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Exploring the Potential of MSC-derived Extracellular Vesicles as Cell-Free Therapy for Osteoarthritis: A Narrative Review

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Abstract

Introduction: Mesenchymal stem/stromal cell (MSC)-based therapies in cartilage repair and osteoarthritis (OA) treatment has gained attention. Recently, there has been increasing evidences to suggest that MSCs secrete a wide range of trophic factors to modulate the injured tissue environment. Extracellular vesicles (EVs) have been suggested to explain the positive and sometimes curative effect, through the paracrine stimulation of the resident progenitor cells to aid in repair.

Objectives: To give a narrative review of the current evidence for MSC-derived EV's potential therapeutic effects and future perspectives for the treatment of OA.

Methods: A literature search was conducted using the MEDLINE databases in November 2023. A total of 24 animal studies describing EV including exosome utilized in cartilage and OA treatments were identified and analyzed.

Results: Preclinical animal studies indicated that the EVs could enhance the therapeutic effects for the treatment of OA, although the purification methods for EVs and their cell sources would be highly relevant to therapeutic efficacy. In contrast, there have been no published clinical studies regarding EVs for the treatment of OA, and thus properly controlled clinical trials and regulations are essential steps in the future clinical applications.

Conclusions: The current evidences suggest that the administration of MSC-derived EVs into damaged joints could effectively reduce cartilage loss and alleviate the progression of OA. In contrast, there are still several potential problems to be solved, including their classification, safety and toxicity in clinical use, as well as optimal dosage and frequency for human administration.

Keywords:

extracellular vesicle, exosome, cartilage, osteoarthritis, cell-free therapy, mesenchymal stem/stromal cell

Introduction

Osteoarthritis (OA) is the most common joint disease affecting 595 million people worldwide [1]. However, repair of damaged joints has remained challenging because cartilage generally has a poor healing capacity [2]. Current treatment approaches range from lifestyle modifications, physical therapy, and symptomatic slow-acting drugs for OA, to more invasive interventions like arthroscopic management, osteotomy, and total knee arthroplasty [3]. Despite the effectiveness of these methods, around 30% of patients express dissatisfaction [4]. Consequently, there is a growing need for less invasive and innovative therapies to cater to an expanding population of active individuals experiencing symptomatic OA [5].

Recently, mesenchymal stem/stromal cells (MSCs) have particularly been in focus of research in the recent decade owing to their property to facilitate regenerative cartilage repair similar to native hyaline cartilage [6-8]. These cells can also be isolated from various tissues such as bone marrow, adipose tissue, and synovial membrane [9-13]. Such cells have several advantages including relatively easy extraction, low cost for isolation and culture, autologous nature, and an immunomodulatory ability which can broaden treatment options to the patient [14]. The efficacy of autologous or allogenic MSCs in cartilage repair has been demonstrated in animal studies as well as clinical trials [7,8,15-17].

As minimally invasive method, intra-articular injection of MSCs has been performed, and showed some positive results with regard to clinical improvement and safety [18-22]. The use of MSCs to repair cartilage tissues was based on the hypothesis that these cells could either differentiate into chondrocytes directly or indirectly through the paracrine stimulation of the resident progenitors to repair the damaged tissue [23]. In recent years, there is increasing evidence to suggest that MSCs secrete a wide range of trophic factors to modulate the injured tissue environment and to orchestrate subsequent regenerative processes including cell migration, proliferation, differentiation, and matrix synthesis [24]. As such interactions between MSCs and the target cells, local secretion of growth factors and cytokines [25], gap junction [26] and nanotube signaling [27] play important roles. Additionally, the part of paracrine effects of MSC is exerted through the release of extracellular vesicles (EVs) that includes exosomes, microvesicles and apoptotic bodies. EVs are considered the natural, efficient transport carrier (e.g., lipid, proteins, mRNA and micro RNA) and can maintain functional characteristics similar to their parent cells. This insight has given rise to a new paradigm wherein EVs are collected from MSCs and used to treat damaged tissue. The cell-free nature of EVs suggests that they may have a more favorable safety profile than cell-based therapies. Thus, this paper aims to

review the current evidence for EV's potential therapeutic effects, regulation and future perspective for the treatment of OA.

