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Review

# Progress in the use of mesenchymal stromal cells for osteoarthritis treatment

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ABSTRACT

*Literature review of MSCs in the treatment of osteoarthritis in the past five years:* Osteoarthritis (OA) is one of the most common chronic joint diseases, with prominent symptoms caused by many factors. However, current medical interventions for OA have resulted in poor clinical outcomes, demonstrating that there are huge unmet medical needs in this area. Cell therapy has opened new avenues of OA treatment. Different sources of mesenchymal stromal cells (MSCs) may have different phenotypes and cellular functions. Pre-clinical and clinical studies have demonstrated the feasibility, safety and efficacy of MSC therapy. Mitogen-activated protein kinase, Wnt and Notch signaling pathways are involved in the chondrogenesis of MSC-mediated treatments. MSCs may also exert effective immunoregulatory and paracrine effects to stimulate tissue repair. Therapy with extracellular vesicles containing cytokines, which are secreted by MSCs, might be a potential treatment for OA.



## Introduction

Osteoarthritis (OA) is one of the most common chronic joint diseases, with prominent symptoms caused by many factors. However, current medical interventions for OA have resulted in poor clinical outcomes, demonstrating that there are huge unmet medical needs in this area. Cell therapy has opened new avenues of OA treatment. Different sources of mesenchymal stromal cells (MSCs) may have different phenotypes and cellular functions. Pre-clinical and clinical studies have demonstrated the feasibility, safety and efficacy of MSC therapy. Mitogen-activated protein kinase (MAPK), Wnt and Notch signaling pathways are involved in the chondrogenesis of MSC-mediated treatments. MSCs may also exert effective immunoregulatory and paracrine effects to stimulate tissue repair. Therapy with extracellular vesicles (EVs) containing cytokines, which are secreted by MSCs, might be a potential treatment for OA.

## OA. and Cell Therapy

### *OA and epidemiology*

In the 16th century, with the rapid development of anatomy, knowledge about joints and surrounding basic structures—including articular cartilage, ligaments that stabilize joints and synovial fluid within joints—solidified, and the clinical disease causing joint pain and stiffness was named OA, literally referring to inflammation of bones and joints [1,2]. OA is caused by many factors, such as degenerative damage to articular cartilage and reactive hyperplasia of joint edges and subchondral bone, which causes slowly developing joint pain, tenderness, stiffness, swelling and deformities, along with limited mobility. OA is the most common type of chronic joint disease and affects all structures of the joint [3,4]. Statistics indicate that there were 303 million patients with OA worldwide in 2017 [5]. The prevalence of symptomatic knee OA (Kellgren and Lawrence score ≥2 and knee pain) in China in 2018 was 8.1%; the prevalence was higher in women than men, and there were obvious regional differences (data from the China health and pension follow-up survey database, China Health and Retirement Longitudinal Study).

### *Clinical manifestations and pathogenesis*

OA is the most common chronic disabling disease affecting elderly individuals. Joint pain and loss of function are the main clinical features of OA. Joint pain and tenderness are the most common clinical manifestations of OA, occurring in 36.8–60.7% of patients. Pain can occur in any joint but is most common in the hips, knees and interphalangeal joints [6]. In addition, OA can present with clinical

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symptoms such as limited joint movement, joint deformity, creaking sensations in the bone and muscle atrophy. Diagnosis depends on both clinical and imaging features. Three typical manifestations of OA on x-rays are asymmetrical joint space narrowing in affected joints, subchondral bone sclerosis and/or cystic degeneration and osteophyte formation at the joint edges. However, nearly half of patients with imaging features of OA are asymptomatic, and more than half of patients without imaging features of OA exhibit symptoms [3]. OA is often symptomatic and has been diagnosed in the late stages of the disease process, with poor treatment results.

OA is a process of not only joint cartilage erosion but also joint tissue remodeling due to various causes, including obesity, joint instability and trauma [7]. Some researchers describe the joint as an organ of common significance, and extensive pathological changes in OA are considered "joint failure". Joint tissue hyperplasia is one of the most significant early features of the disease and includes articular cartilage thickening, chondrocyte proliferation, increased matrix synthesis, subchondral bone densification with low mineralization and increased bone marrow fat content [8]. The relationship between OA and hypertension, hypercholesterolemia and blood glucose suggests that systemic and metabolic components are involved; thus, OA is also considered a systemic musculoskeletal disease (Figure 1) [9].

*OA treatment*

Although many treatment methods for OA have been developed, the clinical results of most are unsatisfactory because cartilage tissue cannot fundamentally be repaired [10]. Many guidelines list paracetamol as the treatment of choice, and other nonsteroidal anti-inflammatory drugs as well as corticosteroid injections and tramadol help relieve symptoms in many patients with OA [11]. Because OA is a chronic disease, long-term medication also confers a series of risks [12]. In addition to traditional anti-inflammatory drugs, researchers have developed new drugs, including trans-capsaicin injection (CNTX-4975) [13] and lutikizumab [14], a humanized IL-1$\alpha/\beta$ dual variable domain immunoglobulin that simultaneously blocks IL-1$\alpha$ and IL-1$\beta$. In addition, investigators have tried to promote tissue repair methods such as MSC injection and platelet-rich plasma (PRP) [15,16]. For advanced hip or knee OA, joint replacement is an effective treatment option. However, an implant is an artificial joint with a certain life span and is not always a once-in-a-lifetime therapy. According to structural risk assessments, the life span of such implants is 25 years [17,18].

*Cell therapy and its application in cartilage injury*

Cell therapy refers to the use of certain cells with specific functions that are obtained by biological engineering methods or through *in vitro* expansion, special culture systems and other treatments. These cells have enhanced immune responses, killed pathogens and tumor cells and promoted tissue and organ regeneration. The use of cell therapy in many refractory diseases is very promising, and it is also one of the most promising strategies for treating tumors. Some researchers have envisaged that by constructing T cells expressing chimeric antigen receptors—synthetic receptors that fuse tumor-specific extracellular antibody fragments with signal chains from primary T-cell receptors—they can target certain molecules to eradicate difficult to treat B-cell malignancies [19]. Since the introduction of induced pluripotent stem cell (iPSC) technology a decade ago, great progress has been made in stem cell biology and regenerative medicine. Researchers have treated Parkinson's disease with cell therapy through allografts of embryonic stem cells or iPSCs [20]. Type 1 diabetes, which usually affects children, is an autoimmune disease that can cause progressive destruction of pancreatic islet $\beta$ cells, eventually leading to loss of insulin secretion, which in turn leads to hyperglycemia. Stem cell therapy promotes a new treatment model by allowing the development of islet $\beta$ cells [21]. Cell therapy also shows considerable therapeutic prospects for spinal muscular atrophy [22], primary immunodeficiency, metabolic diseases and hemoglobin diseases [23], in addition to the aforementioned diseases.

