Review Article
Mesenchymal stem cell related therapies for cartilage lesions and osteoarthritis

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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 turn-

over 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 telomerases 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 transplanta-

tion 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 periostea or 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

Table 1. Preclinical and clinical studies of MSC transplantation for cartilage lesions and OA treatment

Cell type	Scaffold	Subjects	Injured type and position	Results	Reference
Autologous BMSC	Chitosan with TGF-β3	Sheep	Partial-thickness lesions in the internal groove of the patellae	Implantation of BMSCs mixed with a chitosan scaffold and TGF-β3 resulted in hyaline like cartilage filling the defects.	[45]
Autologous BMSCs	β-tricalcium phosphate	Sheep	Osteochondral defects in the media femoral condyle	In the group of BMSCs with scaffold, hyaline-like tissue covered the defective surface. In scaffolds without cells, the defect was clearly visible.	[46]
Allogeneic BMSCs	Self-assembled peptide hydrogels	Rats	OA model by ACLT and medial collateral ligaments transected	The transplantation group showed chondroprotection and reduced subchondral bone mineral density compared to the OA group.	[47]
Autologous BMSCs	N/A	72 patients	Knee articular cartilage defects	Compared with chondrocytes, BMSC-based treatment groups appeared less invasive and reduced both morbidity and operative costs.	[48]
Autologous BMSCs	Collagen gel	2 patients	Full-thickness articular cartilage defects in patellae	Defects were repaired with fibrocartilage. Pain and walking ability of the patients improved significantly.	[49]
Autologous BMSCs	Collagen gel	1 patient	A full-thickness cartilage defect in the medial femoral condyle	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.	[50]
Autologous BMSCs	Collagen gel	3 patients	Full-thickness articular cartilage defects of the patellofemoral joints	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.	[51]
Autologous BMSCs	Platelet-rich fibrin glue	5 patients	Full-thickness cartilage defects of femoral condyles	All patients' symptoms improved over the follow-up period of 12 months. Average Lysholm and RHSSK scores showed significant improvement.	[52]
Autologous BMSCs	Collagen gel	24 patients	Articular cartilage defects in the medial femoral condyle	Both implanted groups underwent a high tibia osteotomy improved functionally but hyaline cartilage was observed only after addition of BMSCs.	[53]
Autologous BMSCs	Collagen gel	41 patients	Knee, hip, elbow, or ankle OA	Neither tumours nor infections were observed between 5 and 137 (mean 75) months of follow-up surveys.	[54]
Autologous AMSCs	N/A	35 patients	Full-thickness articular cartilage lesion in OA knees	The ICRS repair grades at second-look arthroscopic surgery were improved at different degrees in patients filled with AMSC suspensions.	[55]

Table 2. Preclinical and clinical studies of scaffold-free MSC injection for OA treatment

Cell type	Solution medium	Subjects	Injured type and position	Results	Reference
Autologous BMSCs	HA	Minipigs	A cartilage defect in the medial femoral condyle	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.	[56]
Autologous BMSCs	Basal media	Sheep	OA model by ACLT with MMT	Knee OA treated with BMSCs was observed in macroscopical and histological retardation of cartilage destruction.	[57]
Autologous AMSC or BMSCs	Culture media	Sheep	OA model by ACLT with MMT	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.	[58]
Autologous BMSCs	HA	Goats	OA model by ACLT with MMT	Autologous BMSCs injection resulted in regeneration of the medial meniscus and a reduction in osteophyte remodeling, subchondral sclerosis and articular cartilage degradation.	[59]
Allogeneic AMSCs	HA	Rabbits	OA model by ACLT	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.	[63]
Allogeneic AMSCs	PBS	Rats	OA model by ACLT	Intra-articular injection of allogeneic AMSCs in OA rats delayed joint degeneration. AMSCs also protected chondrocytes from the damage induced by inflammatory factors.	[64]
Allogeneic AMSCs	Mouse serum with mouse albumin	Mice	OA model by articular-injection collagenase	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.	[65]
Xenogenic Equine UC-MSC	PBS	Rabbits	OA model by MMT	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 synoviocytes.	[66]
Human MSCs	PBS or HA	Guinea pigs	Spontaneous OA	Injection of human MSCs resulted in the regeneration of articular cartilage in the spontaneous OA animal models.	[67]
Human MSCs or rat MSCs	PBS	Rats	OA model by MMT	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.	[68]
Autologous BMSCs	PBS	A male patient	Keen OA	Patient knees had significant cartilage and meniscus growth, as well as increased range of motion and decreased pain scores.	[70]
Autologous BMSCs	Saline with serum albumin	4 patients	Keen OA	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.	[71]
Autologous BMSCs	Physiological serum	6 female patients	Keen OA	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.	[72]
Allogeneic BMSCs	HA	55 patients	A partial medial meniscectomy	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.	[73]
Autologous AMSCs	HA with PRP	4 patients	Osteonecrosis in the right femoral head or knee OA	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.	[74]
Autologous AMSCs	Saline	18 patients	Knee OA	Intra-articular injection with high-dose (1.0×10^8) 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.	[75]

