Small extracellular vesicles in osteoarthritis: A double-edged sword regulating inflammation and cartilage homeostasis (Review)

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Abstract. Osteoarthritis (OA) is a progressive degenerative joint disease and a leading cause of pain, disability and loss of joint function in older adults worldwide. Small extracellular vesicles (sEVs) are small vesicles secreted by cells and found in various body fluids. They carry a range of bioactive molecules, including nucleic acids, proteins and lipids, and participate in numerous cellular physiological activities, such as cell death, inflammation, immunity, proliferation, migration and differentiation. Depending on their cellular origin, sEVs can exert opposing effects on OA: They may either aggravate inflammation and cartilage damage or suppress inflammation and promote cartilage repair. Therefore, this review focuses on the preclinical research evidence regarding the dual mechanism of 'promoting or treating' by sEVs in the disease of OA, aiming to construct a theoretical framework to provide a theoretical basis for the development and translational application of OA treatment strategies based on sEVs.

Contents

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1. Introduction

Osteoarthritis (OA) is one of the most prevalent chronic joint disorders and a major contributor to pain and disability in the aging population (1). It is characterized by progressive cartilage degradation, synovial inflammation, subchondral bone remodeling and osteophyte formation, and it seriously affects both physical and mental health (2). Statistics indicate that OA affects 250 million people globally. Among individuals >60 years of age, 10% of men and 18% of women suffer from knee OA (3). Current treatment mainly involves drugs and surgical treatments. Drug therapies include systemic agents [such as non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics] and local treatments [such as intra-articular injections of hyaluronic acid (HA) or corticosteroids]. Although these approaches can, to a certain extent, delay OA progression and alleviate patients' discomfort, long-term systemic medication use is associated with several adverse reactions (4). Surgical options, including arthroscopic debridement, osteotomy, joint replacement and total joint arthroplasty, are generally reserved for patients with moderate to severe conditions who have failed conservative treatment. Although surgery can significantly restore joint function, it also comes with substantial trauma, high costs, long recovery times and limited prosthesis longevity (5).

Currently, stem cell therapy has become a hot topic of interest for researchers, but it faces numerous challenges, such as immune rejection, tumorigenicity and unclear cell differentiation, all of which may affect its efficacy (6). Compared to stem cells, small extracellular vesicles (sEVs) possess low immunogenicity, high physicochemical stability and inherent biocompatibility, which have drawn considerable attention (7).

As small vesicles secreted by cells, sEVs can carry and deliver bioactive molecules to regulate various cellular activities. It is worth noting that in the context of OA, sEVs derived from chondrocytes, synovial cells, immune cells and mesenchymal stem cells (MSCs) can exhibit either pathogenic or protective effects, depending on their cell sources and the molecular cargo they carry (8). This review systematically summarizes the current research progress on the mechanism of sEVs as mediators in the progression and treatment of OA diseases. It particularly focuses on the preclinical evidence from *in vitro* and *in vivo* experiments. By integrating these preclinical research results, the study aimed to construct a framework system to provide a basis and guidance for the clinical translation of OA treatment in the future.

2. sEVs

Structure and composition. EVs are a class of nano-sized particles released by cells with a lipid bilayer structure. Their diameters typically range from 10 to 10,000 nm and they are widely distributed in various biological fluids and cell culture supernatants. Conventionally, EVs are classified into three main subtypes: sEVs, microvesicles and apoptotic bodies (9). Of note, the International Society for EVs explicitly recommended in its latest position paper (MISEV2023) that the term 'exosome' should not be used unless the item being referred to can be rigorously experimentally proven to have an endosomal origin (10). Based on this, current scientific literature tends to use 'sEVs' (particle size, <200 nm) to refer to what was previously called 'exosomes'. In this review, spherical vesicles of 30 to 150 nm in diameter were explicitly defined as 'sEVs'. sEVs consist of 'membrane' and 'contents'. The 'membrane' is a lipid bilayer whose surface carries various receptors, allowing targeted recognition and uptake by other cells. Research indicates that sEVs contain approximately four times as much lipid as their parent cells, which facilitates signal transmission and uptake by other cells (11). As for contents, sEVs carry various bioactive molecules such as proteins, lipids and nucleic acids, which can be transported to target cells and exert specific functions (12). Researchers have identified >8,000 proteins and 194 lipids within sEVs, confirming them as crucial carriers and signaling molecules in intercellular communication (13).

Biogenesis, release and uptake. The formation of sEVs involves the following steps (Fig. 1): Phase 1, invagination of the maternal membrane forms early endosomes (EEs). The EEs act as 'sorting center', receiving various 'cargo' from the parent cell membrane and cytoplasm (e.g., nucleic acids and proteins). Phase 2: The EEs gradually mature into the late endosomes, whose membranes invaginate to form multivesicular bodies (MVBs). Phase 3: MVBs face two possible fates: Fusion with lysosomes for degradation or fusion with the plasma membrane for release into the extracellular fluid (14). Subsequently, they are taken up by target cells through paracrine pathways. There are three ways for target cells to acquire them: i) Ligand-receptor binding: Ligands on sEVs bind to receptors on the target cell membrane, directly stimulating downstream signaling pathways; ii) endocytosis: Target cells internalize whole sEVs via phagocytosis or other mechanisms; and iii) direct fusion: The sEV's membrane fuses directly with the target cell membrane, releasing its contents (15).

Extraction and separation. Isolation and extraction are the prerequisites for obtaining highly pure and highly active sEVs, which directly determine the reliability of subsequent experimental results and transformation effects. Currently, commonly used sEVs extraction methods mainly include ultracentrifugation, density gradient centrifugation, immunomagnetic bead separation, polymer precipitation, size exclusion chromatography (SEC) and sEVs kit-based extraction (16). Ultracentrifugation separates sEVs based on density and size. By adjusting rotational speed and duration, impurities are effectively removed to yield highly purified sEVs. Ultracentrifugation is still the most widely used technique for isolating of sEVs from biological fluids and culture supernatants. Although suitable for processing large sample volumes, it has inherent drawbacks: High shear force may disrupt the structural integrity of sEVs. Furthermore, it is time-consuming, labor-intensive and costly (17). Compared with ultracentrifugation, density gradient centrifugation can further enhance purity, but it involves complex operations and limited sample throughput, making it suitable for experiments requiring high purity (18). Polymer precipitation is a simple technique that requires no special equipment. It works by reducing the solubility of target entities to facilitate sEV isolation. Although this method can produce relatively high concentrations of sEVs, it introduces a considerable amount of polymer impurities. This method is easy to operate and suitable for large-scale extraction (19). SEC utilizes a porous packing column to separate sEVs and retain sEV activity. Compared with traditional methods, SEC not only enhances isolation efficiency and purity but also preserves structural integrity of sEVs by obviating physical damage. As a result, SEC is widely regarded as a preferred or benchmark technique in various laboratory settings, and is particularly suitable for small-volume samples. Nevertheless, its relatively low yield may constrain its practicality in large-scale experimental and clinical applications (20). Commercial kit-based extraction procedures, distinguished by their ease of use and high throughput, have been widely adopted across diverse research settings. These kits generally function by altering the solubility of sEVs through polymer-mediated means, enabling isolation under low-speed centrifugation. This method is especially advantageous for processing samples with limited volume (such as synovial fluid aspirates obtained in routine clinical practice). While kit-based extraction approaches can achieve higher yields of sEVs, their purity and specificity are relatively low. Furthermore, they are prone to co-precipitation of non-vesicular impurities (such as proteins and polymeric aggregates), which may significantly interfere with experimental results (21). The benefits and drawbacks of the various separation methods are summarized in Table I (17-22).

Of course, the methods for separating sEVs from different samples vary. Synovial fluid samples are usually collected in very small quantities and have high viscosity, which may interfere with the polymer precipitation process (resulting in excessive co-precipitation) or affect SEC analysis (causing column blockage or co-elution of large molecular aggregates).

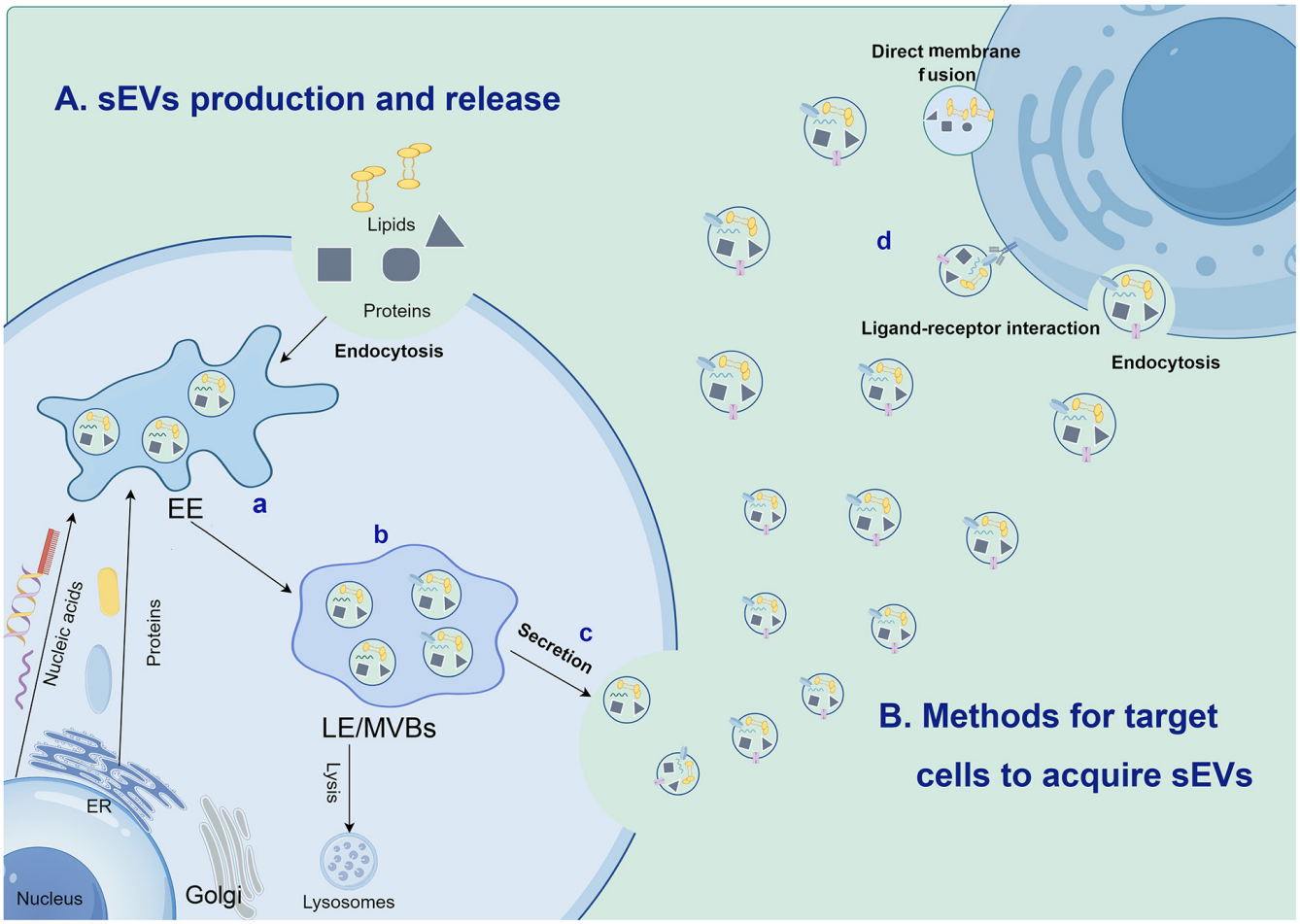

Figure 1. Formation, release and uptake of sEVs. (A) Formation and release of sEVs. a) The cell membrane and associated lipids and proteins invaginate together to form EEs, which contain nucleic acids from the cell nucleus and proteins synthesized by the ER. b) The early endosomes gradually mature into LEs or MVBs. c) MVBs have two fates: They are either degraded by lysosomes or secreted from the cell. When sEVs are secreted, they bind to target cells through paracrine pathways. (B) Mechanisms for target cells to acquire sEVs. d) There are three uptake mechanisms: i) Endocytosis (phagocytosis or other internalization pathways); ii) ligand-receptor binding, which triggers intracellular signal transduction and may subsequently initiate internalization; iii) direct membrane fusion, that is, the sEV membrane fuses with the target cell plasma membrane, releasing the contents into the cytoplasm. MVBs, multivesicular bodies; sEV, small extracellular vesicle; EE, early endosome; LE, late endosome; ER, endoplasmic reticulum.

