Review Article

Mesenchymal stem cell related therapies for cartilage lesions and osteoarthritis

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Received July 28, 2019; Accepted August 9, 2019; Epub October 15, 2019; Published October 30, 2019

Abstract: Osteoarthritis (OA) is a common degenerative joint disease characterized by cartilage degradation, synovitis, subchondral bone sclerosis and osteophyte formation. Current therapeutic approaches for OA are not curative and only temporarily alleviate symptoms. In recent years, pre-clinical experiments and clinical trials have demonstrated that mesenchymal stem cell (MSC) related therapy is a promising option for the treatment of cartilage lesions and OA. MSCs isolated from bone marrow (BMSCs) have been widely used in animal models and clinic practice to demonstrate their chondrogenic potential, however the incidence of BMSC donors is low. Adipose derived mesenchymal stem cells (AMSCs) are a more easily accessible source of stem cells for OA treatment. MSC related therapies for cartilage lesions and OA include tissue engineering of MSC transplantation, scaffold-free injection of stem cells and cell-free injection of exosomes into the injured joints. Although a great deal of effort is required at the basic and clinical research fronts, the promise is that improved cell-based therapies will ultimately lead to the repair of damaged or diseased joints, and MSC exosome therapy for OA could be a safer, cheaper and a more effective treatment modality. MSC related therapy is predicted to become a regular and routine regenerative medicine for OA treatment in future clinical practice.

Keywords: Mesenchymal stem cells, cartilage, osteoarthritis, stem cell tissue engineering, intra-articular injection, exosomes

Introduction

Osteoarthritis (OA) is a common degenerative joint disease characterized by cartilage degradation, synovitis, subchondral bone sclerosis and osteophyte formation [1]. The World Health Organization (WHO) estimate that 10% of men and 18% of women aged 60 years and older have symptoms of OA, 80% of which suffer from movement activity disorders [2]. It is predicted that the population aged over 60 years will expand significantly by the year 2050, reaching well over 2 billion globally [3]. This growing geriatric population will lead to an increased global incidence of OA. Although OA is principally associated with ageing, its etiology is complex and multifactorial, including biological and biomechanical factors [4]. Pathogenic factors include obesity, joint trauma, joint infection, previous rheumatoid arthritis, muscle weakness, metabolic disorders, orthopaedic disorders, disorders of bone turnover and genetics. These factors act alone or in synergy to initiate a cascade of pathophysiological reactions within the joint [5].

OA patients suffer from persistent pain, stiffness and disability. Conventional treatment includes exercise, physical therapy, life style changes and pain medications. For early stage OA, clinical therapy includes nonsteroidal anti-inflammatory drugs (NSAIDs), hyaluronic acid (HA) injections, simple analgesics and corticosteroid injections. These approaches temporarily alleviate the symptoms rather than treat the pathogenesis or reverse the OA process. Joint replacement surgery including osteotomy, arthroscopic surgery and arthroplasty is performed in severe cases [6, 7]. Although these methods are effective, approximately 30% of patients remain unsatisfied. Joint replacement has a limited life span, often requiring complex revisions, and is unsuitable for the ever-growing population of younger patients with early OA.
who have an active life style [8]. To-date, there is no radical curative treatment and novel therapies for OA are urgently required. In recent years, mesenchymal stem cell (MSC) related therapies during the treatment of cartilage lesions and OA have demonstrated promise.

MSCs, a precursor of connective tissue cells, can be isolated from many adult organs. MSCs are multipotent progenitor cells that possess self-renewal capability and can differentiate into multiple lineages including adipocytes, osteoblasts and chondrocytes [9]. Synovial-derived stem cells display greater chondrification but the evidence of their potential is limited to preclinical studies [10]. MSCs isolated from bone marrow (BMSCs) have been widely used in animal models and some clinical cases to investigate their chondrogenic potential for OA treatment. Since a large number of adipose derived mesenchymal stem cells (AMSCs) are accessible and the incidence of BMSC donors is low, AMSCs represent a more readily available source of stem cells. Compared with BMSCs, AMSCs are more easily cultured and grow more rapidly [11]. The main benefits of AMSCs are their ease of isolation, manipulability, potential for proliferation and differentiation, and their telomeres are less affected by age than BMSCs [12]. Further studies have shown that MSCs also possess powerful immunoregulatory and anti-inflammatory activity that is largely mediated by paracrine factors and contributes to tissue repair. The combination of these features makes MSCs attractive seed cells in the treatment of OA.

