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REVIEW

Interdisciplinary MEDICINE

The application of extracellular vesicles in orthopedic diseases

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Abstract

Orthopedic diseases, such as osteoarthritis and fractures, place a significant burden on individuals and healthcare systems worldwide. Extracellular vesicles (EVs), which are membrane-derived particles, have emerged as a novel tool in the field of orthopedics. EVs play a crucial role in diagnosing, regenerating, and treating orthopedic diseases. In terms of diagnosis, EVs serve as potential biomarkers, carrying unique donor cell information and circulating effectively in bodily fluids. Specific biomolecules within EVs, including proteins, nucleic acids, and microRNAs, hold promise as biological markers for the early detection and monitoring of orthopedic diseases. EVs have shown significant potential in promoting bone and cartilage regeneration. They can enhance tissue regeneration by stimulating various stem cells to proliferate, migrate, and differentiate into mature chondrocytes and osteocytes. EVs can also target specific tissues, making them attractive candidates for drug delivery in orthopedic diseases. They can efficiently deliver therapeutic cargo, such as anti-inflammatory agents and growth factors, to the affected sites, enhancing treatment efficacy while minimizing toxicity and adverse effects. In conclusion, EVs have significant potential in diagnosing, regenerating, and treating orthopedic diseases.

KEYWORDS
diagnosis, drug delivery, extracellular vesicles, orthopedic diseases, regeneration

Yuexin Li and Haiyan Zhang contributed equally.

1 | INTRODUCTION

Orthopedic diseases, which rank as the second leading global cause of disability, are an enormous socioeconomic burden.[1] The prevalence of disability resulting from orthopedic diseases is projected to have risen by 45% between 1990 and 2010, with osteoarthritis (OA) being a significant contributor.[1] An epidemiological study predicted that OA will be one of the most common causes of disability in the general population by 2030.[2] An examination of 2019 Global Burden of Disease (GBD) data revealed that around 1.71 billion individuals worldwide are affected by musculoskeletal conditions, such as low back pain, neck pain, fractures, other injuries, OA, amputation, and rheumatoid arthritis.[2,3] Orthopedic diseases frequently demonstrate a persistent and progressive character, causing a significant burden on healthcare systems and financial resources.[3] For example, orthopedic diseases constitute the third most substantial sector of the National Health Service's expenditure in the United Kingdom, totaling £4.7 billion. Notable expenses are linked to procedures, including total joint replacements and other orthopedic surgeries.[4] As the aging population in high-income countries continues to grow, the prevalence of many non-inflammatory musculoskeletal disorders is expected to rise.[4] Orthopedic diseases can lead to persistent pain, limited mobility, and stiffness in bones, joints, and muscles. Especially in older adults and developed nations, the enduring pain and disability linked to orthopedic diseases play a crucial role in determining the quality of life. This often results in frailty, reduced functionality, and ultimately the loss of independence.[5] Hence, prioritizing the prevention, identification, and management of musculoskeletal disorders in this susceptible demographic is of utmost importance.

Extracellular vesicles (EVs) are membrane-derived particles that are naturally secreted by cells. They are currently regarded as a 'novel terminology' within the orthopedic domain.[6,7] EVs are typically categorized into three main classes: microvesicles, exosomes, and apoptotic bodies. The classification is determined by their size and biogenesis. The main function of EVs is to carry proteins, RNA, and DNA and protect them from degradation.[8–10] EVs are relatively stable in bodily fluids due to their similar structure to cells, which includes an extracellular lipid domain, transmembrane proteins on their surface, and cytoplasmic components inside.[11] As EVs are present in numerous biological fluids and are consistently stable, they may be a promising source of biomarkers. In addition, EVs have the ability to alter cell phenotypes, differentiation, and recruitment in a paracrine manner. Therefore, EVs exhibit similar therapeutic properties to parental cells, such as stem cells.[12] Considering the ability of EVs to efficiently transport a wide range of biological molecules across various biofluids while maintaining cellular specificity, EVs demonstrate potential in drug delivery.[13] Extracellular vesicles have been thoroughly researched and proven efficient in diagnosis, regenerative therapy, and targeted drug delivery[14] (Figure 1). Hence, utilizing the cargo transported by EVs may prove beneficial in identifying much-needed biomarkers for musculoskeletal disorders.[15,16] The application of EVs in bone tissue engineering has the potential to promote bone growth and cartilage regeneration, thereby aiding in fracture healing and improving conditions such as OA.[16] In addition, EVs have been demonstrated to possess superior safety and lower immunogenicity characteristics in treating various human diseases. Conventional treatments and stem cell therapies still have numerous limitations and risks.[17,18] Therefore, EVs promise to become an innovative drug delivery carrier for orthopedic medication, overcoming the limitations of traditional drug treatments.[18]

Over the past decade, there has been rapid development in the research field of EVs, encompassing the diagnosis, treatment, and regenerative fields of orthopedic diseases. Thus, this review summarizes the application of EVs in orthopedic diseases and explores their potential value in diagnosis, regenerative medicine, and treatment.

2 | EVS IN THE DIAGNOSIS OF ORTHOPEDIC DISEASES

The early diagnosis of orthopedic diseases is crucial for effective treatment and management. Traditional clinical methods may, in some cases, fail to provide sufficient early diagnostic information, leading to diseases being diagnosed at an advanced stage. EVs, as potential biomarkers, have garnered significant attention in this field[19–21] (Table 1).

EVs show great potential as innovative biomarkers due to their ability to carry unique donor cell information and circulate effectively in bodily fluids.[21] The biomolecules found within EVs, such as proteins, nucleic acids, and microRNAs, have shown great promise as biological markers in cancers, immune disorders, and metabolic diseases.[31–33] In theory, the contents of EVs have the potential to differ across various orthopedic diseases, change with the progression of the disease, and play a role in pathological processes. The cargo within EVs could be employed to screen individuals with a genetic predisposition to developing orthopedic diseases, even before any signs of cartilage or bone damage occur. If EVs were integrated as a complementary tool alongside existing diagnostic methods, early-stage orthopedic