Therefore, pre-treatment steps are usually required before sample separation, such as dilution, centrifugal removal of cell debris or enzymatic digestion with hyaluronidase (23). Blood contains various proteins, which may contaminate sEVs during separation. In addition, platelet activation in plasma releases a large number of additional vesicles, so special attention should be paid to the selection of anticoagulants and centrifugal conditions during collection to minimize platelet contamination (24). Urine contains Tamm-Horsfall protein, which can form aggregates and capture sEVs. Therefore, reducing-reductive pre-treatment is usually required. Brain spinal fluid samples have small volumes and a low yield of sEVs, and accordingly, high-recovery-rate separation methods, such as immunoprecipitation chromatography or microfluidic devices, should be used (25). Saliva contains a large amount of mucin and bacterial vesicles, and additional steps of low-speed centrifugation and density gradient centrifugation are required (26).

Furthermore, the optimal separation method not only depends on the sample type but also on the downstream research goals: In the field of biomarker discovery (such as spectral analysis of low-abundance proteins or nucleic acids in cohort studies), purity and reproducibility are of utmost importance, as contaminating non-vesicular substances may produce false-positive signals. Therefore, even at the expense of yield and throughput, high-resolution techniques such as density gradient ultracentrifugation, immunoprecipitation or sequential SEC-density gradient ultracentrifugation, are often preferred (27). Conversely, for therapeutic applications, maintaining biological activity and safety is the primary consideration, and therefore, size exclusion chromatography is more recommended (28).

In conclusion, there is no single method that is applicable to all detection scenarios. It is necessary to comprehensively consider the sample characteristics (viscosity, volume, interfering substances) and research goals, and adopt complementary detection techniques and strict identification measures, which is crucial for ensuring the reproducibility of experimental results.

Identification. Current methods for characterizing sEVs include nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM) and western blotting (WB) (29).

Table I. Common sEVs isolation methods and their benefits and drawbacks.

Strategy	Principle	Advantages	Disadvantages	(Refs.)
Ultracentrifugation method	Separation based on density and size of sEVs	Low cost, high purity	Time-consuming, labor-intensive, high-quality equipment, low recovery rate	(17,21, 22)
Density gradient centrifugation method	Utilizes sucrose or iodixanol to form density gradients, enriching sEVs through ultracentrifugation	High purity	Time-consuming operation, small sample processing capacity	(18,22)
Kit extraction method	Modifies the solubility of sEVs using polymers to precipitate sEVs during low-speed centrifugation	Convenient and rapid, high recovery rate	High cost, reagent residues, lower specificity and purity	(21)
Polymer precipitation method	Utilizes polyethylene glycol to competitively bind water molecules	Simplifying sEVs precipitation	Low purity, potential impurities	(19)
Size exclusion chromatography	Uses a porous packing column to separate	High efficiency, high purity, good sEV integrity	Limited sample processing capacity, lower production volume	(20)

sEVs, small extracellular vesicles.

NTA is based on laser scattering and Brownian motion tracking technology, which enables precise measurement of sEV size distribution and particle concentration, providing crucial data on basic properties of sEVs. However, NTA cannot distinguish non-vesicular particles with sizes similar to sEVs, and accordingly, complementary methods are needed for validation (30). TEM allows direct visualization of sEV size, bilayer membranes and cup-like structures, which aids in gaining a deeper understanding of their physical properties. However, TEM provides only semi-quantitative assessments (31). WB enables qualitative and quantitative analysis of specific proteins on the sEV surface. These include positive markers such as CD9/CD63/CD81 (tetraspanins) and (tumor susceptibility 101) TSG101/programmed cell death 6 interacting protein (PDCD6IP) (ESCRT complex), along with negative markers such as Calnexin (an endoplasmic reticulum protein) and LaminB (a nuclear protein), thereby elucidating biochemical characteristics. However, WB frequently produces false-positive results due to contamination by cellular debris, and it fails to provide information on the total number or size distribution of sEVs (32). Therefore, a combination of multiple complementary methods is routinely employed in experimental practice for comprehensive identification and characterization of sEVs.

3. Role of sEVs in OA inflammation and cartilage damage

sEVs and inflammation. Synovial inflammation is a typical pathological feature of OA. By disrupting the microenvironment within the joint, it triggers a series of pathological and physiological changes, thereby becoming a key driving factor for the disease (33). As essential mediators of intercellular communication, sEVs play a pivotal role in initiating and perpetuating synovial inflammation (34). sEVs do not function as random messengers. Instead, they regulate their pro-inflammatory effects by acting on several mechanistic pathways within the receptor cells, including the inflammatory signal transduction cascade and macrophage polarization (35-38).

Inflammatory signal transduction cascade reaction. sEVs can actively regulate the classical inflammatory signaling pathways within the OA joint. The synovial fluid of patients with OA contains high levels of pro-inflammatory cytokines, such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), which can be transported and delivered to receptor cells by sEVs (39). These sEVs exert immunostimulatory effects by activating the nuclear factor κB (NF-κB) pathway and the phosphatidylinositol-3-kinase (PI3K)/AKT signaling axis. For instance, the long non-coding RNA (lncRNA) OANCT derived from chondrocytes is packaged into sEVs, targets and binds to the fat mass and obesity-associated protein (FTO protein) and subsequently promotes M1-type macrophage polarization through the PI3K/AKT pathway, thereby amplifying the inflammatory response (40). At the same time, proteins related to sEVs, including IL-1β, IL-6 and various chemokines released by activated synovial macrophages, further drive the inflammatory cascade through paracrine signal transduction (41). It is worth noting that different sEVs carriers can act on the same signaling pathway: Although microRNAs (miRNAs) and lncRNAs usually regulate signal transduction indirectly through target gene inhibition or protein isolation, sEV proteins exert more direct effects on these pathways (42).

Furthermore, different miRNAs, even the same miRNA molecule, can produce different biological effects under different conditions. Although miR-146a and miR-146b-5p have highly similar sequences, they play opposite roles in OA: miR-146a is generally considered to have an anti-inflammatory effect,

while miR-146b-5p-especially secreted by M1-type macrophages-exacerbates the inflammatory response through the ubiquitin specific peptidase 3 (Usp3)/SRY-related high-mobility group box protein B5 (Sox5) pathway (43,44). Remarkably, the elevated level of miR-146a in OA synovial fluid sEVs is often positively correlated with the severity of the disease (45), suggesting the failure of a compensatory anti-inflammatory mechanism. This context-dependent duality reveals a key principle: The functional outcome of sEV-miRNAs is not only determined by their molecular characteristics, but also by the polarization state of the parent cells and the local microenvironment.

Macrophage polarization and immune regulation. The polarization of macrophages towards the pro-inflammatory M1 phenotype is a key event in the pathogenesis of OA, and sEVs play a central role in this process. sEVs from the synovial fluid of patients with OA can be efficiently internalized by macrophages, thereby stimulating the polarization of the M1 phenotype and promoting the release of various inflammatory mediators, including IL-1β, TNF-α and matrix metalloproteinases (MMPs) (46). These findings reveal the important role of sEVs in shaping the immune response associated with OA (47,48). It is notable that sEVs derived from inflamed synovial tissue also carry proteins such as apolipoprotein A1 (a protein closely associated with disease activity), which can act on monocytes and stimulate their production of inflammatory cytokines and exacerbate local inflammatory responses (49).

In conclusion, the pathogenic role of sEVs in OA is not mediated by a single molecule, but is achieved through the synergistic action of multiple interrelated signaling pathways. sEVs form a self-reinforcing vicious cycle: Inflammatory signal transduction promotes the polarization of macrophages to the M1 type, and this polarization further enhances the release of pro-inflammatory sEVs. This comprehensive research perspective emphasizes that the final pathological outcome results from the cumulative effect of multiple RNA and protein cargoes on the common downstream signaling pathways.

sEVs and cartilage damage. In addition to regulating synovial inflammation, sEVs also directly participate in the processes of cartilage damage and matrix degradation during the pathogenesis of OA through multiple interrelated mechanisms. These mechanisms can be broadly classified into three major pathways: i) Extracellular matrix (ECM) degradation and cartilage destruction; ii) regulation of cell survival, autophagy and apoptosis; iii) oxidative stress and ferroptosis; and iv) the regulation of the tissues surrounding the cartilage and neurogenic regulation.

ECM degradation and cartilage destruction. sEVs directly participate in the degradation of ECM and cartilage destruction through two pathways: Indirect regulation and direct enzymatic action. In the indirect regulation aspect, sEVs carry non-coding RNAs, thereby regulating the expression of catabolic enzymes. For instance, treating chondrocytes with sEVs derived from OA synovial fluid can reduce cell viability, decrease the expression of catabolic-related genes (such as type II collagen and aggrecan) and increase the expression of catabolic-related genes [such as MMPs and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)] (39,50). Among the molecules carried by these sEVs, the non-coding RNAs encapsulated in sEVs are key factors driving the inherent inflammatory response and catabolic process in the OA pathological process (51).

At the direct action level, sEV-related enzymes (including MMPs and cathepsins) are transported to the cartilage surface, where they degrade type II collagen and other ECM components (52). This mechanism that directly leads to structural destruction is likely to play a dominant role in the acute phase of OA. Additionally, during the progression of OA, the increase in low density lipoprotein receptor related protein 1 shedding from articular cartilage mediated by endocytosis mechanisms promotes the occurrence of inflammatory responses and damages cartilage integrity (53).

It is noteworthy that certain sEV-carrying molecules can exert protective effects, thereby counteracting their degradation effects. For instance, lncRNA H19 inhibits ECM degradation induced by IL-1β through regulating miR-106b-5p, and promotes chondrocyte proliferation and migration (54). This phenomenon indicates that the effect on ECM integrity reflects the dynamic balance between pathogenic and protective signals transmitted by sEVs.

Regulation of cell survival, autophagy and apoptosis. In addition to affecting the cytoskeleton, sEVs also regulate chondrocytes and synovial cells by modulating autophagy and apoptosis. These processes are closely related to the progression of OA and cartilage loss. The sEV-miR-449a-5p derived from chondrocytes can inhibit the expression of autophagy-related protein 4 homolog B (ATG4B) by targeting its mRNA, thereby blocking the autophagy process of macrophages and promoting the secretion of IL-1β (55). Similarly, in the OA rat model, the overexpression of miR-182 in synovial tissue can target forkhead box O3 (FOXO3)-related mRNAs, suppressing FOXO3 expression and promoting cell apoptosis and inflammatory responses (56). These research results indicate that sEVs can regulate the balance between cell survival and death by targeting the core components of autophagy and apoptosis pathways.

At the functional level, sEV-miRNAs can act on various signaling pathways, such as the autophagy pathway mediated by Usp3, Sox5, FOXP3 and FOXO3 to regulate key biological processes, including inflammatory responses and cell survival (57). It is worth noting that the pathological process of OA is not driven by a single miRNA, but rather results from the synergistic action of multiple molecules. Different miRNAs and even the same miRNA can produce different biological effects under different conditions, which fully demonstrates their 'environmental dependence' (58,59). However, there is still a lack of comprehensive understanding of how sEVs regulate these interacting cellular pathways.

Oxidative stress and ferroptosis. sEVs can reduce the antioxidant stress capacity of chondrocytes in OA animal models and exacerbate cell apoptosis, thereby directly damaging the cartilage (60). However, the effects of sEVs on cartilage are not limited to classical cell apoptosis; they also involve specific cell death programs driven by metabolic disorders, among which ferroptosis has attracted considerable attention. Ferroptosis is a unique cell death mode triggered by intracellular iron accumulation, lipid peroxidation and plasma membrane damage (61). Ferroptosis releases various small molecule substances (such as MMPs), which subsequently cause damage to the cartilage

and matrix (62). Recent evidence indicates that ferroptosis is an important mechanism by which pathological sEVs induce chondrocyte death. Kong *et al* (63) demonstrated that sEVs derived from fibroblast-like synoviocytes would disrupt the antioxidant defense system of chondrocytes, thereby promoting iron overload and lipid peroxidation. This observation suggests that chondrocyte death is not solely attributed to the classical apoptotic pathway but also involves the participation of metabolic regulatory mechanisms centered on iron homeostasis and oxidative stress.

