Sports Injuries
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Primary ACL Repair in Athletes with Mesenchymal Stem Cells and Platelet-Rich Plasma

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Abstract

Anterior cruciate ligament (ACL) injuries are very common, affecting a young, active population and shrouded in controversy regarding the appropriate treatment. New and alternative treatment options need to be investigated to address acute partial ACL tears. There are numerous advantages in repairing the ACL rather than reconstructing it. With bone marrow healing stimulation, introduction of platelet-rich plasma injections, and availability of synthetic and biologic scaffolds, repair of the ACL is no longer an impossible task. Current treatment strategy for ACL rupture, although satisfactory, can be improved in the case of partial tears.

Introduction

Rupture of the anterior cruciate ligament (ACL) is one of the most common knee injuries with an incidence of 1 in 3,000 (Frank and Jackson 1997). As a result of increased participation in recreational and competitive sporting activities, the number of knee ligament injuries has been on a steady rise in the past few decades. According to an ongoing study in the United States, an estimated 200,000 ACL reconstructions (ACLR) are performed annually (National Institutes of Health (NIH) et al. 2011). The sequelae of chronic anterior tibial instability have been well described and documented, including episodic pain and instability, chondral and meniscal injury, and early-onset osteoarthritis. The treatment of acute ACL injury is an area of considerable controversy, although sports medicine has seen rapid advances in the recent years. Despite having a high success rate of 80 %, ACL reconstruction with tendon graft still has some disadvantages. Reduced proprioception, postoperative tendon weakness, inability to restore normal knee kinematics, and premature onset of osteoarthritis are some of the reported shortcomings following ACLR (Gobbi et al. 2005; Lohmander et al. 2007). Considering the fact that ACL tears most commonly affect young people, leading to significant morbidity, newer and alternative therapeutic options should be investigated to effectively address acute partial ACL lesions.

ACL Repair

Suture repair of the torn ACL was first described in 1895; but it was O' Donoghue who popularized this technique in the 1950s (O'Donoghue 1950). Long-term follow-up studies showed that these techniques presented failure rates up to 90 % and were therefore abandoned

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(Feagin and Curl 1976). Despite reports of poor healing potential of the ACL in the past, recent investigations have demonstrated the possibility of ACL healing after primary suture of the ligament augmented with the use of growth factors and bone marrow-derived multipotent, mesenchymal stem cells (BMSCs) (Kaplan et al. 1990; Steadman et al. 2006; Murray et al. 2009) (Figs. 1 and 2). The potential advantage of repair over reconstruction technique is the preservation of the anatomy and kinematics of the ACL, proprioception of the knee, absence of donor-site morbidity, and potentially decreased muscular weakness.

Cellular therapies offer an interesting option in the treatment of the injured ACL by addressing the defect in healing at a molecular level and leading to a more biological way of healing. Steadman's "healing response therapy" (Steadman et al. 2006) was one of the earliest treatments described which extolled the role of BMSCs in aiding the healing of a ruptured ACL in human subjects. The results of this therapy were reported as encouraging and were based on the multipotent nature of the bone marrow cells. The recent finding of similarity between ACL outgrowth cells and BMSCs (Steinert et al. 2011) has presented the possibility to modulate these cells to enhance the healing of a repaired ACL. Bioactive proteins and growth factors play an important role in tissue healing because they can regulate key processes in tissue repair, including cell proliferation, chemotaxis, migration, cellular differentiation, and extracellular matrix synthesis (Gobbi et al. 2012). Platelet-

