

Mesenchymal stem cell-based therapy for cartilage repair: a review

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Received: 23 January 2009 / Accepted: 4 March 2009 / Published online: 31 March 2009
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Abstract Articular cartilage injury remains one of the major concerns in orthopaedic surgery. Mesenchymal stem cell (MSC) transplantation has been introduced to avoid some of the side effects and complications of current techniques. The purpose of this paper is to review the literature on MSC-based cell therapy for articular cartilage repair to determine if it can be an alternative treatment for cartilage injury. MSCs retain both high proliferative potential and multipotentiality, including chondrogenic differentiation potential, and a number of successful results in transplantation of MSCs into cartilage defects have been reported in animal studies. However, the use of MSCs for

cartilage repair is still at the stage of preclinical and phase I studies, and no comparative clinical studies have been reported. Therefore, it is difficult to make conclusions in human studies. This requires randomized clinical trials to evaluate the effectiveness of MSC-based cell therapy for cartilage repair.

Keywords Mesenchymal stem cells · Cartilage repair · Cell transplantation · Chondrocytes

Introduction

Cartilage defects have very limited intrinsic healing capacity. Partial thickness defects that do not penetrate the subchondral bone do not usually repair spontaneously [25], while repair of full thickness cartilage defects that penetrate the subchondral bone depends on the circumstances, such as age, defect size and location [17]. Small defects can repair spontaneously with production of hyaline cartilage, whereas larger defects will only repair with production of fibrous tissue or fibrocartilage which are biochemically and biomechanically different from normal hyaline cartilage. As a result, degeneration subsequently occurs which may progress to osteoarthritic change in some cases [91].

Various surgical methods have been proposed to regenerate articular cartilage. However, they all have inherent problems, leaving many patients with inadequately treated cartilage lesions. Recently, mesenchymal stem cells (MSCs) have been suggested as a source of cells for cell-based treatment of cartilage lesions. MSCs are known to play important roles in development, post-natal growth, repair and regeneration of mesenchymal tissues. They are easily isolated, and retain high expansion potential and multipotentiality that includes chondrogenic

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differentiation potential. Based on these properties, MSCs are potentially an attractive cell source for cartilage regenerative medicine. With regard to *in vivo* studies, transplantation of MSCs into full thickness articular cartilage defects has been attempted under various conditions. Although many successful results have been reported, a number of questions, such as from which tissue MSCs are suitable or what conditions are appropriate for cartilage repair, still exist, limiting clinical applications for cartilage injury. Currently, very few clinical studies of MSC transplantation for cartilage repair have been reported.

This paper briefly describes some of the problems associated with currently used methods for repair of cartilage lesions, followed by a review of the existing literature on MSC-based cell therapy for articular cartilage repair. We focus on three major parts in the process; transplanted cells, scaffolds and growth factors, and their differential performance *in vitro* and in MSC-based cell therapy in animal studies. Finally we review clinical studies on MSC therapy for cartilage repair, and discuss MSC therapy compared with autologous chondrocyte implantation (ACI), which is an established procedure worldwide [7, 10, 59, 62, 73]. The intention of this paper is to review whether MSC-based cell therapy can be an alternative for treatment of articular cartilage injury.

Current surgical treatment for articular cartilage injury

Bone marrow stimulation

Bone marrow stimulation is a technique in which subchondral bone is penetrated. One of the expectations for the penetration is to induce bone marrow-derived chondroprogenitors into the cartilage lesion. The procedure may also enhance the expression of cytokines to promote cartilage repair [57]. This technique includes drilling [75], abrasion [41] and microfracture [61, 93]. At present, these techniques are preferred by the majority of orthopaedic surgeons as they are easy to perform, need no special surgical instruments and are more cost effective than others [103]. However, cartilage defects only repair with fibrous tissue or fibrocartilage by these methods, probably because the number of chondroprogenitors induced from bone marrow is too small to promote repair with hyaline cartilage, and the results are often followed by degeneration in the repaired tissue [93].