Articular cartilage degeneration and subchondral bone deterioration in OA

OA is the clinical syndrome manifested by joint pain and the loss of joint form and function caused by the articular cartilage degeneration and subchondral bone deterioration [13-16]. Articular cartilage is a tenacious and tensile load-bearing connective tissue that covers the surface of joints. The synovitis in the early and late stages of OA is associated with alterations in cartilage. Catabolic and proinflammatory factors are produced by the inflamed synovium and alter the balance of cartilage matrix anabolism and catabolism, leading to the production of redundant proteolytic enzymes, giving rise to cartilage breakdown [17, 18]. The changes in cartilage and subchondral bone cause further synovitis, resulting in a vicious cycle. Progressive synovitis aggravates clinical symptoms and stimulates further joint degeneration [19].

Chondrocytes are the main cell type in cartilage tissue. Articular cartilage does not contain blood vessels, nervous tissue, or lymphatic vessels [20]. Chondrocytes are spatially isolated by a large quantity of extracellular matrix (ECM) and are responsible for the synthesis and maintenance of the ECM [21, 22]. The macromolecular framework of ECM developed by chondrocytes includes collagens (type II collagen), proteoglycans (mainly aggrecan) and bioactive factors. The supply of chondrocyte nutrients and the disposal of metabolic waste occur through the ECM [23-25]. The activity of chondrocytes, including their response to stimuli, controls the synthesis of new ECM components, a process influenced by ageing [26-28]. The ability of cartilage repair declines with increasing age, manifested by a decline in chondrocyte number leading to age-associated changes in ECM composition [29, 30]. These changes result in degeneration of the cartilage and limit its ability of repair [31-33]. In recent years, accumulating evidence suggests that OA should be considered a disease of the whole joint [34]. Articular cartilage and subchondral bone form an integral unit that undergoes uncontrolled catabolic and anabolic remodeling during OA development [35, 36].

Tissue engineering of MSC transplantation for cartilage lesions and OA

Cartilage engineered through autologous chondrocyte implantation (ACI) was first reported by Brittberg and colleagues in 1994 and surgical ACI has since been used to repair chondral lesions for more than 20 years [37, 38]. ACI requires cartilage to be taken either from an intact portion of the damaged joint or from other joints of the patient. The cartilage is then expanded in culture and transplanted into the defective area of the joint [39]. Initial ACI clinical trials proved promising. However, treatment disadvantages included additional donor site morbidity of healthy articular cartilage, poor functionality and quality of the synthesized ECM, and limited technical efficacy in patients older than 40 years [40-42]. In recent years, MSCs have emerged as an alternative cell type to circumvent the drawbacks of ACI.
MSCs can differentiate into chondrocytes in response to several chondrogenic signals including TGF-β superfamily activators and in combination with scaffolds [43, 44]. MSCs have been widely used in tissue engineering during cell transplantation in animal models of cartilage lesions and OA treatment (Table 1). In large animal models of cartilage lesions or OA, cartilage regeneration resulted from tissue engineering using specific biomaterial and MSCs, indicating its promise for clinical application [45, 46]. In small animal models, MSCs combined with biomaterials also facilitated hyaline-like cartilage occurrence. Transplantation of MSCs encapsulated in self-assembled peptide hydrogels showed chondroprotection and reduced subchondral bone mineral density in rat OA models [47].

Recent clinical trials have indicated the potential to improve patient’s symptoms and regenerate articular cartilage using surgical implantation of MSCs into focal articular cartilage defects (Table 1). In a cohort study, Nejadnik and coworkers reported 72 matched (lesion site and age) patients who underwent cartilage repair using chondrocytes or BMSCs. Compared with chondrocytes, BMSC transplantation was equally effective to relieve pain and improve the patient’s quality of life, independently of the patient’s age. Furthermore, BMSC-based treatment was less invasive and reduced both morbidity and operative costs [48]. In clinical practice, using various culture methods, BMSCs that retain their capacity for chondrogenic differentiation have been successfully used to treat cartilage defects. Sporadic case reports have demonstrated that treatment using autologous BMSCs with specific biomaterials leads to significant clinical and radiological improvements in OA patients following surgical transplantation [49-52]. The first large-scale clinical trial was performed in 24 knees of 24 OA patients who underwent a high tibia osteotomy. The autologous BMSCs embedded in collagen gel were implanted into the articular cartilage defect in the medial femoral condyle of 12 patients and covered with autologous periosteum at the time of surgery. The other 12 subjects served as cell-free controls. Both control and BMSC implanted groups functionally improved, but hyaline cartilage was observed only after addition of BMSCs [53]. The same research team further reported the safety and effectiveness of autologous BMSC transplantation for long term cartilage repair. Cell-gel composite was transplanted into 45 joints of 41 patients from January 1998 to November 2008 and followed up to 11 years and 5 months. Neither tumours nor infections were observed between 5 and 137 (mean 75) months of follow-up surveys suggesting autologous BMSC transplantation is safe and applicable for OA treatment [54]. Koh and colleagues transplanted autologous AMSCs to full-thick articular lesions in 37 knees of 35 OA patients and retrospectively evaluated the knees using second-look arthroscopic surgery. The mean International Knee Documentation Committee (IKDC) and Tegner activity scale scores were significantly improved using AMSC implantation. The ICRS (International Cartilage Repair Society) overall repair grades at second-look arthroscopic surgery improved to different degrees and 94% patients manifested good to excellent satisfaction. High body mass index (BMI) and large lesion size were important factors affecting the outcome. These studies indicated that during the early stages of application, AMSC transplantation can improve cartilage repair in OA [55].