MSCs have chondrogenic capacity and have recently been considered a potential tool for OA repair [24]. Intra-articular injection of MSCs can replace autologous chondrocyte implantation for repair of joint surface injuries [25]. The use of iPSC technology, which can generate cells that can self-renew and produce any somatic cell type for an extended time, may overcome these problems. Other iPSC cartilage generation strategies, including tissue engineering techniques to simulate the human cartilage development microenvironment, are also being improved. In this review, the authors summarize the characteristics of MSCs; the differences between various types of MSCs; the clinical research progress made on the use of MSCs for treating OA; the underlying mechanisms of action, including the signaling pathways involved in treating OA; and the latest results of *in vitro* and *in vivo* experiments.

## MSCs for OA Treatment

*Pre-clinical experimental model of MSCs for OA treatment*

In 2003, Murphy *et al.* [26] used a single direct intra-articular injection of autologous bone marrow-derived MSCs (BM-MSCs) suspended in a sodium hyaluronate solution to treat goats in a model of unilateral knee OA. Twenty weeks after the injection, the medial meniscus had regenerated significantly in the injected joints, and the implanted cells were detected in the newly formed tissue. In addition, compared with the joints treated with cell-free carriers alone,

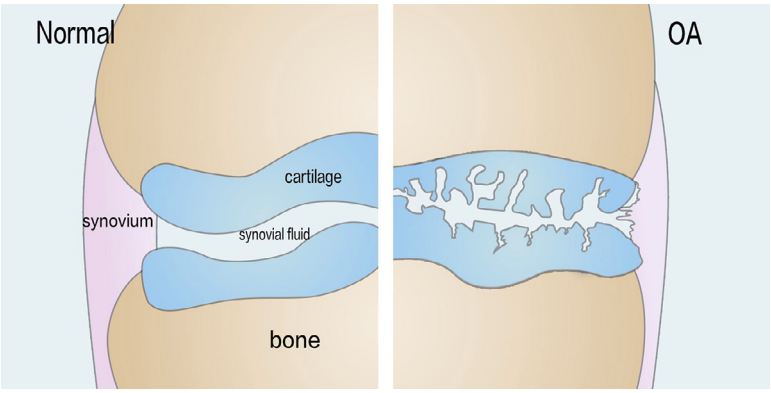


**Fig. 1.** . OA is a systemic musculoskeletal disease exhibiting a process of joint tissue remodeling. Joint tissue hyperplasia includes articular cartilage thickening, chondrocyte proliferation, increased matrix synthesis, subchondral bone densification with low mineralization and increased bone marrow fat content.

the injected joints exhibited decreased articular cartilage degeneration, osteophyte formation and subchondral sclerosis. Since then, in multiple animal models of cartilage defects and anterior cruciate ligament transection (ACLT), the MSC treatment group has shown improvements in cartilage healing both histologically and morphologically (Table 1).

The types of injected cells have also been diversified from the initial BM-MSCs to adipose-derived MSCs (AD-MSCs), umbilical cord-derived MSCs (UC-MSCs), human amniotic membrane-derived MSCs (hAMSCs)/human chorionic membrane-derived MSCs, etc. In a dog model of knee arthritis established in 2018, researchers injected allogeneic UC-MSCs ($1 \times 10^6$ cells), and on day 3, day 7, day 14 and day 28 after treatment, they observed the structure of the dogs' knee joints by magnetic resonance imaging (MRI), ultrasound and x-ray imaging [35]. The high signal in the MRI T2-weighted images of dogs in the treatment group decreased, the echo-free space in the B-mode images basically disappeared and the continuous linear low-echo area of the pulley groove thickened. X-rays showed that the jagged defect in the sacrum of dogs in the treatment group had improved, and the low-density gap between the sacrum and the ventral side of the sacrum gradually increased. After 35 days of treatment, the neocartilage in dogs in the treatment group exhibited visible new tissue, with fibers arranged more irregularly than those in the existing tissues. In addition, more vacuoles were observed in the cartilage of dogs in the untreated control group, but no collagen fibers were seen in this cartilage, and the thickness of the neocartilage was greater in dogs in the treatment group.

Other investigators transplanted hAMSCs into the non-cartilage tissue of mice or implanted them into collagen defects in rat bones to detect type II collagen and cartilage proteoglycans, demonstrating that hAMSCs can differentiate into cartilage both *in vitro* and *in vivo* [47]. In addition to the aforementioned cells, human meniscus stem/progenitor cells (hMeSPCs) have been found to exhibit the characteristics of MSCs and high expression levels of collagen II. Intra-articular injection of hMeSPCs induced meniscus defects in rats 4 weeks after meniscectomy and induced significantly more new tissue formation and extracellular matrix (ECM) deposition than the control treatment [48]. Twelve weeks after meniscectomy, compared with the control treatment, intra-articular injection of hMeSPCs reduced surface irregularities and the expression of OA-labeled collagen I and X but maintained high collagen II expression.

However, in many animal studies of intra-articular injection of MSCs, heterogeneous contaminants (such as fetal calf serum) have caused significant adverse clinical reactions, and such contaminants should be removed. More importantly, in one study involving repeated intra-articular injections of allogeneic MSCs, adverse clinical reactions were seen with a second injection administered 4 weeks after the first, most likely because of adaptive immune activation of allogeneic MSCs [37]. Therefore, researchers studied the rat OA model and found that the treatment of cartilage defects in rats with exosomes injected with heterologous MSCs was associated with complete recovery of cartilage and subchondral bone, which was characterized by the presence of hyaline cartilage with good surface regularity and complete integration with the adjacent cartilage [49]. ECM deposition was very similar to that observed in the age-matched nonsurgical control group. By contrast, only fibrous repair tissue was found in the group with contralateral phosphate-buffered saline-treated defects. This study demonstrated for the first time the efficacy of human embryonic MSC exosomes in cartilage repair.

With the continuous development of tissue engineering and three-dimensional printing technology, it seems that a new solution has been found for large cartilage defects, which were difficult to repair in the past. The combination of MSCs and biomaterials not only promotes the recovery of the cartilage layer but also promotes the regeneration of subchondral bone. In 2017, Chinese researchers seeded autologous BM-MSCs into polyglycolic acid (PGA)/polylactic acid (PLA) scaffolds, which were used to repair articular cartilage defects after 2–12 weeks of cartilage induction. The results showed that in terms of cartilage quality and function, the repaired cartilage in the 4th and 8th week groups reached a level similar to that of natural articular cartilage [46]. Zhang *et al.* [47] seeded MSCs into three-dimensional printed poly-$\varepsilon$-caprolactone scaffolds and found that MSCs could increase the regeneration and mechanical strength of fibrocartilage tissue, providing a functional method of protecting articular cartilage from osteochondral damage after total meniscectomy.

