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**Mesenchymal stem cells as a potent cell source for articular cartilage regeneration**

Eslaminejad MB *et al.* MSCs for articular cartilage regeneration

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**Abstract**

Since articular cartilage possesses only a weak capacity for repair, its regeneration potential is considered as one of the most important challenges for orthopedic surgeons. The treatment options, such as marrow stimulation techniques fail to induce a repair tissue with the same functional and mechanical properties of native hyaline cartilage. Osteochondral transplantation is considered as an effective treatment option, but it is associated with some disadvantages including donor-site morbidity, tissue supply limitation, unsuitable mechanical properties, and thickness of the obtained tissue. Although autologous chondrocyte implantation results in reasonable repair, it requires a two-step surgical procedure. Moreover, chondrocytes expanded in culture gradually undergo dedifferentiation, so loose morphological features and specialized functions. In the search for alterative cells, scientists have found mesenchymal stem cells (MSCs) as an appropriate cellular material for articular cartilage repair. These cells have originally been isolated from bone marrow samples, and further investigations have revealed the presence of the cells in many other tissues. Furthermore, chondrogenic differentiation is an inherent property of MSCs that is noticed at the time of the cell discovery. MSCs are known to exhibit homing potential to the damaged site at which they differentiate into the tissue cells or secrete a wide spectrum of bioactive factors with regenerative properties. Moreover, these cells possess a considerable immunomodulatory potential that make them as the general donor for therapeutic applications. All of these topics will be discussed in this review.

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**Key words:** Mesenchymal stem cells; Regeneration; Articular cartilage; Cell therapy

**Core tip:** Articular cartilage possesses only a weak capacity for repair, therefore regeneration of its defects considered as one of the most important challenges for orthopedic surgeons. On the other hand, mesenchymal stem cells (MSCs) are specified as appropriate cell candidates for regenerating incurable defects of articular cartilage due to the following characteristics: inherent chondrogenic property, easy availability, cell homing potential and immunomodulatory function. In the past, several attempts have been made to exploit MSCs capacity to cure articular cartilage defects developed in osteoarthritis, rheumatoid arthritis, or following trauma. All of these topics are discussed in this review.

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**Introduction**

Articular cartilage covers the ends of bones in diarthrodial joints. This highly specialized tissue reduces joint friction and protects the bone ends from the shear forces associated with high mechanical load. Furthermore, it works as a lubricant and a shock absorber. Histologically, articular cartilage is a hyaline cartilage tissue with no blood, lymphatic, or nerve supply.

An articular cartilage defect is an area of damaged or missing cartilage that is often caused by acute trauma. These defects usually are well defined and surrounded by normal articular cartilage. Cartilage defects may also occur following osteoarthritis (OA), osteonecrosis, osteochondritis dissecans, and other pathologies[1].Defects caused by OA are often ill-defined, large, and surrounded by osteoarthritic tissue of variable quality. If cartilage defects restrict to the articular cartilage, they are termed chondral or partial-thickness defects, and if the defects penetrate into subchondral bone, they are called osteochondral or full-thickness defects.

It has long been known that articular cartilage has only a weak capacity for self-repair[2], which is partially due to its avascularity. In the lack of blood supply, a set of complex biochemical events taking place in order to repair the damage fails to occur. Wound healing in hyaline cartilage is further prevented due to the cartilage dense extracellular matrix impairing migration capacity of chondrocytes[3-5].

In general, while there is no repair process occurring in chondral defects, in osteochondral defects, there would be a repair process initiated by undifferentiated mesenchymal stem cells (MSCs) from the bone marrow tissue of subchondral bone[6,7]. Repair of full-thickness cartilage defects depends mainly on the patient age, defect size and location[8]. Small full thickness defects are repaired by formation of hyaline cartilage, whereas large osteochondral defects are only repaired by formation of scar tissue (fibrous tissue) or fibro cartilage.

