[Orthopaedic Surgery]

Mesenchymal Stem Cell Therapy in the Sports Knee: Where Are We in 2011?

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Background: The relationship between biological tissue healing following knee injury or surgery and long-term clinical outcome has come to the forefront of sports medicine practice. This has led many knee surgeons to incorporate biologically mediated healing factors into the management of knee injuries. In particular, the clinical use of mesenchymal stem cells has opened new horizons.

Evidence Acquisition: Relevant studies were identified through a search of PubMed from January 2000 to April 2011, combining the term *mesenchymal stem cells* with *articular cartilage*, *anterior cruciate ligament*, and *meniscus*. Relevant citations from the reference lists of selected studies were also reviewed.

Results: Knee injury treatment with mesenchymal stem cells shows potential. Most reports represent animal model studies; few advances have been translated to human clinical applications.

Conclusion: Mesenchymal stem cell use to promote healing following knee injury is likely to increase. There are scientific methodological concerns and ethical and legal issues regarding mesenchymal stem cell use for treating knee injuries.

Keywords: biological tissue healing; cell-based therapy; cartilage repair

Many patients who have sustained knee injuries desire treatment options that will enable them to continue sports participation. Over the past several years, the sports medicine community has seen an increase in the use of biological agents, including cell-based therapies for this purpose.[7,8] The introduction of stem cells, specifically mesenchymal stem cells (MSCs), into the clinical setting has opened new knee treatment horizons (Figure 1).[12]

WHAT ARE MSCS?

Stem cells harvested from human embryos and adult tissues have the capacity for self-renewal.[52,76] The utility of adult stem cells is generally restricted to the generation of more of the same tissue from which it was harvested, such as hematopoetic stem cells in blood.[9] However, under certain conditions, some adult stem cells, such as those harvested from mesenchymal tissues, can differentiate into multilineages and become multipotent. MSCs are characterised by surface-specific antigen and the absence of hematopoetic stem cell markers, such as leukocyte common antigen.[15] Friedenstein et al[26] demonstrated that bone marrow cells could differentiate into bone and cartilage. Subsequent research found that MSCs harvested from bone marrow could differentiate into bone, cartilage, tendon, ligament, fat, and other tissues of mesenchymal origin.[42,68] MSCs can also be harvested from synovium,[16,18] periosteum,[27] skeletal muscle,[1] adipose tissue,[21,89,96] trabecular bone,[71] and umbilical cord blood.[54]

MESENCHYMAL STEM CELLS FOR ARTICULAR CARTILAGE REPAIR

Chondrogenesis

One of the primary concerns in articular cartilage repair is the integration of engineered calcified articular cartilage with underlying bone. MSC-mediated chondrogenesis may improve this integration.[42-48,93] Since Ashton et al[5] first reported MSC-mediated chondrogenesis, others have investigated chondrogenic potential when derived from tissues as diverse as human adipose[19] and trabecular bone,[71] as well as rat bone marrow, synovium, periosteum, adipose, and muscle tissues.[11,94] Bone marrow–derived MSCs may allow better differentiation of the deep calcified articular cartilage zone adjacent to healthy bone.[23,62,80] MSCs harvested from bone marrow have

