World Journal of Stem Cells

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4252/wjsc.v6.i3.344

World J Stem Cells 2014 July 26; 6(3): 344-354
ISSN 1948-0210 (online)

REVIEW

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

Mohamadreza Baghaban Eslaminejad, Elham Malakooty Poor

Mohamadreza Baghaban Eslaminejad, Elham Malakooty Poor, Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, 4644 Tehran, Iran
Author contributions: Baghaban Eslaminejad M and Malakooty Poor E equally contributed to this paper.
Correspondence to: Mohamadreza Baghaban Eslaminejad, PhD, Department of Stem Cells and Developmental Biology at Cell Sciences Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, PO Box 19395, 4644 Tehran, Iran. eslami@royaninstitute.org
Telephone: +98-21-22339928 Fax: +98-21-23562681
Received: October 22, 2013 Revised: December 7, 2013
Accepted: April 25, 2014
Published online: July 26, 2014

Abstract

Since articular cartilage possesses only a weak capacity for repair, its regeneration potential is considered 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 an effective treatment option but 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 lose morphological features and specialized functions. In the search for alternative cells, scientists have found mesenchymal stem cells (MSCs) to be an appropriate cellular material for articular cartilage repair. These cells were originally 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 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 the general donor for therapeutic applications. All of these topics will be discussed in this review.

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 is considered 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 were made to exploit MSC capacity to cure articular cartilage defects developed in osteoarthritis, rheumatoid arthritis or following trauma. All of these topics are discussed in this review.

Baghaban Eslaminejad M, Malakooty Poor E. Mesenchymal stem cells as a potent cell source for articular cartilage regeneration. *World J Stem Cells* 2014; 6(3): 344-354 Available from: URL: http://www.wjgnet.com/1948-0210/full/v6/i3/344.htm DOI: http://dx.doi.org/10.4252/wjsc.v6.i3.344

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 a high mechanical load. Furthermore, it works as a lubricant and a shock absorber. Histologically, articular cartilage is 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 are restricted 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. With the lack of blood supply, a set of complex biochemical events that take 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 the migration capacity of chondrocytes[3-5].

In general, while no repair process occurs in chondral defects, in osteochondral defects, a repair process is 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 fibrocartilage.

For a long period of time, the current regenerative treatment option for joint cartilage defects was 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 the osteoarticular transfer system, is the other therapeutic option for cartilage repair. Unfortunately, its clinical application is a technically challenging procedure. 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 an alternative option but it is associated with high cost, risk of immunological rejection and transmission of pathogens[13].

There are two types of cell-based treatments for cartilage defects, the 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, the 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, others believe that even after ACI, some defects have continued to persist in the 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 lose 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: the 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, immunological incompatibility, the possibility of teratoma formation in transplantations, as well as certain ethical concerns make scientists 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 sources for cartilage regeneration, MSCs are considered an appropriate candidate owing to several specific characteristics. These properties will be reviewed and followed by the examples of investigations 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 the wound experi-

mentally 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 a series of bone marrow transplantation experiments, scientists 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 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 and MSCs. MSC is the most frequently used nomination, particularly in recently published investigations.

As with any stem cell type, MSCs possess two important properties, long-term self-renewal ability and the capacity to differentiate along multiple cell lineages, such as bone, cartilage and adipose cells. There is controversy regarding the profile of surface marker expression on MSCs. According to the 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 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. MSC primary culture has been reported to be heterogeneous, containing multiple colonies with various differentiation capacities. Pittenger *et al*[30] showed 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 MSC plasticity or transdifferentiation.

INHERENT CHONDROGENIC POTENTIAL OF MSCS

The 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 uniformly consist of 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 has demonstrated that MSCs hardly differentiate into cartilage cell lineage in a 2D culture system. The current technique for chondrogenic differentiation of MSCs is the micromass culture system which Johnstone used for chondrocyte culture in 1998. These authors reported that chondrocytes from a growth plate cultured in the 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 resulting pellet. Transforming growth factor (TGF)-β3 is the most crucial inducer included in chondrogenic medium[34-38]. This growth factor probably acts by inducing the expression of Sry-related high-mobility-group 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 has 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 the MSC population exists in many tissues in body, they could be considered 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] 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 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] 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 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 a homing potential to the damaged site 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 the 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 MSCs must be transplanted to an adjacent area of injured site following the establishment of the gradient of the chemokine concentration.

IMMUNOMODULATORY FUNCTION OF MSCS

Some scientists consider MSCs a valuable cellular material for applications in a variety of autoimmune and alloimmune diseases since these cells possess a considerable immunomodulatory potential. In this context, research has 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 major histocompatibility complex (MHC) identity between MSC and the target immune effector. Similarly, it has been observed that B lymphocyte neither proliferates nor differentiates into immunoglobulin-producing cells in the 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 cells *in vitro*[67]. MSCs could also inhibit the maturation of monocytes into dendritic cells (DCs) *in vitro*. Mature DCs incubated with MSCs display 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 (TNF)[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 are created 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 has suggested a 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 affects 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 mechanisms are constitutively involved (*i.e.*, production of PGE2), whereas others are induced when MSCs are exposed to an inflammatory environment (*i.e.*, IDO is expressed when MSCs are stimulated with IFNγ). In addition, according to the evidence, 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 trypto-

phan 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 a promising strategy for efficient promotion of regeneration in cartilage defects. 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 the 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 a cartilage defect.