For a long period of time, the current regenerative treatment option for joint cartilage defects have been identified as marrow stimulation techniques including microfracture, Pridie drilling and abrasion arthroplasty, all of which involve punching or drilling holes through the subchondral plate[9]. The main disadvantage of such techniques is the formation of repair tissue that is similar to fibrocartilage rather than hyaline cartilage. Fibrocartilage is a poorly-organized tissue containing significant amounts of collagen type I. It exhibits inferior mechanical and biochemical characteristics compared to normal hyaline articular cartilage. The matrix of fibrocartilage breaks down with time and loading, leading to development of secondary OA in injured cartilage[10].

Autologous osteochondral mosaicoplasty, known also as osteoarticular transfer system, is other therapeutic option for cartilage repair. Unfortunately, it undergoes a technically challenging procedure for the clinical application. Osteochondral tissue is usually obtained from a non-weight bearing area of the patient’s own articular cartilage cells. These methods have some disadvantages including donor-site morbidity, tissue supply limitation, unsuitable mechanical properties, and thickness of the obtained tissue[11,12]. The use of allologous tissue could be considered as an alternative option, but it is associated with high cost, risk of immunologic rejection and transmission of pathogens[13].

There are two types of cell-based treatments for cartilage defects including autologous chondrocyte implantation (ACI) and stem cell-based cell therapy[14]. ACI technique involves a two-step surgical procedure as follows: (1) collecting tissue and (2) transplantation. According to the literature, effectiveness of ACI is still controversial. While some scientists have reported that this technique is more likely to be applicable for small articular cartilage defects, the others believe that even after ACI, some defects have continued to persist in articular cartilage. It is noted that obtaining sufficient chondrocytes from biopsies is challenging; therefore, *in vitro* expansion of chondrocytes is inevitable. It has been reported that expanded chondrocytes in culture gradually undergo dedifferentiation, so loose morphological features and specialized functions[15]. Limitations associated with chondrocyte-based treatment have motivated investigators to search for alternative reliable cellular materials. In this context, embryonic stem cells (ESCs), inducible pluripotent stem cells (iPSCs) and MSCs have gained considerable attention.

ESCs are pluripotent cells derived from a blastocyst inner cell mass. These cells have the characteristics of self-renewal as long as they are exposed to a feeder cell layer or leukemia inhibitory factor (LIF). Differentiation is initiated upon removal of the feeder cell layer or LIF, resulting in the formation of three dimensional cell aggregates known as embryoid bodies (EBs). These EBs can be regionally differentiated into derivatives of three germ layers including mesoderm, ectoderm and endoderm[16]. Thus, ESCs can be a potential stem cell source to fabricate cartilage-like tissue constructs in the field of tissue engineering; however, immunologic incompatibility, possibility of teratoma formation in transplantations, as well as certain ethical concerns which make scientists to be hesitant to use them as cellular materials for tissue regeneration[17]. To consider these concerns, scientists have established ESC-like stem cells, known as iPSCs, from somatic cells by plasmid or adenovirus-based transduction. Actually, iPSCs are patient-specific ESCs without ethical concerns and immunogenicity[18,19].

Among the potential cell source for cartilage regeneration, MSCs are considered as an appropriate candidate owing to the several specific characteristics. In the following lines, these properties will be reviewed and followed by the examples of investigations regarding using MSC-based treatment for articular cartilage defects.

**MSCs**

MSCs, as non-hematopoietic cells, are originally derived from bone marrow tissue. Historically, Cohnheim was the first scientist who suggested the presence of MSCs in bone marrow tissue following some wound-healing experimental studies in rabbits. By intravenous injection of non-soluble aniline stain, this German pathologist found some stained cells at the site of wound, experimentally created in the animal’s distal limb. He concluded that the stained fibroblastic cells would be derived from bone marrow and transferred to the wound site via the circulatory system[20,21]. Many years after this suggestion, through series of bone marrow transplantation experiments, scientists have found that marrow cells are able to produce cartilage and bone-like tissue *in vivo*[22,23], but they were unable to determine the cells responsible for this property. Friedenstein *et* *al*[24] were the first to isolate and to describe a fibroblastic population as the cellular equivalent of chondrogenic and osteogenic features of marrow tissue. They referred to these cells as colony forming unit-fibroblasts. Thus far, the fibroblast-like cells have been referred to as marrow stromal cells, marrow progenitor cells, marrow stromal fibroblasts, as well as MSCs. MSC is the most frequently-used nomination, particularly in recently published investigations.

