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Mesenchymal stem cells and extracellular vesicles for knee osteoarthritis: clinical application, mechanism exploration and prospect

Yujia Li[1,2†], Tiancong Fu[1,2†], Weijia Yu[3†], Haoyang Wen[1,2], Ziqi Wang[1,2], Zhongxi Lyu[1,2,4], Xiaohua Wen[1,2,4], Te Ba[5], Zelin Chen[1,2,4*], Kai Shan[5,6*] and Ningcen Li[1,2,4*]

Abstract

Knee osteoarthritis (KOA) is a prevalent degenerative joint disease characterized by progressive articular cartilage degeneration, synovial inflammation, and abnormal subchondral bone remodeling, with no curative treatment currently available. Mesenchymal stem cells (MSCs) and their extracellular vesicles (MSC-EVs) have emerged as promising therapeutic strategies for KOA due to their anti-inflammatory, regenerative, and immunomodulatory properties. Clinical studies demonstrate that intra-articular MSCs injection significantly alleviates pain, improves joint function, and exhibits a favorable safety profile. MSC-EVs show enhanced therapeutic potential owing to their low immunogenicity, high stability, and targeted delivery capabilities. This review systematically examines the therapeutic role of MSCs and MSC-EVs in KOA treatment. Mechanistic studies reveal that MSC-EVs ameliorate joint inflammatory microenvironments by regulating macrophage polarization, inhibiting key inflammatory pathways (NF-κB, MAPK), and suppressing pro-inflammatory cytokine release (IL-1β, TNF-α). Furthermore, MSC-EVs protect extracellular matrix integrity and promote cartilage regeneration by upregulating chondrogenic markers (Sox9, aggrecan, type II collagen) while downregulating matrix-degrading enzymes (MMP-13, ADAMTS5). Additionally, MSC-EVs enhance chondrocyte proliferation and migration while inhibiting apoptosis and senescence, potentially through activation of YAP and JAK/STAT signaling pathways. These multifaceted mechanisms collectively facilitate cartilage repair and regeneration. Advances in engineered EVs technology and novel delivery systems provide strategies to further enhance MSC-EVs efficacy. Engineered EVs modified with chondrocyte-targeting peptides or loaded with therapeutic molecules (drugs, miRNAs, siRNAs) can deliver bioactive compounds to specific sites and

†Yujia Li, Tiancong Fu and Weijia Yu have contributed equally to this work.

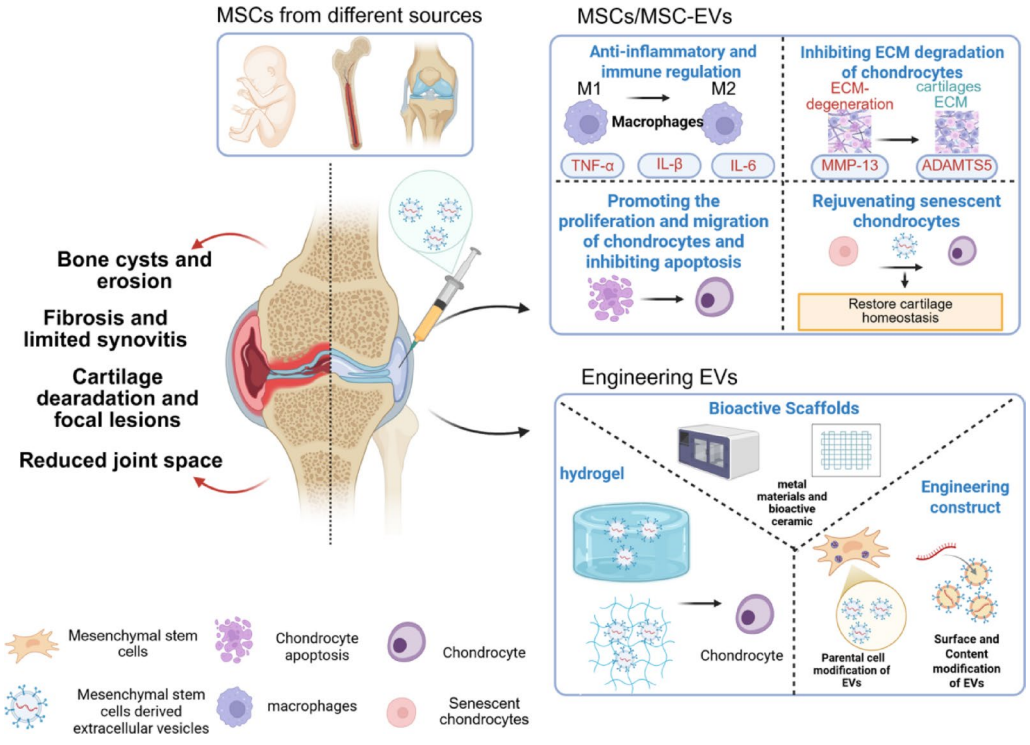
*Correspondence:
Zelin Chen
chenzelin328@163.com
Kai Shan
2646586551@qq.com
Ningcen Li
517654179@qq.com

Full list of author information is available at the end of the article

precisely regulate chondrocyte function, thereby alleviating KOA symptoms. This review comprehensively examines the clinical efficacy and underlying mechanisms of MSCs and MSC-EVs in KOA treatment, discusses current clinical application challenges, and outlines future research directions for advancing precision therapeutic strategies.

Keywords Knee osteoarthritis, Mesenchymal stem cells, Extracellular vesicles, Engineering modification

Graphical Abstract



Background

Knee osteoarthritis (KOA) is a degenerative joint disease with articular cartilage damage as the main manifestation. The clinical manifestations are knee pain, stiffness, swelling, mobility limitation, and deformity [1]. The global burden of KOA has escalated dramatically, with over 360 million affected individuals worldwide. Between 1990 and 2019, the prevalence, incidence, and age-standardized Years Lived with Disability increased substantially across most countries and regions [2], particularly in the Western Pacific region [3]. The incidence of KOA correlates strongly with age, obesity, and socioeconomic status, resulting in high disability rates [4]. Given the dual trends of global population aging and the obesity epidemic, the disease burden imposed by KOA warrants heightened attention.

Current therapeutic approaches for KOA include oral non-steroidal anti-inflammatory drugs, abbreviated as Non-Steroidal Anti-inflammatory Drugs (NSAIDs), intra-articular corticosteroid or viscosupplement injections, and surgical interventions. However, these modalities provide primarily symptomatic relief without halting disease progression. NSAIDs, while offering analgesic and anti-inflammatory benefits, carry significant adverse effects including gastrointestinal complications (nausea, vomiting, dyspepsia), hepatorenal toxicity, and increased cardiovascular risk [5, 6]. Intra-articular corticosteroid injections frequently cause injection-site pain, swelling, and pseudoseptic reactions, with repeated administration accelerating cartilage degradation [7]. Critically, these conservative treatments fail to reverse the progressive articular cartilage degeneration, often necessitating costly total knee arthroplasty (TKA). Although TKA remains the definitive treatment for end-stage KOA, it is associated with substantial risks including infection, deep vein thrombosis, bleeding, periprosthetic fractures, and neurovascular injury [8]. Moreover, TKA demands rigorous preoperative evaluation, meticulous surgical technique, and standardized postoperative management, yet patient satisfaction remains suboptimal [9, 10]. Therefore, it is particularly important to find a treatment that can effectively reverse and alleviate the pathological process.

Mesenchymal stem cells (MSCs) have emerged as a promising regenerative medicine approach for KOA treatment, offering potential disease-modifying capabilities that address the limitations of conventional

therapies. MSCs possess multipotent differentiation capacity, enabling their conversion into osteoblasts, adipocytes, and chondrocytes [11], alongside robust self-renewal potential. As highly responsive mediators of tissue inflammatory responses. As the most sensitive cell population to tissue inflammatory response [12], MSCs exert potent anti-inflammatory and immunomodulatory effects while promoting tissue repair. These multifaceted properties create a favorable microenvironment conducive to articular cartilage regeneration, positioning MSCs as an attractive therapeutic strategy for KOA [13]. The therapeutic potential of MSCs for osteoarthritis has gained global recognition, as evidenced by a 2025 bibliometric analysis of 2,341 studies spanning 26 years, which confirmed the emergence of MSC-based precision therapy as a worldwide trend [14]. Notably, a large-scale phase III clinical trial in China (ChiCTR1800017713) pioneered a dual-endpoint design evaluating both pain relief and functional improvement, marking a significant milestone toward clinical translation of MSC therapy for KOA. However, the limited in vivo survival and engraftment of transplanted MSCs constitute a critical bottleneck for clinical application, prompting investigation of MSC-derived extracellular vesicles (MSC-EVs) as a potentially superior alternative [15].

MSC-EVs are nanosized vesicles secreted by MSCs from diverse tissue sources, including bone marrow, adipose tissue, umbilical cord, and dental pulp. Based on biogenesis and size, EVs are classified into exosomes (30–200 nm), microvesicles (200–1000 nm), and apoptotic bodies (500–2000 nm) [16]. MSC-EVs function as intercellular mediators by transferring bioactive cargoes (including lipids, proteins, and nucleic acids (mRNA, miRNA, long non-coding RNA) to recipient cells, thereby modulating their physiological and pathological processes [17, 18]. Functionally, MSC-EVs recapitulate the immunomodulatory and regenerative properties of parental MSCs while offering distinct advantages as a cell-free therapeutic modality: reduced immunogenicity, enhanced safety profile, and circumvention of ethical concerns associated with cell-based therapies [14, 19]. This review systematically summarizes the clinical application and possible mechanism of MSCs and MSC-EVs in the treatment of KOA, providing a scientific basis for clinical transformation.

Pathological process of KOA

The core performance of KOA is the progressive degeneration of articular cartilage [20]. This process is driven by the destruction of the extracellular matrix, apoptosis of chondrocytes, and inflammatory reactions [21]. It is further associated with synovial lesions, metabolic dysfunction, abnormal bone remodeling, and osteophyte formation, leading to cartilage thinning, surface roughness, and loss of normal structure and function (Fig. 1) [22]. In the early stages of the disease, the remodeling process of subchondral bone undergoes changes, manifested as bone sclerosis and osteophyte formation; As the condition progresses to the later stage, a series of changes such as osteoporosis and changes in trabecular bone structure may occur [23].

As the main load-bearing tissue in adults, articular cartilage is composed of chondrocytes and dense extracellular matrix (ECM), etc [24, 25]. Under physiological conditions, chondrocytes sense mechanical stress, and their pericellular matrix (PCM) resists degradative enzymes like A Disintegrin and Metalloproteinase with Thrombospondin Motifs (ADAMTS) [26]. However, in pathological states, inflammatory factors and mechanical stimuli trigger a vicious cycle of ECM degradation by activating Matrix Metalloproteinases (MMPs) and ADAMTS proteases [27]. ECM constitutes up to 90% of cartilage dry weight, making its degradation a central event in KOA structural damage [28]. Inflammation and abnormal mechanical load activate ECM-degrading enzymes, particularly Matrix Metalloproteinase-13 (MMP-13), ADAMTS4, and ADAMTS5, which disrupt the ECM architecture [29]. This degradation not only directly compromises cartilage's mechanical integrity but also generates breakdown products that fuel further inflammation and chondrocyte apoptosis, creating a vicious cycle that drives KOA progression [30]. Inflammatory response is central to KOA pathology. Early cartilage injury triggers the release of pro-inflammatory factors from chondrocytes and synoviocytes, which induce chondrocytes to produce matrix-degrading enzymes (MMPs, ADAMTS) [31, 32]. This process promotes ECM degradation, inhibits the synthesis of type II collagen (COL II) and proteoglycans, and directly induces chondrocyte apoptosis. Furthermore, these factors recruit immune cells like macrophages and neutrophils into the joint cavity, leading to the further release of inflammatory mediators and exacerbating joint inflammation [33]. Chondrocyte apoptosis, a hallmark of KOA triggered by mechanical stress, oxidative stress, and inflammation, reduces cell numbers and impairs ECM synthesis [34]. This process is exacerbated as apoptotic debris and damage-associated molecular patterns activate immune responses, creating a vicious cycle of destruction, while the downregulation of key factors like Sox9 (cartilage degradation-related gene) further disrupts cartilage homeostasis [35, 36]. Therefore, elucidating these key links is helpful to deeply understand the molecular mechanism of KOA and provide new ideas for targeted therapy.

