Adult Bone Marrow Stem Cells in Cartilage Therapy

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ABSTRACT

Cartilage defect rarely heals spontaneously since cartilage tissue is poorly vascularized and the lesion usually does not penetrate to subchondral bone, and hence it does not have access to progenitor cells of bone marrow. Severe cartilage damage may lead to osteoarthritis (OA). Current surgical and non-surgical therapeutic interventions in OA are limited to symptom relief and/or repair of focal lesion, and later a total knee replacement is still necessary. Cell therapy with chondrocyte implantation requires healthy cartilage for donor of the cells. Adult mesenchymal stem cells (MSCs) have the ability to differentiate into chondrogenic lineage. They can readily be isolated from bone marrow as well as many other adult tissues and have an extensive proliferation capacity. Therefore, MSCs may offer a great potential to be developed as an alternative for cell-based articular cartilage therapy.

Key words: mesenchymal stem cell, cartilage defect.

INTRODUCTION

Articular cartilage defect, especially partial thickness defect, is difficult to heal spontaneously since it does not penetrate to subchondral bone, and hence it does not have access to progenitor cells of bone marrow.¹ Cartilage defects rarely heal since they are poorly vascularized and also lack of local progenitor cells.² Severe cartilage damage due to degeneration or excessive use may lead to osteoarthritis (OA). Various therapeutic interventions, surgical or non-surgical (pharmacological), can be performed to relieve the symptoms.³,⁴ However, none of the current OA treatment show satisfactory outcomes, and these approaches will be ended to a total knee replacement. In 1994, Brittberg reported cell therapy for cartilage lesion using chondrocytes (autologous chondrocyte implantation (ACI))⁵ but the outcome is also not satisfying since the regenerated cartilage often consists of fibrous
tissues and this technique still sacrifices healthy cartilage as donor of chondrocytes. Therefore, it is a challenge for researchers to develop the most ideal therapeutic approach for cartilage lesion.

Adult mesenchymal stem cells (MSCs) can be isolated from a variety of adult tissues including bone marrow, synovium, periosteum, skeletal muscle, adipose tissue, trabecular bone and umbilical cord. They have been shown to have the ability of multilineage differentiation, including chondrogenic lineage, and have an extensive proliferation potential. MSCs can be readily expanded without losing their multilineage differentiation capacity. These characters make MSC a potential cell source for repair of cartilage injury.\(^6\)

**CARTILAGES**

Cartilages can be classified into elastic, fibrocartilage, fibro-elastic, and hyaline cartilage. Synovial joint surface is covered with hyaline cartilage (articular cartilage). The hyaline cartilage provides a low-friction gliding stress. Hyaline articular cartilage is aneural, avascular and alymphatic tissue. Chondrocytes are responsible for synthesizing and maintaining the matrix and form 1-5% volume of articular cartilage.\(^7\) Cartilage mainly consists of collagen type II fibers and much less collagen type IX, X, and XI.\(^8\)

**ARTICULAR CARTILAGE DESTRUCTION**

Generally, articular cartilage defect results from 1 of these following mechanisms: 1) Direct trauma; 2) Chronic degeneration (mechanical overloaded); and 3) Subchondral bone abnormality (avascular necrosis, osteochondritis dissecans).\(^9\) Degenerative joint destruction may be caused by imbalance of anabolic and catabolic metabolisms, which in turn will lead to catabolic destruction of articular cartilage. The cartilage destruction affects mainly the articular surface, which finally needs a total knee replacement.\(^8\)

Cartilage lesions are graded according to the original classification proposed by Outerbridge or the more recent International Cartilage Repair Society (ICRS). The ICRS classification is as follows:\(^9\)
- **Grade 0**: Intact cartilage
- **Grade I**: Superficial (soft indentation or superficial fissures and cracks)
- **Grade II**: Lesion less than half the thickness of articular cartilage
- **Grade III**: Lesion greater than half the thickness of articular cartilage
- **Grade IV**: Lesion extending to subchondral bone

Cartilage damage may also be divided into partial thickness defect and full thickness defect. Partial thickness defect of cartilage resemble clefts and fissures observed in the early osteoarthritis (OA) (Figure 1A). This kind of cartilage defects do not heal spontaneously since they do not have access to the progenitor cells of bone marrow in subchondral zone, although there are certainly other mechanisms involved that need to be elucidated. Full thickness defects pass through calcified cartilage zone and reach subchondral bone, thereby obtaining access to the bone marrow cells including mesenchymal stem cells (Figure 1B).\(^1\) Therefore, full thickness cartilage defect may repair spontaneously depends on the circumstances such as age, defect size and location. Small cartilage defects can heal spontaneously with the synthesis of hyaline cartilage, whereas fibrous tissue or fibrocartilage production, which is biochemically and biomechanically different from normal hyaline cartilage, will repair the large defects. As the result, degeneration subsequently occurs, which in some cases can progress into osteoarthritis.\(^6\)