### *Clinical trials of MSCs for OA treatment*

The aforementioned successful pre-clinical experimental animal model studies led to the start of many clinical trials (Table 2). In numerous prospective randomized controlled clinical trials, the autologous BM-MSCs and AD-MSCs treatment groups achieved significant improvements in Tegner, Lysholm and International Knee Documentation Committee scores and visual analog scale pain scores [50,51]. Unlike BM-MSCs, autologous AD-MSCs are generally obtained from the abdominal fat, which is more convenient for surgery and less damaging to patients. Regarding the injected cell dose, Jo *et al.* [52] constructed three dose gradients of autologous AD-MSCs: low ($1 \times 10^7$ cells), medium ($5 \times 10^7$ cells) and high ($1 \times 10^8$ cells). The groups treated with high doses had significantly improved Western Ontario and McMaster Universities Osteoarthritis Index scores; clinically significant reductions in pain; regeneration of thick, transparent cartilage by histology; and no significant adverse reactions.

Intra-articular injection of human UC-MSCs (once monthly for 2 months) and intra-articular injection of sodium hyaluronate (once weekly for 5 weeks) have also been compared [58]. The two groups exhibited no significant differences in knee joint function scores and SF-36 scale scores after 1 and 2 months of treatment. However, the scores of the cell therapy group were significantly better than those of the control group at 3 and 6 months after treatment. Similar results were observed in a clinical trial with a 7-year follow-up period [59]. Human UC-MSCs showed histological manifestations of hyaline cartilage formation at 1 year of treatment, and MRI at 3 years showed that the regenerating cartilage persisted over the previous 7 years without bone or tumor formation. In addition, intra-articular injection of allogeneic placenta-derived MSCs significantly improved knee OA pain, symptoms, activities of daily living, quality of life, sport and recreation factors and clinical indicators of knee range of motion. During the 24 weeks of follow-up in another study, no differences were observed in evidence of tissue damage, tumor formation, pulmonary embolism or liver or kidney damage [63]. Pain was reduced, but the effects on activities of daily living and symptom improvement began to decrease after 8 weeks of treatment. Therefore, the authors believe that multiple injections may improve and prolong the therapeutic effect of placenta-derived MSCs because OA is a continuous process of joint destruction. Compared with BM-MSCs and AD-MSCs, placenta-derived MSCs and UC-MSCs have two major advantages: (i) they have similar regenerative potential but low immunogenicity and (ii) they are easily available by non-invasive methods.

Regarding MSC scaffolds, the literature has reported the use of PRP and hyaluronic acid (HA). Platelets contain more than 1500 biologically active proteins [69]. These diverse compounds include growth factors, peptide hormones, chemokines, fibrin and proteins with antibacterial and fungicidal properties. However, PRP is limited by variability in its preparation and the amount of biologically active factors it contains. In addition, platelet counts can vary based on the donor's age, health status, hydration and sex. In addition, PRP contains factors—namely, vascular endothelial growth factor (VEGF)—that may adversely affect joints and MSCs. The benefits of HA may go beyond its role as a scaffold. Pre-clinical studies have observed that

**Table 1**
MSC-based treatments in pre-clinical large animal models of OA.

| Species | Animal model | Cell source | Dose, cells | Scaffold | Outcome |
|---|---|---|---|---|---|
| Goat [26] | | | | ACLT + meniscectomy for 6 weeks | Allogeneic BM-MSCs |
| $2 \times 10^6$ | HA | Articular cartilage degeneration, osteophytic remodeling and subchondral sclerosis were reduced in cell-treated joints. | | | |
| Pig [27] | Partial-thickness cartilage defect | Autologous BM-MSCs | $9 \times 10^5$ | HA | At 12 weeks, the Wakitani scores showed marked improvement in the quality of repair tissue in the MSC-treated group. |
| Rabbit [28] | Full-thickness osteochondral defect | Allogeneic synovial MSCs | $1 \times 10^7$ | – | The histological score of the treated group was consistently better at 4 weeks, 12 weeks and 24 weeks than that seen in the control group. Treatment with the MSCs suspension promoted cartilage regeneration. |
| Rabbit [29] | Osteochondral defect | Allogeneic BM-MSCs | $1 \times 10^7$ | OPF (oligo(poly(ethylene glycol) fumarate))/ GMP + TGF-$\beta$1 | Defects were filled with hyaline cartilage-like tissue with zonal organization and intense glycosaminoglycan staining. |
| Guinea pig [30] | Spontaneous OA | Human MSCs | $7 \times 10^6$ | HA | At 5 weeks post-transplantation, partial cartilage repair was noted in the HA-MSC group, with type II collagen around both residual chondrocytes and transplanted MSCs in the OA cartilage. |
| Rabbit [31] | ACLT for 8 weeks | Allogeneic AD-MSCs | $2 \times 10^6$ | HA | Eight weeks after ACLT, loss of cartilage was observed in both the medial and lateral condyles, whereas the cartilage matrix was predominantly retained in AD-MSC-treated knees. |
| Rabbit [32] | MMR | Xenogeneic UC-MSCs | $3.5 \times 10^6$ | – | Early IA injection was effective in preventing OA signs in rabbit knees following MMR. UC-MSCs targeted the synovium and modulated the gene expression pattern of synoviocytes to promote an anti-catabolic environment. |
| Dog [33] | ACLT for 2 months | Autologous AD-MSCs | $1 \times 10^7$ | PRP | The levels of ECM-related genes increased through upregulation, whereas protein expression levels of inflammatory cytokines were decreased through the inhibitory effects of PRP and MSCs on chondrocyte apoptosis and inflammatory cytokine production. |
| Horse [34] | Non-osteochondral defect | Autologous/ allogeneic BM-MSCs | $1 \times 10^7$ | – | Repeated IA injection of allogeneic MSCs resulted in adverse clinical reactions after a second injection administered 4 weeks after the first. It is most likely because of adaptive immune activation of allogeneic MSCs |
| Dog [35] | Patellar and femoral condyle cartilage defect | Allogeneic UC-MSCs | $1 \times 10^6$ | – | Canine UC-MSCs promoted the repair of cartilage and patellar injury in OA, improved healing of the surrounding tissues and reduced the inflammatory response. |
| Horse [36] | OA of the metacarpophalangeal/ metatarsophalangeal joints | Allogeneic UC-MSCs | $1 \times 10^7$ | – | At the end of the study, five horses returned to their intended level of use, eight returned to a lower level and nine remained lame. The mechanism of action of MSCs is currently thought to be an immunomodulatory one rather than any regenerative effects brought about by the MSCs' differentiation into chondrocytes |
| Rabbit [37] | Bilateral section of ACL | Allogeneic BM-MSCs | $1 \times 10^6$ | – | A single injection of MSCs was not enough to restore the condition of joints with OA. This is in contrast to multiple injections of MSCs, which had the ability to replace lost cells as well as reduce inflammation. |
| Porcine [38] | Full-thickness chondral injury of knees | Human UC-MSCs | $5 \times 10^6$ | HA | The treated knees showed significant gross and histological improvements in hyaline cartilage regeneration compared with the control knees. The International Cartilage Repair Society histological score was higher for the treated knees than the control knees. |
| Sheep [39] | Unilateral medial meniscectomy | Autologous BM-MSCs | – | HA | BMC-HA (autologous Bone Marrow Concentrate) treatment showed greater inhibition of OA progression compared with MSC-HA, leading to a reduction in inflammation of cartilage, meniscus and synovium. Both MSC and BMC combined with HA reduced inflammation and contributed to switch-off fibrotic and hypertrophic processes. |
| Porcine [40] | ACL transection | Human BM-MSCs | – | Collagen scaffold | At 5 months after implantation, significant differences in the quality of the regenerated tissue were found between the human BM-MSC-embedded scaffold group and the control group. Newly generated tissue was only observed at the site of implantation with the human BM-MSC-embedded scaffolds. |
| Dog [41] | CR | Autologous BM-MSCs | $5 \times 10^6$ | – | No adverse events after BM-MSCs treatment were detected. Circulating CD8+ T lymphocytes were lower after BM-MSCs injection. Systemic and intra-articular injection of autologous BM-MSCs in dogs with partial CR suppressed systemic and stifle joint inflammation, including CRP concentrations. |