As with any stem cell type, MSCs possess two important properties including long-term-self-renewal ability and the capacity to differentiate along multiple cell lineages such as bone, cartilage and adipose cells. There is a controversy regarding the profile of surface marker expression on MSCs. According to suggestion of the International Society for Cellular Therapy, CD70, CD90, and CD105 have been used as positive markers, while CD34 has been used as a negative marker[25]. In this context, some scientists have believed that the three positive markers are co-expressed in various cells, so they are unable to identify MSCs *in vivo*, whereas expression of the negative marker, CD34, has been shown on native adipose-derived MSCs[26]. Furthermore, Stro-1 is the other frequently-used marker of MSCs[27,28]. This surface epitope has been shown to be an endothelial antigen, but whether it can identify MSCs *in vivo* remains unknown[29].

Investigations have shown that MSCs occur in low quantity in bone marrow aspirate. In spite of their limited numbers, these cells are easily expandable through standard culture techniques. The propagation of MSCs is strongly dependent on the bovine serum content of culture media. The cells assume a spindly-shaped morphology upon cultivation. MSCs primary culture has been reported to be heterogeneous, containing multiple colonies with various differentiation capacities. Pittenger *et al*[30] have shown that nearly one third of these colonies have osteogenic, adipogenic and chondrogenic differentiation potentials, while the other two thirds exhibit either bipotent or unipotent capacity to differentiate into osteogenic/chondrogenic and adipogenic lineages, respectively. In addition to differentiating into bone, cartilage and adipose cells, MSCs have been reported to possess differentiation capacity along non-mesenchymal cell lineages, such as neurons, keratinocytes, liver, intestine and kidney epithelial cells[31,32]. This property is referred to as MSCs plasticity or transdifferentiation.

**Inherent chondrogenic potential of MSCs**

Chondrogenic differentiation property is among the first differentiation capacities of MSCs reported at the time when Friedenstein *et al*[33] isolated and described the cells. These investigators plated marrow cells in plastic dishes and removed non-adherent cells four hours after culture initiation. The adherent cells remained quiescent for two to four days, and then underwent proliferation. The culture tended to consist of uniformly fibroblastic cells after several rounds of subcultures. The most important feature of the cells reported is the capacity of producing small deposits of bone and cartilage-like tissue.

To promote/maintain cartilage differentiation/phenotype in culture, one critical requirement is to provide a 3D cellular condensation in which cells could experience a microenvironment of low oxygen tension. Research works have been demonstrated that MSCs are hardly differentiating into cartilage cell lineage at 2D culture system. Current technique for chondrogenic differentiation of MSCs is the micromass culture system which Jonhstone used for chondrocyte culture in 1998. These authors have reported that chondrocytes from growth plate cultured in micromass system could maintain chondrocytic phenotype without undergoing dedifferentiation. In micromass culture, the cells are placed in a tube and centrifuged into a condensed aggregate. A chondrogenic medium providing appropriate inducers for cell differentiation is then added to the resulted pellet. TGF-beta 3 is the most crucial inducer included in chondrogenic medium[34-38]. This growth factor probably acts by inducing the expression of Sry-related HMG box-9[39], which in turn, regulates the expression of aggrecan and collagen type II, type IX and type XI during chondrocyte differentiation[40]. Furthermore, research works have indicated that addition of bone morphogenetic proteins enhances chondrogenesis under the specific conditions employed by Steinert *et al*[41]. Insulin-like growth factor-1 has also been shown to have a synergistic effect with TGF β1 in promoting chondrogenesis[42]. Furthermore, fibroblast growth factor-2 (FGF-2) may possess a chondrogenic function. It has been demonstrated that in human marrow MSC culture, FGF-2 in combination with dexamethasone enhances production of collagen type II, glycosaminoglycan (GAG) and aggrecan. Platelet-rich plasma has also been reported to possess chondrogenic effects, owing to the presence of FGF-2 and TGF β2[43-46].