In many studies, MSC-mediated gene delivery has been applied for cartilage repair using a variety of chondrogenic growth factors. For example, it has been indicated that overexpression of IGF-1 in concert with TGF-β1 or BMP2 *in vitro* in MSCs could induce greater chondrogenic tissue than either growth factor alone[42,76]. According to this research, overexpression of IGF-1 alone could not induce chondrogenic differentiation of MSCs in culture. In contrast to this finding, Gelse *et al*[77] indicated the differentiation-promoting effect of IGF-1. In this *in vivo* study, MSCs from rib perichondrium of rat were subjected to adenoviral transduction with adenoviral vectors encoding BMP-2 and adenoviral vectors encoding 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 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-1 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 has 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 are concerned about the 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 mature cartilage[84]. However, there have been promising attempts to overcome these issues, such as that 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 an anti-hypertrophic effect is created by the parathyroid hormone-related peptide secreted by mature chondrocytes[86]. Moreover, a recent study indicated that the immature cartilage treated with FGF-2 and TGF-β1 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. Examples of such efforts in animal models and human are described.

MSCS 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 an animal model. In some studies, MSCs have been applied alone without any biomaterial. Im *et al*[88] isolated MSCs from rabbit marrow and transplanted them into a full thickness osteochondral defect which was artificially made on the same rabbit's patellar groove. Evaluation of repair 14 wk post transplantation indicated that the histological score of experimental group was higher than the corresponding value of the 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 knee joint. Cells were combined with collagen I gel and surgically transplanted into the 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, a mechanical test indicated good mechanical strength of repair tissue. Recently, Berninger *et al*[90] attempted to promote regeneration of osteochondral defects in rabbit knee joint by implantation of allogeneic MSC in fibrin clots.

Also, Grigolo *et al*[91] used MSCs with scaffold to promote regeneration in an osteoarthritic defect induced in rabbit knee by cutting the cruciate ligaments. Upon es-

tablishment of an OA model eight weeks post induction, marrow-derived MSCs combined with hyaluronan 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 compared to the scaffold alone.

In addition to rabbit, goat has been also used as an animal model for investigation of the 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 knee joint. About 24 mo after transplantation, evaluation indicated that the defect was filled by a hyaline-like cartilage. According to their findings, the graft tended to integrate with the subchondral bone. About 12-24 mo post-surgery, the 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] reported transplantation of marrow derived MSCs which were suspended in hyaluronan through injection into the cavity of an osteoarthritic knee in a caprine model created by cutting cruciate ligaments and the meniscus. According to their findings, injected cells tended to regenerate meniscus and thereby delayed the formation of OA in the animal knee joint. Recently, an injection approach was evaluated in a sheep model of OA by Al Faqeh *et al*[94]. These authors 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 HUMANS

Cartilage defects following trauma

Articular cartilage of the knee joint is often injured after a fall in 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 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 patellofemoral joints. An undifferentiated MSC/collagen sheet was transplanted into the defects and evaluated for a six month follow-up period, at the end of which the clinical symptoms were significantly improved. The improvements were maintained over a period 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 was unable to determine the nature of the material covering the defect[97]. The formation of 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 the MSC cartilage differentiation process[98].

OA

OA is a group of progressive joint disorders in which biomechanical characteristics of cartilage changes and 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 the 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 the other 12 subjects served as cell-free controls. Outcomes indicated that although clinical improvement was not significantly different, the treatment group showed a better arthroscopic and histological grading score.

In the above-mentioned study, MSCs were introduced through an invasive approach (surgery) into the defective 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 an 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 inflam-

matory disorder that may affect many tissues and organs. It principally attacks synovial joints. This systemic autoimmune disease is associated with progressive reduction of extracellular matrix and joint destruction. Pro-inflammatory cytokines including TNF-α 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 analgesia and anti-inflammatory drugs, including steroids. 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 an efficient method for RA patients due to prevention of cartilage formation by the presence of the inflammatory condition in the joint or destruction of the newly-formed cartilage.

In contrast to chondrocyte-based cell therapy, it has been suggested that injection of an allogeneic 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 has been reported to prevent 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 were made to exploit MSC 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, MSCs will be routinely applicable in articular cartilage defects in the near future. Special attention must be given to improve the quality of repair tissue formed following MSC transplantation into the cartilage defect. First, efficient protocols must be developed to prevent hypertrophy of chondrocytes produced by MSC differentiation. Second, a 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 the 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, a 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*.

REFERENCES

1 **Madry H**, van Dijk CN, Mueller-Gerbl M. The basic science of the subchondral bone. *Knee Surg Sports Traumatol Arthrosc* 2010; **18**: 419-433 [PMID: 20119671 DOI: 10.1007/s00167-010-1054-z]

2 **Zhang L**, Hu J, Athanasiou KA. The role of tissue engineering in articular cartilage repair and regeneration. *Crit Rev Biomed Eng* 2009; **37**: 1-57 [PMID: 20201770 DOI: 10.1615/CritRevBiomedEng.v37.i1-2.10]

3 **Hiraki Y**, Shukunami C, Iyama K, Mizuta H. Differentiation of chondrogenic precursor cells during the regeneration of articular cartilage. *Osteoarthritis Cartilage* 2001; **9** Suppl A: S102-S108 [PMID: 11680673]