Although articular cartilage is the focus of KOA research, a large number of studies in recent years have shown that that aberrant Subchondral Bone (SB) remodeling and pathological angiogenesis are often

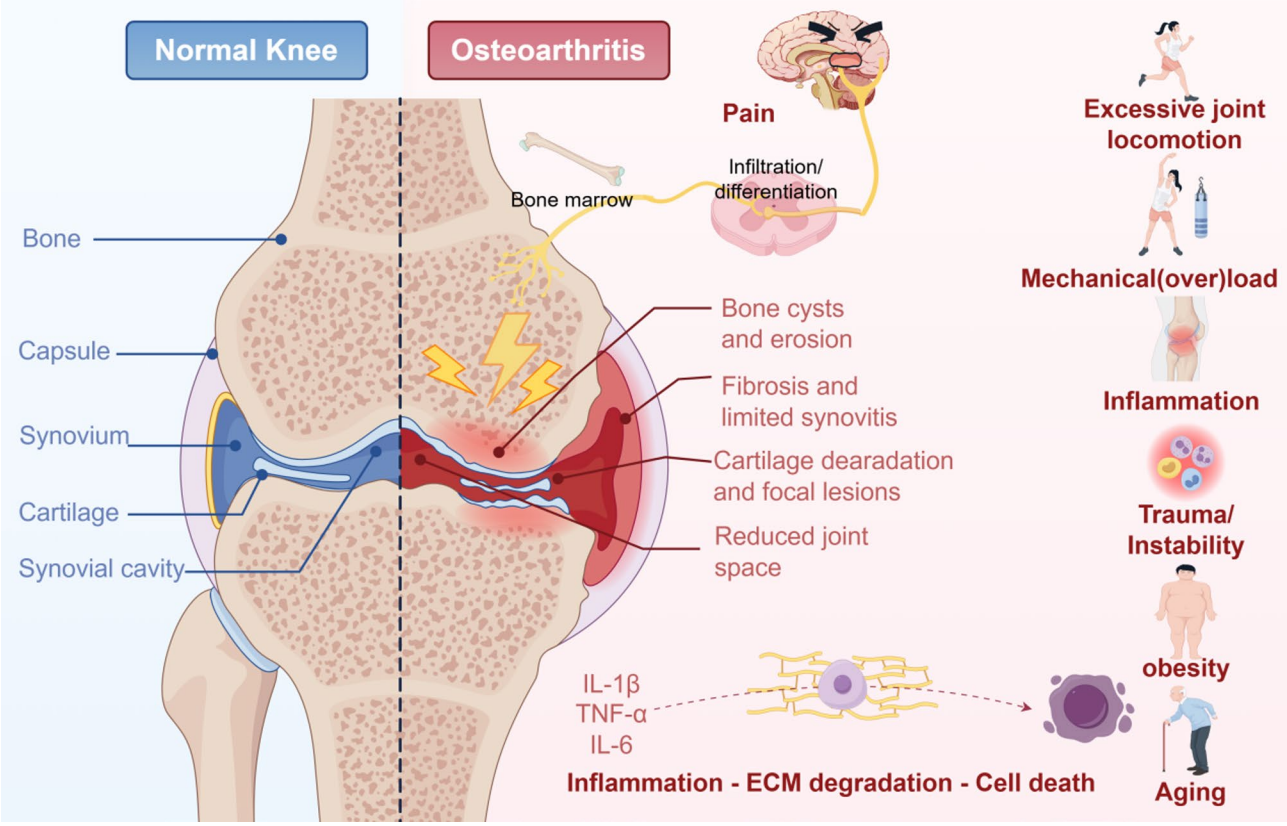


Fig. 1 Comparative illustration of a healthy knee joint versus an osteoarthritic joint. It highlights normal anatomy, KOA pathology, key drivers and risk factors

initiating events that precede and actively drive cartilage degradation [37]. For instance, in the destabilization of the medial meniscus (DMM) mouse model, an increase in specialized Type H vessels (CD31hiEmcnhi) in the SB is detectable as early as 2 weeks post-injury, significantly preceding observable cartilage proteoglycan loss, which only becomes apparent at 4 to 6 weeks [38]. Abnormal subchondral bone remodeling and pathological angiogenesis serve as the starting engines, forming a vicious cycle with cartilage degeneration through both biomechanical and biochemical pathways. Breaking this cycle and targeting H-shaped blood vessels or specific signaling pathways for treatment may become an important direction for developing modified therapies for osteoarthritis in the future.

Clinical application of MSCs and MSC-EVs in the treatment of KOA

The clinical treatment of KOA is mainly to relieve symptoms, and there is a lack of effective means to reverse the disease progression. MSCs and MSC-EVs have emerged as promising alternatives due to their intrinsic tissue repair and immunomodulatory capacities. Clinical MSC therapy typically employs direct or ultrasound-guided intra-articular injection, offering procedural simplicity and feasibility for repeated administration. The most commonly utilized MSC sources include human umbilical cord-derived MSCs (hUC-MSCs), adipose-derived MSCs (ADSCs), and bone marrow-derived MSCs (BMSCs), with clinical-grade resource banks now established for hUC-MSCs [39, 40]. Therapeutic efficacy of intra-articular MSC injection is influenced by both dosing regimens and cell concentration. Clinical evidence demonstrates that hUC-MSCs and ADSCs effectively reduce pain and improve functional outcomes in KOA patients, with repeated hUC-MSCs injections exhibiting superior and more sustained efficacy compared to single administration or hyaluronic acid injection, in a dose-dependent manner [41, 42]. Autologous ADSCs therapy significantly enhances joint range of motion and cartilage morphology [43], while autologous BMSCs derived from iliac crest aspiration markedly alleviate knee pain and improve flexion angles [44]. Notably, allogeneic BMSCs injection not only provides safe symptomatic relief and delays cartilage degeneration but also exerts anti-inflammatory effects by locally counteracting pro-inflammatory factor-mediated osteoclastogenesis and reducing bone resorption markers such as collagen type I C-terminal telopeptide, thereby modulating local immune responses [45]. Recent advances have enabled

standardized production of clinical-grade MSC-EVs, facilitating their translation to KOA therapy. The first-in-human application of hUC-MSC-EVs demonstrated significant improvements in pain and functional recovery following intra-articular injection, with only transient injection-site discomfort and no evidence of cartilage degradation [46]. A subsequent clinical trial employing hUC-MSC-EVs administered at 21-day intervals reported substantial improvements in knee function, pain reduction, and quality of life, accompanied by cartilage thickening on imaging assessment [47].

Despite these preliminary findings, clinical application of MSC-EVs remains in its nascency. More sample sizes are needed to determine the efficacy, extend the follow-up time, and carry out more randomized controlled experiments. While MSCs therapy demonstrates a favorable safety profile and shows promise in pain alleviation and functional improvement, its capacity for cartilage regeneration requires further optimization. Current MSCs-based intra-articular therapy faces several critical translational barriers. First, key treatment parameters remain unstandardized, including optimal cell dosage, administration frequency, delivery protocols, and source tissue selection [48]; Second, cell expansion technologies present scalability limitations; although recent innovations such as self-fabricated microcarrier-stirred bioreactor systems have enhanced hUC-MSCs proliferation efficiency [49], current yields and batch-to-batch consistency fail to meet the demands of large-scale clinical manufacturing. These unresolved challenges directly impede the therapeutic efficacy, reproducibility, and clinical translation of MSCs-based interventions, underscoring the imperative for systematic optimization and standardization.

Potential mechanism of MSCs and MSC-EVs in the treatment of KOA

KOA represents a whole organ disease characterized by pathological crosstalk among all constituent joint tissues: articular cartilage, synovium, menisci, infrapatellar fat pad (IPFP), and SB [50, 51]. Extensive preclinical evidence demonstrates that MSCs and MSC-EVs exert therapeutic effects in KOA by ameliorating the joint microenvironment, promoting cartilage repair, and attenuating inflammatory progression. MSCs mediate tissue repair through three principal mechanisms: direct chondrogenic differentiation, paracrine signaling, and immunomodulation. Notably, comparative studies reveal that MSC-EVs confer superior therapeutic outcomes relative to parental MSCs, exhibiting reduced cartilage degradation, pain-related behaviors, osteophyte formation, and joint inflammation, thereby positioning MSC-EVs as a promising cell-free therapeutic strategy with enhanced safety and efficient cargo delivery [52]. Mechanistically, MSCs and MSC-EVs alleviate KOA pathology in preclinical models through multifaceted pathways: anti-inflammatory activity, immunomodulation, inhibition of ECM degradation, promotion of chondrocyte proliferation and migration, and suppression of apoptosis, and rejuvenate senescent chondrocytes (Table 1; Fig. 2).

Anti-inflammatory and immune regulation

KOA is characterized by chronic inflammation and progressive cartilage degradation. Cartilage damage triggers the intra-articular accumulation of pro-inflammatory mediators, including Tumor Necrosis Factor Alpha (TNF-α), Interleukin-1 Beta (IL-1β), Matrix metalloproteinases-3 (MMP-3) and ADAMTS5 [53]. These inflammatory cytokines stimulate chondrocyte secretion of ECM-degrading enzymes, perpetuating matrix destruction. MSCs and MSC-EVs attenuate this catabolic cascade by suppressing pro-inflammatory cytokine expression while upregulating anti-inflammatory mediators, thereby preserving ECM integrity.

MSCs exert direct anti-inflammatory effects through minimal intrinsic production of pro-inflammatory cytokines and chemokines. For instance, ADSCs significantly reduce Interleukin-6 (IL-6) and Interleukin-8 (IL-8) in chondrocytes and synoviocytes [54]. Additionally, MSCs mediate immunomodulation via paracrine signaling, which concurrently promotes chondrocyte proliferation [55, 56]. By modulating the inflammatory joint microenvironment, MSCs establish a stabilized milieu conducive to cartilage repair and regeneration [57]. However, the immunogenic profile of differentiated MSCs remains controversial; while MSC-derived osteoblasts retain immunoregulatory capacity in vitro, this property is lost following in vivo implantation in rabbit models [58, 59]. In contrast, MSC-EVs demonstrate superior stability in inflammatory environments due to their protective lipid bilayer structure, positioning them as a more robust therapeutic alternative.

MSC-EVs mediate immunomodulation through two principal mechanisms: macrophage polarization and T cell suppression. The imbalance between pro-inflammatory M1 and anti-inflammatory M2 macrophages is implicated in OA pathogenesis, with elevated M1/M2 ratios observed in OA joints [60]. MSC-EVs promote M1 to M2 macrophage polarization, thereby attenuating inflammation [61, 62]. Mechanistically, hUC-MSC-EVs reverse IL-1β induced chondrocyte injury and facilitate M2 polarization in vitro [63], partially through five PI3K/Akt pathway-associated miRNAs including has-miR-122-5p and has-miR-148a-3p [64]. MicroRNA profiling revealed that hUC-MSC-EV-derived miR-1208 targets Methyltransferase-like 3 (METTL3) in macrophages to exert anti-inflammatory effects [65]. Similarly, hUC-MSCs derived apoptotic vesicles (apoVs) vesicles activate

Table 1 Effects and mechanisms of MSC-EVs in KOA

Refs	Models	In Vitro/ In Vivo study	Source of EVs	Dose and route	Biochemical measurements
Manferdini C, 2013 [54]	Chondrocytes and synoviocytes from osteoarthritis patients	In Vitro	ADSCs	ADSCs: Chondrocytes/Synoviocytes = 1 ' 8	IL-6↓, IL-8↓
Sankarana-rayanan J, 2024 [72]	IL-1β-induced chondrocytes	In Vitro	BMSC-EVs	5 μg/mL, 24 h	COX-2↓, IL-6↓, MMP-13↓
Zhou L, 2021 [73]	IL-1β-induced chondrocytes	In Vitro	BMSC-EVs	10 μg/ml, 48 h	COL1A1↑, COL2A1↑, COL3A1↑; TGF-α↓, IL-1β↓, IL-6↓, Versican↓, MMP-13↓, MAPKs↓, NF-kB↓
Dong J, 2021 [74]	IL-1β-induced chondrocytes	In Vitro	BMSC-EVs	20 μg	CDH11↓, Wnt↓, β-catenin↓
Gorgun C, 2022 [99]	Bone marrow macrophages isolated from young and old mice were stimulated with LPS and IFN-γ	In Vitro	Human ADSC-EVs, mouse BMSC-EVs	0.25 μg/mL, 24 h	TNF-α↓, iNOS↓, NADase↓, CD38↓
Li Z, 2022 [67]	Rat model of anterior cruciate ligament reconstruction (ACLR)	In Vivo	BMSC-EVs	Intra-articular injection: 50 μL (10^{11} particles/ml), 3 days and 7 days after the operation	CD163↑, IL-10↑, TGF-β↑, Arg1↑; iNOS↓, IL-6↓, IL-1β↓, TNF-α↓
Zhi Z, 2020 [84]	Wistar OA rats was induced by cutting the medial collateral ligament	In Vivo	BMSCs	Intra-articular injection: 75μL or 1.5 × 10^5 BMSCs + 1.5 × 10^5 articular cartilage chondrocytes, single dose at 1 week post-surgery	lysine-specific demethylase 6 A (KDM6A)↑, Sox9↑, COL II↑, aggrecan↑
Li Q, 2022 [85]	Immunodeficient mice	In Vivo	ADSC-EVs, BMSC-EVs, SMSC-EVs	Subcutaneous injection: 5 × 10^6 BMSCs, 40 μL Matrigel + 10 μL PBS/ EVs, 20 μg	Alpl↑, Ocn ↑, COL I↑, Runx2↑
Chen S, 2019 [86]	Osteochondral defect model in SD rats	In Vivo	ADSC-EVs	50 μg/mL; hydrogel loaded with EVs (miR-375), 50 μg/mL	Bone mass / total bone mass↑,bone mineral density (BMD)↑
Zhang S, 2022 [87]	OA mouse model by medial meniscus instability method	In Vivo	BMSC-EVs	Intra-articular injection 10 μg	Sesn2↑, Nrf2↑; NEAT1↓, miR-122-5p↓
Mao G, 2018 [89]	Collagenase-induced OA mouse model	In Vivo	MSC-miR-92a-3p-EVs	Intra-articular injection 15 μl(500 μg/mL), 28 days	aggrecan↑, COL2A1↑, COL9A1↑, COMP↑, Sox9↑; MMP-13↓, COL10A1↓, Runx2↓, ADAMTS4/5↓
Hu J, 2022 [92]	Rabbit OA model was developed by transecting the anterior cruciate ligament and medial meniscus	In Vivo	BMSC-EVs	10 μg/mL, 2nd to 6th week after surgery	aggrecan↑, COL II↑, Sox9↑, YAP↑, ANKRD1↑, CTGF↑, Cyr61↑; IL-1β↓, TNF-α↓
Li P, 2022 [63]	IL-1β induced chondrocytes; Anterior cruciate ligament transection combined with medial meniscectomy in SD rats (ACLT + pMMx)	In Vitro and In Vivo	hUC-MSCs-EVs	4 × 10^7 particles/mL, 48 h; Intra-articular injection: 10^{11} particles/mL of EVs day 1 and 4 of every week from the 5th to 8th week after surgery	M1 macrophage↑, M2 macrophage↑, COL2A1↑, Sox9↑, aggrecan↑; MMP-13↓, ADAMTS5↓, COL1A2↓
Li K, 2022 [64]	IL-1β induced chondrocytes; Rat OA model induced by the ACLT	In Vitro and In Vivo	hUC-MSCs-EVs	the supernatant of M2 macrophages induced by hUCMSCs-EVs (0, 5, 10, 20, 40, and 80 μg/mL) for 2 days; Intra-articular injection 80 μg/mL, once every week	PI3K↑, Akt↑, M1 macrophage↑, M2 macrophage↑
Zhou H, 2022 [65]	Human articular chondrocytes ; C57BL/6 mouse OA model by surgical destruction of medial meniscus	In Vitro and In Vivo	hUC-MSCs-EVs	4 mg/mL, 24 h; Intra-articular injection 10^{11} particles/mL, twice a week	COL2A1↑, aggrecan↑; ADAMTS5↓, MMP-13↓