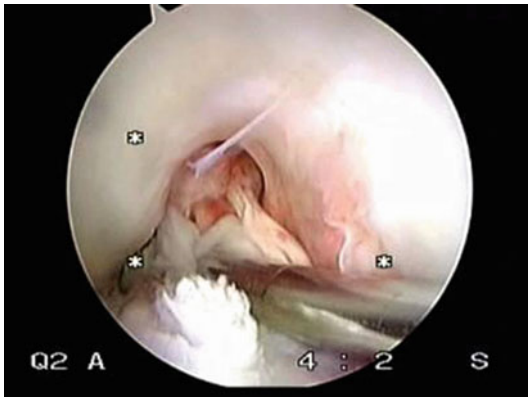

Fig. 1 Torn ACL

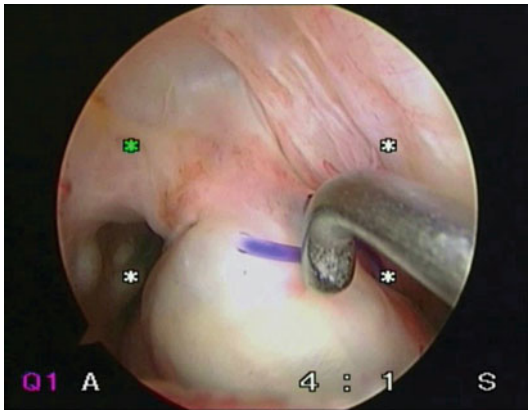

Fig. 2 The healed ACL following primary suture of the ligament augmented with the use of growth factors and bone marrow-derived multipotent, mesenchymal stem cells

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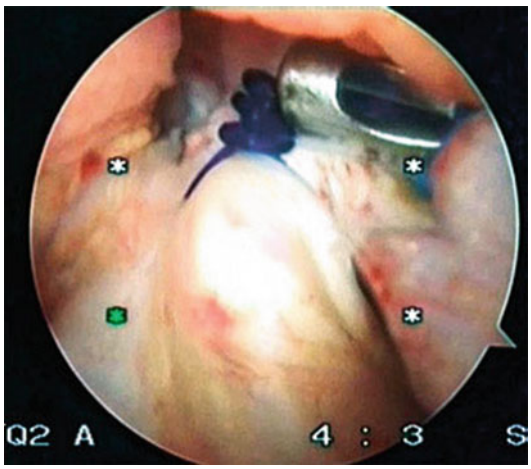

Fig. 3 Primary repair of ACL, suture seen in situ

rich plasma (PRP) contains many important growth factors, and recent studies have proven the beneficial effects of PRP in augmenting the healing of ACL (Murray et al. 2007; Cheng et al. 2010).

For repair of the ACL, therapies can be broadly classified into three methods: cellular, structural, and composite solutions.

Cellular Therapies

Bone Marrow Healing Stimulation

The ideal source of cells for use in an ACL engineering paradigm should provide cells that are readily available for clinical use, show robust proliferation, and possess the potential to elaborate extracellular matrix (ECM) in an organized fashion (Petrigliano et al. 2006). Early studies established the capacity of fibroblasts originating from various mesenchymal tissues to proliferate, synthesize collagen, and respond to mechanical and biochemical growth factors making them a potential source for ligament engineering. Although adult fibroblasts retain many of the phenotypic qualities necessary for collagen synthesis, they are relatively quiescent and have limited potential for further differentiation (Van Eijk et al. 2004). Bone marrow stromal cells (BMSC) are an attractive candidate for ligament engineering because they have the potential to differentiate into cells of multiple mesenchymal lineages, the synthetic and proliferative systems of these cells are robust, and they have the potential to readily adapt to their local niche. BMSC can be easily accessed in most patients without significant additional surgery or the risk of immune reaction (Vunjak-Novakovic et al. 2004). A comparative evaluation of goat BMSCs, ACL fibroblasts, and skin fibroblasts was undertaken by Van Eijk et al. (Van Eijk et al. 2004) to evaluate the optimal cell source for ACL engineering. The cells were seeded onto a degradable suture material and cultured for a maximum of 12 days. Each of the cell types attached, proliferated, and synthesized ECM rich in type I collagen. However, the scaffolds seeded with BMSCs showed the highest DNA content and collagen production.

In 2009, an article published in the American Journal of Sports Medicine showed that primary ACL repair combined with bone marrow healing stimulation could restore satisfactory knee stability and function in athletes with acute ACL incomplete tears (Gobbi et al. 2009) (Figs. 3 and 4).

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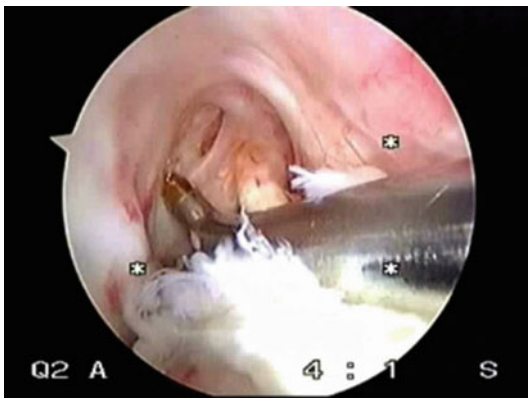

