# Stem Cells and Biological Approaches to Treatment of Wrist Problems

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**Abstract**

Stem cells are being intensively studied for their potential applications in clinical medicine. Mesenchymal stem cells (MSCs) are an important subset of stem cells which are attractive for application in musculoskeletal disorders. In this article, we review the characteristics of these MSCs that are relevant to clinical practice but that are still largely experimental in nature.

**Keywords**

- wrist
- stem cells
- experimental
- therapeutics

Human embryonic stem cells (ESCs) derived from blastocysts are pluripotent stem cells that can differentiate into any cell derivative of the ectoderm, endoderm, and mesoderm. Moreover, ESCs have a nearly unlimited replicating capacity. However, their application in transplantation and tissue engineering is greatly hindered by the risk of tumor formation and political and ethical concerns.[1]

A group of plastic-adherent, fibroblast-like cells (►**Fig. 1**) were first derived from bone marrow in tissue culture plates in the 1980s.[2] These cells had the characteristics of stem cells including self-renewal and differentiation potential. These cells were given many names, including mesenchymal stem cells (MSCs), mesenchymal progenitors, and multipotent stromal cells. The therapeutic potential of MSCs has generated an enormous interest in a wide range of biomedical disciplines. The Mesenchymal and Tissue Stem Cell Committee of International Society for Cellular Therapy proposed a minimal set of standard criteria when using these cells for research to provide a standard definition of human multipotent mesenchymal stromal cells. This definition includes the following: (1) The MSC must be plastic-adherent when maintained in standard culture conditions. (2) The MSC must express specific surface antigen markers CD105, CD73 and CD90 and must not express CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR surface molecules. (3) The MSC must have the capacity to differentiate into osteoblasts, adipocytes, and chondroblasts in vitro.[3] The surface phenotype, in combination with the other functional criteria, best identifies MSC with the current state of knowledge.[3]

Despite this definition of MSCs, it is important to understand that MSCs are a heterogeneous population of cells and lack a unique marker.[4] Besides differentiating into specific lineages of mesoderm origin such as osteocytes, chondrocytes, tenocytes, and muscle cells, MSCs can also transdifferentiate into cell types of the other germ layers such as neurons, hepatocytes, cardiomyocytes, pancreatic, and renal cell types under very defined conditions.[5]

## Isolation and Expansion of MSCs

MSCs from bone marrow aspirate can be isolated by a variety of methods. Two commonly used techniques include the direct plating method and the density centrifugation method. Both methods produce very similar populations of MSCs. In the direct plating method, the bone marrow sample is diluted with an MSC growth medium and cultured without any disturbance at 37°C for 4–5 days. The medium is changed every 3–4 days, and contaminating red cells and other nonreplicating and nonattaching cells are isolated and rinsed away. In the density centrifugation method, an initial isolation of mononuclear cells using a Ficoll density solution is performed, and then mononuclear cells are collected and cultured in an MSC growth medium. In some studies, the MSCs are further purified based on their expression of



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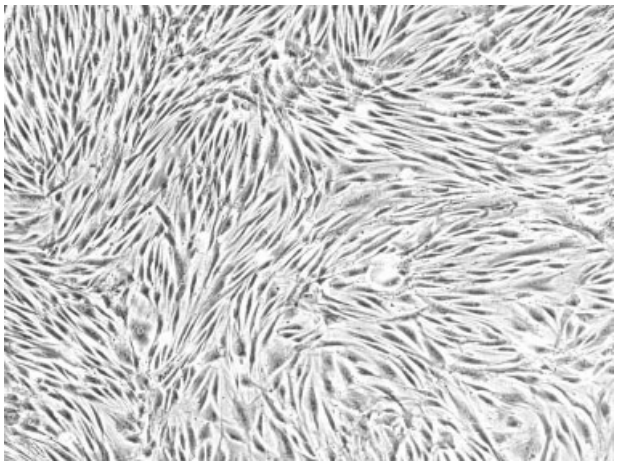

**Fig. 1** The fibroblast-like appearance of bone marrow-derived mesenchymal stem cells after in vitro expansion.

primitive MSC markers.[5] Experiments on rat MSCs have shown that direct plating density may be not critical for maintaining a multipotent MSC population. However, culture time may affect cell characteristics, including cell morphology and differentiation potential, suggesting that cell expansion should be limited.[6]

MSC-like cells have been found in human fetal blood and umbilical cord blood.[7,8] In adults, besides the bone marrow, MSCs can be isolated from multiple tissue sites including adipose tissue, synovium, periosteum, and muscle.[9–11]