(*continued on next page*)

**Table 1** (*Continued*)

| Species | Animal model | Cell source | Dose, cells | Scaffold | Outcome |
|---|---|---|---|---|---|
| Rabbit [42] | ACLT | Human UC-MSCs | – | – | Application of UC-MSCs and CAM enhanced not only the structure and synthesis of proteoglycan and collagen type II but also anti-inflammatory effects in both joint and synovial fluid. |
| Dog [43] | Bilateral elbow OA obtained by diagnostic imaging | Allogeneic AD-MSCs | $5 \times 10^5$ | – | Analyses of SF and ROM were performed on day 0, day 14 and day 42. Concentration levels of MMP-3, TIMP-1, IL-6 and TNF-$\alpha$ in SF showed significant differences before and after treatment ($P < 0.05$). There was a significant improvement in ROM between day 0 and day 42 ($P < 0.001$). |
| Pig [44] | Two cylindrical defects at the weight-bearing area of the medial and lateral condyles of the femur | Autologous BM-MSCs | $6 \times 10^6$ | PGA/PLA scaffold | BEC-*vitro* demonstrated a time-dependent maturation process. The implantation of BEC-*vitro* alone successfully realized tissue-specific repair of osteochondral defects with both cartilage and subchondral bone. Furthermore, the maturity level of BEC-*vitro* had significant influence on the repair results. |
| Rabbit [45] | Total medial meniscectomy of the left knee | Autologous BM-MSCs | $5 \times 10^6$ | Poly $\varepsilon$-caprolactone scaffold | Seeding MSCs in the PCL scaffold increased fibrocartilaginous tissue regeneration and mechanical strength, providing a functional replacement to protect articular cartilage from damage after total meniscectomy. |
| Porcine [46] | Unilateral ACL transection | Human BM-MSCs | $1 \times 10^7$ | – | This study demonstrated the ability of human BM-MSCs to survive and express transgenes within the knee joint of porcine hosts without immunosuppression for at least 2 weeks. |

ACL, anterior cruciate ligament; ACLT, anterior cruciate ligament transection; BEC-*vitro*, BM-MSC-engineered cartilage *in vitro*; CR, cruciate ligament rupture; CRP, C-reactive protein; IA, intra-articular; MMR, medial meniscal release; OPF (oligo(poly(ethylene glycol) fumarate)); ROM, range of motion; SF, synovial fluid.

by combining HA with fibroblast growth factor (FGF) 2, both synovial cell migration and chondrocyte migration can be enhanced [70].

To date, most published clinical trials have reported that MSC therapy is a safe and promising treatment for OA. In addition, researchers in Mexico reported a new type of cell-free bioactive preparation called BIOF2(producted by Esteripharma Mexico (Mexico City, Mexico)), a patented formula composed of corticosteroids, insulin and organic acids [68]. Injection in the knee joint showed a therapeutic effect on OA.

## Potential Mechanism of MSCs in OA Treatment

OA is a disease involving all tissues in the joint, especially the structural integrity of the articular cartilage. Its pathophysiology derives from various tissues, including cartilage, bone, synovium and capsular fibrous tissue. This concept replaces previous views centered on cartilage lesions and encourages exploration of the interactions between the various tissues that comprise joints.

### *Changes in chondrogenic phenotype, ECM and subchondral bone lesions in OA*

Articular chondrocytes are stationary, mature cells that maintain the homeostasis of adult articular cartilage by preserving cell survival and the delicate balance between anabolism and catabolism. In OA, articular chondrocytes respond to the accumulation of noxious biochemical and biomechanical damage by transforming into hypertrophic cells, accompanied by abnormal matrix production and increased aggrecanase and collagenase activity. These pathological changes form a vicious cycle that leads to irreversible damage to cartilage structure and function [71].

Overall dysregulation of the chondrocyte transcription network has been observed in OA. OA chondrocytes are activated by exposure to an abnormal biochemical/biomechanical environment. The signals leading to phenotypic transformation of OA chondrocytes include nuclear factor-kB (NF-$\kappa$B) signals and the effects of the transcription factors *SOX9*, *HIF-2*$\alpha$ and runt-related transcription factor 2 (*RUNX2*). Some studies have reported that *SOX9*, *COL2A1* and *ACAN* messenger RNA levels decrease with increasing severity of OA [72] and that the increased expression of *SOX9* in early disease is associated with decreased production of ADAMTSs (newly identified metalloproteinases) and increased expression of *COL2A1*, cartilage oligomeric protein and *ACAN* [73]. These abnormally expressed mediators have anti-anabolic and pro-catabolic functions in chondrocytes and can disrupt the *SOX9*-CBP/p300 interaction [74,75]. NF-$\kappa$B signaling pathways also play a central role in cartilage degradation [76] and articular chondrocyte differentiation to hypertrophic cells [77]. IKK$\beta$ and IKK$\alpha$, kinases that control the canonical and non-canonical NF-$\kappa$B pathways, respectively, are also necessary for inducing chondrocyte hypertrophy *in vitro*. In summary, stress and pro-inflammatory signals activate canonical NF-$\kappa$B pathways, and by disrupting key regulatory networks, OA drifts toward hypertrophic changes.

Recent research has identified changes in *SIRT1*, *DOT1L* and other histone modifications in OA articular chondrocytes [78,79]. DNA methylation is the main mechanism by which cells maintain a stable phenotype. This process converts 5-methylcytosine to 5-hydroxymethylcytosine (5hmC). Evidence indicates that 5hmC is not only an intermediate product of the demethylation process but also an independent epigenetic marker that facilitates gene transcription and a "ready-to-go" epigenetic state [80]. IL-1$\beta$ can increase the level of 5hmC in OA chondrocytes through TET1 [81]. Many genes related to OA, such as matrix metalloproteinase (MMP) 3 in OA cartilage, may be regulated by a gain in 5hmC. Total methylation is increased and expression of transcription factors is decreased in OA chondrocytes compared with healthy chondrocytes [82].