Pathology of OA

OA is characterized by degenerative changes, such as articular cartilage loss, subchondral bone thickening, and osteophyte formation [28,29]. The primary morphologic changes include thinning, fissuring, and fragmentation of articular cartilage. With the progression of the disease comes a continuous loss of articular cartilage, accompanied by a decrease of type-II collagen and aggrecan, leading to exposure of subchondral bone [30,31]. Secondary changes are frequently seen in the underlying bone including fibrosis, cystic change, and new bone formation. These changes are considered to be triggered by a multitude of factors, including ageing, trauma, obesity, mechanical overload, congenital disorders, genetics and infection, which fail to heal spontaneously once damaged has started.

On the molecular level, OA development is associated with loss of homeostatic balance between degradation and repair mechanisms in the articular cartilage [30,32]. Such an imbalance induces senescence, differentiation, proliferation, and death in joint cells through gene and/or protein expression networks that switch from anabolic to catabolic

outcomes [33]. Cartilage-degrading enzymes, such as a disintegrin and metalloproteinases with thrombospondin motifs (ADAMTS)-4 and ADAMTS-5, and matrix metalloproteinase (MMP)-13, play critical roles in OA pathogenesis [30]. Periarticular bone formation like osteophyte is generated by a process of endochondral ossification that recapitulates the cellular mechanisms of bone growth that occur during skeletal growth and development in which new bone is formed by replacement of a cartilaginous matrix [34].

Also, it would be important to identify OA subtypes concerning the selection of corresponding interventions for treatment responders. With a recent advance in bioinformatics-based OA subtype analyses, Yuan et al. reported four distinct OA subtypes based on the knee joint tissue transcriptome atlas: a glycosaminoglycan metabolic disorder subtype (C1), a collagen metabolic disorder subtype (C2), an activated sensory neuron subtype (C3), and an inflammation subtype (C4) [35]. These findings revealed distinct molecular subtypes in knee OA patients and may allow for precise diagnosis and treatment of OA as an alternative of traditional OA diagnosis by medical imaging [33].

Extracellular vesicles (EVs)

EVs are membrane-bound vehicles classified based on their biogenesis and size into exosomes (30–150 nm in diameter), microvesicles/microparticles, and apoptotic bodies (both considered to be >100 nm) [36]. Exosomes are secreted to the extracellular environment through the fusion of multivesicular bodies with the plasma membrane. The last two types of vesicles are released through the forward budding of the plasma membrane in living and dying cells, respectively. Among them, exosomes are presently considered more important as evidenced by the exponentially increasing number of exosome-related publications in recent years [36]. EVs are generally recognized to be intercellular communication vehicles and function to transfer lipids, nucleic acids (mRNAs and micro RNAs) and proteins between cells to elicit biological responses in recipient cells that are reflective of the cargo contents [37].

As nomenclature, the International Society for Extracellular Vesicles (ISEV) endorses “extracellular vesicle” (EV) as the generic term for particles naturally released from the cell that are delimited by a lipid bilayer and cannot replicate, i.e. do not contain a functional nucleus [38]. Since consensus has not yet emerged on specific markers of EV subtypes, such as endosome-origin “exosomes” and plasma membrane-derived “ectosomes” (microparticles/microvesicles), assigning an EV to a particular biogenesis pathway remains extraordinarily difficult unless e.g. the EV is caught in the act of

release by live imaging techniques [39]. Thus, the term is unified as EV in this article, according to the ISEV.

EVs for potential therapeutic applications in OA can be obtained from various sources [40]. The sources of the unmodified EVs include: 1) Stem cells, including bone marrow MSCs (BMSCs), adipose MSCs (AMSCs), synovial MSCs (SMSCs), and others; 2) Adult cells - immune cells (macrophages, neutrophils), and various components of joint structures (chondrocytes, synoviocytes, tenocytes); 3) Body fluids - blood-derived EVs like platelet-rich plasma exosomes (PRP-Exos) and synovial fluid EVs and 4) Other species - biomaterials from other species such as milk.