Mosaicplasty

Mosaicplasty is a procedure whereby autologous osteochondral plugs are transplanted into the cartilage defect. By

this method, the repaired tissue is predominantly composed of hyaline cartilage [33, 60]. However, donor site morbidity [78] and the limited availability of autologous osteochondral plugs limit the usefulness of this method, particularly for repair of large lesions.

Autologous chondrocyte implantation

The clinical use of ACI was first reported by Brittberg et al. in 1994 [10], following animal studies which had shown its effectiveness [29]. In this method, chondrocytes are obtained from a biopsy taken from a non-weight bearing part of the cartilage of the patients, and are expanded *in vitro*, followed by the injection of a suspension of expanded chondrocytes into cartilage defects, covered with autologous periosteal flap. Although clinical results of the original ACI were reported as promising [62, 73], this procedure has some potential disadvantages, such as leakage of transplanted cells, invasive surgical method, hypertrophy of periosteum [32, 50] and loss of chondrogenic phenotype of expanded chondrocytes in monolayer culture [8]. Second-generation ACI was introduced to improve these problems, and biomaterials such as collagen type I gel [69], hyaluronan-based scaffold [59] and collagen type I/III membrane [7] were applied to secure cells in the defect area, to restore chondrogenic phenotype by way of three-dimensional culture [28] and to replace the periosteum as defect coverage. At present, only two prospective studies comparing the original and second-generation ACI are available [7, 59] and both studies showed no significant differences in the short term clinical outcomes. As for the first generation ACI, the newly regenerated cartilage often consists of fibrous tissues [35, 96] possibly due to limited number of chondrocytes and their low proliferation potential. Bony overgrowth which causes thinning of the regenerated cartilage and violation of the tidemark is also of concern [1]. Moreover, this method still sacrifices healthy cartilage. These aspects limit ACI in the treatment of large defects and may increase the long-term risk of developing osteoarthritis.

Comparison of treatments currently in use for cartilage repair

Jakobsen et al. [40] evaluated quality and outcome of 69 clinical cartilage repair studies using microfracture, autologous osteochondral transplantation (mosaicplasty), autologous periosteal transplantation or ACI. Data from 3,987 surgical procedures in these studies were assessed. More than half of the studies were retrospective, and only four studies were prospective, randomized and controlled trials [35, 44]. No significant differences in outcome were found between the four techniques, and large variations in the reported outcomes were seen within each treatment

modality. The low-methodological quality found in these studies indicates that caution is required when interpreting results after surgical cartilage repair. It was concluded that firm recommendations on which procedure to choose cannot be provided at this time.

Mesenchymal stem cells

Friedenstein [23] first established the existence of MSCs and showed that bone marrow contains cells that can differentiate into bone and cartilage. His initial work has been extended by a number of investigators and it has been reported that MSCs isolated from bone marrow have self-renewal potential and multilineage differentiation potential including chondrogenesis [42, 74, 76, 81]. MSCs can be isolated from a variety of adult mesenchymal tissues, have extensive proliferation potential and are easily expanded without loss of their multilineage differentiation potential within several passages. Therefore, MSCs are perceived as an attractive cell source for regenerative medicine for cartilage injury.

Chondrogenesis of MSCs

Chondrogenesis of MSCs was first reported by Ashton et al. [5] and a defined medium for *in vitro* chondrogenesis of MSCs was first described by Johnstone et al. [42], who used micromass culture with transforming growth factor-beta (TGF- β) and dexamethasone. Sekiya et al. [85, 88] reported that addition of bone morphogenetic proteins (BMPs) enhanced chondrogenesis under the conditions employed by Johnstone et al. [42]. Currently, the micromass culture is widely used to evaluate chondrogenic potential of MSCs *in vitro*.

However, this *in vitro* chondrogenesis does not mimic cartilage formation during development. During micromass culture, MSCs increase expressions of both collagen type II (chondrocyte marker) and type X (hypertrophic chondrocyte marker) [6, 39]. Furthermore, MSCs continue to express collagen type I [94]. Other cytokines such as insulin-like growth factor (IGF) [71] and parathyroid hormone-related peptide (PTHrP) [43] have been tried for better differentiation cocktails, but it is still difficult to obtain *in vitro* MSC-based cartilage formation comparative with native cartilage tissue.