## MSCs in Articular Cartilage

Articular cartilage, which consists mostly hyaline cartilage, has a limited capacity to regenerate or self-repair. Osteoarthritis often affects joints that are subjected to high-loading conditions, which may lead to a dramatic loss of articular cartilage. Autologous chondrocyte implantation (ACI) is currently the most common reconstructive technique for replacing articular cartilage.[12] ACI includes the following steps: (1) Harvest a small piece of articular cartilage from the patient. (2) Subsequently, this biopsy is enzymatically treated to isolate the chondrocytes. The chondrocytes are expanded in vitro. (3) The patient then undergoes a second operation, in which the expanded chondrocytes are applied on the damaged area of cartilage. The implanted autologous chondrocytes are presumed to regenerate the damaged cartilage. Biopsy specimens from patients who underwent autologous chondrocyte transplantation tissue after 2 years showed evidence of fibrous tissue and fibrocartilage formation instead of typical hyaline cartilage. Viable chondrocytes have been seen in the biopsy samples.[13,14] The expansion of chondrocytes in vitro for ACI induces the dedifferentiation of chondrocytes, characterized by the loss of expression of type II collagen and aggrecan in favor of fibrotic type I and III collagens.[15]

In an attempt to improve the outcomes, the use of stem cells to regenerate cartilage have been investigated. It is well established that MSCs can manipulated in vitro to undergo chondrogenic or osteogenic differentiation. Comparative analysis has shown that bone marrow–derived MSCs have a slightly superior chondrogenic potential when compared with adipose tissue–derived MSCs.[16] Synovium and bone marrow MSCs had a greater in vivo chondrogenic potential than adipose and muscle MSCs, but synovium MSCs had the advantage of a greater proliferation potential.[17] Saw et al have found that arthroscopic subchondral drilling, followed by postoperative intraarticular injections of autologous peripheral blood stem cells in combination with hyaluronic acid, improved the quality of articular cartilage repair on knees, as shown by histologic and magnetic resonance imaging (MRI) evaluation.[18,19]

Bone marrow–derived MSCs have been used in attempts to regenerate articular cartilage in vivo in humans. Kuroda et al treated a 20 $\times$ 30-mm full-thickness articular cartilage defect in the femoral condyle of a judo athlete with autologous bone marrow stromal cells.[20] Bone marrow was aspirated from the iliac crest of the patient 4 weeks before surgery, and mesenchymal stem cells were expanded in vitro before transplantation into a collagen gel. Gel–cell composite was covered with an autologous periosteal flap. The autologous periosteal patch was affixed to the surrounding rim of the normal cartilage with interrupted absorbable sutures. Seven months after surgery, arthroscopy revealed that the defect was covered with smooth tissues. Histologically, the defect was filled with hyaline-like cartilage tissue that stained positively with both safranin-O and toluidine blue. Immunohistochemistry for type II collagen revealed a positive staining. One year after surgery, the clinical symptoms had improved significantly. Gianinni et al used a one-step repair technique to repair talar osteochondral lesions (chronic type II $<$1.5 $cm^2$ in area, $<$5 mm deep) in 48 patients, using a malleable paste that was composed of collagen, bone marrow concentrate, and platelet-rich fibrin.[21] Two years after surgery, functional scores using the American Orthopaedic Foot and Ankle Society (AOFAS) scores were improved. However, histologic and immunohistologic results showed the presence of new cartilaginous tissues with various degrees of tissue remodeling toward hyaline cartilage. Haleem et al used MSCs transplanted on a scaffold of platelet-rich fibrin glue in the treatment of articular cartilage defects (defect size: 3 to 12 $cm^2$) in the knees of five patients. Symptoms and knee scores (Lysholm and Revised Hospital for Special Surgery Knee scores) were improved in all patients.[22] Controlling MSCs' differentiation to regenerate hyaline cartilage instead of fibrocartilage in vivo remains a major challenge.

## MSCs in Bone Repair

Bone has a much superior regeneration capacity compared with cartilage. This difference is probably due to a large number of cells involved in the bone innate repair response, including osteoclasts, osteoblasts, periosteum, and bone marrow–derived stem cells.[23] The extensive vascularity in bone also provides sufficient nutrition and growth factors during the self-repair process.[23] The limits of bone regeneration are reached when there is significant bone loss or in situations where vascularity of the bone is tenuous, for example in the

scaphoid. In those situations, there is a clinical need to enhance bone repair and regeneration. MSCs provide an osteoblast supply during adulthood; therefore they are the cells of particular interest in research for bone regeneration.