**Different sources of MSCs**

Since MSC population exists in many tissues in body, they could be considered as readily available cells for application in regenerative medicine. Besides bone marrow, multiple tissues have been reported to contain MSCs. These include adipose tissue[47], trabecular bone[48], periosteum[49], synovial membrane[50], skeletal muscle[51], as well as teeth[52], among which bone marrow and adipose tissue are widely-used sources. Furthermore, some researchers have paid special attention to synovial membrane as a potent source of stem cells with good chondrogenic potential.

Unlike bone marrow MSCs, adipose MSCs can be isolated in large quantities with minimal morbidity and discomfort[53,54]. Moreover, the frequency of MSCs in the whole bone marrow of skeletally mature adults ranges from 1 in 50000 to 1 in 100000 cells, corresponding to a yield of a few hundred MSCs/milliliter of marrow. Fraser *et al*[54] have reported that the frequency of MSCs in adipose tissue is in the order of 1 in 100 cells, about 500-fold more than that found in bone marrow[55]. In view of these practical advantages, MSC from adipose tissue could be considered as an alternative option for bone marrow MSCs in cell-based cartilage regeneration strategies.

MSCs derived from synovial membranes have been shown to possess multilineage potential. These cells can be stimulated to undergo chondrogenesis *in vitro* with appropriate inducers. The study by Shirasawa *et al*[56] has showed that human synovial derived cells have greater chondrogenic potential than bone marrow MSCs, adipose MSCs, as well as periosteal- or muscle-derived cells from the same patients. Furthermore, a follow-up study by the same authors has indicated that synovial-derived MSCs produce consistently larger cartilage than bone marrow MSCs from the same patients[57].

**Homing property of MSCs**

MSCs are known to have homing potential to the damaged site at which could possibly help to repair in two ways: (1) differentiation to tissue cells and restoration of lost morphology and function; and (2) secretion of a wide range of bioactive factors and creation of a repair environment with anti-apoptotic effects, immunoregulatory function and the stimulation of endothelial progenitor cell proliferation[58].

The precise mechanisms of MSC homing process have not been thoroughly understood. In this regards, it has been proposed that chemokines and their receptors on the surface of MSCs are the key players[59], which enable MSCs to migrate towards chemokine gradients secreted by injured tissues[60] or tumors[61]. MSCs express multiple chemokine receptors allowing their migration in response to the chemokine-attractive gradients created by the inflamed injured site. Some chemokine receptors expressed by MSCs include CCR1, CCR7, CCR9, CXCR3, CXCR4, CXCR5 and CX3CR1[62]. To consider the relationship between gradient of chemokine concentration and cell migration, it can be concluded that MSC must be transplanted to adjacent area of injured site following the establishment of the gradient of the chemokine concentration.

**Immunomodulatory function of MSCs**

Some scientists consider MSCs as a valuable cellular material for applications in variety of autoimmune and alloimmune diseases since these cells possess a considerable immunomodulatory potential. In this context, research works have indicated that MSCs can suppress proliferation and activity of CD4+ and CD8+ T lymphocytes, as well as T memory cells[63,64]. This is directed mainly by targeting the inhibition of cyclin D2, which leads the T cells into cell cycle arrest regulating anergy[65]. Furthermore, for this effect, there is no need for MHC identity between MSC and the target immune effector. Similarly, it has been observed that B lymphocyte neither proliferates nor differentiates into immunoglubin-producing cells in presence of MSCs[66]. Moreover, MSCs have been shown to inhibit the proliferation and cytotoxicity of interleukin (IL)-2 or IL-15-stimulated natural killer (NK) cells *in vitro*[67]. MSCs could also inhibit the maturation of monocytes into dentritic cells (DCs) *in vitro.* Mature DCs incubated with MSCs displays a decreased cell-surface expression of MHC class II molecules, CD11c, CD83 and co-stimulatory molecules, resulting in impaired antigen-presenting cell function. In addition, MSCs have been shown to inhibit pro-inflammatory potential of DCs by inhibiting their production of tumor-necrosis factor[66-69].