4 **Duynstee ML**, Verwoerd-Verhoef HL, Verwoerd CD, Van Osch GJ. The dual role of perichondrium in cartilage wound healing. *Plast Reconstr Surg* 2002; **110**: 1073-1079 [PMID: 12198420 DOI: 10.1097/00006534-200209150-00011]

5 **Xian CJ**, Foster BK. Repair of injured articular and growth plate cartilage using mesenchymal stem cells and chondrogenic gene therapy. *Curr Stem Cell Res Ther* 2006; **1**: 213-229 [PMID: 18220868 DOI: 10.2174/157488806776956904]

6 **Dhinsa BS**, Adesida AB. Current clinical therapies for cartilage repair, their limitation and the role of stem cells. *Curr Stem Cell Res Ther* 2012; **7**: 143-148 [PMID: 22023635 DOI: 10.2174/157488812799219009]

7 **Frenkel SR**, Di Cesare PE. Degradation and repair of articular cartilage. *Front Biosci* 1999; **4**: D671-D685 [PMID: 10525475]

8 **Vijayan S**, Bentley G, Briggs T, Skinner J, Carrington R, Pollock R, Flanagan A. Cartilage repair: A review of Stanmore experience in the treatment of osteochondral defects in the knee with various surgical techniques. *Indian J Orthop* 2010; **44**: 238-245 [PMID: 20697474 DOI: 10.4103/0019-5413.65136]

9 **Behery O**, Siston RA, Harris JD, Flanigan DC. Treatment of cartilage defects of the knee: expanding on the existing algorithm. *Clin J Sport Med* 2014; **24**: 21-30 [PMID: 24157464 DOI: 10.1097/JSM.0000000000000004]

10 **Hunziker EB**. Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. *Osteoarthritis Cartilage* 2002; **10**: 432-463 [PMID: 12056848 DOI: 10.1053/joca.2002.0801]

11 **Bartha L**, Vajda A, Duska Z, Rahmeh H, Hangody L. Autologous osteochondral mosaicplasty grafting. *J Orthop Sports Phys Ther* 2006; **36**: 739-750 [PMID: 17063836 DOI: 10.2519/jospt.2006.2182]

12 **Rose T**, Craatz S, Hepp P, Raczynski C, Weiss J, Josten C, Lill H. The autologous osteochondral transplantation of the knee: clinical results, radiographic findings and histological aspects. *Arch Orthop Trauma Surg* 2005; **125**: 628-637 [PMID:

