Application of mesenchymal stem cells in joint diseases

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Abstract
Introduction
Multilineage differential potentials and immunomodulatory function of mesenchymal stem cells have led to the birth of this subfield of regenerative medicine. More than a thousand clinical trials have been conducted, including clinical trials treating joint diseases, e.g., osteoarthritis and inflammatory arthritis. This review is to summarize preclinical studies of mesenchymal stem cells or mesenchymal stem cells-like cells in arthritis, with a focus on currently unsolved issues in utilising the regenerative and immunomodulatory properties of mesenchymal stem cells.

Conclusion
Numerous issues remain in utilising mesenchymal stem cells in treating joint diseases. Quantitative measurement of chondrogenic capacity, demonstration of long-term survival and engraftment after transplantation, further understanding the mechanism of immunomodulatory function are pivotal for the application of mesenchymal stem cells in treating joint diseases.

Introduction
Friedenstein and coworkers, in a series of seminal studies in the 1960s and 1970s1, showed that the osteogenic potential of bone marrow (BM) cells was associated with a minor subpopulation of the cell of the bone marrow isolate. These cells were distinguishable from the majority of hematopoietic cells by their rapid adherence to tissue culture vessels and by the fibroblast-like appearance of their progeny in culture, pointing to their origin from the stromal compartment of BM. The currently popular, though perhaps inaccurate, term mesenchymal stem cells (MSCs) was first coined in 1991 by Dr. Arnold Caplan2. Work by Darwin Prockop and others3,4 further defined the cells and their adipogenic, osteogenic, and chondrogenic differentiation potentials in vitro. Cells with similar properties from other tissues (Figure 1) were reported, such as synovium5, umbilical cord blood6, placenta7, adipose tissue8, dental pulp9, etc. MSC can modulate the activity of immune cells by secreting soluble factors and via cell–cell contact. This property has two potential implications: substitution of autologous MSCs with off-the-shelf allogenic MSC products, and treatment of immune-mediated diseases, like acute graft-versus-host-disease (GvHD), Crohn’s disease, multiple sclerosis, or other autoimmune diseases.

The multilineage differentiation potentials and immunomodulatory function of MSCs, and their ability to obtain and grow as autologous cells in large quantities has led to the birth of an entire subfield of regenerative medicine. More than a thousand trials have been conducted and more than fifty companies offer commercial MSC products from different sources for treatment of various diseases.

In the field of musculoskeletal diseases, MSCs have been proposed to treat osteoarthritis and inflammatory arthritis. Eighteen clinical trials

Figure 1: MSC for joint disease: Restorative or immune modulatory.
using MSCs for arthritis treatment are currently registered at clinicaltrial.gov; among them, 16 trials (Table 1) investigate the application of MSCs in osteoarthritis or chondral defects, using its regenerative property, with three trials of allogenic MSCs and two trials exploring its use in rheumatoid arthritis with its immunomodulatory property.

This review is to summarize preclinical studies of MSC or MSC-like cells in arthritis, with focus on currently unsolved issues in utilising the regenerative and immunomodulatory properties.

**Discussion**

**Chondrogenic capacity of MSCs**

Injury to chondral tissue eventually leads to degeneration of joints

Table 1 Current clinical trials of mesenchymal stem cells in osteoarthritis or chondral defects