Regulation of the tissues surrounding the cartilage and neurogenic regulation. In addition to the regulatory effects of inflammatory cells and synovial cells on cartilage changes, subchondral osteoblasts can also secrete sEVs that promote catabolic metabolism of chondrocytes. These sEVs inhibit the energy metabolism of chondrocytes and thereby induce cartilage degradation (64). This finding suggests that sEVs derived from subchondral bone actively participate in the pathogenesis of OA by targeting the metabolic adaptability of chondrocytes, rather than simply acting on actinomorphin, and by regulating the metabolism of chondrocytes.

Furthermore, nerve stimulation can affect the process of cartilage destruction mediated by sEVs, revealing a new type of neural-immune-bone axis. Guan *et al* (65) confirmed that in the context of OA, sympathetic nerve activity is enhanced, leading to an increase in norepinephrine levels. This can activate the β1-adrenergic receptor signaling pathway on chondrocytes. This activation process promotes the release of exosomes rich in miR-125 from chondrocytes, and subsequently, these sEVs are transported to the subchondral bone, where they promote osteoblast differentiation, disrupt the homeostasis of the subchondral bone and ultimately exacerbate cartilage damage in aged mice (65). It is worth noting that changes in the expression level of sirtuin 6 were also observed in chondrocytes, which is related to the aforementioned process. These observations indicate that the OA pathological process is not solely dominated by internal joint mechanisms; instead, sEVs from surrounding tissues (including bone and nerves) also play a non-negligible promoting role. Furthermore, these findings also imply that the metabolic communication between bone, nerves and cartilage is partially achieved through intercellular signal transduction mediated by sEVs.

In summary, the cartilage damage in OA is driven by four interrelated sEV-mediated mechanisms: i) ECM degradation caused by both indirect genetic regulation and direct enzymatic action; ii) cartilage cell survival imbalance triggered by inhibition of autophagy and induction of apoptosis; iii) oxidative damage ultimately leading to ferroptosis; and iv) neurogenic regulation. These signaling pathways do not function independently but are likely to amplify the cartilage degradation process through synergistic effects. For instance, cartilage cells that undergo ferroptosis may release damage-associated molecular patterns, which can then stimulate synovial inflammation; and inflammatory sEVs can, in turn, make cartilage cells more sensitive to oxidative stress (66). The identification of this multi-dimensional regulatory network highlights the need for future research to deeply analyze the relative contributions of each pathway and to explore whether sEVs from different cell sources exhibit different characteristics of cartilage damage.

Interaction between inflammation and bone changes in OA. OA is a systemic joint disease involving interactions between cartilage and synovium, which collectively drives its progression (67). In the early stages of OA, inflammation initiates and develops, but the cartilage surface remains relatively intact due to the body's compensatory mechanisms. In the late stages, intra-articular decompensation occurs, inflammation worsens, cartilage is destroyed and even bone changes occur, simultaneously releasing large amounts of pro-inflammatory mediators that further exacerbate inflammation. This vicious cycle ultimately leads to joint disruption, causing pain, swelling and stiffness. Inflammation persists throughout the process. Degradation products from joint cartilage can trigger inflammation, leading to synovial fibrosis, vascularization and immune cell infiltration (68). Subsequently, immune cells, particularly M1-polarized macrophages, secrete pro-inflammatory cytokines, accumulating these factors and accelerating cartilage destruction (69). Concurrently, subchondral bone thickens and hardens, thereby impairing cartilage nutrient supply. Additionally, subchondral bone cells secrete pro-inflammatory cytokines, driving cyclic degradation and destruction of cartilage (70). Nevertheless, the 'vicious cycle' is mainly based on causal inferences and temporal correlations. To date, limited investigation has adopted a longitudinal approach to monitor the evolution of sEV signatures spanning the transition from initial inflammatory events to advanced structural deterioration within an identical animal cohort (71). This lack of such longitudinal research data is the greatest deficiency in supporting this theoretical evidence.

In summary, sEVs participate in the cartilage damage process of OA through multiple molecular mechanisms: They can not only directly degrade and disrupt ECM structure by regulating the expression of genes related to matrix metabolism, but also regulate the balance of autophagy and apoptosis, mediate oxidative stress and ferroptosis to directly induce the death of chondrocytes, and receive pathological signals from surrounding tissues such as subchondral bone and nerves to amplify the effect of cartilage damage. Furthermore, the inflammatory response in OA and the apoptosis of chondrocytes can mutually reinforce each other, forming a vicious cycle, ultimately leading to irreversible disease progression (Fig. 2).

4. Applications of sEVs in anti-inflammation and cartilage regeneration

Application in anti-inflammatory therapy. Inflammation is known to permeate the entire process of OA development. Therefore, suppressing inflammation is a major therapeutic measure to counteract OA and protect joints from damage. The primary clinical approach to suppressing OA inflammation currently involves NSAIDs. These drugs exert anti-inflammatory and analgesic effects by inhibiting cyclooxygenase activity, thereby reducing prostaglandin synthesis (4). However, NSAIDs may bring side effects, such as gastrointestinal discomfort and adverse cardiovascular reactions (72). In addition, as the disease progresses, NSAIDs may fail to sustain their initial efficacy (73). In contrast, sEVs are small, immunogenicity-low and relatively stable cargo carriers. They can be taken up by target cells and, through various regulatory mechanisms, avoid the toxic side effects of drugs.

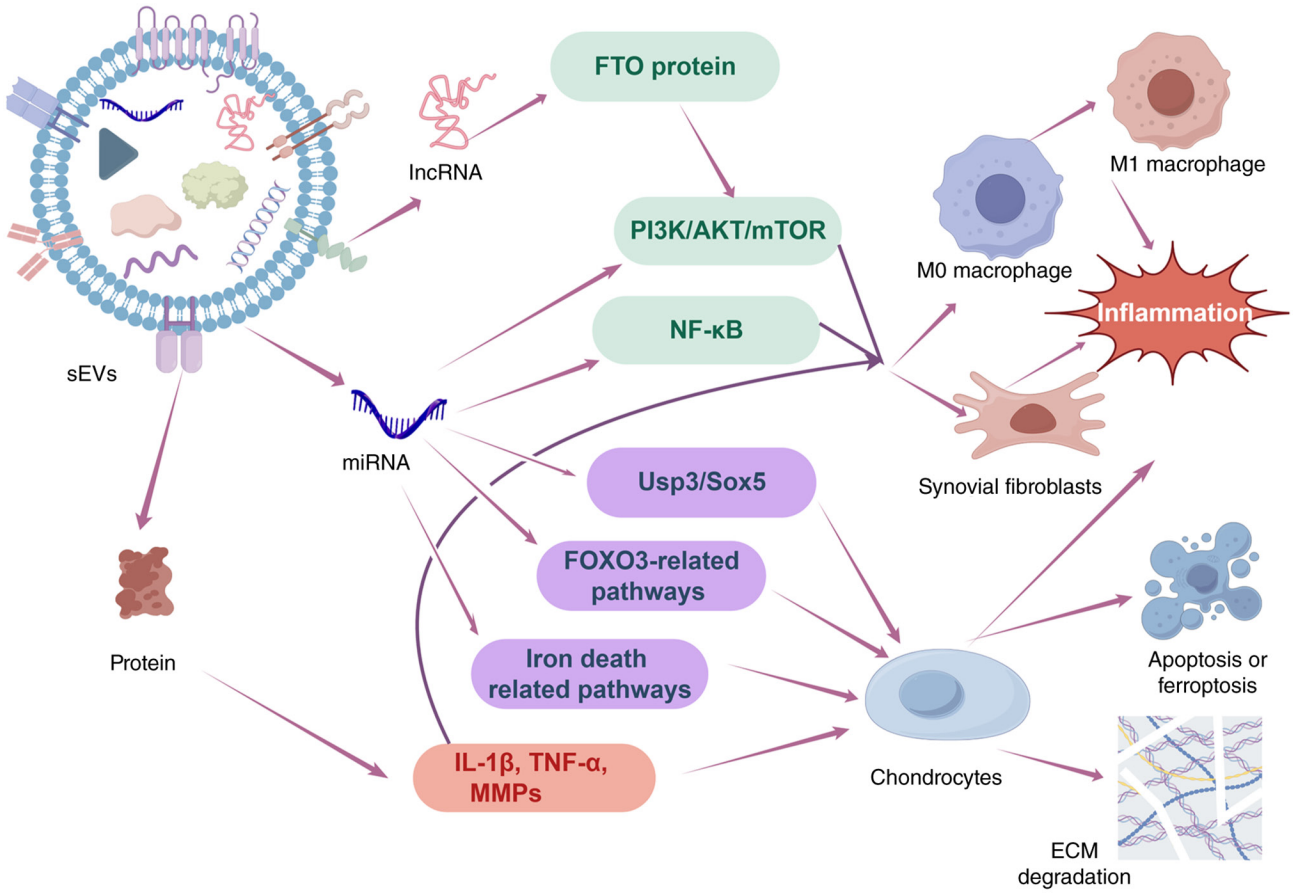

Figure 2. sEVs promote inflammation and cartilage damage. Under the condition of OA, various small molecules released by tissues and cells, such as miRNA, lncRNA and proteins (such as IL-1β, TNF-α and MMPs), can activate NF-κB, PI3K/AKT, Usp3/Sox5 and ferroptosis-related signal pathways, and target intracellular molecules and pathways such as FTO protein and FOXO3 mRNA, thereby promoting the transformation of M0 macrophages into M1 type (pro-inflammatory), and inducing the secretion of pro-inflammatory cytokines by synovial fibroblasts, apoptosis or ferroptosis of chondrocytes, and degradation of ECM, in order to promote the development of OA. OA, osteoarthritis; sEV, small extracellular vesicle; ECM, extracellular matrix; miRNA, microRNA; lncRNA, long non-coding RNA; FOXO3, forkhead box O3; FTO, fat mass and obesity-associated; Usp3, ubiquitin specific peptidase 3.

Consequently, sEVs have unique advantages and potential in anti-inflammatory treatment of OA (74). The following table summarizes experimental findings on the anti-inflammatory effects of sEVs over the last 5 years (Table II) (75-98).

Regulation of intracellular signal pathways. sEVs, as natural nanocarriers, can carry various small molecules and bind to target cells to regulate multiple intracellular singaling pathways (such as PI3K/AKT/mTOR and NF/κB pathways), inhibiting the release of inflammatory mediators such as IL-1β, TNF-α and inducible nitric oxide synthase, thereby alleviating the inflammatory response in OA joints (99).

The NF-κB signaling pathway is a key pathway associated with inflammatory progression and tissue destruction in OA (100). Proinflammatory cytokines can activate the NF-κB pathway, thereby suppressing chondrocyte anabolic metabolism and promoting the secretion of various MMPs to accelerate articular cartilage destruction. Furthermore, activation of this pathway induces chondrocyte release of inflammatory cytokines and chemokines, including TNF-α, IL-1β, IL-6, IL-8 and receptor activator of nuclear factor-κB ligand, thereby amplifying inflammatory responses (101). Consequently, sEVs can target NF-κB pathway inhibition to suppress host inflammation. For instance, sEVs derived from bone marrow mesenchymal stem cells (BMSCs) have been proven to significantly inhibit the NF-κB pathway, and the physical pre-treatment can enhance this inhibitory effect (80,82). Nevertheless, it must be acknowledged that the NF-κB pathway is a fundamental signaling axis for host immune defense and inflammatory resolution. Systemic blockade of this pathway has potential safety risks, such as increased susceptibility to pathogens and impaired immune surveillance function (102).

By comparison, the PI3K/Akt/mTOR signaling pathway presents a more favorable and selective intervention window. It inhibits chondrocyte apoptosis by regulating downstream pathways (including Bad, Caspase-3 and NF-κB) and is therefore crucial for regulating chondrocyte proliferation, apoptosis and matrix homeostasis (103). In the early stage of OA, the PI3K/AKT/mTOR pathway can be activated by TNF-related apoptosis-inducing ligand, IL-15, IL-22 and various growth factors, including AKT and mTOR phosphorylation to exacerbate the inflammatory response and promote chondrocyte apoptosis (104). sEVs can act as various inhibitors within this signaling pathway, such as negative regulators of mTOR and TNF receptor-associated factor 6 (TRAF6), thereby suppressing OA inflammation (105). For instance, Lou *et al* (83) utilized engineered sEVs to target TRAF6 and inhibit the PI3K/AKT/mTOR pathway, which not only reduced

Table II. Application of sEVs in anti-inflammatory therapy for OA.