**Table 2**
Clinical studies of MSCs for OA treatment.

| Sample size (indications) | Study design | Cell source | Dose, cells | Scaffold | Outcome |
|---|---|---|---|---|---|
| One (degenerative joint disease) [53] | Follow-up visit at 24 weeks after IA injection | Autologous BM-MSCs | $4.56 \times 10^7$ | HA | The patient had statistically significant cartilage and meniscus growth, as assessed by MRI, as well as increased ROM and decreased modified VAS pain score. |
| 50 (mild to moderate knee OA) [50] | Injection with MSC concentrate along with arthroscopic debridement | Autologous BM-MSCs | – | – | Overall OA outcome score, especially QoL, was improved. |
| Four (moderate to severe knee OA) [54] | IA injection of cultured MSCs | Autologous BM-MSCs | $8–9 \times 10^6$ | – | Walking time and pain improved for three patients and remained unchanged for one patient. |
| 25 (knee OA) [51] | IA injection of cultured MSCs combined with arthroscopic debridement | Autologous AD-MSCs | $1.89 \times 10^6$ | PRP | The mean Lysholm, Tegner activity scale and VAS scores of patients in the study group improved significantly. |
| 12 (unicompartmental knee OA) [55] | IA injection of MSCs with HA 3 weeks after HTO and microfracture | Autologous BM-MSCs | $1.5 \times 10^7$ | HA | Treatment was effective in improving both short-term clinical and MOCART outcomes. |
| 12 (chronic knee pain) [56] | Follow-up visit 1 year after IA injection | Autologous BM-MSCs | $4 \times 10^7$ | – | Patients exhibited rapid and progressive improvement (approaching 65–78% after 1 year) on algo-functional indices. |
| 18 (knee OA) [52] | IA injection in three dose-escalation cohorts and a follow-up visit at 6 months after high-dose injection | Autologous BM-MSCs | $1–10 \times 10^7$ | – | High-dose IA injection improved knee joint function and pain without causing adverse events and reduced cartilage defects via regeneration of hyaline-like articular cartilage. |
| 55 (partial medial meniscectomy) [25] | Single injection of cultured MSCs 7–10 days after meniscectomy | Allogeneic BM-MSCs | $(5–15) \times 10^7$ | HA | Treated patients had significant reductions in pain and significant increases in meniscal volume. |
| 18 (symptomatic severe knee OA) [57] | 6 months of follow-up after a single intraarticular injection | Autologous AD-MSCs | $2–50 \times 10^6$ | – | The procedure was found to be safe and resulted in clinical improvement, with a reduction in pain levels and WOMAC scores in all three groups. |
| 36 (moderate or severe degenerative knee OA) [58] | 6 months of follow-up after injection | Allogeneic UC-MSCs | $2–3 \times 10^7$ | – | In the cell treatment group, Lysholm score at 1–6 months after treatment and WOMAC and SF-36 scale scores at 2–6 months after treatment were significantly improved, and no recurrence of knee pain was observed. |
| Seven (knee OA with full-thickness cartilage defects) [59] | 7 years of follow-up after injection | Allogeneic UC-MSCs | $1.15–2 \times 10^7$ | HA | The improved clinical outcomes were stable over 7 years of follow-up. There were no incidences of osteogenesis or tumorigenesis over the 7 years of follow-up. |
| 17 (unilateral ACL reconstruction) [60] | 26 weeks of follow-up after injection | Allogeneic MPCs | $7.5 \times 10^7$ | HA | The MPC + HA group showed greater improvements in KOOS pain scores, symptoms, ADLs and SF-36 bodily pain scores; reduced medial and lateral tibiofemoral joint space narrowing; less tibial bone expansion; and a trend toward reduced tibial cartilage volume loss. |
| 18 (knee OA) [61] | 96 weeks of follow-up | Autologous AD-MSCs | $1–5 \times 10^7$ | – | IA injections of human AD-MSCs were safe and improved knee joint pain, function and cartilage volume. The group treated with the dose of $5 \times 10^7$ AD-MSCs exhibited the greatest improvement. |
| 30 (knee OA) [62] | 4 years of follow-up | Autologous BM-MSCs | $1–10 \times 10^7$ | HA | No adverse effects were reported after BM-MSCs administration or during follow-up. BM-MSC-treated patients improved according to the median value (IQR) of the VAS score. |
| 20 (knee OA) [63] | 24 weeks of follow-up | Allogeneic PL-MSCs | $5–6 \times 10^8$ | – | Allogeneic PL-MSCs improved clinical measures of pain, symptoms, ADLs, QoL, S/R factors and knee ROM, but improvements were sustained until only 8 weeks post-treatment. |
| 30 (knee OA) [64] | 12 months of follow-up | Autologous AD-MSCs | $1 \times 10^8$ | – | The AD-MSC-treated groups showed clinically significant improvements in pain and function at the end of the 12-month follow-up period. Radiological analysis indicated modification of disease progression. |
| 40 (knee OA) [65] | 52 weeks of follow-up after injection | UC–MSCs | $2 \times 10^7$ | HA | Only MSC-treated patients experienced significant pain and functional improvements from baseline ($P = 0.001$). In a phase 1/2 trial (NCT02580695), repeated UC-MSC treatment was safe and superior to active comparator in knee OA at 1-year follow-up. |

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**Table 2** (*Continued*)

| Sample size (indications) | Study design | Cell source | Dose, cells | Scaffold | Outcome |
|---|---|---|---|---|---|
| 3 (knee OA) [66] | One patient lost to follow-up at 2 years and three patients followed for 5 years | Autologous BM-MSCs | $8–9 \times 10^6$ | – | All parameters improved in transplant knees at 6 months (walking time, stair climbing, gelling pain, patellar crepitus, flexion contracture and VAS pain score). The patients then started to gradually deteriorate, but at 5 years they were still better than at baseline. |
| 30 (knee OA) [67] | 12 months of follow-up | Autologous BM-MSCs | $1–10 \times 10^7$ | HA | A single intra-articular injection of *in vitro*-expanded autologous BM-MSCs, together with HA, was a safe and feasible procedure that resulted in a clinical and functional improvement in knee OA, especially when $100 \times 10^6$ cells were administered (NCT02123368). |
| 43 (knee OA) [68] | 6 months of follow-up | Autologous BM-MSCs | $4 \times 10^7$ | Human serum albumin | This randomized, triple-blind, placebo-controlled RCT demonstrated the safety and efficacy of a single intra-articular implantation of $40 \times 10^6$ autologous MSCs in patients with knee OA. Intra-articular implantation of MSCs provided significant and clinically relevant pain relief over 6 months versus placebo. |