When bone marrow-derived mesenchymal cells were subcutaneously transplanted in special diffusion chambers, some cells differentiated into cartilage ectopically [5]. Also, MSCs implanted into osteochondral defects differentiated into chondrocytes [99]. On the other hand, after cartilage pellets differentiated from MSCs *in vitro* were

transplanted subcutaneously, these pellets disappeared [19] or calcified with vascular invasion [72]. This indicates the importance of signals from the microenvironment to induce cartilage formation of MSCs and to maintain its phenotypes [15, 47, 99].

MSCs from various mesenchymal tissues

Although bone marrow is considered to be a well-accepted source of MSCs, stem cells are present in a variety of mesenchymal tissues other than bone marrow and can be isolated from them, such as synovium [18], periosteum [24], skeletal muscle [11], adipose tissue [110], trabecular bone [82] and umbilical cord blood [55]. These MSCs are similar irrespective of their origin in that they have colony-forming ability and *in vitro* chondrogenic, osteogenic and adipogenic potentials [20, 30]. Recently, there are increasing number of studies describing the specific properties of MSCs, including chondrogenic potential, dependent on their origin [16, 37, 48, 56, 70, 77, 79, 83, 86, 105, 109].

Some comparative studies showed that MSCs from bone marrow had more *in vitro* chondrogenic potential than those from adipose tissue [16, 37, 56, 77, 79, 86, 105]. Sakaguchi et al. [83] harvested human bone marrow, synovium, periosteum, muscle and adipose tissue, and isolated and expanded MSCs in a similar condition. They demonstrated that MSCs derived from synovium had higher chondrogenic potential than those from other mesenchymal tissues. Yoshimura et al. [109] also demonstrated higher chondrogenic differentiation potential of MSCs from synovium in rats in a similar way.

One drawback of these studies is that the evaluation of *in vitro* chondrogenesis may not represent the chondrogenic potential of MSCs transplanted into cartilage defects. Park et al. [70] showed that MSCs from bone marrow and periosteum were superior to cells isolated from fat with respect to forming hyaline cartilaginous tissue when transplanted into cartilage defect in rats. Koga et al. [48] compared *in vivo* chondrogenic potential among various MSCs in rabbits and demonstrated that MSCs from synovium and bone marrow had a higher potential to repair cartilage defect than those from skeletal muscle and adipose tissue.

Which is a better MSC source for cartilage regeneration, bone marrow or synovium? Nimura et al. [67] reported that MSCs from synovium expanded much faster than those from bone marrow when cultured with autologous human serum. This is an advantage of MSCs from synovium. However, bone marrow is easier to harvest than synovium, which is a reason why bone marrow is more widely accepted as an MSC source.

Suitable conditions for cartilage repair with MSCs

Cell density

In ACI, chondrocyte density used for clinical treatment was 10^6 cells/ml or less when they chondrocytes embedded in a gel [7, 59, 69]. In MSC transplantation, higher density appears to be required. Koga et al. reported that 5×10^7 MSCs/ml embedded in collagen type I gel repaired cartilage defect successfully whereas 10^6 MSCs/ml resulted in failure in rabbit [48]. MSCs divided sparsely during in vitro [38, 87] and in vivo [47] chondrogenesis, whereas viable MSCs were decreased by apoptosis [38].

