Diagrams showing the differences in intrinsic healing response of the anterior cruciate ligament (ACL; top) and medial collateral ligament (MCL; bottom), highlighting the lack of provisional scaffold (blood clot) formation within the ACL wound site as the key mechanism for ACL healing failure (reproduced with permission from Murray and Fleming[63]).

Porcine small intestinal submucosa (SIS) was among the first scaffolds used to enhance the regeneration and repair of ligaments and tendons.[166-171] SIS is a collagen based (90% of dry weight) bio-absorbable scaffold which contains a small number of cytokines and growth factors such as FGF and TGF-β.[170] In addition to the collagenous structure, which works as a provisional scaffold, it can also deliver the essential supplies (i.e., FGF-2, TGF-β, VEGF and PDGF) needed for tissue healing.[166] Using a goat stifle joint model of ACL injury, Fisher et al[172] reported significant improvement in tissue mechanical and histological properties using a primary repair technique supplemented with SIS bio-scaffold and hydrogel. Using a tissue-engineered collagen-I scaffold, Robayo et al[64] demonstrated improved ACL fibroblasts activity (i.e., migration) *in vitro* supporting the collagen-based scaffolds as proper bedding for ACL tissue regeneration.

Recent *in vivo* work by Fleming et al[173] reported no significant improvement of suture repair when supplemented with a collagen scaffold alone used for complete ACL tears in a porcine model. However, by combining a collagen scaffold with autologous platelets, Murray et al[75,158,174] demonstrated significantly improved ACL repair outcomes in a series of large animal studies. They showed superior tissue mechanical properties using primary repair augmented with collagen-PRP hydrogel, compared with suture repair alone.[158] It was further reported that the augmented ACL repair can result in enhanced tissue properties

similar to ACL reconstruction, the current gold standard of treatment.[174] Additional studies have also now demonstrated that the combination of an ECM-based collagen scaffold and PRP is substantially more effective than the application of each of these factors alone.[173,175] The mechanism behind this remains unclear, but it may be due to a synergic effect between the collagen, PRP and other ECM molecules.

