Stem cell-based therapy in anterior cruciate ligament repair

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Abstract: In the past, the suture repair of anterior cruciate ligament (ACL) has been reported with poor results, which indicated the poor healing potential of ACL. However, the poor results were attributable not only to a low intrinsic healing potential of the ligament, but also to other environmental factors that include mechanical environment, inflammatory condition, blood supply, nutrient delivery, and the supply of growth factors. Recently, biological manipulation could be an available option among several potential options. This includes the use of growth factors, platelet-rich plasma (PRP), stem cells, and biological scaffolds. With these tools, augmented ACL repair has been investigated to accelerate repair and regeneration. Specifically, stem cell-based therapy has been paid great attention based on the potential of stem cells to regenerate tissue. This review describes the current understanding of capacity of ACL healing and summarizes the current status of related stem cell therapy. Future limitations and perspectives are also discussed.

Keywords: Stem cell; anterior cruciate ligament (ACL) repair; cell-based therapy

Introduction

The frequency of anterior cruciate ligament (ACL) injuries are increasing and an estimated 200,000 ACL reconstructions performed per year in the United States (1). In the past, the suture repair of ACL has been reported with poor results, which indicated the poor healing potential of ACL (2-7). Therefore, ACL reconstruction has been replaced for the treatment of ACL injury. Nowadays, with the improvement on surgical techniques such as anatomic tunnel placement, graft selection, and optimal initial tension during graft fixation (8,9), ACL reconstruction is considered as a gold standard with a high success rate up to 80% (10-14). However, according to a meta-analysis, only 40% of patients could achieve full recovery independent of surgical techniques (15). Another multicenter cohort study showed that a median 7 months (range, 5–24 months) was needed for return to play of soccer athletes after ACL

reconstruction (16). Moreover, the microscopic findings of autogenous patellar tendon graft for ACL reconstruction indicated that the autogenous grafts were different from natural ACL fiber and still immature structure even at 1 year postoperatively (17). Some studies reported several concerns related to ACL reconstruction such as poor proprioception, postoperative muscular weakness, donor harvest morbidity, inability to restore normal kinematics, and potential development of early osteoarthritis (18-21). Considering these limitations associated with ACL reconstruction, novel therapeutic options are needed.

Among several potential options, biological manipulation could be an available option. This includes the use of growth factors, platelet-rich plasma (PRP), stem cells, and biological scaffolds. With these tools, augmented ACL repair have been investigated to accelerate repair and regeneration. Specifically, stem cell-based therapy has been paid great attention based on the potential of stem cells

to regenerate tissue. There are various types of stem cells such as embryonic stem cell, induced pluripotent stem cell and mesenchymal stem cell (MSC). Among these stem cells, MSCs are most widely investigated because of their isolation with relative ease, and safety, along with high multipotency as well as high proliferative capacity (22-24). Thus, the purpose of this review article was to overview the current concepts on stem cell-based ACL repair.

Capacity of ACL healing

The suture repair of torn ACL was first reported in 1895 (2). This initial report was followed by several studies. The results of these reports indicated that primary ACL repair had good outcomes in only one-third of patients (3-5). In addition, long-term follow-up studies showed that the failure rates of up to 90% (6,7) and were therefore largely replaced by ACL reconstruction for the past four decades. Conversely, a recent study reported that the patients who initially had good outcomes at 5 years preserved the good outcomes at 30 years postoperatively (25). This suggests the potential of ACL repair by optimizing the healing environment.

There here are likely both intrinsic and environmental factors to be considered for optimized repair (26). Regarding the intrinsic factor, reparable capacity of ACL was thought to be poor *in situ*. In the past, several studies have shown a decrease in growth factors, cellularity and expression of molecules in ACL repair process, sometimes compared with other ligaments and tendons (27-33). However, *in vitro* healing study of human ACL showed that the injured ACL with preserved synovium had comparable healing capacity with that of semitendinosus tendon (34). Moreover, within the remnant, there were some cells including synovial cells that have healing potential for injured soft tissues, but no tissue bridging between the femoral and tibial remnant in another *in vitro* study (28). These results suggest that the human ACL possesses some intrinsic healing capacity and the synovium could play an important role in initial ACL healing. Therefore, the poor clinical outcome of ACL repair was attributable not only to a low intrinsic healing potential of the ligament, but also to other environmental factors that include mechanical environment, inflammatory condition, blood supply, nutrient delivery, and the supply of growth factors (28,35-39).