A range of mechanisms have been proposed to explain MSC immunomodulatory capacity. For example, it has been reported that MSCs exert their immunomodulatory effects through secretion of soluble inflammatory mediators, including IL-6, IFN-g, TNF-a, IL-1a and IL-1b[70]. These effects create through enzymatic action such as the expression of inducible nitric oxide synthase (iNOS) and indoleamine 2,3-dioxygenase (IDO), and through production of human leukocyte antigen class I molecule HLA-G and prostaglandin E2 (PGE2)[70,71]. Moreover, it has been indicated that MSCs can mediate immunosuppression (modulation of T cell proliferation, gene expression and cell migration) by releasing galectin-1, an intracellular and cell surface protein, in a soluble-form[72]. Furthermore, research works has suggested relationship between MSC immunosuppressive function and the expression of Toll-like receptors (TLR). MSCs have been shown to express a range of functional TLRs, specifically TLR-2 through TLR-8, leading to the production of IL-6 and IL-8 which subsequently affect T-cell function. In support of this idea, some authors have demonstrated that the inhibition of these receptors *in vitro* is conversely associated with a reduction in immunosuppressive activity of MSCs[72,73].

Regarding the mechanism of MSC-mediated immunosuppression, it must be emphasized that some of mechanisms are constitutively involved (*i.e.* production of PGE2), whereas others are induced when MSCs are exposed to inflammatory environment (*i.e.* IDO is expressed when MSCs are stimulated with IFNγ). In addition, according to evidences, cooperation of several molecules (rather than a single molecule) is responsible for the MSC immunomodulatory function[66]. Finally, there are differences among species regarding the mechanism of immunosuppression. For example, in human MSCs, IDO-mediated suppression is one of the most prominent mechanisms. This enzyme depletes the cellular microenvironment of the essential amino acid tryptophan that is required for T-cell proliferation. In contrast, in murine MSC, immunosuppression is mediated by iNOS[74].

**MSCs and cartilage gene therapy**

Gene therapy approaches could be considered as promising strategy for efficient promotion of regeneration in cartilage defect. In this context, MSCs could readily be transduced by viral vectors. Also, specific liposomal formulations have been reported as a safe gene delivery system into MSCs with some efficiency[75]. MSC-based gene therapy offers some advantages for articular cartilage repair. Using this approach, therapeutic proteins could be designed to overexpress in MSCs transplanted into articular cartilage defect. This, in turn, could enhance the structural features of the repair tissue formed at defect site. Furthermore, MSC-based gene therapy is an applicable approach to deliver genes with complementary mechanisms of action (*i.e.* chondrogenic and proliferative factors) into cartilage defect.

In many studies, MSC-mediated gene delivery has been applied for cartilage repair using variety of chondrogenic growth factors. For example, it has been indicated that overexpression of IGF-I in concert with TGFβ1 or BMP2 *in vitro* in MSCs could induce greater chondrogenic tissue than either growth factor alone[42,76]. According to this research work, overexpression of IGF-I alone could not induce chondrogenic differentiation of MSCs in culture. In contrast to this finding, Gelse *et al*[77] have indicated the differentiation-promoting effect of IGF-I. In this *in vivo* study, MSCs from rib perichondrium of rat were subjected to adenoviral transduction with adenoviral vectors encoding BMP-2 (Ad.BMP-2) and adenoviral vectors encoding IGF-1 (Ad.IGF-1). The cells were then mixed with fibrin glue matrix and delivered to cartilage partial thickness lesions of the patellar groove. Both treatments with BMP-2 and with IGF-1 have been shown to improve repair tissue as compared with the naïve and Ad.LacZ controls after eight weeks. However, the majority of BMP-2 treated joints showed signs of ectopic bone formation and osteophytes, which were not present in the knees of the IGF-1 treated defects[77]. In addition to IGF-I and BMP-2, some other growth factors including BMP-4[78] and growth differentiation factor 5[79] were also employed in MSC-based gene delivery to cartilage defects which resulted in an enhanced cartilage repair.

