Potential of Exosomes as Cell-Free Therapy in Articular Cartilage Regeneration: A Review

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Abstract: Treatment of cartilage defects such as osteoarthritis (OA) and osteochondral defect (OCD) remains a huge clinical challenge in orthopedics. OA is one of the most common chronic health conditions and is mainly characterized by the degeneration of articular cartilage, shown in the limited capacity for intrinsic repair. OCD refers to the focal defects affecting cartilage and the underlying bone. The current OA and OCD management modalities focus on symptom control and on improving joint functionality and the patient’s quality of life. Cell-based therapy has been evaluated for managing OA and OCD, and its chondroprotective efficacy is recognized mainly through paracrine action. Hence, there is growing interest in exploiting extracellular vesicles to induce cartilage regeneration.

In this review, we explore the in vivo evidence of exosomes on cartilage regeneration. A total of 29 in vivo studies from the PubMed and Scopus databases were identified and analyzed. The studies reported promising results in terms of in vivo exosome delivery and uptake; improved cartilage morphological, histological, and biochemical outcomes; enhanced subchondral bone regeneration; and improved pain behavior following exosome treatment. In addition, exosome therapy is safe, as the included studies documented no significant complications. Modifying exosomal cargos further increased the cartilage and subchondral bone regeneration capacity of exosomes. We conclude that exosome administration is a potent cell-free therapy for alleviating OA and OCD. However, additional studies are needed to confirm the therapeutic potential of exosomes and to identify the standard protocol for exosome-based therapy in OA and OCD management.

Keywords: extracellular vesicle, exosome, chondrocyte, cartilage, osteoarthritis

Introduction

Osteoarthritis (OA) is a common disease linked to mobility-related disability. In 2020, an estimated 654 million people worldwide had knee OA.1 Rising obesity and population aging are the main contributors to the increasing prevalence and incidence of OA.2 OA is a chronic inflammatory disease that causes the deterioration of articular cartilage, which leads to joint pain and stiffness.3 OA is not confined to the articular cartilage, but also affects the synovium, subchondral bone, and joint ligaments.4 Osteochondral defect (OCD) develops when cartilage lesions caused by OA affect the subchondral bone.5 However, OCD can also be caused by traumatic injury.6

Currently, no treatment can halt the progression of OA. However, treatments are available for relieving the symptoms. Treatments for OA can be classified into non-pharmacologic, pharmacologic, surgical, and alternative therapies such as regenerative therapy. Non-pharmacologic treatments are recommended for OA caused by...
modifiable risk factors (e.g., obesity). Weight loss is associated with improvement in cartilage structure by reducing the joint load. Exercise has also been proven to relieve pain and improve muscle strength in OA patients. Nevertheless, these treatments are more effective for lower limb OA. Pain is the hallmark symptom of OA. Therefore, pharmacologic treatment is mainly used for managing pain. The classical pain-relieving treatments for OA include acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs). However, the usage of these drugs increases the risk of gastrointestinal and cardiovascular toxicity. The surgical approach is preferred when conservative treatments are not effective. Although most patients have improved quality of life after surgery, potential complications such as pain, infection, and poorer knee function must be taken into consideration when opting for surgical intervention. In addition, knee replacement implants have limited lifespans and may fail eventually. OCD treatment can be categorized into non-surgical and surgical. Non-surgical treatment includes rest, joint immobilization, and pain-relieving medication. OCD can also be corrected surgically by removing the lesion, microfracture drilling, retrograde drilling, antegrade drilling, cancellous bone graft implantation, osteochondral transplantation, and autologous chondrocyte implantation.

Regenerative therapy is a rapidly growing approach used for treating OA and OCD due to conventional therapies being unsatisfactory, only managing to provide symptom control and short-term functional improvement. The conventional therapies fail to address the underlying problem of cartilage and osteochondral bone loss. Several preclinical studies have proven that intra-articular injection of mesenchymal stem/stromal cells (MSCs) enhances cartilage and meniscal tissue regeneration, and slows OA progression by attenuating synovial membrane inflammation. Similarly, a few preclinical studies have reported OCD regeneration by MSCs. Clinically, intra-articular injection of adipose-derived MSCs (AD-MSCs) is safe and produces significant functional improvement in OA and OCD patients.

Recently published systematic reviews have shown that MSC therapy is safe and can improve pain and joint function significantly, but the improvement in cartilage regeneration, based on structural assessment by magnetic resonance imaging (MRI), is not statistically significant. Although no major adverse effects have been reported, we should remain aware of the possible risks associated with cell therapies. These include differentiation into undesirable cell types or tissues and the promutagenic effect of MSCs. The transplantation of autologous AD-MSCs caused renal fibrosis and inflammatory cell infiltration in the interstitium of a patient with chronic kidney disease, and the reason might be the differentiation of the multipotent stem cells recruited by the MSCs into myofibroblasts or the differentiation of the transplanted MSCs into myofibroblasts. A paradigm shift has recently occurred, as MSCs and their conditioned medium were found to have similar therapeutic effects. Many papers have summarized that the primary mechanism of action of MSC therapy is paracrine signaling via extracellular vesicles (EVs). The use of EVs can mitigate the risk of transdifferentiation of transplanted MSCs into the wrong cells in response to the local milieu while retaining the beneficial therapeutic effects exerted by MSC paracrine secretion. Furthermore, the use of EVs can also minimize the risk of rejection of donor stem cells and tumor formation (especially for embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs)).

EVs can be categorized into three groups based on their biogenesis, i.e., microvesicles, exosomes, and apoptotic bodies. Lai et al. found that the exosome is the active component in MSC-conditioned medium. Exosomes are nanosized intraluminal vesicles in multivesicular bodies (MVBs) secreted by various cell types upon MVB fusion with the plasma membrane. They play a major role in cell–cell communication by transferring their contents, including proteins, lipids, and nucleic acids. MSC-derived exosomes have drawn much attention recently for their broad therapeutic effect on various diseases such as myocardial infarction, liver fibrosis, cutaneous wound healing, and OA. A recent study reported that exosomes aid bidirectional signaling between MSCs and chondrocytes for chondrogenesis, ECM deposition, and cell proliferation, suggesting that they are important in communication between native heterogeneous cell populations of cartilage tissue, and thus are the crucial paracrine factors in cartilage repair.

Apart from identifying the biogenesis pathway, a concrete means of distinguishing the EV subtypes is absent due to the lack of techniques for purifying and specific markers for the EV subtypes. Moreover, the size of EVs is heterogeneous and overlap among the subtypes. In fact, the generic term “extracellular vesicles” or “EVs” should be used. In the present systematic review, the
term “exosomes” essentially refers to 50–200 nm EVs, without demonstration of their origin and/or purity, with regard to the nomenclature used by the authors.

Recently, several systematic reviews reported the potential of using MSC-derived EVs to promote cartilage regeneration in preclinical in vivo studies. However, EVs derived from other cell sources have also been used for promoting cartilage regeneration. Thus, the present review was aimed at summarizing the broad literature and evidence on the effects of exosome therapy derived from all cell sources on cartilage repair. Only results from preclinical in vivo studies were considered.

**Literature Search, Article Selection and Data Extraction**

The literature search was conducted from December 30, 2020, to January 6, 2021, on the PubMed and Scopus databases using the following keywords: (exosome OR extracellular vesicle) AND (cartilage OR osteoarthritis OR osteochondral). We included all preclinical and clinical studies reporting the safety and efficacy of EVs on cartilage regeneration. Studies reporting only in vitro findings were excluded. We also excluded secondary literature, studies only available in abstract form, conference/proceeding papers, letters to the editor, theses, and articles written in languages other than English. Figure 1 shows the article identification and selection process. In brief, a total of 160 articles from PubMed and 284 articles from Scopus were identified. After deduplication and title and abstract screening, 79 articles were obtained for full-text screening. Finally, a total of 29 articles published between 2016 and 2021 were included in this review. All included articles reported in vivo findings without any clinical data. Two reviewers (C.Y.N. and J.Y.C.) conducted the literature search, article selection, and data extraction independently. The methodological quality of the studies was assessed using the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) risk of bias tool. Meta-analysis was not performed due to the lack of quantitative data for pooling because of the

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**Figure 1** Flow diagram of article selection process.
heterogeneity in the scoring systems and exosome isolation methods used. Thus, the results extracted were analyzed qualitatively.

**Results and Discussion**

**Quality of Studies**

The included studies were graded using the SYRCLE risk of bias assessment tool.\textsuperscript{55} Table 1 summarizes the risk of bias analysis. There is no high risk of bias among the 29 included studies. All the included studies have low risk of selective outcome for reporting bias and other sources of bias were not detected. However, only nine studies (31.0%) were assigned low risk of bias and 20 studies (69.0%) had unclear risk of bias for baseline characteristics (ie animal species, age, gender, and weight) in selection bias. Furthermore, all studies showed unclear risk in most of the domains, including sequence generation and allocation concealment for selection bias, random housing and blinding for performance bias, random outcome assessment and blinding for detection bias, and incomplete outcome data for attrition bias. The lack of detailed documentation in the included articles resulted in a high unclear risk of bias and ambiguous methodological quality.

**Source of Exosomes**

Exosomes were extracted from a variety of cell sources. Seventeen studies used human exosomes,\textsuperscript{56–72} nine studies used murine exosomes,\textsuperscript{73–81} one study used rabbit exosomes,\textsuperscript{82} and two studies did not mention the cell origin (Table 2).

Regarding cell type, 25 studies reported the use of exosomes from stem cells, namely ESC-derived MSCs (ESC-MSCs),\textsuperscript{61,63,68–70} iPSC-derived MSCs (iPSC-MSCs),\textsuperscript{58,72} amniotic fluid stem cells (AFSCs),\textsuperscript{67} and other adult stem cells, including bone marrow-derived MSCs (BM-MSCs),\textsuperscript{56,57,59,71,73–76,78,83,84} synovial membrane-derived MSCs (SM-MSCs),\textsuperscript{50,62,72} infrapatellar fat pad-derived MSCs (IPFP-MSCs),\textsuperscript{64} umbilical cord-derived MSCs (UC-MSCs),\textsuperscript{65,66} polydactyly BM-derived MSCs (pBM-MSCs),\textsuperscript{71} and commercial MSCs of unknown tissue origin.\textsuperscript{79} Exosomes from primary chondrocytes,\textsuperscript{81} platelet-rich plasma (PRP),\textsuperscript{82} dendritic cells,\textsuperscript{77} and serum\textsuperscript{80} were also studied.