Fig. 4 Microfracture at the ACL insertion to facilitate healing stimulation

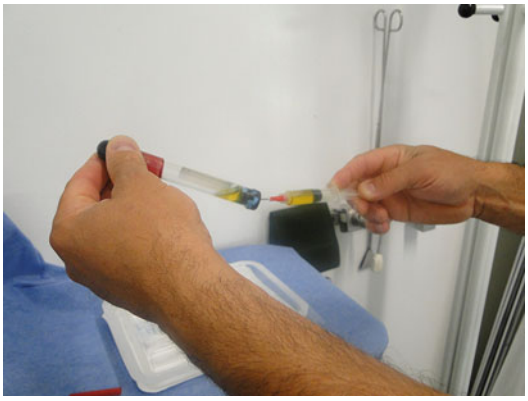

Fig. 5 PRP being aspirated into a syringe after its preparation

Platelet-Rich Plasma (PRP) and Growth Factors

PRP is defined as the volume of the plasma fraction from autologous blood with a platelet concentration above the baseline count (200,000 platelets/μL). Platelets contain many important bioactive proteins and growth factors (GFs). These factors regulate key processes in tissue repair, including cell proliferation, chemotaxis, migration, cellular differentiation, and extracellular matrix synthesis. The rationale for the use of PRP is to stimulate the natural healing cascade and tissue regeneration by a "supraphysiologic" release of platelet-derived factors directly at the site of treatment.

Autologous PRP can be obtained from simple blood extraction with a commercially available kit. Once the blood is collected into a tube containing anticoagulant, it undergoes a centrifugation process to produce PRP (Fig. 5). For PRP gel preparations, platelets are normally activated by thrombin (autologous or animal derived), calcium chloride, or procoagulant enzyme (i.e., batroxobin), which works as a fibrinogen-cleaving enzyme inducing rapid fibrin clot formation. When PRP solutions are injected directly for topical treatment, platelets are activated by endogenous thrombin and/or intra-articular collagen. Based on the preparation methods, PRP can be referred to as leukocyte-rich PRP, leukocyte-poor PRP (LP-PRP), or platelet-poor PRP (PPP) (Dohan Ehrenfest et al. 2008). A PRP preparation rich in growth factors is the preferred preparation to aid in tendon healing and improving functional outcomes in patients. As per the absolute number of platelets obtained, method of activation, and presence or absence of white blood cells (PAW) classification

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system, the PRP obtained was classified as P2 Bβ (P2 = platelets > baseline levels to 750,000 platelets/*u*L, B = WBCs below or equal to baseline level, β = neutrophils equal or below baseline, if activated "x" is added) (DeLong et al. 2012). PRP is rich in growth factors such as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), and IGF-1 which are known to have a role in the biological healing process.

However, researchers have questioned the beneficial effects of isolated PRP use in tissue healing. Murray et al. (Murray et al. 2009) contested the role of PRP in ACL healing following their results in skeletally immature animals. They performed ACL repair in 15 pigs and ACL repair with PRP injection in another 15 pigs, but the addition of PRP to the suture repairs did not improve AP knee laxity maximum tensile load or linear stiffness of the ACL repairs after 14 weeks in vivo. However, the use of collagen-platelet composites has been reported to have beneficial effects (Vunjak-Novakovic et al. 2004). Growth factors have been proven in in vitro studies to enhance cellular proliferation and migration and increase collagen production. Among the growth factors, PDGF, fibroblast growth factor (FGF), bone morphogenic protein (BMP), and TGF-β have shown to enhance the healing of ligaments. Kobayashi et al. (Kobayashi et al. 1997) noted improved healing and vascularity following instillation of FGF in the canine ACL, while Aspenberg and Forslund (Aspenberg and Forslund 1999) reported the use of BMP 14 in the Achilles tendon and showed improved healing. These growth factors can be used along with synthetic scaffolds to enhance the process of repair of ACL. Chen (Chen 2009) reported the use of BMP 2 along with hydrogel and periosteum to stimulate tendon-bone healing in an ACL reconstruction model. Moreau et al. (Moreau et al. 2005) have attempted to establish specific media formulations and growth factor combinations that support BMSC differentiation toward a fibroblast phenotype. They reported that media supplemented with ascorbate-2-phosphate was potent in promoting BMSC proliferation, and they cited three growth factors and media combinations that enhanced fibroblast differentiation: (1) EGF and TGF, (2) bFGF and TGF, and (3) growth factor-free advanced Dulbecco's minimal essential medium (ADMEM).