**Potential pitfalls of use of MSCs**

In spite of the above-mentioned potential, there are some pitfalls associated with MSC application for articular cartilage regeneration. Some research works have indicated the expression of cartilage hypertrophy markers such as collagen type X, matrix metalloproteinase-13, alkaline phosphatase, parathyroid hormone-related protein receptor and vascular endothelial growth factor after inducing MSCs to undergo chondrogenesis. Since hypertrophy could finally lead to ossification of cartilage tissue, scientists have concerned clinical application of MSCs for regenerating articular cartilage defects[80-83]. Furthermore, it has been reported that the thickness of the regenerated cartilage by MSC transplanted into cartilage defects was too thin to resemble a mature cartilage[84]. However, there has been promising attempts to overcome these issues, like co-culture of MSCs with mature chondrocytes has been reported to result in decreased expression of hypertrophy markers[85]. Further investigation has revealed that such anti hypertrophic effect is created by the parathyroid hormone-related peptide secreted by mature chondocytes[86]. Moreover a recent study has indicated that the immature cartilage treated with FGF-2 and TGFβ1 displays increased nano-compressive stiffness, decreased surface adhesion, decreased water content, increased collagen content and smoother surfaces, indicating characteristics of mature cartilage[87].

**Regeneration of articular cartilage with MSC transplantation**

During the past years, valuable attempts have been made to evaluate MSC potential in regeneration of articular cartilage defects. At the following lines, examples of such efforts in animal models and human are described.

**MSCs-mediated for cartilage regeneration in animal models**

In order to study the regenerative potential of MSCs in cartilage defects *in vivo*, rabbit has frequently been used as animal model. In some study, MSCs have been applied alone, without any biomaterial. Im *et al*[88] isolated MSCs from rabbit marrow and transplanted them into full-thickness osteochondral defect which was artificially made on the same rabbit’s patellar groove. Evaluation of repair, 14 wk post transplantation, indicated that histological score of experimental group was higher than corresponding value of control group (untreated); therefore, they concluded that repair of cartilage defects can be enhanced by the implantation of cultured MSCs.

Most investigators preferred transplantation of cells combined with scaffold. Wakitani *et al*[89] used this strategy to create regeneration of full-thickness articular cartilage following experimentally-created defects in rabbit’s knee joint. Cells were combined with collagen I gel and surgically transplanted into animal’s medial femoral condyle defect. Two weeks post-surgery, evaluations indicated that MSCs differentiated into chondrocytes contribute to regenerate damaged tissue and within 24 wk, the defect was completely repaired. Interestingly, mechanical test indicated a good mechanical strength of repair tissue. Recently, Berninger *et al*[90] have attempted to promote regeneration of osteochondral defects in rabbit’s knee joint by implantation of allogenic MSC in fibrin clots.

Also, Grigolo *et al*[91] used MSCs with scaffold to promote regeneration in osteoarthritic defect induced in rabbit’s knee by cutting the cruciate ligaments. Upon establishment of osteoarthritis (OA) model eight weeks post induction, marrow-derived MSCs combined with hyaluronan (Hyaff-11) were transplanted into the osteoarthritic knee. Six months post transplantation, a statistically significant difference in the quality of the regenerated tissue was found in the implants with scaffolds carrying MSCs as compared to the scaffold alone.

In addition to rabbit, goat has been also used as animal model for investigation of regenerative potential of MSCs for cartilage defects. Guo *et al*[92] loaded goat marrow-derived MSC in tricalcium phosphate scaffolds and transplanted them into a cartilage defects of 4 mm × 8 mm dimensions created in femur articular surface at the animal’s knee joint. About 24 mo after transplantation, evaluation indicated that the defect was filled by a hyaline-like cartilage. According to their finding, the graft tended to integrate with the subchondral bone. About 12-24 mo post-surgery, GAG content of the repair tissue increased significantly.