On the other hand, modified EVs for OA treatment covers various strategies, categorized into modifying donor cells and modifying EVs directly. Donor cell modification includes 1) biochemical approaches like a) co-incubation with anti-inflammatory substances (e.g., curcumin), b) gene transfection (e.g., micro RNA overexpression), and c) hypoxic methods and 2) mechanical approaches, like low intensity pulsed ultrasound (LIPUS), and 3D culture methods. Direct EV modification involves 1) loading exogenous cargoes by methods such as a) electroporation (creating a transport hole in the EVs membrane through an electric field, allowing the entry of exogenous cargo) and b) direct mixing; 2) modifying the EV membrane for

enhanced targeting and delivery and 3) biomimetic EVs (synthetic EVs), such as hybrid EVs and EV-like nanoparticles.

The mechanisms and pathways associated with EV uptake has been studied to understand the intercellular communication via EVs [41,42]. When EVs reach recipient cells, they will bind to the cell surface and can undergo various fates. Depending on the cell type, EVs can remain bound to the surface, that initiating intracellular signaling pathways [43]. Other than that, EVs may also be internalized by multiple pathways, including macropinocytosis, phagocytosis, caveolar endocytosis, clathrin-mediated endocytosis, lipid rafts-mediated endocytosis, and membrane fusion [43]. Internalized exogenous EVs reach multivesicular endosomes (MVEs), in which they will be likely to mix with endogenous intraluminal vesicles (ILVs). Fusion of MVEs with the lysosome will lead to the degradation of EVs and the recycling of their contents to promote the metabolism of the recipient cell. EVs docked at the plasma membrane of MVEs can release their intraluminal contents into the cytoplasm of the recipient cell by fusion. Most of the detail process has been still unknown but it would be import to understand the delivery of intraluminal cargoes.

Therapeutic effects

MSC-based therapy has been extensively studied and shown to be promising for cartilage defect repair [44,45]. Additionally, intra-articular injection of MSCs into the knee joint with OA has exhibited reliable safety, and (partially) alleviated knee pain and function [46-48]. Although the detailed mechanisms for MSC-based OA treatments have not been well clarified, an increasing number of studies have suggested that the therapeutic effects of stem cells are mainly dependent on the paracrine function of stem cells, including the secretion of EVs [36,49]. To date, EVs from different types of MSCs have been revealed to regulate cartilage regeneration and attenuate OA progression [50].

The EVs derived from BMSCs exerted chondroprotective and anti-inflammatory function in vitro, by enhancing the expression of chondrocyte markers (type II collagen and aggrecan) and inhibiting catabolic (MMP-13, ADAMTS5) and inflammatory (iNOS) markers, and protected mice knees from developing OA [51]. Also, BMSC-derived EVs could relieve pain via abrogation of aberrant CGRP-positive nerve and abnormal H-type vessel formation in the subchondral bone, and attenuate cartilage degeneration and facilitate subchondral bone remodeling by inhibiting tartrate-resistant acid phosphatase expression and RANKL-RANK-TRAF6 signaling activation in a mouse lumbar facet joint osteoarthritis model [52]. AMSC-derived EVs were demonstrated to have potent capability for cartilage regeneration via upregulating micro

RNAs (miRNA)-145 and miRNA-221 and inflammatory modulation by reducing the production of inflammatory mediators tumor necrosis factor-α, IL-6, prostaglandin E2 (PGE2) and nitric oxide (NO), and thus those are considered an excellent cell source for OA treatment [53,54]. EVs derived from SMSCs could effectively promote cartilage regeneration, protect subchondral bone and attenuate OA progression through miRNA-140-5p/RalA-mediated increase of SOX9 and aggrecan [55-57].

Although studies with EVs demonstrate excellent properties such as biocompatibility, low immunogenicity and low toxicity, some barriers still limit its application as a potential treatment, since EVs present low yields, complex contents and poor homogeneity [58]. Alternatively, biomimetic EVs produced through synthetic methods and different fabrication strategies are being studied to mitigate the aforementioned problems. The application of methods such as chemical modification, genetic engineering and physical methods, and the use of nanomaterials to prepare the EVs, were able to improve targeting efficiency [59].