Authors, year	Source	*In vivo* model	Mechanism of action	(Refs.)
Song *et al*, 2024	M2 macrophages	Surgically induced rat knee OA	Enhancing lymphatic endothelial cell activity, proliferation, migration and capillary formation and reducing apoptosis	(75)
Liang *et al*, 2025	ADSCs	NR	Promoting FOXC1 deubiquitination to induce macrophage M2 polarization	(76)
Zhang *et al*, 2025	BMSCs	Sodium iodate-induced rat knee OA model	Inhibiting NF-κB to control inflammatory mediators	(77)
Zhang *et al*, 2025	M2 macrophages	Sodium monoiodoacetate-induced rat knee OA model	Reducing the expression of inflammatory factors and promoting the transformation of M1 type macrophages into M2 type	(78)
Tao *et al*, 2021	BMSCs	Rat OA model	Inhibiting NF-κB signaling pathway	(79)
Liao *et al*, 2021	BMSCs	Rat OA model	Inhibiting the activation of the NF-κB signaling pathway	(80)
Chen *et al*, 2025	hUC-MSCs	Sodium iodacetate-induced rat OA model	Inhibiting the MAPK4/NF-κB signaling pathway	(81)
Kim *et al*, 2021	MSCs	NR	Inhibiting the miR-147b-mediated NF-κB pathway to suppress inflammatory mediator expression	(82)
Lou *et al*, 2023	BMSCs	Rat OA model	Inhibiting the expression of TRAF6 to suppress inflammation	(83)
Liao *et al*, 2025	Platelet-rich plasma	Medial meniscus-induced mouse OA model	Activating PI3K/Akt signaling pathway to enhance synovial lymphocyte function, promoting clearance of inflammatory cells and associated cytokines	(84)
Cheng *et al*, 2025	Milk	NR	Inhibiting the TLR4/NF-κB signaling pathway and the PI3K/AKT signaling pathway	(85)
Li *et al*, 2024	MSCs	Surgically induced rat knee OA	Modulating PI3K-Akt pathway to inhibit degradative enzymes MMP3 and MMP9 expression and activate antioxidant enzymes	(86)
Li *et al*, 2025	M2 macrophages	Surgically induced rat knee OA	Inhibition of miR-124-3p/STAT3 axis	(87)
Karaturk *et al*, 2025	DPSCs	NR	NR	(88)
Kang *et al*, 2025	ADSCs	Surgically induced rat knee OA	Inhibiting chondrocyte hypertrophy and inflammatory response via CRIM1/BMP signaling pathway	(89)
Chen *et al*, 2024	Cartilage-derived stem cells	Sodium acetate-induced rat knee OA	Reducing inflammatory cytokine production and improving chondrocyte microenvironment	(90)
Cao *et al*, 2024	Synovial fibroblasts	Surgically induced mouse knee OA	SOD3 in EVs prevents ROS accumulation and reduces proinflammatory cytokines	(91)
Zhou *et al*, 2021	Synovial fibroblasts	Surgically induced rat knee OA	Reducing inflammatory cytokine production	(92)
Wan *et al*, 2023	BMSCs	Surgically induced mouse knee OA	Suppression of OA-associated inflammatory and gene expression, rescue of transcriptional responses	(93)
Qin *et al*, 2025	hUC-MSCs	Surgically induced mouse knee OA	Inhibiting Wnt/β-catenin pathway, enhancing autophagy	(94)
Liu *et al*, 2025	Garlic	Surgically induced rat knee OA	Inhibiting MAPK pathway activation and suppressing expression of degradative enzymes MMP3 and MMP9	(95)
Klyucherev *et al*, 2024	hUC-MSCs rat knee OA	Surgically induced	Downregulating inflammatory markers and increasing Arg-1 expression	(96)
Jiang *et al*, 2025	BMSCs	NR	Inhibiting the expression of pro-inflammatory and anabolic genes	(97)

Table II. Continued.

Authors, year	Source	*In vivo* model	Mechanism of action	(Refs.)
El-Din *et al*, 2024	BMSCs	Rat OA model	Regulation of circYAP1/miRNA-21/TLR7 axis	(98)

ADSCs, adipose-derived mesenchymal stem cells; OA, osteoarthritis; sEVs, small extracellular vesicles; FOXC1, forkhead box C1; BMSCs, bone marrow mesenchymal stem cells; NF-κB, nuclear factor-κB; TRAF6, tumor necrosis factor receptor-associated factor 6; TLR4, Toll-like receptor 4; MAPK4, mitogen-activated protein kinase 4; hUC-MSCs, human umbilical cord mesenchymal stem cells; PI3K, phosphatidylinositol-3-kinase; mTOR, mammalian target of rapamycin; MMP3, matrix metallopeptidase 3; DPSCs, dental pulp mesenchymal stem cells; CRIM1, cysteine-rich transmembrane module containing 1; BMP, bone morphogenetic protein; SOD3, extracellular superoxide dismutase; ROS, reactive oxygen species; mTORC1, mechanistic target of rapamycin complex 1; Arg-1, Arginase-1; circYAP1, circular RNA YAP1; miR, microRNA; NR, information was not reported in the literature or no animal models were used.

inflammatory mediators but also promoted autophagy. This observation implies that, relative to mere suppression of inflammatory signaling, activation of endogenous pathways such as autophagy may confer superior therapeutic benefits.

It is worth noting that most current studies on the regulation of the NF-κB and PI3K/AKT/mTOR pathways have used pathway inhibitors or agonists to indirectly infer the role of these signaling cascades. To date, only a few studies have verified the necessity of these signaling pathways through gene knockout techniques. For instance, conditional gene knockout of the phosphatase and tensin homolog in chondrocytes has been shown to constitutively activate the PI3K/AKT signaling pathway and promote the development of OA (106). Other studies have used alternative genetic approaches, such as gene knockdown, overexpression or drug regulation, to assess the involvement of NF-κB-related molecules (107,108). However, it should be noted that these studies explored the role of these signaling pathways in the pathogenesis of OA independently of sEV-mediated effects. Therefore, although these studies have provided valuable insights into the functional significance of these signaling pathways in OA, they do not constitute direct genetic evidence for the specific signal regulation of sEV, and their applicability to sEV-mediated therapy remains to be established. Furthermore, there is complex cross-regulation between the NF-κB and PI3K/AKT/mTOR pathways. However, to date, to the best of our knowledge, relatively few studies have evaluated the synergistic or antagonistic effects of sEVs when simultaneously acting on both pathways, which limits a comprehensive understanding of the therapeutic mechanisms (109).

Modulating the polarization of immune cells. sEVs can also modulate immune cell polarization, promoting the transition of M0 or M1 macrophages into M2 macrophages, thereby improving the intra-articular immune microenvironment and alleviating inflammatory responses (110). Under normal conditions, synovial macrophages within the joints remain quiescent; however, during OA inflammation, they polarize toward the pro-inflammatory M1 phenotype, thereby initiating or amplifying the release of a series of pro-inflammatory cytokines (111). By contrast, the anti-inflammatory M2 phenotype secretes IL-10, arginase-1 and transforming growth factor (TGF)-β, suppressing inflammation and promoting tissue repair (110). Therefore, using sEVs to polarize macrophages from M0 or M1 to M2 type is expected to alleviate synovial inflammation and mitigate cartilage degeneration, presenting a promising therapeutic approach for OA (112). To enhance this polarization effect, sEVs can be engineered to carry specific cytokines or proteins. For instance, Liang *et al* (76) successfully modified adipose-derived MSC-sEVs with ubiquitin-specific protease 15 (USP15). This enhanced the release of anti-inflammatory and chemotactic factors by inducing macrophage polarization toward the M2 phenotype through promoting FOXC1 protein deubiquitination (76). Qian *et al* (112) found that sEVs from M2 macrophages could target Toll-like receptor 3 (TLR3) and collagen type X α 1 chain (COL10A1) through miR-26-5p, also driving the M1 to M2 phenotypic transformation. However, it is important to note that although these studies have confirmed the feasibility of the sEV-induced M2 polarization mechanism, it remains to be further investigated whether the polarized M2 macrophages will revert to the M1 phenotype.

Application of engineered sEVs in anti-inflammatory therapy. Although sEVs possess strong barrier penetration and high circulatory stability, their entry into joints is rapidly cleared by intracellular cells or enzymatic reactions, thereby limiting their clinical application (35). To address these obstacles, two principal engineering approaches have been developed in recent years: Engineered sEVs and hydrogel delivery systems. Comparative evaluations have revealed that both modalities markedly enhance treatment outcomes (Table III) (113-118). Among them, engineering sEVs with exogenous materials can effectively increase their retention time and targeting accuracy in the joint cavity, thereby amplifying the anti-inflammatory effect (119). Hydrogels, with their unique biocompatibility, stability and degradability, are widely employed to enhance sEV retention and adhesion in OA joints (120). For instance, encapsulating M2 macrophage-derived sEVs within a thermosensitive Pluronic hydrogel significantly prolonged sustained release up to 12 days. This formulation effectively promotes synovial lymphatic vessel formation and enhances synovial lymph drainage, thereby suppressing inflammation (75). Another study demonstrated that, compared with the pure sEVs group, HA-alginate hydrogel-loaded sEVs (ALG-M2-sEVs hydrogel) could adhere to cartilage surfaces, enabling sustained delivery of M2-sEVs and extending its therapeutic duration. Rat models treated with ALG-M2-sEVs hydrogel exhibited significantly enhanced cartilage damage repair, highlighting the potential

Table III. Comparative analysis of delivery strategies for sEVs-based osteoarthritis therapy.

Therapeutic strategy	Modification method	Pharmacokinetic feature	Advantages	Limitations	(Refs.)
Native sEVs	Unmodified	Passive targeting, rapid clearance	High biocompatibility, simple preparation, low immunogenicity	Poor stability, low bioavailability, lack of targeting	(113)
Engineered sEVs	Surface modification (e.g. targeting peptides, antibodies) or cargo loading	Active targeting, moderately prolonged retention	Enhanced precision, increased cellular internalization, capability for drug loading	Complex engineering process, stability issues	(114,115)
Hydrogel-based delivery systems	Physical encapsulation of sEVs within hydrogel matrices	Passive retention, sustained release	Biocompatibility, biodegradability, significantly prolongs intra-articular time	Lacking active targeting, limited penetration into dense cartilage matrix	(116,117)
Engineered sEVs-hydrogel composite systems	Combination of hydrogel encapsulation technology with the surface/content material engineering of sEVs	Dual-targeting, controlled release	Synergistically addresses retention (hydrogel), specificity (engineering), bioactivity	Most complex fabrication, higher cost, regulatory challenges for clinical translation	(115,118)

sEVs, small extracellular vesicles.

of this hydrogel composite system for anti-inflammatory and cartilage repair applications (78). In contrast to free sEVs, this hydrogel-based delivery platform substantially extends the articular retention duration of sEVs, thus facilitating the maintenance of elevated local concentrations and subsequently attenuating inflammation. It is worth noting that recent research has combined these two strategies to address the two challenges of prolonged presence and organizational infiltration. Wan *et al* (93) developed spherical gelatin methacrylate (GelMA) hydrogels encapsulating collagen II-targeted peptide WYRGRL and small inhibitor leucine-rich repeat kinase 2 (LRRK2)-IN-1-modified engineered sEVs. Unlike the passive release of conventional hydrogels, this design overcomes dense collagen barriers and rapid sEVs clearance, enhancing targeting to chondrocytes and enabling delivery of the encapsulated LRRK2-IN-1 deep into cartilage. By inhibiting LRRK2, this approach regulates mitochondrial homeostasis, inflammation and autophagy, thereby suppressing inflammation and promoting cartilage repair. Through the integration of hydrogel-mediated sustained release with the active targeting capacity of engineered sEVs, this approach enables effective modulation of mitochondrial homeostasis and autophagic flux, thereby conferring superior therapeutic benefits relative to non-targeting delivery systems (93). This integrated approach epitomizes the advanced development in sEV-based therapeutic strategies for OA to date. In conclusion, sEVs inhibit the progression of inflammatory response by regulating a variety of signaling pathways and macrophage polarization processes, thereby inhibiting the expression of pro-inflammatory cytokines. Furthermore, engineered sEVs can prolong their retention time in local tissues and enhance anti-inflammatory effects; when coupled with targeting peptides, these sEVs can achieve better targeting specificity, which represents a more advanced technological improvement in the development of OA treatment strategies based on sEVs.