Experience with Cellular Therapies

In a study conducted at Orthopaedic Arthroscopic Surgery International (OASI) Bioresearch Foundation, Milan, 50 athlete patients were prospectively followed up for 5 years. All of them underwent an arthroscopic primary ACL repair combined with BMS technique and growth factors after an acute partial ACL injury. Included in this study were patients less than 40 years old presenting with a documented acute ACL injury (<4 weeks from the time of trauma), with a history of giving away sensation, and positive Lachman and pivot shift tests. Exclusion criteria were lesions not amenable to primary repair, mid-substance ACL tears, associated chondral lesions > grade 3 on the ICRS classification system, partial or complete tears of the lateral (fibular) collateral ligament (LCL) or posterior cruciate ligament (PCL), grade 3 medial collateral ligament (MCL) injury, patients with contralateral knee ligament injury, severe lower limb malalignment, and history of previous surgery on the same knee. Anterior translation of the knee was objectively assessed with the use of Rolimeter® (Aircast ®, Boca 100 Raton, FL, USA), and the pivot shift was measured before surgery under anesthesia according to the IKDC objective knee score. Knee function and activity level were assessed using Marx, Noyes, Tegner, single assessment numeric evaluation (SANE), Lysholm, and IKDC objective scoring systems which were assessed preoperatively and followed up at 1, 2, and 5 years. ACL repair was performed by passing No. 1 polydioxanone (PDS) sutures (Ethicon, Piscataway, New Jersey) using a Clever Hook or Express suture passer (DePuy Mitek, Raynham, Massachusetts) through the torn portions of the ACL and tied using a Duncan loop. The aim of this step was re-approximation of the torn ends of the ligament, thereby reducing

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the gap between the residual ends and providing continuity to the ligament, allowing the BMSCs recruited from the penetration of the bone marrow to promote healing. Using a 45° microfracture awl, several holes (1.5 mm in diameter, 3–4 mm apart, and 3 mm deep) were made around the anatomic femoral insertion of the ACL. Bleeding was confirmed under direct visualization. PRP glue was then injected at the repaired site to biologically augment the healing process. In cases of a partial tear of both the AM and PL bundles, a microfracture was performed around the femoral insertion of the ACL followed by pasting the PRP glue along the tendon; no suture repair was performed in these lesions. The rehabilitation protocol was standardized for all patients.

Four patients had a re-tear during sporting activity and underwent ACL reconstruction within 2 years from primary repair surgery; their last evaluation scores at 2 years follow-up were included in the final results. Three patients out of the four were involved in high-risk sports. One patient presented with loss of ROM greater than 15° at final follow-up. One patient had episodes of giving away of the operated knee despite a lack of any significant injury, but an MRI revealed an intact but lax ligament. Anterior translation was significantly reduced (<3 mm side to side difference) at 5 years follow-up. Pre-injury and final Tegner scores were comparable, while the final SANE score was significantly lower than the pre-injury value. The final Marx, Noyes, and Lysholm scores were similar to the preoperative values. The final IKDC objective score was normal in 78 % of patients, nearly normal in 20 %, and severely abnormal in 2 %. Thirty-nine patients (78 %) fully resumed sport activity. The return to sport was reached at a mean of 5.9 (SD = 1.3) months after surgery. Eleven patients (22 %) did not return to sport at pre-injury level; in four of them, this was a personal choice. A second look arthroscopy was performed in six out of 50 patients and revealed a healed ACL which was stable on probing and had minimal fibrous tissue.

It was concluded that primary ACL repair with BMS and growth factors represents an effective procedure in the treatment of acute partial ACL tears. However, careful patient selection, accurate surgical technique, and proper functional rehabilitation are essential to achieve good results. This treatment does not burn any bridges so that conversion to a standard ACLR can be done in the event of a failure.