In summary, MSCs with scaffold were implanted into fixed and damaged sites and could repair defects in tissue engineering for cartilage lesions and OA treatment. Few studies have transplanted MSCs without scaffolds to the specific defects covered with periosteum according to the local adherent technique. Preclinical and clinical studies have verified that tissue engineering can successfully repair cartilage lesions and the damage of cartilage and subchondral bone in OA. This therapy is comparatively suitable for the treatment of relatively large defects and severe OA. Larger cohorts of OA patients are required before MSC-based tissue engineering can be used in large-scale clinical applications.

**Scaffold-free MSC injections for cartilage lesions and OA**

Recently, animal experiments and clinical trials have highlighted the potential of percutaneous intra-articular MSC injections in treating articular cartilage degeneration in OA (Table 2). In animal models, OA is primarily induced by surgery, such as anterior cruciate ligament transection (ACLT) or combined medial meniscus transection (MMT) [31]. In an adult minipig
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</thead>
<tbody>
<tr>
<td>Autologous BMSC</td>
<td>Chitosan with TGF-β3</td>
<td>Sheep</td>
<td>Partial-thickness lesions in the internal groove of the patellae</td>
<td>Implantation of BMSCs mixed with a chitosan scaffold and TGF-β3 resulted in hyaline-like cartilage filling the defects.</td>
<td>[45]</td>
</tr>
<tr>
<td>Autologous BMSCs</td>
<td>β-tricalcium phosphate</td>
<td>Sheep</td>
<td>Osteochondral defects in the media femoral condyle</td>
<td>In the group of BMSCs with scaffold, hyaline-like tissue covered the defective surface. In scaffolds without cells, the defect was clearly visible.</td>
<td>[46]</td>
</tr>
<tr>
<td>Allogeneic BMSCs</td>
<td>Self-assembled peptide hydrogels</td>
<td>Rats</td>
<td>OA model by ACLT and medial collateral ligaments transected</td>
<td>The transplantation group showed chondroprotection and reduced subchondral bone mineral density compared to the OA group.</td>
<td>[47]</td>
</tr>
<tr>
<td>Autologous BMSCs</td>
<td>N/A</td>
<td>72 patients</td>
<td>Knee articular cartilage defects</td>
<td>Compared with chondrocytes, BMSC-based treatment groups appeared less invasive and reduced both morbidity and operative costs.</td>
<td>[48]</td>
</tr>
<tr>
<td>Autologous BMSCs</td>
<td>Collagen gel</td>
<td>2 patients</td>
<td>Full-thickness articular cartilage defects in patellae</td>
<td>Defects were repaired with fibrocartilage. Pain and walking ability of the patients improved significantly.</td>
<td>[49]</td>
</tr>
<tr>
<td>Autologous BMSCs</td>
<td>Collagen gel</td>
<td>1 patient</td>
<td>A full-thickness cartilage defect in the medial femoral condyle</td>
<td>Arthroscopy revealed the defect was filled with a hyaline-like type of cartilage tissue. Clinical symptoms improved significantly. The patient could regain his previous activity levels and experienced less pain or other complications.</td>
<td>[50]</td>
</tr>
<tr>
<td>Autologous BMSCs</td>
<td>Collagen gel</td>
<td>3 patients</td>
<td>Full-thickness articular cartilage defects of the patellofemoral joints</td>
<td>The patients’ clinical symptoms improved and the improvements have been maintained over the follow-up periods (17-27 months). The defects were repaired with the regeneration tissue.</td>
<td>[51]</td>
</tr>
<tr>
<td>Autologous BMSCs</td>
<td>Platelet-rich fibrin glue</td>
<td>5 patients</td>
<td>Full-thickness cartilage defects of femoral condyles</td>
<td>All patients’ symptoms improved over the follow-up period of 12 months. Average Lysholm and RHSSK scores showed significant improvement.</td>
<td>[52]</td>
</tr>
<tr>
<td>Autologous BMSCs</td>
<td>Collagen gel</td>
<td>24 patients</td>
<td>Articular cartilage defects in the medial femoral condyle</td>
<td>Both implanted groups underwent a high tibia osteotomy improved functionally but hyaline cartilage was observed only after addition of BMSCs.</td>
<td>[53]</td>
</tr>
<tr>
<td>Autologous BMSCs</td>
<td>Collagen gel</td>
<td>41 patients</td>
<td>Knee, hip, elbow, or ankle OA</td>
<td>Neither tumours nor infections were observed between 5 and 137 (mean 75) months of follow-up surveys.</td>
<td>[54]</td>
</tr>
<tr>
<td>Autologous AMSCs</td>
<td>N/A</td>
<td>35 patients</td>
<td>Full-thickness articular cartilage lesion in OA knees</td>
<td>The ICRS repair grades at second-look arthroscopic surgery were improved at different degrees in patients filled with AMSC suspensions.</td>
<td>[55]</td>
</tr>
</tbody>
</table>
### Table 2. Preclinical and clinical studies of scaffold-free MSC injection for OA treatment