![Figure 1. Illustration of a partial thickness focal defect of articular cartilage (A) and a full thickness defect that penetrates the subchondral bone (adapted from Redman et al., 2005).]
Osteoarthritis (OA) is a consequence of mechanical and biological events, including oxidative stresses and aging that destabilize articular cartilage homeostasis. Osteoarthritis occurs mostly (over 70%) among people over 65 years, and the prevalence of this disease may increase in the future with the increase of aged population. The etiology of OA remains to be elucidated but multiple factors such as obesity, age, history of joint trauma and joint dysplasia are suggested to be involved.

TREATMENT OPTIONS OF ARTICULAR CARTILAGE DEFECTS

Cartilage defect could be treated by conservative treatment or surgical procedure. Non-steroidal anti-inflammatory drugs (NSAIDs) are often used in conservative management to relieve pain. Food supplements containing chondroitin sulphate, glucosamine, and hyaluronic acid could also relieve pain, although their long term effect is not known. Physical therapy, rehabilitation, and the use of brace may provide short-term pain relief. Currently, surgical procedures available for articular cartilage injury may be categorized as follows: • Palliative (debridement arthroscopy) • Intrinsic repair enhancement/marrow stimulation (microfracture, abrasion arthroplasty, drilling) • Cell-based repair (using chondrocytes, mesenchymal stem cells) • Scaffold-based repair (synthetic osteoarticular reconstruction) • Cell- and scaffold-based repair • Whole-tissue transplantation (mosaicplasty, osteochondral allograft)

Stimulation of intrinsic repair by microfracture and drilling of the subchondral bone facilitates the formation of fibrocartilage. However, these fibrous or fibrocartilage tissues are often followed by degeneration in the repaired tissue. Autologous chondrocyte implantation (ACI) for treatment of cartilage was first reported by Brittberg et al. in 1994 and is indicated for cartilage defect of 2-10 cm². Although chondrocytes are considered as the appropriate cells for regenerating cartilage tissue, culture expansion can cause dedifferentiation of these cells. Limited sources and donor site morbidity for harvesting cartilage tissue are also major concern and limitation to the application of ACI. Osteoarthritis patients are recently excluded from receiving chondrocytes transplantation since they are considered of being lack of healthy chondrocytes. Therefore, mesenchymal stem cells might be potential as an alternative source for cells with chondrogenic differentiation capacity. Stem cells have two distinguished characteristics, i.e. they renew themselves without differentiating, and under certain physiological or experimental conditions, they can be induced to become cells producing cartilage extracellular matrix.

Yan et al. (2007) reported a study in rabbit comparing the qualities of repaired tissue in full-thickness cartilage defects after implantation of four types of cells, i.e. chondrocytes, MSCs, fibroblasts, and human umbilical chord blood (hUCB), seeded in a scaffold. A histologic evaluation was performed up to 12 weeks after transplantation of each type of cells. They concluded that full-thickness defects treated with chondrocyte or MSCs transplantation were repaired with hyaline-like cartilage tissue. The quality of repaired tissue after MSCs or chondrocytes implantation was significantly better than that attained with fibroblast or hUCB. Whereas repaired tissue treated with MSCs showed superior histological characteristics to that treated with chondrocyte implantation. Therefore, MSCs may be the most suitable source of cells for cartilage repair.

MESENCHYMAL STEM CELLS FOR ARTICULAR CARTILAGE REGENERATION

Three types of stem cell have been identified: embryonic stem cells, stem cells derived from umbilical cord blood, and adult or somatic stem cells. Adult stem cells may be derived from bone marrow, adipose tissue, and muscle. Those somatic stem cells can be induced to differentiate into different mesenchymal lineages such as bone, cartilage, fat, ligament, tendon, and other connective tissues. To clarify the terminology of mesenchymal stem cells (MSCs) the International Society for Cellular Therapy has proposed minimal criteria to define human MSCs: • The cells must be plastic-adherent in standard culture conditions using tissue culture flasks • ≥ 95% of the cell population must express CD105, CD73 and CD90. These cells must lack expression (≤ 2% positive) of CD45,
CD34, CD14 or CD11b, CD79α or CD19 and HLA class II

- The cells must be able to differentiate to osteoblasts, adipocytes and chondrocytes under standard in vitro differentiating conditions.