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 $1.0×10^8$ 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 ($5.0×10^7$ AMSCs) showed improvement in some clinical outcomes, but those in the low-dose group ($1.0×10^7$ 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-
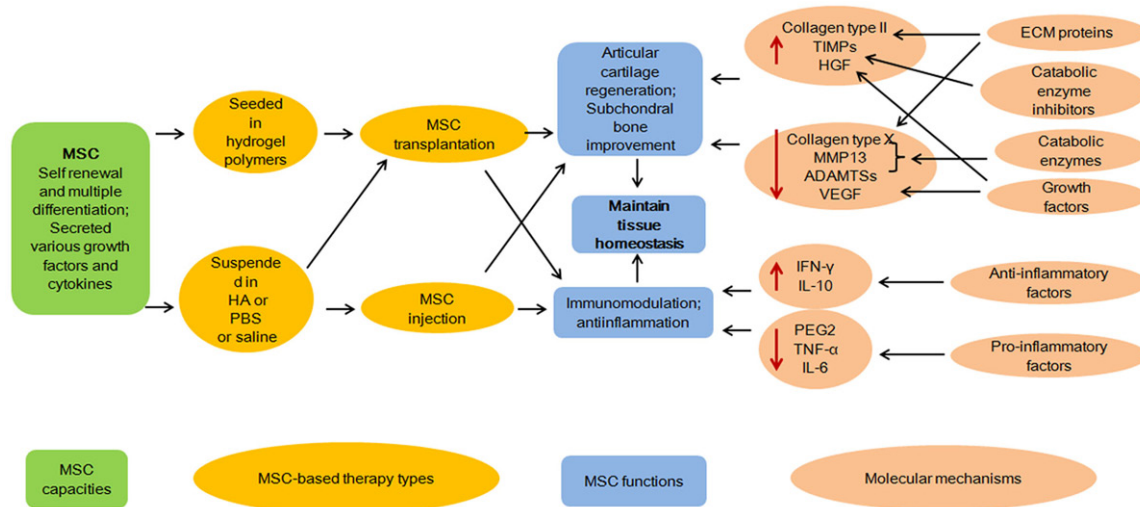
Figure 1. Schematic of MSC-based therapies for cartilage lesions and OA. The benefits of MSCs include their capacity to self-renew, differentiate, and to secrete growth factors and cytokines. The tissue engineering of MSCs seeded in hydrogel polymers induce cartilage regeneration and subchondral bone 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.

lation and anti-inflammatory effects through regulating several molecules such as prostaglandin E2 (PGE2), interferon (IFN)-γ, interleukins (ILs) et al [80, 81]. The capacity of MSCs, MSC-based therapies, MSC functionality, and the potential mechanisms by which MSCs improve cartilage lesions and OA treatment are summarized in **Figure 1**. In recent years, it was reported that exosomes play a leading role in the efficacy of MSC-based therapies in mediating tissue repair. MSC exosomes provide new perspectives for the development of cell-free and ready-to-use therapy for for treatment of cartilage lesions and OA.

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 liq-

uid, 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-
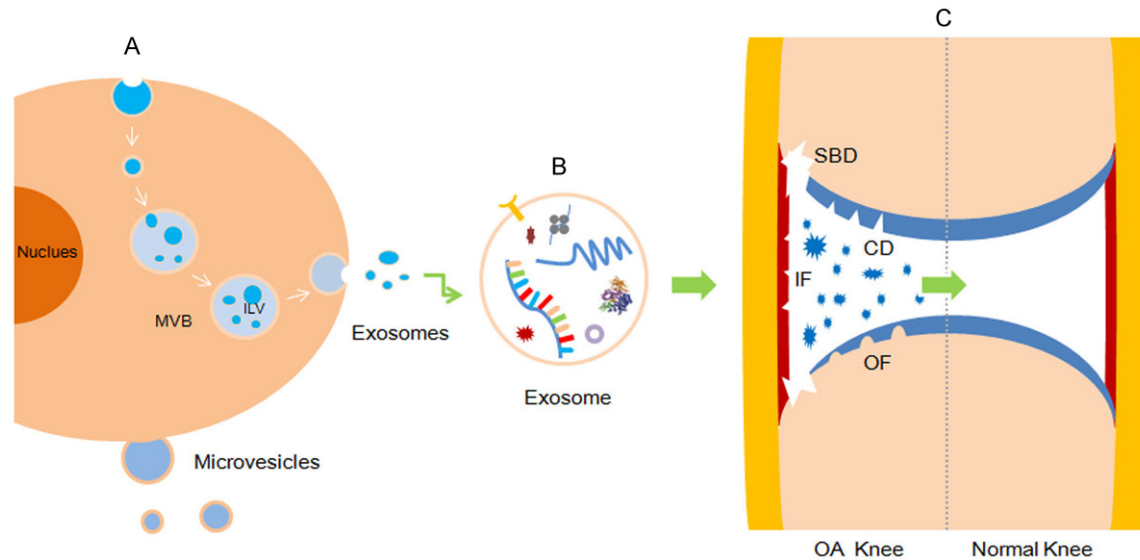
Figure 2. Biogenesis of MSC exosomes and the function of intra-articular injection of MSC exosomes for OA treatment. A. MSC exosome biogenesis. Exosomes originate from the inward invagination of the plasma membrane, and are formed as multivesicular bodies (MVBs) which composed of intraluminal vesicles (ILVs). Exosomes are secreted by fusion of the MVB with the plasma membrane, while microvesicles are secreted through the forward budding of plasma membrane. B. The contents of exosomes include transmembrane proteins, internal proteins (cytosolic, cytoskeletal, and growth factors), internal lipids, internal miRNAs and mRNAs. C. The function of intra-articular injection of MSC exosomes for OA treatment including inhibiting cartilage degeneration (CD) and subchondral bone deterioration (SBD), reducing osteophyte formation (OF) and resisting synovial inflammation (IF).