Cell-based therapy with ACL repair

In order to promote healing capacity of ACL, cell-based ACL repair has been investigated by *in vitro* and animal

studies (40-42). MSCs are adult stem cells populated in various tissues with the multipotentiality and the capacity of self-renewal. MSCs can differentiate into progenitors of mesoderm-associated cells such as chondrocytes, adipocytes, or osteoblasts. *In vivo*, it was confirmed that MSCs are often localized in the perivascular area (40). It is accepted that MSCs are also present in the ACL (43,44).

In vitro studies

Past engineering approaches using ACL-derived fibroblasts have been reported and promising (29,32,45). But slow growth rate of such fibroblasts *in vitro* may limit their practical application (22,46). Especially, comparison of intra- and extra-articular ligament-derived cells *in vitro* (29,31), ACL-derived cells exhibited lower rates of cell division and migration than those derived from the medial collateral ligament.

Recent studies have documented bone mesenchymal stem cells (BMSCs) had the higher proliferation ability comparing to ACL-derived fibroblasts (22,47). Indeed, BMSCs have been capable of ligamentogenic differentiation with growth factors (23,48-53).

The adipose-derived stem cells (ASCs) have been also proposed as an alternative MSC for ACL repair. But the use of ASCs is controversy and still remains relatively unexplored. *In vitro* studies, porcine ASCs could stimulate ACL-fibroblast proliferation and procollagen production (54), whereas human ASCs with growth factors could not stimulate their ligament differentiative potential (55).

In terms of mesenchymal stem cells derived from synovium (SMSCs), there have been no reports of ACL repair except one report of ACL reconstruction (56). However, the higher proliferation and differentiation potentials of SMSCs have than MSCs derived other tissues were confirmed (57-60) and thus SMSCs can be an alternative for ACL repair.

Using ACL-derived stem cells for ACL repair, there were a few reports of *in vitro* researches. Only two studies (43,44) reported that, under suitable culture conditions, both ACL-derived stem cells are similar to human BMSCs, which suggested that these ACL-derived cells could be viable alternative source for use in ACL repair.

Animal experiments

Intra-articular injection of MSCs

Morito *et al.* (61) evaluated the localization of rabbit MSCs

after synovial fluid-derived MSCs intra-articular injection. They found that MSCs enter the synovial fluid after ACL and more MSCs were found in injured ACL compared to normal ACL. Their findings suggested that MSCs are not normally present in the intact ACL, but in injured ACL. In the other previous animal studies, intra-articular injection of BMSCs was applied for animals with partially injured ACL as a biologic treatment (26,62). Kanaya *et al.* (26) found that partially transected ACL gap was covered with repair tissue in which injected BMSCs were detected at 4 weeks after injection, whereas transected area without BMSCs injection retracted with increasing time and the gap remained. In their report, the ultimate failure load of the femur-ACL-tibia complex after BMSCs injection was significantly higher than that after injection without BMSC at 4 weeks after surgery. Oe *et al.* (62) also found that fresh BMSCs was injected into the knee joint after transection of medial halves of ACL and both histological and biomechanical outcomes were almost same as normal ACL at 4 weeks after injection. In both studies, BMSCs remained in repair ACL until 4 weeks after injection (26,62). It is not clear whether the injected MSCs directly participated in repair tissue by matrix synthesis or acted to exert tropic effect to modulate repair environment. However, it is likely that the intra-articular injection of MSCs could accelerate the healing of partially torn ACLs and thus this treatment using MSCs could be an option for promoting ACL repair, specifically in partially torn cases.

Scaffold seeded with MSCs

There were several reports demonstrated the evidence of scaffold seeded with MSCs for regeneration of ruptured ACL (51,63-65). Most studies showed the positive effect of BMSCs and scaffold for repair of ACL. BMSCs and scaffold have much clearer and distinct advantages over ACL fibroblasts, with respect to cell proliferation, GAG excretion, gene and protein expression for ligament-related extracellular matrix (ECM) markers, and *in vivo* survivability (63). Histological observation also showed that MSCs were distributed throughout the regenerated ligament and exhibited fibroblast morphology and, furthermore, direct ligament-bone insertion was reconstructed (50). In another past study, the use of BMSCs seeded in a collagen type I scaffold in the treatment of ACL injuries was associated with an enhancement of ligament regeneration, whereas regeneration was not observed in the group treated with suture alone or in the group associated with collagen type I scaffold without cells (64). Conversely, Proffen *et al.* (65) showed no significant improvements of ACL healing in the biomechanical or histological properties with the addition of ASCs and ECM scaffold.