A new paradigm in ACL repair

The low capacity of the ACL to heal compared with other extra-articular tissues, such as the MCL, has long been attributed to the intrinsic differences in cell behaviour and insufficient blood supply following injury.[71,85,86,88,90,92,94] However, extensive *in vitro* cell culture and *in vivo* histological and immunohistochemical studies of the ACL and MCL have revealed that both ligaments have a comparable proliferative vascular and neurogenic reaction to injury.[66,75,176-180] It has also been shown that, similar to the MCL, collagen production continues within the ACL up to one year post-injury.[179] However, germinal observations showed that the provisional scaffold (fibrin-platelet clot) found within the wound site of extra-articular ligaments was missing in the ACL (Fig. 2).[178] The prevention of clot formation is mainly due to the continuous flow of the synovial fluid within the knee joint, dispersing the blood as a haemarthrosis.[178] It was further demonstrated that this lack of provisional scaffold leads to a decreased presence of critical ECM proteins and cytokines
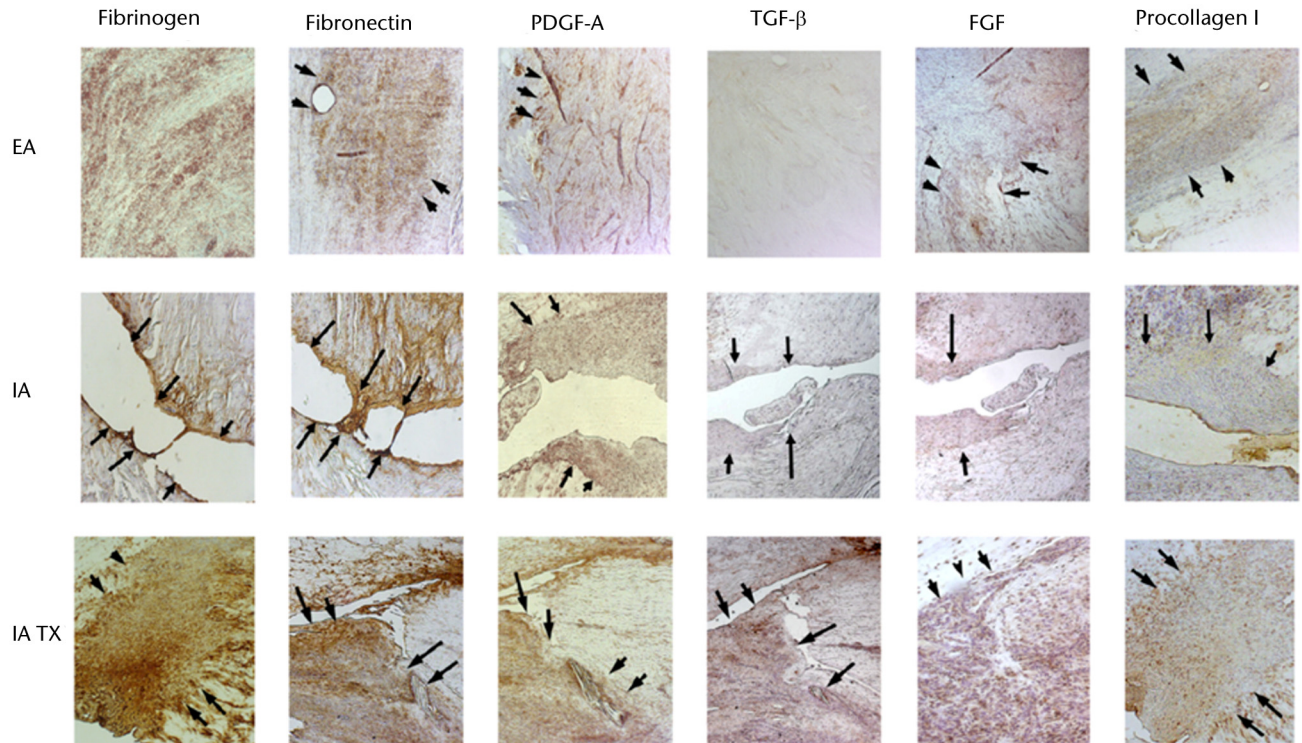
Fig. 3

Representative photomicrographs of patellar ligament wounds (extra-articular, EA), untreated anterior cruciate ligament (ACL) wounds (intra-articular, IA) and treated ACL with collagen-platelet scaffold (IA Tx) at 21 days after injury (10x). Treated ACLs show similar distribution of protein presence as the patellar ligament. The untreated ACL wounds remain with almost no substratum (PDGF-A: platelet-derived growth factor; TGF-β: transforming growth factor; FGF: fibroblast growth factor) (adapted and modified with permission from Murray et al[75]).
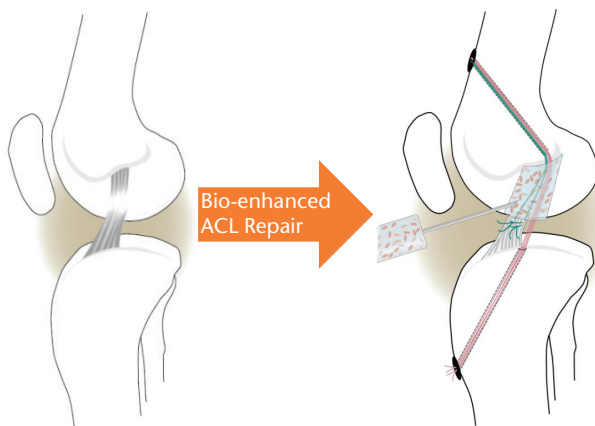

Fig. 4

Schematic of bio-enhanced anterior cruciate ligament (ACL) repair method (adapted and modified with permission from Murray and Fleming[182]).