16172863 DOI: 10.1007/s00402-005-0010-8]
13 **Williams RJ**, Ranawat AS, Potter HG, Carter T, Warren RF. Fresh stored allografts for the treatment of osteochondral defects of the knee. *J Bone Joint Surg Am* 2007; **89**: 718-726 [PMID: 17403792 DOI: 10.2106/JBJS.F.00625]
14 **Viste A**, Piperno M, Desmarchelier R, Grosclaude S, Moyen B, Fessy MH. Autologous chondrocyte implantation for traumatic full-thickness cartilage defects of the knee in 14 patients: 6-year functional outcomes. *Orthop Traumatol Surg Res* 2012; **98**: 737-743 [PMID: 23026726 DOI: 10.1016/j.otsr.2012.04.019]
15 **Dehne T**, Schenk R, Perka C, Morawietz L, Pruss A, Sittinger M, Kaps C, Ringe J. Gene expression profiling of primary human articular chondrocytes in high-density micromasses reveals patterns of recovery, maintenance, re- and dedifferentiation. *Gene* 2010; **462**: 8-17 [PMID: 20433912 DOI: 10.1016/j.gene.2010.04.006]
16 **Itskovitz-Eldor J**, Schuldiner M, Karsenti D, Eden A, Yanuka O, Amit M, Soreq H, Benvenisty N. Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. *Mol Med* 2000; **6**: 88-95 [PMID: 10859025]
17 **Undale AH**, Westendorf JJ, Yaszemski MJ, Khosla S. Mesenchymal stem cells for bone repair and metabolic bone diseases. *Mayo Clin Proc* 2009; **84**: 893-902 [PMID: 19797778 DOI: 10.4065/84.10.893]
18 **Takahashi K**, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126**: 663-676 [PMID: 16904174 DOI: 10.1016/j.cell.2006.07.024]
19 **Park IH**, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, Lerou PH, Lensch MW, Daley GQ. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* 2008; **451**: 141-146 [PMID: 18157115 DOI: 10.1038/nature06534]
20 **Chonheim JF**. Über Entzündung und Eiterung. *Arch Path Anat Physiol Klin Med* 1867; **40**: 1-79
21 **Ross R**, Everett NB, Tyler R. Wound healing and collagen formation. VI. The origin of the wound fibroblast studied in parabiosis. *J Cell Biol* 1970; **44**: 645-654 [PMID: 5415241 DOI: 10.1083/jcb.44.3.645]
22 **Friedenstein AJ**, Piatetzky-Shapiro II, Petrakova KV. Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol* 1966; **16**: 381-390 [PMID: 5336210]
23 **Petrakova KV**, Tolmacheva AA, Fridenshtein AIa. [Bone formation occurring in bone marrow transplantation in diffusion chambers]. *Biull Eksp Biol Med* 1963; **56**: 87-91 [PMID: 14149787 DOI: 10.1007/BF00784048]
24 **Friedenstein AJ**. Chapter 9: Detrimined and inducible osteogenic precursure cells. In: Elliott K, FitzsimonsHard DW. Ciba Foundation Symposium 11 - Hard Tissue Growth, Repair and Remineralization. Wiley Online Library, 1973: 169-185 [DOI: 10.1002/9780470719947.ch9/summary]
25 **Dominici M**, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; **8**: 315-317 [PMID: 16923606 DOI: 10.1080/14653240600855905]
26 **Lin CS**, Ning H, Lin G, Lue TF. Is CD34 truly a negative marker for mesenchymal stromal cells? *Cytotherapy* 2012; **14**: 1159-1163 [PMID: 23066784 DOI: 10.3109/14653249.2012.729817]
27 **Ning H**, Lin G, Lue TF, Lin CS. Mesenchymal stem cell marker Stro-1 is a 75 kd endothelial antigen. *Biochem Biophys Res Commun* 2011; **413**: 353-357 [PMID: 21903091 DOI: 10.1016/j.bbrc]
28 **Yoshiba N**, Yoshiba K, Ohkura N, Shigetani Y, Takei E, Hosoya A, Nakamura H, Okiji T. Immunohistochemical analysis of two stem cell markers of α-smooth muscle actin and STRO-1 during wound healing of human dental pulp. *Histochem Cell Biol* 2012; **138**: 583-592 [PMID: 22673840 DOI: 10.1007/s00418-012-0978-4]
29 **Lin CS**, Xin ZC, Dai J, Lue TF. Commonly used mesenchymal stem cell markers and tracking labels: Limitations and challenges. *Histol Histopathol* 2013; **28**: 1109-1116 [PMID: 23588700]
30 **Pittenger MF**, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; **284**: 143-147 [PMID: 10102814 DOI: 10.1126/science.284.5411.143]
31 **Sugaya K**. Potential use of stem cells in neuroreplacement therapies for neurodegenerative diseases. *Int Rev Cytol* 2003; **228**: 1-30 [PMID: 14667041 DOI: 10.1016/S0074-7696(03)28001-3]
32 **Chapel A**, Bertho JM, Bensidhoum M, Fouillard L, Young RG, Frick J, Demarquay C, Cuvelier F, Mathieu E, Trompier F, Dudoignon N, Germain C, Mazurier C, Aigueperse J, Borneman J, Gorin NC, Gourmelon P, Thierry D. Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multiorgan failure syndrome. *J Gene Med* 2003; **5**: 1028-1038 [PMID: 14661178 DOI: 10.1002/jgm.452]
33 **Friedenstein AJ**, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. *Cell Tissue Kinet* 1970; **3**: 393-403 [PMID: 5523063]
34 **Johnstone B**. Mesenchymal stem cells and chondrogenesis. *Europ Cell Mat* 2002; **4**: 27
35 **Bosnakovski D**, Mizuno M, Kim G, Ishiguro T, Okumura M, Iwanaga T, Kadosawa T, Fujinaga T. Chondrogenic differentiation of bovine bone marrow mesenchymal stem cells in pellet cultural system. *Exp Hematol* 2004; **32**: 502-509 [PMID: 15145219 DOI: 10.1016/j.exphem.2004.02.009]
36 **Indrawattana N**, Chen G, Tadokoro M, Shann LH, Ohgushi H, Tateishi T, Tanaka J, Bunyaratvej A. Growth factor combination for chondrogenic induction from human mesenchymal stem cell. *Biochem Biophys Res Commun* 2004; **320**: 914-919 [PMID: 15240135 DOI: 10.1016/j.bbrc.2004.06.029]
37 **Eslaminejad MB**, Nikmahzar A, Taghiyar L, Nadri S, Massumi M. Murine mesenchymal stem cells isolated by low density primary culture system. *Dev Growth Differ* 2006; **48**: 361-370 [PMID: 16872449 DOI: 10.1111/j.1440-169X.2006.00874.x]
38 **Eslaminejad MB**, Nikmahzar A, Piriea A. The structure of Human Mesenchymal Stem Cells differentiated into cartilage in micro mass culture system. *Yakhteh Med J* 2006; **3**: 162-171
39 **Magne D**, Vinatier C, Julien M, Weiss P, Guicheux J. Mesenchymal stem cell therapy to rebuild cartilage. *Trends Mol Med* 2005; **11**: 519-526 [PMID: 16213191 DOI: 10.1016/j.molmed.2005.09.002]
40 **Sekiya I**, Larson BL, Vuoristo JT, Reger RL, Prockop DJ. Comparison of effect of BMP-2, -4, and -6 on in vitro cartilage formation of human adult stem cells from bone marrow stroma. *Cell Tissue Res* 2005; **320**: 269-276 [PMID: 15778851 DOI: 10.1007/s00441-004-1075-3]
41 **Steinert AF**, Palmer GD, Pilapil C, Nöth U, Evans CH, Ghivizzani SC. Enhanced in vitro chondrogenesis of primary mesenchymal stem cells by combined gene transfer. *Tissue Eng Part A* 2009; **15**: 1127-1139 [PMID: 18826340 DOI: 10.1089/ten.tea.2007.0252]
42 **Solchaga LA**, Penick K, Porter JD, Goldberg VM, Caplan AI, Welter JF. FGF-2 enhances the mitotic and chondrogenic potentials of human adult bone marrow-derived mesenchymal stem cells. *J Cell Physiol* 2005; **203**: 398-409 [PMID: 15521064 DOI: 10.1002/jcp.20238]
43 **Stewart AA**, Byron CR, Pondenis H, Stewart MC. Effect of fibroblast growth factor-2 on equine mesenchymal stem cell monolayer expansion and chondrogenesis. *Am J Vet Res* 2007; **68**: 941-945 [PMID: 17764407 DOI: 10.2460/ajvr.68.9.941]