Exosomes have similar biological functions as parental cells.\textsuperscript{39,85} Most of the studies included in this review used MSCs as the source of exosomes due to the predominant therapeutic benefits of MSCs in tissue repair and regeneration. MSCs have self-renewal, differentiation, anti-apoptotic, anti-fibrotic, pro-mitotic, anti-oxidative, and immunomodulatory properties.\textsuperscript{86–88} Besides, MSCs can be harvested from many tissue sources using minimally invasive techniques.\textsuperscript{89} MSCs can also be expanded easily for many passages without significant changes in characteristics and functionality, and produce more exosomes.\textsuperscript{90,91} Currently, the most ideal cell source of exosomes for promoting cartilage regeneration remains ambiguous. In the present review, only two studies compared the efficacy of exosomes secreted by different cell sources.\textsuperscript{71,72} Zhu et al\textsuperscript{72} used exosomes secreted by iPSC-MSCs and SM-MSCs, and found that iPSC-MSC-secreted exosomes were more effective in supporting cartilage regeneration. Neotissue of the iPSC-MSC-derived exosome-treated group presented typical hyaline features and intense type II collagen staining in the superficial and deep zones of cartilage tissue, which were comparable to the healthy cartilage in the control group. On the other hand, the SM-MSC-derived exosome-treated group exhibited moderate cartilage repair and very weak type II collagen staining in the superficial cartilage zone, but the results were nevertheless better compared to the untreated cartilage in the OA group. Zhou et al\textsuperscript{71} reported that pBM-MSC-secreted exosomes were more potent in facilitating cartilage repair compared to those secreted by BM-MSCs, as demonstrated by the lower Osteoarthritis Research Society International (OARSI) scores. Exosomes secreted by MSCs of different tissue sources show distinctive therapeutic results, as the exosomal cargo varies according to the tissue origin.\textsuperscript{92–94} The therapeutic potential of exosomes secreted by MSCs isolated from different tissues has been summarized in the reviews of Álvarez-Viejo,\textsuperscript{95} Nikfarjam et al,\textsuperscript{96} Yin et al,\textsuperscript{97} and Tang et al.\textsuperscript{98}

**Modification of Exosomal Cargo for Enhancing Efficacy**

Exosomes mediate cell–cell communication by transporting bioactive lipids, proteins, and RNAs, including mRNAs, and non-coding RNAs such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs).\textsuperscript{99} We identified 15 studies that only used exosomes derived from naïve cells.\textsuperscript{58,61,63,67–75,81,82,84} All naïve exosomes promoted the repair and regeneration of damaged cartilage. Zhang et al\textsuperscript{69,70} reported that exosomal CD73 from naïve ESC-MSCs contributed to cartilage repair by inducing AKT and ERK phosphorylation in chondrocytes. Chen
### Table 1 Summary of Risk of Bias Analysis Using SYRCLE Tool

| References       | Selection Bias | Baseline Characteristics | Allocation Concealment | Random Housing | Blinding | Random Outcome Assessment | Blinding | Incomplete Outcome Data | Selective Outcome Reporting |
|------------------|----------------|--------------------------|------------------------|----------------|---------|----------------------------|---------|-------------------------|-------------------------------|
| Zhang et al \(^68\) | Unclear        | Unclear                  | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Cosenza et al \(^44\) | UnPearl        | Unclear                  | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Liu et al \(^18\)  | Unclear        | Unclear                  | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Tao et al \(^60\)  | Unclear        | Low risk                 | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Wang et al \(^11\)  | Unclear        | Unclear                  | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Zhu et al \(^72\)  | Unclear        | Unclear                  | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Mao et al \(^59\)  | Unclear        | Unclear                  | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Wang et al \(^79\)  | Unclear        | Low risk                 | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Zhang et al \(^79\)  | Unclear        | Unclear                  | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Chen et al \(^73\)  | Unclear        | Unclear                  | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Liu et al \(^82\)  | Unclear        | Low risk                 | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Wu et al \(^64\)  | Unclear        | Unclear                  | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Zhang et al \(^70\)  | Unclear        | Low risk                 | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Zheng et al \(^81\)  | Unclear        | Unclear                  | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Chen et al \(^86\)  | Unclear        | Unclear                  | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| He et al \(^75\)  | Unclear        | Unclear                  | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Jin et al \(^76\)  | Unclear        | Low risk                 | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Jin et al \(^77\)  | Unclear        | Low risk                 | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Liang et al \(^77\) | Unclear        | Low risk                 | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Liu et al \(^78\)  | Unclear        | Low risk                 | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Qiu et al \(^83\)  | Unclear        | Unclear                  | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Wong et al \(^83\)  | Unclear        | Low risk                 | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Yan and Wu \(^66\) | Unclear        | Unclear                  | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Zavatti et al \(^67\) | Unclear        | Unclear                  | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Zhang et al \(^84\)  | Unclear        | Low risk                 | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Zhou et al \(^71\)  | Unclear        | Unclear                  | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Wang et al \(^80\)  | Unclear        | Unclear                  | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Wang et al \(^72\)  | Unclear        | Unclear                  | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| Yan et al \(^65\)  | Unclear        | Unclear                  | Unclear                | UnPearl        | Unclear | Unclear                   | Unclear | Unclear                 | Low risk                       |
| References       | Cell Sources | Cell Types                      | Exosome Isolation                             | Exosome Characterization                      |
|------------------|--------------|---------------------------------|------------------------------------------------|-----------------------------------------------|
| Zhang et al\(^{68}\) | Human        | HuES9 ESC-MSCs                  | Size fractionation and TFF                     | NTA: Homogeneously sized with modal size of 100 nm |
|                  |              |                                 | Exosomes were stored at −20 °C until use       | Western blot: Positive for CD81, ALIX, and TSG101 |
|                  |              |                                 |                                                | RNA detection: Majority less than 300 nucleotides |
| Cosenza et al\(^{74}\) | Murine      | BM-MSCs from C57BL/6 mice       | Ultracentrifugation                           | DLS: 96 nm                                     |
|                  |              |                                 |                                                | NTA: 112 ± 6.6 nm                              |
|                  |              |                                 |                                                | Flow cytometry: Positive for CD9 and CD81      |
| Liu et al\(^{68}\) | Human        | iPSC (iPSC-01)-MSCs             | Ultracentrifugation and ultrafiltration       | TEM: Spherical morphology; 30 to 60 nm         |
|                  |              |                                 |                                                | TRPS: 50 to 150 nm                             |
|                  |              |                                 |                                                | Western blot: Positive for CD9, CD63, and CD81 |
| Tao et al\(^{60}\) | Human        | Normal SM-MSCs and miR-140-5p-overexpressing SM-MSCs from knee joint synovial membrane tissue of 30 to 35 years old donors | Ultrafiltration and ultracentrifugation on 30% sucrose/D2O cushion Used freshly | TEM: Hollow spherical microvesicles |
|                  |              |                                 |                                                | DLS: 30 to 150 nm                              |
|                  |              |                                 |                                                | Western blot: Positive for CD63, CD9, CD81, and ALIX |
|                  |              |                                 |                                                | RT-qPCR: Increased miR-140-5p expression level in miR-140-5p overexpressed SM-MSC-Exos |
| Wang et al\(^{61}\) | Human        | Male H1 ESC-MSCs                | Ultracentrifugation                            | TEM: Round lipid bilayer vesicles; 38 to 169 nm |
|                  |              |                                 | Exosomes were stored in PBS at −80 °C          | Western blot: Positive for CD63 and CD9        |
| Zhu et al\(^{72}\) | Human        | iPSC (C1P33)-MSCs               | Ultrafiltration                                | TEM: Cup or round-shaped                        |
|                  |              |                                 | The exosomes aliquot was stored −80 °C or used | TPRS: 50 to 150 nm                              |
|                  |              |                                 | freshly                                        | Western blot: Positive for CD9, CD63, and TSG101 |
| Mao et al\(^{59}\) | Human        | SM-MSCs from 3 donors (2 males and 1 female, age range 22 to 28 years) | Ultrafiltration                                | TEM: Cup or round-shape                        |
|                  |              |                                 | The exosomes aliquot was stored −80 °C or used | TPRS: 50 to 150 nm                              |
|                  |              |                                 | freshly                                        | Western blot: Positive for CD9, CD63, and TSG101 |
| Mao et al\(^{59}\) | Human        | Normal BM-MSCs and miR-92a-3p-overexpressing BM-MSCs from 6 donors (3 males and 3 females, ranged of 32 to 38 years old) | Ultrafiltration                                | TEM: Cup-shaped or round morphology; 50 to 150 nm |
|                  |              |                                 | The exosomes aliquot was stored −80 °C or used  | NTA: 50 to 150 nm                              |
|                  |              |                                 | freshly                                        | Western blot: Positive for CD9, CD63, CD81, and HSP70 |
| Wang et al\(^{79}\) | Murine       | Normal MSCs, TGF-β1 stimulated MSCs and miR-135b inhibited MSC from SD rats | Exosome extraction kit                         | TEM: Hollow-spherical morphology              |
|                  |              |                                 |                                              | Western blot: Positive for CD63, CD9, and CD81 |
|                  |              |                                 |                                              | higher CD81 and ALIX levels in TGF-β1-MSC-Exos |
|                  |              |                                 |                                              | RT-qPCR: Increased miR-135b expression level in TGF-β1-MSC-Exos |

(Continued)
| References     | Cell Sources          | Cell Types                                                                 | Exosome Isolation                                                                 | Exosome Characterization                                                                 |
|---------------|-----------------------|----------------------------------------------------------------------------|---------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| Zhang et al   | Human                 | Immortalized E1-MYC 16.3 ESC-MSCs                                           | Size fractionation and TFF Exosomes were stored at −20 °C until use              | Size determination: Homogenously sized particles; 100 nm model size Western blot: Positive for CD81, ALIX, and TSG101 Flotation density: 1.10 to 1.19 g/mL |
| Chen et al    | Murine                | BM-MSCs from articular cartilage from femoral condyles and tibial plateau of C57BL/6 mice | Ultrafiltration and ultracentrifugation on 30% sucrose/D2O cushion              | TEM: Hollow spherical vesicles DLS: 40 to 110 nm in diameter Western blot: Positive for TSG101, CD9, and CD63 |
| Liu et al     | Rabbit                | PRP isolated from whole blood of New Zealand white rabbits                  | Membrane-based affinity binding step using exoEasy Maxi Kit Exosomes were stored at −80 °C until used | TEM: Round-shaped morphology NTA: 145.6 ± 50.4 nm Western blot: Positive for CD9, CD63, CD81, and HSP101 |
| Wu et al      | Human                 | IPFP-MSCs from patients with primary knee OA                                | Precipitation (ExoQuick reagent kit) or ultrafiltration Exosomes were stored at −80 °C for further use | No significant differences between exosomes isolated from precipitation and ultrafiltration TEM: Sphere-shaped bilayer membrane structure about 100 nm in diameter NTA: 121.9 nm Western blot: Positive for CD81, CD9, and CD63 Flow cytometry with CiO-labeling: 30 nm to 150 nm |
| Zhang et al   | Human                 | Immortalized E1-MYC 16.3 ESC-MSCs                                           | Size fractionation and TFF Exosomes were stored at −20 °C until use              | Size determination: 100 to 200 nm Western blot: Positive for CD81, ALIX, and TSG101 Flotation density: 1.10 to 1.19 g/mL |
| Zheng et al   | Murine                | Primary chondrocytes from knee articular cartilage of 5 to 6 days old C57BL/6 mice cultured in standard or inflammatory environment (degenerative chondrocytes) | Ultrafiltration and ultracentrifugation with 30% sucrose/D2O                    | TEM: Hollow and spherical-like morphology DLS: 40 to 110 nm Western blot: Positive for TSG101, CD9, and CD63 Mass spectrometry: 2409 and 2077 proteins were identified in primary chondrocytes and degenerative chondrocytes, respectively |
| Chen et al    | Human                 | Normal BM-MSCs and miR-136-5p-overexpressed BM-MSCs from femur bone marrow of traumatized patients | Ultrafiltration and ultracentrifugation with 30% sucrose/D2O                    | TEM: Hollow spherical microvesicles DLS: 50 to 150 nm Western blot: Positive for CD63, CD9, CD81, and ALIX; but very low TSG101 RT-qPCR: Upregulated miR-136-5p expression level in miR-136-5p overexpressed BM-MSC-Exos |