<table>
<thead>
<tr>
<th>Cell type</th>
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</thead>
<tbody>
<tr>
<td>Autologous BMSCs</td>
<td>HA</td>
<td>Minipigs</td>
<td>A cartilage defect in the medial femoral condyle</td>
<td>In the MSCs with HA treated groups, the tissue of the filling of the defects was hyaline-like, with good integration, thickness and surface regularity. Cells resembled well-differentiated chondrocytes and were surrounded by a metachromatic matrix.</td>
<td>[56]</td>
</tr>
<tr>
<td>Autologous BMSCs</td>
<td>Basal media</td>
<td>Sheep</td>
<td>OA model by ACLT with MMT</td>
<td>Knee OA treated with BMSCs was observed in macroscopical and histological retardation of cartilage destruction.</td>
<td>[57]</td>
</tr>
<tr>
<td>Autologous AMSC or BMSCs</td>
<td>Culture media</td>
<td>Sheep</td>
<td>OA model by ACLT with MMT</td>
<td>The tested knee injected intra-articularly AMSCs or BMSCs showed regenerated cartilage. The injected cells had been filled in areas of cartilage damage and the regeneration cartilage produced extracellular matrix.</td>
<td>[58]</td>
</tr>
<tr>
<td>Autologous BMSCs</td>
<td>HA</td>
<td>Goats</td>
<td>OA model by ACLT with MMT</td>
<td>Autologous BMSCs injection resulted in regeneration of the medial meniscus and a reduction in osteophyte remodeling, subchondral sclerosis and articular cartilage degradation.</td>
<td>[59]</td>
</tr>
<tr>
<td>Allogeneic AMSCs</td>
<td>HA</td>
<td>Rabbits</td>
<td>OA model by ACLT</td>
<td>The cartilage defect and surface wear of the AMSC injection group was lower and the histological scoring and cartilage components were significantly better compared with the control group injected with HA alone.</td>
<td>[63]</td>
</tr>
<tr>
<td>Allogeneic AMSCs</td>
<td>PBS</td>
<td>Rats</td>
<td>OA model by ACLT</td>
<td>Intra-articular injection of allogeneic AMSCs in OA rats delayed joint degeneration. AMSCs also protected chondrocytes from the damage induced by inflammatory factors.</td>
<td>[64]</td>
</tr>
<tr>
<td>Allogeneic AMSCs</td>
<td>Mouse serum with mouse albumin</td>
<td>Mice</td>
<td>OA model by articular-injecction collagenase</td>
<td>Thickening of the synovial lining, formation of osteophytes were significantly inhibited, and the destruction of cartilage was inhibited in the groups treated with cell injection.</td>
<td>[65]</td>
</tr>
<tr>
<td>Xenogenic Equine UC-MSC</td>
<td>PBS</td>
<td>Rabbits</td>
<td>OA model by MMT</td>
<td>Cartilage fibrillation was lower in the early treatment group. OA synovium exhibited reduced expression of MMP13 in the early cell-treated group. In vitro, UC-MSC paracrine exerted anti-inflammatory and anti-catabolic effects on synovioocytes.</td>
<td>[66]</td>
</tr>
<tr>
<td>Human MSCs</td>
<td>PBS or HA</td>
<td>Guinea pigs</td>
<td>Spontaneous OA</td>
<td>Injection of human MSCs resulted in the regeneration of articular cartilage in the spontaneous OA animal models.</td>
<td>[67]</td>
</tr>
<tr>
<td>Human MSCs or rat MSCs</td>
<td>PBS</td>
<td>Rats</td>
<td>OA model by MMT</td>
<td>Human MSCs enhanced meniscal regeneration in a manner similar to rat MSCs, and human MSC injection increased the expression of rat type II collagen and inhibited OA progression.</td>
<td>[68]</td>
</tr>
<tr>
<td>Autologous BMSCs</td>
<td>PBS</td>
<td>A male patient</td>
<td>Keen OA</td>
<td>Patient knees had significant cartilage and meniscus growth, as well as increased range of motion and decreased pain scores.</td>
<td>[70]</td>
</tr>
<tr>
<td>Autologous BMSCs</td>
<td>Saline with serum albumin</td>
<td>4 patients</td>
<td>Keen OA</td>
<td>The number of stairs climbed and the pain on the VAS improved for all 4 patients. The improvement for physical examinations was mainly for crepitus. The improvement of the motion range was minor.</td>
<td>[71]</td>
</tr>
<tr>
<td>Autologous BMSCs</td>
<td>Physiological serum</td>
<td>6 female patients</td>
<td>Keen OA</td>
<td>Pain, functional status of the knee and walking distance were improved. MRI displayed an increase in cartilage thickness, extension of the repair tissue and a considerable decrease in the size of edematous subchondral patches.</td>
<td>[72]</td>
</tr>
<tr>
<td>Allogeneic BMSCs</td>
<td>HA</td>
<td>55 patients</td>
<td>A partial medial meniscectomy</td>
<td>Patients receiving BMSCs experienced significant reductions in pain and reduced OA progression. Subchondral sclerosis and osteophyte formation were also reduced. The results of MRI revealed regrowth of the meniscus and reduced OA progression.</td>
<td>[73]</td>
</tr>
<tr>
<td>Autologous AMSCs</td>
<td>HA with PRP</td>
<td>4 patients</td>
<td>Osteonecrosis in the right femoral head or knee OA</td>
<td>All patients injected with autologous AMSCs showed positive changes. Probable bone formation was clear in osteonecrosis patients, and cartilage regeneration was observed in the OA patients.</td>
<td>[74]</td>
</tr>
<tr>
<td>Autologous AMSCs</td>
<td>Saline</td>
<td>18 patients</td>
<td>Knee OA</td>
<td>Intra-articular injection with high-dose (1.0×10⁶) AMSCs into the OA knee improved function and pain of the knee joint without causing adverse events, and reduced cartilage defects by regeneration of hyaline-like articular cartilage.</td>
<td>[75]</td>
</tr>
</tbody>
</table>
model of cartilage lesions, BMSC injection enhanced cartilage healing both morphologically and histologically [56]. In OA sheep models, intra-articular injection of autologous BMSCs or AMSCs could slow the progression of OA and regenerate cartilage through the production of ECM [57, 58]. Autologous BMSC injection in a goat OA model resulted in the regeneration of the medial meniscus, a reduction in osteophyte remodeling, subchondral sclerosis and articular cartilage degradation [59]. In addition, other preclinical experiments in large OA animal models, including porcine or beagle dogs, demonstrated that intra-articular injection of autologous or allogeneic MSCs alone or synergistically with other factors ameliorated induced OA [60-62]. Likewise, MSCs improved the symptoms in small OA animal models, including rabbit, rat and mouse [63-65]. In addition, direct transplantation of xenogeneic MSCs or human MSCs into the knee joints of OA animal models could prevent cartilage degradation and promote meniscal regeneration [66-68].