Non-surgical administration of MSCs for articular cartilage repair has also been investigated. Using this strategy, Murphy *et al*[93] have reported transplantation of marrow derived MSCs which were suspended in hyaluronan through injection into cavity of osteoarthritic knee at caprine model created by cutting cruciate ligaments and meniscus. According to their findings, injected cells tended to regenerate meniscus, and thereby, delayed the formation of OA in the animal knee joint. Recently, injection approach was evaluated in sheep model of OA by Al Faqeh *et al*[94]. These authors have reported marrow MSCs transplanted either as undifferentiated cells or chondrogenically induced cells could retard the progression of OA. According to their findings, the induced cells indicated better results, especially in meniscus regeneration.

**MSCs-mediated cartilage regeneration in human**

***Cartilage defects following trauma***

Articular cartilage of knee joint is often injured after a fall among athletes, which is considered a challenging surgery for orthopedists. In this context, some authors have tried to apply the regenerative potential of MSCs. For example, Kuroda *et al*[95] attempted to reconstruct a 20 mm × 30 mm full-thickness cartilage defect [International Cartilage Repair Society Classification (ICRS) grade IV] in the weight-bearing area of the medial femoral condyle of the right knee in a 31-year-old male judo athlete. They transplanted MSC/collagen gel into the cartilage defects and observed the formation of hyaline cartilage in the histological sections. The patient returned to a normal life seven months post-implantation. Similarly, Wakitani *et al*[96] transplanted autologous MSC combined with collagen gel into two patients with patellar full thickness articular cartilage defects and observed significant improvements in patient pain and walking ability six months post-transplantation.

In another clinical study, Wakitani *et al*[97] tried to treat three patients including a 31-year-old female, a 44-year-old male and a 45-year-old male with full-thickness articular cartilage defects in their patello-femoral joints. Undifferentiated MSC/collagen sheet was transplanted into the defects, and evaluated for a six-month follow-up period, at the end of which the patients' clinical symptoms were significantly improved. The improvements were maintained over a periods of 17-27 mo. One year post-transplantation, histological examination of the repair tissue from one patient revealed that the defect was repaired by fibrocartilaginous tissue. Magnetic resonance imaging of the second patient revealed a complete coverage of the defect, but they were unable to determine the nature of the material covered the defect[97]. The formation of a repair tissue with fibrocartilage nature, in this study, would be due to the inappropriate microenvironment (*i.e.*, collagen type I solution) that was used for transplantation of MSCs. Hyaline cartilage naturally contains plenty of collagen type II and hyaluronic acid (HA) macromolecules. In this study, the addition of matrix substance in the form of HA could provide chemical signals for right matrix production by the cells. The effect of HA-synthetic hydrogel matrix has been recently emphasized in MSC cartilage differentiation process[98].

**OA**

OA is a group of progressive joint disorders in which biomechanical characteristics of cartilage changes, so results in patient disability[99]. This disease progressively involves articular cartilage, subchondral bone, ligaments and synovial membrane. Some attempts have been made to treat osteoarthritic joints using MSCs. In this context, a report about treatment of 24 patients with knee OA revealing MSC transplantation by Wakitani *et al*[100]. is remarkable. In this clinical trial, adherent cells from bone marrow aspirates were embedded in collagen gel and transplanted into articular cartilage defects in the medial femoral condyle of 12 patients, while other 12 subjects were served as cell-free controls. Outcomes indicated that although clinical improvement was not significantly different, the treatment group showed better arthroscopic and histologic grading score.

In the above-mentioned study, MSCs were introduced through an invasive approach (surgery) into the defected area. Some authors have attempted to introduce the cells by injection. Using this approach, Centeno *et al*[101] applied culture-expanded autologous MSCs and transplanted the cells through an intra-articular injection into the knee of a 46-year-old OA patient. They reported that 90% of the patient’s pain was reduced two years post-injection. Furthermore, Davatchi *et al*[102] used this strategy to introduce the cells into knee joints of four OA patients and reported the strategy as encouraging method. Using this strategy, Emadedin*et al*[103] injected autologous MSCs in six female volunteer patients with knee OA and observed more satisfactory outcomes.