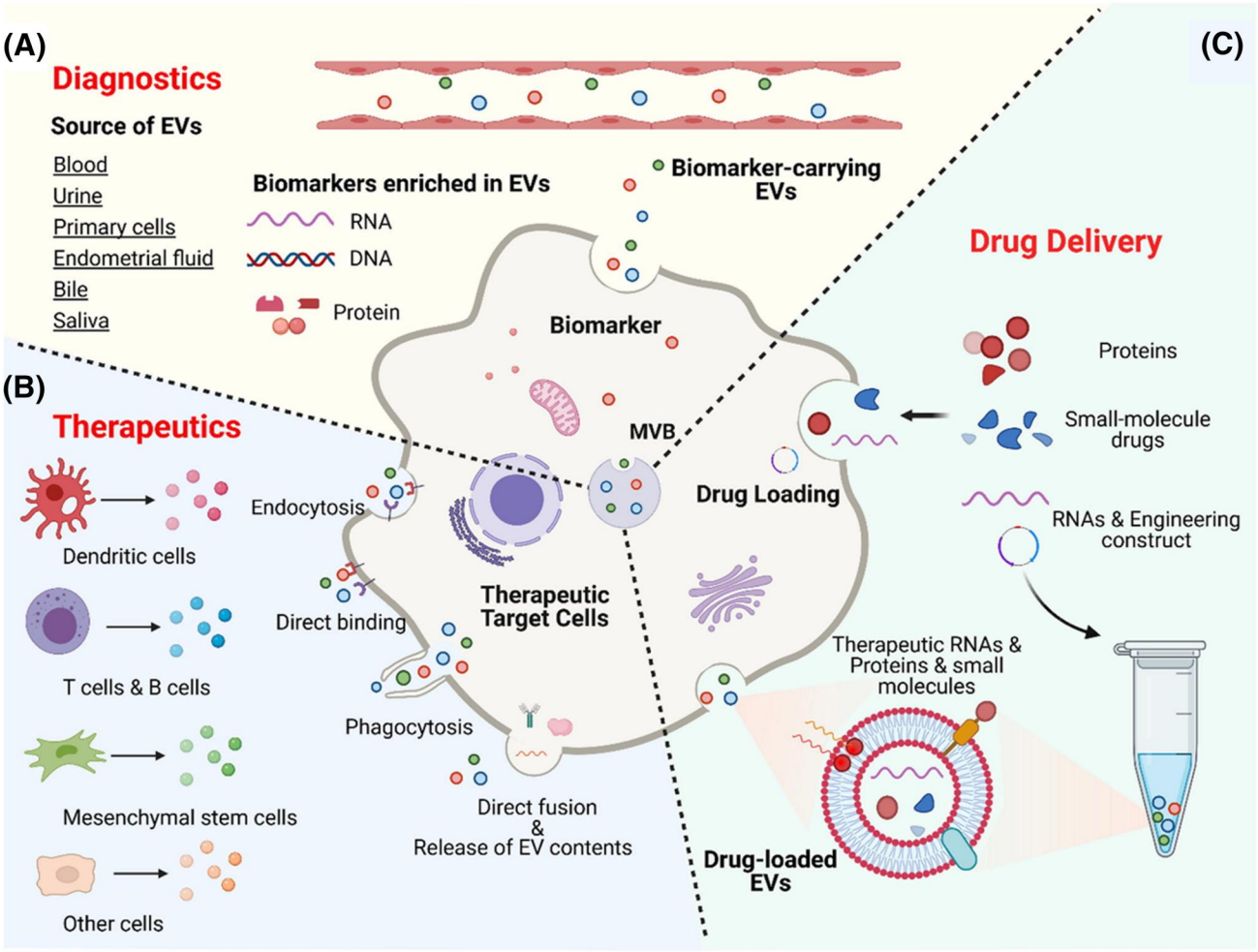

FIGURE 1 Potential applications of EVs. (A) Diagnostic (and prognostic) potential of EVs obtained from various sources. EVs generated under pathological microenvironments are able to capture complex intracellular molecular signatures that are unique to specific disease stages or injuries. As such, they are an attractive reservoir of biomarkers. (B) Therapeutic potential of EVs. EVs derived from multiple cells can interact with the target cells via various pathways, including endocytosis, direct binding, phagocytosis, and direct fusion, imparting specific therapeutic effects. (C) EVs as a potential DDS. EVs can be loaded with therapeutics such as RNAs, proteins, and small-molecule drugs, delivering the cargo to target cells. Reproduced with permission.[14] Copyright 2021, Elsevier.

TABLE 1 EVs in the diagnosis of orthopedic diseases.

Disease types	Source	CArgos	Biological functions	Reference
OA	Serum	miR -193b-3p	Biomarker	22
OA	Plasma and synovial fluid	HLA-DR, HLA-DP and HLA-DQ; CD34	Biomarker	23
OA	Synovial fluid	lncRNA PCGEM1	Biomarker	24
RA	Serum	miR-451a and exomiR-25-3p	Biomarker	25
RA	Plasma	miR-204-5p	Biomarker	26
RA	Serum	Proneuregulin-3, alpha-1-antitrypsin, and TLR3	Biomarker	27
Osteoporosis	Serum	Vinculin, filamin A, and profilin 1. Profilin 1	Biomarker	28
Osteoporosis	Plasma	tRF-25, tRF-38 and tRF-18	Biomarker	29
Osteoporosis	Plasma	PSMB9, AARS, PCBP2, and VSIR	Biomarker	30

diseases with detectable EV manifestations would be the clear focus for such screening efforts.[34] By analyzing EVs in the patient's blood or other bodily fluids, specific biomarkers could be identified, and changes in the presence or expression levels of these biomarkers might be associated with the development and progression of specific orthopedic conditions (Figure 2). This offers a new approach to the early screening and diagnosis of orthopedic diseases.

2.1 | EVs in the diagnosis of joint disorders

In joint disorders such as OA, EVs from plasma and synovial fluid have shown tremendous potential in diagnostics. Prior research has demonstrated a negative correlation between serum miR-193b expression and inflammation, suggesting that miR-193b holds potential as a diagnostic marker for OA.[22] Furthermore, EVs containing HLA-DR, -DP, and -DQ are the predominant subpopulations in synovial fluid compared with plasma, indicating a substantial contribution from infiltrating immune cells in OA joints. Conversely, CD34+ medium and small EVs, which reflect hematopoietic stem cells, progenitor cells, and endothelial cells, are significantly more abundant in plasma than in synovial fluid. The ratios of EVs from neutrophils and lymphocytes are closely related in both synovial fluid and plasma, suggesting that plasma EVs could indicate the severity of OA and could be used as systemic biomarkers for the development of OA in joints. Certain subsets of plasma EVs may also be used as advanced autologous biological products for intra-articular therapy of OA joints.[23] Zhao et al. examined exosomal lncRNAs in plasma and synovial fluid from patients with OA and revealed that exosomal lncRNA PCGEM1 holds promising potential as a robust biomarker for distinguishing early-stage OA from late-stage OA.[24]

EVs serve as biomarkers in OA and hold tremendous diagnostic potential in rheumatoid arthritis (RA). Researchers have also identified a series of dysregulated exosomal miRNAs in early RA, which are predicted to target YHWAB and may play a significant role in the development of RA. A combination panel consisting of exosomal miR-451a, exosomal miR-25-3p, and serum levels of sTWEAK was developed. This panel surpasses the conventional anti-citrullinated protein antibody biomarker in accurately diagnosing early RA, thereby enhancing the preclinical detection of the disease.[25] Wu et al. have also discovered a plasma exosomal miRNA-204-5p that is associated with RA and facilitates the communication between immune cells and synovial fibroblasts, suggesting its potential as a biomarker in diagnosing and treating RA.[26] The varying protein expression in plasma EVs can also be used as diagnostic evidence. For instance, the proteomic analysis of serum exosomes from patients with RA and healthy controls revealed increased levels of serum pro-neuregulin-3, alpha-1-antitrypsin, and TLR3 and decreased levels of

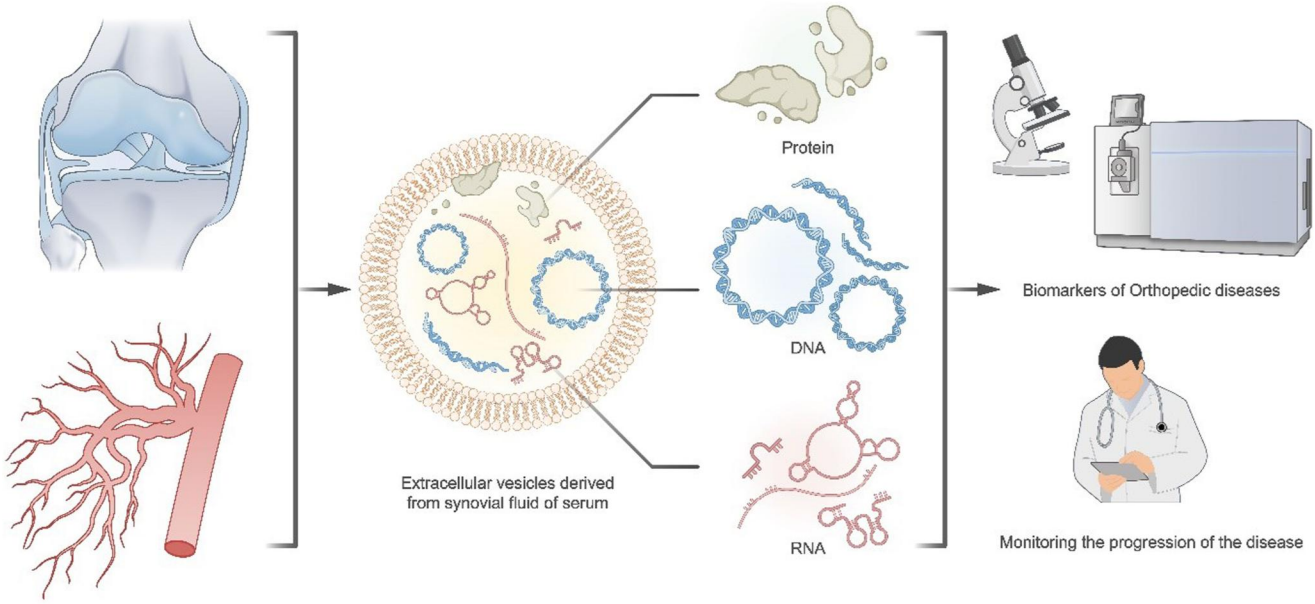

FIGURE 2 By analyzing EVs carrying proteins, DNA, and RNA in the patient's blood or other bodily fluids, specific biomarkers can be identified, and changes in the presence or expression levels of these biomarkers may be associated with the development and progression of specific orthopedic conditions.

type II cytoskeletal one in exosomes from RA patients.[27] In summary, EVs in serum and synovial fluid can be considered as potential biomarkers for joint diseases.