44 **Wang CY**, Chen LL, Kuo PY, Chang JL, Wang YJ, Hung SC. Apoptosis in chondrogenesis of human mesenchymal stem cells: effect of serum and medium supplements. *Apoptosis* 2010; **15**: 439-449 [PMID: 19949977 DOI: 10.1007/s10495-009-0431-x]

45 **Eppley BL**, Woodell JE, Higgins J. Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing. *Plast Reconstr Surg* 2004; **114**: 1502-1508 [PMID: 15509939 DOI: 10.1097/01.PRS.0000138251.07040.51]

46 **Pires de Carvalho P**, Hamel KM, Duarte R, King AG, Haque M, Dietrich MA, Wu X, Shah F, Burk D, Reis RL, Rood J, Zhang P, Lopez M, Gimble JM, Dasa V. Comparison of infrapatellar and subcutaneous adipose tissue stromal vascular fraction and stromal/stem cells in osteoarthritic subjects. *J Tissue Eng Regen Med* 2012; Epub ahead of print [PMID: 22807102 DOI: 10.1002/term.1565]

47 **Tuli R**, Tuli S, Nandi S, Wang ML, Alexander PG, Haleem-Smith H, Hozack WJ, Manner PA, Danielson KG, Tuan RS. Characterization of multipotential mesenchymal progenitor cells derived from human trabecular bone. *Stem Cells* 2003; **21**: 681-693 [PMID: 14595128 DOI: 10.1634/stemcells.21-6-681]

48 **Fukumoto T**, Sperling JW, Sanyal A, Fitzsimmons JS, Reinholz GG, Conover CA, O'Driscoll SW. Combined effects of insulin-like growth factor-1 and transforming growth factor-beta1 on periosteal mesenchymal cells during chondrogenesis in vitro. *Osteoarthritis Cartilage* 2003; **11**: 55-64 [PMID: 12505488 DOI: 10.1053/joca.2002.0869]

49 **Wickham MQ**, Erickson GR, Gimble JM, Vail TP, Guilak F. Multipotent stromal cells derived from the infrapatellar fat pad of the knee. *Clin Orthop Relat Res* 2003; (**412**): 196-212 [PMID: 12838072 DOI: 10.1097/01.blo.0000072467.53786.ca]

50 **Jankowski RJ**, Deasy BM, Huard J. Muscle-derived stem cells. *Gene Ther* 2002; **9**: 642-647 [PMID: 12032710 DOI: 10.1038/sj.gt.3301719]

51 **Bakopoulou A**, Leyhausen G, Volk J, Tsiftsoglou A, Garefis P, Koidis P, Geurtsen W. Comparative analysis of in vitro osteo/odontogenic differentiation potential of human dental pulp stem cells (DPSCs) and stem cells from the apical papilla (SCAP). *Arch Oral Biol* 2011; **56**: 709-721 [PMID: 21227403 DOI: 10.1016/j.archoralbio.2010.12.008]

52 **Parker AM**, Katz AJ. Adipose-derived stem cells for the regeneration of damaged tissues. *Expert Opin Biol Ther* 2006; **6**: 567-578 [PMID: 16706604 DOI: 10.1517/14712598.6.6.567]

53 **Zuk PA**, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 2002; **13**: 4279-4295 [PMID: 12475952 DOI: 10.1091/mbc.E02-02-0105v]

54 **Fraser JK**, Wulur I, Alfonso Z, Hedrick MH. Fat tissue: an underappreciated source of stem cells for biotechnology. *Trends Biotechnol* 2006; **24**: 150-154 [PMID: 16488036 DOI: 10.1016/j.tibtech.2006.01.010]

55 **Sakaguchi Y**, Sekiya I, Yagishita K, Muneta T. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. *Arthritis Rheum* 2005; **52**: 2521-2529 [PMID: 16052568 DOI: 10.1002/art.21212]

56 **Shirasawa S**, Sekiya I, Sakaguchi Y, Yagishita K, Ichinose S, Muneta T. In vitro chondrogenesis of human synovium-derived mesenchymal stem cells: optimal condition and comparison with bone marrow-derived cells. *J Cell Biochem* 2006; **97**: 84-97 [PMID: 16088956 DOI: 10.1002/jcb.20546]

57 **Granero-Moltó F**, Weis JA, Miga MI, Landis B, Myers TJ, O'Rear L, Longobardi L, Jansen ED, Mortlock DP, Spagnoli A. Regenerative effects of transplanted mesenchymal stem cells in fracture healing. *Stem Cells* 2009; **27**: 1887-1898 [PMID: 19544445 DOI: 10.1002/stem.103]

58 **Chamberlain G**, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. *Stem Cells* 2007; **25**: 2739-2749 [PMID: 17656645 DOI: 10.1634/stemcells.2007-0197]

59 **Mirotsou M**, Jayawardena TM, Schmeckpeper J, Gnecchi M, Dzau VJ. Paracrine mechanisms of stem cell reparative and regenerative actions in the heart. *J Mol Cell Cardiol* 2011; **50**: 280-289 [PMID: 20727900 DOI: 10.1016/j.yjmcc.2010.08.005]