Applications in promoting cartilage repair. Beyond the occurrence and progression of inflammation, another crucial pathological feature of OA is the persistent degradation of articular cartilage accompanied by concomitant bone changes (68). The regenerative capacity of cartilage and bone tissue is limited. Furthermore, articular cartilage lacks vascularization and the supply of essential nutrients for oxygenation, and this condition is further exacerbated by continuous inflammatory damage caused by OA, thereby accelerating cartilage degeneration and bone spur formation (2). Currently, surgical interventions such as arthroscopic procedures and joint replacement remain the primary clinical approaches (5). However, high costs and postoperative complications continue to impose significant burdens on patients (121). The repair-promoting sEVs can inhibit the apoptosis of chondrocytes and the degradation of ECM, and also promote the proliferation and migration of chondrocytes as well as the differentiation of stem cells into chondrocytes. Therefore, as a means of cartilage repair, it is expected to play an immeasurable role in clinical practice in the future (122).

Inhibition of chondrocyte apoptosis and matrix degradation. On the one hand, sEVs can inhibit chondrocyte apoptosis, promote autophagy and suppress cartilage matrix degradation to inhibit cartilage degeneration (Table IV) (88,123-140).

Apoptosis is a cell death pathway triggered by external environmental stimuli [e.g., inflammatory cytokines, reactive oxygen species (ROS) and TNF-α]. It is executed through caspase-dependent signaling cascades, in which caspase-8 activates downstream executioner caspases (such as caspase-3 and caspase-7) to orchestrate programmed cell death (141). Stem cell sEVs can effectively inhibit chondrocyte apoptosis and subsequent cartilage degeneration by suppressing of multiple key genes or proteins in the apoptotic pathway and blocking the activation of NF-κB and other signaling cascades (140,142). Therefore, targeting these signaling pathways to prevent chondrodegeneration by modulating this pathway may become a future treatment approach for OA. At the apoptotic level, the pro-apoptotic effector BAX acts on the mitochondrial outer membrane, promoting its permeabilization and facilitating the release of cytochrome C and other pro-apoptotic factors into the cytoplasm. These factors, in turn, activate the Caspase family (caspase-3/7-9), triggering apoptosis and cell death (143). Accordingly, inhibiting BAX activation may be crucial for reducing chondrocyte apoptosis following injury. Studies have indicated that treatment of inflamed chondrocytes with human plasma sEVs downregulates BAX expression, thereby suppressing caspase-3 activation and inhibiting chondrocyte apoptosis. Concurrently, it enhances SOX9 activity, promoting chondrogenesis (127). Nevertheless, it should be noted that the human plasma sEVs employed in this investigation were obtained from healthy donors. In clinical practice, however, patients with OA often have multiple comorbid conditions, including diabetes, hypertension and hyperlipidemia. Whether such systemic metabolic disturbances modify the compositional profile and functional properties of circulating sEVs remains to be evaluated (144). Consequently, the true therapeutic effect of healthy donor-derived sEVs in the OA patient population may be subject to overestimation.

Autophagy serves as a protective mechanism in normal chondrocytes. Through autophagy, chondrocytes can self-clear defective organelles and macromolecules, enabling maintenance of nutrition and metabolism (145). This process promotes cell death induced by aging and trauma, thereby sustaining cellular homeostasis. sEVs can intrinsically activate autophagy programs in chondrocytes or serve as delivery carriers to promote senescent or injured chondrocyte autophagy (146). For instance, Matrilin-3 (MATN3), a non-collagenous chondrospecific ECM protein, alleviates autophagy defects in osteoblasts by binding to IL-17 and activating the PI3K/AKT/mTOR pathway, as demonstrated by Long *et al* (134). Their team successfully delivered MATN3 to OA models using sEVs extracted from synovial MSCs as carriers, thereby promoting chondrocyte autophagy and maintaining cellular homeostasis. However, autophagy is a double-edged sword, as excessive activation of autophagy can also lead to cell death, namely autophagic cell death. The study did not investigate whether the level of autophagy induced by MATN3 falls within a safe and beneficial range, nor did it establish the relationship between autophagic activity and cell survival. Furthermore, mitochondrial autophagy, a crucial form of cellular autophagy, participates in the pathogenesis of OA. Deficiency in mitochondrial autophagy results in chondrocyte death, ECM homeostasis imbalance and cartilage degeneration. Therefore, it has increasingly become a research hotspot. BMSCs-sEVs

Table IV. Application of sEVs in inhibiting chondrocyte apoptosis.

Authors, year	EVs source	Processing	Animal model	Mechanism	(Refs.)
Mao *et al*, 2021	BMSCs	No special treatment	Surgically induced mouse knee OA	Enhancing Col2a1 and Sox9 expression, suppressing MMP13 expression	(123)
Zhao *et al*, 2023	ADSCs	Hypoxia treatment	Lumbar arthritis in mice induced by spinal instability	Enhancing sEVs' promotion of chondrogenic anabolism and prevention of catabolism	(124)
Zhang *et al*, 2024	Expi293F cells	CAP-EVs/siMMP13	Surgically induced rat knee OA	Reducing MMP13 levels, increasing cartilage collagen COL2A1 and proteoglycans	(125)
Wang *et al*, 2022	ADSCs	miR-486-5p transfection	Surgically induced rat knee OA	Suppressing endoplasmic reticulum stress	(126)
Shi *et al*, 2023	Plasma	No special treatment	NR	Inhibiting chondrocyte apoptosis by suppressing BAX protein, downregulating TNF-α and upregulating TGF-β1, IL-4 and IL-10	(127)
Lu *et al*, 2025	BMSCs	Quercetin treatment	Surgically induced mouse knee OA	Reducing expression of proinflammatory genes and iNOS, TNF-α, MMPs	(128)
Karaturk *et al*, 2025	DPSCs	Hypoxia treatment	NR	Enhancing sEVs' suppression of inflammatory factors	(88)
Li *et al*, 2023	ADSCs	miR-376c-3p transfection	Sodium monoiodoacetate-induced rat knee OA	Targeting WNT3 or WNT9a to inhibit WNT/β-catenin pathway	(129)
Wang *et al*, 2025	Human amniotic epithelial stem cells	No special treatment	Surgically induced rat knee OA	Delivery of ACTA2-AS1 downregulates ACSL4, inhibiting lipid peroxidation and ferroptosis	(130)
Li *et al*, 2022	hUC-MSCs	No special treatment	Surgically induced rat knee OA	Proliferation and migration, inhibiting apoptosis, suppressing proinflammatory factor secretion, promoting anti-inflammatory factor expression, modulating macrophage polarization	(131)
Xu *et al*, 2024	Osteoblasts	No special treatment	Surgically induced mouse knee OA	Promoting chondrocyte migration and ECM production, inhibiting apoptosis by upregulating DLX2 to inactivate the Wnt pathway	(132)
Meng *et al*, 2023	ADSCs	No special treatment	Sodium iodacetate-induced rat knee OA	miR-429 promotes autophagy by targeting FEZ2	(133)
Long *et al*, 2023	Synovial mesenchymal stem cells	MATN3 treatment	Surgically induced mouse knee OA	MATN3 inhibits IL-17A/PI3K/AKT/mTOR pathway	(134)
Xu *et al*, 2023	Platelets	No special treatment	Surgically induced mouse knee OA	Modulating immune and inflammatory responses, promoting chondrocyte proliferation and migration	(135)
Liu *et al*, 2025	Human urinary stem cells	miR140 transfection	Surgically induced rat knee OA	Downregulating CAPN1 expression, improving mitochondrial morphology, reducing ROS accumulation, enhancing mitochondrial membrane potential, promoting mitochondrial autophagy	(136)
Chen *et al*, 2025	BMSCs	No special treatment	MIA-induced TMJOA rats	Inhibiting apoptosis and activating the PI3K/AKT pathway	(137)

Table IV. Continued.

Authors, year	EVs source	Processing	Animal model	Mechanism	(Refs.)
Cao *et al*, 2023	hUC-MSCs	CAP-EVs hydrogel	Surgically induced rat knee OA	Negative regulation of p53 signaling pathway inhibits OA chondrocyte senescence and inflammation	(138)
Yang *et al*, 2025	hUC-MSCs	miR-148a-3p transfection, CAP loading	Surgically induced mouse knee OA	miR-148a targets ROBO2 to maintain cartilage homeostasis	(139)
Xu *et al*, 2021	BMSCs transfection	miR-326 mimetic	Sodium iodate-induced knee OA in rats	Inhibiting pyroptosis in chondrocytes by targeting HDAC3 and activating STAT1/NF-κB p65 signaling pathways	(140)

BMSCs, bone marrow mesenchymal stem cells; OA, osteoarthritis; sEVs, small extracellular vesicles; Col2a1, collagen, type II; Sox9, SRY-box transcription factor 9; MMP13, matrix metallopeptidase 13; ADSCs, adipose-derived mesenchymal stem cells; CAP, cartilage affinity peptide; BAX, BCL2-associated X protein; TNF-α, tumor necrosis factor-α; TGF-β1, transforming growth factor-β1; iNOS, inducible nitric oxide synthase; DPSCs, dental pulp mesenchymal stem cells; WNT, wingless-type MMTV integration site family; HDAC3, histone deacetylase 3; NF-κB, the nuclear factor-κB; ACTA2-AS1, actin α 2, smooth muscle antisense RNA1; ACSL4, acyl-CoA synthetase long-chain family member 4; hUC-MSCs, human umbilical cord mesenchymal stem cells; ECM, extracellular matrix; DLX2, distal-less homeobox 2; FEZ2, fasciculation and elongation protein zeta 2; MATN3, matrilin-3; IL-17A, interleukin-17A; PI3K, phosphatidylinositol-3-kinase; mTOR, mammalian target of rapamycin; CAPN1, calpain-1 large subunit; ROS, reactive oxygen species; miR, microRNA; TMJOA, the temporomandibular joint OA; ROBO2, roundabout homolog 2; NR, information was not reported in the literature or no animal models were used; No special treatment, treatment with EVs alone.

have been confirmed to promote mitochondrial autophagy in chondrocytes, thereby inhibiting chondrocyte apoptosis (147). Additionally, Liu *et al* (136) engineered human urinary stem cells (hUSCs)-140-sEVs by transfecting hUSC sEVs with miR-140. These engineered sEVs delivered miRNA-140 to target and downregulate calpain 1 expression, thereby decreasing ROS accumulation, strengthening mitochondrial membrane potential and promoting mitochondrial autophagy (136). However, the detection methods used to assess mitochondrial autophagy in current studies vary significantly. Certain studies rely solely on immunoblotting analysis of microtubule-associated protein 1A/1B light chain 3 and p62, while others utilize the mito-Keima transgenic reporter system. The former cannot distinguish between mitochondrial autophagy and classical autophagy, while the latter faces considerable technical challenges in *in vivo* applications. This methodological discordance markedly undermines the comparability and reproducibility of conclusions drawn from different studies.

The ECM of articular chondrocytes is primarily composed of type II collagen synthesized and secreted by chondrocytes. It provides cushioning, wear resistance and lubrication; transports nutrients and metabolic waste to chondrocytes; and maintains joint structural integrity to prevent degradation. Following arthritis onset, various intra-articular cells secrete chondromatrix-degrading enzymes such as MMPs and ADAMTS5 to degrade type II collagen and disrupt cartilage integrity. Therefore, sEVs control of ECM degradation to preserve its integrity and stability represents another crucial therapeutic approach for OA. For example, sEVs derived from human umbilical cord MSCs can reduce the expression of MMP-13 and ADAMTS5 in chondrocytes induced by IL-1β (131), while miR-7704-modified sEVs can enhance the expression of type II collagen and reduce the level if MMP-13, which is helpful for maintaining ECM homeostasis (148).

In conclusion, sEVs exhibit multiple synergistic effects in cartilage repair, but their inherent repair capabilities still have clear boundaries. Although sEVs can inhibit apoptosis (for instance, through the BAX/Caspase-3 axis) and promote ECM synthesis, relying solely on sEVs to induce endogenous repair is often insufficient for large-area cartilage defects in advanced OA. Hence, future research priorities may shift toward sEV-based combination therapies, such as integrating sEVs with bioengineering strategies or combining sEVs with pharmaceutical agents.