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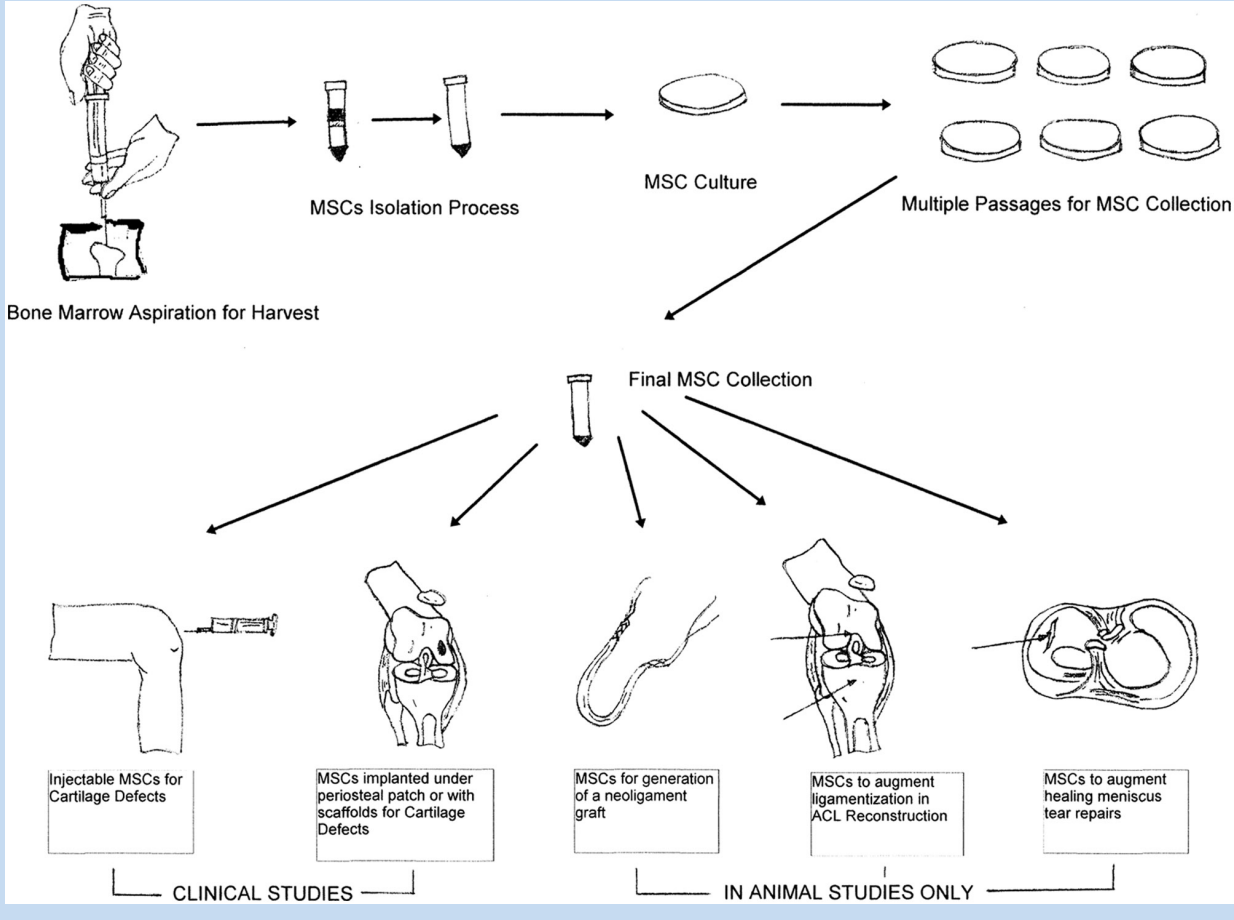

Figure 1. Mesenchymal stem cell (MSC) harvest, isolation, and culturing. Clinical studies have been performed with MSC injections under a periosteal patch or in combination with a scaffold to treat articular cartilage defects. Intra-articular MSC injections have also been performed clinically to treat knee articular cartilage defects. MSC use to create neoligaments, to augment graft ligamentization following anterior cruciate ligament reconstruction, and to enhance meniscal repair healing has been performed using only animal models.

been the primary source in most animal model and human chondrogenesis research studies.[13,14,33] MSCs derived from rat synovium may have greater proliferation and chondrogenesis potential than rat bone marrow, periosteum, adipose, or muscle sources. In humans, bone marrow has a high MSC density, providing similar culture expansion potential compared with other tissue sources.[61,72,94] A rabbit study by Koga et al[47] reported that MSCs harvested from bone marrow and synovium had a greater potential to repair articular cartilage defects than cells from skeletal muscle and adipose tissue.[37]

Enhancing Articular Cartilage Repair

Cytokines and growth factors are important for chondrogenesis.[30,38,59,90] The cytokines and growth factors that promote chondrogenic differentiation also contribute to osteogenic differentiation.[25,59] The most potent chondrogenic differentiation inducers are transforming growth factor β, bone morphogenic protein, fibroblast growth factor, and insulin-like growth factor 1.[38,57,64,90] The induction process is enhanced in the presence of dexamethasone.[15]