Table 1 (continued)

Refs	Models	In Vitro/ In Vivo study	Source of EVs	Dose and route	Biochemical measurements
Tian G, 2024 [66]	In vitro macrophage model; Osteochondral defect model in SD rats	In Vitro and In Vivo	hUMSCs-apoVs	Co-culture: 250 ng/mL, 2.5 μg/mL, 5 μg/mL; Intra-articular injection: 20 μL of a 1 μg/mL apoVs, every 3 days	CD206↑, Arginase-1↑, TGF-β1↑; iNOS↓, TNF-α↓, IL-6↓
Li B, 2024 [68]	LPS induced RAW264.7 cells; Anterior cruciate ligament transection combined with partial medial meniscectomy in SD rats (ACLT + pMMx)	In Vitro and In Vivo	BMSC-EVs	Co-culture: 100 μg/mL, 24 h; Intra-articular injection: 100 μg/50 μL	IL-10↑; PINK1↓, Parkin↓, IL-6↓, IL-1β↓, TNF-α↓
Sun W, 2023 [69]	LPS induced macrophages / chondrocytes The model of KOA was produced in SD rats by unilateral intraarticular injection of 8% sodium iodoacetate.	In Vitro and In Vivo	SMSC-EVs	Co-culture: 17.2×10^7 particles, 48 h; Intra-articular injection: 1×10^{11} EVs particles/mL, 100 μL every time, once every three days	iNOS/CD206↓
Zhang S, 2018 [70]	Primary Chondrocytes; Osteochondral defect model in SD rats	In Vitro and In Vivo	human embryonicstem cell-derived MSCs-EVs	Co-culture: 10 μg/ml; Intra-articular injections of 100 μg	M1 macrophage↑, M2 macrophage↑
Cosenza S, 2018 [71]	$CD4^+$ or $CD8^+$ T lymphocytes were isolated from spleen; Delayed type hypersensitivity (DTH) and collagen induced arthritis (CIA) models	In Vitro and In Vivo	BMSC-EVs	Co-culture: 100 μL; Intravenously injection: 30 μL, at day 18 and 24	IL-6↓, IL-1β↓
		In Vitro and In Vivo			
Chen X, 2020 [77]	Chondrocytes from patients with traumatic KOA and post-traumatic amputation; Mouse model of post-traumatic OA	In Vitro and In Vivo	SMSCs	600μL, 37°, 12 h; Intra-articular injection: 100 μL of 10^{11} particles/mL	COL II↑, aggrecan↑, Sox9↑; MMP-13↓
Tang S, 2021 [78]	IL-1β induced chondrocytes; Rat OA model induced by the ACLT	In Vitro and In Vivo	hUC-MSC-sEVs	Co-cultured: 12 h; Intra-articular injection: 200 μL, 4 weeks	COL II↑; MMP-13↓, ADAMTS5 ↓
Liu Y, 2022 [79]	IL-1β induced chondrocytes; Rat KOA model induced by the ACLT + DMM	In Vitro and In Vivo	hUSC-EVs	Co-cultured: 48 h; Intra-articular injection: 100 μL, 10^{11} particles/mL once a week	COL II↑, aggrecan↑
Wang Z, 2021 [80]	SMSCs from OA patients during total knee arthroplasty; OA mouse model induced by 4 °C cold water stimulation for 2 h twice a day to and injected with normal saline into the articular cavity	In Vitro and In Vivo	SMSC-EVs	Co-cultured: 12 h; Intra-articular injection: 30 μL, 10^{11} particles/mL	Runx2↓
Ye P, 2022 [88]	IL-1β induced chondrocytes; OA mouse model induced by the DMM	In Vitro and In Vivo	MSC-EVs	Co-cultured: 12 h; Intra-articular injection: 10 μL once a week, 3 weeks.	PHLDA2↑; SDC1↓, Wnt↓, β-catenin↓
Zhang B, 2022 [90]	Modified Hulth method induced the OA model and chondrocytes	In Vitro and In Vivo	BMSC-EVs	(5, 10, 20 μg/mL) EVs, 600 μ, 37°, 12 h; Intra-articular injection: 100 μg, 4 weeks	Chondrocytes↑
Rong Y, 2021 [91]	IL-1β-induced chondrocyte, The chondrocytes were transfected with lentiviral vectors (Vector, JAK2, shNC, shJAK2, and siHIF-1α) Lentiviral-mediated shRNA knockdown mouse model, FemaleOA SD rats induced by the DMM	In Vitro and In Vivo	MSCs-sEVs	150 μg/mL were co-grown with chondrocytes, 24 h; 200 μg, the 4th week after operation	Chondrocytes↑, COL II↑, Sox9↑; MMP-13↓

Table 1 (continued)

Refs	Models	In Vitro/ In Vivo study	Source of EVs	Dose and route	Biochemical measurements
Li F, 2023 [93]	IL-1β induced chondrocytes, IL-1β or TGF-β1 induced synovial fibroblast; Monosodium iodoacetate (MIA)-induced rat OA model	In Vitro and In Vivo	hADSC-EVs	10 μg/ml, 37°, 1 h; Intra-articular injection: After 2 weeks of MIA injection, (100 μg/250 μl), 2 weeks	WNT3↓, WNT9↓, β-catenin↓
Cao H, 2023 [97]	OA chondrocytes from patients during total knee arthroplasty; Rat OA model induced by the ACLT	In Vitro and In Vivo	UCMSC-EVs	Co-cultured for 3 and 7 days; Intra-articular injection: 30 μg/mL, 3–7 days	P53↓

↑ is upregulated by EVs, ↓ is downregulated by EVs

ADSCs: Adipose-Derived Stem Cells; IL-6: Interleukin-6; IL-8: Interleukin-8; IL-1β: Interleukin-1 Beta; BMSCs: Bone Marrow Mesenchymal Stem Cells; BMSC-EVs: Bone Marrow Mesenchymal Stem Cell-Derived Extracellular Vesicles; COX-2: Cyclooxygenase-2; MMP-13: Matrix Metalloproteinase-13; COL1A1: Collagen Type I Alpha 1; COL2A1: Collagen Type II Alpha 1; COL3A1: Collagen Type III Alpha 1; TGF-α: Transforming Growth Factor Alpha; MAPK: Mitogen-Activated Protein Kinases; NF-kB: Nuclear Factor Kappa B; CDH11: Cadherin-11; Wnt: Wingless-Related Integration Site; β-catenin: Beta-Catenin; ACLR: Anterior Cruciate Ligament Reconstruction; CD163: Cluster of Differentiation 163; IL-10: Interleukin-10; TGF-β: Transforming Growth Factor Beta; Arg1: Arginase-1; iNOS: Inducible Nitric Oxide Synthase; TNF-α: Tumor Necrosis Factor Alpha; OA: Osteoarthritis; KDM6A: Lysine-Specific Demethylase 6 A; Sox9: SRY-Box Transcription Factor 9; COL II: Collagen Type II; ADSC-EVs: Adipose-Derived Stem Cell-Derived Extracellular Vesicles; SMSC-EVs: Synovial Membrane Mesenchymal Stem Cell-Derived Extracellular Vesicles; Alpl: Alkaline Phosphatase, Liver/Bone/Kidney; Ocn: Osteocalcin; Col I: Collagen Type I; Runx2: Runt-Related Transcription Factor 2; SD rats: Sprague-Dawley Rats; BMD: Bone Mineral Density; Sesn2: Sestrin 2; Nrf2: Nuclear Factor Erythroid 2-Related Factor 2; NEAT1: Nuclear Paraspeckle Assembly Transcript 1; MSC-miR-92a-3p-EVs: Mesenchymal Stem Cell-Derived miR-92a-3p-Enriched Extracellular Vesicles; COL9A1: Collagen Type IX Alpha 1; COMP: Cartilage Oligomeric Matrix Protein; COL10A1: Collagen Type X Alpha 1; ADAMTS4: A Disintegrin and Metalloproteinase with Thrombospondin Motifs 4; ADAMTS5: A Disintegrin and Metalloproteinase with Thrombospondin Motifs 5; YAP: Yes-Associated Protein; ANKRD1: Ankyrin Repeat Domain 1; CTGF: Connective Tissue Growth Factor; Cyr61: Cysteine-Rich Angiogenic Inducer 61; hUC-MSCs-EVs: Human Umbilical Cord Mesenchymal Stem Cells-Derived Extracellular Vesicles; COL1A2: Collagen Type I Alpha 2; ACLT: Anterior Cruciate Ligament Transection; pMMx: Partial Medial Meniscectomy; PI3K: Phosphatidylinositol 3-Kinase; Akt: Protein Kinase B; hUMSCs-apoVs: Human Umbilical Cord Mesenchymal Stem Cells-Derived Apoptotic Vesicles; CD206: Cluster of Differentiation 206; TGF-β1: Transforming Growth Factor Beta 1; LPS: Lipopolysaccharide; RAW264.7: Mouse Macrophage Cell Line; PINK1: PTEN-Induced Kinase 1; Parkin: Parkin RBR E3 Ubiquitin Protein Ligase; SMSCs: Synovial Membrane Mesenchymal Stem Cells; KOA: Knee Osteoarthritis; human embryonic stem cells-derived MSCs-EVs: Human Embryonic Stem Cell-Derived Mesenchymal Stem Cells-Extracellular Vesicles; CD4 + T lymphocytes: CD4 Positive T Lymphocytes; CD8 + T lymphocytes: CD8 Positive T Lymphocytes; DTH: Delayed Type Hypersensitivity; CIA: Collagen Induced Arthritis; hUC-MSC-sEVs: Human Umbilical Cord Mesenchymal Stem Cell-Derived Small Extracellular Vesicles; hUSC-EVs: Human Urine-Derived Stem Cell-Derived Extracellular Vesicles; DMM: Destabilization of Medial Meniscus; PHLDA2: Pleckstrin Homology Like Domain Family A Member 2; SDC1: Syndecan-1; JAK2: Janus Kinase 2; shRNA: Short Hairpin RNA; shNC: Short Hairpin RNA Negative Control; shJAK2: Short Hairpin RNA Targeting JAK2; siHIF-1α: Small Interfering RNA Targeting HIF-1α; HIF-1α: Hypoxia-Inducible Factor 1 Alpha; hADSC-EVs: Human Adipose-Derived Stem Cell-Derived Extracellular Vesicles; WNT3: Wnt Family Member 3; WNT9: Wnt Family Member 9; MIA: Monosodium Iodoacetate; UCMSC-EVs: Umbilical Cord Mesenchymal Stem Cell-Derived Extracellular Vesicles; P53: Tumor Protein p53; IFN-γ: Interferon Gamma; NADase: NAD Glycohydrolase; CD38: Cluster of Differentiation 38; PBS: Phosphate Buffered Saline

the Mitogen-activated protein kinase/Extracellular regulated protein kinases (MAPK/ERK) pathway to promote M2 polarization [66]. BMSC-EVs achieve comparable effects by delivering miR-23a-3p to suppress Nuclear factor kappa B (NF-κB) activation [67] or by inhibiting the PTEN Induced Kinase 1/Parkin (PINK1/Parkin) pathway, thereby reducing IL-6, IL-1β, and TNF-α levels and decelerating OA progression [68]. BMP-7-modified SMSC-EVs enhance macrophage and chondrocyte proliferation while promoting M2 polarization [69], and human embryonic stem cell-derived MSCs facilitate M1 to M2 polarization of synovial macrophages, suppressing inflammatory cytokine secretion and cartilage damage [70]. Beyond macrophage modulation, MSC-EVs dose-dependently inhibit T cell proliferation and promote regulatory T cell expansion, as demonstrated in delayed-type hypersensitivity models [71].