(Continued)
| References | Cell Sources | Cell Types | Exosome Isolation | Exosome Characterization |
|------------|--------------|------------|-------------------|--------------------------|
| He et al\(^{75}\) | Murine | BM-MSCs from SD rats | Ultracentrifugation | TEM: Oval in shape  
DLS: 153 nm  
Western blot: Positive for flotillin-1,  
TSG101, and CD63; negative for calnexin |
| Jin et al\(^{76}\) | Murine | Normal BM-MSCs and miR-9-5p overexpressed BM-MSCs | Ultracentrifugation  
Exosomes were stored at \(-80^\circ\text{C}\) | TEM: Round or tea-shaped with outer membrane; 40 to 100 nm  
Western blot: Positive for CD9,  
TSG101, and CD63; negative for calnexin |
| Jin et al\(^{77}\) | Human | Normal BM-MSCs and miRNA-26a-5p overexpressed-BM-MSCs from ilium of healthy volunteers | Precipitation (ExoQuick-TC reagent kit) | TEM: Round or oval-shaped; 30 to 100 nm  
DLS: 50 to 100 nm  
Western blot: Positive for CD63,  
Hsp70, and CD9  
RT-qPCR: Upregulated miR-26a-5p expression level in miR-26a-5p overexpressed BM-MSC-Exos |
| Liang et al\(^{77}\) | Murine | CAP-Lamp2b dendritic cells | Ultracentrifugation  
Exosomes were stored at \(-80^\circ\text{C}\)  
miR-140 mimic is introduced into exosomes by electroporation | TEM: Cup-shaped morphology; 40 to 200 nm  
NTA: 40 to 200 nm  
Western blot: Positive for CD63 and CD9  
Confocal microscope: Fluorescence signals of CAP-GFP-Exos |
| Liu et al\(^{78}\) | Murine | Normal BM-MSCs from SD rats and BM-MSCs treated with kartogenin | TFF and HPLC  
Exosomes were stored at \(-80^\circ\text{C}\) | TEM: Cup or round-shaped morphology  
NTA: 50 to 200 nm  
Western blot: Positive for CD63 and CD81 |
| Qiu et al\(^{83}\) | Not reported | Normal BM-MSCs and curcumin pre-treated BM-MSCs | Ultrafiltration | TEM: Round-shaped morphology; 50 to 150 nm  
Western blot: Positive for CD9,  
CD63, and CD81 |
| Wong et al\(^{63}\) | Human | E1-MYC 16.3 ESC-MSCs | Size fractionation and TFF  
Exosomes were stored at \(-20^\circ\text{C}\) until use | Size determination: 100 to 200 nm  
Western blot: Positive for CD9,  
CD63, ALIX, and TSG101 |
| Yan and Wu\(^{66}\) | Human | UC-MSCs cultured in 2D and 3D (hollow-fiber bioreactor) | Ultracentrifugation  
Exosomes were stored at \(-80^\circ\text{C}\) until used | TEM: Cup-shaped morphology  
NTA: 120 nm  
Western blot: Positive for CD63,  
CD81, and TSG101; negative for calnexin |
| Zavatti et al\(^{67}\) | Human | AFSCs from pregnant women (mean age of 35.7) between the 16th and 17th weeks of gestation | Precipitation (total exosome isolation solution) | Western blot: Positive for CD9,  
CD63, CD81, and Rab5 |

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et al.\(^7\) suggested that BM-MSC-derived exosomes restored the mitochondrial dysfunction of degenerated chondrocytes by supplementing mitochondrial-related proteins. Another study noted that normal chondrocyte-derived exosomes carried proteins that involved in mitochondrial function and immune system process, which are important in alleviating OA progression.\(^3\) On the contrary, 14 articles modified and loaded exosomes with specific therapeutic agents, and reported enhanced therapeutic efficacy of cartilage regeneration compared to naïve

| References | Cell Sources | Cell Types | Exosome Isolation | Exosome Characterization |
|------------|--------------|------------|-------------------|-------------------------|
| Zhang et al.\(^8\) | Not reported | BM-MSCs | Ultrafiltration and ultracentrifugation with sucrose cushion Exosomes were stored at -80 °C or used freshly | TEM: Cup-like shape NTA: 140 nm Western blot: Positive for CD63, CD81, and CD9; negative for cerulean |
| Zhou et al.\(^7\) | Human | Polydactyly BM-MSCs | Precipitation (total exosome isolation kit) | TEM: 30 to 150 nm Western blot: Positive for CD63 and CD9 |
| | Human | BM-MSCs | Precipitation (total exosome isolation kit) | TEM: 30 to 150 nm Western blot: Positive for CD63 and CD9 |
| Wang et al.\(^6\) | Murine | OA serum from OA mice and sham serum from sham mice | Norgen’s proprietary resin-based purification (Serum Exosome Purification Mini Kit) ATF4-OA-Exos were constructed by introducing ATF4 mRNA into OA-Exos through electroporation. | TEM: Spherical-shaped morphology NTA: 0 to 150 nm Western blot: Positive for ALIX and CD63 RT-qPCR: Highest expression level of ATF4 in ATF4-OA-Exos, followed by OA-Exos and sham-Exos |
| | Human | Normal SM-MSCs and miR-155-5p-overexpressing SM-MSCs | Not reported | TEM: Cup-shaped morphology NTA: 100 to 120 nm Western blot: Positive for CD63 and CD81; negative for TFIIB RT-qPCR: 60-fold higher miR-155-5p expression in miR-155-5p overexpressed SM-MSC-Exos compared to SM-MSC-Exos |
| Yan et al.\(^6\) | Human | UC-MSCs cultured in static environment and rotary cell culture system | Ultracentrifugation Exosomes were stored at -80 °C | TEM: Cup-shaped morphology NTA: 120 nm Western blot: Positive for CD63, CD81, and TSG101; low calnexin RT-qPCR: Increased lncRNA H19 expression level in exosomes collected from cells cultured in rotary cell culture system |

Abbreviations: AFSC, amniotic fluid stem cell; BM-MSC, bone marrow-derived mesenchymal stem/stromal cell; CAP-Lamp2b, chondrocyte-affinity peptide-lysosome-associated membrane glycoprotein 2b; D2O, deuterium oxide; DLS, dynamic light scattering; ESC-MSC, embryonic stem cell-derived mesenchymal stem/stromal cell; Exo, exosome; IPFP-MSC, infrapatellar fat pad-derived mesenchymal stem/stromal cell; iPSC-MSC, induced pluripotent stem cell-derived mesenchymal stem/stromal cell; IncRNA, long non-coding RNA; miR, microRNA; MSC, mesenchymal stem/stromal cell; NTA, nanoparticle tracking analysis; PBS, phosphate-buffered saline; PRP, platelet-rich plasma; RT-qPCR, reverse transcription quantitative polymerase chain reaction; SD rat, Sprague Dawley rat; SM-MSC, synovial membrane-derived mesenchymal stem/stromal cell; TEM, transmission electron microscopy; TFF, tangential flow filtration; TGF-β1, transforming growth factor beta 1; TPRS, tunable resistive pulse sensing; UC-MSC, umbilical cord-derived mesenchymal stem/stromal cell.
Exosomal cargo can be modified via preconditioning of the exosome-secreting cells; incubating the exosome-secreting cells with the therapeutic cargo (e.g., drugs, proteins, RNAs, nanomaterials); transfection of the exosome-secreting cells; and physical treatments such as electroporation, sonication, extrusion, surfactant treatment, dialysis, and freeze-thawing.

The genomic molecules within exosomes aid the regulation of gene expression. Valadi et al. were the first to discover the presence of mRNAs and miRNAs in exosomes, and indicated that exosomes could modify the protein production and gene expression of target cells by transferring exosomal mRNAs or miRNAs. Wang et al. investigated the therapeutic effect of the activating transcription factor 4 (ATF4) gene in cartilage regeneration by introducing ATF4 mRNA into exosomes via electroporation. The effects of exosomes derived from OA serum (OA-Exos) and ATF4 mRNA-overexpressing exosomes (ATF4-OA-Exos) were compared, and showed that the ATF4-OA-Exos were more potent compared to the OA-Exos in preventing and alleviating cartilage degeneration via the stimulation of autophagy.

miRNAs play a critical role in the post-transcriptional regulation of a wide range of physiological processes, including cartilage homeostasis, and the pathological processes in disorders such as OA. In the past few years, there has been growing emphasis on determining the biological function of miRNAs in regenerative medicine. Given the eminent role of miRNAs in cartilage regeneration, six studies included here used transfection to upregulate specific miRNAs, i.e., miR-9-5p, miR-26a-5p, miR-92a-3p, miR-136-5p, miR-140-5p, and miR-155-5p, in exosomes. All miRNA-overexpressing exosomes showed superior therapeutic potential compared to the naïve exosomes by regulating the target genes and their downstream signaling pathways in the recipient cells. Mao et al. revealed that exosomal miR-92a-3p downregulated the Wnt signaling pathway via WNT5A, eventually causing lower ECM degradation. Comparing the results of exosome-treated groups with and without antagonim-miR-100-5p, Wu et al. reported that IPFP-MSC-derived exosomes ameliorated cartilage damage and the gait patterns of OA mice by delivering miR-100-5p into the recipient chondrocytes, leading to activation of the miR-100-5p-mediated mTOR autophagy pathway. In another study, miR-140 was loaded into exosomes through electroporation, and the miR-140-overexpressing exosomes were more effective compared to the naïve exosomes in suppressing the progression of cartilage degeneration and in enhancing cartilage regeneration. Additionally, miR-135b was increased in exosomes secreted by transforming growth factor (TGF)-β1-stimulated MSCs. The exosomal miR-135b downregulated Sp1 protein expression, leading to better cartilage regeneration in rats with OA.

LncRNAs are involved in the transcriptional and post-transcriptional regulation of many biological processes related to cartilage development, degeneration, and regeneration, and can be transferred by exosomes to recipient cells. Yan et al. studied the role of exosomal lncRNA H19 in cartilage regeneration. The exosomal lncRNA H19 played an important role in cartilage regeneration by promoting chondrocyte proliferation and matrix production whilst suppressing apoptosis. In vivo, injecting exosomes rich in lncRNA H19 led to greater improvement in cartilage repair, with uniform tissue, obscured boundaries, and lower T2 values on MRI analysis compared to that of cartilage tissue treated with exosomes secreted by UC-MSCs transfected with small interfering RNA (siRNA) against H19. In that study, the 10-fold increase in exosomal lncRNA H19 was achieved by exposing the cells to mechanical stimulus via culture in a rotary cell culture system (RCCS).