A number of clinical trials assessing the benefits of BMSCs or AMSCs for OA treatment have been initiated [69]. In a patient with severe OA of the knee that ordinarily requires a total knee replacement, significant cartilage and meniscus growth on MRI, decreased modified visual analogue scale (VAS) pain scores, and increased range of motion were observed in response to six months of BMSC injection. The treatment course included the injection of cultured BMSCs suspended in phosphate buffered saline (PBS), with supplementary 10% platelet lysate (PL) and 10 ng dexamethasone injections for chondrogenic stimulation [70]. Other preliminary studies reported a reduction in pain and improvement in 4 of 6 patients with knee OA after injection autologous BMSCs [71, 72]. A long term treatment and follow-up study was reported by Vangsness and coworkers in phase I/II trials including 55 patients from 7 institutions receiving single injections of BMSCs. Cells were injected into the knee joint 7-10 days after operative meniscectomy and all patients were followed-up for 2 years. Those who received BMSCs experienced a significant reduction in pain and reduced OA progression. Subchondral sclerosis and osteophyte formation were also reduced compared to placebo controls. The results of MRI revealed regrowth of the meniscus and OA progression had been reduced [73]. AMSC injections have also been investigated for the treatment of OA. Injections of AMSCs in two patients with osteonecrosis in the right femoral head or in two patients with knee OA, led to significant positive changes as assessed by MRI. Probable bone formation was clear in the osteonecrosis patients, and cartilage regeneration was visible in OA patients [74]. The clinical and radiological improvements with AMSC injections were also directly related to the specific number of cells injected. An intra-articular injection of $1.0 \times 10^8$ AMSCs (high-dose group) into the OA knee significantly improved pain and function without causing adverse events, and reduced the size of cartilage defects through the regeneration of thick hyaline-like articular cartilage. Patients in the mid-dose group ($5.0 \times 10^7$ AMSCs) showed improvement in some clinical outcomes, but those in the low-dose group ($1.0 \times 10^7$ AMSCs) showed no improvement in the majority of outcome measures [75].