MSC-EVs further regulate inflammation by modulating key signaling pathways. BMSC-EVs attenuate IL-1β-induced catabolic responses in chondrocytes by targeting NF-κB and Mitogen-Activated Protein Kinases (MAPK) pathways [72, 73]. Some people took BMSC-EVs to treat IL-1β-induced OA rats, and found that miR-127-3p contained in EVs targeted to inhibit the expression of CDH11 in chondrocytes, thereby blocking the activation of the Wingless-Related Integration Site/Beta-Catenin (Wnt/β-catenin) pathway, thus achieving an anti-inflammatory effect [74].

In summary, MSC-EVs ameliorate the inflammatory joint microenvironment through macrophage polarization, T cell suppression, and modulation of inflammatory signaling cascades, creating a stabilized tissue milieu that facilitates cartilage repair and regeneration. Under optimal conditions, MSC-EVs and the inflammatory milieu achieve bidirectional regulatory.

Inhibiting ECM degradation

Articular cartilage exhibits limited regenerative capacity due to its avascular structure [75]. To prevent the structural destruction of articular cartilage, delaying the degradation of the ECM is crucial in the progression of KOA pathology. Pro-inflammatory cytokines, particularly IL-1β and TNF-α, accelerate ECM degradation by dual mechanisms: IL-1β suppresses the expression of structural proteins (COL2A1 and aggrecan) while simultaneously inducing chondrocyte production

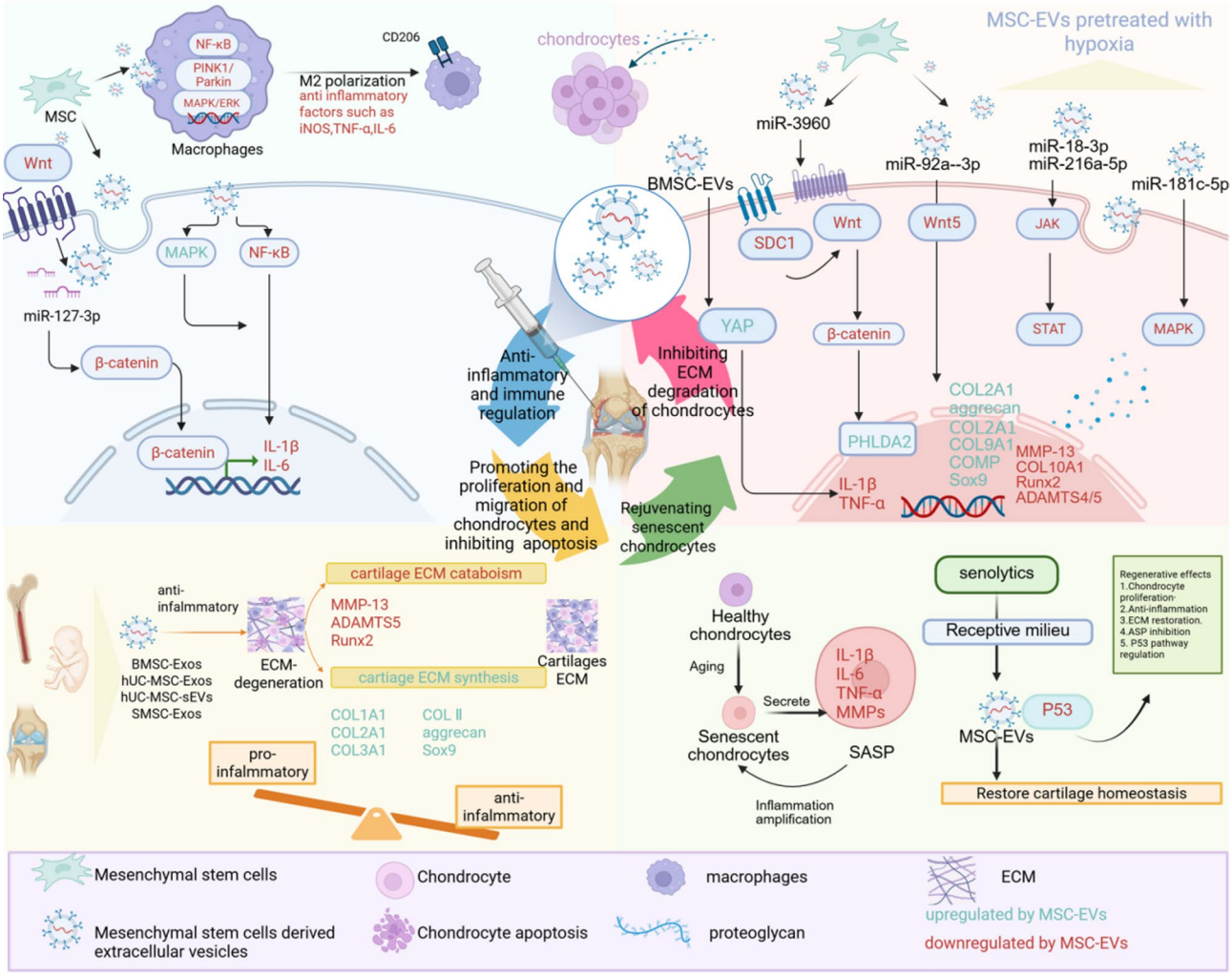


Fig. 2 Therapeutic mechanisms of MSC-EVs in KOA. MSC-EVs alleviate KOA by: (1) exerting anti-inflammatory and immunomodulatory effects (e.g., suppressing pro-inflammatory cytokines); (2) inhibiting ECM degradation in chondrocytes (e.g., downregulating MMP-13 and ADAMTS5); (3) promoting chondrocyte proliferation/migration while suppressing apoptosis; (4) rejuvenating senescent chondrocytes

of ECM-degrading enzymes, including MMP-13 and ADAMTS5 [29].

MSCs counteract ECM degradation by secreting endogenous protease inhibitors, such as tissue inhibitors of metalloproteinases, which attenuate MMPs proteolytic activity and preserve collagen and aggrecan integrity [76], reduce the degradation of collagen and aggrecan, and protect the structural integrity of articular cartilage. BMSC-EVs further enhance ECM synthesis by upregulating miR-136-5p, which increases Sox9 gene expression and promotes glycosaminoglycan (GAG) and type II collagen (COL II) production, thereby maintaining ECM content under inflammatory conditions and mitigating cartilage damage [77]. Beyond anti-inflammatory effects, MSC-EVs directly inhibit catabolic enzyme activity. Studies demonstrate that IL-1β-stimulated chondrocytes treated with human BMSC-EVs exhibit elevated expression of Collagen type I alpha 1 chain (COL1A1), Collagen type II alpha 1 chain (COL2A1), and Collagen type II alpha 1 chain (COL3A1), alongside reduced MMP-13 mRNA levels, indicating that BMSC-EVs counteract inflammation-induced upregulation of ECM-degrading enzymes [73]. Comparative analyses reveal that hUC-MSC-derived small EVs (hUC-MSC-sEVs) contain higher concentrations of cartilage repair-associated proteins and demonstrate superior COL II expression relative to parental hUC-MSCs, though they exhibit reduced inhibitory effects on MMP-13 and ADAMTS5 [78]. Engineered MSC-EVs overexpressing specific miRNAs demonstrate enhanced therapeutic targeting. For instance, lentiviral transfection of hUC-MSC-EVs with miR-140 not only preserves native hUC-MSC-EVs' functions but also amplifies ECM secretion (COL II and aggrecan) by targeting vascular endothelial gnrowth factor A (VEGFA) [79]. Similarly, while unmodified SMSC-EVs exert negligible effects on chondrocyte ECM secretion, SMSC-EVs

overexpressing miR-155-5p substantially enhance ECM production by targeting Runt-related transcription factor 2 (Runx2) [80]. These findings underscore the potential of genetically engineered EVs to augment cartilage repair efficacy.

In summary, the avascular architecture of articular cartilage necessitates ECM protection as a cornerstone strategy in OA management. MSCs and MSC-EVs preserve cartilage integrity through multifaceted mechanisms, including Tissue Inhibitor of metalloproteinases (TIMP) secretion, catabolic enzyme inhibition, and anabolic pathway activation. Genetic engineering of MSC-EVs to overexpress therapeutic miRNAs represents a promising advancement, offering enhanced targeting precision and ECM regenerative capacity that provides robust theoretical support for next-generation engineered EVs therapeutics.

Promoting the proliferation and migration of chondrocytes and inhibiting apoptosis

In healthy articular cartilage, orderly chondrocyte proliferation compensates for daily cellular turnover, maintaining stable cell populations that continuously synthesize ECM components [81]. MSCs and their EVs have been shown to effectively enhance the regenerative capacity of articular cartilage. Articular cartilage contains multiple progenitor populations, including mechanosensitive Protein C receptor^{+} cells in the superficial zone that sense Piezo1-mediated stimuli and proteoglycan 4 lubricin-expressing cells with heterogeneous chondrogenic capacity [82, 83]. Additional progenitors reside in deeper zones and adjacent tissues, collectively maintaining cartilage homeostasis and facilitating repair.

MSCs promote cartilage regeneration primarily through chondrogenic differentiation, guided by surface markers including CD90 (regulating differentiation), Stro-1 (migration), CD44 (hyaluronan-mediated migration), and CD49b (integrin α-2, mediating adhesion and osteogenic differentiation) [76]. MSCs can directly secrete relevant pro-repair factors to repair chondrocytes, such as insulin-like growth factor 1, fibroblast growth factor 2, epidermal growth factor, platelet-derived growth factor, and other growth factors to drive chondrogenic differentiation [76]. Additionally, MSCs exert synergistic effects via paracrine signaling; co-culture of articular chondrocytes with BMSCs significantly enhances chondrocyte proliferation while reducing apoptosis [84]. Thus, MSCs leverage differentiation potential and trophic factor secretion to accelerate cartilage repair.

MSC-EVs exhibit source-dependent efficacy in promoting chondrocyte proliferation and migration. Comparative studies demonstrate that ADSC-EVs outperform BMSC-EVs and SMSC-EVs in stimulating BMSC migration, proliferation, chondrogenesis, and osteogenic differentiation in vitro, as well as cartilage and bone regeneration in vivo [85]. Notably, human ADSC-EVs overexpressing miR-375 markedly enhance calvarial defect regeneration [86]. BMSC-EVs engineered to overexpress Nuclear paraspeckle assembly transcript 1 (NEAT1) activate chondrocyte autophagy and suppress apoptosis via the miR-122-5p/Sesn2 axis, thereby attenuating OA progression [87]. MSC-EVs can also regulate chondrocyte proliferation through miRNA-mediated modulation of key signaling pathways, including Wnt/β-catenin, Yes-Associated Protein (YAP), Janus kinase/signal transducer and activator of trancription (JAK/STAT), and MAPK. MSC-EV-delivered miR-3960 inhibits chondrocyte injury by downregulating the pleckstrin homology like domain family A member 2 Gene (PHLDA2)/Syndecan-1 (SDC1)/Wnt/β-catenin axis [88], while miR-92a-3p overexpression enhances chondrocyte viability and proliferation at 24 and 48 h [89]. Hypoxia-preconditioned MSC-EVs stimulate chondrocyte proliferation and migration via miR-18-3p/JAK/STAT and miR-181c-5p/MAPK pathways [90]. Similarly, hypoxic BMSC-sEVs promote proliferation, migration, and apoptosis resistance through the miR-216a-5p/JAK2/STAT3 pathway with superior efficacy compared to normoxic conditions [91]. BMSC-EVs activate YAP signaling, enhancing proliferation, migration, and phenotype maintenance while reducing IL-1β and TNF-α expression [92]. Conversely, ADSC-EVs mitigate chondrocyte degradation and synovial fibrosis by inhibiting Wnt/β-catenin signaling in OA models [93].

The limited self-repair capacity of chondrocytes necessitates therapeutic strategies to enhance proliferation, migration, and survival. MSCs promote cartilage regeneration through chondrogenic differentiation, trophic factor secretion, and paracrine signaling. MSC-EVs regulate chondrocyte proliferation and migration via miRNA modulation of key signaling pathways, thereby providing targeted cartilage regeneration therapy.

Rejuvenating senescent chondrocytes

In addition, Chondrocytes in osteoarthritis typically exhibit features of ageing and senescence [94]. It is reported that hallmarks of aging (genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, disabled macroautophagy, chronic inflammation, and dysbiosis) have been observed in articular cartilage, which may contribute to the development of OA in part by promoting cellular senescence [95]. Therefore, targeting senescent chondrocytes is also a feasible therapeutic strategy [31]. The accumulated aging chondrocytes in KOA joints secrete a large amount of pro-inflammatory cytokines, chemokines,

and proteases, forming harmful senescence-associated secretory phenotype (SASP), further exacerbating local inflammation and damaging surrounding healthy cells [96]. MSCs and their EVs have strong immune regulation and anti-inflammatory properties. They may inhibit the production of SASP by delivering bioactive substances, break the vicious cycle of inflammation amplification mediated by SASP, and create a favorable microenvironment for cartilage repair. For example, studies have found that umbilical cord mesenchymal stem cell-derived EVs (UCMSC-EVs) can restore the vitality of aging chondrocytes and treat OA by regulating the P53 signaling pathway [97]. Beyond chondrocyte-specific effects, hUC-MSC-EVs contain abundant Proliferating Cell Nuclear Antigen (PCNA), rejuvenating adult BMSCs, promoting osteogenic differentiation, and reducing bone degeneration in aged mice [98]. Hypoxia-preconditioned MSC-EVs exert anti-aging effects by attenuating inflammation, reducing TNF-α, Nducible nitric oxide synthase (iNOS), and Cluster of Differentiation 38 (CD38) expression, and restoring macrophage mitochondrial function [99], providing mechanistic support for MSC-EVs-mediated inhibition of chondrocyte apoptosis through macrophage immunomodulation and mitochondrial repair.