Structural Therapies

Supplementing a ruptured ACL with a scaffold is an interesting method to aid in the healing of the ACL. An ideal scaffold must be biocompatible with the cell source and recipient, while allowing for cell adhesion and proliferation. The scaffold must be strong enough to withstand mechanical stress, yet it must degrade over time and yield to the progress of native tissue ingrowth. Moreover, the scaffold structure will influence the transport of nutrients, metabolites, and regulatory molecules to and from the cells seeded within the scaffold (Gobbi et al. 2009) (Fig. 6). Many varieties of scaffold materials have been considered, including biologic materials such as collagen, silk, and biodegradable polymers and composite materials (Lin et al. 1999; Steadman et al. 2006). Collagen received a great deal of early interest and has been in clinical use for decades. Although the fibrous collagen scaffolds support cell attachment, spreading, and fiber coverage with ECM, the capacity of the construct to support mechanical loading decreases over time (Goulet et al. 1997). This has led investigators to study alternative scaffold materials. Molecules such as hyaluronic acid, chitosan, and alginate, which have inherent biocompatibility and cell-adhesion properties, have been modified to make them more appropriate for ligament engineering applications (Majima et al. 2005). Wiig et al. (Wiig et al. 1990) reported the use of intra-articular hyaluronic acid as a scaffold in a central defect rabbit model with a ruptured ACL and reported a greater angiogenic response, more

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Fig. 6 Collagen-based scaffold

pronounced repair, and higher type III collagen when compared to saline-treated controls. However, the biomechanical strength of the healing tendons was not assessed in their study. In recent times, use of hydrogel either as a scaffold alone or as a composite with collagen, platelet-rich plasma (PRP) (Moreau et al. 2005), and periosteum (Majima et al. 2005) has shown good outcomes in experimental studies. The results of the clinical application of this product in humans are awaited.

Cell Scaffold Composites

A combination of cell therapy embedded in a scaffold seems to be a promising treatment of ACL rupture. This is based on the premise that while PRP/BMSCs from the bone marrow will act as the source of growth factors and precursor cells, the scaffold would act both as a matrix in the cellular process and as a biomechanical support following primary repair of ACL and provide a secure environment for the cells away from the effects of plasmin. In an in vitro study by Cheng et al. (Cheng et al. 2010), the addition of PRP to collagen hydrogel enhanced ACL cell viability, metabolic activity, and collagen synthesis and has been purported to stimulate ACL healing. In an experimental study conducted by Murray et al. (Murray et al. 2009), supplementation of suture repair with collagen-platelet composite resulted in the formation of a large scar mass in the region of the ACL in a porcine ACL model. Load at yield, maximum load, and ACL tangent modulus were all significantly higher in the suture repairs augmented with collagen-platelet composite than in repairs performed with suture alone. Other laboratory studies have also supported this concept and elucidated the potential benefits of the cell-scaffold composites (Fan et al. 2008).

After conducting laboratory studies in Sweden, the OASI Bioresearch Foundation is starting a new project where a group of patients will undergo primary ACL repair augmented with a synthetic slowly degradable scaffold enriched with bone marrow aspirate concentrate (BMAC) embedded in platelet-rich plasma (Fig. 7). The BMAC is expected to act as a source of BMSCs and PRP as a source of growth factors, and the biodegradable scaffold should augment the repaired ligament and provide a matrix for the tissue ingrowth giving both cellular and biomechanical support to the injured tissue.

Conclusions

With the modern emphasis on fitness and athleticism, the prevalence of ACL injuries is on the rise, and patients' expectations have also increased. The current strategies applied to ACL reconstruction

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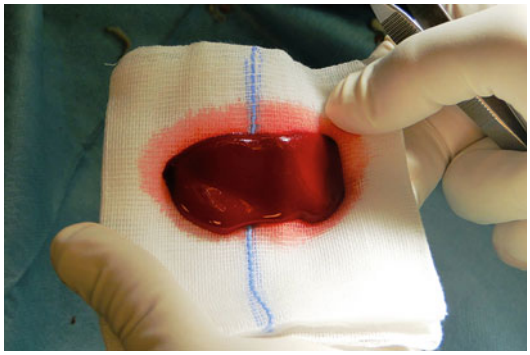

Fig. 7 Appearance of BMAC once prepared and ready for use

are satisfactory. However, the goal should remain to improve this procedure further and eliminate its associated complications. ACL primary repair and a healing stimulation technique can be a viable treatment in acute partially torn ACL. Careful patient selection and proper functional rehabilitation are essential to achieve good results. Current research has found that natural and synthetic scaffolds can sustain cell adhesion, growth, and matrix deposition under appropriate conditions and that growth factors and mechanical stimuli have a role in modulating cellular response. Although these advancements are promising, many obstacles persist. Only through perseverance and human trials will we succeed in this endeavor to achieve successful ACL repairs.

Cross-References

- ▶ ACL Augmentation in Partial Ruptures
- ▶ ACL Injuries and Surgery Current Evidence and Modern Development
- ▶ Arthroscopic Repair of Partial ACL Tears: My Viewpoint

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