These results clearly show that exosomal cargo can be modulated by modifying the culture condition. The changes in exosomal cargo, such as higher miR-135b expression in TGF-β1-stimulated MSC-derived exosomes and higher lncRNA H19 expression in exosomes secreted by UC-MSCs exposed to mechanical stimulus, enhanced cartilage regeneration in vivo. Additionally, preconditioning BM-MSCs with kartogenin and curcumin improved the therapeutic potential of the secreted exosomes for treating OA. However, the studies did not examine the differences in the exosomal cargos of preconditioned and naïve cells.

Apart from modulating the exosomal cargo, cell preconditioning can also stimulate exosome secretion. Yan and Wu collected exosomes secreted by UC-MSCs cultured in 2D and 3D (using a hollow-fiber bioreactor) conditions. The 3D culture had increased exosome yield (7.5-fold higher), and the exosomes secreted by 3D-culture UC-MSCs showed more potent therapeutic effects in promoting cartilage repair compared to the exosomes secreted by the 2D-culture UC-MSCs, as indicated by the greater surface regularity and better thickness of OA cartilage in vivo. Similarly, Yan et al. also found that UC-MSCs...
cultured in 3D conditions using a RCCS secreted exosomes with increased yield and therapeutic potential in treating OA. Consistent with this, Yang et al.\(^{109}\) and Cao et al.\(^{116}\) attained higher exosome yields from cells cultured in 3D conditions, and the exosomes showed improved therapeutic potential, albeit for different indications.

The above results demonstrate the importance of exosomal cargo modification for achieving better performance. Additionally, exosomal cargo profiling is crucial for elucidating the mechanism of action.

**In vivo Delivery and Exosome Uptake**

Cartilage is an avascular and alymphatic tissue.\(^{111}\) Thus, unlike in other tissue injuries, exosomes are not suitable for infusing intravenously for treating cartilage defects. In fact, all of the included studies administered exosomes directly to the affected joint, as intra-articular administration increases bioavailability and reduces off-target effects, thereby improving the delivery of exosomes to the cartilage and decreasing the dose of exosomes needed.\(^{112}\) The exosomes were either injected or encapsulated in a scaffold. Some studies used a single injection and others used multiple injections to treat cartilage injuries. Currently, it remains unclear if multiple injections are more effective compared to single injection for promoting cartilage regeneration, as none of the studies compared the therapeutic effect of single and multiple injections. Cosenza et al.\(^{74}\) and Wang et al.\(^{80}\) showed that the effect of exosomes from a single intra-articular injection may be month-long. This is most probably due to the direct exosome administration to the target site (cartilage is avascular and alymphatic; thus, the clearance of the injected exosomes is slower) and the exosomes might induce cellular reprogramming and remodel resident or injured cells by activating regenerative mechanisms by transferring bioactive molecules.\(^{113–115}\)

The findings of the 29 studies are summarized in Table 3 and illustrated in Figure 2. To date, direct injection appears to be a more popular cell delivery method, as only a few studies\(^ {58,73,78}\) used a scaffold to deliver exosomes to the injured cartilage tissues. A scaffold is beneficial for sustaining the delivery of exosomes for a longer period and also aids exosome homing to the injury site. Additionally, combining exosomes and biomaterial can create a synergistic effect to promote cartilage regeneration. Liu et al.\(^ {58}\) found that the implantation of in situ hydrogel glue with iPSC-MSC-derived exosomes was more potent for promoting cartilage regeneration compared to in situ hydrogel glue implantation and iPSC-MSC-derived exosome injection, supported by the formation of a smoother surface with fully filled regenerated tissue that integrated with the surrounding cartilage. The histological findings were supported by the results of optical coherence tomography (OCT), which displayed a uniform and well-organized articular cartilage structure in the in situ formed iPSC-MSC-exosome–hydrogel tissue patch implantation group.

In vitro studies have shown that exosomes promote chondrocyte proliferation and migration, impede chondrocyte apoptosis and the expression of pro-inflammatory markers, and restore the balance between chondrocyte catabolism and anabolism in a dose-dependent manner.\(^ {69,74,78,82,116}\) However, no article included in the present review reported on the in vivo response to exosome dosage.

We also noted that the studies used several exosome quantitation methods, ie nanoparticle analyzer, protein quantitation assay, and enzyme-linked immunosorbent assay (ELISA). The number of exosome particles (particles/mL) was detected using nanoparticle analyzers such as nanoparticle tracking analysis (NTA) and tunable resistive pulse sensing (TRPS). Some of the studies used an ExoELISA kit to quantify exosomes based on the presence of exosome markers such as CD63. The exosome concentration was also determined by exosome protein mass concentration (µL/mL) using the bicinchoninic acid (BCA) or Bradford assay. Although all included studies reported that exosomes promoted cartilage repair and prevented lesion progression compared to the control group, none conducted in vivo study for identifying the optimal dosage and number of injections. Based on the different quantification methods used in the 29 included studies, the quantity of exosomes used ranged from \(8 \times 10^7–1 \times 10^{10}\) particles and 7.5–200 µg in mouse models; \(1 \times 10^8–1 \times 10^{10}\) and 1.25–100 µg in rat models; and \(2 \times 10^9–5 \times 10^9\) and 100–200 µg in rabbit models, and the injection number and time varied in each study.

Intercellular communication via exosomes mainly takes place through three mechanisms: (1) direct interaction of exosome ligands and receptors on target cells that activate the intracellular signaling cascades; (2) direct fusion; or (3) endocytosis. Direct fusion and endocytosis resulted in the release of exosomal contents into the cytosol.\(^ {117}\) Wu et al.,\(^ {64}\) He et al.,\(^ {75}\) Liang et al.,\(^ {77}\) and Zhang et al.\(^ {84}\) monitored exosome uptake in joint tissues. Fluorescent labeled exosomes were mainly observed in the
| References        | Animal Models         | Method of Delivery               | Treatment Groups                                                                 | Euthanasia                  | Efficacy Outcome                                                                 | Safety Outcome                                                                 |
|-------------------|-----------------------|----------------------------------|----------------------------------------------------------------------------------|-----------------------------|----------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Zhang et al.      | SD rats               | Surgically induced OCD           | Multiple intra-articular injections after surgery and thenceforth weekly          | Week 6 or 12 post-surgery  | ● Exo group demonstrated almost complete neotissue filling with good surface regularity and complete integration of neotissue with surrounding cartilage by 12 weeks | No detrimental responses were observed in all animals |
| Cosenza et al.    | C57BL/6 mice         | Collagenase-induced OA          | Single intra-articular injection at day 7 after OA induction                     | Day 42 post-collagenase induction | ● CLSM showed structural improvement in articular cartilage which comparable with healthy control group in all treatment groups  
● µCT showed higher bone volume, lower bone degradation, lower osteophyte formation and lower calcification of menisci and ligaments in all treatment groups compared to the OA group | ● Exo group showed the greatest improvement with the lowest OA scores |
| Liu et al.        | New Zealand rabbits  | Surgically created OCD          | Scaffold implantation or single intra-articular injection immediately after surgery | Week 12 post-surgery       | ● EHG group showed the best repair with smooth surface with white regenerated tissue fully filled the defects and integrated with surrounding cartilage  
● OCT displayed uniform and well-organized articular cartilage structure in EHG group | ● EHG group has the highest ICRS score and the neotissue was almost entirely hyaline cartilage (strong safranin O and Col II staining, weak Col I staining) |
| Author | Model | Methodology | Intervention | Outcome | Results |
|--------|-------|-------------|--------------|---------|---------|
| Tao et al | SD rats | Surgically induced OA | Multiple intra-articular injections on the first day of week 5 to 8 after surgery | Week 12 post-surgery | Exo-miR-140-5p group showed the lowest OARSI assessment scores; Significant increased chondrocyte count, higher Col II and aggrecan expression, and lower Col I in the Exo-miR-140-5p group compared to the Exo and OA groups |
| Wang et al | C57BL/6 J mice | DMM induced OA | Intra-articular injections at week 4 after surgery (once for Cell and Cell-OA group; multiple injections every 3 days for 4 weeks for Exo and Exo-OA group) | Week 8 post-surgery | Exo group exhibited similar regenerative effect as Cell group; Exo group revealed milder OA pathology compared to the OA group which was concomitant with lower OARSI scores and stronger Col II staining and weaker ADAMTS5 and aggrecan neoepitope staining |
| Zhu et al | C57BL/10 mice | Collagenase-induced OA | Multiple intra-articular injections on day 7, 14 and 21 after collagenase administration | Day 28 post-collagenase induction | No significant differences in ICRS macroscopic analysis scores among the normal, iPSC-MSC-Exo and SM-MSC-Exo groups, but significant higher than the OA group |

(Continued)
Table 3 (Continued).

| References | Animal Models | Method of Delivery | Treatment Groups | Euthanasia | Efficacy Outcome | Safety Outcome |
|------------|---------------|--------------------|------------------|------------|-----------------|----------------|
|            |               |                    |                  |            | Macroscopic and Functional Analysis | Imaging | Histological and Biochemical Analysis | Outcome |
| Mao et al⁶⁹ | C57BL/10 mice | Collagenase-induced OA | Multiple intra-articular injections on day 7, 14 and 21 after collagenase administration | 1. Exo group: 15 µL of 500 µg/mL BM-MSC-Exos 2. Exo-miR-92a-3p group: 15 µL of 500 µg/mL miR-92a-3p overexpressed BM-MSC-Exos 3. OA group: 15 µL of PBS 4. Healthy control: 15 µL of PBS | Day 28 after collagenase induction | Not reported | Not reported | Exo-miR-92a-3p group demonstrated significant reduced severity of cartilage matrix loss with higher Col II and aggrecan, and lower Wnt5a and MMP13 expressions in both gene and protein levels compared to the Exo and OA groups |
| Wang et al⁷⁰ | SD rats | Surgically induced OA | Intra-articular injection | 1. Exo group: 100 µL of 1×10¹¹ particles/mL MSC-Exos 2. TGF-β1-Exo group: 100 µL of 1×10¹¹ particles/mL TGF-β1-MSC-Exos 3. TGF-β1-NC-Exo group: 100 µL of 1×10¹¹ particles/mL TGF-β1-MSC-NC-Exos 4. TGF-β1-miR135b inhibitor-Exo group: 100 µL of 1×10¹¹ particles/mL TGF-β1-MSC-miR135b inhibitor-Exos | Week 12 post-surgery | Not reported | Not reported | Significant lower OARSI scores in the TGF-β1-Exo group than the Exo group, with high OARSI scores in the TGF-β1-miR135b inhibitor-Exo group |