Methods

There has been growing interest in utilizing EVs as potential therapies for cartilage regeneration and OA treatment. To give a narrative review of the current evidence for

MSC-derived EV's potential therapeutic effects and future perspectives for the treatment of OA, we performed the literature search as follows. The literature search was conducted from January 1, 2016 to November 15, 2023, on the MEDLINE databases using the following keywords: (exosome OR extracellular vesicle) AND (cartilage OR osteoarthritis OR osteochondral), and a total of 808 articles were identified from the databases. We included all preclinical animal and clinical studies reporting the EVs on cartilage repair and OA treatment. In vitro studies were excluded. After the careful screening, a total of 24 articles were analyzed in this review.

MSCs versus EVs

As mentioned above, recent evidence indicates that the therapeutic efficacy of stem cells is attributed to the paracrine action of the secreted factors like EVs, contrary to the initial paradigm of cell differentiation and replacement as the mechanism by which stem cells exhibit a therapeutic effect. To assess the feasibility of EVs as a cell-free therapy, several studies have compared the efficacy of stem cells with EVs. Muhammad, et al. designed to identify data for systematic review and meta-analysis of stem cell and secretome interventions and to compare the therapeutic efficacy of stem cells and secretome in animal models of cartilage defects [60]. Based on the results, the authors

showed a similar therapeutic benefit of secretome to stem cell transplantation in preclinical animal studies. Oh, et al. prepared the supernatants of adipose-derived stromal cell (ADSC) cultures (the secretome), which were pressure-concentrated (ca. 50-fold) [61], and compared the therapeutic efficacies of ADSCs and their secretome in terms of rabbit auricular cartilage regeneration. They showed ADSCs significantly enhanced new cartilage formation, but their secretome did not. Zavatti, et al. compared the efficacy of amniotic fluid stem cells (AFSC) with their secreted exosomes, in an MIA-induced rat model of osteoarthritis [62]. These authors demonstrated that intra-articular injection of human AFSC exosomes showed enhanced pain tolerance levels and improved histological scores than that of AFSC. These results indicate that the purification methods for EVs and their cell sources would be highly relevant to therapeutic efficacy. Thus, it is very important to figure out optimal conditions, in which the purified EVs could enhance the therapeutic effects for the treatment of OA.

A summary of the comparison between MSCs and EVs in animal studies is shown in Table 1.

In vivo studies

Several studies examined the efficacy of EVs for cartilage and osteoarthritic joint repair. A variety of cell sources and EV purification methods were employed in animal studies. Cosenza, et al. compared the respective role of exosomes or microvesicles/ microparticles (MPs) in OA, which were isolated from bone marrow MSCs [51]. These authors showed that exosomes and MPs exerted similar chondroprotective and anti-inflammatory functions in vitro and protected mice from developing OA in vivo. Hanai, et al. established isolation methods for small EVs using a new clinical grade chemically-defined media and showed that small EVs derived from AMSCs cultured in a chemically-defined media without detectable contaminants demonstrated enhanced biological effects on human chondrocytes and the progression of OA [63]. Zhou et al. investigated the effects of exosomal miRNA derived from synovial fibroblasts on cartilage degeneration in a surgically-induced rat OA model [64]. The authors revealed that rat synovial fibroblast-derived exosomal miRNA-126-3p was sufficient to suppress the formation of osteophytes, prevent cartilage degeneration, and exert anti-apoptotic and anti-inflammatory effects on articular cartilage. Zhang et al. validated the safety and efficacy of human MSC-derived exosomes for osteochondral repair in a micro pig model [65]. This study showed that intra-articular injections of MSC-derived exosomes combined with hyaluronic acid promoted functional cartilage and subchondral bone

repair, with significantly improved morphological, histological, and biomechanical outcomes, while at the same time demonstrating its safety in terms of tumor formation and infection.

Taken together, these results suggest that the administration of MSC-derived EVs into damaged joints could effectively reduce cartilage loss and alleviate the progression of OA. However, it is important to establish the purification method of clinical grade EVs and verify the proper amount of EVs used in clinical practice, which does not ruin the methodology for future clinical application [66]. Moreover, the validation in large-animal models are needed in order to assess therapeutic efficacy, biosafety, kinetics and biodistribution of MSC-derived EVs [59].

Details of the therapeutic effects of EVs in animal studies are shown in Table 2.