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Phase</th>
<th>Enrolment</th>
<th>Indication</th>
<th>Cell type</th>
<th>Outcome measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT01300598</td>
<td>I, II</td>
<td>18</td>
<td>Degenerative arthritis</td>
<td>Autologous ADSC</td>
<td>Safety; WOMAC Index</td>
</tr>
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<td>NCT00891501</td>
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<td>25</td>
<td>Degenerative arthritis</td>
<td>Autologous BMSC</td>
<td>Clinical and radiological improvement</td>
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<td>NCT01159899</td>
<td>0</td>
<td>50</td>
<td>OA</td>
<td>Fresh non-culture expanded autologous BMSC</td>
<td>International knee score</td>
</tr>
<tr>
<td>NCT01183728</td>
<td>I, II</td>
<td>12</td>
<td>Knee OA</td>
<td>Autologous BMSC</td>
<td>Feasibility and safety; efficacy</td>
</tr>
<tr>
<td>NCT01504464</td>
<td>II</td>
<td>40</td>
<td>OA</td>
<td>Autologous BMSC</td>
<td>Physical function improvement; change in pain density</td>
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<tr>
<td>NCT01436058</td>
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<td>6</td>
<td>OA</td>
<td>Autologous BMSC</td>
<td>Safety</td>
</tr>
<tr>
<td>NCT00850187</td>
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<td>Knee OA</td>
<td>Autologous BMSC mixed with collagen I scaffold</td>
<td>Knee cartilage defects</td>
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<tr>
<td>NCT01499056</td>
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<td>6</td>
<td>Hip OA</td>
<td>Autologous BMSC</td>
<td>Clinical improvement and safety</td>
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<td>Knee OA</td>
<td>Allogenic MSC</td>
<td>Safety</td>
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<td>NCT01733186</td>
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<td>OA</td>
<td>CARTISTEM</td>
<td>Safety</td>
</tr>
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<td>NCT01041001</td>
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<td>104</td>
<td>Knee cartilage injury osteoarthritis</td>
<td>CARTISTEM vs. Microfracture treatment</td>
<td>ICRS score</td>
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<tr>
<td>NCT01626677</td>
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<td>Cartistem vs. Microfracture treatment</td>
<td>Degree of improvement in knee assessments</td>
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<tr>
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<td>Autologous BMSC</td>
<td>Change in cartilage thickness by MRI</td>
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<tr>
<td>NCT01227694</td>
<td>I, II</td>
<td>15</td>
<td>Osteoarthritis</td>
<td>Autologous bone marrow MSC</td>
<td>Safety and Feasibility</td>
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<tr>
<td>NCT00557635</td>
<td>II</td>
<td>50</td>
<td>Tibia or Femur pseudo-arthrosis</td>
<td>Co-implantation of osseous matrix and mesenchymal progenitors cells from autologous bone marrow</td>
<td>Evaluate osseous setting at 3-months follow-up and compare our results with past studies</td>
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<tr>
<td>NCT01585857</td>
<td>I</td>
<td>18</td>
<td>OA</td>
<td>Autologous ADSC</td>
<td>Recording of serious adverse events</td>
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</table>

MSC, mesenchymal stem cell; ADSC, adipose tissue derived stem cell; BMSC, bone marrow stromal cells; OA, osteoarthritis; CARTISTEM®, allogeneic-unrelated, umbilical cord blood-derived MSC, combined with sodium hyaluronate; WOMAC, Western Ontario and McMaster Universities Arthritis Index; ICRS, International Cartilage Repair Society.
and osteoarthritis. As cartilage is a tissue with very poor self-regeneration ability, transplantation of chondrocytes or progenitor cells is a potential approach to repair cartilage defect. Autologous chondrocyte implantation (ACI) is the first cell-based tissue engineering product used in clinics to treat local articular cartilage defects smaller than 4 cm². However, it requires a cartilage biopsy via arthroscopic procedure to harvest autologous cartilage from non-weight bearing areas. So cells with chondrogenic potential, which are easier to acquire, become an attractive alternative, including BMSCs and MSCs from other tissues, and progenitor cells with chondrogenic potentials isolated from perichondrium and cartilage. Although all of these cells have been demonstrated to differentiate into chondrocytes in vitro, no direct comparison of chondrogenic capacity of different cell preparations is available, mainly due to lack of quantitative measurement of chondrogenic process, as well as the variation of donors, ex vivo culture, and differentiation conditions. One recent paper demonstrated that, measured with gene expression profile, chondrogenic differentiations of MSCs from the same donor were comparable even when MSCs were isolated and cultured in different laboratories and with different protocols. The second question is how to translate in vitro chondrogenic capacity into in vivo cartilage repair function. In a rabbit model of cartilage defect, synovium-derived MSC and bone marrow stromal cells demonstrated superior chondrogenic capacity on histology, both in vivo and in vitro, when compared to adipose-derived stem cells and muscle-derived stem cells. The consistency of in vitro and in vivo results is encouraging; however, limited conclusions can be drawn due to lack of quantitative measurement.