In addition, when MSC-EVs are used in combination with other senolytics drugs, such as Dasatinib and Quercetin, they may exhibit better synergistic therapeutic effects [100]. The combination of MSC-EVs and senolytics presents a transformative therapeutic outlook for KOA, founded on a sequential strategy that first employs senolytics to clear the joint microenvironment of senescent cells and their deleterious SASP, thereby creating a receptive milieu for the subsequent administration of MSC-EVs, which can then more effectively exert their multifaceted regenerative effects by promoting chondrocyte proliferation, modulating inflammation, and restoring extracellular matrix homeostasis, thereby addressing the core limitation of each monotherapy where senolytics lack regenerative capacity and MSC-EVs efficacy is diminished in a senescence-rich environment; future directions should focus on optimizing this synergy through engineering MSC-EVs for enhanced cartilage targeting and controlled cargo delivery, establishing standardized protocols for the timing and dosing of the sequential treatment, and validating the long-term safety and disease-modifying effects in large-scale clinical trials, which could ultimately pave the way for a precision medicine approach to halt KOA progression.

Modification of MSC-EVs for KOA

However, most of the current clinical therapies are intra-articular injection of MSCs or a combination therapy. MSC-EVs are mostly limited by their low production, insufficient circulation stability, insufficient targeting ability, low survival rate, and suboptimal efficacy. Moreover, due to their relatively large size and negative charge of the lipid bilayer, they will also be hindered by steric and electrostatic obstacles in the process of delivery to cartilage, which can produce superimposed effects when combined with biomaterials. However, engineered EVs can overcome the above limitations because of their editable and strong targeting. Engineering EVs refers to the modification of EVs by physical, chemical, or biotechnological means to enhance their functions or endow them with new characteristics, to be applied in disease treatment, drug delivery, diagnosis, and other fields. Engineered EVs are divided into pre-loading, that is, the modification of parental cells, the content modification of EVs, and the surface modification of EVs. Engineering modification of EVs shows strong therapeutic potential for KOA (Table 2; Fig. 3).

Parental cell genetic modification of MSCs

During the biogenesis of EVs, donor cells such as MSCs can be genetically modified to produce EVs that intrinsically contain specific therapeutic molecules. Through genetic engineering approaches, target nucleic acids including miRNAs, siRNAs, and mRNAs can be delivered to target tissues and cells [101, 102]. These therapeutic molecules become naturally incorporated into EVs during the secretory process.

The EVs of gene edited MSCs can maintain the homeostasis of chondrocytes more significantly than ordinary EVs. As in a study on senescent chondrocytes, MSC-EVs with multifunctional modification can more effectively eliminate senescent chondrocytes and maintain matrix metabolic homeostasis, enhance cartilage penetration ability, and prolong retention time [103]. The EVs of gene edited MSCs can maintain the homeostasis of chondrocytes more significantly than ordinary EVs. For example, The EVs of human urine-derived stem cells (hUSC-EVs) can enhance the proliferation and migration ability of chondrocytes treated with IL-1β and inhibit apoptosis, but the secretion of ECM is less. hUSC-EVs transfected with miR-140 by lentiviru not only retain the ability of hUSC-EVs, but also increase the secretion of ECM by targeting vascular endothelial growth factor A, including COL II and aggrecan [79]. The same is true for SMSC-EVs, which have no effect on the secretion of ECM from chondrocytes. However, SMSC-EVs overexpressing miR-155-5p showed common features of EVs in vitro and further promoted ECM secretion by targeting Runx2 [80]. Matrilins are adaptor proteins within the ECM. In one study, SMSCs were transduced with a lentiviral vector overexpressing matrilin-3 (MATN3). The resulting SMSC-EVs carrying MATN3 demonstrated the ability to inhibit chondrocyte degradation by suppressing the

Table 2 Comparative analysis of engineered EVs for the treatment of KOA

Engineering Strategy	Specific Method	Drug/Modification Molecule	Therapeutic Effects	Refs
Parental Cell Genetic Modification	Lentiviral transfection	miR-140	COL II↑, aggrecan↑, chondrocyte proliferation↑	[79]
	Lentiviral transfection	miR-155-5p	ECM secretion↑, Runx2↑; Caspase-3↓	[80]
	Lentiviral transfection	MATN3 protein	COL II↑, aggrecan↑; IL-6↓,TNF-α↓, MMP-13↓, ADAMTS5↓, IL-17 A↓, PI3K↓, Akt↓, mTOR ↓	[104]
	Hypoxic preconditioning	miR-216a-5p	Chondrocyte proliferation and migration↑; chondrocyte apoptosis↓, IL-1β↓, JAK2↓	[91]
	3D culture system	-	Chondrocyte proliferation↑, VEGF↑, hepatocyte growth factor↑, IL-6↑, IL-8↑	[107]
	3D culture system agomir/lentivirus transfection, electroporation, and Exo-Fect transfection	miR-455-3p	Hyaline cartilage regeneration↑; PAK2↓	[108]
	3D culture system mimics transfection	miR-365a-5p	Chondrogenic potential↑, M2 macrophage↑	[109]
	Hypoxic preconditioning	-	Chondrocyte proliferation and migration↑, COL II↑, aggrecan↑; Chondrocyte apoptosis↓, IL-1β↓, NF-κB↓, TNF-α↓, IL-6↓	[90]
	Hypoxic preconditioning	-	angiogenesis↑, Chondrocyte proliferation and migration↑, HIF-1α↑, Ras↑, Erk↑; Sprouty-Related EVH1 Domain-Containing Protein 1 (SPRED1)↓	[110]
	Hypoxic preconditioning	-	MMP-13↑, COL II↑	[91]
	Mechanical loading primed BMSCs	-	COL II↑, aggrecan↑; MMP-13↓, MMP-3↓, ADAMTS5↓	[114]
	Quercetin-primed BMSCs	-	COL II↑, Sox9↑; MMP-3↓, COX-2↓	[115]
Surface Modification	CAP peptide modification	Chondrocyte affinity peptide	Chondrogenesis↑; Senescent↓, SASP↓, P53↓	[97]
	LAMP-2B fusion	CAP-miR-140	miR-140↑, COL II↑, aggrecan↑, Sox9↑; MMP-13↓, IL-1β↓, ADAMTS5↓	[129]
	Charge reversal design	Cationic amphiphilic macromolecule	COL2A1↑, Sox9↑, aggrecan↑; IL-1β↓, IL-6↓, TNF-α↓, MMP-13↓, MMP-3↓, ADAMTS5↓, Runx2↓	[137]
Content Loading	Electroporation	Curcumin	miR-124↑, miR-143↑; NF-κB↓, ROCK1↓,TLR9↓, IL-1β↓, TNF-α↓	[138]
	Electroporation	miR-361-5p	COL2A1↑, Sox9↑, aggrecan↑; DDX20↓, NF-κB↓, IL-1β↓, TNF-α↓, MMPs↓, ADAMTS5↓	[140]
	Ultrasonic	KGN small molecule	TGF-β↑, Smad4↑, COL II↑, Sox9↑, aggrecan↑; COL10A1↓, Runx2↓, MMP-13↓, MMP-13↓	[130]
	Electroporation	siMDM2	COL II↑, Sox9↑, aggrecan↑; chondrocyte senescence↓	[103]
	Sonication technology	CAP and si-STING	STING↓	[144]
Biomaterial Composite	PCL scaffolds	Silver nanoparticles	ALP↑, Runx2↑, COL1A1↑; IL-6↓, TNF-α↓	[147]
	Functionalized 3D-printed Ti-6Al-4 V "Cell Climbing Frame"	E7 peptide	Bone Regeneration (COL1A1)↑, Cell proliferation rate and migration↑, Bone formation rate↑; Cell senescence markers (p16, p21) ↓, TNF-α↓, IL-6↓, Elastic modulus ↓	[148]
	3D-printed biomimetic trabecular porous Ti-6Al-4 V scaffold	-	MAPK↑, mTOR↑, HIF-1↑, VEGF↑, Runx2↑, Osteogenesis-related markers (COL1, Runx2)↑	[149]
	Hierarchical Mesoporous Bioactive Glass (MBG) Scaffold	-	Key Exosomal miRNAs (miR-328a-5p, miR-31a-5p) ↑, BMP Receptor 2↑, Phosphorylated Smad1/5/9 ↑, Osteogenic Markers (COL1, Runx2)↑; SMAD2↓	[151]
	β-TCP	-	Bone Volume/Tissue Volume Ratio↑, Bone Mineral Density↑, Runx2↑, COL1A1↑, Osteocalcin↑, PI3K/Akt↑	[153]
	Polyethylene Glycol Maleate Citrate (PG)/TCP Hydrogel	-	Bone regeneration↑, angiogenesis	[154]
	Injectable GelMA hydrogels	-	Chondrocyte/human BMSCs proliferation and migration↑, M2 macrophage↑	[112]
	Hyaluronic acid hydrogel microspheres	-	Chondrocyte proliferation↑, Chondrocyte ECM production↑, M2 macrophage↑, IL-10 ↑, TGF-β ↑, IL-1β↓, TNF-α↓	[120]

Table 2 (continued)

Engineering Strategy	Specific Method	Drug/Modification Molecule	Therapeutic Effects	Refs
	ECM-mimic hydrogel	-	COL II ↑, Sox9 ↑, Arg-1↑, IL-10↑; MMP-13↓, iNOS↓, TNF-α↓, IKBα↓, p65↓	[157]
	Mussel-inspired multifunctional hydrogel	Icariin (ICA)	Cartilage histological Improvements↑; MMP-13↓, IL-1β↓	[160]
	HA-SH (Thiolated Hyaluronic Acid) microgels	Cholesterol-PEG-CAP	COL2A1↑, COL II↑, Sox9 ↑, aggrecan↑, GAG↑, COL1A1↑,COL10A1↑; P53↓, IL-1↓, IL-8↓, MMP-13↓, NF-κB↓, ADAMTS5↓	[97]
	Glycyrrhizic acid (GA)/ Methacrylate-acylated hyaluronic acid (HA) scaffold	GDF-5	Sox9↑, aggrecan↑, COL II↑	[161]
	Stereolithography 3D-printing ECM/GelMA/EVs scaffold	-	Chondrocyte migration↑, M2 macrophage↑, COL2A1↑, aggrecan↑; MMP-13↓, ADAMTS5↓	[162]
	3D-printed bioinspired hydrogel scaffold	-	COL II↑, Sox9↑, aggrecan↑, Alkaline Phosphatase (ALP)↑, Osteocalcin (OCN)↑, Bone Volume/Total Volume↑; IL-1β↓	[163]
	Hydrogel (Gel-CEKT)	KGN TGF-β1	Sox9↑, aggrecan↑, COL II↑	[164]

↑ is upregulated by EVs, ↓ is downregulated by EVs

COL II: Collagen Type II; ECM: Extracellular Matrix; Runx2: Runt-Related Transcription Factor 2; MATN3: Matrilin-3; IL-6: Interleukin-6; TNF-α: Tumor Necrosis Factor Alpha; MMP-13: Matrix Metalloproteinase-13; ADAMTS5: A Disintegrin and Metalloproteinase with Thrombospondin Motifs 5; IL-17 A: Interleukin-17 A; PI3K: Phosphatidylinositol 3-Kinase; Akt: Protein Kinase B; mTOR: Mammalian Target of Rapamycin; IL-1β: Interleukin-1 Beta; JAK2: Janus Kinase 2; 3D culture: Three-Dimensional Culture; VEGF: Vascular Endothelial Growth Factor; IL-8: Interleukin-8; PAK2: P21-Activated Kinase 2; TLR2: Toll-Like Receptor 2; Myd88: Myeloid Differentiation Primary Response 88; NF-κB: Nuclear Factor Kappa B; COL2A1: Collagen Type II Alpha 1; HIF-1α: Hypoxia-Inducible Factor 1 Alpha; Erk: Extracellular Signal-Regulated Kinase; SPRED1: Sprouty-Related EVH1 Domain-Containing Protein 1; MMP-3: Matrix Metalloproteinase-3; BMSCs: Bone Marrow Mesenchymal Stem Cells; Sox9: SRY-Box Transcription Factor 9; COX-2: Cyclooxygenase-2; CAP: Chondrocyte Affinity Peptide; SASP: Senescence-Associated Secretory Phenotype; P53: Tumor Protein p53; LAMP-2B: Lysosomal-Associated Membrane Protein 2B; MMPs: Matrix Metalloproteinases; ROCK1: Rho-Associated Coiled-Coil Containing Protein Kinase 1; TLR9: Toll-Like Receptor 9; DDX20: DEAD-Box Polypeptide 20; KGN: Kartogenin; TGF-β/TGF-β1: Transforming Growth Factor Beta; Smad4: SMAD Family Member 4; COL10A1: Collagen Type X Alpha 1; siMDM2: siRNA mouse double minute 2 homologue; si-STING: Small Interfering RNA Targeting STING; STING: Stimulator of interferon genes; EVs: Extracellular Vesicles; PCL: Polycaprolactone; ALP: Alkaline Phosphatase; COL1A1: Collagen Type I Alpha 1; E7 peptide: E7 Peptide; Ti-6Al-4 V: Titanium-6Aluminum-4Vanadium Alloy; p16: Cyclin-Dependent Kinase Inhibitor 2 A; p21: Cyclin-Dependent Kinase Inhibitor 1 A; MAPK: Mitogen-Activated Protein Kinase; HIF-1: Hypoxia-Inducible Factor 1; MBG: Mesoporous Bioactive Glass; miR-328a-5p: microRNA-328a-5p; miR-31a-5p: microRNA-31a-5p; BMP Receptor 2: Bone Morphogenetic Protein Receptor 2; Smad1/5/9: SMAD Family Members 1/5/9; COL1: Collagen Type I; SMAD2: SMAD Family Member 2; β-TCP: Beta-Tricalcium Phosphate; BV/TV: Bone Volume/Total Volume Ratio; OCN: Osteocalcin; TCP: Tricalcium Phosphate; PG: Polyethylene Glycol Maleate Citrate; GelMA: Gelatin Methacrylate; HA: Hyaluronic Acid; IL-10: Interleukin-1; Arg-1: Arginase-1; iNOS: Inducible Nitric Oxide Synthase; IKBα: Inhibitor of NF-κB Alpha; p65: NF-κB p65 Subunit; ICA: Icariin; HA-SH: Thiolated Hyaluronic Acid; Cholesterol-PEG-CAP: Cholesterol-Polyethylene Glycol-Chondrocyte Affinity Peptide; GAG: Glycosaminoglycan; GA: Glycyrrhizic Acid; GDF-5: Growth Differentiation Factor 5; Gel-CEKT: Gelatin-based Composite Hydrogel; PEG: Polyethylene Glycol

