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Current Stem Cell Applications in Anterior Cruciate Ligament Repair

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ABSTRACT

This paper will detail the current research pertaining to stem cell use in anterior cruciate ligament repair. Stem cells have expanded treatment options to include injection of stem cells into allograft ligament, and bioengineered scaffolding. There are numerous types of stem cells that can be used in these procedures, but the most common type used is bone marrow mesenchymal stem cells (BM-MSCs), because of their ability to differentiate into chondrocytes, adipocytes, and hematopoietic cells, all necessary products in the ligament healing process. Issues with the injection method include number of viable cells in the cell count, the correct angle and location of injection, and binding of the BM-MSCs to appropriate target once in vivo. The scaffolding method can involve a few treatment options: three-dimensional printing of a bioengineered scaffold that is afterwards coated in BM-MSCs, allograft scaffold that is laced with BM-MSCs and inserted during the surgery, or decellularized allografts that BM-MSCs have been added to. Many of the same issues arise in this method, such as viable stem cell count, but with the scaffolding there is more success of correct location placement and stem cell differentiation in vivo. In both of these methods it is also important to discuss the appropriate growth factors that must be important for differentiation of the targeted stem cell. Overall, the allograft scaffold laced with BM-MSCs shows to be the most effective repair method, and direct injection appears to aid in the healing of the autograft and promotes exponential differentiation of the laced stem cells.

Keywords

Stem cells, Anterior cruciate ligament reconstruction, Bioengineered scaffolding, Autograft, Allograft, Animal studies.

List of Abbreviations

BM-MSC: Bone marrow mesenchymal stem cell; ACL: Anterior cruciate ligament; OA: Osteoarthritis; hACL: Human anterior cruciate ligament; hMCL: Human medical collateral ligament; haMPCs: human adipose-derived mesenchymal progenitor cells; DIS: Dynamic Intraligamentary Stabilization; IBLA: Internal Brace Ligament Augmentation; COII: Collagen type II; TDSCs: Tendon derived stem cells; GFP: Green fluorescent protein; hUBC-MSC: Human umbilical cord mesenchymal stem cell; H&E stain: Hematoxylin and Eosin stain; BPTB: Bone-patellar tendon-bone.

Introduction

Anterior cruciate ligament (ACL) reconstruction has been around for years, and initially involved the use of an allograft, which has remained the gold standard for some time now [1]. With the advent of stem cell technology and 3D printing, though, strategies have been suggested that may further the healing capacity and functionality in patients who may experience this injury multiple times in their lifetime, like avid athletes and younger individuals. The annual incidence rate of an ACL rupture is 70 cases per 100,000 person-years, and ranks among one of the most common orthopedic injuries [2]. There has also been a shift in focus to preserving the functionality of remaining tissues, which can be enhanced with the use of injected mesenchymal stem cells [3]. An ACL tear can happen in a variety of patients, but significant risk factors are present in athletes and patients who put great strain on this ligament regularly [4]. The hope of these patients is that they would be able to return to preinjury state with surgical intervention, but unfortunately 50% of patients present with knee osteoarthritis (OA) within 10 years of the procedure [5]. The mechanical disruption that is caused by an ACL tear, can then cause damage to the meniscus of patients, which results in an even greater risk of OA of the knee [3]. Thus, ACL reconstruction may help in the short term with the regain of movement, but functionality and the possibility of a re-tear is still in question.

Two new types of surgical reconstruction that increase the mechanical stability of the ligament involve a Dynamic Intraligamentary Stabilization (DIS) (Figure 1) and an Internal Brace Ligament Augmentation (IBLA) (Figure 2). DIS provides stability in the ligament to aid in ligamentous healing. The procedure involves using a threaded sleeve with preloaded spring mechanism to secure the spring in the tibia. As shown in Figure 1, a 1.8mm braided polyethylene (PE) wire is attached to the tibial component, traverses the knee joint, and through the middle of the torn ACL. IBLA, made popular by Arthrex, involves the acquisition of a 2.5mm PE tape that bridges the anatomical attachments to the mid-bundle position of the ACL on both the femur and the tibia [6].