Stem cell therapy has achieved fracture healing in several different animal models. Kadiyala et al implanted human bone marrow–derived MSCs with a ceramic carrier into critical-sized segmental defects in the femurs of adult athymic rats.[24] Histological evaluation demonstrated increasing bone formation by MSC implantation through 12 weeks. Femurs implanted with MSC-loaded ceramics were significantly stronger biomechanically than those that received cell-free ceramics. Petite et al implanted MSCs with a coral scaffold in large segmental bone defects in sheep.[25] Clinical union never occurred when the defects were left empty or filled with the coral scaffold alone (seven operated limbs in each group). In contrast, clinical union was obtained in three out of seven operated limbs when the defects were filled with the tissue-engineered bone. Lee et al treated a case of canine nonunion fracture with autologous adipose-derived MSCs seeded on a hydroxyapatite/chitosan composite scaffold.[26] Three months after surgery, the nonunion successfully healed with no complications.

Percutaneous injection of autologous bone marrow has been used to promote bone healing in humans in several clinical studies since the 1990s.[27–29] In these studies, bone union was obtained in ~75–90% patients. However, no statistical comparisons were made. Bone marrow serves as a promptly renewable and reliable source of osteogenic stem cells, and its efficacy appears to be related to the number of progenitors in the graft.[28] Administration of MSCs has also shown promising results in human. Successful healing of a 9-year old tibial nonunion resistant to six previous surgical procedures was achieved by combination of in vitro–expanded bone marrow–derived MSCs and calcium sulfate pellets two months after implantation.[30] The clinical use of enriched bone marrow MSCs combined with porous β-tricalcium phosphate had a 95.1% union rate in spinal fusion; however, in that study no controls were used.[31] Stem cell implantation, or stem cells coupled with gene therapy, may have the potential to treat nonunion of the scaphoid[32]; however, direct evidence in animal models or in clinical studies is not available yet to the best of our knowledge.

## MSCs in Wrist Disorders

Arthrosis is characterized by degeneration of the articular cartilage and, ultimately, joint destruction. In a donkey model of osteoarthritis induced by amphotericin-B, MSCs labeled with green fluorescent protein were detected in all examined articular cartilages, indicating successful homing after intra-articular injection of stem cells.[33] MSCs only partially delayed cartilage deterioration in this model.[33] Similarly in a horse model of osteoarthritis induced arthroscopically, MSCs did not show a significant therapeutic effect. Further study is required to validate the effect of stem cell treatment in arthrosis.[34]

Kienböck disease, or avascular necrosis of the lunate, was first described in 1910. Osteonecrosis of the lunate will lead to wrist pain and loss of function. In the early stage, this disorder has been treated by a joint-leveling procedure in an attempt to prevent collapse of the lunate. In the late stages, when there is fragmentation and lunate collapse, salvage procedures are performed such as a proximal row carpectomy or lunate excision and partial wrist fusion. Stem cell–based tissue engineering strategies might provide an alternative treatment method. In a rabbit lunate excision model, implantation of bone marrow–derived MSCs with a gelatin and hyaluronan scaffold maintained carpal height by 6 weeks, compared with the control group.[35] Abundant bone formation as well as evidence of neovascularization was observed by 12 weeks.[35] Implantation of MSCs preincubated in a chondrogenic medium with a scaffold also promoted bone and cartilage formation and stimulated vascularization in a rabbit model after 12 weeks.[36] Similarly, insertion of hybrid tendon rolls with a core of collagen–ceramic composite seeded with MSCs induced bone formation in the central part and cartilage formation at the peripheral part of the lunate.[37] In other studies, bone necrosis in rabbits was created by freezing and thawing the bone with liquid nitrogen. Drilling and bone marrow injection into the necrotic lunate accelerated bone formation and remodeling compared with the control group.[38] In a dog model, liquid nitrogen–treated scapholunates showed no regeneration of bone tissue, demonstrating severe collapse and deformity as observed in human Kienböck disease.[39] However, liquid nitrogen–treated scapholunates that were subsequently seeded with MSCs showed no collapse and deformity, and the cavity was completely filled with normal cancellous bone within 4 weeks.[39]

The limitations of these studies in animal models include the following: (1) Biomechanical features of regenerated tissues are unknown. It is still not clear whether they can bear the pressure from surrounding bones during active movement in human. (2) The follow-up time is relatively short. The long-term effect of MSCs has not been investigated. (3) The studies use an acute model of chronic phenomena.[40] It is unclear whether the findings will be translated to actual clinical situations. Future clinical studies are crucial for gaining convincing evidence of therapeutical potential of MSCs in wrist disorders.

**Conflict of Interest**
None

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