2.2 | EVs in the diagnosis of bone diseases

Numerous researchers have found that EVs play a crucial role in osteoporosis diagnosis. Comparative proteomics analysis revealed differential protein profiles in EVs among normal subjects, osteopenia patients, and osteoporosis patients. Approximately 200 proteins were identified and quantified from serum EVs. Among these, 19 proteins showed increased levels, while five proteins exhibited decreased levels in both the osteopenia and osteoporosis groups compared to the normal group. Three protein candidates were selected for initial validation: vinculin, filamin A, and profilin-1. Profilin-1 was subsequently confirmed in an independent sample set to distinguish the osteoporosis group from the osteopenia and normal groups.[28] Zhang et al. also found that plasma exosomal tRF-25, tRF-38, and tRF-18 could serve as diagnostic biomarkers for the detection of osteoporosis. Quantitative proteomics analysis was conducted to characterize and compare the plasma exosome-derived protein profiles across various stages of osteoporosis. The research identified and validated the presence of four exosomal proteins, namely PSMB9, PCBP2, VSIR, and AARS, all of which might be biomarkers of osteoporosis.[30]

In summary, EVs demonstrate significant advantages in the early screening, diagnosis, and monitoring of orthopedic disease progression.

3 | EVS IN THE REGENERATION OF CARTILAGE AND BONE

The bone and cartilage possess an inherent ability to regenerate; however, in certain cases, this capacity is compromised, mandating clinical intervention. Such instances include OA, osteoporosis, non-union fractures, and other orthopedic diseases.[35,36] Considering that numerous orthopedic diseases are linked to the depletion of chondrocytes or loss of bone, using EVs in therapy has been proposed as a viable alternative. EVs obtained from distinct cell types and under specific conditions have proven to enhance tissue regeneration across a broad spectrum of organs, including the heart, blood vessels, kidneys, liver, lungs, skin, neural tissue, and reproductive tissue.[37] The use of EVs in orthopedic diseases is notably attractive, given that their inherent complexity empowers them to engage in numerous complementary signaling pathways, thus promoting both osteogenic and angiogenic reactions in diverse cell types crucial for bone development[38,39] (Table 2).

3.1 | EVs in the regeneration of bone

EVs can promote bone regeneration by stimulating osteogenic genes and inhibiting bone loss. The repair ability of bone tissue has been demonstrated by researchers through in vivo and in vitro experiments, whereby milk-derived EVs (milk-EVs) enhanced the expression of the osteogenic gene GJA1 via the transcript AP3B1.[40] Researchers have also examined the paracrine functional role of EVs derived from naive (M0), M1, and M2 polarized macrophages in bone repair. They discovered that M0 and M2 EVs boost repair/regeneration, while M1 EVs hinder bone repair. When mesenchymal stem cells (MSCs) were treated with an M2 macrophage EV-enriched miR-378a mimic, their osteoinductive gene expression increased compared to the control group.[41] Exosomes in MSC-conditioned medium have been implicated in promoting fracture healing and have been deemed a novel component of MSC paracrine signaling, which plays a crucial role in tissue repair.[64] Due to the potential of EVs derived from bone marrow mesenchymal stem cells(BM-MSCs) to mitigate oxidative stress, expedite DNA damage repair, and reduce the expression of proliferation-inhibiting and cell senescence-related proteins, researchers are considering it as a promising treatment approach for bone loss.[42] Investigations also revealed that the systemic infusion of umbilical cord mesenchymal stem cell-derived extracellular vesicles (hucMSC-EVs) preserved bone integrity and strength in osteoporotic mice. This was achieved through augmented bone formation, decreased medullary adipose deposition, and reduced bone resorption. Proteomic profiling identified a potent osteogenic protein, CLEC11A (C-type lectin domain family 11, member A), which was highly enriched in the hucMSC-EVs. Furthermore, by delivering CLEC11A in an in vitro setting, hucMSC-EVs facilitated a shift from adipogenic toward osteogenic differentiation of bone marrow stromal cells (BMSCs). Importantly, CLEC11A was found to be essential in the inhibitory actions of hucMSC-EVs on the formation of osteoclasts.[43] Researchers also found that extracellular vesicles derived from human umbilical cord mesenchymal stromal cells display robust bone-protective properties, which are mediated by the modulation of bone metabolism expression through the CLEC11A pathway. When subjected to an in vivo assessment in a rat model with defects in the calvarial bone, it was observed that the MSC-derived EVs that underwent TNFa preconditioning

TABLE 2 EVs in the regeneration of bone and cartilage.

Biological functions	Source	CArgos	Working mechanisms	References
Bone regeneration	Milk	AP3B1	Enhancing the expression of the osteogenic gene GJA1 via the transcript AP3B1	40
Bone regeneration	M2 macrophage	miR-378a	Increasing osteoinductive gene expression	41
Bone regeneration	Bone marrow mesenchymal stem cells	None	Expediting DNA damage repair, and reducing the expression of proliferation-inhibiting and cell senescence-related proteins	42
Bone regeneration	Human umbilical cord mesenchymal stromal cells	CLEC11A	Preserving bone integrity and strength	43
Bone regeneration	Mesenchymal stem cells	Oncostatin M	Improving bone regeneration process	44
Bone regeneration	Bone marrow mesenchymal stem cells	Ubiquitin-specific peptidase 7	Maintaining the pro-osteogenic and anti-osteoclastogenic properties	45
Bone regeneration	Human-induced pluripotent stem cells	None	Enhancing bone regeneration and angiogenesis	46
Bone regeneration	Endothelial progenitor cells	miR-126	Promoting endothelial cell proliferation, migration, and angiogenic potential, accelerating bone regeneration	47
Bone regeneration	Serum	None	Suppressing macrophage inflammation by upregulating vascular endothelial adhesion factor 1 and facilitating angiogenesis	48
Bone regeneration	Hypoxic mesenchymal stem cells	miR-210-3p	Potentiating angiogenesis by suppressing EFNA3 expression and by stimulating the phosphorylation of the PI3K/AKT pathway	49
Bone regeneration	Human umbilical cord-MSCs	miR-23a-3p	Facilitating vascularized bone regeneration through the gradual release of sEVs	50
Bone regeneration	Bone marrow mesenchymal stem cells	TIM3	Promoting osseointegration through the activation of the Bmp2 promoter to enhance BMP2 secretion in macrophages	51
Bone regeneration	Macrophage	None	Facilitating MSC osteogenesis through the BMP2/Smad5 pathway	52
Cartilage regeneration	Bone marrow mesenchymal stem cell	None	Stimulating the proliferation and chondrogenic differentiation of tendon stem/progenitor cells (TSPCs) into fully developed chondrocytes	53
Cartilage regeneration	Mesenchymal stem cell	KLF3-AS1	Mitigating IL-1β-induced chondrocyte injury	54
Cartilage regeneration	Mesenchymal stem cell	miR-92a-3p	Enhancing proliferation of cartilage cells and upregulated expression of matrix genes in both MSCs and primary human chondrocytes	55
Cartilage regeneration	Kartogenin-preconditioned mesenchymal stem cells	miR-381	Resulting in the inhibition of the hippo signaling pathway and promotion of chondrogenesis	56
Cartilage regeneration	Synovium mesenchymal stem cells	miR-140-5p	Enhancing cartilage tissue regeneration	57