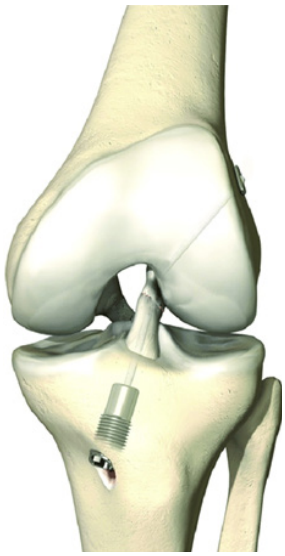

Figure 1: Dynamic Intraligamentary Stabilisation (Mathys Medical™) [6].

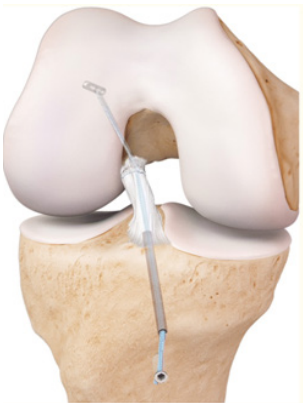

Figure 2: Internal Brace Ligament Augmentation (Arthrex) [6].

To address the functionality issue post operation, stem cells are being studied in great detail. Each stem cell category elicits its own benefit in the repair process of specific tissues. For example, some target the healing of tissue membranes, while others go deeper into the root of the graft and provide effective nutrients for the other stem cells to proliferate. The table below summarizes the functioning of each type of stem cell, and how it is purposed to help in healing of the damaged ligament.

Stem Cell Type	Harvest Site	Benefits
Ligament Derived/ progenitor cells (LSPCs)	Human Ligament	Produce more of a tendon/ligament matrix that can contribute to tissue repair [7].
Human adipose-derived mesenchymal progenitor cells (haMPCs)	Adipose Tissue	Promoted cartilage repair and expressed human leukocyte antigen I (HLA-I) [8]
Bone marrow mesenchymal stem cells (BM-MSCs)	Bone marrow	Accelerated healing in rat models and increased ultimate failure load of ligament in biomechanical testing [9]
Human Anterior Cruciate Ligament (hACL)—ACL derived CD34+ cells	Anterior cruciate ligament	When added to natural or biodegradable scaffold, they promote collagen type I and type III production [10]
Human Medial Collateral Ligament Stem Cells (hMCL-SCs)	Medial ligament	Made larger colonies than hACL-SCs in culture, and grew in a timelier manner [11]
Human muscle-derived stem cells (hMDSCs)	Gracilis and semitendinosus muscle obtained in vitro	In early bone differentiation, hMDSCs have excellent differentiation potential. Growth time in culture was expedited compared to tendon-derived stem cells [12]

Table 1: Types of Stem Cells and their individual harvest site and benefits.

Types of Grafts

Macaulay et al. discuss in depth the current evidence relating to graft choice and the benefits they each present. There are two main categories: allografts and autografts [13]. Autografts can be broken into three subcategories that include: bone-patellar tendon-bone (BPTB), Semitendinosus and gracilis tendons (quadrupled hamstring tendon [HT]), and quadriceps tendon (QT). Allografts would theoretically eliminate donor site morbidity and include shorter rehabilitation times, but this is not always the case [14]. With the primary goal of ACL repair being stability of the knee, it is important to consider follow up rupture rates of both types of grafts. There have been three-meta-analyses performed so far on the stability of autografts versus allografts. Two of these studies found no statistically significant difference, and one study conducted by Foster et al. showed that there was a statistically significant difference, of 1.4 ± 0.2 mean allograft laxity and the mean autograft laxity being 1.8 ± 0.1 ($P < 0.02$) [14].