Ongoing clinical studies and regulations

To date, there have been no published clinical studies regarding EVs for the treatment of OA. A search of “ClinicalTrials.gov” using the keyword “exosome” or “extracellular vesicle” for all ongoing clinical trials identified 265 clinical studies (as of November 2023). These trials mostly include the fields of oncology, respiratory, and gastrointestinal studies, and one study with MSC-derived EVs for OA treatment was

identified (ID: NCT05060107). The purpose of this clinical study is to evaluate the safety of allogeneic MSC-derived EVs administered by intra-articular injection into the knee of patients with mild to moderate symptomatic OA, starting with 10 patients with a follow-up of up to 12 months.

Currently, there are no approved EV or exosome-based products worldwide. As mentioned above, there is ongoing research to establish the safety of administering any type of experimental therapy to patients and to investigate whether that therapy is effective for the treatment of a specific condition. Additionally, no country has established laws, regulations, guidelines, etc., and each regulatory authority deals with EVs by applying existing legal frameworks. In the United States and Europe, the regulatory authorities classify EVs as pharmaceuticals or biological products. On the other hand, EVs should continue to be discussed with regard to the regulations, since some people might be still convinced that EVs, which contain no cells, will be less strictly regulated. Among them, EVs isolated from blood products like PRP might be less regulated than those from expanded MSCs in terms of the regulations. Additionally, manufacturing and release criteria would be required for an EV product.

Properly controlled clinical trials and regulations are essential steps in translating innovation or novel technology from the bench to the bedside. The initial step consists

of the definition of a problem to be solved and there is a real opportunity when it comes to cartilage damage and osteoarthritis. The development of EVs have been recognized as a viable alternative for treating these conditions and, in order to prove this concept. Collaboration between industry, academia and other institutions is crucial and can provide different perspectives during the research and development process, and facilitates the carrying out of all experimental studies necessary to prove the effectiveness and safety of EVs for later application in clinical practice [67].

Limitations

It is essential to acknowledge the current limitations and potential risks associated with EV-based therapies, including minor concerns about MSCs pro-tumorigenic properties and differentiation into undesired cell types. Although disease-modifying effects have been established with the utility of MSC-derived EVs, different cell sources might exert response differently. Moreover, OA is a disease of the whole joint and combined usage of EVs from different cell sources might be considered an alternate strategy. However, the pore size of the extracellular matrix of articular cartilage is estimated at around 6.0 nm [68]. This lays down a biological barrier that could be counteracted only by small cationic nanocarriers of size less than 15 nm in diameter [69].

While EVs like exosomes range in size from 30-150 nm, it is prudent to work on improving the delivery efficacy of its contents into the chondrocytes. Further, the thickness of the cartilage adds to the barrier affecting its permeability to act on the subchondral resident progenitors [70]. Direct subchondral targeting could be an option although technically and logistically more challenging and costly to achieve. Although encapsulating EVs in a scaffold appears as a reasonable strategy to achieve controlled release in the vicinity to reduce the number of injections needed, the material properties and its pharmacokinetics need further evaluation [71]. Further research is needed to address these concerns and optimize the therapeutic potential of exosomes in OA treatment [66]. While preclinical studies have shown promising results, translating these findings into clinically effective treatments for OA in humans and animals is an ongoing challenge. Further research and clinical trials are needed to establish the safety and efficacy of EV-based therapies in diverse populations. The lack of standardized protocols for EV isolation, characterization, and administration poses a challenge to the reproducibility and comparability of research findings. Establishing standardized procedures in terms of dosage and frequency of MSC-derived EV administration is essential for advancing the field and ensuring the reliability of EV-based therapies.

With their regenerative and anti-inflammatory properties, EVs hold significant potential for addressing the underlying causes of OA. Continued research into the mechanisms of action and optimization of EV-based therapies could lead to breakthroughs in regenerative medicine. The shift towards cell-free products, such as EVs, offers advantages in reduced tumorigenic potential and immunologic reactions compared to traditional cell-based therapies. This could pave the way for safer and more accessible treatment options. While challenges and limitations exist, the prospects for EV-based therapies in the treatment of osteoarthritis are promising. Continued research, addressing safety concerns, and standardizing protocols will contribute to advancing this field, potentially revolutionizing the landscape of OA treatment in both human and veterinary medicine.