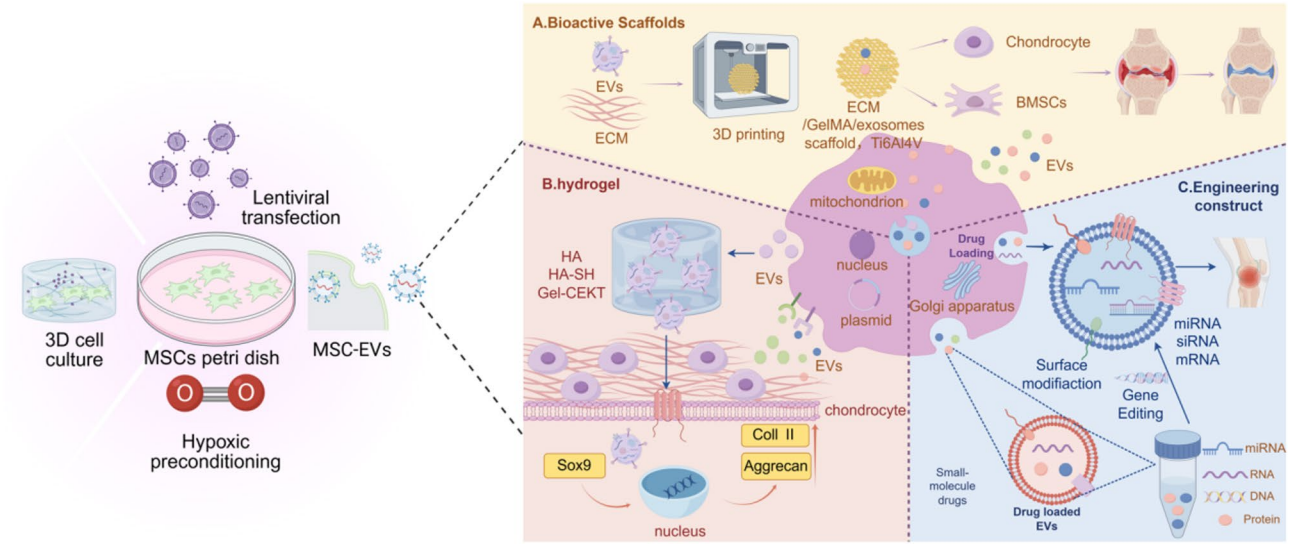


Fig. 3 Engineered modification of MSC-EVs for treatment of KOA. The modification approaches for MSC-EVs can be primarily categorized into two aspects: parental cell pretreatment and MSC-EVs engineering

interleukin-17 A (IL-17 A)/Phosphoinositide 3-Kinase/Protein Kinase B (PI3K/ Akt)/mammalian target of rapamycin (mTOR) pathway, thereby exerting synergistic therapeutic effects combining the inherent properties of SMSC-EVs with the functional benefits of MATN3 [104]. SMSCs transfected with miR-140-5p were isolated and characterized. Unlike unmodified SMSC-EVs which showed negligible effects, SMSC-140-EVs significantly enhanced ECM synthesis, promoted Sox9 expression, and stimulated chondrocyte proliferation and migration in vitro [105]. Recent advances in transfection methodology include the development of acoustothermal transfection technology, which significantly enhances transfection efficiency while preserving the therapeutic potency and homing capacity of BMSCs in vivo. Although this technology has been primarily validated in mouse models of middle cerebral artery occlusion, it offers promising translational potential for osteoarthritis therapeutic applications [106].

Furthermore, MSCs proliferation and EVs secretion can be enhanced through strategic modifications of the culture environment, thereby increasing MSC-EVs yield. 3D culture systems not only augment EVs production but also enhance their therapeutic efficacy. Investigators have established 3D culture platforms for hUC-MSCs utilizing chitin nanoscaffolds. Compared to conventional 2D monolayer cultures, 3D cultured hUC-MSCs exhibited superior proliferative capacity and enhanced paracrine activity [107]. Researchers have similarly applied 3D culture methodologies to expand ADSCs. Compared to 2D culture systems, EVs secreted by 3D cultured ADSCs contained significantly elevated levels of chondrogenesis-related miRNAs, thereby enhancing the chondrogenic potential of ADSCs [108]. Studies have demonstrated that 3D culture-derived MSC-EVs promote macrophage M2 polarization through inhibition of the toll-like receptor 2 Gene (TLR2)/Myeloid Differentiation Primary Response 88 (MyD88)/NF-κB signaling axis with superior efficacy compared to conventional 2D-EVs, providing a promising therapeutic avenue for MSC-EVs based interventions in KOA [109].

Additionally, hypoxic preconditioning has been demonstrated to enhance the therapeutic properties of MSC-EVs in promoting chondrocyte proliferation and migration while inhibiting apoptosis [91]. Investigations have revealed that EVs derived from hypoxia-preconditioned BMSCs stimulate chondrocyte proliferation and migration through activation of miR-18-3p/JAK/STAT and miR-181c-5p/MAPK signaling pathways, thereby facilitating cartilage repair [90]. hUC-MSC-EVs cultured under hypoxic conditions effectively promoted angiogenesis, as evidenced by increased vascular volume and density during fracture healing [110]. Consistently, hypoxia-conditioned BMSC-EVs promoted chondrocyte proliferation, migration, and survival through the miR-216a-5p/JAK2/STAT3 signaling pathway, demonstrating superior therapeutic efficacy compared to normoxic controls. Therefore, hypoxic preconditioning represents an effective strategy for augmenting the therapeutic potential of MSC-EVs [91].

Abnormal mechanical stimuli activate inflammatory cascades. In the ACLT mouse model, Transforming Growth Factor-Beta (TGF-β) signaling is activated in subchondral bone in response to mechanical stimuli, and inhibition of TGF-β signaling in subchondral bone MSCs effectively alleviates osteoarthritis progression [111]. Conversely, under optimal mechanical conditions, controlled mechanical loading can enhance the capacity of MSC-EVs to maintain matrix homeostasis and preserve chondrocyte function [112, 113]. Specifically, investigations have demonstrated that EVs produced by BMSCs subjected to continuous cyclic sinusoidal tensile strain (8%, 1 Hz for 6 h) exhibited altered miRNA profiles that mediated protective effects. The upregulated miR-27b-3p was shown to promote matrix homeostasis and chondrocyte proliferation while inhibiting cellular senescence, thereby attenuating osteoarthritis progression in vivo [114]. Pharmacological preconditioning of MSCs also demonstrates therapeutic potential. EVs secreted by BMSCs following co-culture with quercetin not only reduced the expression of IL-1β induced pro-inflammatory genes, including MMP-9 and cyclooxygenase-2 (COX-2), but also prevented cartilage matrix degradation [115]. In addition, mechanical stimulation can not only serve as abnormal pathological input, but mechanical transduction may also be the process by which cells convert physical forces into biochemical signals that alter EV secretion. That means mechanotransduction, the process by which cells convert physical forces into biochemical signals, represents a transformative biomanufacturing strategy for programming MSCs to produce EVs with enhanced therapeutic cargo [116, 117]. This approach leverages mechanically sensitive ion channels, which initiate intracellular signaling cascades upon stimulation, ultimately altering EV biogenesis, release, and composition. By applying controlled mechanical preconditioning—such as cyclic stretch, fluid shear stress, or compression—MSCs can be directed to secrete EVs enriched with specific therapeutic molecules, for instance, miR-27b-3p, which demonstrates potent anti-senescence and anti-inflammatory effects by modulating pathways like Receptor tyrosine kinase-like orphan receptor 1 (ROR1)/NF-κB. Furthermore, this biomechanical programming can be combined with genetic engineering, such as overexpressing specific microRNAs or decorating EVs with cartilage-targeting peptides, to create advanced “designer” EVs with improved targeting and functionality [114]. The use of mechanotransduction

to program MSCs to produce functionally enhanced EVs represents a paradigm shift in integrating physical intelligence into biomanufacturing.

Regarding cartilage tissue, pro-inflammatory factors do not exert exclusively detrimental effects. Under specific conditions, pro-inflammatory stimuli can prime MSCs to produce EVs with enhanced cartilage-protective properties. Pre-stimulation of MSCs with IL-1β results in the secretion of EVs containing cartilage-protective miRNA cargo and anti-inflammatory mediators, facilitating their enhanced penetration into collagen-rich cartilage regions [118, 119]. Investigators have characterized the differentially expressed miRNA profiles in EVs derived from IL-1β-pre-stimulated hUC-MSCs, revealing that these miRNAs regulate critical biological processes including cell proliferation, inflammatory immune modulation, and cartilage matrix regeneration, with particular emphasis on immune regulatory mechanisms [120]. Furthermore, EVs secreted by lipopolysaccharide (LPS)-preconditioned MSCs significantly enhanced chondrocyte proliferation and migration while inhibiting apoptosis [121]. The pro-inflammatory cytokine TNF-α serves as a key coordinator of the osteoarthritic inflammatory response [122]. Investigations have demonstrated that TNF-α preconditioning significantly augmented EVs production in IPFP-derived MSCs. Intra-articular administration of TNF-α preconditioned IPFP-MSC-EVs demonstrated superior therapeutic efficacy in ameliorating gait abnormalities and pathological changes [123]. This enhanced therapeutic effect was attributed to the significant enrichment of low-density lipoprotein receptor-related protein 1 (LRP1), which serves as the primary endocytic receptor for MMPs and ADAMTS in chondrocytes [124]. Additionally, TGF-β1 stimulated BMSCs promoted synovial macrophage polarization toward the M2 phenotype through delivery of exosomal miR-135b, which suppressed MAPK6 expression [125].

In conclusion, the superior therapeutic performance of MSCs and their EVs may be attributed to their responsiveness to controlled and quantifiable external stimuli. Appropriately calibrated mechanical and inflammatory stimuli can synergistically amplify the therapeutic efficacy of MSCs and their EVs, achieving enhanced regenerative outcomes.

Surface modification of MSC-EVs

Surface modification strategies can enhance the therapeutic efficiency of EVs by improving tissue targeting, cargo loading, and therapeutic payload delivery. These approaches can be categorized into three main methodologies [126].

Chemical conjugation approaches utilize polymer linkers to attach targeting moieties onto EVs surfaces post-isolation [127]. For instance, CLS-PEG-CAP polymer was employed to conjugate chondrocyte affinity peptide (CAP) onto UCMSC-EV surfaces, significantly enhancing EVs enrichment in cartilage lesions while reducing off-target distribution to other organs [97]. Similarly, MSC-sEVs functionalized with the cartilage-targeting peptide WYRGRL-PEG2K-DSPE (WPD) and loaded with siRNA mouse double minute 2 homologue (siMDM2) demonstrated amelioration of chondrocyte senescence and maintenance of matrix homeostasis through this multifunctional modification strategy [103].

Genetic engineering approaches enable endogenous display of targeting ligands during EVs biogenesis by creating fusion proteins with EVs membrane proteins [128]. CAP was genetically fused to the N-terminus of lysosome-associated membrane protein-2 isoform B (LAMP-2B), generating CAP-displaying EVs that achieved targeted miR-140 delivery to chondrocytes, where it inhibited ECM-degrading enzymes and demonstrated significant therapeutic efficacy following intra-articular administration [129]. Similarly, MSC-binding peptide E7 was fused with LAMP-2B to generate E7-displaying EVs that exhibited targeting specificity toward synovial fluid-derived mesenchymal stem cells (SF-MSCs), enhanced bioavailability, and achieved significant cartilage regenerative efficacy in OA rat models [130].