Discussion

Animal Trials

Bone marrow mesenchymal stem cells

Lim et al. [15] showed the effects of BM-MSCs in 48 adult rabbits using a bilateral ACL reconstruction with a hamstring tendon autograft. The procedure involved coating the grafts with MSCs, that were encased in a fibrin glue mixture, on one limb of the rat. The MSCs were harvested 3 to 4 weeks before the ACL reconstruction from the iliac crest of the rats. The control in the experiment was the other rat limb autograft that received only the fibrin glue mixture. Results from this experiment showed that the MSC-enhanced reconstruction sites have cartilage cell formation

at the tendon-bone junction at 2 weeks, and by 8 weeks a zone of mature cartilage was seen that blended from the bone to the tendon graft. There was no mechanical difference between MSC-enhanced reconstruction at 2 and 4 weeks, but by 8 weeks load failure and stiffness did occur. BM-MSC were also found to enhance tendon-bone healing when injected around the autograft tendon and caused the promotion of chondrogenesis genes and proteins: collagen type II (COII), aggrecan and three osteogenic genes and proteins [16]. Soon et al. [17] found similar findings in their study that used a soft tissue allograft in a bilateral ACL reconstruction of 36 rabbits, coated in MSCs. The results at 8 weeks showed significantly higher load-to-failure rates than the control, but a decreased stiffness and Young's modules (Figure 3). The multilineage ability of MSCs allows it to differentiate into an array of connective tissue cells including bone, cartilage, tendon, muscle and adipose tissue. This variety allows the cells to be isolate with ease from the bone marrow and used in different functioning capacities [18].

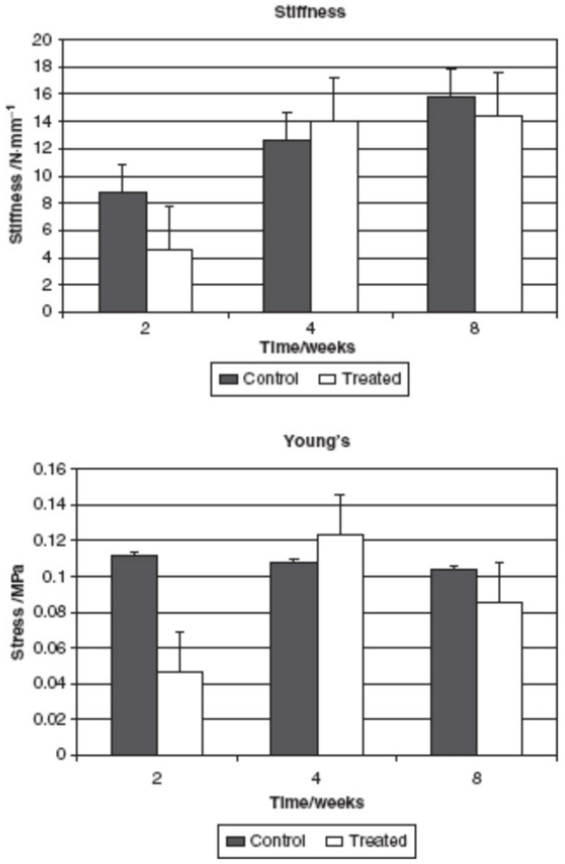

Figure 3: Stiffness and Young's modules at the ultimate load to failure in mesenchymal stem cell-treated and control reconstructions at each time frame [17].

Human adipose-derived mesenchymal progenitor cells (haMPCs)

Wang et al. [19] conducted a study that involved injecting haMPCs into rabbit models via intra-articular injection. The data demonstrated that haMPCs were an effective method to treat OA in rabbit models.