ADLs, activities of daily living; HTO, high tibial osteotomy; IA, intra-articular; IQR, interquartile range; KOOS, Knee Injury and Osteoarthritis Outcome Score; MOCART, magnetic resonance observation of cartilage repair tissue; MPCs, mesenchymal precursor cells; PL-MSCs, placenta-derived MSCs; QoL, quality of life; RCT, randomized controlled trial; ROM, range of motion; S/R, sport and recreation; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

Abnormal activation of OA chondrocytes and the drift toward hypertrophy induce the secretion of abnormal types and quantities of matrix proteins and increase the expression and activity of matrix-degrading enzymes, which irreversibly changes the structural and functional integrity of articular cartilage [71,83]. During the development of OA, articular chondrocytes express matrix proteins that do not belong to the normal cartilage matrix, such as type X collagen. MMP-13 and collagenase-3 are the major cartilage-degrading collagenases [84]. Overexpression of these enzymes leads to characteristic changes in OA in mice, and results demonstrate that excessive MMP-13 activity can result in articular cartilage degradation and joint pathology of the kind observed in OA [85]. Similar to other proteases, MMP-13 is regulated at multiple levels, including transcription, activation and suppression. Chondrocytes can produce various tissue inhibitors of matrix metalloproteinases (TIMPs)—specific inhibitors of MMPs. TIMPs are present in articular cartilage and specifically balance and regulate the activity of matrix-degrading enzymes. In addition, TIMPs are considered good therapeutic targets for altering disease progression and structural damage [86,87]. In OA chondrocytes, MMP-13 gene expression can occur through mechanical stress and the actions of inflammatory cytokines and adipokines, which signal through different signaling cascades, including the MAPK cascade. These pathways affect a complex transcriptional network that includes transcription factors such as *RUNX2*, *CEBPβ*, *ELF3*, *HIF-2α*, CREB and ATF. Transactivation of MMP-13 in articular chondrocytes is also regulated by epigenetics [88].

In OA, osteoblasts in subchondral bone respond to hypoxia in numerous ways. For example, osteoblasts upregulate *HIF-1α*. When osteoblasts are hypoxic (at oxygen partial pressures of 35–40 mmHg), the expression profile of their cytokines, proteins and growth factors (e.g., VEGF, insulin-like growth factor [IGF] II, transforming growth factor [TGF]-$\beta$1, type I collagen and TIMP-1) changes. These changes, which are related to bone remodeling and increased cartilage degeneration, are hallmarks of histopathological OA [89]. In addition, OA osteoblasts express high levels of cytokines related to osteogenesis, especially osteocalcin, alkaline phosphatase and IGF-1, and are involved in inducing remodeling of the subchondral plate and subchondral trabecula. In one study, OA osteoblasts induced chondrocytes to acquire an OA-associated matrix mineralization and hypertrophy phenotype [90]. In turn, normal subchondral osteoblast formation was inhibited, and chondrocytes isolated from OA joints were co-cultured with osteoblasts to significantly activate ERK 1/2 phosphorylation, suggesting that OA chondrocytes may avoid MAPK signaling pathway-induced changes in the formation of subchondral bone osteoblasts [91]. However, whether subchondral bone thickening precedes the occurrence of cartilage fibrosis remains controversial.

### *Synovial lesions in OA*

Usually, the joint synovium is only a few cell layers thick and is composed mainly of fibroblast-like synovial cells (FLSs). Proliferation of synovial cells and synovitis are among the main characteristics of OA. Histological analysis of the OA synovium shows that characteristic presentations in most OA patients are infiltration of immune cells such as dendritic cells (DCs), macrophages, natural killer (NK) cells and T cells [92]; synovial hyperplasia; MMP and ADAMTS production; and enhanced macrophage activity. Moreover, OA FLSs undergo phenotypic changes. Some researchers believe that the synovial reaction in OA is characterized by fibrosis accompanied by increased reactivity to TGF-$\beta$ [93]. Any damage to the meniscus or subchondral bone results in the release of ECM fragments, such as molecular fragments (e.g., fibronectin, collagen type II and cartilage oligomeric protein), with increased MMP and ADAMTS activity. At the same time, such fragments of molecules acts as a damage-associated molecular pattern to further activate FLSs via integrin or Toll-like receptor pathways [94]. Activated OA FLSs can secrete cytokines such as IL-1,

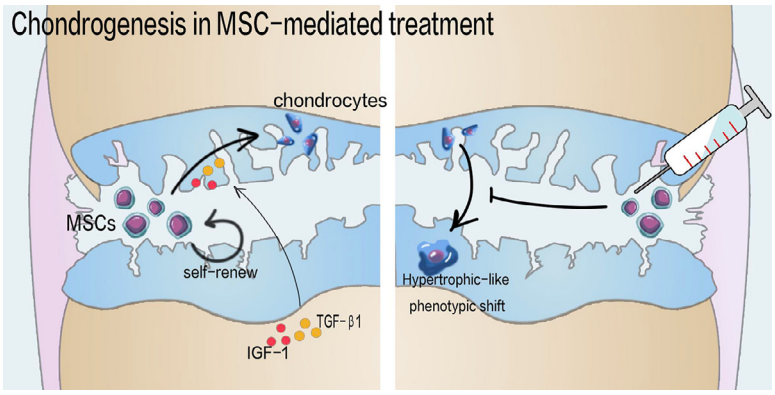


**Fig. 2.** . Chondrogenesis in MSC-mediated treatment. The synergistic effect of TGF-$\beta$1 and IGF-1, which is mediated by the MAPK and Wnt signaling pathways, can stimulate cartilage formation. Stimulation with certain cytokines, such as TGF-$\beta$, FGF2, FGF9 and FGF18, and selection of the most controlled and chondrogenic cell subpopulation may serve as the approaches to address the limiting utility of MSCs in cartilage repair.

tumor necrosis factor alpha (TNF-$\alpha$) and IL-6 and can activate a variety of cellular signaling pathways, including the IL-6/STAT3 signaling pathway and Toll-like receptor surface receptor signaling, causing further cellular infiltration and angiogenesis while also providing catabolic signals to articular chondrocytes, thus stimulating articular chondrocytes [95] and damaging bone and cartilage [96,97].

In addition to these damage-related molecules, accumulating evidence indicates that factors directly related to the cartilage disease process affect the synovium, thereby promoting specific synovial reactions in FLSs. Indeed, Wnt/$\beta$-catenin pathway inhibition was recently shown to reduce the severity of disease in a model of traumatic OA by inhibiting not only the degradation of chondrocytes but also fibrosis mediated by synovial fibroblasts [98]. These data not only solidify the concept of the synovial membrane but also reveal the mechanism by which FLSs are involved in the development of OA. Moreover, these findings may facilitate the development of new treatment strategies for OA.