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| Study Authors | Animal Model | Induction Method | Study Design | Treatment Group Details | Week | Outcome Measures | Notes |
|---------------|--------------|-----------------|--------------|-------------------------|------|-----------------|-------|
| Zhang et al<sup>69</sup> | SD rats | Surgically induced OCD | Multiple intra-articular injections immediately after surgery on weekly basis | 1. Exo group: 100 µg/100 µL ESC-MSC-Exos 2. Contralateral control: 100 µL PBS | Week 2, 6 or 12 | Not reported | Not reported |
| Chen et al<sup>73</sup> | New Zealand white rabbits | Surgically induced OCD | 3D printed scaffold implantation | 1. ECM/GelMA group: ECM/GelMA scaffold 2. GelMA group: GelMA scaffold 3. ECM/GelMA/Exo group: 3D printed ECM/GelMA/BM-MSC-Exo scaffold 4. OA group: untreated | Week 2 or 12 post-surgery | ● ECM/GelMA/Exo group exhibited smooth and intact tissues and gave the highest ICRS macroscopic analysis scores  ● MRI scanning demonstrated smooth neo-cartilage and great defect filling in both ECMGelMA and ECM/GelMA/Exo groups  ● µCT displayed increased ratio of bone volume to tissue volume, trabecular thickness and ossified tissues in the subchondral bone of both ECMGelMA and ECM/GelMA/Exo groups  ● ECM/GelMA and ECM/GelMA/Exo groups showed hyaline-like cartilage regeneration in the defect sites  ● ECM/GelMA/Exo group has higher ICRS visual histological scores at week 12  ● Increased Col II and decreased of MMP13 expression in the synovial membrane of ECM/GelMA and ECM/GelMA/Exo groups  ● ECM/GelMA/Exo group expressed the lowest MDA levels | No apparent pathological effects in myocardium, liver, and kidney 1 to 2 weeks after transplantation |
| Liu et al<sup>82</sup> | New Zealand white rabbits | Surgically induced OA | Multiple intra-articular injections once a week | 1. Exo group: 100 µg/mL PRP-Exos 2. PRP-As group: 100 µg/mL activated PRP 3. OA group: normal saline 4. Control: normal saline | Week 6 after surgery | ● Exo group exhibited more regular arrangement of chondrocytes, clearer tidal line, reduced hyperplasia on articular cartilage surface and lower OARSI scores than the PRP-As and OA groups  ● Exo group showed increased expression of Col II and RUNX2 | No adverse events occurred |
| Wu et al<sup>64</sup> | C57BL/6 mice | DMM induced OA | Multiple intra-articular injections twice a week, starting at week 4 after surgery | 1. Exo group: 10 µL of 10<sup>10</sup> particles/mL IPPP-MSC-Exos 2. OA group: 10 µL PBS 3. Sham control: 10 µL PBS | Week 8 post-surgery | ● Improved gait pattern (CatWalk gait analysis) after 6 weeks of exosome treatment  ● Obvious green fluorescent dots (DiO-labeled IPPP-MSC-Exos) can be found at the defect sites  ● Exo group showed integration of cartilage with smooth surface and lower OARSI scores confirming cartilage lesion was healed  ● Exo group showed higher expression of Col II as well as lower expression of ADAMTS5 and MMP13 | Not reported |

(Continued)
| References       | Animal Models | Method of Delivery | Treatment Groups                                                                 | Euthanasia | Efficacy Outcome | Safety Outcome |
|------------------|---------------|--------------------|----------------------------------------------------------------------------------|------------|------------------|----------------|
| **Zhang et al.** | SD rats       | Multiple intra-articular injections once a week, starting 2 weeks after OA induction | 1. Exo group: 100 µg/mL of ESC-MSC-Exos  
2. OA group: 50 µL of PBS  
3. Sham control: needle pricks | Week 2, 4 or 8 post-treatment | ● HWT improved gradually in the Exo group and reached the baseline level of the sham group at week 5  
● µCT showed Exo group restored subchondral bone volume and architecture at week 8  
● Exo group revealed significant reduced gene expressions of pro-inflammation (IL-1β), apoptosis (BAX), fibrosis (α-SMA) and pain (Substance P, CGRP, NGF, P75NTR and TrkA) and upregulated TIMP 2 and downregulated ADAMTS5 in condylar cartilage tissues  
● Significant lower Mankin scores at week 4 and 8 in the Exo group  
● Exo group showed smoother cartilage surface, improved cellularity, reduced fibrous cartilage thickening, minimal depletion of s-GAG in condylar cartilage lesion at week 4 and marked restoration of TMJ condylar structure at week 8  
● Exo group has lesser MMP13+ cells in condyle region at week 4; lesser IL-1β+ and iNOS+ cells, higher proliferative PCNA+ cells and lesser CCP3 + apoptotic cells at week 4 and 8  
● No adverse immune reactions observed | Not reported |
| **Zheng et al.** | C57BL/6 mice  | Surgically induced OA | Multiple intra-articular injections once per week, starting 10 days after surgery | Week 4, 6 or 8 post-surgery | ● µCT demonstrated lower subchondral bone mineral density and smaller osteophyte formation at the joint margins in the Exo group  
● Both femoral and tibia cartilage of the Exo group showed nearly complete preservation  
● No obvious synovitis appearance in all groups  
● Exo group expressed marked repression in MMP13 staining and elevated Col II staining in joint samples  
● Significant lower OARSI scores in the Exo group  
● Exo group exhibited higher level of M2 macrophages infiltration in the synovium and cartilage tissues | Not reported |
| Study | Model | Induction/Injury | Group/Intravenous Injection | Time | Key Observations |
|-------|-------|-----------------|----------------------------|------|-----------------|
| Chen et al<sup>56</sup> | C57BL/6 mice | Mechanical load induced OA | 1. Exo group: 100 µL of 10^11 particles/mL BM-MSC-Exos 2. Exo-miR-136-5p group: 100 µL of 10^11 particles/mL miR-136-5p overexpressed BM-MSC-Exos 3. OA group: Untreated 4. Normal control: Untreated | One hour after injury | Exo-miR-136-5p group showed lesser loss of cartilage matrix followed by the Exo and OA groups  Exo-miR-136-5p group revealed higher level of Col II and aggrecan gene and protein expressions, decreased in ELF3 and MMP13 gene and protein expressions |
| He et al<sup>57</sup> | SD rats | MIA-induced OA | 1. Exo group: 40 µg/100 µL BM-MSC-Exos 2. OA group: 100 µL of normal saline 3. Sham control: 100 µL of normal saline | Week 6 post-treatment | Joint injuries were alleviated in the Exo group  Thermal PWL and mechanical PWT improved at weeks 2, 4 and 6 in the Exo group  In vivo imaging showed accumulation of PKH26 labeled BM-MSC-Exos in the joint cavity  Articular cartilage of the Exo group regenerated, with small number of defects and fractures on cartilage surface  Exo group has significantly reduced OARSI scores  Exo group demonstrated notably increased in Col 2 protein expression level in chondrocytes and ECM, and decreased of MMP13 and Coll protein levels in cartilage tissue  Exo group showed reduced expression of CGRP and iNOS in DRG  Lower inflammatory cytokines (IL-1β, IL-6 and TNF-α) and higher anti-inflammatory cytokine (IL-10) in serum of Exo group |
| Jin et al<sup>58</sup> | SD rats | Surgically induced OA | 1. Exo group: BM-MSC-Exos 2. OA group: Normal saline | Week 7 post-treatment | Lower observation and Mankin scores in the cartilage of the Exo group  Notably decreased in inflammatory factors, AKP content and oxidative stress injury indicators, but increased in SOD in synovial fluid of the Exo group  Cartilage tissue of Exo group showed significant reduced MMP13, OCN and COMP gene and protein expressions |
### Table 3 (Continued).

| References | Animal Models | Method of Delivery | Treatment Groups | Euthanasia | Efficacy Outcome | Safety Outcome |
|------------|---------------|--------------------|------------------|------------|-----------------|----------------|
| Jin et al  | Wistar rats   | Surgically induced OA | 1. Exo group: BM-MSC-Exos | Not reported | Not reported | Not reported |
|            |               |                    | 2. Exo-miR-9-5p group: miR-9-5p overexpressed BM-MSC-Exos |            | ● Exo-miR-9-5p group exhibited the lowest observation and Mankin scores among the treated groups |
|            |               |                    | 3. Exo-miR-9-5p inhibitor group: miR-9-5p inhibited BM-MSC-Exos |            | ● Lower OA infiltration in the Exos. Exo-miR-mimic-NC and Exo-miR-inhibitor-NC group, and improved cartilage regeneration in the exo-miR-9-5p group |
|            |               |                    | 4. Exo-miR-mimic-NC group: miR-9-5p mimic NC BM-MSC-Exos |            | ● Exo-miR-9-5p group has the lowest expression level of inflammatory factors, AKP content, and oxidative stress injury indicators, as well as highest level of SOD in synovial fluid among all the treated groups |
|            |               |                    | 5. Exo-miR-inhibitor-NC group: miR-9-5p inhibitor NC BM-MSC-Exos |            | ● Exo-miR-9-5p group showed lowest gene and protein expressions of MMP13, OCN and COMP in cartilage tissue compared to all the treated groups |
|            |               |                    | 6. Liposomes miR-9-5p group: miR-9-5p-embedded liposomes |            |                |                |
|            |               |                    | 7. Sham control |            |                |                |
|            |               | One-week intra-articular injection of treatments after surgery | Week 8 post-surgery | Not reported | Not reported | Not reported |
| Ng et al   |               |                    | 1. Exo-miR-26a-5p group: 250 ng/5 µL of miR-26a-5p overexpressed BM-MSC-Exos |            | ● Exo-miR-26a-5p group has less pathological changes with the concomitant of reduced synovial tissue proliferation and suppressed inflammation |
|            |               |                    | 2. Exo-miR-NC group: 250 ng/5 µL of miR-NC BM-MSC-Exos |            | ● Exo-miR-26a-5p group has lower MMP3 and MMP13 expression and higher apoptotic index in synovial cells |
|            |               |                    | 3. OA group: Untreated |            | ● Notably increased of miR-26a-5p expression and declined of PTGS2 expression in synovial tissue of the Exo-miR-26a-5p group |
|            |               |                    | 4. Sham control: Untreated |            | ● Exo-miR-26a-5p group showed reduced serum IL-1β levels |
|            |               |                    | 5. Healthy control: Untreated |            |                |                |
| Authors | Rats | OA method | Intra-articular | Injection protocol | Week post-surgery | Findings | Toxicity |
|---------|------|------------|----------------|-------------------|------------------|----------|---------|
| Liang et al | SD rats | DMM induced OA | Multiple intra-articular injections post-injury, once per week | 1. Exo group: 100 µg of Exos particles in 100 µL PBS  
2. Exo-miR140 group: 100 µg of Exo-miR140 particles in 100 µL PBS  
3. CAP-Exo-miR140 group: 100 µg of CAP-Exo-miR140 particles in 100 µL PBS  
4. OA group: Untreated  
5. Sham control: Untreated | Week 8 post-surgery | Not reported  
- Fluorescence microscopy showed that CAP-Exo-miR-140 mainly stayed in the articular cavity, while Exo-miR-140 distributed to other body parts and enriched in kidney  
- CAP-Exo-miR140 group displayed smooth and flat cartilage surface, small joint space, proper cell alignment, normal subchondral bone and dense proteoglycan which was almost identical to the sham control and comparable OARSI scores with sham control  
- CAP-Exo-miR140 group suppressed the MMP13 and Adamts5 protein levels in cartilage tissue  
- Upregulation of miR-140 and downregulation of MMP13 gene expression in cartilage tissue in the CAP-Exo-miR140 group  
- Non-toxic to major organs (heart, liver, kidney, lung, and spleen) | |
| Liu et al | SD rats | Surgically induced OCD | Intra-articular implantation | 1. Exo group: Col-Tgel hydrogel with BM-MSC-Exos  
2. KGN-Exo group: Col-Tgel hydrogel with KGN-BM-MSC-Exos  
3. Gel group: Col-Tgel hydrogel  
4. OA group: untreated  
5. Normal control: untreated | Week 4, 6 and 8 post-surgery | Not reported  
- Better cartilage regeneration in the KGN-Exo group as indicated by smoother articular surface and better integration of newly formed cartilage with adjacent host cartilage  
- KGN-Exo group exhibited higher ICRS macroscopic scores which were comparable with the normal group at 8 weeks  
- KGN-Exo group revealed better cartilage reconstruction with hyaline cartilage predominantly, corresponding to notable high ICRS visual histological scores  
- KGN-Exo group showed increased s-GAG in cartilage tissue started at week 2  
- More lubricin and Col II positive cells and less Col I positive cells in KGN-Exo group compared to other groups except for the normal control group | Non-toxic to major organs (heart, liver, kidney, lung, and spleen) |
| Qiu et al | Mice | Surgically induced OA | Not mentioned | 1. Exo-Cur group: Curcumin pre-treated BM-MSC-Exos  
2. Exo group: BM-MSC-Exos  
3. OA group  
4. Sham control | Not reported | Not reported  
- Exo-Cur group showed higher gene expression of miR-124 and miR-143 as well as lower protein expression of ROCK1, NF-kB and TLR9 that were similar to the sham control group  
- Exo-Cur group has less apoptotic chondrocytes compared to the OA and Exo groups | Non-toxic to major organs (heart, liver, kidney, lung, and spleen) |