***Rheumatoid arthritis***

Rheumatoid arthritis (RA) is a chronic [systemic](http://en.wikipedia.org/wiki/Systemic_disease) [inflammatory disorder](http://en.wikipedia.org/wiki/Inflammation) that may affect many tissues and organs. It principally attacks [synovial joints](http://en.wikipedia.org/wiki/Synovial_joints). This [systemic autoimmune disease](http://en.wikipedia.org/wiki/Systemic_autoimmune_disease) is associated with progressive reduction of extracellular matrix and joint destruction. Pro-inflammatory cytokines including TNF-alpha and IL-6 are believed to be responsible for the creation of RA symptoms[104,105]. Current therapy is based mainly on suppressing the symptoms using a[nalgesia](http://en.wikipedia.org/wiki/Analgesia) and [anti-inflammatory](http://en.wikipedia.org/wiki/Anti-inflammatory) drugs, including [steroids](http://en.wikipedia.org/wiki/Glucocorticoid). Although such therapy is effective in relieving pain and inflammation, it is not able to regenerate damaged cartilage. Furthermore, it has been reported that cartilage-regenerating methods including cell-based treatment strategies using autologous chondrocytes is not considered as an efficient method for RA patients, which is due to that prevention of cartilage formation by presence of the inflammatory condition in the joint or that destruction of the newly-formed cartilage.

In contrast to chondrocyte-based cell therapy, it has been suggested that injection of an allogenic MSC results in a considerable reduction in inflammation and a formation of new cartilage in RA due to their immunosuppressive and anti-inflammatory features[106]. In support of this concept, injection of MSCs in the mouse animal model with collagen-induced arthritis (CIA) has been reported to prevent from severe arthritis and to lower the serum level of inflammatory cytokines[107].

**Conclusion**

MSCs are specified as appropriate cell candidates for regenerating incurable defects of articular cartilage due to the following characteristics: inherent chondrogenic property, easy availability, cell homing potential and immunomodulatory function. In the past, several attempts had been made to exploit MSCs capacity to cure articular cartilage defects developed in OA, rheumatoid arthritis, or following trauma. Taken together, the outcomes of these trials show promising results. Furthermore, many clinical trials have been registered at www.clinicaltrial.gov regarding application of MSCs for regenerating articular cartilage. With a worldwide extensive effort, MSC will be routinely applicable in articular cartilage defects in near future. Especial attention must be given to improve the quality of repair tissue formed following MSC transplantation into cartilage defect. First, efficient protocols must be developed to prevent hypertrophy of chondrocytes produced by MSC differentiation. Second, practical solution must be explored regarding production of mature cartilage by MSC differentiation. Third, optimal biomaterial mimicking the matrix of hyaline cartilage must be developed in order to provide appropriate chemical signals for right matrix production by MSCs following transplantation. Fourth, in most clinical trials, MSCs are applied in undifferentiated state. This approach exhibits a major potential drawback. MSCs represent a heterogeneous population containing multiple colonies with various differentiation capacities; therefore, to improve MSC regenerative outcome in cartilage defects, the cell population must be enriched for chondrogenic cells. Otherwise, pre-differentiation of MSCs will be essential in clinical applications in order to ensure appropriate lineage commitment and to avoid undesired heterotopic tissue formation. Finally, gene therapy approach offers the potential of addressing most of these issues (*i.e.* chondrocyte hypertrophy, production of immature cartilage and pre-differentiation of MSCs), but this approach requires further improvement for MSC engraftment. More importantly, in this context, a safe highly efficient gene delivery system into MSCs with sustained duration of transgene expression and the optimal therapeutic gene(s) for cartilage repair must be identified. Moreover, determination of an optimized combination of genetically modified MSCs with scaffolds is of utmost importance for producing a high quality repair tissue in *vivo*.

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