Chondrogenesis can be confirmed using alcian blue staining to detect articular cartilage-specific proteoglycans and by microscopy to identify chondrocytes and type II collagen. Additionally, molecular testing can detect MSC chondrogenic-specific transcription factor Sox 9 and β_1 integrin.[52] With autologous chondrocyte implantation (ACI), the chondrocyte density required for treatment is 10^6 cells per milliliter or slightly less when embedded in a scaffold gel.[63] The cell density required to achieve chondrogenesis with MSCs is greater than that for ACI.[46-48] Similar concentrations eventually failed in animal models, while 10^7 cells per milliliter embedded in a collagen gel successfully repaired articular cartilage defects.[46-48] Yokoyama et al[92] reported that a MSC density of 5×10^7 or 5×10^8 cells per milliliter embedded in a collagen gel had more proteoglycans than lower cell densities, better

facilitating chondral defect healing and supporting the need to identify a MSC source with a high proliferation potential.[47]

Johnstone et al[42] first described a 3-dimensional micromass culture medium of bone marrow–derived MSCs for in vitro chondrogenesis to investigate regulatory factors and signaling events. In vitro high-density micromass culture enhances cell-cell interaction, aggregating into a high-density precartilaginous core. The extracellular matrix that develops resembles those seen in vivo compared with single-layer methods.[91] The culture medium used for in vitro MSC expansion and clinical use has traditionally been fetal bovine serum.[35,89-92] However, disease transmission and immune reaction issues exist with fetal bovine serum use in human clinical trials.[58,76] Studies by Tateishi et al[81] and Nimura et al[61] have shown that human serum culture medium has the potential to increase MSC proliferation without the disease transmission or immune reaction risk of an animal source.

Implantation Methods for Articular Cartilage Repair

The method used to deliver MSCs to a chondral defect is an important consideration. The goal is to create a 3-dimensional environment that optimizes cell proliferation and differentiation.[16] First-generation MSCs for chondral repair involved direct implantation under a periosteal patch, like early ACI procedures.[60] Second-generation techniques differentiate MSCs in vitro within a matrix or bioscaffold and implant the construct into a chondral defect at cellular maturity.[91] This approach does not mimic natural articular cartilage formation.[6,7,36,79] MSCs differentiated in vitro and transplanted subcutaneously fail to produce articular cartilage or become calicified.[16,18,66] Creation of an appropriate in vivo microenvironment is essential for inducing articular cartilage formation of the desired phenotype when MSCs are used.[16,66]

A third-generation approach using a bioscaffold seeded with MSCs is similar to second-generation ACI techniques.[40] Bioscaffolds can reduce cell leakage and complications from periosteal hypertrophy.[20] MSCs can differentiate and adhere to scaffolds and matrices[56,69,75] consisting of synthetic polymers, poly(L-lactide), poly(glycolide), poly(D,L-lactide-co-glycolide), alginate, or biomaterials such as collagen, fibrin, hydrogel, hyaluron, and chitosan.[4,10,20,31,50,51,92] Scaffold-free MSC tissue-engineered constructs in porcine models and humans retain chondrogenic potential and display adequate mechanical properties without scaffold support.[2,3] Intra-articular MSC injections have been investigated for treating chondral defects and knee osteoarthritis.[53,74] In a porcine model, MSCs injected intra-articularly for medial femoral chondral defects resulted in better tissue morphology and histology scores compared with controls injected with saline or hyaluronic acid.[53]

Saw et al[74] created 4-mm full-thickness chondral defects in goat stifle joints followed by microfracture. Group A did not receive intra-articular injections. Group B received weekly injections of 1 mL sodium hyaluronate for 3 weeks. Group C received the same injections as group B but with the addition of 2 mL of autologous bone marrow aspirate (carrying MSCs). At 24 weeks postsurgery, group C had a histologically superior articular cartilage repair.[74] Electromagnetic fields may guide intra-articularly injected MSCs directly to the chondral defect.[44]