Tendon Derived Stem Cells and Human Umbilical Cord Mesenchymal Stem Cells

Lui et al. [20] illustrated the use of tendon derived stem cells (TDSCs) in rat models, to promote early graft healing. Green fluorescent protein (GFP) rat TDSCs were treated with connective tissue growth factor and ascorbic acid to stimulate the TDSCs to secrete extra-cellular matrix and form a cell sheet. The tendon graft was wrapped in a GFP-TDSC sheet before graft insertion. Computed tomography imaging and histological/biomechanical testing was performed at weeks 2, 6, and 12 after the reconstruction. Results showed that cell counts, and vascularity increased in the control group, but there was a loss in cell alignment (Figure 4). The TDSC group stayed intact throughout the graft advancement and shows clear increase in vascularity with visualized alignment.

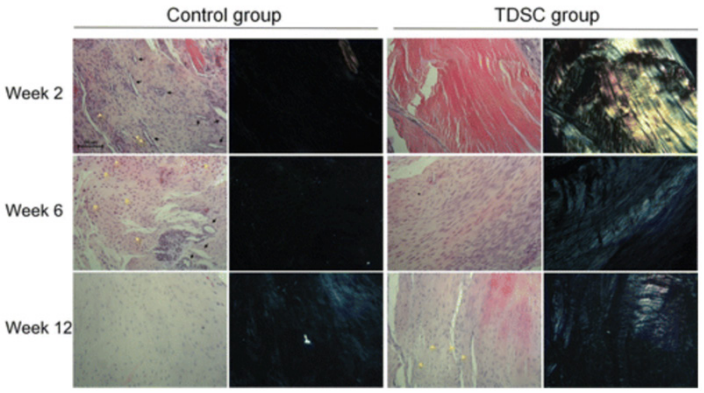

Figure 4: Intra-articular graft mid-substance remodeling after the ACL reconstruction. Photomicrographs show the intra-articular graft mid-substance in the control and TDSC groups at weeks 2, 6, and 12. The left images show hematoxylin and eosin staining, while the right shows polarized images of the same view. Black arrows indicate blood vessels, and yellow arrowheads indicate chondrocyte-like cells [20].

Human Umbilical Cord Mesenchymal Stem Cell (hUCB-MSC) enhanced ACL reconstruction was performed by Jang et al. [21] on 30 adult rabbits. The bone tunnels were treated with hUCB-MSCs and the control group was untreated. Specimens were collected at 4, 8, and 12 weeks to perform histological assessment using Haemotoxylin and Eosin staining (H&E stain) as well as immunohistochemical staining to test for COII. Results from this study showed enhanced tendon-bone healing through fibrocartilage formation and high histological scores (Figure 5 and Figure 6) and decreased femoral tunnel widening (Figure 7) in the treatment group compared to the control group (79.2% and 80% respectively, at 12 weeks). The worry of using this type of cell lineage was immune rejection, but that did not occur in this study.

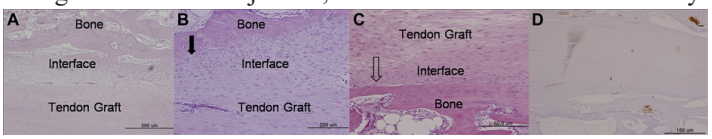

Figure 5: Histological findings for the control group. (A) At 4 weeks, distinctive and broad interface zones were noticed. Sharpey-like fibers crossing the tendon-bone interface were rarely found along the interface zone (H&E stain at 100x magnification). (B) The interface zones at 8 weeks were narrowed and Sharpey-like fibers (arrow) were identified

partially (H&E stain, original magnification x100). (C) At 12 weeks, increased Sharpey like collagen fibers (arrow) were noticed around the tendon graft (H&E stain at original magnification x100). (D) The control group showed limited immunohistochemical staining even at 12 weeks (immunohistochemical stain for COII, original magnification x20) [21].