TABLE 2 (Continued)

Biological functions	Source	CArgos	Working mechanisms	References
Cartilage regeneration	Synovium mesenchymal stem cells	circRNA3503	Preventing OA progression	59
Cartilage regeneration	Chondrocytes	miR-95-5p	Enhancing the chondrogenic differentiation of MSCs and stimulating the expression of cartilage matrix in chondrocytes	60
Cartilage regeneration	Chondrocytes	miR-8485	Facilitating the chondrogenic differentiation of BMSCs	61
Cartilage regeneration	Mesenchymal stem cell	Mitochondrial proteins	Mitigating nucleus pulposus cells death in a three-dimensional hydrogel culture model and slowing the progression of intervertebral disc degeneration	62
Cartilage regeneration	Mesenchymal stem cell	Antioxidant proteins	Inhibiting inflammatory mediators and NLRP3 inflammasome activation, restoring damaged mitochondria	63

were able to diminish inflammation at 1-, 3-, and 7-day intervals after the initial injury. This consequently led to an improved bone regeneration process at 4 and 8 weeks after the injury. This positive outcome may have been achieved by regulating the expression of oncostatin M (OSM).[44] Investigators discovered an enhancement in the osteogenic differentiation of MC-3T3 cells and simultaneous inhibition of osteoclastic differentiation of RAW264.7 cells upon treatment with EVs derived from BMSCs. Microarray analysis revealed a significant increase in the expression of ubiquitin-specific peptidase 7 (USP7) in mouse bone tissue after the administration of EVs. It was further revealed that USP7 interacted with Yes1-associated transcriptional regulator (YAP1) and stabilized YAP1 protein levels through deubiquitination modification. YAP1-related genes were found to be enriched in the Wnt/β-catenin signaling pathway, and the overexpression of YAP1 led to the translocation of β-catenin into the nucleus. Functional experiments confirmed the pivotal role of USP7, YAP1, and β-catenin in maintaining the pro-osteogenic and anti-osteoclastogenic properties of BMSC-derived extracellular vesicles.[45]

EVs not only directly accelerate bone regeneration but also enhance bone vascularization to promote bone regeneration. In vivo experiments on ovariectomized rats demonstrated that human-induced pluripotent stem cells (hiPSCs)-MSC-EVs could significantly enhance bone regeneration and angiogenesis in critical-sized calvarial defects.[46] Further in vitro studies analyzed by western blot and quantitative real-time PCR(qRT- PCR) demonstrated that exosomes secreted by endothelial progenitor cells, in an miR-126-dependent fashion promoted endothelial cell proliferation, migration, and angiogenic potential, accelerating bone regeneration.[47] Researchers also found that serum-Exo suppressed macrophage inflammation by upregulating vascular endothelial adhesion factor 1 (VCAM1) in human umbilical vein endothelial cells (HUVEC), facilitating angiogenesis. It enhanced bone regeneration and angiogenesis and mitigated macrophage inflammation during the repair of scaffold-based bone defects. These findings suggest that autologous serum-Exo may be a potential candidate for application in the repair of scaffold-based critical-sized bone defects.[48] Researchers have identified that minute extracellular vesicles, originating from hypoxic mesenchymal stem cells facilitate the regeneration of vascularized bone. In contrast to sEVs derived from normoxic MSCs (nor-sEVs), sEVs from hypoxia-preconditioned MSCs(hypo-sEVs) facilitated the enhancement of HUVEC proliferation, migration, and angiogenesis, culminating in the augmentation of bone regeneration and neovascularization within a critical-sized calvarial

bone defect model. Sequencing of miRNAs and subsequent validation revealed an elevation of miR-210-3p in hypo-sEVs, which was mediated by HIF-1α under hypoxic conditions. The elevated expression of miR-210-3p in hypo-sEVs potentiated angiogenesis by suppressing EFNA3 expression and stimulating the phosphorylation of the PI3K/AKT pathway.[49] When assessing the outcomes of regenerative research, it is crucial to know that bone is a vascular-rich tissue, and the approaches that integrate osteogenesis and angiogenesis are likely the most effective for enhancing bone regeneration.[65]

Recently, the field of tissue engineering has concentrated on deploying specialized materials capable of selectively modulating the regional immune milieu and enhancing tissue regeneration.[66] As an efficient vector for conveying biological data, they possess lower immunogenicity and better stability than synthetic nanoparticles.[67] The combination of EVs with biomaterials holds tremendous prospects for promoting bone regeneration (Figure 3). Numerous studies have already been conducted in this field. The investigations revealed that hUC-MSCs-sEVs effectively enhanced angiogenesis and osteogenesis by delivering miR-23a-3p to activate the PTEN/AKT signaling pathway in vitro. Moreover, the BG-gel-sEV composite scaffold facilitated vascularized bone regeneration through the gradual release of sEVs.[50] Besides, EVs of high expression TIM3 with immunosuppressive properties have been utilized to modulate the early immune microenvironment in cases of bone injury, with a specific focus on macrophages. Engineered EVs with high TIM3 expression acted as mediators for the release of anti-inflammatory cytokines by inhibiting the p38/MAPK pathway. They also promoted osseointegration by activating the Bmp2 promoter to enhance BMP2 secretion in macrophages. The engineered EVs were evenly loaded into a hydrogel, enabling their continuous and slow release, which resulted in the recruitment of more anti-inflammatory macrophages during the initial stages of bone defect repair. This simultaneous regulation of the immune microenvironment served to counteract excessive inflammation and its detrimental effects.[51] Researchers have also assessed the role of intracellular communication via small extracellular vesicles (sEVs) and its impact on the endogenous bone regeneration

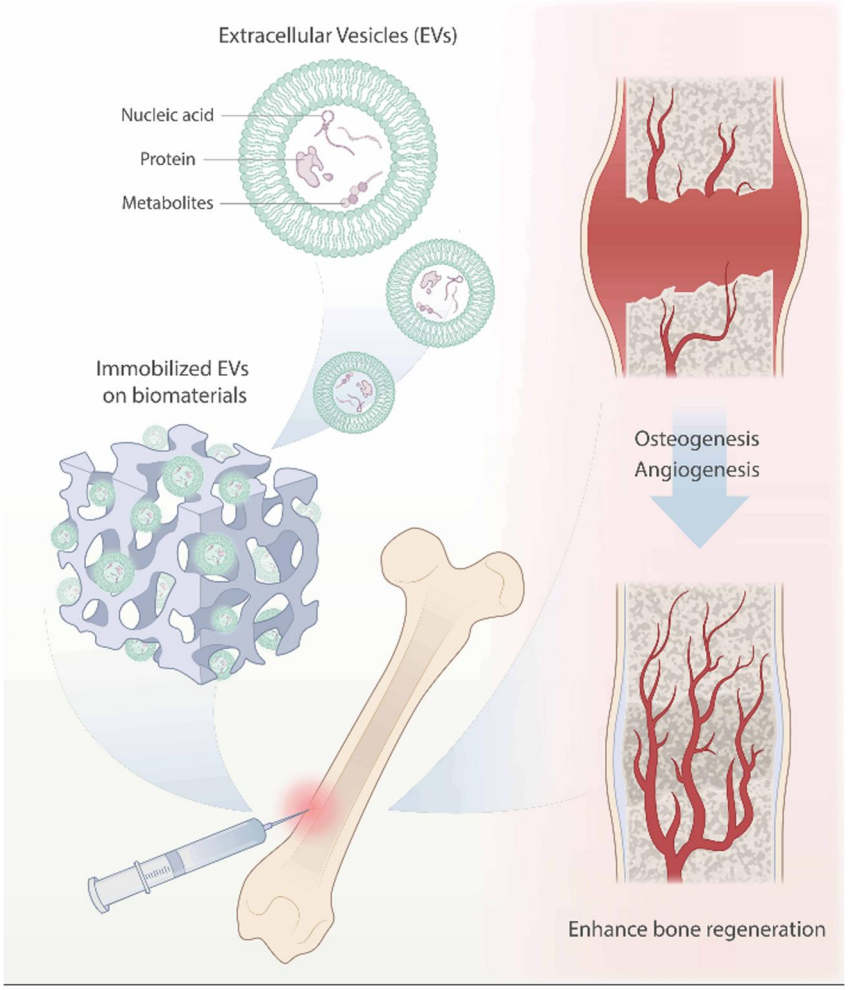

FIGURE 3 Schematic overview of EVs with biomaterials for promoting bone regeneration. When EVs are combined with biomaterials and injected, they promote bone regeneration by enhancing osteogenic gene expression and angiogenesis.