Conclusions and Future perspective

With recent advancements in the field of EVs, we may have new bespoke therapeutic options on the horizon for addressing cartilage injuries and osteoarthritis in clinical practice. On the other hand, there are still several potential problems to be solved, including their classification, safety and toxicity in clinical use, as well as optimal dosage and frequency for human administration. These questions are crucial in order to

satisfy international health regulations before MSC-derived EVs can be safely progressed to a clinical product. Indeed, MSC-derived EVs were shown to be generally well-tolerated and to have minimal risk of immunogenicity and toxicity [36,72]. Also, a promising new fabrication method for clinical grade EVs has been developed for future clinical use [63], which will allow large-scale production and off-the-shelf use.

On the other hand, purification methods for EVs are varied , and thus their product specifications will need to be standardized for future clinical studies. In addition, there are still much to be verified, such as differences in effects depending on preconditioning and cell origin types. Therefore, further studies are needed to elucidate these issues in the near future.

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Authors	Animal	Cell source	EV type and size	Model	Biological effects
Cosenza, et al. (2017)[51]	Mouse	Bone marrow MSC	Exosome 112 ± 6.6 nm	Collagenase-induced OA model	Bone marrow MSCs and exosomes equally protected mice from joint damage.
Oh, et al. (2020)[61]	Rabbit	Adipose-derived stem cells	Pressure-concentrated supernatant of ADSC culture	Auricle cartilage defect model	ADSCs significantly enhanced new cartilage formation, but their secretome did not.
Zavatti, et al. (2020)[62]	Rat	Amniotic fluid stem cells	Exosome	MIA-induced OA model	Exosome enhanced pain tolerance level and improved OA histological scores than the AFSC-treated defects.
Tang, et al. (2021)[73]	Rat	Human umbilical cord-derived MSC	sEV 70-90 nm	ACL transection OA model	sEVs had effective therapeutic properties similar to MSCs in suppressing inflammatory responses and subsequently ameliorated OA.
Chen et al. (2022)[74]	Rat	Wharton's jelly MSC	sEV 100-200 nm	ACL transection OA model	MSC and sEVs equally promoted cartilage and subchondral bone repair, as well as enhanced extracellular matrix synthesis.

Chang et al. (2023)[75]	Rat	Adipose-derived stem cells	Hypoxia-induced exosome Approx. 130 nm	ACL transection OA model	Hypoxia-ADSC-Exos and ADSCs had a chondroprotective effect that suppressed cartilage erosion and reversed proteoglycan and type II collagen in OA cartilage.
Warmink et al. (2023)[76]	Rat	Bone marrow MSC	EV Avg. 125 nm	Cartilage defect model	MSC-EV resulted in lower cartilage degeneration, less pain behavior, osteophytosis and joint inflammation, than MSC.

Table 1. Comparison of therapeutic effects between MSCs and EVs in the animal studies.

ACL, anterior cruciate ligament; ADSC, adipose-derived stromal cell; AFSC, Amniotic fluid stem cells; EV, extracellular vesicle; Exo, exosome; MIA, monoiodoacetate; MSC, mesenchymal stem/stromal cell; OA, osteoarthritis; sEV, small extracellular vesicle

Authors	Animal	Cell source	EV type and size	Purification method	Model	Assessment	Biological effects	Mechanism of actions
Cosenza, et al. (2017)[51]	mouse	BMSC	Exosome 112±6.6nm	Ultracentrifugation	Collagenase-induced OA model	Histology, μCT, confocal laser microscopy	Protected mice from developing OA.	Enhanced type II collagen, aggrecan, and

								inhibiting MMP-13, ADAMTS 5, iNOS.
Wang Y, et al. (2017)[77]	mouse	MSC derived from ESC	Exosome 38-169 nm	Ultracentrifugation	Destabilization of MM model	Histology, immunohistochemistry	Exerted a beneficial therapeutic effect on OA by balancing the synthesis and degradation of chondrocyte ECM, and alleviated OA development.	Maintained the chondrocyte phenotype by increasing collagen type II synthesis and decreasing ADAMTS 5 expression.
Zhu Y, et al. (2017)[56]	mouse	SMSC and iPSC-derived MSC	Exosome 50–150 nm	Ultracentrifugation	Collagenase-induced OA model	Macroscopic, histology, immunohistochemistry	iMSC-Exos had a greater therapeutic effect on OA than SMSC-Exos.	Stimulated chondrocyte migration and proliferation.
Wang R, et al. (2020)[78]	mouse	Chondrogenic progenitor cell	EV 50–150 nm	Ultracentrifugation	Destabilization of MM model	Histology, immunohistochemistry	Prevented the development of OA.	Connected OA repair to processes such as MAPK