(Continued)
Table 3 (Continued).

| References | Animal Models | Method of Delivery | Treatment Groups | Euthanasia | Efficacy Outcome | Safety Outcome |
|------------|---------------|--------------------|------------------|------------|------------------|----------------|
| **Macroscopic and Functional Analysis** | Imaging | Histological and Biochemical Analysis | |
| **Wong et al** | New Zealand white rabbits | Surgically induced OCD | Multiple intra-articular injections at day 0, day 7 and day 14 post-surgery | 1. Exo + HA group: 1 mL of 3% (w/v) hyaluronic acid with 200 µg ESC-MSC-Exos 2. HA group: 1 mL of 3% (w/v) hyaluronic acid | Week 6 or 12 post-treatment | ● Significant improvement in ICRS scores of Exo + HA group at week 6 and 12, associated with marked improvements of neotissue integration at the border zone  ● Exo + HA group displayed improved mean Young’s moduli and stiffness of the repaired cartilage that approximated the normal tissue | No reported | ● Exo + HA group showed improvements in cartilage regeneration over time as confirmed by complete defect coverage by neotissue characterized by the presence of hyaline cartilage, normal cellularity and chondrocytic-like cells, high GAG and Col II deposition, and lower Col I deposition at week 12  ● Exo + HA group exhibited higher modified O’Driscoll scores than the HA group at week 6 and 12 | No adverse events |
| **Yan and Wu** | New Zealand rabbits | Surgically induced cartilage defect | Multiple intra-articular injections on a weekly interval | 1. 2D-Exo group: 500 µL of 1×10^10 particles/mL 2D cultured UC-MSC-Exos 2. 3D-Exo group: 500 µL of 1×10^10 particles/mL 3D cultured UC-MSC-Exos 3. OA group: 500 µL PBS | Week 4 post-treatment | ● Significant improvement in ICRS macroscopic scores in the 3D-Exo group compared to the 2D-Exo and OA groups  ● 3D-Exo group demonstrated more neotissue formation with smoother surface and better integration with the native hyaline cartilage at the surrounding | Not reported | ● 3D-Exo group displayed partly hyaline cartilage and the defects showed greater surface regularity and better thickness of cartilage than the 2D-Exo group  ● 3D-Exo group exhibited lower Wakitani score than the 2D-Exo and OA groups | Not reported |
| **Zavatt et al** | CD rats | MIA-induced OA | Intra-articular injection 3 weeks after OA induction (once for Cell and OA groups; twice for Exo group with 10 days interval) | 1. Cell group: 50 µL of 5×10^5 AFSCs 2. Exo group: 50 µL of 100 µg AFSC-Exos 3. OA group: 50 µL of glucose/PBS 4. Contralateral control | Week 3 post-treatment | ● Cell and Exo groups have higher pain tolerance, and the results were comparable to the normal control group | Not reported | ● Exo group showed better cartilage regeneration as indicated by complete neotissue formation with good surface regularity compared to the cell-treated defects which exhibited few fissures on the cartilage surface  ● Uniformed GAG distribution was demonstrated in the Exo group  ● Both Exo and Cell groups exhibited improved OARSI scores  ● Cell group displayed more intense Col II staining, whereas Exo group showed more regular distribution of Col II | No adverse inflammatory responses were observed |
| Author(s) | Species | Model | Treatment | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Week | Findings |
|----------|---------|-------|-----------|---------|---------|---------|---------|---------|-------|----------|
| Zhang et al. | SD rats | Surgically induced OA | Intra-articular injection every 3 days for 4 weeks, starting at week 4 post-operation | Exo group: 10 µL 10^{10} particles/mL BM-MSC-Exos | OA group: 10 µL PBS | Sham control | Week 4 post-treatment | Not reported | µCT showed less cartilage degradation, normal chondrocyte morphology and distribution, and less osteophyte formation around the joint treated with Exo | Not reported |
| Zhou et al. | C57BL/6j mice | Collagenase VII induced OA | Multiple intra-articular injections at day 7, 14 and 21 after collagenase induction | Exo group: BM-MSC-Exos | pExo group: polydactyly BM-MSC-Exos | OA group: Saline | Normal control | Day 28 post-collagenase VII injection | Not reported | pExo group alleviated cartilage damage evidenced by the significant lower OARSI scores than the OA and Exo groups | Not reported |
| Wang et al. | C57BL/6j mice | Surgically induced OA | Single intra-articular injection at week 4 post-operation | Sham-Exo group: 200 µg sham-Exos | OA-Exo group: 200 µg OA-Exos | ATF4-OA-Exo group: 200 µg ATF4-OA-Exos | OA group | Sham control | Week 8 post-surgery | µCT showed reduced osteophyte formation in Sham-Exo and ATF4-OA-Exo groups, while OA-Exo group displayed enlarged osteophytes | Not reported |

(Continued)
Table 3 (Continued).

| References | Animal Models | Method of Delivery | Treatment Groups | Euthanasia | Efficacy Outcome | Safety Outcome |
|------------|---------------|-------------------|------------------|------------|-----------------|----------------|
| Wang et al  | BALB/C mice   | Multiple intra-articular injection once a day, starting from day 20 after OA induction | 1. Exo group: 30 µL of 10 \(^{11} \) particles/mL SM-MSC-Exos  
2. Exo-155-5p group: 30 µL of 10 \(^{11} \) particles/mL miR-155-5p overexpressed SM-MSC-Exos  
3. OA group: normal saline  
4. Normal control | Two weeks post-treatment | Not reported | Not reported |
|            | 4°C water stimulated OA | | | | | |
| Yan et al  | SD rats       | Surgically induced cartilage defects | 1. S-Exo group: 100 µL of 1 mg/mL rotary cell culture system cultured UC-MSC-Exos  
2. si-Exo group: 100 µL of 1 mg/mL rotary cell culture system cultured siRNA H19 transfected UC-MSC-Exos  
3. OA group: 100 µL PBS | Week 4 or 8 | ● S-Exo group revealed reduced pain with higher LWT at week 3  
● Defects covered with neotissue at week 4 and were filled with uniform tissue and obscured boundaries at week 8 in the S-Exo group  
● S-Exo group showed the highest ICRS scores  
● Defects of S-Exo group displayed similar intensity to the adjacent cartilage through MRI at week 8  
● S-Exo group exhibited the lowest T2 values at week 4 and 8  
● S-Exo group has the best surface regularity, highest glycosaminoglycan, most orderly tissues and best subchondral bone repair among the groups  
● S-Exo group has significant lower Wakitani scores compared to the other groups  
● S-Exo group displayed the highest matrix synthesis which consists mainly of Coll II | Not reported |

**Abbreviations:** µCT, micro computed tomography; ADAMTS, ADAM metallopeptidase with thrombospondin motifs; AFSC, amniotic fluid stem cell; AKP, alkaline phosphatase; BAX, Bcl-2 associated X-protein; BM-MSC, bone marrow-derived mesenchymal stem/stromal cell; CAP, chondrocyte-affinity peptide; CCP3, cleaved caspase-3; CGRP, calcitonin gene-related peptide; CLSM, confocal laser scanning microscopy; col I, type I collagen; col II, type II collagen; COMP, cartilage oligomeric matrix protein; DMM, destabilization of medial meniscus; DRG, dorsal root ganglion; ECM, extracellular matrix; EH4, exosome encapsulating hydrogel; ELF3, E74-like factor 3; ESC-MSC, embryonic stem cell-derived mesenchymal stem/stromal cell; Exo, exosome; GAG, glycosaminoglycan; GelMA, gelatin methacrylate; HWT, head withdrawal threshold; ICRS, International Cartilage Repair Society; IL, interleukin; iNOS, inducible nitric oxide synthase; IPFP-MSC, infrapatellar fat pad-derived mesenchymal stem/stromal cell; KGN, kartogenin; LWT, leg withdrawal threshold; MDA, malondialdehyde; MIA, monosodium iodoacetate; MMP, matrix metalloproteinase; MRI, magnetic resonance imaging; MSC, mesenchymal stem/stromal cell; NC, negative control; NGF, nerve growth factor; OA, osteoarthritis; OARSI, Osteoarthritis Research Society International; OCD, osteochondral defect; OCN, osteocalcin; OCT, optical coherence tomography; P75NTR, p75 neurotrophin receptor; PBS, phosphate-buffered saline; PCNA, proliferating cell nuclear antigen; PRP, platelet-rich plasma; PWL, paw withdrawal latency; PWT, paw withdrawal threshold; RUNX2, runt-related transcription factor 2; SD rat, Sprague Dawley rat; SMA, smooth muscle actin; SM-MSC, synovial membrane-derived mesenchymal stem/stromal cell; SOD, superoxide dismutase; TGF-β1, transforming growth factor beta 1; TIMP, tissue inhibitor of metalloproteinase; Tm7, temporomandibular joint; TNF-α, tumor necrosis factor-alpha; TrkA, tropomyosin receptor kinase A; UC-MSC, umbilical cord-derived mesenchymal stem/stromal cell.
Figure 2 Overview of the studies. The exosomes tested in the included studies were derived from human, murine, or rabbit amniotic fluid stem cells (AFSCs), embryonic stem cell-derived mesenchymal stem/stromal cells (ESC-MSCs), induced pluripotent stem cell-derived MSCs (iPSC-MSCs), bone marrow-derived MSCs (BM-MSCs), polydactyly BM-MSCs, synovial membrane-derived MSCs (SM-MSCs), infrapatellar fat pad-derived MSCs (IPFP-MSCs), umbilical cord-derived MSCs (UC-MSCs), chondrocytes, dendritic cells, platelet-rich plasma (PRP), and serum. The exosomes were administrated to the osteoarthritic joint through intra-articular injection or scaffold implantation. The exosomal bioactive compounds played an important role in cartilage and subchondral bone repair and regeneration. Overall, exosome therapy restored joint function, reduced joint pain, and improved the joint macroscopic, histological, and biochemical features.
articul cavity, suggesting that exosomes acted locally after intra-articular injection. Zhang et al\textsuperscript{64} noted BM-MSC-derived exosomes could be taken up by synovial cells in vivo. Liang et al\textsuperscript{77} reported that dendritic cell-derived exosomes were distributed to other body parts and enriched in the kidney. Exosome retention in the articular cavity was improved by fusing a chondrocyte-affinity peptide on the exosome surface. The peptide confined the exosomes to the articular cavity and facilitated exosome penetration into the middle zone of the cartilage tissue to achieve targeted delivery of exosomes to chondrocytes for better therapeutic efficacy.\textsuperscript{77} Another study reported on the improved tissue-targeting ability of modified exosomes.\textsuperscript{18}