Hybrid engineering strategies combine multiple modification techniques to optimize both targeting and cargo loading. CAP-LAMP-2B engineered EVs were further fused with liposomes to form hybrid CAP-EVs, which were then loaded with Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9 (CRISPR/Cas9) system components via membrane fusion. These hybrid vesicles successfully delivered gene-editing machinery to the deep cartilage matrix in arthritic rats, achieving MMP-13 knockdown and attenuating ECM degradation [131]. Another hybrid approach conjugated CAP peptide onto EV surfaces while employing electroporation to load exogenous MMP-13 siRNA, demonstrating the ability to suppress MMP-13 expression in osteoarthritic chondrocytes and attenuate OA pathological progression [132].

Zeta potential quantifies particle surface charge density and serves as a direct indicator of colloidal stability [133]. Typically, the zeta potential of EVs ranges from – 10 to -40 mV, indicating that EVs predominantly exhibit negative surface charges [134]. The surface charge distribution of EVs critically influences their biodistribution, cellular targeting specificity, and functional efficacy in vivo [135]. Specifically, surface charge affects EV-cell membrane interactions, thereby modulating cellular uptake efficiency. Strategic modification of EVs surface charge can enhance their targeting capacity toward specific cell types or tissues.

Recent investigations have explored charge reversal engineering of EVs as a targeting strategy. Cationic EVs demonstrate superior targeting efficiency toward the negatively charged extracellular matrix of early arthritic cartilage compared to native anionic EVs. Investigators have utilized buffer pH as a charge reversal switch, successfully reversing EVs surface charge from – 25.4 ± 1.1 mV to -2.5 ± 1.0 mV. Currently, this approach has been primarily demonstrated using milk-derived EVs [136]. Charge-reversed EVs exhibit enhanced delivery capabilities compared to native EVs, enabling efficient transmembrane delivery of mRNA cargo. The surface charge of MSC-sEVs was successfully reversed through modification with a novel cationic amphiphilic macromolecule, ε-polylysine-diphenyl phosphatidylethanolamine (PPD). Compared to unmodified MSC-sEVs, charge-reversed PPD-sEVs demonstrated significantly enhanced bioavailability. In ACLT mouse models, intra-articular administration of PPD-sEVs increased pain thresholds and downregulated pro-inflammatory mediators including IL-1β, TNF-α, and nerve growth factor [103]. Furthermore, the prolonged intra-articular retention time of PPD-sEVs eliminated the necessity for repeated injections, thereby reducing infection risk and minimizing soft tissue trauma [137].

Collectively, surface charge modification represents a versatile strategy to enhance EVs targeting and retention, complementing targeting approaches based on ligands in optimizing MSC-EVs therapeutic efficacy for KOA treatment.

Content modification of MSC-EVs

Following EVs secretion, therapeutic molecules including drugs, siRNAs, miRNAs, and other bioactive compounds can be incorporated into EVs through physical, chemical, or biological post-loading methodologies to modify EVs cargo and enhance targeted therapeutic efficacy. Post-loading techniques encompass electroporation, co-incubation, extrusion, and ultrasonic loading; however, these methods may compromise EVs membrane integrity and result in cargo leakage.

Curcumin-loaded MSC-EVs modulate miR-143/Rho-associated coiled-coil containing protein kinase 1 (ROCK1)/Toll-like receptor 9 Gene (TLR9) and miR-124/NF-kB signaling pathways, thereby attenuating apoptosis in osteoarthritic chondrocytes [138]. Similarly, puerarin-loaded BMSC-EVs effectively stimulated chondrocyte proliferation and migration [139]. Investigators incorporated miR-361-5p into MSC-EVs via electroporation. In vitro studies demonstrated that miR-361-5p, which targets Asp-Glu-Ala-Asp-box polypeptide 20 (DDX20, a key factor upregulated in osteoarthritis), exhibited superior efficacy in ameliorating IL-1β induced chondrocyte injury and inflammation [140]. Previous investigations established that kartogenin (KGN) induces chondrogenic differentiation of SF-MSCs in vivo. Electroporation-mediated loading of KGN into surface-modified MSC-EVs enhanced intracellular KGN concentrations and significantly promoted SF-MSCs chondrogenic differentiation capacity both in vitro and in vivo [130].

Because siRNA can bind to target mRNA, the target mRNA is degraded by cellular nucleases, and the expression of target genes is blocked [141]. In recent years, targeted delivery of siRNA has become a new direction of gene therapy research [142]. Investigators synthesized siMDM2 and loaded it into MSC-sEVs. This siRNA effectively delayed chondrocyte senescence. Additionally, the study developed a novel cationic peptide WPD targeting COL II to functionalize Mesenchymal Stem Cells-derived small extracellular vesicles (MSC-sEVs), thereby enhancing cartilage penetration. These engineered MSC-sEVs effectively reversed chondrocyte senescence phenotypes and maintained matrix homeostasis [103]. In addition, as the Cyclic GMP-AMP Synthase (cGAS)-Stimulator of interferon genes (STING) pathway is the core of chronic inflammation and aging related functional decline [143], inhibition of the STING signaling pathway can delay cellular senescence, so there are studies that combine BMSC-EVs with CAP and si-STING through sonication technology to remodel the senescent microenvironment [144].

Engineered EVs demonstrate superior therapeutic versatility compared to native EVs. Although technical challenges including membrane structural disruption exist when introducing therapeutic molecules into EVs, these modifications effectively enhance the targeted therapeutic capacity of EVs, enabling more efficient maintenance of chondrocyte homeostasis and promotion of ECM secretion. Simultaneously, engineered EVs exhibit significantly prolonged in vivo retention times and improved treatment efficacy, establishing them as a promising therapeutic strategy for osteoarthritis [80].

Biomaterial based modifications of MSC-EVs

Metal materials and bioactive ceramic modification

The correct design of biomaterial scaffolds for intra-articular delivery of cells will contribute to the transformation of MSC-based OA therapy in the future [145], especially when cooperation with ECM-derived scaffolds can improve the repair ability of cartilage [146]. Related studies have shown that silver nanoparticles and BMSC-EVs modified polycaprolactone (PCL) scaffolds can effectively promote cartilage regeneration and play an immunomodulatory role [147]. Although the traditional Ti-6Al-4 V titanium alloy scaffold has strong mechanical strength and superior biocompatibility, its elastic modulus is much higher than that of natural bone, which is not conducive to vascular regeneration. Someone has designed a

3D-printed titanium alloy (Ti-6Al-4 V) scaffold with high porosity and connectivity. This scaffold can effectively recruit BMSCs and promote their proliferation, migration, and osteogenic differentiation. Combined with the repair effect of EVs, it enhances the osteogenic potential of BMSCs and the viability of chondrocytes, showing significant efficacy in osteoporosis models [148]. Recently, research teams designed trabecular bone-mimicking porous Ti-6Al-4 V scaffolds (BTPS) via 3D printing based on the Voronoi algorithm and image data. Subsequently, they encapsulated hypoxia-induced human umbilical vein endothelial cell EVs within Polyethylene Glycol Diacrylate (PEGDA)/Methacrylated Gelatin (GelMA) hydrogel microspheres using microfluidic technology. The PGHExo EVs sustained-release microspheres were loaded on the BTPS scaffold by pDA coating, achieving dual biomimetic properties. Finally, in vivo and in vitro experiments showed that it can significantly enhance bone volume, density, and neovascularization by activating MAPK, mTOR, Hypoxia-inducible factor (HIF-1), and vascular endothelial growth factor (VEGF) signaling pathways to regulate gene expression, showing a strong osteogenic and angiogenic promotion [149].

Bioactive ceramics include bioactive glass and β-tricalcium phosphate (β-TCP), which have been applied in the field of bone regeneration for a long time and have good biocompatibility [150]. The optimized osteogenic BMSC-EVs were loaded onto the hierarchical mesoporous bioactive glass scaffold to maintain the bioactivity of EVs and achieve their sustained release, thereby effectively enhancing the bone formation ability and accelerating the process of bone regeneration [151]. β-TCP is similar to human bone tissue components and is one of the most effective bone graft substitutes [152]. Previous studies found that induced pluripotent stem cells derived EVs (hiPS-MSC-EVs) combined with β-TCP significantly promoted bone regeneration after skull defects in rats. EVs released from hiPS-MSC-EVs/β-TCP scaffolds can promote the recruitment, proliferation, and differentiation of endogenous BMSCs [153]. It has also been found that polyethylene glycol maleate citrate combined with β-TCP loaded rat BMSC-EVs not only has a high osteogenic rate, but also indirectly promotes angiogenesis [154].

The application prospects of MSC-EVs combined with metal materials and Bioceramics in the field of bone tissue engineering are broad. It can not only realize the continuous release of MSC-EVs activity but also enhance the formation and regeneration ability of bone tissue, enhance the viability of chondrocytes, and achieve a significant curative effect in osteoporosis and skull defect models. To a certain extent, there is still room to explore the influence of material composition and matrix mechanics on the secretion, diffusion, and therapeutic ability of EVs, which opens up a new research avenue in the future [145].

Hydrogels modification

Hydrogel is a three-dimensional network structure polymer chain with excellent mechanical strength, which has been widely used in the field of bone regeneration [155]. Typically, the variable compositions including collagen, gelatin, and polyethylene glycol (PEG)-based hydrogels correspond to fibrous, macroporous, and nanoporous architectures, respectively [156]. Hydrogels have unique characteristics highly similar to cartilage ECM, providing a 3D microenvironment for cell growth, differentiation, and migration [157, 158].

The integration of hydrogels with MSC-EVs addresses critical limitations inherent to free EVs administration. Relaxing hydrogel stress facilitates EVs distribution throughout the network structure [159], while the encapsulation prolongs intra-articular retention and prevents rapid systemic clearance. Furthermore, hydrogels combined with cells, growth factors, and EVs demonstrate synergistic therapeutic potential for addressing OA cartilage defects [160]. The functional interplay between hydrogel physical properties (lubrication, mechanical support) and EVs biological activities (anti-inflammatory, regenerative) amplifies overall therapeutic efficacy.

Injectable hydrogel formulations offer minimally invasive administration and conformal filling of irregular defects. IL-1β-prestimulated hUC-MSCs-derived EVs (C-EVs) were loaded onto hyaluronic acid (HA) hydrogel microspheres, where HA not only enhanced joint lubrication but also prolonged C-EVs bioactivity through synergistic anti-inflammatory and matrix-promoting effects [120]. A BMSC-EVs-loaded ECM-mimicking hydrogel demonstrated cartilage repair through direct ECM supplementation and sustained anti-inflammatory activity [157]. Mussel-inspired multifunctional hydrogels achieved codelivery of MSC-EVs and icariin (ICA), ensuring effective intra-articular delivery and sustained therapeutic efficacy [160]. GelMA hydrogels containing MSCs nanovesicles (MSC-NVs) achieved sustained local release with excellent biocompatibility and mechanical properties [112].

To overcome rapid clearance and low retention efficiency, advanced release mechanisms have been developed. A “two-phase” release system utilizing thiolated hyaluronic acid (HA-SH) microgels employed dual mechanisms of disulfide bonding and physical blending to encapsulate EVs. Unbound EVs were rapidly released and enriched on the articular cartilage surface, while disulfide-bonded EVs gradually released during hydrogel degradation, achieving chondrocyte-specific targeting and sustained intra-articular release exceeding 14 days [97]. Growth Differentiation Factor 5 (GDF-5)-preconditioned

SMSC-EVs (G-EVs) were loaded into 3D-printed glycyrrhizic acid/methacrylate-acylated hyaluronic acid (GA/HA) scaffolds, maintaining biological activity with sustained release lasting up to 20 days. Rat models demonstrated superior cartilage repair with increased bone mineral density via micro-CT and histological scores approaching normal cartilage with orderly collagen arrangement [161].

Three-dimensional bioprinting enables precise spatial control of EVs distribution and biomimetic microenvironment recreation. A 3D-printed ECM/GelMA/EVs scaffold maintained MSC-EVs viability for 2 weeks and significantly promoted cartilage regeneration in rabbits, offering advantages over repeated intra-articular injections [162]. A dual-network hydrogel scaffold mimicking natural osteochondral organization was fabricated from 9% GelMA, 2% dopamine-conjugated HA, and 2% oxidative HA, incorporating decellularized porcine cartilage/bone ECM and human ADSC-EVs. This scaffold enhanced BMSCs chondrogenic differentiation in vitro and promoted hyaline cartilage regeneration with seamless tissue integration in vivo [163]. Recently, a dynamic EV-crosslinked hydrogel (Gel-CEKT) was developed by encapsulating kartogenin and TGF-β1 in MSC-EVs, coating them with succinylated chitosan to generate positively charged EVs, and crosslinking with oxidized chondroitin sulfate (OCS) and Wharton's jelly. In vitro, Gel-CEKT recruited BMSCs and upregulated Sox9 expression alongside beneficial cartilage matrix components (COL II and aggrecan), while in vivo experiments demonstrated significant cartilage structural repair and enhanced chondrogenesis [164].

The combination of MSC-EVs and hydrogel breaks through the space-time limitations of traditional treatment through the trinity strategy of "local sustained release, targeted regulation and mechanical adaptation", and provides a programmable treatment platform for arthritis regenerative medicine.