								signaling, regulation of autophagy, and insulin signaling.
Duan A, et al. (2021)[79]	mouse	SMSC	EV 50–200 nm	Ultracentrifugation	Destabilization of MM model	Histology, immunohistochemistry	LPS-preconditioned EVs had better cartilage protection compared with EVs.	Promoted proliferation and migration of chondrocytes and inhibited the apoptosis of chondrocytes.
Hanai H, et al. (2023)[63]	mouse	AMSC	sEV 50–100nm	Tangential flow filtration and concentrated	Collagenase-induced OA model	Macroscopic, histology	Prevention of OA progression	Promoted the cellular proliferation, migration, chondrogenic differentiation, and anti-apoptotic activity.
Zhang S, et al. (2016)[80]	rat	embryonic MSC	Exosome	Tangential flow filtration	Osteochondral	Macroscopic, histology,	Promoted repair of critical-	N/A

			100 nm	and concentrated	defect model	immunohistochemistry	sized osteochondral defects.	
Tao SC, et al. (2017)[57]	rat	miR-140-5p-overexpressing SMSC	Exosome 30-150 nm	Supernatant (culture media)	Transected MCL, MM, ACL model	Histology, immunohistochemistry	Successfully prevented OA.	Enhanced the proliferation and migration of articular chondrocytes.
Chen W, et al. (2019)[81]	rat	BMSC	N/A	Supernatant (culture media)	ACL transection and destabilization of MM model	Macroscopic, histology, immunohistochemistry, μCT	Remarkable articular-protective effect, well-maintained subchondral bone structure, and significantly more abundant cartilage matrix were observed.	Decreased ratio of MMP-13 to TIMP-1, and inhibited chondrocyte apoptosis with enhanced autophagy.
He L, et al. (2020)[82]	rat	BMSC	Exosome Avg. 153 nm	Ultracentrifugation	OA model by injection of sodium iodoacetate	Macroscopic, histology, immunohistochemistry, pain assessment	Effectively promoted cartilage repair and extracellular matrix synthesis,	Upregulated COL2A1 protein and downregulated

							as well as alleviated knee pain.	MMP13 protein
Zavatti M, et al. (2020)[62]	rat	Amniotic fluid stem cell	Exosome	Centrifugal Filter Units	MIA-induced OA model	Histology, immunohistochemistry, behavioral scoring	Enhanced pain tolerance level and improved OA histological scores.	Promoted the anti-inflammatory M2 macrophage.
Zhou Y, et al. (2021)[64]	rat	miR-126-3p-overexpressing synovial fibroblasts	Exosome 100 ± 10 nm	Ultracentrifugation	Transecting ACL and resecting MM model	Macroscopic, histology, immunohistochemistry, μMRI, μCT	Suppressed the formation of osteophytes, prevented cartilage degeneration, and exerted anti-apoptotic and anti-inflammatory effects on articular cartilage.	Promoted chondrocyte migration and proliferation. Suppressed apoptosis and IL-1β, IL-6, and TNF-α expression.
Liu Y, et al. (2022)[83]	rat	Urine-derived stem cells transfected with	Exosome Avg. 135.5 nm	Ultracentrifugation	Transecting ACL and resecting MM model	Behavioral, macroscopic, histology, immunohistochemistry, μCT	Enhanced cartilage regeneration and subchondral bone	Suppressed the progression of OA in part mediated