Mesenchymal stem cells isolated from various adult mesenchymal tissues has extensive proliferation potential and are easily expanded without loosing their multilineage differentiation potential within several passages. Bone marrow is one of MSCs sources that is widely studied. However, there are also increasing reports that MSCs can be isolated from other mesenchymal tissues, such as adipose tissue, synovium membrane, synovial fluid, and muscle. An in vitro study by Sakaguchi et al. (2005) compared the properties of MSCs isolated from bone marrow, synovium, periosteum, skeletal muscle, and adipose tissue. Among mesenchymal tissue-derived MSCs examined, synovium-derived MSCs have the greatest chondrogenic potential. Although many studies showed that bone marrow-derived MSCs have great expansion ability, this study demonstrated that expansion ability of synovium-derived MSCs was comparable with that of bone marrow MSCs.

ADVANTAGES AND DISADVANTAGES OF MESENCHYMAL STEM CELL THERAPY IN ARTICULAR CARTILAGE DEFECTS

Mesenchymal stem cells implantation in articular cartilage defects has some advantages over ACI. Mesenchymal stem cells can be isolated from various tissues without causing morbidity to healthy articular cartilage. They also can be easily expanded without loosing their chondrogenic potential at early passages. Mesenchymal stem cells isolated from a 2-mL bone marrow aspirate can be expanded 500-fold in about 3 weeks. These cells retain their pluripotency for at least further 6-10 passages in culture.

However, MSCs application in articular cartilage repair has also some disadvantages. Several studies reported that MSCs-derived chondrocytes expressed hypertrophy-related genes leading to cell death or calcification followed by vascularization when implanted subcutaneously or intramuscularly. Other studies showed that the thickness of regenerated cartilage produced by MSCs-chondrogenic differentiation was thinner than the original thickness and the tidemark was violated. Other disadvantage is a concern that adult human MSCs may be prone to malignant transformation. However, an in vitro study showed that human bone marrow-derived MSCs cultured for up to 44 weeks maintained a normal karyotype, without showing expression of telomere maintenance mechanisms. The bone marrow-derived MSCs, in agreement with previous studies on cultured MSCs, showed a progressive decline in their proliferative capacity resulting in the development of a senescence phase after variable culture periods (6-44 weeks). The susceptibility to malignant transformation found in murine bone marrow-derived MSCs by Miura et al., might be related to the species of origin of the cells and also connected to the origin of the tissue. Therefore, the biological properties of human bone marrow-derived MSCs after ex vivo expansion remain suitable for clinical use, although it is also strongly recommended to test the characteristics of MSCs for safety guarantee for the patient.

The age of the MSC-donor affects the quantity and quality of these cells. The MSC titers in elderly are low. It is shown in Caplan (2007) that MSC titers in 80-year-old subjects decreased three orders-of magnitude compared to newborn. It was also suggested that age-related changes in MSCs including also loss of differentiation potential and proliferation potential. Chondrogenic differentiation declined in "aged" (over 40 years old donors) MSC compared to "young" (7-18 years) and "adult" (19-40 years) MSCs, although this was not statistically significant. The in vitro aging changes the quality of MSCs. Bonab et al. (2006) has showed physiological, functional, and molecular changes of MSCs that occurred during long-term cultures, e.g. gradual decrease of proliferation potential, telomere shortening, and impairment of cell functions. To evaluate the long-term growth kinetics of MSC culture, this group measured cumulative population doublings (PDs) and found that MSC proliferation potential decreased faster after 120 days in vitro expansion. Therefore, MSCs should be considered for cell and gene therapy at the early stages of in vitro culture.
Concerning the application of MSC in osteoarthritis (OA), there are factors that should be considered. Researchers should ascertain whether chondrogenic capacity and longevity of MSCs obtained from OA patients differ functionally from those which were from healthy donors. The altered capacity of these MSCs from OA patients perhaps is related to their exposure to elevated levels of proinflammatory cytokines and/or antiinflammatory drugs. Another factor that may be associated with MSCs in OA is advanced age since OA is commonly found in elderly and several studies showed an age-dependent reduction of progenitor cells number. However, some studies have also demonstrated that sufficient numbers of MSCs with adequate chondrogenic capacity can be isolated from OA patients, irrespective of their age or the ethiology of the OA. Therefore, the potential use of autologous MSC therapy in OA is feasible and the future development should be well considered.