Articular Cartilage Repair: Human Clinical Studies

Although basic science MSC research is abundant, few studies have been translated directly into human clinical practice. Bone marrow MSCs were used to treat knee osteoarthritis in 24 patients with medial femoral condyle articular cartilage defects, including those treated previously with high tibial osteotomy and articular cartilage resurfacing.[86] Patients were divided into 2 groups. Group 1 received MSCs suspended in a type I collagen gel scaffold covered with an autologous periosteal flap. Group 2 received a cell-free scaffold covered with an autologous periosteal flap.[86] At 16 months, both groups had improved knee rating scores, and group differences were not evident. Nine of 12 patients in the MSC group (group 1) and 6 of 12 patients in the cell-free group (group 2) had second-look arthroscopy at a mean of 42 weeks postimplantation (range, 28-95). Histologic assessment revealed better scores for the MSC group. The main criticism of this study is that patients with preexisting knee osteoarthritis are generally not considered articular cartilage resurfacing candidates. Reports such as these have spurred additional MSC chondral defect studies.[29,49,60,87,88] In 5 patients with isolated patellofemoral joint chondral defects treated with MSCs, decreased pain and improved walking ability at 6 months was reported lasting up to 4 years postsurgery.[87,88] Clinical symptom improvement following bone marrow–derived MSC treatment of an isolated femoral chondral defect has been reported.[49] At 6 and 12 months following bone marrow–derived MSCs delivered on a platelet-rich fibrin glue scaffold for femoral condyle articular cartilage defects, 5 patients had decreased knee pain and improved function.[29] Two patients underwent second-look arthroscopy at 12 months postsurgery and had International Cartilage Repair Society evaluation scores of 8 and 11 (of 12; ie, nearly normal). Magnetic resonance imaging at 12 months postsurgery in 3 patients showed complete chondral defect filling and restored surface congruity. Two patients had incomplete chondral surface congruity; however, both had significantly decreased knee pain.

Despite these good results at 1 year postsurgery, it is unknown how they would compare with ACI or microfracture. A matched patient cohort of a MSC-derived chondrocyte group implanted with a periosteal patch (n = 36) were compared with an ACI group (n = 36) for the treatment of patella, trochlea, and femoral condyle chondral defects.[60] Both groups had improved scores at 3, 6, 9, 12, 18, and 24 months postsurgery. MSCs cultured in human serum may provide cell proliferation and chondrogenesis that surpasses ACI articular cartilage–resurfacing capability.[81] At 1 week after arthroscopic subchondral drilling of grade III and IV knee chondral lesions, 180 patients received a series of 5 weekly intra-articular injections (autologous peripheral blood-derived stem cells and hyaluronic acid).[73,74] Five patients from this series underwent second-look arthroscopy between 10 and

26 months postsurgery. Histologic testing of the chondral core confirmed articular cartilage regeneration and hyaline cartilage formation.[73]

Several reports have described chondral drilling followed by bone marrow type I/III collagen patch use.[41,56,65] At 24 months postsurgery, the median International Knee Documentation Committee Subjective Knee Evaluation score of 30 improved to 83 (176.7% improvement); the Lysholm Knee Scale score of 54 improved to 98 (81.5% improvement); and magnetic resonance imaging revealed decreased chondral defect size.[65] At 8 months, an equine model using bone marrow aspirate concentrate and thrombin for femoral trochlea chondral defects treated with microfracture showed superior chondral defect filling and improved integration with the adjacent articular cartilage based on magnetic resonance imaging and histologic evaluations compared with the control group that underwent microfracture alone.[24]

Potential Benefits of MSC Use Compared With ACI

The advantage of bone marrow MSCs compared with ACI would be that of eliminating the need to harvest articular cartilage as a treatment source. A comparison of ACI and MSCs for chondral defect repair in a rabbit model showed ACI articular cartilage degeneration within 36 weeks postsurgery, while MSC-derived articular cartilage remained intact.[34]

Martin and Buckwalter[58] suggested that autologous chondrocytes harvested from older patients would produce inferior results. In a rat study, bone marrow MSCs of older rats produced less extracellular matrix.[17,95] Patients < 45 years of age had superior clinical outcomes (International Cartilage Repair Society, Short Form–36, International Knee Documentation Committee, Lysholm, and Tegner) than patients > 45 years of age for the ACI.[60] Age did not influence the clinical outcome of patients that received bone marrow–derived MSCs.