		miR-140s					remodeling.	by VEGFA.
Hosseinzadeh M, et al. (2023)[84]	rat	Chondrocytes and BMSC	EV 44.25nm and 112.1nm	Ultracentrifugation	MIA-induced OA model	micro-X-ray, histology, immunohistochemistry	EVs from the higher ratio of chondrocyte to MSC co-culture had superior chondrogenic potential and resulted in fully regenerated osteoarthritic cartilage.	Enhanced type II collagen, aggrecan, and decreased type X collagen.
Liang H, et al. (2023)[85]	rat	Synovial fluid MSC	EV 117 nm	Ultracentrifugation	Transecting ACL and resecting MM model	Histology, immunohistochemistry, serum cytokines concentrations	Provided chondroprotective effects that were dose-dependent.	Displayed more COL2A1 and less MMP13. Decreased serum proinflammatory cytokines.
Wong KL, et al. (2020)[86]	rabbit	ESC-derived MSC	Exosome 100-200 nm	Tangential flow filtration and concentrated	Osteochondral defect model	Macroscopic, histology, immunohistochemistry, biomechanical.	Combination of MSC exosomes and HA could promote sustained	Mediated and maintained cell migration, proliferation and

							and functional cartilage repair.	GAG synthesis.
Yang H, et al. (2022)[87]	rabbit	BMSC	Exosome Avg. 131.2nm	Commercially available kit	Osteochondral defect model	Macroscopic, histology	Facilitated cartilage regeneration as evidenced by gross view and histology.	Promoted cell proliferation and migration in chondrocytes.
Hsueh YH, et al. (2023)[88]	rabbit	iPSC	EV Avg. 136.8 nm	Ultracentrifugation	ACL transection model	Macroscopic, histology, immunohistochemistry	Inflammation, subchondral bone protrusion, and articular cartilage destruction, were ameliorated	Reduced cartilage destruction by the upregulation of collagen II and down-regulation of MMP13 and ADAMTS 5.
Zhang S, et al. (2022)[65]	porcine	ESC-derived MSC	Exosome Avg. 147.4 nm	Tangential flow filtration and concentrated	Osteochondral defect model	Macroscopic, histology, immunohistochemistry, MRI, μCT, biomechanical	Combination of MSC exosomes and HA promoted functional cartilage and subchondra	N/A

	l bone repair.

Table 2. Summaries of therapeutic effects of EVs in the animal studies.
ACL, anterior cruciate ligament; ADAMTS, a disintegrin and metalloproteinases with thrombospondin motifs; AMSC, adipose MSC; BMSC, bone marrow MSC; CT, Computed Tomography; ECM, extracellular matrix; ESC, embryonic stem cell; Exo, exosome; EV, extracellular vesicle; GAG, glycosaminoglycan; HA, hyaluronic acid; IL, interleukin; iNOS, inducible nitric oxide synthase; iPSC, induced pluripotent stem cell; LPS, lipopolysaccharide; MCL, medial collateral ligament; MIA, monoiodoacetate; MM, medial meniscus; MMP, matrix metalloproteinase; MRI, Magnetic Resonance Imaging; MSC, mesenchymal stem/stromal cell; OA, osteoarthritis; sEV, small extracellular vesicle; SMSC, synovial MSC; TIMP, tissue inhibitor metalloproteinase; TNF, tumor necrosis factor; VEGFA, vascular endothelial growth factor A

Ethics approval

As this article is a narrative review and no direct patients were involved, it was exempt from obtaining patient consent by our institutional review board.

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Declaration of competing interest

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Author contribution

K.S.: Concept of manuscript, manuscript first drafting, revision and proofing. K.L.W.: Manuscript drafting, revision and proofing. S.S.: Manuscript drafting, revision and proofing. S.M.: Manuscript drafting, revision and proofing. S.C.: Revision and proofing. T.L.F.: Manuscript drafting, revision and proofing. A.M.: Manuscript drafting, revision and proofing.

Declaration of interests

☐ The authors declare that they have no known competing financial interests or

personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Ansar Mahmood reports a relationship with Arthrex and J&J. that includes: consulting or advisory. Sathish Muthu reports a relationship with AO Spine Knowledge Forum LV Award Travel Grant that includes: travel reimbursement. All authors are members of ICRS NextGen Committee. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed Patient Consent

☒The authors declare that informed patient consent was not provided for the following reason:

As this article is a narrative review and no direct patients were involved, it was exempt from obtaining patient consent by our institutional review board.