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-

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

Cell type	Solution medium	Subjects	Injured type and position	Results	Reference
Human EMSCs	PBS	Rats	Cartilage defects on the trochlear grooves of distal femurs	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.	[90]
Human EMSCs	PBS	Mice	OA model induced by DMM surgery on the knee joints	Intra-articular injection of EMSCs alleviated cartilage destruction and matrix degradation. Further *in vitro* 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.	[91]
Allogeneic BMSCs	Saline	Mice	OA model induced by articular-injection of collagenase	Microvesicles/microparticles (MPs) and exosomes exerted similar chondroprotective and anti-inflammatory functions *in vitro* and protected mice from developing OA *in vivo*.	[92]
Human SMSCs overexpressing miR-140-5p	Saline	Rats	OA model induced by ACLT and MMT	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+SMSC-Exos group.	[94]
Huaman BMSCs overexpressing miR-92a-3p	PBS	Mice	OA model induced by articular-injection of collagenase	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.	[95]
Human MSCs knocking down lnc-KLF3-AS1	PBS	Rats	OA model induced by articular-injection of collagenase	Exosomes derived from MSC transfected with scramble Lentivirus (MSCscramble-Exos group) promoted cartilage repair and chondrocyte proliferation better than MSCsi-KLF3-AS1-Exos group in OA rats.	[96]

fected with scramble lncRNA-KLF3-AS1 promoted cartilage repair significantly better than si-lncRNA-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 lncRNAs 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, aggrencan, 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, aggrencan, 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 overex-

pression promote proliferation and inhibit apoptosis of chondrocytes via lncRNA-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 storage 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 issue 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 exsome 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 exosomes may represent the most effective clinical strategy once trials have been fully controlled and their benefits and safety fully assessed. In the future, MSC related therapy could ultimately become a regular and routine regenerative medicine for OA treatment in clinic practice.

Acknowledgements

Disclosure of conflict of interest

None.

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References

[1] Arden NK and Leyland KM. Osteoarthritis year 2013 in review: clinical. Osteoarthritis Cartilage 2013; 21: 1409-1413.

[2] Gao SG, Li KH, Zeng KB, Tu M, Xu M and Lei GH. Elevated osteopontin level of synovial fluid and articular cartilage is associated with disease severity in knee osteoarthritis patients. Osteoarthritis Cartilage 2010; 18: 82-87.

[3] Richardson SM, Kalamegam G, Pushparaj PN, Matta C, Memic A, Khademhosseini A, Mobasheri R, Poletti FL, Hoyland JA and Mobasheri A. Mesenchymal stem cells in regenerative medicine: focus on articular cartilage and in-tervertebral disc regeneration. Methods 2016; 99: 69-80.

[4] Glyn-Jones S, Palmer AJ, Agricola R, Price AJ, Vincent TL, Weinans H and Carr AJ. Osteoarthritis. Lancet 2015; 386: 376-387.

[5] Abramson SB and Attur M. Developments in the scientific understanding of osteoarthritis. Arthritis Res Ther 2009; 11: 227.

[6] Zhang W, Nuki G, Moskowitz RW, Abramson S, Altman RD, Arden NK, Bierma-Zeinstra S, Brandt KD, Croft P, Doherty M, Dougados M, Hochberg M, Hunter DJ, Kwoh K, Lohmander LS and Tugwell P. OARSI recommendations for the management of hip and knee osteoarthritis: part III: changes in evidence following systematic cumulative update of research published through January 2009. Osteoarthritis Cartilage 2010; 18: 476-499.