process facilitated by biomimetic intrafibrillarly mineralized collagen (IMC). In the IMC group, the inclusion of macrophage-derived sEVs resulted in an improved Young's modulus and demonstrated positive effects on the osteogenic differentiation of bone marrow MSCs. This was evident through the upregulation of important markers associated with osteoblastic differentiation, including BMP2, BGLAP, COL1, and OSX, as well as enhanced formation of calcium nodules.[52]

3.2 | EVs in the regeneration of cartilage

The application of EVs in cartilage regeneration has been widely researched. The potential roles of EVs in cartilage regeneration are discussed in both in vitro and in vivo settings.

Currently, many studies are focused on the effects of EVs derived from MSCs on cartilage repair. EVs derived from MSCs stimulate the proliferation and chondrogenic differentiation of tendon stem/progenitor cells (TSPCs) into fully developed chondrocytes, which promote cartilage recovery.[53] MSC-Exos demonstrated a protective effect by reducing IL-1β-induced inhibition of chondrocyte proliferation and preventing apoptosis. Moreover, the use of MSCs overexpressing KLF3-AS1 (a long non-coding RNA) in the production of exosomes (MSCKLF3-AS1-Exos) has shown promising results in mitigating IL-1β-induced chondrocyte injury. Mechanistically, these findings revealed that KLF3-AS1 acted as a competitive endogenous RNA (ceRNA) that sequestered miR-206, leading to an increase in GIT1 expression. Further experiments demonstrated that enhancing miR-206 expression and suppressing GIT1 expression reversed the protective effects of MSCKLF3-AS1-Exos on chondrocyte injury.[54] Treatment with MSC-miR-92a-3p-enriched exosomes (MSC-miR-92a-3p-Exos) resulted in enhanced proliferation of cartilage cells and upregulated expression of matrix genes in both MSCs and primary human chondrocytes (PHCs). Further analysis using a luciferase reporter assay revealed that miR-92a-3p directly inhibited the activity of a reporter construct containing the 3′-untranslated region (3′-UTR) and suppressed the expression of WNT5A in both MSCs and PHCs. In addition, in OA mouse model, MSC-miR-92a-3p-Exos exhibited the ability to inhibit cartilage degradation.[55] Another finding emphasized that sEVs, derived from the supernatants of KGN-preconditioned human umbilical cord mesenchymal stem cells (hUCMSCs) and isolated using gradient ultracentrifugation, were effectively internalized by native hUCMSCs, resulting in chondrogenic differentiation. Through in vitro and in vivo studies involving overexpression and inhibition, it was determined that the chondrogenesis-inducing potential of sEVs from KGN-preconditioned hUCMSCs stems primarily from the presence of miR-381-3p, which is one of the most abundant microRNAs found in sEVs. Dual-luciferase reporter assays demonstrated that miR-381-3p directly suppressed the expression of TAOK1 by targeting its 3′-untranslated region, inhibiting the Hippo signaling pathway and promoting chondrogenesis.[56] Cartilage tissue regeneration was also enhanced through an alternative Wnt signaling pathway by EVs derived from human synovial mesenchymal stem cells that overexpressed miR-140-5p.[57] Previous studies have also reported the development of EV-encapsulating gels that are suitable for injection and aimed at promoting cartilage regeneration[58] (Figure 4). Researchers have successfully produced and isolated circRNA3503-loaded sEVs (circRNA3503-OE-sEVs) from synovium mesenchymal stem cells(SMSCs) and then used poly(D,L-lactide)-b-poly(ethylene glycol)-b-poly(D,L-lactide) (PDLLA-PEG-PDLLA, PLEL) triblock copolymer gels as carriers for sEVs. Through in vivo and in vitro experiments, it has been shown that PLEL@-circRNA3503-OE-sEVs are a highly effective therapeutic strategy for preventing OA progression. This therapy involves the transfer of circRNA3503 into sEVs, which are

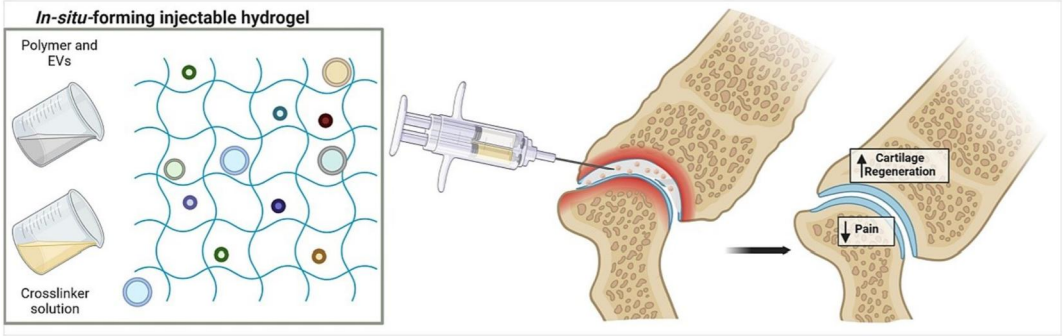

FIGURE 4 Schematic overview of the injectable EV-loaded hydrogels for cartilage regeneration in vivo. Polymer and crosslinker solutions were added together with EVs, after which they were injected and crosslinked in situ by means of a dual-chamber syringe. Reproduced with permission.[58] Copyright 2023, Elsevier.

then administered to patients to target OA progression. The therapeutic effect of PLEL@circRNA3503-OE-sEVs has been demonstrated to be superior to that of other existing therapeutic strategies for OA.[59]

Moreover, EVs produced by chondrocytes also contribute to cartilage repair. Additional investigation demonstrated that exosomes derived from chondrocytes overexpressing miR-95-5p enhance the chondrogenic differentiation of MSCs and stimulate the expression of cartilage matrix in chondrocytes. The overexpression of miR-95-5p also suppresses the expression of histone deacetylase 2/8 (HDAC2/8), which is known to be upregulated in OA.[60] miR-8485 was identified as an exosomal microRNA originating from chondrocytes and transferred to bone marrow-derived mesenchymal stem cells (BMSCs). Notably, the silencing of miR-8485 in chondrocytes significantly impaired the ability of exosomes to induce chondrogenic differentiation in BMSCs. Mechanistically, exosomal miR-8485 directly targeted GSK3B, leading to the suppression of GSK-3b expression, and also targeted DACT1, resulting in the induction of p-GSK-3b (Ser9). This activation of the Wnt/ß-catenin signaling pathway facilitated the chondrogenic differentiation of BMSCs.[61] Researchers have implanted rabbit constructs consisting of cartilage progenitor cell (CPC)-alginate subcutaneously in nude mice. Following surgery, chondrocytes-Exos were injected into the constructs at a consistent dose of 30 μg exosomes per 100 μL injection, with subsequent weekly injections administered for 12 weeks. The injections of chondrocytes-Exos enhanced collagen deposition and reduced vascular ingrowth within the engineered constructs, facilitating their efficient and consistent development into cartilage.[36]