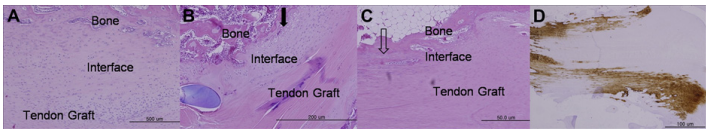

Figure 6: Representative histologic findings in a treatment-group rabbit. (A) At 4 weeks, immature cartilage cells were partially arranged in disorganized clusters in the interface zone (H&E stain, original magnification ×100). (B) Increasing organization of cartilage cells in the interface zone was noticed in some parts (arrow) at 8 weeks (H&E stain, original magnification ×40). (C) At 12 weeks, a smooth transition was noticed from bone to tendon through the fibrocartilage resembling the chondral-like enthesis in a normal ACL (arrow) (H&E stain, original magnification ×40). (D) The interface zone in the treatment group showed abundant COOII production by immunohistochemical staining at 12 weeks (immunohistochemical stain for type II collagen, original magnification ×20) [21].

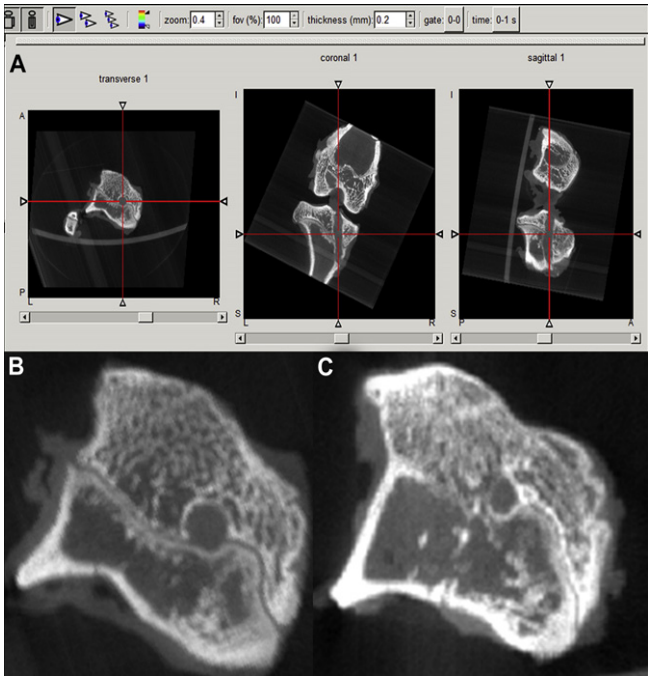

Figure 7: Micro-computer tomography of bone tunnel enlargement. (A) The area of the bone tunnel in the perpendicular plane to the longitudinal axis of the tibial tunnel at a 5-mm depth from the tunnel inlet was assessed using AMIDE (a medical imaging data examiner). (B) Control-group specimen. (C) Treatment group specimen. Tibial tunnel enlargements were significantly smaller in the treatment group than in the control at 12 weeks. Femoral tunnel enlargement was significantly smaller in the treatment group at 8 and 12 weeks [21].

ACL derived CD34+ stem cells

Matsumoto et al. [22] reported an effect of using ruptured human ACL to obtain vascular stem cells that can contribute to tendon-bone healing in an immunodeficient rat model. Two rat studies have been conducted using hACL CD34+ cells derived from the site of ACL rupture. These cell lineages showed enhanced expression of COII and increased angiogenesis and osteogenesis in the hACL CD34+ cell-treated group at week two [23].

Summary and Conclusions

Currently, the most applicable type of graft is an allograft, due to low donor site morbidity and high rehabilitation time in animal studies, as well as human studies. The only downside of this graft type is re-tearing of the ACL, but this outcome is also present in autograft studies, and is not shown to be significantly different. It has also been brought to light in recent studies about functionality/rigidity of allografts, but the data is inconclusive at this time. BMSC's have the greatest efficacy at this point because of they are easily retrievable and can differentiate into multiple cell lineages including bone, cartilage, tendon, muscle and adipose tissue. Vastness of differentiation can lead to a more holistic healing of the knee joint as a whole as well as the injured ligaments. Overall, more work needs to be done on the injection side of stem cells and their future applications, but promise was shown in the study completed by Wang et al., which reduced OA in rat models, which also happens to be a side effect of allograft usage in the long run.

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