60 **Song C**, Li G. CXCR4 and matrix metalloproteinase-2 are involved in mesenchymal stromal cell homing and engraftment to tumors. *Cytotherapy* 2011; **13**: 549-561 [PMID: 21171825 DOI: 10.3109/14653249.2010.542457]

61 **Granero-Molto F**, Weis JA, Longobardi L, Spagnoli A. Role of mesenchymal stem cells in regenerative medicine: application to bone and cartilage repair. *Expert Opin Biol Ther* 2008; **8**: 255-268 [PMID: 18294098 DOI: 10.1517/14712598.8.3.255]

62 **Di Nicola M**, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, Grisanti S, Gianni AM. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood* 2002; **99**: 3838-3843 [PMID: 11986244 DOI: 10.1182/blood.V99.10.3838]

63 **Le Blanc K**, Rasmusson I, Götherström C, Seidel C, Sundberg B, Sundin M, Rosendahl K, Tammik C, Ringdén O. Mesenchymal stem cells inhibit the expression of CD25 (interleukin-2 receptor) and CD38 on phytohaemagglutinin-activated lymphocytes. *Scand J Immunol* 2004; **60**: 307-315 [PMID: 15320889 DOI: 10.1111/j.0300-9475.2004.01483.x]

64 **Glennie S**, Soeiro I, Dyson PJ, Lam EW, Dazzi F. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. *Blood* 2005; **105**: 2821-2827 [PMID: 15591115 DOI: 10.1182/blood-2004-09-3696]

65 **Dazzi F**, Lopes L, Weng L. Mesenchymal stromal cells: a key player in 'innate tolerance'? *Immunology* 2012; **137**: 206-213 [PMID: 22804624 DOI: 10.1111/j.1365-2567.2012.03621.x]

66 **Spaggiari GM**, Capobianco A, Becchetti S, Mingari MC, Moretta L. Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. *Blood* 2006; **107**: 1484-1490 [PMID: 16239427 DOI: 10.1182/blood-2005-07-2775]

67 **Shi M**, Liu ZW, Wang FS. Immunomodulatory properties and therapeutic application of mesenchymal stem cells. *Clin Exp Immunol* 2011; **164**: 1-8 [PMID: 21352202 DOI: 10.1111/j.1365-2249.2011.04327.x]

68 **Yagi H**, Soto-Gutierrez A, Parekkadan B, Kitagawa Y, Tompkins RG, Kobayashi N, Yarmush ML. Mesenchymal stem cells: Mechanisms of immunomodulation and homing. *Cell Transplant* 2010; **19**: 667-679 [PMID: 20525442 DOI: 10.3727/096368910X508762]

69 **Selmani Z**, Naji A, Zidi I, Favier B, Gaiffe E, Obert L, Borg C, Saas P, Tiberghien P, Rouas-Freiss N, Carosella ED, Deschaseaux F. Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4+CD25highFOXP3+ regulatory T cells. *Stem Cells* 2008; **26**: 212-222 [PMID: 17932417 DOI: 10.1634/stemcells.2007-0554]

70 **Bouffi C**, Djouad F, Mathieu M, Noël D, Jorgensen C. Multipotent mesenchymal stromal cells and rheumatoid arthritis: risk or benefit? *Rheumatology* (Oxford) 2009; **48**: 1185-1189 [PMID: 19561159 DOI: 10.1093/rheumatology/kep162]

71 **Gieseke F**, Böhringer J, Bussolari R, Dominici M, Handgretinger R, Müller I. Human multipotent mesenchymal stromal cells use galectin-1 to inhibit immune effector cells. *Blood* 2010; **116**: 3770-3779 [PMID: 20644118 DOI: 10.1182/blood-2010-02-270777]

72 **Pevsner-Fischer M**, Morad V, Cohen-Sfady M, Rousso-Noori L, Zanin-Zhorov A, Cohen S, Cohen IR, Zipori D. Toll-like receptors and their ligands control mesenchymal stem cell functions. *Blood* 2007; **109**: 1422-1432 [PMID: 17038530 DOI: 10.1182/blood-2006-06-028704]

73 **Tomchuck SL**, Zwezdaryk KJ, Coffelt SB, Waterman RS, Danka ES, Scandurro AB. Toll-like receptors on human mesenchymal stem cells drive their migration and immunomodulating responses. *Stem Cells* 2008; **26**: 99-107 [PMID: 17916800 DOI: 10.1634/stemcells.2007-0563]

74 **Meisel R**, Zibert A, Laryea M, Göbel U, Däubener W, Dilloo D. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. *Blood* 2004; **103**: 4619-4621 [PMID: 15001472 DOI: 10.1182/blood-2003-11-3909]

75 **Haleem-Smith H**, Derfoul A, Okafor C, Tuli R, Olsen D, Hall DJ, Tuan RS. Optimization of high-efficiency transfection of adult human mesenchymal stem cells in vitro. *Mol Biotechnol* 2005; **30**: 9-20 [PMID: 15805572]

76 **Longobardi L**, O'Rear L, Aakula S, Johnstone B, Shimer K, Chytil A, Horton WA, Moses HL, Spagnoli A. Effect of IGF-I in the chondrogenesis of bone marrow mesenchymal stem cells in the presence or absence of TGF-beta signaling. *J Bone Miner Res* 2006; **21**: 626-636 [PMID: 16598383 DOI: 10.1359/jbmr.051213]