Promoting the proliferation, migration, and differentiation of chondrocytes. On the other hand, sEVs can accelerate cartilage repair by promoting chondrocyte proliferation and migration. Chondrocyte proliferation and migration are crucial pathways for repairing damaged cartilage, which is essential for restoring the morphology and function of joints damaged by OA. sEVs can directly promote chondrocyte proliferation and migration (35,149). For instance, M2-sEVs have been proven to induce M1 macrophages to polarize towards the M2 phenotype, thereby alleviating the inhibition of chondrocyte proliferation by OA and reducing apoptosis (78). Furthermore, sEVs also stabilize the chondral microenvironment by promoting chondrocyte secretion of ECM. Human umbilical cord MSC sEVs secrete various immunomodulatory and reparative factors. They were shown to suppress the upregulation of MMP-13 and ADAMTS-5 while promoting increased type II collagen expression. Concurrently, they were observed to enhance type II collagen synthesis, thereby promoting ECM synthesis of chondrocytes (150). Nevertheless, that study did not evaluate the ultrastructure or mechanical modulus of the newly formed matrix, and thus could not confirm whether its quality is equivalent to that of native hyaline cartilage.

Additionally, sEVs can promote the differentiation of MSCs into chondrocytes to facilitate cartilage repair. sEVs

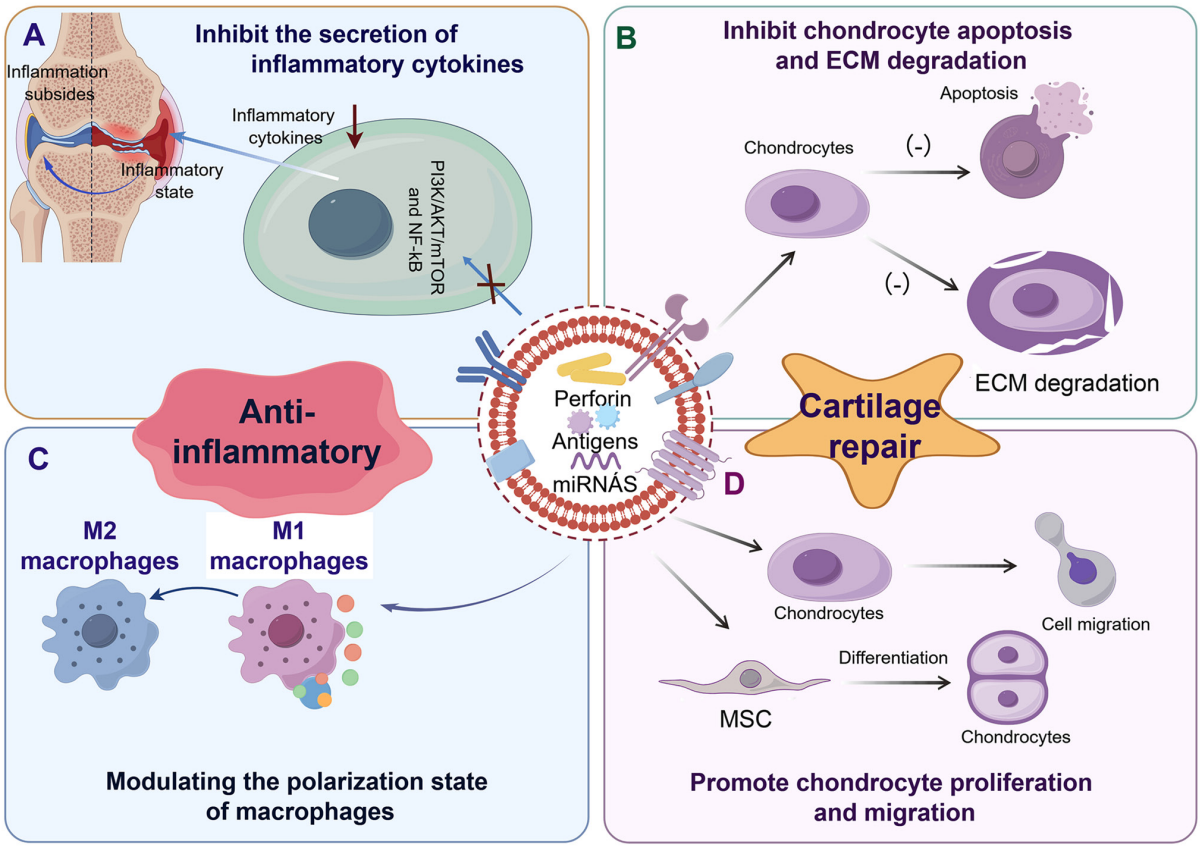

Figure 3. Applications of sEVs in anti-inflammation and cartilage. (A) Inhibition of inflammatory cytokines: sEVs deliver anti-inflammatory non-coding RNAs and proteins to block PI3K/AKT/mTOR or NF-κB signaling, reducing local inflammatory mediators and alleviating synovitis. (B) Inhibition of chondrocyte apoptosis and ECM degradation: sEVs protect chondrocytes from apoptotic stimuli and maintain cartilage matrix stability. (C) Modulation of macrophage polarization: sEVs deliver specific miRNAs to shift macrophages from the pro-inflammatory M1 to the anti-inflammatory M2 phenotype. (D) Promotion of chondrocyte proliferation and migration: sEVs stimulate resident chondrocyte proliferation and migration, and recruit MSCs to differentiate into chondrocytes, facilitating cartilage regeneration. Parts A and C represent the anti-inflammatory capacity of sEVs; and parts B and D represent the cartilage-repair capacity. sEV, small extracellular vesicle; ECM, extracellular matrix; miRNA, microRNA; MSC, mesenchymal stem cell.

are rich in or loaded with various growth factors, including TGF-β1, vascular endothelial growth factor and stromal cell-derived factor-1. These factors can induce various MSCs to differentiate into chondrocytes, thereby promoting cartilage repair (151). For example, TGF-β1 in sEVs suppressed mothers against decapentaplegic homolog 2/3 (Smad2/3), extracellular signal-regulated kinase 1/2 (ERK1/2) and p38 signaling pathways, thereby promoting BMSC differentiation (152). Wu *et al* (153) successfully engineered pH-responsive sEVs loaded with VSV-G and hyaluronan synthase 2 (HAS2) (V-H-sEVs). Under acidic conditions, the low pH responsiveness of the VSV-G protein not only effectively promoted sEVs fusion with the cell membrane but also increased the amount of HAS2 delivered to cells via sEVs. Chondrocytes induced by V-H-sEVs produced HA and promoted BMSC chondrogenic differentiation while preserving the chondrocyte phenotype (153). Special caution is warranted because during the differentiation of MSCs into chondrocytes, improper microenvironmental regulation, such as excessively high TGF-β concentrations or abnormal mechanical stimulation, can readily drive cells toward a hypertrophic chondrocyte phenotype, as indicated by high COL10A1 expression, rather than a stable hyaline chondrocyte phenotype. The latter may ultimately lead to endochondral ossification and rapid degeneration of the graft (154).

In summary, sEVs can prevent chondrocyte apoptosis and ECM degradation while promoting chondrocyte proliferation and migration, and facilitating MSCs to differentiate into chondrocytes. This offers tremendous potential for future cartilage repair and regeneration in OA (Fig. 3). However, a fundamental paradox still exists: The characteristic of end stage OA is severe disruption of the microenvironment, including persistent inflammation, oxidative stress, acidic pH value and excessive proteolytic activity (155). Whether exogenous sEVs can preserve their biological functionality stability in such harsh environments remains uncertain. Notably, most studies on sEVs promoting cartilage repair to date have adopted an experimental model in which inflammation is pretreated before sEVs administration. This experimental design, to a certain extent, avoids the most severe clinical reality, that is, the destructive impact that the microenvironment of advanced OA may have on the sEVs themselves that are administered. Therefore, more research is still needed in the future to evaluate the therapeutic effect of sEVs in cartilage repair.

5. The double-edged sword effect of sEVs: Causing disease or providing treatment

sEVs play a dual role in the occurrence, progression and treatment of OA, and this role depends on their cellular

origin and the surrounding environmental background (156). Furthermore, the bioactive molecules carried by sEVs do not act independently but rather work together through specific signaling pathways to mediate complex interactions between cells in the joint microenvironment. Their effects are bidirectional: They can either exacerbate the inflammatory response or participate in tissue repair, and the specific direction depends on the integration of the microenvironmental state and regulatory signals (56). This phenomenon is not random, but is governed by sophisticated regulatory mechanisms.

Causing disease: Disrupting homeostasis through inflammatory and catabolic pathways. In the pathological microenvironment of OA, sEVs derived from senescent chondrocytes, inflammatory synovial fibroblasts and M1 macrophages serve as important carriers for transmitting 'decompositional metabolic signals', markedly exacerbating local inflammation and cartilage degeneration (44,157-160). These sEVs not only contain abundant pro-inflammatory miRNAs (such as miR-146a and miR-155) and degradation enzymes like MMP-13, but also reduce or lack some protective anabolic miRNAs, such as miR-140-5p and matrix proteins. This imbalance lays the foundation for their pathogenic effects (161). Specifically, their pathogenic mechanism involves cross-regulation of multiple signaling pathways. First, sEVs derived from M1 macrophages can deliver specific miRNAs to chondrocytes, activating the NF-κB signaling pathway and weakening autophagy by targeting the Usp3/Sox5 axis or suppressing ATG4B, thereby disrupting chondrocyte metabolic homeostasis and promoting matrix degradation (44). Second, sEVs secreted by synovial cells carry lncRNA OANCT, which can bind to FTO protein and amplify the inflammatory response through the PI3K/AKT/mTOR pathway, while promoting the polarization of synovial macrophages to the M1 phenotype (40). At the same time, pro-inflammatory factors released by M1 macrophages and various enzymes (such as MMPs and collagenases) directly act on chondrocytes (41,52); and sEVs derived from synovial cells can also induce chondrocytes to undergo ferroptosis, further exacerbating tissue damage (63). These pathways ultimately synergistically activate NF-κB, inhibit autophagy and induce ferroptosis, forming a malignant microenvironment characterized by excessive catabolism and chronic inflammation at the joint site, continuously accelerating the degradation of ECM (162).

Providing treatment: Restoring homeostasis via anti-inflammatory and anabolic pathways. In contrast, 'therapeutic sEVs' derived from MSCs exert 'reparative effects'. They are capable of reprogramming recipient cells toward a state conducive to cellular well-being. These sEVs are primarily derived from MSCs or M2 macrophages and possess distinctive molecular signatures: They are enriched with anti-inflammatory miRNAs, such as miR-140-5p and miR-23a, and growth factors, while simultaneously suppressing pro-inflammatory cytokines, such as miR-155, and catabolic enzyme activity (52). Their therapeutic mechanisms are primarily achieved through the following pathways. First, MSC-derived EVs carrying 'therapeutic' miRNAs such as miR-26b-5p can target and inhibit the TLR3/NF-κB axis, promoting the polarization of M1 macrophages toward the anti-inflammatory M2 phenotype, thereby secreting IL-10 and TGF-β and reversing the inflammatory microenvironment (112). This accomplishes immunomodulation. Second, engineered sEVs, such as those carrying miR-149 or miR-140-5p, can target the PI3K/AKT/mTOR and RAS/ERK pathways within chondrocytes, suppressing apoptosis through downregulation of the BAX/Caspase-3 signaling cascade while simultaneously activating autophagic pathways to clear damaged organelles (163,164). This protects chondrocytes and eliminates damaged cellular components. Furthermore, these sEVs can modulate Smad signaling pathways to upregulate the expression of SOX9 and type II collagen, thereby promoting ECM synthesis and repair (165,166). This indicates that therapeutic sEVs can effectively reverse the deleterious microenvironment and restore joint homeostasis. The differences between these two types of sEVs are presented in Table V (40, 44,52,63,112,129,157-166) and Fig. 4. This distinction highlights the importance of developing sEV-based therapeutic strategies in research.

In summary, the fundamental distinction between pathogenic sEVs and therapeutic sEVs lies in the differential regulation of key signaling pathways, such as NF-κB and PI3K/AKT/mTOR, by their respective cargo compositions, as well as their distinct cellular origins. This difference highlights the critical importance of in-depth elucidation of their molecular profiles and downstream mechanisms in the development of sEV-based therapeutic approaches.

6. Limitations

Limitations of current in vitro models. sEVs have excelled in the treatment of a wide range of diseases due to their low immunogenicity, high stability and good biocompatibility (7). However, the clinical application of sEVs in treating OA still faces various challenges (Fig. 5A and B). First, most research is based on *in vivo* rat or mouse OA models. These physiological structures of rats or mice differ significantly from those of large animals or even clinical patients, and most animal models are artificially constructed, making it challenging to simulate the natural progression of OA in clinical patients (167).