[7] Vargas Negrin F, Medina Abellan MD, Hermosa Hernan JC and de Felipe Medina R. Treatment of patients with osteoarthritis. Aten Primaria 2014; 46 Suppl 1: 39-61.

[8] Dieppe P, Lim K and Lohmander S. Who should have knee joint replacement surgery for osteoarthritis? Int J Rheum Dis 2011; 14: 175-180.

[9] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S and Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284: 143-147.

[10] De Bari C, Dell'Accio F, Karystinou A, Guillot PV, Fisk NM, Jones EA, McGonagle D, Khan IM, Archer CW, Mitsiadis TA, Donaldson AN, Luyten FP and Pitzalis C. A biomarker-based mathematical model to predict bone-forming potency of human synovial and periosteal mesenchymal stem cells. Arthritis Rheum 2008; 58: 240-250.

[11] Lindroos B, Suuronen R and Miettinen S. The potential of adipose stem cells in regenerative medicine. Stem Cell Rev 2011; 7: 269-291.

[12] Mirsaidi A, Kleinhans KN, Rimann M, Tiaden AN, Stauber M, Rudolph KL and Richards PJ. Telomere length, telomerase activity and osteogenic differentiation are maintained in adipose-derived stromal cells from senile osteoporotic SAMP6 mice. J Tissue Eng Regen Med 2012; 6: 378-390.

[13] Buckwalter JA and Mankin HJ. Articular cartilage: degeneration and osteoarthritis, repair, regeneration, and transplantation. Instr Course Lect 1998; 47: 487-504.

[14] Buckwalter JA and Mankin HJ. Articular cartilage: tissue design and chondrocyte-matrix interactions. Instr Course Lect 1998; 47: 477-486.

[15] Buckwalter JA, Martin J and Mankin HJ. Synovial joint degeneration and the syndrome of osteoarthritis. Instr Course Lect 2000; 49: 481-489.

[16] Zhang L, Hu H, Tian F, Song H and Zhang Y. Enhancement of subchondral bone quality by alendronate administration for the reduction of cartilage degeneration in the early phase of experimental osteoarthritis. Clin Exp Med 2011; 11: 235-243.

[17] Berenbaum F, Eymard F and Houard X. Osteoarthritis, inflammation and obesity. Curr Opin Rheumatol 2013; 25: 114-118.

[18] Goldring MB and Berenbaum F. The regulation of chondrocyte function by proinflammatory mediators: prostaglandins and nitric oxide. Clin Orthop Relat Res 2004; Suppl: S37-46.

[19] Sellam J and Berenbaum F. The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. Nat Rev Rheumatol 2010; 6: 625-635.

[20] Bora FW Jr and Miller G. Joint physiology, cartilage metabolism, and the etiology of osteoarthritis. Hand Clin 1987; 3: 325-336.

[21] Archer CW and Francis-West P. The chondrocyte. Int J Biochem Cell Biol 2003; 35: 401-404.

[22] Muir H. The chondrocyte, architect of cartilage. Biomechanics, structure, function and molecular biology of cartilage matrix macromolecules. Bioessays 1995; 17: 1039-1048.

[23] Mobasheri A, Richardson S, Mobasheri R, Shakibaei M and Hoyland JA. Hypoxia inducible factor-1 and facilitative glucose transporters GLUT1 and GLUT3: putative molecular components of the oxygen and glucose sensing apparatus in articular chondrocytes. Histol Histopathol 2005; 20: 1327-1338.

[24] Millward-Sadler SJ and Salter DM. Integrin-dependent signal cascades in chondrocyte mechanotransduction. Ann Biomed Eng 2004; 32: 435-446.

[25] Fosang AJ and Beier F. Emerging Frontiers in cartilage and chondrocyte biology. Best Pract Res Clin Rheumatol 2011; 25: 751-766.

[26] Hudelmaier M, Glaser C, Hohe J, Englmeier KH, Reiser M, Putz R and Eckstein F. Age-related changes in the morphology and deformational behavior of knee joint cartilage. Arthritis Rheum 2001; 44: 2556-2561.

[27] Mobasheri A, Carter SD, Martin-Vasallo P and Shakibaei M. Integrins and stretch activated ion channels; putative components of functional cell surface mechanoreceptors in articular chondrocytes. Cell Biol Int 2002; 26: 1-18.

[28] Ralphs JR and Benjamin M. The joint capsule: structure, composition, ageing and disease. J Anat 1994; 184: 503-509.

[29] Mobasheri A, Bondy CA, Moley K, Mendes AF, Rosa SC, Richardson SM, Hoyland JA, Barrett-Jolley R and Shakibaei M. Facilitative glucose transporters in articular chondrocytes. Expression, distribution and functional regulation of GLUT isoforms by hypoxia, hypoxia mimetics, growth factors and pro-inflammatory cytokines. Adv Anat Embryol Cell Biol 2008; 200: 1 p following vi, 1-84.