the ACL to heal, a collagen-based scaffold has been used to fill the gap between the two ends of the torn ligament.[63] This bio-active scaffold could then be used as a carrier for cells, growth factors and enzymes required to optimise tissue healing. In the first *in vivo* studies, platelets maintained with their physiological plasma were placed within the collagen-based scaffold, and the loaded scaffold used to repair torn ACL using multiple established large animal models.[157-160,174,181-183] These studies further demonstrated the improved biological and mechanical healing of the ACL using this novel technique (bio-enhanced repair) (Fig. 4). In a recent randomised trial in a large animal model, the biomechanical outcome of bio-enhanced ACL repair was found to be equal to that of ACL reconstruction (Fig. 5).[174] More importantly, while 80% of the knees treated with ACL reconstruction developed post-traumatic OA by one year post-operatively, OA was not seen in those knees treated with bio-enhanced repair within the same time period (Fig. 6).[182]

Conclusion

A successful ACL repair can theoretically provide the patient with multiple advantages over surgical reconstruction, including preservation of the proprioceptive function of the ligament and the complex ligament insertion sites.
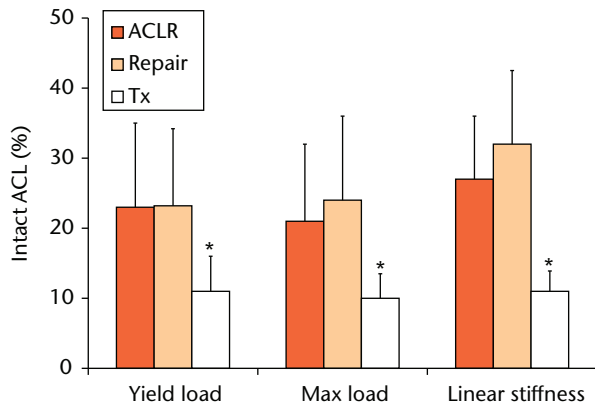
such as fibrinogen, fibronectin, PDGF-A, TGF-β1, FGF-2, and von Willebrand's factor (vWF) within the ACL wound site (Fig. 3).[75,159]

In order to test the hypothesis that the missing provisional scaffold was a key mechanism behind the failure of

Fig. 5

Bar chart showing identical mechanical properties of bio-enhanced repaired anterior cruciate ligament (ACL) (Repair) compared with the surgically reconstructed samples (ACLR) (p > 0.6 for all comparisons), with significantly lower mechanical properties (*) within the untreated ACL rupture group (Tx) (reproduced with permission from Murray and Fleming[63]).
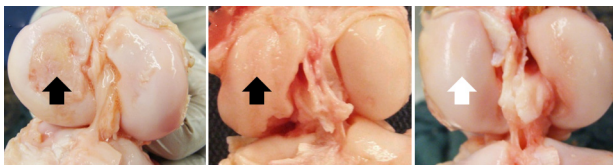

Fig. 6

Photographs showing the distal femoral cartilage at one year after a) an untreated anterior cruciate ligament (ACL) rupture, b) after conventional ACL reconstruction, and c) bio-enhanced ACL repair. Note the damage to the medial femoral condyle in the untreated and ACL-reconstructed knee (black arrows). No damage to the medial femoral condyle in the bio-enhanced ACL-repair knee (white arrow) was observed (adapted and modified with permission from Murray and Fleming [182]).

However, previously reported high failure incidences of primary repair and the relative robustness of ACL reconstruction led the clinical switch to use of a graft to replace, rather than repair, the ACL. Recent advances in the area of tissue engineering and regenerative medicine coupled with an improved understanding of the requirements for ACL healing, has led to the emergence of novel biologically augmented ACL repair techniques. Despite being in their infancy, these methods have resulted in repeated stepwise improvements in ACL repair and become a promising future candidate for ACL injury treatment. One such approach, bio-enhanced repair, has shown comparable structural and biomechanical outcomes with the current gold standard of treatment, ACL reconstruction. Bio-enhanced repair using a collagen-based scaffold and autologous blood has also resulted in significant decreases in risk of post-traumatic OA, which makes it the first and so far only possible ACL injury treatment with the potential to lower the risk of OA after an ACL injury. Despite promising

results obtained from *in vitro* and *in vivo* animal studies, well-controlled human trials are needed to assure the ultimate efficacy of these novel approaches. Future work should focus on further refinement of these techniques in an effort to improve the outcomes, along with successful translation to humans.

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