To summarize, intra-articular injection of MSCs into damaged joints is comparatively simple and easy method for OA treatment. Preclinical and clinical studies verify that MSCs can successfully inhibit the degeneration of cartilage and subchondral bone in OA. MSC therapy is suitable for use in mild or moderate OA patients, with comparable benefits. Further studies are now warranted to promote and improve MSC injections for their application to routine clinical treatment.

MSC functional mechanisms and potential therapeutics of MSC exosomes

The use of MSCs to repair cartilage tissue is based on their ability to act as chondroprogenitors to replace injured cartilage or as regenerative cells to stimulate cartilage repair by endogenous cells. Increasing evidence suggests that MSCs secrete a wide range of trophic factors that modulate the injured tissue environment to orchestrate subsequent regenerative processes including cell migration, proliferation, differentiation and matrix synthesis [76, 77]. MSCs reduce tissue damage, inhibit fibrous remodeling and apoptosis, stimulate stem cell proliferation, promote angiogenesis and decrease oxidative stress through regulating TGF-β, VEGF, ADAMTSs MMPs, TIMPs et al [78, 79]. MSCs not only contribute to tissue regeneration, but also have an efficacious immune regu-
MSC and OA

Figure 1. Schematic of MSC-based therapies for cartilage lesions and OA. The benefits of MSCs include their capacity to self-renew, differentiate, and to secrete growth factors and cytokines. The tissue engineering of MSCs seeded in hydrogel polymers induce cartilage regeneration and subchondral bone improvement; in very rare case, the cell transplantation is performed without the scaffold. The intra-articular injection of MSCs suspended in media play important roles in immunomodulation and reduced inflammation. Both of these therapies maintain cartilage and bone hemostasis and inhibit OA progression. The molecular mechanisms of MSC function involve the differential expression of anabolic and catabolic genes including collagen type II, MMP13, ADAMTS and VEGF, and secreted factors including IFN-γ, IL-10, TNF-α and IL-6. Upward red arrows indicate increased gene expression and downward red arrows indicate decreased gene expression.

Exosomes are extracellular vesicles with a diameter range of 30-150 nm that in essence are intraluminal vesicles (ILVs) formed by the inward budding of endosomal membranes during the maturation of multivesicular bodies (MVBs). Exosomes are secreted through the fusion of multivesicular endosomes with the cell membrane, while microvesicles (diameter range of 50-5000 nm) are secreted through forward budding of the plasma membrane [82, 83] (Figure 2A). A large number of cells secrete exosomes which can be found in most bodily fluids including blood, urine, cerebrospinal fluid, breast milk and saliva, and this process is conserved throughout evolution from bacteria to humans and plants [84, 85]. MSC exosomes are derived from adipose tissue, bone marrow, fetal tissues, the umbilical cord and embryo. Mass spectrometry and microarray analysis revealed that MSC exosomes carry a complex cargo of nucleic acids (mRNA and miRNA), proteins and lipids [86] (Figure 2B).

Recent studies have shown that MSC exosomes can promote the repair of heart, liver and skin tissue [87-89]. MSC exosomes have also been reported to mediate cartilage repair and regeneration in recent preclinical studies (Table 3). Zhang and colleagues first demonstrated the effects of human embryonic MSC (EMSC) exosomes on cartilage repair. In these studies, cartilage defects were induced on the trochlear grooves of distal femurs of 12 adult rats. After 12 weeks, the exosome-treated defects showed complete cartilage and subchondral bone recovery, and other characteristic features including hyaline cartilage with regular surface, complete adherence to the adjacent cartilage, and ECM deposition that closely resembled that of age-matched controls without operation. In contrast, the contra-
lateral PBS-treated defects exhibited only fibroid repair cartilage [90]. Using destabilization of medial meniscus (DMM) surgery, Wang and coworkers performed two animal section experiments including 32 mice, which followed intra-articular injection of either human EMSCs or their exosomes. The results of Osteoarthritis Research Society International (OARSI) scores and molecular mechanisms demonstrated that both intra-articular injections alleviated cartilage destruction and matrix degradation in the DMM model. Further in vitro experiments illustrated that these effects were exerted through EMSC-derived exosomes [91]. In collagenase-induced OA mouse models, Cosenza and colleagues found that exosomes derived from allogeneic BMSCs protected mice from developing OA by protecting cartilage and bone from degradation [92]. The in vitro studies also revealed chondroprotective and anti-inflammatory functions of exosomes in OA, as observed in chondrocyte models [91, 92]. In OA osteoblast models, Vian and coworkers indicated that exosomes from AMSCs downregulated the senescence features and corrected the metabolism of abnormal osteoblasts [93]. Interestingly, further study indicated that exosomes from gene vector or chemical synthetic modified MSCs could alter the expression of certain miRNA or long noncoding RNA (lncRNA) and affect OA treatment. For example, in rat models established by ACLT and MMT, exosomes from miR-140-5p-overexpressing human synovial mesenchymal stem cells (SMSCs) successfully slowed the progression of early OA and prevented severe damage to knee articular cartilage. In the OA+SMSC-140-Exos group, the cartilage matrix consisting of type II collagen was significantly better than that of the OA+SMSC-Exos group. Functional and molecular analysis in vitro indicated that SMSC-140-Exos enhanced the proliferation and migration of articular chondrocytes without influencing ECM secretion [94]. In collagenase-induced OA mouse models, exosomes from miR-92a-3p-overexpressing human MSCs (MSC-miR-92a-3p-Exos) inhibit the progression of early OA and prevented the severe damage to knee articular cartilage better than MSC-Exos. The study in vitro demonstrated MSC-miR-92a-3p-Exos promoted cartilage proliferation and matrix genes expression more effectively in MSCs and primary human chondrocytes, respectively [95]. In addition, exosomes from human MSC trans-
# MSC and OA