[30] Goggs R, Carter SD, Schulze-Tanzil G, Shakibaei M and Mobasheri A. Apoptosis and the loss of chondrocyte survival signals contribute to articular cartilage degradation in osteoarthritis. Vet J 2003; 166: 140-158.

[31] Setton LA, Elliott DM and Mow VC. Altered mechanics of cartilage with osteoarthritis: human osteoarthritis and an experimental model of joint degeneration. Osteoarthritis Cartilage 1999; 7: 2-14.

[32] Sarzi-Puttini P, Cimmino MA, Scarpa R, Caporali R, Parazzini F, Zaninelli A, Atzeni F and Canesi B. Osteoarthritis: an overview of the disease and its treatment strategies. Semin Arthritis Rheum 2005; 35 Suppl 1: 1-10.

[33] Buckwalter JA. Sports, joint injury, and post-traumatic osteoarthritis. J Orthop Sports Phys Ther 2003; 33: 578-588.

[34] Lories RJ and Luyten FP. The bone-cartilage unit in osteoarthritis. Nat Rev Rheumatol 2011; 7: 43-49.

[35] Pan J, Wang B, Li W, Zhou X, Scherr T, Yang Y, Price C and Wang L. Elevated cross-talk between subchondral bone and cartilage in osteoarthritic joints. Bone 2012; 51: 212-217.

[36] Hu X, Ji X, Yang M, Fan S, Wang J, Lu M, Shi W, Mei L, Xu C, Fan X, Hussain M, Du J, Wu J and Wu X. Cdc42 is essential for both articular cartilage degeneration and subchondral bone deterioration in experimental osteoarthritis. J Bone Miner Res 2018; 33: 945-958.

[37] Brittberg M, Faxen E and Peterson L. Carbon fiber scaffolds in the treatment of early knee osteoarthritis. A prospective 4-year followup of 37 patients. Clin Orthop Relat Res 1994; 155-164.

[38] Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O and Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med 1994; 331: 889-895.

[39] Phull AR, Eo SH, Abbas Q, Ahmed M and Kim SJ. Applications of chondrocyte-based cartilage engineering: an overview. Biomed Res Int 2016; 2016: 1879837.

[40] Brittberg M, Peterson L, Sjogren-Jansson E, Tallheden T and Lindahl A. Articular cartilage engineering with autologous chondrocyte transplantation. A review of recent developments. J Bone Joint Surg Am 2003; 85-A Suppl 3: 109-115.

[41] Horas U, Pelinkovic D, Herr G, Aigner T and Schnettler R. Autologous chondrocyte implantation and osteochondral cylinder transplantation in cartilage repair of the knee joint. A pro-

spective, comparative trial. J Bone Joint Surg Am 2003; 85-A: 185-192.

[42] Minas T. Autologous chondrocyte implantation for focal chondral defects of the knee. Clin Orthop Relat Res 2001; Suppl: S349-361.

[43] Demoor M, Ollitrault D, Gomez-Leduc T, Bouyoucef M, Hervieu M, Fabre H, Lafont J, Denoix JM, Audigie F, Mallein-Gerin F, Legendre F and Galera P. Cartilage tissue engineering: molecular control of chondrocyte differentiation for proper cartilage matrix reconstruction. Biochim Biophys Acta 2014; 1840: 2414-2440.

[44] Sakata R, Iwakura T and Reddi AH. Regeneration of articular cartilage surface: morphogens, cells, and extracellular matrix scaffolds. Tissue Eng Part B Rev 2015; 21: 461-473.

[45] Mrugala D, Bony C, Neves N, Caillot L, Fabre S, Moukoko D, Jorgensen C and Noel D. Phenotypic and functional characterisation of ovine mesenchymal stem cells: application to a cartilage defect model. Ann Rheum Dis 2008; 67: 288-295.

[46] Guo X, Wang C, Zhang Y, Xia R, Hu M, Duan C, Zhao Q, Dong L, Lu J and Qing Song Y. Repair of large articular cartilage defects with implants of autologous mesenchymal stem cells seeded into beta-tricalcium phosphate in a sheep model. Tissue Eng 2004; 10: 1818-1829.

[47] Kim JE, Lee SM, Kim SH, Tatman P, Gee AO, Kim DH, Lee KE, Jung Y and Kim SJ. Effect of self-assembled peptide-mesenchymal stem cell complex on the progression of osteoarthritis in a rat model. Int J Nanomedicine 2014; 9 Suppl 1: 141-157.

[48] Nejadnik H, Hui JH, Feng Choong EP, Tai BC and Lee EH. Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: an observational cohort study. Am J Sports Med 2010; 38: 1110-1116.