To promote chondrogenic capacity, genetically modified MSCs are currently being investigated. A number of growth factors and transcriptional factors have been shown to influence chondrogenic differentiation of MSCs. Members of transforming growth factor-beta (TGF-β) superfamily, including TGF-β1, TGF-β3, bone morphogenetic protein 2 (BMP-2), BMP-4, BMP-7, and growth differentiation factor-5 (GDF-5), increase proliferation and cartilaginous extracellular matrix production, promote chondrogenic differentiation of MSCs, and/or decrease type I collagen gene expression; insulin-like growth factor 1 (IGF-1), fibroblast growth factor 2 (FGF-2), and FGF-18 have been shown to have similar effects. Overexpression of Sox9, alone, or in combination with Sox5 and Sox6, has been shown to enhance chondrogenesis. A phase I clinical trial using genetically modified chondrocytes expressing TGF-β1 has been carried out and completed; recently, it has been advanced to a phase II study in the U.S. and a phase IIb study in Korea. However, genetically modified chondrogenitor cells, including mesenchymal stem cells, have not been studied extensively in animal models or used in any clinical studies. Long-term survival and integration of MSCs have not been thoroughly evaluated in animal models. In a study of direct intra-articular injection of human bone marrow stromal cells mixed with hyaluronic acid into the knee joints of Hartley strain guinea pigs with spontaneous osteoarthritis, partial cartilage repair was observed after five weeks; migration and differentiation into chondrocytes was demonstrated using carboxyfluorescein diacetate succinimidyl ester (CFSE) labelled cells. In this study, expression of type II collagen was observed not only in the injected MSCs, but also in the residual layer of chondrocytes as well. This observation raised the question whether the cartilage was regenerated from the injected MSCs or the paracrine effect of MSCs on endogenous chondrogenic cells.

Pathogenesis of osteoarthritis is far more complicated than cartilage defect; it involves decreased matrix synthesis, increased proteolytic degradation of matrix, and increased chondrocyte apoptosis. Clinically, besides articular cartilage loss, it has features of osteophytes, bone sclerosis, and subchondral cysts. Therefore, preclinical studies of MSC transplantation into osteoarthritis animal models, rather than into animals with chondral defects, are essential to help us predict the results of clinical studies in osteoarthritis. The first study in a large animal model of osteoarthritis was conducted in 2003 in caprine model. Twenty weeks after MSC injection, degeneration of the articular cartilage, osteoarthritic remodelling, and subchondral sclerosis were reduced in the injected joints compared to control joints. The immunomodulatory property of MSCs Understanding the immunomodulatory function of MSCs is pivotal in the application of allogenic MSCs and developing “off-the-shelf” MSC products. However, it is controversial whether these cells can escape immune surveillance of the recipient and achieve long-term engraftment. It has been well-known for a decade that MSCs can modulate the activity of immune cells and secrete cytokines and chemokines. In vitro studies show that cultured MSCs can suppress T cell proliferation, inhibit maturation of monocytes into dendritic cells and its antigen presentation function, and inhibit respiratory burst in neutrophils. MSCs have very limited expression of MHC class II molecules in cell cultures, and do not express co-stimulatory molecules, including CD40, CD80, or CD86. The immunosuppressive function is likely to be conducted via soluble factors secreted by MSCs, including transforming growth factor-beta (TGF-β).
hepatocyte growth factor (HGF), nitric oxide (NO), and indolamine dioxygenase (IDO) \(^21\); and cell–cell contact also seems involved\(^22\). In mixed lymphocyte reaction experiment, an inhibitory effect on effector T cell proliferation was observed when T cells were co-cultured with allogenic or even xenogenic MSCs\(^23\). In hematopoietic stem cell transplant (HSCT) recipients who received MSC infusions for treatment of acute GVHD, it was found that immune unresponsiveness was restricted to MSCs and HSCT recipients maintained an allogeneic response to allostimuli from MSC donors\(^24\).

Although these evidences demonstrate that MSCs have immune privilege, recent studies indicate that immune function of MSCs is more complicated than previously described. At low level of interferon-gamma (IFN-γ), MSCs was found to express MHC class II molecules and have antigen presenting property\(^25\); at high level of IFN-γ, MHC class II molecules are down regulated and the expression of programmed cell death 1 ligand (PD-L1), an inhibitory co-stimulatory molecule, is observed\(^22,26\). MSCs express toll-like receptors (TLRs), including TLR-3 and TLR-4, and stimulation of TLR-3 leads to the expression of immunosuppressive factors in MSCs while stimulation of TLR-4 induces pro-inflammatory mediators\(^27\). This finding indicated that the cellular environment of the recipient will affect the balance between pro-inflammatory and anti-inflammatory properties of MSC, and may further influence its engraftment and/or differentiation capacity.