**Cartilage Gross Morphological and Morphometric Analysis**

Macroscopic assessment of cartilage degradation in small animals, especially mice, is technically difficult due to their small size.\textsuperscript{119} Hence, most of the included studies used rat and rabbit OA models to conduct cartilage gross morphological analysis. Eight studies graded the gross appearance of treated joints according to the International Cartilage Repair Society (ICRS) macroscopic assessment scale.\textsuperscript{58,63,65,66,68,72,73,78} Higher ICRS macroscopic assessment scores indicated morphological improvement in exosome-treated defects. Generally, the cartilage defects of the negative control group exhibited incomplete neotissue coverage, surface irregularity, and distinct border areas even at 12 weeks. On the other hand, exosome-treated cartilage defects showed much better and improved gross appearance. For example, Zhang et al\textsuperscript{68} stated that exosome-treated cartilage defects displayed almost complete neotissue filling, with good surface regularity and full integration with the surrounding cartilage at 12 weeks. Another study used confocal laser scanning microscopy (CLSM) analysis to evaluate cartilage defects, and reported improvement in articular cartilage volume and thickness, as well as lower cartilage degradation in collagenase-induced defects treated with BM-MSCs, BM-MSC-derived exosomes, and BM-MSC-derived microsicles as compared to the untreated control defects.\textsuperscript{74}

Apart from gross morphological assessment, Wong et al\textsuperscript{63} also performed biomechanical assessment of regenerated cartilage tissue. The mean Young’s moduli and stiffness of the repaired cartilage at the center and periphery of the defects improved significantly over time in the hyaluronic acid (HA) + ESC-MSC-derived exosome group in comparison to the HA group, suggesting that exosomes not only promote cartilage tissue morphology repair, but also contribute to functional improvement, ie better mechanical strength.

**Cartilage Histological Analysis**

Histological assessment is vital for determining tissue regeneration, repair, and pathological changes at the microscopic level. Various histological stains are used for identifying different cellular and tissue components.\textsuperscript{120} Hematoxylin–eosin (H&E) staining is widely used for revealing cell and tissue structure, and has become a common practice in histological study. Safranin O/fast green, toluidine blue, and Alcian blue are used for examining cartilaginous and chondrogenic differentiated tissues by visualizing the proteoglycans.\textsuperscript{121} In all 29 included studies, exosome treatment contributed to notable improvements in cartilage regeneration at the histological level. The studies used multiple histological grading systems for joint repair to grade the defects, ie the OARSI,\textsuperscript{60–62,64,67,71,72,74,75,77,79,81,82,84} ICRS visual histological assessment scale,\textsuperscript{58,73,78} O’Driscoll histological cartilage repair score,\textsuperscript{63,68} Wakitani histological scoring system,\textsuperscript{65,66,69} and Mankin scoring system.\textsuperscript{70,76,80} Other than that, Jin et al\textsuperscript{57} scored the pathological changes of condylar joint and synovial tissues using a 0–3 subjective grading system with the parameters of synovitis inflammation, synovial thickening, and subchondral bone erosion. All studies recorded improved histological scores after exosome treatment. For example, Zhang et al\textsuperscript{68} reported that ESC-derived exosome-treated defects demonstrated good cartilage and subchondral bone restorations by week 6 postsurgery. Near complete regeneration and bonding of cartilage with the underlying subchondral bone, which was very similar to that of the age-matched native control, were recorded at week 12. The O’Driscoll histological cartilage repair scores were significantly higher at week 6 and 12 in the exosome-treated group compared to the phosphate-buffered saline (PBS)-treated group. Similarly, Liu et al\textsuperscript{82} reported that, at week 6, articular cartilage defects treated with PRP-derived exosomes had regularly arranged chondrocytes, clearer tidal line, reduced hyperplasia, and better OARSI scores compared to defects treated with activated PRP.

Immunohistochemistry is used for detecting specific antigens in tissue sections by incubating the tissue sections with the appropriate antibody. Anabolic and catabolic markers of cartilage metabolism in regenerated tissue are identified to analyze the cartilage regeneration properties.
Type II collagen and aggrecan, a major cartilage ECM component and a core proteoglycan of articular cartilage, respectively, are always determined for evaluating cartilage regeneration.\textsuperscript{122,123} Cartilage degradation is mostly indicated by key enzymes in cartilage matrix degradation: matrix metalloproteinases (MMPs) and ADAM metallopeptidase with thrombospondin motifs (ADAMTS).\textsuperscript{124} Generally, exosome-treated cartilage defects had upregulated type II collagen and aggrecan expression and downregulated MMP13 and ADAMTS5 expression. Wong et al\textsuperscript{65} treated surgically induced OCD with ESC-MSC-derived exosomes and found that the regenerated tissue was mainly hyaline cartilage with high glycosaminoglycan (GAG) and type II collagen, as well as low type I collagen deposition. The accumulation of type I collagen is a marker of fibrocartilage formation.

Zhang et al\textsuperscript{66} reported increased proliferating cell nuclear antigen (PCNA)\textsuperscript{+} cells in reparative cartilage and synovium, and decreased cleaved caspase-3 (CCP3)\textsuperscript{+} apoptotic cells on reparative cartilage, suggesting that exosomes derived from ESC-MSCs mediate cartilage repair by promoting cell proliferation and suppressing apoptosis. The research team reported similar results in a subsequent study using ESC-MSC-derived exosomes for treating temporomandibular joint OA.\textsuperscript{70} Chondrocyte apoptosis may abrogate cartilage homeostasis, eventually leading to cartilage degeneration.\textsuperscript{125} Qiu et al\textsuperscript{63} and Jin et al\textsuperscript{57} used the TUNEL (terminal deoxynucleotidyl transferase dUTP nick-end labeling) assay to examine apoptosis. Qiu et al\textsuperscript{63} stated that curcumin-treated BM-MSC-derived exosomes were more effective in inhibiting chondrocyte apoptosis compared to naïve BM-MSC-derived exosomes. Jin et al\textsuperscript{57} found that miR-26a-5p overexpression in BM-MSC-derived exosomes enhanced synovium fibroblast apoptosis to alleviate cartilage damage.

Furthermore, Zhang et al\textsuperscript{69} indicated that MSC-derived exosomes mediate cartilage repair by modulating the pro-inflammatory environment in defects, as demonstrated by the increase in M2 macrophages (CD163\textsuperscript{+} cells) and reduction in M1 macrophages (CD86\textsuperscript{+} cells) in both cartilage and the overlying synovium in the ESC-MSC-derived exosome group, with a concomitant reduction in M1-associated cytokines, ie interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), in synovial fluid. Aside from this, exosomes induced regenerative M2 macrophage infiltration and attenuated inflammatory M1 macrophage infiltration in defect sites, evident in the higher abundance of CD163\textsuperscript{+} or arginase-1\textsuperscript{+} cells and scarcity of CD86\textsuperscript{+}, inducible nitric oxide synthase (iNOS)\textsuperscript{+} or IL-1β\textsuperscript{+} cells.\textsuperscript{67,70,81,84} The results are consistent with studies reporting that exosomes have anti-inflammatory and immunomodulatory functions for attenuating disease progression.\textsuperscript{126–129}

Subchondral Bone Regeneration Analysis

OA may lead to pathophysiological changes in subchondral bone and cartilage, also known as the bone–cartilage unit, that perform complementary work in the load-bearing of joints.\textsuperscript{130,131} Microstructural alterations of subchondral bone and the osteochondral junction, including osteophytes, subchondral sclerosis, subchondral cysts, or bone marrow lesions, are observed in the different stages of OA.\textsuperscript{132} Increasing evidence has shown that subchondral bone disturbance is associated with OA initiation and progression.\textsuperscript{133–136} There is molecular crosstalk between cartilage and subchondral bone, and cartilage is partly dependent on the neighboring subchondral bone for its nutrient supply.\textsuperscript{137,138} Subchondral bone migration is involved in OCD repair.\textsuperscript{139}

Among the 29 included studies, six evaluated subchondral bone regeneration.\textsuperscript{70,73,74,80,81,84} Bone parameters including bone volume (bone volume/tissue volume); bone degradation (bone surface/bone volume); osteophyte formation; meniscal or ligament calcification; and trabecular thickness, separation, and number were quantified. The six studies used micro-computed tomography (µCT) to examine the joint microarchitecture and reported that exosome-treated groups showed a lower extent of osteophyte formation, whereas the groups treated with normal saline exhibited a rough joint surface with osteophytes on the bone edge.\textsuperscript{70,73,74,80,81,84}

Interestingly, osteophytes formed in the OA serum-derived exosome-treated group were impeded by the introduction of ATF4 mRNA into the OA serum-derived exosomes.\textsuperscript{80} Apart from that, Cosenza et al\textsuperscript{74} reported higher epiphyseal and subchondral bone volume, lower bone degradation, and decreased calcification of menisci and ligaments in the groups treated with BM-MSCs, BM-MSC-derived exosomes, and BM-MSC-derived microvesicles. Zhang et al reported notable differences in bone volume, trabecular thickness, trabecular separation, and trabecular number at week 8 post-treatment,\textsuperscript{70} proving that ESC-MSC-derived exosomes restored subchondral bone volume and architecture in monosodium iodoacetate (MIA)-induced OA. Moreover, subchondral bone regeneration has been reported in cartilage defects treated with exosomes loaded in a scaffold. Chen et al\textsuperscript{73} reported that more ossified tissues were

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regenerated in OCD that received implantation of a 3D-printed cartilage ECM/gelatine methacrylate (gelMA)/BM-MSC-derived exosome scaffold.