### *Chondrogenesis in MSC-mediated treatment*

MSCs can form cells of the mesoderm lineage and can differentiate into three lines of osteoblasts, chondrocytes and adipocytes. In *in vitro* experiments, multiple approaches to steer MSC differentiation in the direction of cartilage formation have been identified. The synergistic effect of TGF-$\beta$1 and IGF-1, which is mediated by the MAPK and Wnt signaling pathways, can stimulate cartilage formation [99,100]. *In vitro*, MSC-derived chondrocytes can produce type II collagen and proteoglycan, similar to mature adult chondrocytes [100]. Other molecules found to steer MSC differentiation toward chondrocytes include dexamethasone [101] and some bone morphogenetic proteins, mainly bone morphogenetic protein 7 [102] and FGF2 [103].

Notch signaling is overactivated during OA progression, and intra-articular treatment with normal MSCs downregulates the Notch signaling pathway [104]. The transient receptor potential vanillin 4 ion channel, a calcium ion-permeable channel, has also been found to play an important role in the progression of mouse OA [105]. In addition, studies have shown that in animal models of OA, intra-articular injection of MSCs may inhibit the expression of MMP-13, TNF-$\alpha$ and IL-1$\beta$ [106].

However, the reduction in cartilage cells and inadequate nutrient supply, coupled with the inefficiency of the true cartilage-type hyaluronic ECM produced by MSCs, result in the inability to perform functional healing responses, thereby limiting the utility of MSCs in cartilage repair [107]. In addition, mineralized nodules are produced in *in vitro* cultures of MSCs [108], and after ectopic implantation in mice, MSC clusters have been shown to gradually evolve from a phenotype of mineralization and angiogenesis to one of trabecular bone formation [109]. Stimulation with certain cytokines is one approach to addressing these key limitations. TGF-$\beta$-mediated inhibition of differentiation and hypertrophy in nascent cartilage is necessary for 7 weeks. However, alkaline phosphatase, type X collagen, parathyroid hormone 1 receptor, MMP13, VEGF and *RUNX2* are simultaneously upregulated [110,111]. Alternatively, continuous exposure to FGF2, FGF9 and FGF18 enhances the tendency of MSCs to differentiate into cartilage and prevents further development into a terminal hypertrophic phenotype [112]. Another strategy is to select the most controlled and chondrogenic cell subpopulation. In a mouse model of collagen-induced arthritis, intra-articular injection of CD146+ MSCs more strongly inhibited Th17 cell activation and promoted cartilage protection compared with CD146− cells [113]. In addition, synovial membrane-derived MSCs rich in CD271, CD73 or CD105 have also proven to be a better choice for the treatment of focal chondropathy compared with other MSCs (Figure 2) [114–116].

### *Immunoregulatory and paracrine effects of MSC treatment*

MSCs not only aid tissue regeneration but also exert beneficial effects through immunoregulatory and paracrine mechanisms and thus the control of disease processes. MSCs exert effective immunoregulatory and anti-inflammatory effects by regulating multiple cytokines, such as prostaglandin E2, interferon gamma and interleukins [117,118]. Prostaglandin E2 secreted by MSCs can promote the production of immunosuppressive IL-10 by binding to EP2 and EP4 receptors on macrophages and participate in the regulation of CD4+ effector T cells [119]. In addition, MSCs have been shown to inhibit T-cell proliferation and induce T-cell apoptosis, thereby stimulating phagocytic cells to produce TGF-$\beta$ and increasing the number of regulatory T cells [120]. MSCs also regulate innate immunity by inhibiting DC maturation and reducing NK cell cytotoxicity [121]. Moreover, MSCs can change the polarization of macrophages from the pro-inflammatory (M1) phenotype to the anti-inflammatory (M2) phenotype [122]. MSCs can interact with macrophages and inhibit their activation and the secretion of IL-1$\beta$, TGF-$\alpha$ and other inflammatory factors [52].

In addition to exhibiting immunoregulatory and differentiation potential, MSCs express essential cytokines, including TGF-$\beta$, VEGF, epidermal growth factor and a range of mediators that stimulate local tissue repair. Bioactive molecules promote chondrocyte proliferation and ECM deposition and repair damaged bone and cartilage [123]. Moreover, the secretion of cytokines by mature chondrocytes can in turn promote the differentiation of MSCs into chondrocytes. Most recent studies have shown that MSCs participate in tissue repair by regulating local inflammation, apoptosis and cell proliferation through mainly paracrine mechanisms rather than via direct

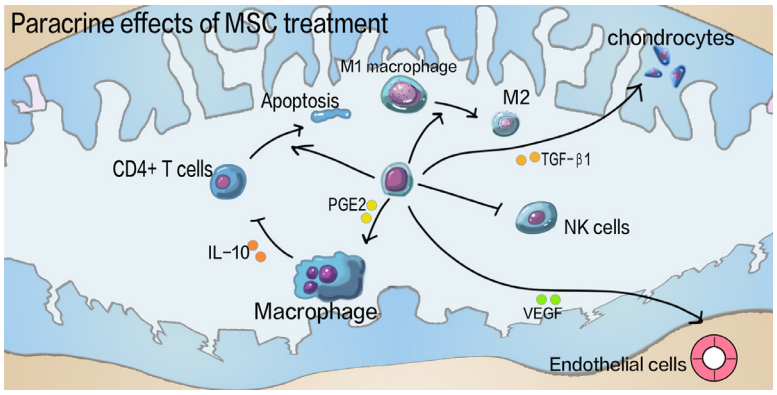


**Fig. 3.** . Immunoregulatory and paracrine effects of MSC treatment. MSCs exert effective immunoregulatory and anti-inflammatory effects by regulating multiple cytokines, such as PGE2, IFN-$\gamma$ and ILs. PGE2 secreted by MSCs can promote the production of immunosuppressive IL-10 by binding to EP2 and EP4 receptors on macrophages. MSCs also regulate innate immunity by inhibiting DC maturation and reducing NK cell cytotoxicity. MSCs can change the polarization of macrophages from the pro-inflammatory (M1) phenotype to the anti-inflammatory (M2) phenotype. In addition to exhibiting immunoregulatory and differentiation potential, MSCs express essential cytokines, including TGF-$\beta$, VEGF and EGF, and a range of mediators that stimulate local tissue repair. EGF, epidermal growth factor; IFN-$\gamma$, interferon gamma; ILs, interleukins; PGE2, prostaglandin E2.

differentiation into chondrocytes. The potential paracrine effects of MSCs in OA treatment are summarized in Figure 3.

*Exosomes from MSCs*

Recently, the therapeutic potential of cytokine secretion by MSCs has been attributed to the release of exosomes. Exosomes are defined as nanoscale extracellular lipid bilayer vesicles derived from endocytosis. They transfer lipids, proteins and different kinds of RNAs, including regulatory circular RNAs and microRNAs (miRNAs), between cells. They fuse with the plasma membrane of target cells, are internalized by endocytosis or interact with cell surface receptors to activate intracellular signaling pathways. More and more studies have shown that the paracrine mechanism of MSC is through exosomes [124].