Whether the immunomodulatory function is unique to MSCs remains an intriguing question. In a mixed lymphocyte reaction (MLR) assay, when cocultured with TCRβ+ CD44- lymphocyte reaction (MLR) assay, an intriguing question. In a mixed function is unique to MSCs, and may further influence its and anti-inflammatory properties of recipient will affect the expression of immuno suppressive molecule, is observed\(^22,26\). MSCs express toll-like receptors (TLRs), including TLR-3 and TLR-4, and stimulation of TLR-3 leads to the expression of immunosuppressive factors in MSCs while stimulation of TLR-4 induces pro-inflammatory mediators\(^27\). This finding indicated that the cellular environment of the recipient will affect the balance between pro-inflammatory and anti-inflammatory properties of MSC, and may further influence its engraftment and/or differentiation capacity.

Clinical trials or case series of MSC in arthritis

The first study of MSC treatment in OA was published in 2002. Twelve patients received transplantation of culture expended, autologous BMSC embedded in collagen gel, into the articular cartilage defect in the medial femoral condyle. Compared with twelve control patients who received cell-free products, at 46 weeks, the study group demonstrated a better arthroscopic and histological grading score, without a significant clinical improvement\(^29\). A follow-up case report from the same authors reported MRI and histology aspects of transplanted tissue in patients who had significant improvement of clinical symptoms. The authors found that defects were repaired with fibrocartilage, and MRI of the defect area showed decreased intensity with irregularity compared to normal cartilage\(^21,22\). Safety data of mesenchymal stem cell transplantation is scarce; in one study of 41 patients who received autologous BMSC transplantation, no tumours or infections were observed between 5 to 137 months of follow-up, with a mean of 75 months\(^31\). Unfortunately, outcome measurements of osteoarthritis were not commented on in the report. Currently, there are 16 clinical trials registered at ClinicalTrial.gov applying MSC in the treatment of osteoarthritis or chondral defect by intra-articular injection (Table 1), but no peer-reviewed literature is available as of now.

The characteristics of cellular therapy and its delivery procedure require special considerations in clinical trials when compared to conventional drugs. Potential risks of cellular therapy include differentiation into undesired cell types, ectopic tissue formation, transformation into tumour, potential immune response in allogenic cell transplant, and transplanted cells may cause unpredicted adverse events. In 2011, U. S. Food and Drug Administration issued a document of preparation of investigational device exemption applications (IDEs) and investigational new drug applications (INDs) for Products Intended to Repair or Replace Knee Cartilage, providing a broad outline of major features to consider in designing a clinical trial.

Conclusion

Chondrogenic differentiation property and immunomodulatory function of MSCs can be potentially used to treat arthritis, including osteoarthritis and inflammatory arthritis. A better understanding of the biology of MSCs at cellular level and through preclinical animal models will facilitate application of “off-the-shelf” MSCs products in a real clinical setting. Numerous issues remain in utilising the regenerative and immunomodulatory properties of mesenchymal stem cells in treating human diseases. For its application in osteoarthritis, quantitative measurement of in vitro chondrogenic capacity of MSCs and its correlation with in vivo cartilage repair is one of the essential steps. Long-term survival and engraftment have not been demonstrated; various delivery methods of mesenchymal stem cells in joint diseases. OA Musculoskeletal Medicine 2013 Dec 01;1(3):26.
methods have not been compared. Understanding the mechanism of immunomodulatory function of MSCs is pivotal in the application of allogenic MSCs in transplant and its application in treating inflammatory arthritis. Genetic modification that allows MSCs to express chondrogenic factors and anti-inflammatory molecules in a safe way will enhance this process. Risk of cellular therapy will require special consideration when designing a clinical trial. In the long run, safety, effectiveness, and cost will determine its utility in treating arthritis.

Acknowledgement

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Abbreviations list

ACI, autologous chondrocytes implantation; ADSC, adipose-derived stem cells; BM, bone marrow; BMP-2, bone morphogenic protein 2; CFSE, carboxyfluorescein diacetate succinimidyl ester; FB, fibroblast; FGF-2, fibroblast growth factor 2; GDF-5, growth differentiation factor-5; GVHD, graft-versus-host-disease; HGF, hepatocyte growth factor; HSCT, hematopoietic stem cell transplant; HUVEC, human umbilical vein endothelial cells; IDEs, investigational device exemption applications; IDO, indolamine 2,3-dioxygenase; IFN-γ, interferon-gamma; IGF-1, insulin-like growth factor 1; INDs, investigational new drug applications; MLR, mixed lymphocyte reaction; MSCs, mesenchymalstem cells; NO, nitric oxide; PD-L1, programmed cell death 1 ligand; TGF-β, transforming growth factor-beta; TLRs, toll-like receptors.

References