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

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

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

[52] Haleem AM, Singergy AA, Sabry D, Atta HM, Rashed LA, Chu CR, El Shewy MT, Azzam A and Abdel Aziz MT. The clinical use of human culture-expanded autologous bone marrow mesenchymal stem cells transplanted on platelet-rich fibrin glue in the treatment of articular cartilage defects: a pilot study and preliminary results. Cartilage 2010; 1: 253-261.

[53] Wakitani S, Imoto K, Yamamoto T, Saito M, Murata N and Yoneda M. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. Osteoarthritis Cartilage 2002; 10: 199-206.

[54] Wakitani S, Okabe T, Horibe S, Mitsuoka T, Saito M, Koyama T, Nawata M, Tensho K, Kato H, Uematsu K, Kuroda R, Kurosaka M, Yoshiya S, Hattori K and Ohgushi H. Safety of autologous bone marrow-derived mesenchymal stem cell transplantation for cartilage repair in 41 patients with 45 joints followed for up to 11 years and 5 months. J Tissue Eng Regen Med 2011; 5: 146-150.

[55] Koh YG, Choi YJ, Kwon OR and Kim YS. Second-look arthroscopic evaluation of cartilage lesions after mesenchymal stem cell implantation in osteoarthritic knees. Am J Sports Med 2014; 42: 1628-1637.

[56] Lee KB, Hui JH, Song IC, Ardany L and Lee EH. Injectable mesenchymal stem cell therapy for large cartilage defects--a porcine model. Stem Cells 2007; 25: 2964-2971.

[57] Al Faqeh H, Nor Hamdan BM, Chen HC, Aminuddin BS and Ruszymah BH. The potential of intra-articular injection of chondrogenic-induced bone marrow stem cells to retard the progression of osteoarthritis in a sheep model. Exp Gerontol 2012; 47: 458-464.

[58] Ude CC, Sulaiman SB, Min-Hwei N, Hui-Cheng C, Ahmad J, Yahaya NM, Saim AB and Idrus RB. Cartilage regeneration by chondrogenic induced adult stem cells in osteoarthritic sheep model. PLoS One 2014; 9: e98770.

[59] Murphy JM, Fink DJ, Hunziker EB and Barry FP. Stem cell therapy in a caprine model of osteoarthritis. Arthritis Rheum 2003; 48: 3464-3474.

[60] Yun S, Ku SK and Kwon YS. Adipose-derived mesenchymal stem cells and platelet-rich plasma synergistically ameliorate the surgical-induced osteoarthritis in Beagle dogs. J Orthop Surg Res 2016; 11: 9.

[61] Dutton AQ, Choong PF, Goh JC, Lee EH and Hui JH. Enhancement of meniscal repair in the avascular zone using mesenchymal stem cells in a porcine model. J Bone Joint Surg Br 2010; 92: 169-175.

[62] Hatsushika D, Muneta T, Nakamura T, Horie M, Koga H, Nakagawa Y, Tsuji K, Hishikawa S, Kobayashi E and Sekiya I. Repetitive allogeneic

intraarticular injections of synovial mesenchymal stem cells promote meniscus regeneration in a porcine massive meniscus defect model. Osteoarthritis Cartilage 2014; 22: 941-950.

[63] Chiang ER, Ma HL, Wang JP, Liu CL, Chen TH and Hung SC. Allogeneic mesenchymal stem cells in combination with hyaluronic acid for the treatment of osteoarthritis in rabbits. PLoS One 2016; 11: e0149835.

[64] Mei L, Shen B, Ling P, Liu S, Xue J, Liu F, Shao H, Chen J, Ma A and Liu X. Culture-expanded allogenic adipose tissue-derived stem cells attenuate cartilage degeneration in an experimental rat osteoarthritis model. PLoS One 2017; 12: e0176107.

[65] ter Huurne M, Schelbergen R, Blattes R, Blom A, de Munter W, Grevers LC, Jeanson J, Noël D, Casteilla L, Jorgensen C, van den Berg W and van Lent PL. Antiinflammatory and chondroprotective effects of intraarticular injection of adipose-derived stem cells in experimental osteoarthritis. Arthritis Rheum 2012; 64: 3604-3613.

[66] Sato M, Uchida K, Nakajima H, Miyazaki T, Guerrero AR, Watanabe S, Roberts S and Baba H. Direct transplantation of mesenchymal stem cells into the knee joints of Hartley strain guinea pigs with spontaneous osteoarthritis. Arthritis Res Ther 2012; 14: R31.

[67] Saulnier N, Viguier E, Perrier-Groult E, Chenu C, Pillet E, Roger T, Maddens S and Boulocher C. Intra-articular administration of xenogeneic neonatal Mesenchymal Stromal Cells early after meniscal injury down-regulates metalloproteinase gene expression in synovium and prevents cartilage degradation in a rabbit model of osteoarthritis. Osteoarthritis Cartilage 2015; 23: 122-133.