ACL reconstruction with MSCs

Regarding the promotion of ACL reconstruction using MSCs, there have been some studies investigated (56,66-70). In rabbit model, coating of semitendinosus tendon grafts with BMSCs results in the restoration of the chondral enthesis of normal ACL insertions rather than collagen fibers and scar tissue (66). The BMSC-enhanced ACL reconstruction also showed significantly better biomechanical properties than ACL reconstruction only. Conversely, Ju *et al.* (56) implanted SMSCs into tendon-bone interface and showed SMSCs could enhance collagen production for strong connection between tendon and bone without formation of fibrocartilage. The cell career in their study was not fibrin sealant but the atelocollagen gel, which was one of the reasons for no fibrocartilage formation, different from the results using BMSCs. Thus, the results are inconsistent and additional studies, preferably with large animal models, are needed whether MSCs promote the osteointegration of ACL grafts.

On allogenic ACL reconstruction, there is one study evaluating the effect of cell and gene therapy technique (71). Histological observation showed that the implantation of MSCs or PDGF-B transfected MSCs accelerated cellular infiltration into the ACL and enhanced collagen deposition in the wound. Similar to their findings, Nakamura *et al.* (72) reported an increased vascularity and enhanced collagen deposition in the wound of a patellar ligament after direct *in vivo PDGF-B* gene transfer in a rat model. Thus, gene transfer technique in combination with MSC implantation could further effectively optimize ACL repair and graft remodeling. Further studies are needed to accumulate evidences until clinical application of such combination therapies

Clinical applications

Likewise, *in vitro* and *in vivo* animal studies, there were not sufficient numbers of reports on clinical applications of MSC-based therapy in ACL repair. To date, cell-based therapies were applied as only by percutaneous intra-articular injection of autologous BMCs, or BMCs delivered by micro-fracture of the femoral condyle. Centeno *et al.* (73) reported a small case series of intra-articular injection of autologous BMCs for the patients with partial tear or complete tear retracted less than 1 cm. Based on good

magnetic resonance imaging (MRI) and clinical results, they claimed the feasibility of this treatment. However, no objective data such as anterior laxity was shown. There were also studies on the effectiveness of BMCs application to ACL healing by micro-fracture technique. First report was by Steadman *et al.* (74), reporting that the repair of complete proximal ACL tear in skeletally immature athletes with an averaged follow-up of 69 months. Micro-fracture reportedly leads to the formation of a blood clot and subsequent hematoma formation with BMSCs. Postoperative clinical score and activity level were equivalent to those before ACL injury. Anterior laxity evaluated by instrument was sufficiently improved (5 mm preoperatively to 2 mm postoperatively); 23% of 13 patients had a re-injury and underwent subsequent ACL reconstruction. More recently, same group showed that active middle-aged patients after same procedures with an average follow-up of 7.6 years (75). Similar to their previous report, good clinical results and only 8.9% of 48 patients required subsequent ACL reconstruction. These studies suggested that the healing response by micro-fracture could restore stability and knee function, with proper patient selection. Gobbi *et al.* (76) reported the suture repair of proximal partial ACL tear combined with micro fracture. These procedures were reapproximation of the torn ends of the ligament, thereby reducing the gap between the residuals, and creation of a continuity of the ligament, thus allowing the BMSCs recruited from the penetration of bone marrow to promote healing. Moreover, Gobbi *et al.* (77) evaluated the outcome after the suture repair of proximal partial ACL tear combined with micro fracture and injection of PRP glue at repair site. BMSCs and injected PRP might act as the source of precursor cells and growth factors. According their middle-term results, 78% of 50 athletes could return to their sports activities, a significant decrease in the side-to-side differences in anterior laxity (4.1 mm preoperatively to 1.4 mm postoperatively). Clinical scores were sufficient, but four patients experienced re-tear and one patient had residual laxity resulting in a survival rate of 90% at the 5-year follow-up. Even though good clinical results were found, they highlighted that not all ACL lesions can be treated with this technique; patient selection is essential and strict inclusion criteria should be followed. Finally, they concluded that this surgical technique of ACL primary repair utilized in severely selected patients with acute partial ACL lesions could offer good clinical outcomes (77).

All these studies were only case series, but MSCs could promote the healing potential of injured and repaired ACL. Further researches and more evidences are necessary for expanding the indication of these approaches.