Pain Behavior Analysis

Joint pain is the major symptom of OA. Recent studies indicate that sensory innervation in osteoarthritic subchondral bone or neuronal hypersensitivity as a result of aberrant subchondral bone remodeling during OA progression may contribute to arthritic pain.\(^{140,141}\) Hence, behavioral and functional assessments are important in OA evaluation. Pain cannot be measured directly in animals, but can be estimated through pain-like behaviors.\(^{142}\) Withdrawal response, a pain-like behavior towards a nociceptive stimulus, is the most commonly used method for examining pain in animals.\(^{143}\) Yan et al,\(^{65}\) Zhang et al,\(^{70}\) and He et al\(^{75}\) measured the withdrawal threshold of OA animals using the von Frey microfilament procedure, while Zavatti et al used a pressure application measurement device to do so.\(^{67}\) He et al\(^{75}\) also tested the paw withdrawal latency of rats treated with BM-MSC-derived exosomes using a plantar test and measured the thermal threshold. The results showed that exosome-treated OA animals have high pain tolerance levels and that the results were comparable to that of healthy control animals. Furthermore, increasing evidence supports the notion that neuropathic pain also contributes to OA pain.\(^{144-146}\) He et al\(^{75}\) reported neuropathic pain in OA rats, and demonstrated that exosome injection relieved inflammatory and neuropathic pain, as shown by the decreased protein levels of calcitonin gene-related peptide (CGRP) and iNOS in the dorsal root ganglion tissue.

In addition, OA animals may have altered gait patterns to reduce the pain from movement or loading force.\(^{147}\) Wu et al\(^{64}\) performed gait analysis using the CatWalk method to measure motion-related pain in OA mice, and found that IPFP-MSC-derived exosomes ameliorated gait disturbance, indicated by the marked increase in duty cycle at week 10.

Safety Profile

Exosomes have low immunogenicity and potent immunoregulatory properties.\(^{148,149}\) So far, in vivo studies using single or repeated doses of exosomes have not reported severe immune reactions.\(^{150,151}\) Among the included studies, nine reported the safety outcome.\(^{50,63,67,68,70,73,77,82}\) No adverse events or inflammatory responses occurred in those studies, regardless of the cell source, ie MSC- or non-MSC-derived exosomes, and method of administration, ie intra-articular administration in solution form or encapsulated within a scaffold. Liang et al\(^{77}\) demonstrated via H&E staining that dendritic cell-derived exosome injections did not cause toxicity to major organs such as the heart, kidney, lung, and spleen. Similarly, no apparent pathological effects were shown in the myocardium, liver, and kidney 1–2 weeks after 3D printed ECM/gelMA/exosome scaffold implantation.\(^{73}\)

The majority of the included studies used human exosomes to promote cartilage repair in animal models. No study reported adverse events related to the use of xenogeneic and allogeneic exosomes. Thus, allogeneic exosomes may be considered safe for use in humans. Nonetheless, clinical trials are needed to validate the safety of allogeneic exosomes.

Cells or Exosomes?

Previously, stem cell therapy was believed to exert its tissue repair mechanism through the replacement of injured cells. However, many studies have shown that most of the MSCs injected are trapped and cleared in the blood circulation and that only a small fraction of the transplanted cells reach the target tissues.\(^{152}\) Despite the low cell homing to the injured tissues, the therapeutic effect of MSCs remains significant, indicating that MSCs support tissue repair and regeneration via the secretion of paracrine factors.\(^{153}\)

Several studies showed that exosomes have comparable therapeutic effects to cell-based therapy in managing OA. Wang et al\(^{61}\) reported that ESC-MSC-derived exosomes were as effective as ESC-MSCs for treating DMM (destabilization of medial meniscus)-induced OA animal models. However, the experiments were carried out sequentially, and the study compared single cell suspension injection versus multiple exosome injections. Thus, it might not be a fair comparison. According to Cosenza et al\(^{154}\) and Zavatti et al,\(^{67}\) cell therapy using BM-MSCs and AFSCs, respectively, promoted cartilage regeneration. However, more spectacular results were recorded when exosomes derived from these cells were used. Zavatti et al\(^{67}\) stated that both AFSCs and AFSC-derived exosomes improved the pain threshold of OA rats, whereby the results were comparable to that of the healthy group. However, histological and immunohistochemical analyses showed that the exosome-treated group displayed better cartilage regeneration compared to the AFSC-treated group. Additionally, the lower OARSI grading scores and higher volume of cartilage tissue...
with lower fibrous connective tissue composition in the neotissue in exosome-treated defects indicated that AFSC-derived exosomes were superior for repairing and regenerating cartilage tissue as compared to AFSC therapy. The AFSC-derived exosomes were also more potent for reducing osteoarthritic joint inflammation compared to AFSCs, as indicated by the presence of more M2 macrophages. Nonetheless, the OA animals received two intra-articular injections of exosomes whilst the AFSCs were only administered once, as the authors postulated that the transplanted cells may exert their therapeutic effects for a longer period, i.e., up to 3 weeks. Cosenza et al reported that single injection of BM-MSCs, BM-MSC-derived exosomes, and BM-MSC-derived microvesicles exhibited similar chondroprotective effects. Yet, exosome treatment led to greater improvement in modified Pritzker OARSI scores and reduction in osteophyte formation compared to BM-MSC and microvesicle treatments. These results suggest that exosomes might be a more effective and safer alternative to cell-based therapy for OA. Nevertheless, more studies, especially clinical trials, are needed to validate the safety and efficacy of exosome therapy.

Nowadays, many researchers advocate the transition from cell-based therapy to cell-free therapy, driven by several factors. Generally, the heterogeneity of living cells often leads to inconsistent curative effects. Long-term in vitro expansion might lead to cell dedifferentiation and senescence, thus compromising the therapeutic potential. Furthermore, stem cells carry the risk of mutation and tumor formation. Tumor formation is a hurdle to overcome for the clinical use of ESCs and iPSCs. Exosome therapy is a cell-free therapeutic option available for overcoming the drawbacks of cell-based therapy. Exosomes are easier to handle and store, and are less costly and less time-consuming to produce compared to cells. Furthermore, exosome dosage and potency are easier to optimize. Apart from that, exosomes can be developed as nanosized carriers to deliver the desired therapeutic cargo to the target cells. However, the most prominent shortcoming of exosomes is that they cannot replicate or reproduce in vivo. Coupled with their short half-life, multiple dosages might be required to achieve the desired treatment results.

**Conclusion and Perspective**

The promising in vivo findings indicate that exosomes promote cartilage repair and regeneration, and modulate the pro-inflammatory environment, subchondral bone regeneration, and pain behavior in both OA and OCD models. More importantly, none of the included studies reported adverse events, indicating the low immunogenicity and excellent immunomodulatory property of exosomes. The modification of exosomal cargo further enhanced the therapeutic efficacy of exosomes.

On the other hand, despite many preclinical studies reporting that exosomes enhance cartilage regeneration, research on the therapeutic efficacy of exosomes for OA is still in its infancy. The exact mechanism and detailed signaling cascade mediated by exosomes in cartilage repair and regeneration are not fully understood. Hence, there should be more studies examining the mechanism of action. Moreover, exosomes demonstrate dose-dependent therapeutic efficacy in terms of promoting chondrocyte migration and proliferation, reducing chondrocyte apoptosis and pro-inflammatory markers, and restoring cartilage anabolic–catabolic marker equilibrium in vitro. However, the optimum source and dose of exosomes for OA management have not been determined in animal models. Furthermore, multiple intra-articular injections of exosomes cause pain and discomfort to the recipients and increase the risk of other complications such as inflammation, arthritis, and neuropathy. Encapsulating exosomes within a scaffold might reduce the number of injections needed, as it allows the controlled release of exosomes. A scaffold also provides an ECM that mimics the native cartilage tissue to facilitate regeneration. However, more studies are needed to identify a scaffold with all the desired biological and physicochemical properties that could hasten the regeneration of damaged cartilage without causing adverse events. Finally, the term “exosome” used in the studies is controversial, as the identity of exosomes cannot be confirmed through size, density, or protein markers due to the lack of specific isolation methods and characterization techniques. “Exosome” is used mostly due to perceived popularity. To be more precise, EVs should be defined based on their physical characteristics, biochemical composition, culture condition, and cell of origin.

The low yield of EV production with existing harvesting methods is a challenge for clinical application. Large-scale EV production can be achieved by manipulating the culture condition, such as with a microcarrier-based 3D culture system and human platelet lysate supplementation. Batch-to-batch biological variation of EV therapy also cannot be ignored. To address this issue, Chen et al transformed human ESC-MSCs into immortalized cells, which enabled the consistent supply of therapeutic EVs or delivery vesicles. Besides, Lian et al proposed a protocol for producing
human ESC-MSCs that are reproducible and able to generate consistent batches of cells and conditioned medium on a large scale. iPSCs, iPSC-derived MSCs, and iPSC-derived MSC-like mesenchymal progenitor cells could also be alternative inexhaustible EV production sources due to their unlimited proliferative potential. In addition, there is no standard protocol for ensuring exosomes or EV quality.

In summary, studies examining the therapeutic efficacy of exosomes on cartilage regeneration remain limited to small-animal models. Therefore, studies using large-animal models that are more clinically relevant should be carried out in the future to validate the safety and efficacy of EV therapy. The standardization of EV therapy is needed to achieve consistent and optimum therapeutic outcomes. More efforts are required to identify the most ideal cell source, culture condition, exosome dosage, and frequency of administration, as well as the method of administration, to achieve the best therapeutic results without causing adverse events.

**Abbreviations**

2D, two-dimensional; 3D, three-dimensional; ADAMTS, ADAM metallopeptidase with thrombospondin motifs; AFSC, amniotic fluid stem cell; ATF4, activating transcription factor 4; BM-MSC, bone marrow-derived mesenchymal stem/stromal cell; ECM, extracellular matrix; ELISA, enzyme-linked immunosorbent assay; ESC-MSC, embryonic stem cell-derived mesenchymal stem/stromal cell; EV, extracellular vesicle; GelMA, gelatin methacrylate; H&E, hematoxylin-eosin; ICRS, International Cartilage Repair Society; IL-1β, interleukin-1 beta; iNOS, inducible nitric oxide synthase; IPFF-MSC, infrapatellar fat pad-derived mesenchymal stem/stromal cell; iPSC-MSC, induced pluripotent stem cell-derived mesenchymal stem/stromal cell; lncRNA, long non-coding RNA; miR/miRNA, microRNA; MMP, matrix metalloproteinase; MRI, magnetic resonance imaging; MSC, mesenchymal stem/stromal cell; MV, multi-vesicular bodies; OA, osteoarthritis; OARSI, Osteoarthritis Research Society International; OCD, osteochondral defect; PRP, platelet-rich plasma; RCCS, rotary cell culture system; SM-MSC, synovial membrane-derived mesenchymal stem/stromal cell; SYRCLE, Systematic Review Center for Laboratory Animal Experimentation; TGF-β1, transforming growth factor beta 1; UC-MSC, umbilical cord-derived mesenchymal stem/stromal cell.

**Data Sharing Statement**

All data generated or analyzed during this study are included in this published article.

**Author Contributions**

C.Y.N. and J.Y.C. conducted the literature search, article selection, and data extraction. All authors contributed to data analysis, drafting, or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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**Disclosure**

The authors report no conflicts of interest in this work.

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