[68] Horie M, Choi H, Lee RH, Reger RL, Ylostalo J, Muneta T, Sekiya I and Prockop DJ. Intra-articular injection of human mesenchymal stem cells (MSCs) promote rat meniscal regeneration by being activated to express Indian hedgehog that enhances expression of type II collagen. Osteoarthritis Cartilage 2012; 20: 1197-1207.

[69] Barry F and Murphy M. Mesenchymal stem cells in joint disease and repair. Nat Rev Rheumatol 2013; 9: 584-594.

[70] Centeno CJ, Busse D, Kisiday J, Keohan C, Freeman M and Karli D. Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells. Pain Physician 2008; 11: 343-353.

[71] Emadedin M, Aghdami N, Taghiyar L, Fazeli R, Moghadasali R, Jahangir S, Farjad R and Baghaban Eslaminejad M. Intra-articular injection of autologous mesenchymal stem cells in six

[72] Davatchi F, Abdollahi BS, Mohyeddin M, Shahram F and Nikbin B. Mesenchymal stem cell therapy for knee osteoarthritis. Preliminary report of four patients. Int J Rheum Dis 2011; 14: 211-215.

[73] Vangsness CT Jr, Farr J 2nd, Boyd J, Dellaero DT, Mills CR and LeRoux-Williams M. Adult human mesenchymal stem cells delivered via intra-articular injection to the knee following partial medial meniscectomy: a randomized, double-blind, controlled study. J Bone Joint Surg Am 2014; 96: 90-98.

[74] Pak J. Regeneration of human bones in hip osteonecrosis and human cartilage in knee osteoarthritis with autologous adipose-tissue-derived stem cells: a case series. J Med Case Rep 2011; 5: 296.

[75] Jo CH, Lee YG, Shin WH, Kim H, Chai JW, Jeong EC, Kim JE, Shim H, Shin JS, Shin IS, Ra JC, Oh S and Yoon KS. Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a proof-of-concept clinical trial. Stem Cells 2014; 32: 1254-1266.

[76] Toh WS, Foldager CB, Pei M and Hui JH. Advances in mesenchymal stem cell-based strategies for cartilage repair and regeneration. Stem Cell Rev 2014; 10: 686-696.

[77] Meirelles Lda S, Fontes AM, Covas DT and Caplan AI. Mechanisms involved in the therapeutic properties of mesenchymal stem cells. Cytokine Growth Factor Rev 2009; 20: 419-427.

[78] Shabbir A, Zisa D, Suzuki G and Lee T. Heart failure therapy mediated by the trophic activities of bone marrow mesenchymal stem cells: a noninvasive therapeutic regimen. Am J Physiol Heart Circ Physiol 2009; 296: H1888-1897.

[79] Lozito TP and Tuan RS. Mesenchymal stem cells inhibit both endogenous and exogenous MMPs via secreted TIMPs. J Cell Physiol 2011; 226: 385-396.

[80] Gray A, Marrero-Berrios I, Weinberg J, Manchikalapati D, SchianodiCola J, Schloss RS and Yarmush J. The effect of local anesthetic on pro-inflammatory macrophage modulation by mesenchymal stromal cells. Int Immunopharmacol 2016; 33: 48-54.

[81] Yi T and Song SU. Immunomodulatory properties of mesenchymal stem cells and their therapeutic applications. Arch Pharm Res 2012; 35: 213-221.

[82] Lai RC, Yeo RW and Lim SK. Mesenchymal stem cell exosomes. Semin Cell Dev Biol 2015; 40: 82-88.

[83] Pan BT, Teng K, Wu C, Adam M and Johnstone RM. Electron microscopic evidence for externalization of the transferrin receptor in vesicular form in sheep reticulocytes. J Cell Biol 1985; 101: 942-948.

[84] Yáñez-Mó M, Siljander PR, Andreu Z, Zavec AB, Borràs FE, Buzas EI, Buzas K, Casal E, Cappello F, Carvalho J, Colás E, Cordeiro-da Silva A, Fais S, Falcon-Perez JM, Ghobrial IM, Giebel B, Gimona M, Graner M, Gursel I, Gursel M, Heegaard NH, Hendrix A, Kierulf P, Kokubun K, Kosanovic M, Kralj-Iglic V, Krämer-Albers EM, Laitinen S, Lässer C, Lener T, Ligeti E, Linē A, Lipps G, Llorente A, Lötvall J, Manček-Keber M, Marcilla A, Mittelbrunn M, Nazarenko I, Nolte-'t Hoen EN, Nyman TA, O'Driscoll L, Olivan M, Oliveira C, Pállinger É, Del Portillo HA, Reventós J, Rigau M, Rohde E, Sammar M, Sánchez-Madrid F, Santarém N, Schallmoser K, Ostenfeld MS, Stoorvogel W, Stukelj R, Van der Grein SG, Vasconcelos MH, Wauben MH and De Wever O. Biological properties of extracellular vesicles and their physiological functions. J Extracell Vesicles 2015; 4: 27066.