77 **Gelse K**, von der Mark K, Aigner T, Park J, Schneider H. Articular cartilage repair by gene therapy using growth factor-producing mesenchymal cells. *Arthritis Rheum* 2003; **48**: 430-441 [PMID: 12571853 DOI: 10.1002/art.10759]

78 **Kuroda R**, Usas A, Kubo S, Corsi K, Peng H, Rose T, Cummins J, Fu FH, Huard J. Cartilage repair using bone morphogenetic protein 4 and muscle-derived stem cells. *Arthritis Rheum* 2006; **54**: 433-442 [PMID: 16447218 DOI: 10.1002/art.21632]

79 **Katayama R**, Wakitani S, Tsumaki N, Morita Y, Matsushita I, Gejo R, Kimura T. Repair of articular cartilage defects in rabbits using CDMP1 gene-transfected autologous mesenchymal cells derived from bone marrow. *Rheumatology* (Oxford) 2004; **43**: 980-985 [PMID: 15187242 DOI: 10.1093/rheumatology/keh240]

80 **Sekiya I**, Vuoristo JT, Larson BL, Prockop DJ. In vitro cartilage formation by human adult stem cells from bone marrow stroma defines the sequence of cellular and molecular events during chondrogenesis. *Proc Natl Acad Sci USA* 2002; **99**: 4397-4402 [PMID: 11917104 DOI: 10.1073/pnas.052716199]

81 **Mwale F**, Girard-Lauriault PL, Wang HT, Lerouge S, Antoniou J, Wertheimer MR. Suppression of genes related to hypertrophy and osteogenesis in committed human mesenchymal stem cells cultured on novel nitrogen-rich plasma polymer coatings. *Tissue Eng* 2006; **12**: 2639-2647 [PMID: 16995797 DOI: 10.1089/ten.2006.12.2639]

82 **Mwale F**, Stachura D, Roughley P, Antoniou J. Limitations of using aggrecan and type X collagen as markers of chondrogenesis in mesenchymal stem cell differentiation. *J Orthop Res* 2006; **24**: 1791-1798 [PMID: 16779832 DOI: 10.1002/jor.20200]

83 **Pelttari K**, Winter A, Steck E, Goetzke K, Hennig T, Ochs BG, Aigner T, Richter W. Premature induction of hypertrophy during in vitro chondrogenesis of human mesenchymal stem cells correlates with calcification and vascular invasion after ectopic transplantation in SCID mice. *Arthritis Rheum* 2006; **54**: 3254-3266 [PMID: 17009260 DOI: 10.1002/art.22136]

84 **Koga H**, Engebretsen L, Brinchmann JE, Muneta T, Sekiya I. Mesenchymal stem cell-based therapy for cartilage repair: a review. *Knee Surg Sports Traumatol Arthrosc* 2009; **17**: 1289-1297 [PMID: 19333576 DOI: 10.1007/s00167-009-0782-4]

85 **Bian L**, Zhai DY, Mauck RL, Burdick JA. Coculture of human mesenchymal stem cells and articular chondrocytes reduces hypertrophy and enhances functional properties of engineered cartilage. *Tissue Eng Part A* 2011; **17**: 1137-1145 [PMID: 21142648]

86 **Fischer J**, Dickhut A, Rickert M, Richter W. Human articular chondrocytes secrete parathyroid hormone-related protein and inhibit hypertrophy of mesenchymal stem cells in coculture during chondrogenesis. *Arthritis Rheum* 2010; **62**: 2696-2706 [PMID: 20496422]

87 **Khan IM**, Francis L, Theobald PS, Perni S, Young RD, Prokopovich P, Conlan RS, Archer CW. In vitro growth factor-induced bio engineering of mature articular cartilage. *Biomaterials* 2013; **34**: 1478-1487 [PMID: 23182922 DOI: 10.1016/j.biomaterials.2012.09.076]

88 **Im GI**, Kim DY, Shin JH, Hyun CW, Cho WH. Repair of cartilage defect in the rabbit with cultured mesenchymal stem cells from bone marrow. *J Bone Joint Surg Br* 2001; **83**: 289-294 [PMID: 11284583 DOI: 10.1302/0301-620X.83B2.10495]

89 **Wakitani S**, Goto T, Pineda SJ, Young RG, Mansour JM, Caplan AI, Goldberg VM. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. *J Bone Joint Surg Am* 1994; **76**: 579-592 [PMID: 8150826]

90 **Berninger MT**, Wexel G, Rummeny EJ, Imhoff AB, Anton M, Henning TD, Vogt S. Treatment of osteochondral defects in the rabbit's knee joint by implantation of allogeneic mesenchymal stem cells in fibrin clots. *J Vis Exp* 2013; (**75**): e4423 [PMID: 23728213 DOI: 10.3791/4423]

91 **Grigolo B**, Lisignoli G, Desando G, Cavallo C, Marconi E, Tschon M, Giavaresi G, Fini M, Giardino R, Facchini A. Osteoarthritis treated with mesenchymal stem cells on hyaluronan-based scaffold in rabbit. *Tissue Eng Part C Methods* 2009; **15**: 647-658 [PMID: 19249964 DOI: 10.1089/ten.TEC.2008.0569]