Discussion and conclusion

Globally, KOA currently affects over 300 million individuals. Conventional treatments, including pharmacotherapy and surgical intervention provide solely symptomatic relief without reversing cartilage degeneration, underscoring the urgent need for regenerative therapies. Furthermore, the knee joint constitutes a complex and critical structure comprising articular cartilage, synovium, subchondral bone, menisci, and associated tissues [165], where daily mechanical loading complicates regeneration and repair processes. KOA pain manifestations exhibit acute, unpredictable patterns with substantial inter-patient variability. Factors such as obesity and physical activity can trigger pain episodes, profoundly diminishing quality of life. Consequently, comprehensive assessment through patient-reported outcomes, biomarkers, and biomechanical evaluations is essential to characterize the frequency and severity of disease exacerbations [166]. Notably, synovial fluid derived EVs show promise as diagnostic biomarkers, with surface markers such as Chondroitin Sulfate Proteoglycan 4 (CSPG4), V-set and immunoglobulin domain-containing 4 (VSIG4) and miRNA signatures (miR-126-3p) correlating with disease severity and radiographic progression [167], thereby enabling early diagnosis, patient stratification for precision medicine, and objective monitoring of therapeutic responses in clinical trials.

Despite the therapeutic potential of MSCs, safety concerns persist among clinicians and patients. Although major adverse events following MSCs injection remain rare, some patients experience transient joint swelling, pain, and mobility restrictions [43]. MSC-EVs, as a cell-free therapeutic modality, retain the regenerative capacity of parental MSCs while offering distinct advantages, including reduced immunogenicity and enriched paracrine factors. However, the immunogenicity profile warrants careful consideration, while native MSC-EVs exhibit lower immunogenic potential than whole cells, engineered EVs may present novel epitopes capable of eliciting immune responses upon repeated administration. MSC-EVs encapsulate bioactive cargoes including nucleic acids (mRNA, miRNAs), proteins, and lipids that recapitulate key MSCs functions. Through paracrine signaling, MSC-EVs deliver these molecular cargoes to target cells, modulating cellular behaviors and tissue homeostasis. Accumulating evidence demonstrates that EVs exhibit superior therapeutic efficacy compared to MSCs in osteoarthritis treatment [78, 168]. MSC-EVs promote cartilage repair through multifaceted mechanisms: inhibition of extracellular matrix degradation, regulation of chondrocyte proliferation and apoptosis, attenuation of inflammatory cascades, and enhancement of autophagy. Specifically, MSC-EVs suppress pathological apoptosis while stimulating chondrocyte proliferation in KOA, thereby decelerating disease progression. Furthermore, MSC-EVs circumvent the safety concerns and ethical controversies inherent to cell-based therapies while preserving MSCs reparative functions. Collectively, these findings establish MSC-EVs as a promising therapeutic platform that addresses critical translational barriers in KOA treatment.

Clinical translation of MSC-EVs requires systematic optimization of physicochemical properties, drug loading capacity, encapsulation efficiency, and membrane integrity when employing physical or chemical modification strategies. This is particularly critical for KOA therapy, where sustained delivery of macromolecular therapeutics (including anti-inflammatory cytokines

and cartilage-regenerating proteins) presents formidable challenges. Current MSC-EVs delivery systems face several obstacles: First, systemic administration results in poor joint targeting, insufficient therapeutic specificity, and limited treatment duration due to non-specific biodistribution. Intra-articular injection or surface modification strategies may overcome these limitations. Preclinical studies have demonstrated that MSC-EVs attenuate inflammatory responses and promote cartilage repair and functional recovery. Additionally, MSC-EVs can serve as drug carriers or be integrated with biomaterials for combination therapy, enhancing local drug concentrations and amplifying therapeutic efficacy. Engineered EVs exhibit enhanced safety profiles and improved targeting precision, showing considerable promise for KOA treatment. Surface modification strategies enhance EVs tissue tropism and bioavailability, while biomaterial-assisted delivery systems further optimize anatomical targeting [169]. Second, free MSC-EVs undergo rapid dilution in synovial fluid and macrophage-mediated clearance, resulting in inadequate joint retention and therapeutic durability. Although biomaterial encapsulation prolongs retention time and enhances cartilage targeting [120, 157], optimizing in vivo degradation kinetics remains essential for sustaining therapeutic efficacy. Third, clinical grade MSC-EVs production requires addressing substantial manufacturing and standardization challenges. Transitioning from laboratory-scale 2D culture to industrial-scale 3D bioreactors (which enhance yields 10–50 fold) necessitates process optimization to minimize variability in EVs composition and potency. Serum-free, xeno-free culture media are mandatory for Current Good Manufacture Practices (cGMP) compliance to eliminate xenogeneic contamination, though these formulations may alter MSCs phenotypes and EVs cargo profiles. Scalable isolation methods such as Tangential Flow Filtration offer improved throughput but require optimization to preserve EVs integrity while achieving adequate purity and recovery [170]. Currently, isolation, purification, and characterization protocols lack standardization across the field. Simple particle enumeration is insufficient, the International Society for Extracellular Vesicles (ISEV)'s MISEV2018 guidelines mandate comprehensive characterization encompassing particle size distribution, protein markers (positive: CD9, CD63, CD81; negative: calnexin, GM130), purity quantification, and critically, functional potency assays [171]. U.S. Food and Drug Administration (FDA) emphasizes functional bioassays that correlate with clinical outcomes (e.g., chondrocyte proliferation, anti-inflammatory activity) as essential for demonstrating batch-to-batch consistency and supporting regulatory approval. Heterogeneity arising from diverse MSCs sources (bone marrow, adipose tissue, umbilical cord) and production methods (2D/3D culture, hypoxic preconditioning) may compromise therapeutic reproducibility. Allogeneic EVs may trigger immune rejection, potentially exacerbating KOA's chronic inflammatory microenvironment [172]. International consortia, particularly the ISEV, must establish comprehensive Good Manufacturing Practice specifications encompassing particle concentration, purity standards, and functional marker validation. Additionally, long-term safety monitoring through phase III/IV randomized controlled trials is essential to detect delayed adverse events and establish evidence-based dosing regimens, while individualized treatment protocols based on patient molecular phenotyping will optimize clinical outcomes. From a regulatory perspective, MSC-EVs occupy a complex classification space between cell therapy and biological drugs, necessitating clear guidelines for informed consent, donor screening protocols, and traceability systems to ensure ethical compliance and patient safety throughout the clinical translation process [173].

In conclusion, MSCs and MSC-EVs extends beyond cartilage repair to include restoring overall joint homeostasis by influencing synovial tissue and SB. Advancing MSC-EVs for KOA treatment requires a multifaceted approach encompassing engineering optimization, biomaterial integration, and rigorous clinical translation. MSC-EVs represent a transformative shift from conventional symptom management toward genuine regenerative medicine through cell-free therapy. These nanoscale vesicles offer dual advantages: efficient penetration of the dense cartilage extracellular matrix for targeted delivery, and endogenous bioactive cargoes (including miRNAs) that synergistically promote chondrocyte regeneration, thereby addressing longstanding bioavailability limitations of traditional therapies. Despite persistent challenges in mechanistic elucidation, cGMP-compliant scale-up, and comprehensive quality control implementation per MISEV2018 and FDA guidelines, the convergence of bioengineering innovations, standardized manufacturing protocols, SF-EVs biomarker integration for precision medicine, and interdisciplinary collaboration positions MSC-EVs as a cornerstone of next generation KOA therapeutics with the potential to fundamentally transform patient outcomes worldwide.

Abbreviations

KOA	Knee Osteoarthritis
NSAIDs	Nonsteroidal Anti-inflammatory Drugs
TKA	Total knee arthroplasty
MSCs	Mesenchymal Stem Cells
MSC-EVs	Mesenchymal Stem Cell-Derived Extracellular Vesicles
EVs	Extracellular vesicles
ECM	Extracellular Matrix
PCM	Pericellular matrix
ADAMTS	A disintegrin and metallo-proteinase with thrombospondin motifs
MMPs	Matrix Metalloproteinases
MMP-13	Matrix metalloproteinases-13

COL II	Type II collagen
Sox9	SRY-Box Transcription Factor 9
DMM	Destabilization of the medial meniscus
SB	Subchondral bone
hUC-MSCs	Human Umbilical Cord Mesenchymal Stem Cells
ADSCs	Adipose-Derived Mesenchymal Stem Cells
BMSCs	Bone Marrow Mesenchymal Stem Cells
IPFP	Infrapatellar fat pad
TNF-α	Tumor Necrosis Factor Alpha
IL-1β	Interleukin-1 Beta
MMP-3	Matrix metalloproteinases-3
IL-6	Interleukin-6
IL-8	Interleukin-8
METTL3	Methyltransferase-like 3
apoVs	Apoptotic vesicles
NF-kB	Nuclear Factor Kappa B
MAPK	Mitogen-Activated Protein Kinases
Wnt/β-catenin	Wingless-Related Integration Site/Beta-Catenin
GAG	Glycosaminoglycan
COL II	Type II collagen
COL1A1	Collagen type I alpha 1 chain
COL2A1	Collagen type II alpha 1 chain
COL3A1	Collagen type III alpha 1 chain
VEGFA	Vascular endothelial growth factor A
Runx2	Runt-related transcription factor 2
TIMP	Tissue Inhibitor of metalloproteinases
NEAT1	Nuclear paraspeckle assembly transcript 1
YAP	Yes-Associated Protein
JAK/STAT	Janus Kinase/Signal Transducer and Activator of Transcription
PHLDA2	Pleckstrin homology like domain family A member 2 Gene
SDC1	Syndecan-1
SASP	Senescence-associated secretory phenotype
UCMSC-EVs	Umbilical cord mesenchymal stem cell-derived EVs
PCNA	Proliferating Cell Nuclear Antigen
iNOS	Nducible nitric oxide synthase
CD38	Cluster of Differentiation 38
hUSC-EVs	The EVs of human urine-derived stem cells
MATN3	Matrilin-3
PI3K/AKT	Phosphoinositide 3-Kinase/Protein Kinase B
mTOR	Mammalian target of rapamycin
TLR2	Toll-like receptor 2 Gene
MyD88	Myeloid Differentiation Primary Response 88
TGF-β	Transforming Growth Factor-Beta
COX-2	Cyclooxygenase-2
ROR1	Receptor tyrosine kinase-like orphan receptor 1
LPS	Lipopolysaccharide
LRP1	Lipoprotein receptor-related protein 1
CAP	Chondrocyte affinity peptide
WPD	WYRGRL-PEG2K-DSPE
siMDM2	siRNA mouse double minute 2 homologue
LAMP-2B	Lysosome-Associated Membrane Protein-2B
SF-MSCs	Synovial fluid-derived mesenchymal stem cells
CRISPR/Cas9	Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9
PPD	ε-Polylysine-Diphenyl Phosphatidylethanolamine
ROCK1	Rho-associated coiled-coil containing protein kinase 1
TLR9	Toll-like receptor 9 Gene
DDX20	Asp-Glu-Ala-Asp-box polypeptide 20
KGN	Kartogenin
MSC-sEVs	Mesenchymal Stem Cells-derived small extracellular vesicles
cGAS	Cyclic GMP-AMP Synthase
STING	Stimulator of interferon genes
PCL	Poly-ε-caprolactone
BTPS	Trabecular bone-mimicking porous Ti-6Al-4 V scaffolds
PEDGA	Polyethylene Glycol Diacrylate
GelMA	Methacrylated Gelatin
HIF-1	Hypoxia-inducible factor
VEGF	Vascular endothelial growth factor
β-TCP	β-tricalcium phosphate
hiPS-MSC-EVs	Induced pluripotent stem cells derived EVs
PEG	Polyethylene glycol
C-EVs	IL-1β-prestimulated hUC-MSCs-derived EVs
HA	Hyaluronic acid
ICA	Icariin
MSC-NVs	MSCs Nanovesicles
HA-SH	Hyaluronic acid
GDF-5	Growth Differentiation Factor 5
GA/HA	Glycyrrhizic acid/methacrylate-acylated hyaluronic acid
CEKT	One of positively charged EVsomes
Gel-CEKT	Dynamic EVsome crosslinked hydrogel
CSPG4	Chondroitin Sulfate Proteoglycan 4
VSIG4	V-set and immunoglobulin domain-containing 4
FDA	U.S. Food and Drug Administration
cGMP	Current Good Manufacture Practices
ISEV	International Society for Extracellular Vesicles

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Data availability
No datasets were generated or analysed during the current study.

Declarations

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Competing interests
The authors declare no competing interests.

Author details
[1]Research Center of Experimental Acupuncture Science, Tianjin University of Traditional Chinese Medicine, Tianjin, China
[2]School of Acupuncture & Moxibustion and Tuina, Tianjin University of Traditional Chinese Medicine, No.10 Poyang Lake Road, Tuanbo New Town, Jinghai District, Tianjin 301617, People's Republic of China
[3]Traditional Chinese Medicine Department, China-Japan Union Hospital of Jilin University, Jilin, China
[4]National Clinical Research Center for Chinese Medicine Acupuncture and Moxibustion, Tianjin, China
[5]Shanxi University of Traditional Chinese Medicine, Taiyuan, Shanxi, China
[6]Qingdao Traditional Chinese Medicine Hospital, Qingdao Hiser Hospital Affiliated of Qingdao University, Qingdao 260000, Shandong, People's Republic of China

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