## Table 3. Preclinical studies of MSC exosome injection for cartilage lesion and OA treatment

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<tr>
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<tbody>
<tr>
<td>Human EMSCs</td>
<td>PBS</td>
<td>Rats</td>
<td>Cartilage defects on the trochlear grooves of distal femurs</td>
<td>Exosome-treated defects showed cartilage and subchondral bone recovery, and the regenerated hyaline cartilage and ECM deposition closely resembled the age-matched controls without operation. Contralateral PBS-treated defects exhibited only fibroid repair cartilage.</td>
<td>[90]</td>
</tr>
<tr>
<td>Human EMSCs</td>
<td>PBS</td>
<td>Mice</td>
<td>OA model induced by DMM surgery on the knee joints</td>
<td>Intra-articular injection of EMSCs alleviated cartilage destruction and matrix degradation. Further <em>in vitro</em> studies illustrated that these effects were exerted through EMSC-derived exosomes. These exosomes maintained the chondrocyte phenotype. Intra-articular injection of exosomes successfully impeded cartilage destruction.</td>
<td>[91]</td>
</tr>
<tr>
<td>Allogeneic BMSCs</td>
<td>Saline</td>
<td>Mice</td>
<td>OA model induced by articular injection of collagenase</td>
<td>Microvesicles/microparticles (MPs) and exosomes exerted similar chondroprotective and anti-inflammatory functions <em>in vitro</em> and protected mice from developing OA <em>in vivo</em>.</td>
<td>[92]</td>
</tr>
<tr>
<td>Human SMSCs overexpressing miR-140-5p</td>
<td>Saline</td>
<td>Rats</td>
<td>OA model induced by ACLT and MMT</td>
<td>Exosomes derived from SMSCs transfected with miR-140-5p lentivectors (SMSC-miR-140-exos) successfully prevented OA in rats. In OA+SMSC-miR-140-exos treated group, the cartilage matrix consisted of type II collagen which was significantly better than the OA+MSC-exos group.</td>
<td>[94]</td>
</tr>
<tr>
<td>Human BMSCs overexpressing miR-92a-3p</td>
<td>PBS</td>
<td>Mice</td>
<td>OA model induced by articular injection of collagenase</td>
<td>Exosomes derived from BMSCs transfected with miR-92a-3p mimics (MSC-miR-92a-3p-exos) inhibit the progression of early OA and prevented the severe damage to knee articular cartilage better than MSC-exos group in OA mice.</td>
<td>[95]</td>
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<tr>
<td>Human MSCs knocking down Inc-KLF3-AS1</td>
<td>PBS</td>
<td>Rats</td>
<td>OA model induced by articular injection of collagenase</td>
<td>Exosomes derived from MSC transfected with scramble Lentivirus (MSC-scramble-exos group) promoted cartilage repair and chondrocyte proliferation better than MSCs-KLF3-AS1-exos group in OA rats.</td>
<td>[96]</td>
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ected with scramble IncRNA-KLF3-AS1 promoted cartilage repair significantly better than si-IncRNA-KLF3-AS1 group in a rat model of OA [96]. Therefore, intra-articular injection of MSC exosomes impeded the progression of OA and partly restored the injured joint to a normal state. The function of intra-articular injection of MSC exosomes for OA treatment included the ability to inhibit cartilage degeneration and subchondral bone deterioration, reducing osteophyte formation and resisting synovial inflammation (Figure 2C). Thus, MSC exosomes provide a new opportunity for OA treatment and drug-delivery therapy. Intra-articular injection of chemosynthetic miRNA mimics are relatively safe and efficient for OA treatment and MSC exosomes provided optimal media to package and transport them.