EVs also have significant potential in the regeneration of intervertebral disc degeneration. Liao et al. discovered that EV transport antioxidant proteins to protect nucleus pulposus cells (NPCs) against pyroptosis. The therapeutic effect of EVs was reduced in TNF-α-treated NPCs, which was attributed to the impaired caveolae-mediated endocytosis pathway. Transcriptome sequencing and functional verification revealed the important role of caveolae-associated protein 2 (Cavin-2) in the uptake process of EVs. EVs modified with Cavin-2 by gene editing parental MSCs have been engineered. These modified EVs exhibited a higher uptake rate in TNF-α-treated NPCs, effectively mitigating NPC death in a three-dimensional hydrogel culture model and slowing the progression of intervertebral disc degeneration (IDD) in the ex vivo organ culture model.[62] Exosomes exert an anti-inflammatory effect on pathological nucleus pulposus cells (NPCs) by inhibiting inflammatory mediators and NLRP3 inflammasome activation. Exosomes may also provide mitochondrial proteins to NPCs, potentially restoring damaged mitochondria. In a rabbit IDD model, exosomes notably halted the advancement of degenerative changes.[63]

Collectively, these findings suggest that EVs derived from stem cells, milk, or different types of cells can stimulate the migration, proliferation, and differentiation of both transplanted and native stem/progenitor cells. Moreover, they enhance the regeneration of bone and cartilage, highlighting the potential of EVs in the cell-free treatment of cartilage defects.[68]

4 | EV-BASED TARGETING DRUG DELIVERY

There is an increasing need for further research, preventative measures, nutritional support, and mental health assistance to enhance the quality of life of individuals suffering from orthopedic diseases. The treatment of multiple underlying pathologies associated with orthopedic diseases often cannot be effectively addressed solely through conventional conservative treatments or surgical interventions.[34] The application of bioengineering in treating musculoskeletal disorders has recently gained considerable attention, especially in EV research. EVs play a crucial role in facilitating intercellular communication. They allow donor cells to transfer exogenous substances, including proteins, mRNAs, microRNAs (miRNAs), and lipids, to recipient cells. These naturally occurring nanocarriers, modified by genetic engineering or surface chemistry approaches, have been used for drug delivery.[69] A comprehensive overview of various parameters, such as payloads, drug loading strategies, EV surface modifications, administration routes, animal models, and disease indications are illustrated in Figure 5.[14]

The advantage of using extracellular vesicles (EVs) as drug delivery systems is multi-faceted. Firstly, EVs derived from patients' cells exhibit superior biocompatibility and reduced toxicity compared to synthetic drug carriers.[70] These characteristics increase their compatibility with the human body and reduce the risk of adverse reactions. To mitigate immunogenicity and prevent rapid clearance of EVs from the bloodstream, polyethylene glycol (PEG) surface-coating is commonly employed, resulting in enhanced accumulation within the desired tissue.[71] Moreover, an intriguing strategy entails the preferential selection of EV subsets harboring specific surface proteins, such as CD47, which serves as a 'do not eat me signal' and enables EVs to evade the mononuclear phagocyte system, thereby prolonging their circulation time.[72] Secondly, these EVs demonstrate the ability to permeate tissues, diffuse within the bloodstream, and

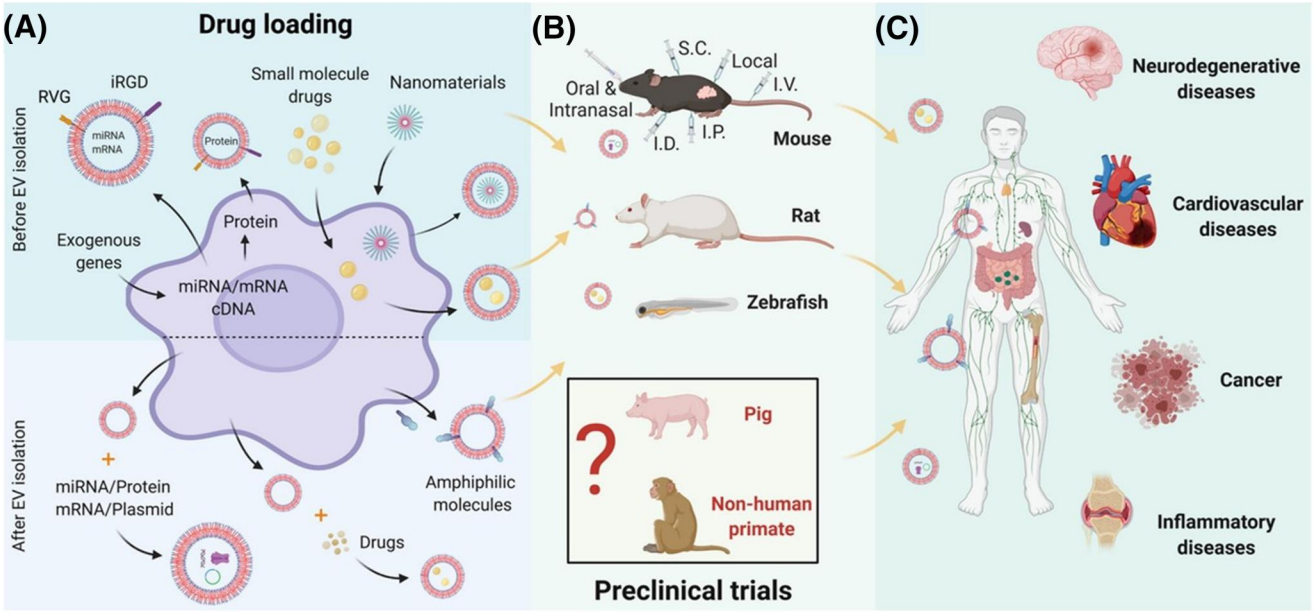

FIGURE 5 Schematic overview of EVs as drug delivery systems in preclinical animal models. (A) General simplification of EV contents, drug loading procedures, and surface ligand incorporation before or after EV isolation. (B) Various animal models and administration routes for preclinical testing of EVs for drug delivery. (C) Examples of disease indications for drug delivery via EVs. Reproduced with permission.[14] Copyright 2021, Elsevier.

traverse the blood-brain barrier.[70] Therefore, this characteristic makes targeted drug delivery via extracellular vesicles particularly useful for targeting many areas involved in orthopedic diseases. Thirdly, EV-mediated delivery provides a mechanism to overcome drug resistance by circumventing the P-glycoprotein drug efflux system.[73] This system often limits the effectiveness of conventional drug delivery methods. By utilizing EVs, drugs can reach their target cells more efficiently. Lastly, compared to other methods, engineered EVs can efficiently target and deliver drugs to specific cells.[74] This level of precision allows for targeted therapy, reducing potential side effects and enhancing treatment effectiveness. Therefore, using EVs as drug delivery systems offers several advantages, including their natural ability to facilitate communication between cells, superior biocompatibility, tissue permeation, circumvention of drug resistance mechanisms, and targeted delivery. These advantages make EVs a prominent area of research in the treatment of orthopedic diseases (Table 3).

4.1 | EV-based targeting drug delivery in joint diseases

Tissue-targeting EVs have been extensively studied in joint diseases. In the 2020s, Liang et al. successfully generated chondrocyte-affinity peptide (CAP)-exosomes by fusing the CAP with glycoprotein 2b, a membrane protein associated with lysosomes, on the surface of exosomes. These exosomes efficiently encapsulate miR-140. In a rat model, the CAP-exosomes transported miR-140 to the innermost layers of the cartilage by penetrating the thick mesochondrium. This inhibited the proteases responsible for cartilage degradation and subsequently provided relief from the progression of OA. These encouraging findings highlight the potential of utilizing organelle-based, cell-free therapy as a potential treatment approach for OA.[75] Zhao et al. also transfected CAP to the MSCs and produced CAP-labeled exosomes (CAP-MSCsSC-Exos). The MSCsSC-Exos could enhance the restoration of damaged cartilage in an OA model by elevating the autophagy levels, primarily through exosomal miR-199a-3p.[76] Cao et al. formulated a chondrocyte-targeting polymer that effectively links a CAP and a cholesterol group at both extremities of the PEG chain. The rejuvenating impact of UCMSC-EXOs on OA chondrocytes and the possibility of integrating with chondrocyte-targeted and sustained-release approaches for a forthcoming cell-free OA therapy have also been emphasized.[84] In addition to CAP, researchers have also invented a hydrogel that can target the cartilage. The novel photo-crosslinking spherical gelatin methacryloyl hydrogel (GelMA)-encapsulated cartilage affinity WYRGRL (W) peptide-modified engineered exosomes (Exo) were developed for treating OA. The performance of the engineered exosomes loaded with a small inhibitor LRRK2-IN-1 (W-Exo-L@GelMA) was assessed both in

TABLE 3 EV-based targeting drug delivery.