Growth and differentiation factors

Growth and differentiation factors including members of the TGF- β superfamily, IGF-1 or FGF have been shown to stimulate the chondrogenic differentiation of MSCs. Although some studies have reported that transplantation of undifferentiated MSCs into cartilage defects provided good results [1, 47, 99], these factors such as TGF- β [31, 54], BMPs [66], IGF-1 [26] and, particularly, a combination of TGF- β and BMPs [88] have been shown to enhance cartilage repair in combination with MSCs in vitro and in animal studies. Such stimulations could be achieved by direct administration of recombinant growth and differentiation factors or by transfer of the respective genes, and each study showed that cartilage defects transplanted with enhanced MSCs led to better repair than those with untreated MSCs. These results suggest that the use of appropriate differentiation factors could improve cartilage repair by MSC transplantation. Presently, the differentiation factors cannot be ranked according to efficiency. There are some disadvantages with the use of growth factors. Direct administration into the injured site requires high dosages or repeated injections due to relatively short half-lives of these proteins in vivo. This technique is very expensive and may lead to unanticipated adverse effects [3, 98]. Gene therapy has been suggested as an alternative method by which these proteins can be provided for a prolonged period of time directly at the site of cell transplantation [27]. However, a large amount of work still has to be carried out to prove that gene therapy is sufficiently safe to allow clinical use [22]. Even if the growth factors are used only during in vitro differentiation, there are problems regarding availability of good manufacturing practice format, price and the fact that no combination of differentiation factors has been able to turn off collagen type I and collagen type X genes [6, 39, 94]. A better strategy might be to construct scaffolds which are able to deliver differentiation factors directly to the cells embedded in the scaffold. Currently some bioscaffolds exist that can elicit a controlled action and reaction to the

surrounding tissue environment (bioactive), and others that exhibit a controlled chemical breakdown and resorption with ultimate replacement by regenerating tissue (resorbable). New generation biomaterials are also being designed to stimulate regeneration of living tissues using tissue engineering and in situ tissue regeneration methods. These materials will lead to limitless possibilities for cartilage regeneration [34, 108]. However, they require further investigations for clinical use.

Culture serum

MSCs have been expanded with foetal bovine serum (FBS) for research as well as for clinical use [36, 99]. However, supplementation with FBS has several risks, such as disease transmission and immune reaction [58, 89, 104]. Increasing the safety of MSC transplantation requires the use of safe and effective substitute for FBS. Autologous human serum has been investigated as a substitute for FBS. Several studies have compared the proliferative effects of autologous human serum and FBS on MSCs from bone marrow with variable results. Some reported that FBS was superior to human serum [52]. Others reported that their proliferative effects were similar [92, 106], and still others demonstrated that MSCs proliferated more in human serum than in FBS [45, 64, 90, 95]. The discrepancies among them may be from serum dose difference, from variations among donor sera, from differences in sample numbers and also from differences in harvested sites [2]. For other MSCs, Nimura et al. [67] reported that MSCs from synovium expanded more in human serum than in FBS through platelet-derived growth factor signalling, while opposite results were obtained with MSCs from bone marrow. As for chondrogenic potential, MSCs from bone marrow [90] and synovium [67] precultured in autologous human serum showed lower in vitro chondrogenic potential than in FBS, whereas in vivo chondrogenic potential of rabbit MSCs from synovium was similar [67].

Human platelet lysate (hPL) has also been reported as a substitute. Some reports showed that MSCs from bone marrow proliferated more in hPL than in FBS and retained their differentiation potentials including chondrogenesis, while there is a disadvantage that the amount of hPL obtained from a patient is less than half that of human serum [12, 13, 21, 53, 84]. Although there is no comparative study between human serum and hPL, they might be an effective and more beneficial substitute for FBS.

Clinical studies on MSC transplantation for cartilage repair

Presently only one prospective clinical study of MSC transplantation for cartilage repair has been published, in

Table 1 Clinical studies on MSC transplantation for cartilage repair

Authors	Year	No. of patients	Results and comments
Wakitani et al.	2002	24	Prospective, randomized study Bone marrow MSC versus cell-free scaffold No significance in clinical results Better arthroscopic and histological score in MSC group
Wakitani et al.	2004	2	Case reports transplanted in patellar defects Bone marrow MSC All are clinically improved
Wakitani et al.	2007	3	Three case reports involving nine defects in five knees Bone marrow MSC All are clinically improved
Kuroda et al.	2007	1	A case report Bone marrow MSC Clinically improved

which bone marrow-derived MSCs were resuspended in a collagen type I gel and transplanted with autologous periosteal flap [100]. In this study, patients with knee osteoarthritis who underwent a high tibial osteotomy were treated with a cell-containing scaffold with a periosteal flap transplanted into a cartilage defect in the medial femoral condyle and compared with patients who were transplanted with cell-free scaffold with a periosteal flap transplanted into a similar lesion. Although the cell-treated group showed no significant improvement clinically compared with the control group, the arthroscopic and histological score was better in MSC-transplanted group. There were also three case reports from the same group which reported that patients' clinical symptoms had improved [51, 101, 102] (Table 1). However, there is still no comparative clinical study with other surgical methods.