Other biological treatment of ACL repair

Along with MSC-based approaches, several growth factors including transforming growth factor beta1 (TGF-β1), fibroblast growth factor-2 (FGF-2), growth and basic-FGF (bFGF) could potentially improve ACL healing by manipulating cellular activities, such as proliferation and differentiation of MSCs into ligament progenitor cells in repair process (78-82). In addition, PRP is known to contain these growth factors and the effect of PRP on ACL healing has been also investigated. Although no clear positive effects of PRP on ACL healing were reported (83,84), one human clinical study demonstrated that PRP promoted the repair of acute partial ACL tear (85). This clinical study showed the complete integrity of remnant on MRI and normalization of anterior laxity evaluated by KT-1000 in all patients.

Recently, bioscaffolds combined with PRP were used for ACL repair. *In vivo* animal studies showed no positive effect of only collagen scaffold on ACL repair (86), however, there was significant improvement of the outcomes in using combination of collagen scaffold and autologous platelets (87). In a clinical study, Murray *et al.* (88) reported the results of ACL repair with augmentation using ECM-based collagen scaffold saturated with autologous whole blood. They confirmed the continuity of repair ACL substance by MRI and the clinical outcomes after ACL repair were equivalent compared to those after ACL reconstruction using hamstring autograft at 3 months postoperatively. They also showed that, the hamstring strength at 3 months postoperatively was significantly better in the ACL repair group than that in the ACL reconstruction group. This study only included fresh ACL tear less than 1 month following injury, which had at least 50% of the length of the ACL attached to the tibia on the preoperative MRI. Thus, with careful selection of the cases, this bio-scaffold technique could be applicable to some ACL injuries. More detailed, controlled studies are needed to validate the feasibility of this treatment.

Generally, the scaffolds provide mechanical stability to the injured site while allowing for cell attachment and proliferation under a protected three-dimensional environment (89). However, several concerns still remain
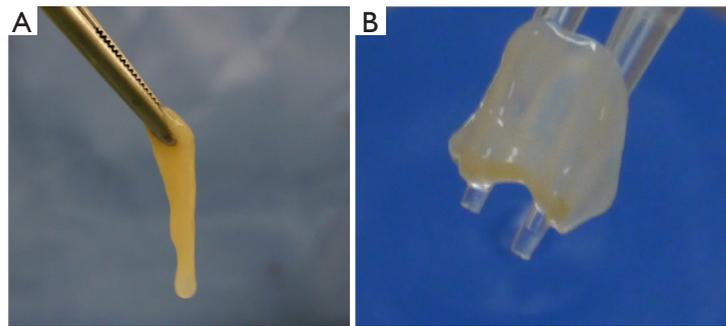
Figure 1 Macroscopic view of TEC. (A) Basic TEC; (B) chondro-TEC which was thick and hard tissue (cartilage-like). TEC, tissue-engineered construct.

associated with animal or chemical polymer-derived materials contained in scaffolds that could affect the long-term durability and safety (90-93). Therefore, the concept of scaffold-free tissue engineering has been paid attention. Recent studies demonstrated the feasibility of a scaffold-free tissue-engineered construct (TEC) derived from synovial MSCs to cartilage and meniscus repair (*Figure 1*) (94-98). The TEC contains undifferentiated MSCs at high density in a three-dimensional matrix that has been synthesized by the MSCs themselves and promoted cartilage repair with comparable mechanical properties at 6 months in porcine cartilage defect model (99,100). Deie *et al.* (34) suggested that the synovium could play an important role in enhancing ligament-healing capacity. In this regard, it is reasonable to use the TEC derived from synovium to promote ligament healing as well. Absolutely, further researches are needed for clinical application of these biologic approaches including TEC to promote ACL repair and graft remodeling.

Conclusions

ACL reconstruction is considered as a gold standard for ACL injury. But, clinically, several months are needed for achievement of full return to play sports in limited patients. Furthermore, microscopically, the autogenous tendon graft for ACL reconstruction was immature and different from normal ACL fiber at 1 year postoperatively. Therefore, new biological techniques using MSCs should be applied for ACL repair, owing to the development of tissue engineering. Historically, ACL suture repair has not succeeded in clinical results, because of the environmental factors, poor surrounding tissue and hypovascularity as well as poor healing capacity of ACL. Recent experimental and clinical studies associated with cell-based therapy using

MSCs, with or without scaffold, PRP or the other biologic agents, suggested good results in healing of acute and partial ACL tears. These cell-based therapies using MSCs may be a potentially useful tool for improving ACL healing. However, patient selection is essential and strict inclusion criteria should be important. Not all patients with ACL tear can be treated with these cell-based therapies. To repair the complete ACL tear using MSCs, further researches and more evidences are necessary in the future.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/aoj.2017.11.03). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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