The mechanisms underlying cartilage regeneration by MSC exosomes have not been elucidated as for other therapeutic efficacies reported for MSC exosomes. MSCs are stromal support cells that function to maintain a homeostatic tissue microenvironment. MSC exosomes are rich in ECM proteins and enzymes that can modulate and restore ECM homeostasis [82, 86]. The immunomodulation of MSCs is mediated largely through the secretion of trophic factors. However, this activity is not limited to a single secreted factor and most likely occurs through the synergism of multiple factors [97], reflected by the ability of some MSC exosomes to contain over 200 proteins [82, 98]. Many of Noncoding RNA including miRNAs and IncRNAs packaged in MSC exosomes are potent regulators of some key genes and signal transduction pathways in OA [96, 99-101]. For example, exosomes that highly-expressed miR-140-5p derived from human SMSCs block alternative Wnt signaling via repressing RalA and activating SOX9 in vitro, and regulate Col II, aggrecan, Col I expression to enhance cartilage tissue regeneration in vivo [94]. Exosomes derived from miR-92a-3p overexpressing increased the expression levels of COL2A1, COL9A1, COMP and SOX9 and decreased COL10A1, RUNX2, MMP13 via targeting WNT5A in vitro, and alter the expression of Wnt5a, ColII, aggrecan, Mmp13 to enhance chondrogenesis and suppress cartilage degradation in vivo [95]. LncRNA-KLF3-AS1 promotes chondrocyte proliferation and cartilage repair and MSC exosomes derived from lncRNA-KLF3-AS1 overexpression promote proliferation and inhibit apoptosis of chondrocytes via IncRNA-KLF3-AS1/miR-206/GIT1 axis in osteoarthritis [96, 101]. Exosomes from rat MSCs stimulated by TGF-β1 promote chondrocyte proliferation and cartilage repair through TGF-β1/miR-135b/Sp1 pathway [102].

In a word, MSC exosomes are now widely accepted as the principal therapeutic agents that mediate the many therapeutic efficacies of MSCs. Exosome production is more amenable to cell culture techniques and genetic manipulation, ensuring their cost-effective production. MSC exosome therapy for OA represents a potentially safer, cheaper and more effective treatment modality than MSC-based cell therapy.

**Conclusion**

To-date, the two major types of pre-clinical and clinical approaches for OA therapies are based on either tissue-engineering implantation of MSCs or scaffold-free injection into the injured joint. Increasing experimental and clinical data have emerged to support the use of MSCs for the repair of cartilage lesions and OA treatment. MSC exosomes, especially with certain miRNA packaged demonstrate great potential as therapeutics for articular cartilage defects and OA treatment. BMSCs have been widely used in animal models and in the clinic to investigate their chondrogenic potential and treatment effects for OA. AMSCs now represent a potential source of stem cells for OA treatment, demonstrated in a small number of preclinical and clinical studies.

However, MSC associated treatment for OA is not used routinely in the clinic and many problems restrict its use. In pre-clinical studies, the optimized conditions for MSC culture in vitro and the mechanisms of MSC treatment in vivo require further studies. In clinical trials, the different types of therapy according different classes of OA patients, how to culture MSC, how to select the types of MSCs, how to store and transport MSCs, how to select the media to transport MSCs to the injured joint, how to evaluate the therapeutic efficacy and safety of MSCs, and how to execute blind investigations in large cohorts are all issues that need to be solved. In addition, in the context of cartilage repair, important questions regarding ther-
apeutic efficacy and safety of MSC exosomes in large animal studies remain. The treatment effect of MSC related therapies will be enhanced with those problems resolved. At present, MSC-based therapies are not suitable for regeneration of large cartilage lesions in severe OA patients and the criteria of optimal scaffold, cell dose, injected times and intervals are not definite. In addition, MSC exosome therapy has not been used in clinical trials.

Although a great deal of effort is required at the basic and clinical research fronts, the promise of MSCs in cartilage tissue engineering or cell therapy is clear. MSC exosome therapy has not been used in clinical trials. Although a great deal of effort is required at the basic and clinical research fronts, the promise of MSCs in cartilage tissue engineering or cell therapy is clear. MSC exosome therapy has not been used in clinical trials. Although a great deal of effort is required at the basic and clinical research fronts, the promise of MSCs in cartilage tissue engineering or cell therapy is clear. MSC exosome therapy has not been used in clinical trials.

Acknowledgements

This study was supported by funding from the Natural Science Foundation of Shaanxi Province (No. 2018JM7039, No. 2018JM7081; the National Natural Science Foundation of China (No. 31700730, No. 81601937).

Disclosure of conflict of interest

None.

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MSC and OA


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