Disease types	Source	Surface modifications	Target	CArgos	Working mechanisms	References
OA	Dendritic cells	Chondrocyte-affinity peptide	Chondrocyte	miR-140	Inhibiting the proteases responsible for cartilage degradation and subsequently provided relief from the progression of OA	75
OA	Subcutaneous fat stromal cells	Chondrocyte-affinity peptide	Chondrocyte	miR-199a-3p	Enhancing the restoration of damaged cartilage in an OA model by elevating the autophagy levels	76
OA	Bone marrow mesenchymal stromal cell	Collagen II-targeting peptide	Chondrocyte	LRRK2-IN-1	Inhibiting of OA-related inflammation and immune gene expression	77
RA	Neutrophils	Oxidants-specific antibody	Arthritic joint	IL-10 and anti-TNF	Accelerating the reduction of clinical and synovial inflammation	78
RA	RAW 264.7	Folic acid-polyethylene glycol-cholesterol	Arthritic joint	Dexamethasone sodium phosphate	Suppressing the pro-inflammatory cytokines and increasing the production of anti-inflammatory cytokines	79
RA	M2 macrophages	None	Activated macrophages	IL-10 and the betamethasone sodium phosphate.	Reducing the secretion of pro-inflammatory cytokines (IL-1β, TNF-α) and increasing the expression of IL-10 cytokine	80
Osteoporosis	Endothelial cell	None	Bone	miR-155	Inhibiting osteoclast activity and enhancing osteoporosis recovery	81
Osteoporosis	Red blood cell	TBP-CP05	Osteoclasts	Anti-miR-214	Reducing osteoclast activity, enhancing osteoblast activity, and improving bone density	82
Osteoporosis	Platelet	Alendronate	Bone	Platelet-derived growth factors	Influencing the osteogenic differentiation of bone marrow mesenchymal stem cells and the angiogenic differentiation of endothelial progenitor cells	83

vitro and in vivo. The W-Exo-L@GelMA demonstrates effective targeting of chondrocytes and a significant impact on suppressing catabolism and promoting anabolism in vitro. It exhibits notable inhibition of OA-related inflammation and immune gene expression, rescuing the transcriptomic responses induced by IL-1β. In the OA murine model, W-Exo-L@GelMA displayed superior anti-OA activity and cartilage repair ability thanks to its enhanced retention in the joint. These therapeutic effects have been validated in cultured human OA cartilage.[77]

Tissue-targeting EVs have also been designed for RA. Topping et al. identified an antibody specifically targeting damaged arthritic cartilage (anti-ROS-CII) that allows for the targeted delivery of treatments exclusively to arthritic joints, resulting in effectiveness in experimental arthritis. To deliver targeted anti-inflammatory treatments to arthritic joints, they used EVs derived from human neutrophils (PMNs) known for their intrinsic anti-inflammatory properties and ability to penetrate the inflamed arthritic cartilage. They fortified the EVs with

anti-ROS-CII (EV/anti-ROS-CII) to ensure specific binding and retention in the damaged cartilage. In vivo systemic administration of EV/anti-ROS-CII demonstrated two significant findings: (a) specific localization within the arthritic joint and (b) the ability to selectively target single (viral IL-10 or anti-TNF) or combination (viral IL-10 and anti-TNF) anti-inflammatory treatments to the arthritic joint, effectively accelerating the reduction of clinical and synovial inflammation.[78] Activated macrophages express folic acid receptors in RA. Therefore, researchers successfully developed an active targeting drug delivery system consisting of a biomimetic exosome (Exo) enclosing dexamethasone sodium phosphate (Dex) nanoparticles (Exo/Dex). They modified the surface with a folic acid (FA)-polyethylene glycol (PEG)-cholesterol (Chol) compound. The in vitro investigation demonstrated that this system exhibited improved endocytosis and remarkable anti-inflammatory effects on RAW264.7 cells. These effects were achieved by suppressing the pro-inflammatory cytokines and increasing the production of anti-inflammatory cytokines. The in vivo biodistribution experiment revealed that the FPC-Exo/Dex targeted drug delivery system resulted in good bone and cartridge preservation in the collagen-induced arthritis (CIA) mice and significantly reduced joint inflammation. Subsequent in vivo safety assessment demonstrated that this biomimetic drug delivery system demonstrated no noticeable hepatotoxicity and displayed excellent biocompatibility.[79] Furthermore, as previous studies indicated that M2 Exo could be captured by activated macrophages,[85] Li et al. loaded biomimetic vector M2 exosomes (M2 Exo) derived from M2-type macrophages with a plasmid DNA encoding the anti-inflammatory cytokine IL-10 (IL-10 pDNA) and the chemotherapeutic drug betamethasone sodium phosphate (BSP). The exosomes loaded with IL-10 pDNA and BSP efficiently targeted and mitigated inflammation. These findings offer a promising approach for combined therapy against RA by diminishing the production of pro-inflammatory cytokines (such as IL-1β and TNF-α) and enhancing the expression of IL-10.[80]

4.2 | EV-based targeting for drug delivery in bone diseases

Researchers have also focused on the targeted delivery of EVs to promote bone mass increase. Song et al. found that endothelial cell (EC)-secreted Exos (EC-Exos) exhibits a specific affinity toward bone tissue and enhances osteoporosis recovery both in vitro and in vivo, without any toxicity, utilizing the delivery of miR-155.[81] Researchers have reported a novel approach for the targeted delivery of anti-miR-214 to osteoclasts using red blood cell EVs (RBCEVs) as the carrier. The delivery strategy is guided by a bi-functional peptide called TBP-CP05, which can bind to CD63 on RBCEVs and receptors on osteoclasts. TBP-CP05 facilitates RBCEV binding through CP05, which in turn displays the TRAP-binding peptide (TBP) on the surface of RBCEVs. This unique design confers RBCEVs with the ability to specifically target osteoclasts, both in vitro and in vivo. The administration of osteoclast-targeting RBCEVs (OT-RBCEVs) via intravenous injection resulted in the accumulation of EVs in the skeletal system, leading to a substantial reduction in osteoclast activity, enhanced osteoblast activity, and improved bone density in osteoporotic mice.[82] Zheng et al. isolated exosomes derived from platelet lysate (PL-Exo) to concentrate platelet-derived growth factors (GFs). The PL-Exo was then conjugated with alendronate (ALN) grafted PEGylated phospholipid (DSPE-PEG-ALN) to create bone-targeting PL-Exo (PL-Exo-ALN). The modification with ALN greatly enhanced the hydroxyapatite binding affinity of PL-Exo in vitro and the aggregation of PL-Exo at the bone site in vivo. In addition to directly influencing the osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) and the angiogenic differentiation of endothelial progenitor cells, PL-Exo-ALN also facilitated their interaction under the stimulation of glucocorticoids (GCs). Additionally, intravenous injection of PL-Exo-ALN has successfully ameliorated glucocorticoid-induced osteoporosis (GIOP) in animal models.[83]

5 | CHALLENGES OF EVS IN CLINICAL TRANSLATION

While EVs hold tremendous potential in diagnosing and monitoring orthopedic diseases, there are also significant challenges to address. For instance, the selection of biomarkers, standardization of analysis methods, and the need for large-scale validation studies are all hurdles that must be overcome. Absence of standardized methods for purification hinders reproducibility and significantly impacts the identification of biomarkers. This lack of consensus has resulted in the absence of specialized EVbiobanks, which could allow for the correlation of specific biofluid/biopsy samples with the patient's medical record.[86] In addition to the purification protocol, other factors such as the sample collection procedure, processing time, and sample storage conditions can also affect the outcome of biomarker identification studies.[87] The complexity in the pathophysiology of some

orthopedic diseases also limits the sensitivity of a single biomarker, making it necessary to use multiple complementary diagnostic methods. Interdisciplinary collaboration will contribute to advancing this field and offer advantages for the early diagnosis of orthopedic diseases. Establishing standardized protocols for validating EV biomarkers in different laboratories and samples is essential for expediting the clinical application of EVs as markers. Therefore, the direction of future research may encompass further exploration of more reliable EVs as biomarkers for orthopedic diseases and the development of more accurate and dependable diagnostic and monitoring methods.