Second, most experiments focus exclusively on the knee joint. While the knee is the most common site for OA, clinically, OA can occur in other joints as well. Although joint anatomy and physiology are broadly consistent, the human body's complex environment and physiological functions confer unique characteristics to each joint site. For example, the temporomandibular joint (TMJ) is the only bilateral connecting joint with a complex structure, precise regulation and intricate functions. This characteristic dictates that any stimulus affecting the TMJ will trigger a 'chain reaction' throughout the rest of the oromandibular system (168). Another distinction lies in neural distribution: The 3 cm sphere centered on the knee joint meniscus contains no sensory structures. Conversely, a 3 cm sphere centered on the TMJ disc encompasses multiple crucial anatomical structures and sensory nerves, rendering patients with TMJ-OA susceptible to neurological symptoms (169). Consequently, the clinical application of sEVs in OA treatment remains a distant prospect.

Current challenges in manufacturing and therapeutic delivery. Although the sEV-based treatment approach has

Table V. Comparative analysis of pathogenic and therapeutic sEVs in OA.

Feature	Pathogenic sEVs (drivers of OA)	Therapeutic sEVs (inhibitors of OA)	(Refs.)
Primary sources	Senescent chondrocytes, synovial fibroblasts (inflamed), M1 macrophages	MSCs, M2 macrophages, engineered cells	(44,52,157-160)
Key molecular cargo	Upregulated: Pro-inflammatory miRNAs (miR-146a, miR-155), catabolic enzymes (MMP-13, ADAMTS-5), inflammatory cytokines (IL-1β, TNF-α); Downregulated: Anabolic miRNAs (miR-140-5p, miR-23a), matrix proteins (collagen II, aggrecan)	Upregulated: Anti-inflammatory miRNAs (miR-140-5p, miR-23a), growth factors (TGF-β, IGF-1), immunomodulators (PD-L1, TSG-6); Downregulated: Pro-inflammatory miRNAs (miR-155), catabolic enzymes (low levels of MMP-13)	(44,52,157-161)
Target pathways	Activation: NF-κB MAPK/ERK; Promotion: Inflammation and catabolism	Inhibition: NF-κB, Wnt/β-catenin; Activation: PI3K/Akt, autophagy; Promotion: Anabolism and survival	(40,44,63,112, 129,162-166)
Biological effects	Degenerative: Inducing chondrocyte apoptosis, accelerating ECM degradation, spreading inflammation (SASP)	Regenerative: Promoting cell proliferation, enhancing matrix synthesis, modulation of immune response (M2)	(44,112, 157-160, 162-166)

OA, osteoarthritis; sEVs, small extracellular vesicles; MSCs, mesenchymal stem cells; miRNAs, microRNAs; MMP-13, matrix metallopeptidase-13; ADAMTS-5, a disintegrin and metalloproteinase with thrombospondin motif-5; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; TGF-β, transforming growth factor-β; IGF-1, insulin-like growth factor 1; PD-L1, programmed death ligand 1; TSG-6, TNF-stimulated gene-6 protein; NF-κB, the nuclear factor-κB; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; SASP, the senescence-associated secretory phenotype; PI3K, phosphatidylinositol-3-kinase.

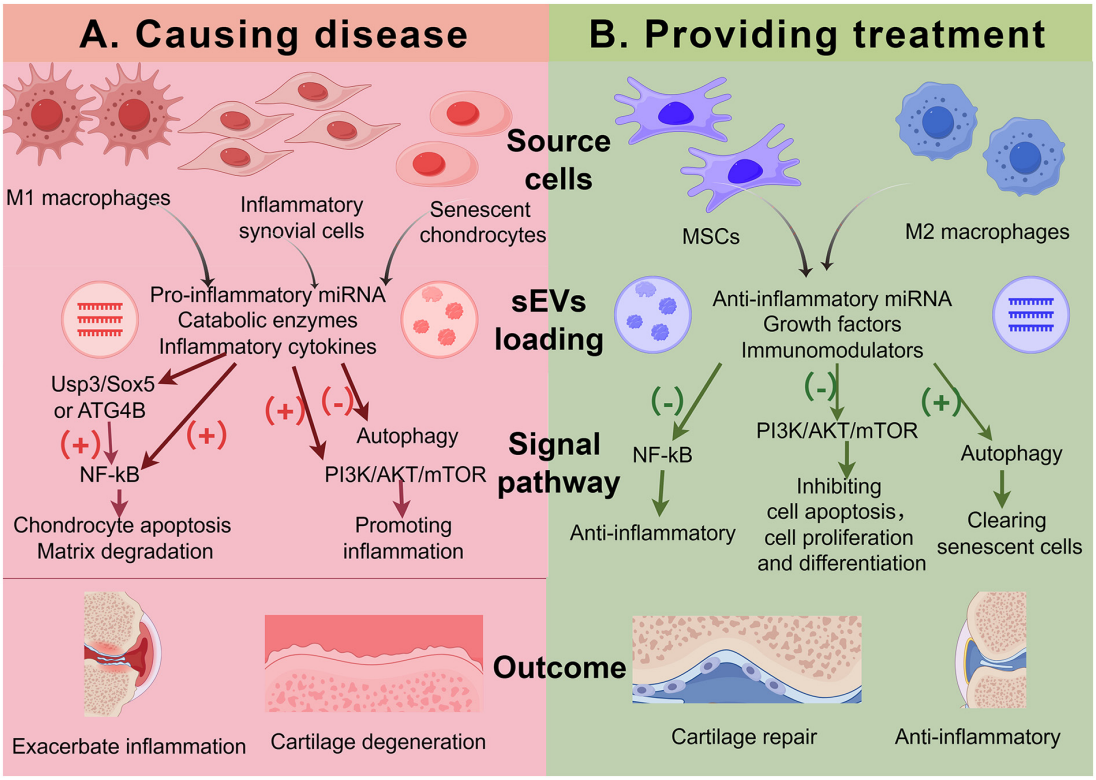

Figure 4. The dual roles of sEVs in OA: Promotion or treatment. (A) Pathogenic sEVs derived from M1 macrophages and inflammatory synovial cells carry pro-inflammatory miRNAs, proteins and cytokines. These sEVs target Usp3/Sox5 or ATG4B to indirectly activate NF-κB, or directly activate NF-κB and PI3K/AKT/mTOR, while suppressing autophagy, ultimately promoting inflammation, apoptosis and matrix degradation. (B) Therapeutic sEVs derived from MSCs and M2 macrophages carry anti-inflammatory miRNAs, growth factors and immunomodulators. These sEVs inhibit NF-κB and PI3K/AKT/mTOR or promote autophagy, thereby exerting anti-inflammatory effects, inhibiting apoptosis, promoting cell proliferation and migration, and inducing MSC differentiation into chondrocytes, culminating in cartilage repair. sEV, small extracellular vesicle; miRNA, microRNA; MSC, mesenchymal stem cell; ATG4B, autophagy related 4B cysteine peptidase; Usp3, ubiquitin specific peptidase 3.

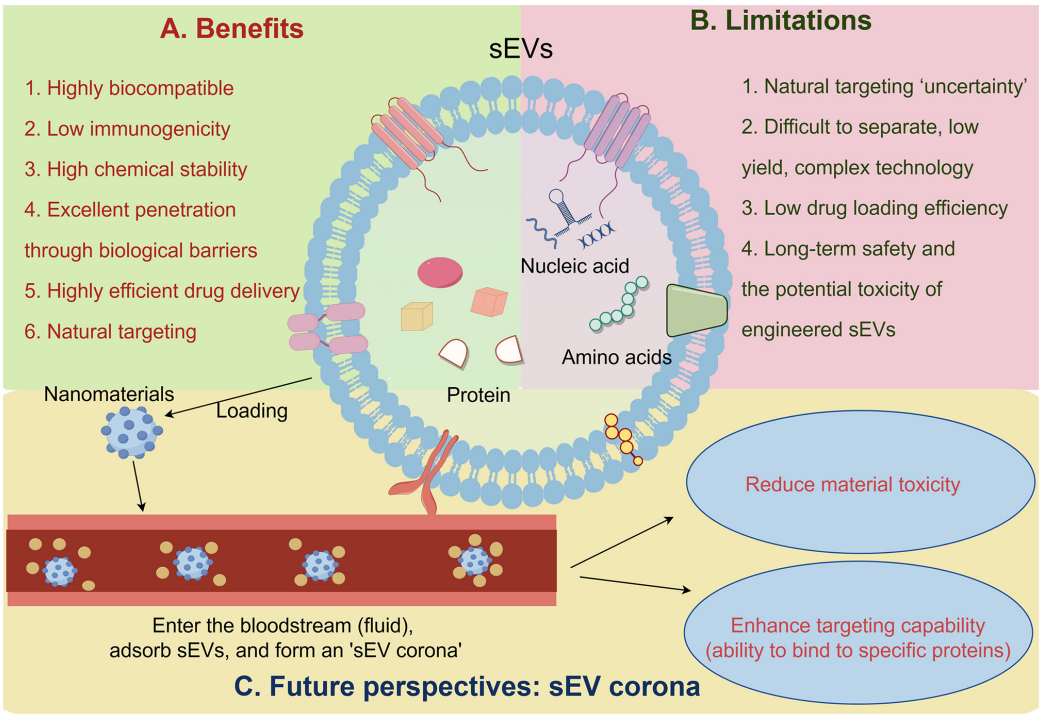

Figure 5. Benefits, limitations and future perspectives of sEVs (sEV corona). (A) Benefits: sEVs have low immunogenicity and high biocompatibility; they can penetrate biological barriers and deliver bioactive molecules (nucleic acids and proteins); they can serve as diagnostic biomarkers and natural drug delivery carriers, and can be engineered to enhance targeting specificity and therapeutic efficacy. (B) Limitations: The natural targeting properties of sEVs remain largely elusive; in addition, the separation techniques and production yields still restrict their large-scale preparation and clinical application; the drug loading efficiency has not reached the optimal level and the long-term safety of sEVs still needs further verification. (C) Future prospects (sEV shell layer): sEVs can form an 'sEV shell layer' by adsorbing surrounding biomolecules, which significantly changes their surface properties, biological distribution and cell uptake efficiency. sEV, small extracellular vesicle.

opened up new horizons for the treatment of OA, its manufacturing and delivery still face numerous interrelated challenges. These challenges include the following: A scalable production process that meets Good Manufacturing Practices (GMP) regulations and ensures consistency between batches, validated potency determination methods and product release standards, poor biological distribution characteristics and the rapid clearance phenomenon within the joint, as well as issues related to immunogenicity. Therefore, this section mainly focuses on these several challenges to promote the standardization of the practical application of sEVs.

Extensible and GMP-compliant manufacturing process with batch-to-batch consistency. The transition from laboratory-scale, research-grade materials to large-scale, clinical-grade production scale highlights a key bottleneck. This process requires optimization of both the upstream process (such as cell source selection, culture conditions, bioreactor systems) and the downstream purification steps (such as separation, concentration and formulation preparation) (170). Automated closed bioreactors have emerged as a promising platform for preparing GMP-grade sEVs derived from MSCs, enabling validation of each process step, selection of appropriate release criteria and ensuring consistency among batches (171). However, the inherent heterogeneity of sEVs makes the purification and scale-up production process complex. Key bottlenecks include donor cell source control, medium composition, culture system design, separation methods, batch yield, sterilization or biological load control, and storage stability (172). Due to the lack of standardized detection protocols, the quality of sEVs from different batches varies, which not only increases the difficulty of obtaining regulatory approval but may also affect the verification of clinical efficacy reproducibility (173). Therefore, it is necessary to strictly determine product characteristics to ensure that the sEVs used in clinical applications meet the minimum MISEV 2023 standards in terms of production, separation and characterization.

Potency testing and product-release criteria. Efficacy testing, as a crucial step in product release, currently lacks standardized testing methods. The basic batch release standards typically include sterility, endotoxin levels, total protein content and the size and concentration of sEVs particles, but these indicators cannot fully reflect the biological efficacy. Functional testing is increasingly regarded as a core component of the manufacturing strategy, shifting from post-event remedial measures to the cornerstone of early process development stages (174). Storage and product release standards present additional challenges: sEV therapeutic drugs must be transported under controlled conditions (typically 2-8˚C) and administered within a strict time window. For most sEV products, long-term stability studies under different storage conditions have not been fully characterized (175).