IMPLANTATION OF MESENCHYMAL STEM CELLS

Treatment of cartilage defect with cell therapy (MSCs or chondrocytes) requires more development studies to obtain a minimal-invasive implantation procedure. Currently, implantation of MSCs needs periosteal coverage. However, the procedure of harvesting the periostium and suturing it to the neighbouring cartilage is quite invasive. If periosteum patch is not used, then a scaffold is required to keep the cells at the cartilage lesion. Scaffolds are derived from animals, thereby the risk of disease transmission and immune reaction increases. Many biomaterials have been tested to be used as scaffold for cell therapy or tissue regeneration. Some characteristics of ideal scaffold have been suggested such as having similar characteristics to the native tissue, being a source of cells that could promote tissue regeneration, highly porous to permit cells penetration and tissue impregnation, permeable to allow nutrient delivery and gas exchange, biocompatible, and biodegradable once the functional tissue has been formed. Various substances have been used to develop scaffold such as poly-lactic-co-glycolic acid (PLGA) spheres, agarose, alginate, chitosan. There are also protein-based polymer scaffolds like fibrin, collagen and gelatin. Other substances used for scaffold include corals, TCP (tricalcium phosphate), ceramics, silk, and hydroxyapatite.

A study investigated the possibility of MSC that were transferred to a hyaluronan scaffold hours prior to the implantation for treating the osteochondral defect of rabbit knee. The scaffold with MSC was implanted in one knee while the empty scaffold was implanted in the contralateral knee as control. Although it was not statistically significant, the evaluation showed a tendency for a better quality of repair in the MSC treated knee. Thus, MSC in hyaluronan scaffold may be a promising treatment approach, but further studies are needed to optimise the scaffold and cells preparations before implantation and to increase hyaline cartilage synthesis following the MSC implantation.

The easy and less invasive implantation method might be intra-articular MSCs injection. However, there is a risk that the injected MSCs adhered to synovial tissues, which might increase the risk of synovial proliferation. In addition, if most of the cells adhered to the synovial tissues then less cells would adhere to the cartilage defects. An animal study of intra-articular injection of MSCs showed that MSC injection improved cartilage healing at 12 weeks as compared with control groups. The injected MSCs were labeled with fluorescence substance and could be traced in the neocartilage showing that these cells could “home” and adhere in the site of lesion to regenerate the cartilage. In other animal study, MSCs were suspended in hyaluronic acid and injected intra-articularly to the injured knee of a caprine model of osteoarthritis. They found that this local delivery of MSCs stimulates regeneration of meniscal tissue and retards the progressive destruction of OA.

In the development of minimal invasive technique of MSC delivery system, it is now investigated for the use of cells coupled with magnetic beads in association with an external magnetic force to direct the cells to the desired location. It has been shown that magnetically labeling does not alter function and differentiation capacity of MSCs. The magnetically labeled MSCs was injected into the knee joint using arthroscopic control and under the influence of external magnetic force. The knee was kept in that position under magnetic force for 4 hours. They found accumulation of magnetically labeled MSCs in the osteochondral defect.
(2004) showed that magnetically labeled MSCs injected intravenously could be directed to the region of interest by using external magnet.33 An in vitro study investigated the efficacy of transforming growth factor-β (TGF-β)-immobilized magnetic beads for chondrogenesis using MSC delivery system and an external magnet. They found that chondrogenesis was achieved from magnetically labeled MSCs in the presence of TGF-β-immobilized magnetic beads. They also demonstrated that the TGF-β-immobilized magnetic beads system could lower the concentration of TGF-β required for chondrogenesis of MSC-magnetic beads complexes.36

CONCLUSION
The use of transplanted MSCs from bone marrow is very potential for the treatment of degenerative diseases including osteoarthritis. MSC can be isolated from a large number of adult tissues, can easily be expanded in culture without loss of their multilineage differentiation capacity including chondrogenic differentiation potential. The capability of the MSCs in repair and regeneration of mesenchymal tissues has also been widely studied including cartilage defect regeneration. However, there are still unknown mechanisms of tissue repair using MSCs; for instance, it is not yet known whether the transplanted MSCs directly fill the lesion and regenerate the defect articular cartilage or they indirectly stimulate the secretion of bioactive factors such as cytokines and growth factors. Clinical studies on MSC application for cartilage regeneration are also still limited. Therefore, although many studies have reported promising results on the potential of MSC in regenerative medicine, more preclinical and clinical studies are necessary to establish the appropriate conditions and techniques of MSC application in human.

REFERENCES