Even though previous clinical studies have shown promising results regarding the therapeutic effects of EVs on cartilage and bone regeneration, several challenges need to be addressed in order to translate these findings into clinical applications. Firstly, it is important to consider the therapeutic efficacy and safety of different cargo types and optimal dosages when utilizing EVs as a therapeutic agent. Using rotational thromboelastometry and thrombodynamic tests, Silachev et al. unveiled the robust procoagulant properties of MSCs on human blood and platelet-free plasma. Remarkably, a comparable enhancement of clotting was observed in the case of EVs derived from MSCs. Furthermore, the investigation identified the presence of annexin V on certain MSCs and EVs, indicating the existence of phosphatidylserine on their surfaces. This characteristic holds the potential to augment clot formation.[88] The specific cargo contents (proteins, RNA, and DNA) responsible for the observed therapeutic effects are still not completely understood. Thus, efforts in future studies should focus on improving the effectiveness and safety of EVs and investigating the specific therapeutic effects of the cargo contents within these EVs.

Although EV-based targeted delivery has shown significant potential in skeletal muscle therapeutics, several challenges still need to be addressed. Firstly, maintaining the homogeneity of EVs represents a significant challenge. Due to the early stage of EV research, the lack of fundamental biological understanding restricts the available methodological approaches to effectively isolate uniform EV populations for targeted drug delivery.[89] Physical characteristics such as size and density are commonly employed for EV separation and isolation, requiring techniques such as size-exclusion chromatography and ultracentrifugation. However, given that EVs range in size from 30 to 2000 nm, obtaining a consistently homogeneous EV population based on physical parameters alone is extremely challenging. In addition, the significant variation in the biological composition of EV membranes (including proteins and lipids) further introduces unpredictable factors to consider in the context of targeted drug delivery.[90] Secondly, the efficient loading of drugs into MSC-derived EVs poses a challenge for their use as drug delivery vehicles. For example, the electrostatic repulsion resulting from the negative charge of nucleic acids and the negative charge of the EV membrane presents significant obstacles in achieving efficient nucleic acid loading into EVs. Direct loading of nucleic acids into EVs using electroporation causes nucleic acids to become insoluble and precipitate. A study conducted by Kooijmans et al observed that even in the absence of EVs, substantial amounts of electroporated siRNA with a size of 100 nm persisted as aggregates after isolation through ultracentrifugation.[91] Although the efficient loading of drugs into MSC-derived EVs poses a challenge, the field is actively exploring strategies and techniques to overcome this hurdle. The development of techniques, such as electroporation, sonication, and extrusion, can enhance the drug loading efficiency by promoting the permeability of EV membranes and facilitating drug entry into the vesicles. Thirdly, surface engineering, which confers cell-type targeting specificity, has yet to be thoroughly investigated. Despite its promising prospects, comprehensive exploration and understanding of surface engineering techniques for EVs are still lacking. Various factors, such as the choice of targeting ligands, the methods of surface modification, and the impact of these modifications on EV stability and cargo integrity, need to be thoroughly investigated. Furthermore, it is important to consider the challenges associated with the large-scale production and clinical translation of surface-engineered EVs. The scalability and reproducibility of manufacturing methods need to be evaluated for successful implementation in therapeutic settings. Future studies should focus on developing techniques that can effectively load sufficient amount of drugs into sEVs without compromising physical integrity or biological activity. Extensive studies on the surface modification of EVs for targeting should be conducted.

6 | CONCLUSION AND OUTLOOK

EVs have emerged as a prominent focus of research in orthopedics, garnering extensive and in-depth investigation, especially in the diagnosis, regenerative fields, and treatment. EVs derived from blood, synovial fluid, or various other cells have demonstrated significant promise as biomarkers. EVs play a crucial role in the early detection of orthopedic diseases. They also possess predictive capabilities in identifying individuals with a

heightened susceptibility to developing orthopedic diseases. Numerous studies have also highlighted the potential role of exosomes in promoting bone and cartilage regeneration. Exosomes derived from stem cells, milk, or different types of cells are able to stimulate osteogenic differentiation and the differentiation of progenitor and stem cells into mature chondrocytes in vitro. These exosomes have shown their capability in vivo to induce osteogenic differentiation and the migration, proliferation, and differentiation of endogenous stem/progenitor cells into chondrocytes. Surface engineering techniques have been employed to enhance the accumulation of EVs at the site of disease, minimizing toxicity and adverse effects while optimizing therapeutic efficacy. EV-mediated delivery provides a mechanism to overcome drug resistance.

Significant challenges must be addressed in order to advance the development of EVs in the diagnosis of orthopedic diseases. These challenges include enhancing yield and purity, reducing costs, simplifying procedures, and establishing standardization protocols. The aspiration is to obviate the necessity for EV isolation prior to analytical assessment, thereby pursuing a more streamlined and cost-effective integrated approach. Innovative strategies have emerged, paving the way for detecting EVs from minuscule clinical specimens. Electrochemical biosensor platforms, exemplified by the iMEX system, have demonstrated the capability of quantifying EVs from microliter volumes of unaltered plasma, heralding the potential for liquid biopsy to become a routine-of-care diagnostic in orthopedic diseases and a valuable tool for monitoring therapeutic response.[92] The presence of EVs in various body fluids, particularly urine and saliva, underscores the promise of noninvasive EV-based liquid biopsies for precision medicine.

The role of EVs in future therapeutic interventions for the regeneration of bone and cartilage is anticipated to be significant, especially considering them as a straightforward and secure substitute for the existing cellular-based treatment modalities. Moreover, the concomitant use of EVs with scaffolds, either by adsorption on the scaffold's surface or incorporation within the scaffold's matrix, would facilitate the modulated release of defined EV subsets. Bioengineering strategies could potentially refine the targeting precision of EV delivery by means of three-dimensional fabrication of structured tissue constructs.[19] Investigations into the biomaterial-mediated release of meticulously characterized EV populations are still in their preliminary stages. Yet, it promises substantial utility. Nonetheless, the efficacy of EVs in therapy necessitates the implementation of rigorous isolation and analytical protocols to ensure that the production of EV preparations is reliable and replicable. Safety assurance during its application is also of paramount importance. Additional and reliable research should be conducted on the side effects of EVs during therapy.

EVs present a potential strategy for delivering diverse drugs in various applications. Systematically administering EVs to rodents has shown reduced immune clearance and efficient transport of functional cargo compared to conventional delivery methods.[93] Nevertheless, to adequately evaluate the risk-benefit ratio, further investigation in clinically relevant systems and direct quantitative comparisons with liposome-based alternatives are necessary. For the successful translation of EVs, it is crucial to establish cost-effective large-scale production methods and efficient isolation and characterization techniques that exhibit high sensitivity to detecting batch-to-batch variations and the subsequent biological implications. Developing widely applicable drug-loading methods is also pivotal to ensure broad applicability.[94]

In summary, EVs have been extensively studied and proven effective in diagnosis, regenerative therapy, and targeted drug delivery. With further development, EVs have tremendous potential in the clinical applications in orthopedics.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interests.

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