First, exosomes from MSCs (MSC-exos) can promote articular cartilage regeneration and improve ECM. Some researchers combined human iPSC-MSC-exos with photoinduced imine crosslinking hydrogels to form acellular tissue patches—EHG tissue patch (hiPSC-MSCs-derived exosomes, HA-NB and Gelatin), that could fix exosomes and exert a positive role in cellular regulation and also implanted EHGs into the cartilage-deficient site in a rabbit model, finding after 12 weeks that the EHGs could accurately permeate and repair the cartilage defect [125]. Recent studies have found that BM-MSC-exo transplantation upregulates the SOX9 and Wnt7a genes related to chondrocyte regeneration and significantly downregulates the expression of genes related to hypertrophic chondrocyte differentiation, such as RUNX2, COL10A1 and ALP [126]. With regard to ECM, MSC-exos can promote glycosaminoglycan synthesis influenced by IL-1$\beta$ and inhibit the production of nitric oxide and MMP-13 induced by IL-1$\beta$ [31].

Second, MSC-exos have shown an immunosuppressive effect in many diseases. They can inhibit pro-inflammatory cytokines such as IL-1$\beta$, IL-6 and TNF-$\alpha$. In addition, exosomes may induce Th1 cells to transform into Th2 cells and inhibit their ability to differentiate into Th17 cells [127]. Moreover, MSC-exos may play a beneficial role in the progression of OA through specific miRNAs. One study has shown that MSC-exos can reverse pathological inflammation through three specific miRNAs: miRNA21, miRNA-146a and miRNA-181c [128]. Exosome miRNA-125a can promote angiogenesis by targeting Dll4 [129]. MiRNA-100-5p inhibits mechanistic target of rapamycin autophagy as well as chondrocyte apoptosis by balancing anabolism and catabolism [130]. In the rat model, MSC exogenous miRNA-135b can promote cartilage repair by regulating TGF-$\beta$ [131].

However, the amount of miRNA in MSC-exos is relatively low and is affected by cell type, cell viability and cell environment. The exosomes of human embryonic stem cells are rich in pre-miRNA. By contrast, mature MSCs contain more mature miRNA than immature MSCs [132].

## Summary and Outlook

*Problems that remain to be solved*

Despite the promise of MSCs for the treatment of OA, many issues have yet to be resolved. First, the mechanism of OA requires further clarification. Approaches to mimicking joint biomechanical changes have been used in numerous studies, suggesting that biomechanical forces play a key role in regulating cartilage growth and maintaining physiological cartilage homeostasis. Many other studies have shown that the inflammatory microenvironment at the time of OA development, including the synovial inflammation characterized by structural cartilage changes, is characterized by infiltration of immune cells, such as DCs, macrophages, NK cells and T cells. These effects may be the result of mild inflammation caused by metabolic syndrome, innate immunity or aging-related inflammation. This possibility requires further study.

Second, many questions remain about the biological behavior of MSCs. The precise microlocalization of MSCs *in vivo* remains a topic of debate. When tissue from a specific organ, such as bone marrow, is cultured, it can proliferate and differentiate into adherent cells with mature mesenchymal phenotypes, such as adipocytes, osteoblasts and chondrocytes. If these adherent cell populations meet the required standards, they can technically be called MSCs; however, whether these cells are representative of MSCs *in vivo* is undetermined. The properties of cells removed from the body are likely to change under culture conditions that do not mimic those found in their original niche [133]. With regard to inflammation, MSCs are thought to exhibit a dual behavior pattern in inflamed tissues; that is, they exhibit a unique ability to transform from a pre-inflammatory phenotype to an anti-inflammatory phenotype and interfere with innate and adaptive immune responses. Through this mechanism, MSCs promote homoeostasis between these opposing pathways [134].

Finally, the treatment of OA with MSCs focuses mainly on (i) cell sources (autologous/allogeneic), (ii) doses (tens to millions of MSCs), (iii) delivery systems (joint or surgical MSC stent transplantation/intra-articular MSC injection), (iv) number of injections and (v) combinations of MSCs with co-stimulatory factors (PRP, HA or growth factors). In addition, regarding the effectiveness and safety of MSC treatment, many qualitative and often subjective clinical measures are currently used to evaluate its efficacy, and data obtained from these measures may not truly represent the efficacy of treatment [135]. Although many

researchers were initially concerned about MSC therapy, systematic reviews of clinical trials have shown that intravascular and intra-articular injections of MSCs are relatively safe; however, their long-term therapeutic efficacy and safety need further research. In addition, more reliable studies with larger sample sizes and randomized controls are needed to obtain more supportive evidence and to fully standardize and optimize the use of MSCs for the treatment of OA disease.

*Prospects*

In terms of cell preparation procedures, differentiation potential and long-lasting effects of MSCs, the best sources of MSCs are still speculative. Therefore, standardized cell sources and preparation methods are essential to assess the outcomes of regenerative therapy. Five MSC products have entered the market, but only two—Cartistem (producted by Medipost Co., Ltd. (Seongnam, South Korea)) and Stempeucel (producted by Stempeutics Research Pvt. Ltd (Bangalore, India))—have been approved as advanced therapy medicinal products for clinical application in the European Union.

Recently, the therapeutic potential of cytokine secretion by MSCs has been attributed to the release of EVs. EVs consist of small particles from the endolysosomal system and include exosomes (50–150 nm) and large vesicles (100–500 nm) that bud from the plasma membrane. EVs transfer lipids, proteins and different kinds of RNAs, including regulatory circular RNAs and miRNAs, between cells. Compared with similar cell-based drugs, the ultimate acellular therapy has multiple advantages, including lower product variability; higher repeatability; simpler storage; and better safety, dosage regimens and potency. In addition, this therapy can be tested by approaches similar to those used to test drugs [136].

MSC/biomaterial constructs for articular cartilage tissue engineering have also been designed. Among protein-based biomaterials, type I and type III collagens have been extensively tested because of their low immunogenicity and ability to mimic the proper structure of the natural cartilage microenvironment and support the adhesion, proliferation and differentiation status of MSCs. Fibrin is another protein-based biomaterial widely used clinically because of its immunocompatibility. Although fibrin is characterized by low mechanical strength and rapid biodegradation, it can effectively support the proliferation of MSCs and their differentiation into cartilage [137]. In addition, silk fibroin is a biopolymer with superior biomechanical properties compared with those of synthetic polymers, with low immunogenicity after transplantation *in vivo*. Synthetic polymers, including PLA, PGA, PLA and PGA co-polymers, polylactide-coglycolide and poly-ε-caprolactone biomaterials such as lactone and polyethylene glycol, have good biocompatibility and biodegradability [138].

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## Author Contributions

Conception and design of the study: WW. Acquisition of data: XZ, JH and WW. Analysis and interpretation of data: XZ, JH and WW. Drafting or revising the manuscript: XZ. All authors have approved the final article.

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