Biological distribution and joint retention. The rapid clearance rate of sEVs in the body and their ability to achieve precise targeted delivery are also key issues that need to be addressed urgently (119). A preclinical biodistribution study showed that after intra-articular injection, sEVs can remain in the joint cavity for at least 72 h. This characteristic, due to the

absence of significant leakage to other organs, further confirms their safety (170). However, sEVs administered systemically or accidentally distributed are prone to accumulate in the liver, spleen and lungs, thereby increasing the possibility of triggering unexpected tissue reactions (176). Furthermore, the effusion clearance effect and lymphatic drainage can rapidly reduce the vesicle concentration in the joint cavity, particularly in large joints. A single injection may not maintain a sustained and effective therapeutic concentration, and these drawbacks severely limit the clinical application of sEVs (177).

Immunogenicity and engineering-related safety. Although sEVs have relatively low immunogenicity on their own, through engineering modifications such as cross-linking, targeted peptide conjugation or surface modification, new toxicity or antigenicity that does not exist in the natural state may be introduced, especially in repeated exposure scenarios (178). Allogeneic donor-derived sEVs, due to the possibility of carrying membrane proteins from the donor source, may trigger immune recognition reactions (179). Minor changes in the production process, especially when introducing new surface groups or payloads, may affect their immunogenicity and must be strictly evaluated. For example, the photoinitiator used in gel synthesis (such as Irgacure 2959) may have cytotoxicity and can induce DNA damage and metabolic inhibition in chondrocytes, thereby converting potentially therapeutic materials into cytotoxic preparations (180). Therefore, the biological safety of engineered sEVs needs to be strictly evaluated.

Long-term safety and regulatory environment. The long-term safety profile of sEV-based therapies has not been fully elucidated, yet. Most published studies have employed single-dose or short-term dosing regimens in small animal models, which has led to a significant lack of in-depth exploration of the long-term persistence and safety of sEV-based interventions (178). Numerous key issues (including the immune response that may be triggered by repeated administration of sEV, the risk of off-target effects and the chronic toxicity characteristics of engineered sEV) remain unclarified (175). Currently, the US Food and Drug Administration has explicitly stipulated under the Public Health Service Act that engineered EVs are regarded as biological products, requiring submission of an Investigational New Drug (IND) application, complete chemical, manufacturing and control (CMC) documents, potency determination and strict virus safety testing before conducting clinical trials (181,182). The regulatory classification of sEV-based products remains rather complex, and comprehensive guidance documents and standardized production process specifications need to be formulated urgently.

Given the aforementioned challenges, future research should focus on developing sEV delivery strategies suitable for different joint sites, improving their *in vivo* utilization efficiency, continuously optimizing separation and purification techniques, establishing unified quality control standards in accordance with GMP requirements and promoting the conduct of more high-quality clinical studies to verify their feasibility.

Clinical translation and current research landscape. Although many preclinical studies have confirmed the potential of MSC-derived sEVs in the treatment of OA, their translation into clinical practice is still at an early stage, albeit with rapid development momentum. A systematic review and meta-analysis that included 28 animal experiments demonstrated that injecting such sEVs into the joint cavity can effectively alleviate cartilage damage and inflammatory responses, manifested by significant decreases in Osteoarthritis Research Society International and Mankin scores, thereby providing strong preclinical evidence for subsequent human trials (183).

Encouragingly, several clinical studies have been conducted in recent years. A landmark Phase I clinical trial conducted by Wang *et al* (150) in 2025 evaluated the safety and efficacy of human umbilical cord (hUC)-MSC-sEVs in 41 patients with knee OA. The study confirmed that the intra-articular injection of these sEVs did not cause systemic toxicity or serious adverse events. Notably, patients receiving high-dose treatment exhibited a significant improvement in the Western Ontario and McMaster Universities OA Index pain scores at 21 days after injection. During the 270-day follow-up period, the joint function of these patients improved, and magnetic resonance imaging showed that joint effusion and bone marrow edema were both alleviated. This trial was the first systematic clinical evidence that hUC-MSC-sEVs can effectively improve OA pathology by modulation of the IL-6/MMP13/COL2A1 signaling pathway, while also demonstrating the short-term safety of sEVs. Table VI summarizes the currently ongoing and registered clinical trials related to this. These clinical trials will mark a new era of clinical translation for the treatment of OA using sEVs.

However, based on the current experimental research, the dose optimization and administration barrier for sEVs in the treatment of OA remain a challenge for true clinical translation. Although various administration protocols have been used in current preclinical studies, simply increasing the dosage does not always lead to improved efficacy (184). In the sequential design of clinical trials, further exploration of the administration frequency and dose response is still needed. Additionally, the dense cartilage matrix constitutes a significant structural barrier in the drug delivery process: The pore size of healthy cartilage is only ~5-6 nm, while the diameter range of sEVs is 30 to 150 nm. This size mismatch results in most sEVs being unable to penetrate the cartilage matrix, significantly reducing their therapeutic effect (185). These challenges should not be overlooked in real clinical applications.

In conclusion, future research should focus on establishing unified quality control standards that comply with GMP and regulatory requirements, designing more rigorous clinical trial protocols with longer follow-up periods, exploring delivery strategies that can cross physiological barriers and systematically evaluating biodistribution, immunogenicity and long-term safety. The aim is to promote sEV therapy to truly become a safe and effective cell-free regenerative treatment method.

7. Future perspectives

Although sEVs show great promise in the treatment research of OA, their rapid clearance in the body, poor targeting ability and the challenges in personalized therapy still hinder clinical translation (119). To overcome these obstacles, future research

Table VI. Ongoing and completed registered clinical trials of sEV-based therapies for OA.

Registration no.	Title	Phase	Status	Estimated enrolled subjects, n	Intervention	Injection method and frequency
NCT06466850	Mesenchymal Stem Cells Derived Exosomes in Osteoarthritis Patients	NA	Recruiting	20	Allogenic MSC-derived sEVs	Intra-articular injection, two times (day 1 and day 90)
NCT06431152	Intra-articular Injection of UC-MSC Exosome in Knee Osteoarthritis (EXO-OA01)	Early Phase 1	Recruiting	12	UC-MSC sEV	Intra-articular injection, single dose
NCT06463132	Phase 1b Clinical Trial to Evaluate PEP and EUFLEXXA for Knee Osteoarthritis (KOA)	Phase 1	Not yet recruiting	24	PEP, PEP-Eufflexxa	Intra-articular injection, single dose
NCT06937528	Use of Extracellular Vesicles (EV) for Knee Osteoarthrosis	Phase 1	Recruiting	50	EV	Intra-articular injection, two doses
NCT05060107	Intra-articular Injection of MSC-derived Exosomes in Knee Osteoarthritis (ExoOA-1)	Phase 1	Not recruiting	10	Allogeneic MSC sEVs	Intra-articular injection, single dose

The data were retrieved from ClinicalTrials.gov on September 4th, 2026. 'Not recruiting' indicates the trial is closed for enrollment. sEVs, small extracellular vesicles; OA, osteoarthritis; MSCs, mesenchymal stem cells; UC-MSC, umbilical cord MSC; PEP, purified exosome product; Exo, exomoses; EUFLEXXA, a commercial cross-linked hyaluronic acid product; NA, not applicable.

needs to integrate the advantages of interdisciplinary fields such as bioengineering and materials science to explore comprehensive solutions.

In the field of targeted delivery, genetic engineering provides new ideas for enhancing the functions of sEVs. By loading specific miRNAs, small molecule drugs or targeting peptides into sEVs, they can be endowed with diverse therapeutic functions. For instance, Zhao *et al* (186) used sEVs derived from subcutaneous adipose stem cells and genetically engineered them to enable specific delivery of miR-199a-3p. After intra-articular injection in the OA model, this approach effectively improved cartilage damage by regulating the mTOR autophagy pathway. This strategy transforms natural sEVs into engineered tools with targeting capabilities and potent therapeutic activity, opening up new pathways for precise intervention in the key pathological processes of OA.

In terms of prolonging the retention time within the joint, the gel-based sEV delivery system demonstrates significant advantages. Pei *et al* (187) developed a biomimetic gel scaffold, integrating sEVs into a cartilage-like ECM composed of GelMA, chondroitin sulfate methacrylate and HA methacrylate. In the rat OA model, this scaffold significantly promoted cartilage repair by creating a regenerative microenvironment conducive to the recruitment of endogenous MSCs and cartilage differentiation (187). Future research can further explore intelligent responsive hydrogel systems, such as designing materials sensitive to pH changes or enzyme activity, enabling sEVs to release on demand according to the requirements of the inflammatory microenvironment, thereby achieving a balance between targeting and timing (153,188). Additionally, targeting ligands, such as cartilage-affinity peptides, could be installed on the surface of such hydrogels (125,136,189). These strategies collectively demonstrate that controlled release and targeting capabilities of sEVs can be achieved through rational design.

Third, personalized medical approaches, including the concept of the 'sEV corona', represent a promising direction for optimizing therapeutic outcomes by tailoring delivery systems to match patient-specific pathological characteristics. The sEV corona refers to a protein crown wrapped around the sEV lipid bilayer and formed by electrostatic interactions and protein aggregation (Fig. 5C). When combined with nanomaterials, it forms a layer of sEV corona at the periphery of nanomaterials, which is expected to change the physicochemical properties of nanomaterials (190). Önal Acet *et al* (191) showed that binding of peptide-based Fmoc-lysine nanomaterials to cancer cell-derived sEVs formed a layer of sEV corona, which reduced the toxicity of the nanomaterials. In addition, sEVs can interact with specific endogenous proteins to form 'individual' sEV coronas, thus improving their targeting and bringing great potential for future breakthroughs in the field of individualized diagnosis and treatment of clinical OA (192).

Finally, the deep integration of multi-omics technologies and artificial intelligence (AI) is expected to open up new paths for the discovery of biomarkers for OA. High-throughput multi-omics analysis (covering transcriptomics, proteomics and metabolomics) can systematically interpret the complex molecular load of sEVs, helping identify key molecular events in the progression and treatment response of OA. At the same time, AI algorithms have the ability to handle large-scale data

and can extract biomarkers with early diagnostic value. For instance, Shin *et al* (193) combined surface-enhanced Raman spectroscopy with deep neural networks to evaluate the severity of OA using type II collagen in sEVs from synovial fluid as the detection indicator, and the results showed that the accuracy of disease severity prediction reached 95.3%, significantly superior to traditional detection methods. You *et al* (194) integrated four OA-related transcriptome datasets and combined LASSO, random forest and XGBoost algorithms to screen out 10 key genes closely related to immune-metabolic disorders. These findings underscore the immense potential of computers in identifying sEV-related biomarkers and potential therapeutic targets. Furthermore, AI technology can also be applied to the risk assessment of OA, automatic image scoring and the discovery of multiple biomarkers (195). Overall, these studies fully demonstrate the great promise of AI in enabling personalized OA management and therapy.

In summary, the aforementioned research directions are jointly driving the OA therapeutic strategies based on sEVs from an empirical exploration towards a more rigorous and personalized management paradigm. In the future, the effective integration of genetic engineering modification, optimization of sEVs delivery systems and AI-assisted analysis is expected to translate into practical and feasible individualized treatment plans, providing more precise treatment options for patients with OA.

8. Conclusion

Overall, sEVs hold significant research and clinical application value in the progression and treatment of OA. sEV RNA and proteins promote OA inflammation. Furthermore, sEVs secreted by various intra-articular cells can degrade the ECM and induce chondrocyte necrosis, thereby accelerating cartilage damage (35). However, sEVs derived from diverse cellular and humoral sources, either via intrinsic bioactivity or as delivery carrier, exert anti-inflammatory effects through multiple mechanisms. These include inhibiting or regulating immune cell polarization via various signaling pathways (196). They can also promote chondrocyte proliferation and migration, inhibit chondrocyte necrosis and ECM degradation, and even induce various MSCs to differentiate into chondrocytes, thereby effectively facilitating cartilage repair and regeneration (152,197).

This paper provides a brief overview of the composition, generation, isolation and identification of sEVs, primarily elucidating the mechanisms by which sEVs mediate inflammation and cartilage damage in OA, as well as their various pathways for anti-inflammatory effects and the promotion of cartilage regeneration. This provides a clearer direction for potential future sEVs-based therapies for OA.

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Authors' contributions

YY participated in writing-original draft, design of the review search strategy and framework, literature screening and selection, conceptualization and data curation. JL contributed to writing-original draft, literature screening and selection, and data curation. XYS and AN performed literature screening, data extraction and synthesis of evidence, formal analysis and data curation. RG and XW contributed to writing-review & editing and literature screening and validation. XG, XTS and KC performed writing-review & editing and data curation. BL and RL participated in writing-review & editing, validation, supervision, resources, development of the review methodology and analytical framework, funding acquisition, visualization and project administration. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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