[85] Deatherage BL and Cookson BT. Membrane vesicle release in bacteria, eukaryotes, and archaea: a conserved yet underappreciated aspect of microbial life. Infect Immun 2012; 80: 1948-1957.

[86] Colombo M, Raposo G and Thery C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu Rev Cell Dev Biol 2014; 30: 255-289.

[87] Lai RC, Arslan F, Lee MM, Sze NS, Choo A, Chen TS, Salto-Tellez M, Timmers L, Lee CN, El Oakley RM, Pasterkamp G, de Kleijn DP and Lim SK. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. Stem Cell Res 2010; 4: 214-222.

[88] Tan CY, Lai RC, Wong W, Dan YY, Lim SK and Ho HK. Mesenchymal stem cell-derived exosomes promote hepatic regeneration in drug-induced liver injury models. Stem Cell Res Ther 2014; 5: 76.

[89] Zhang B, Yin Y, Lai RC, Tan SS, Choo AB and Lim SK. Mesenchymal stem cells secrete immunologically active exosomes. Stem Cells Dev 2014; 23: 1233-1244.

[90] Zhang S, Chu WC, Lai RC, Lim SK, Hui JH and Toh WS. Exosomes derived from human embryonic mesenchymal stem cells promote osteochondral regeneration. Osteoarthritis Cartilage 2016; 24: 2135-2140.

[91] Wang Y, Yu D, Liu Z, Zhou F, Dai J, Wu B, Zhou J, Heng BC, Zou XH, Ouyang H and Liu H. Exosomes from embryonic mesenchymal stem cells alleviate osteoarthritis through balancing synthesis and degradation of cartilage extracellular matrix. Stem Cell Res Ther 2017; 8: 189.

[92] Cosenza S, Ruiz M, Toupet K, Jorgensen C and Noel D. Mesenchymal stem cells derived exosomes and microparticles protect cartilage and bone from degradation in osteoarthritis. Sci Rep 2017; 7: 16214.

[93] Tofino-Vian M, Guillen MI, Perez Del Caz MD, Castejon MA and Alcaraz MJ. Extracellular vesicles from adipose-derived mesenchymal stem cells downregulate senescence features in osteoarthritic osteoblasts. Oxid Med Cell Longev 2017; 2017: 7197598.

[94] Tao SC, Yuan T, Zhang YL, Yin WJ, Guo SC and Zhang CQ. Exosomes derived from miR-140-5p-overexpressing human synovial mesenchymal stem cells enhance cartilage tissue regeneration and prevent osteoarthritis of the knee in a rat model. Theranostics 2017; 7: 180-195.

[95] Mao G, Zhang Z, Hu S, Zhang Z, Chang Z, Huang Z, Liao W and Kang Y. Exosomes derived from miR-92a-3p-overexpressing human mesenchymal stem cells enhance chondrogenesis and suppress cartilage degradation via targeting WNT5A. Stem Cell Res Ther 2018; 9: 247.

[96] Liu Y, Zou R, Wang Z, Wen C, Zhang F and Lin F. Exosomal KLF3-AS1 from hMSCs promoted cartilage repair and chondrocyte proliferation in osteoarthritis. Biochem J 2018; 475: 3629-3638.

[97] Liu H, Lu K, MacAry PA, Wong KL, Heng A, Cao T and Kemeny DM. Soluble molecules are key in maintaining the immunomodulatory activity of murine mesenchymal stromal cells. J Cell Sci 2012; 125: 200-208.

[98] Lai RC, Tan SS, Teh BJ, Sze SK, Arslan F, de Kleijn DP, Choo A and Lim SK. Proteolytic potential of the MSC exosome proteome: implications for an exosome-mediated delivery of therapeutic proteasome. Int J Proteomics 2012; 2012: 971907.

[99] Chen TS, Lai RC, Lee MM, Choo AB, Lee CN and Lim SK. Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs. Nucleic Acids Res 2010; 38: 215-224.

[100] Sun H, Hu S, Zhang Z, Lun J, Liao W and Zhang Z. Expression of exosomal microRNAs during chondrogenic differentiation of human bone mesenchymal stem cells. J Cell Biochem 2019; 120: 171-181.

[101] Liu Y, Lin L, Zou R, Wen C, Wang Z and Lin F. MSC-derived exosomes promote proliferation and inhibit apoptosis of chondrocytes via lncRNA-KLF3-AS1/miR-206/GIT1 axis in osteoarthritis. Cell Cycle 2018; 17: 2411-2422.

[102] Wang R, Xu B and Xu H. TGF-beta1 promoted chondrocyte proliferation by regulating Sp1 through MSC-exosomes derived miR-135b. Cell Cycle 2018; 17.