92 **Guo X**, Wang C, Duan C, Descamps M, Zhao Q, Dong L, Lü S, Anselme K, Lu J, Song YQ. Repair of osteochondral defects with autologous chondrocytes seeded onto bioceramic scaffold in sheep. *Tissue Eng* 2004; **10**: 1830-1840 [PMID: 15684691 DOI: 10.1089/ten.2004.10.1830]

93 **Murphy JM**, Fink DJ, Hunziker EB, Barry FP. Stem cell therapy in a caprine model of osteoarthritis. *Arthritis Rheum* 2003; **48**: 3464-3474 [PMID: 14673997 DOI: 10.1002/art.11365]

94 **Al Faqeh H**, Nor Hamdan BM, Chen HC, Aminuddin BS, Ruszymah BH. The potential of intra-articular injection of chondrogenic-induced bone marrow stem cells to retard the progression of osteoarthritis in a sheep model. *Exp Gerontol* 2012; **47**: 458-464 [PMID: 22759409 DOI: 10.1016/j.exger.2012.03.018]

95 **Kuroda R**, Ishida K, Matsumoto T, Akisue T, Fujioka H, Mizuno K, Ohgushi H, Wakitani S, Kurosaka M. Treatment of a full-thickness articular cartilage defect in the femoral condyle of an athlete with autologous bone-marrow stromal cells. *Osteoarthritis Cartilage* 2007; **15**: 226-231 [PMID: 17002893 DOI: 10.1016/j.joca.2006.08.008]

96 **Wakitani S**, Mitsuoka T, Nakamura N, Toritsuka Y, Nakamura Y, Horibe S. Autologous bone marrow stromal cell transplantation for repair of full-thickness articular cartilage defects in human patellae: two case reports. *Cell Transplant* 2004; **13**: 595-600 [PMID: 15565871 DOI: 10.3727/000000004783983747]

97 **Wakitani S**, Nawata M, Tensho K, Okabe T, Machida H, Ohgushi H. Repair of articular cartilage defects in the patello-femoral joint with autologous bone marrow mesenchymal cell transplantation: three case reports involving nine defects in five knees. *J Tissue Eng Regen Med* 2007; **1**: 74-79 [PMID: 18038395 DOI: 10.1002/term.8]

98 **He J**, Jiang B, Dai Y, Hao J, Zhou Z, Tian Z, Wu F, Gu Z. Regulation of the osteoblastic and chondrocytic differentiation of stem cells by the extracellular matrix and subsequent bone formation modes. *Biomaterials* 2013; **34**: 6580-6588 [PMID: 23787112 DOI: 10.1016/j.biomaterials.2013.05.056]

99 **Saarakkala S**, Julkunen P, Kiviranta P, Mäkitalo J, Jurvelin JS, Korhonen RK. Depth-wise progression of osteoarthritis in human articular cartilage: investigation of composition, structure and biomechanics. *Osteoarthritis Cartilage* 2010; **18**: 73-81 [PMID: 19733642 DOI: 10.1016/j.joca.2009.08.003]

100 **Wakitani S**, Imoto K, Yamamoto T, Saito M, Murata N, Yoneda M. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. *Osteoarthritis Cartilage* 2002;

10: 199-206 [PMID: 11869080 DOI: 10.1053/joca.2001.0504]
101 **Centeno CJ**, Busse D, Kisiday J, Keohan C, Freeman M, Karli D. Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells. *Pain Physician* 2008; **11**: 343-353 [PMID: 18523506]
102 **Davatchi F**, Abdollahi BS, Mohyeddin M, Shahram F, Nikbin B. Mesenchymal stem cell therapy for knee osteoarthritis. Preliminary report of four patients. *Int J Rheum Dis* 2011; **14**: 211-215 [PMID: 21518322 DOI: 10.1111/j.1756-185X.2011.01599.x]
103 **Emadedin M**, Aghdami N, Taghiyar L, Fazeli R, Moghadasali R, Jahangir S, Farjad R, Baghaban Eslaminejad M. Intra-articular injection of autologous mesenchymal stem cells in six patients with knee osteoarthritis. *Arch Iran Med* 2012; **15**: 422-428 [PMID: 22724879]
104 **Taylor PC**, Feldmann M. Anti-TNF biologic agents: still the therapy of choice for rheumatoid arthritis. *Nat Rev Rheumatol* 2009; **5**: 578-582 [PMID: 19798034]
105 **Nishimoto N**. Interleukin-6 as a therapeutic target in candidate inflammatory diseases. *Clin Pharmacol Ther* 2010; **87**: 483-487 [PMID: 20182422 DOI: 10.1038/clpt.2009.313]
106 **Ringe J**, Sittinger M. Tissue engineering in the rheumatic diseases. *Arthritis Res Ther* 2009; **11**: 211 [PMID: 19232063 DOI: 10.1186/ar2572]
107 **Augello A**, Tasso R, Negrini SM, Cancedda R, Pennesi G. Cell therapy using allogeneic bone marrow mesenchymal stem cells prevents tissue damage in collagen-induced arthritis. *Arthritis Rheum* 2007; **56**: 1175-1186 [PMID: 17393437 DOI: 10.1002/art.22511]

P- Reviewers: Asahina K, Gharaee-Kermani M **S- Editor**: Ma YJ
L- Editor: Roemmele A **E- Editor**: Liu SQ

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com