Comparison of chondrocyte and MSC transplantation for cartilage repair

As mentioned before, MSC transplantation has some advantages in cartilage repair over ACI. MSCs can be isolated from various tissues without harvesting healthy articular cartilage and are easily expanded without loss of their chondrogenic potential at early passages. Therefore, MSCs are an attractive alternative cell source not only for patients with focal cartilage lesion but also for those with osteoarthritis [65, 68]. However, there are also some disadvantages and risks to use MSCs for cartilage repair. Some reports showed that MSCs-derived chondrocytes expressed hypertrophy-related genes leading to cell death or calcification followed by vascularization when implanted subcutaneously or intramuscularly, whereas articular chondrocytes resisted calcification and vascular invasion [19]. When MSCs were transplanted into cartilage defects, while they could differentiate into chondrocytes according

to local microenvironment in articular joints, the thickness of the regenerated cartilage became thinner than the original thickness and the tidemark was violated [1, 47, 99]. Transformation of MSCs is also one of the concerns. It has been reported that MSCs can undergo spontaneous transformation after long-time culture [80, 97], although they can be managed safely during the standard ex vivo expansion period and such transformation is considered to be very rare [9]. Further investigation to solve these problems is required.

A few animal studies have been published comparing ACI and MSC transplantation for cartilage repair. In these studies, no significant differences were observed in histological score between the group transplanted with chondrocytes and with MSCs [1, 107], although repaired tissue with MSCs appeared better in cell arrangement, subchondral bone remodelling, and integration with surrounding cartilage [107].

Less invasive technique for MSC transplantation

Treatment with MSCs (and chondrocytes) requires the transplantation of a cell and scaffold composite. If periosteal coverage is needed, the method is quite invasive as it requires harvesting the periosteum and fixation with suturing to the neighbouring cartilage. Moreover, hypertrophy and ossification are of concern [32, 50, 69]. Without periosteum, a scaffold is needed to keep the cells at the injured site; the scaffolds are derived from animals, thereby increasing the risk of disease transmission and immune reaction [14]. The easiest and the least invasive method might be intra-articular injection; however, with this technique, most of the injected cells adhered to synovial tissues [63], which might increase the risk of adverse effects such as synovial proliferation, and only a small portion of the cells adhered to the cartilage defects [49]. Recently, some

papers reported less invasive methods without scaffolds to adhere transplanted cells effectively [4, 46, 49]. If clinically successful, such methods may extend the indications for MSC-based cell therapy for cartilage repair.

Conclusions

As articular cartilage defects have very limited intrinsic healing capacity, development of new methods for treatment for cartilage defects is of major importance for orthopaedic surgeons. Although various surgical methods have been attempted, including bone marrow stimulation technique, mosaicplasty and ACI, each of them has some disadvantages. MSC-based cell therapy is expected to be an alternative for cartilage repair because MSCs are easily isolated from a variety of mesenchymal tissues, have high proliferative potential and have chondrogenic potential. In animal studies, transplantation of MSCs into cartilage defects has been attempted under different circumstances, and a number of publications exist on cell source, cell density, growth and differentiation factors, culture serum and scaffold. Recently, less invasive techniques for MSC transplantation into cartilage defects have also been developed, and the clinical use of MSCs may well have a bright future. However, the technique is still in the state of preclinical and phase I studies, and there is no comparative clinical study with other surgical methods. Moreover, some concerns still exist about the chondrogenesis and genetic stability of MSCs. Randomized clinical trials are needed to evaluate the effectiveness of MSC-based cell therapy for cartilage repair.

Acknowledgment H.K. was supported by a grant from Research Fund of Mitsukoshi Health and Welfare Foundation 2007.

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