Concerns Regarding MSCS for Articular Cartilage Repair

There are 4 primary concerns regarding MSCs. First, MSC-derived chondrocytes may express hypertrophy-related genes, leading to cell death or calcification, followed by vascularization postimplantation.[16] Second, the ultimate articular cartilage thickness is less than healthy tissue, and the tidemark may be violated.[1,46-48,85-88] Third, MSCs may transform long after culturing (during the standard ex vivo expansion period, these transformations can be safely managed).[8,70,82] Fourth, the mechanical integrity of the regenerated tissue is unknown.[47,84] Based on mechanical tests, biochemical alterations may be needed before MSC chondrogenesis can produce results comparable with primary autologous chondrocytes.[47,84]

STEM CELLS IN ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION OR REPAIR

Although MSC use for anterior cruciate ligament (ACL) reconstruction has been studied using animal models,[43,55,77] we did not identify any human clinical studies. Surgically created partial ACL lesions in rat studies were treated with intra-articular stem cell injections to accelerate ACL healing.[43] At 4 weeks postsurgery, the MSC group had superior histological scores and withstood greater mechanical loads at ultimate failure, compared with the control group.[43] In a rabbit study, hamstring ACL autografts were coated with a fibrin glue laden with bone marrow–derived MSCs or with fibrin glue. The experimental graft withstood greater loads at failure.[55] Similar results were obtained with fresh-frozen rabbit Achilles tendon allografts coated with bone marrow MSCs for ACL reconstruction.[77]

MSC might be used to create an ACL "neoligament": The prerequisites are (1) a suitable cell source, (2) a biocompatible scaffold, and (3) a biomechanical environment that promotes safe healing and organized maturation.[67] Adult fibroblasts retain many phenotypic qualities necessary for collagen synthesis but are relatively quiescent biologically and have limited potential for further differentiation.[83] Comparison of goat bone marrow MSC, ACL, and skin fibroblasts found that bioscaffolds seeded with bone marrow–derived MSC had the highest DNA content and collagen production.[83] In a rat study, MSC on a fascial wrap scaffold was compared with knotted poly-L-lactic-acid and poly-lactic-co-glycolic acid scaffolds.[28] MSC–fascial wrap scaffold generated more type I and type III collagen essential to neoligament development. However, the increased collagen content did not translate into improved maximal tensile load or stiffness.

MSC USE FOR AUGMENTING MENISCUS REPAIR

In a rat study, a surgically-created meniscal defect was treated with MSC embedded in fibrin glue, with fibrin glue alone, or was left untreated. At 12 weeks, an abundant extracellular matrix with meniscal-like tissue was observed in the MSC–fibrin glue group.[39] In a porcine model, MSC and fibrin glue augmented meniscus suturing in avascular zone radial tears.[22] When no fibrin or MSCs were used, healing did not occur. With MSCs and fibrin glue, 21 of 28 specimens (75%) had complete healing; 5 (17.9%) had incomplete healing; and 2 (7.1%) had no healing. With only fibrin glue augmentation, no healing occurred in 12 of 19 specimens (63.2%), and incomplete healing was observed in 7 (36.8%). Horie et al[32] reported that synovium-derived MSC injected intra-articularly to heal massive meniscal tears adhered directly to the lesion and differentiated into meniscal cells.

CONCLUSIONS

Numerous animal model studies have been performed to determine the ideal MSC source, culture medium, and implantation technique. In the right combination and with timely growth factor availability, articular cartilage healing may occur with MSCs, producing histologic differentiation and biomechanical characteristics of healthy tissue. Unfortunately, few animal model MSC study advances have been translated into the human clinical setting.

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