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REVIEW

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Mesenchymal stem cell-derived extracellular vesicles in musculoskeletal regeneration: mechanisms, applications, and future prospects

Fatemeh Aziziyan[1], Shiva Sarani Asl[2], Mohammadreza Mahdipour[3], Rahil Nasari Fard[3] and Mohsen Sheykhhasan[4*]

Abstract

Mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) have emerged as promising cell-free therapeutic strategies for musculoskeletal regeneration. MSC-EVs, which are enriched with diverse cargos, exert multifaceted biological effects, including the modulation of inflammation, the promotion of angiogenesis, and the regulation of immune responses. They also activate key regenerative signaling pathways, such as the PI3K/Akt, Wnt/β-catenin, TGF-β/Smad, and NF-κB pathways, thereby promoting osteogenesis, chondrogenesis, tenogenesis, and muscle repair to support the repair of bone, cartilage, tendon, and muscle tissues. In addition to their intrinsic activity, advances in bioengineering, including surface modification, cargo engineering, and integration with biomaterial scaffolds, have further increased their therapeutic potential and delivery. Preclinical studies consistently demonstrate efficacy across diverse musculoskeletal tissues, and early clinical trials highlight their translational promise. Nevertheless, clinical application remains constrained by challenges in large-scale production, standardization, and long-term safety evaluation. This review summarizes current knowledge on the mechanisms, therapeutic applications, engineering strategies, delivery systems, and clinical progress of the use of MSC-EVs in musculoskeletal regeneration while highlighting critical obstacles and future directions for their clinical implementation.

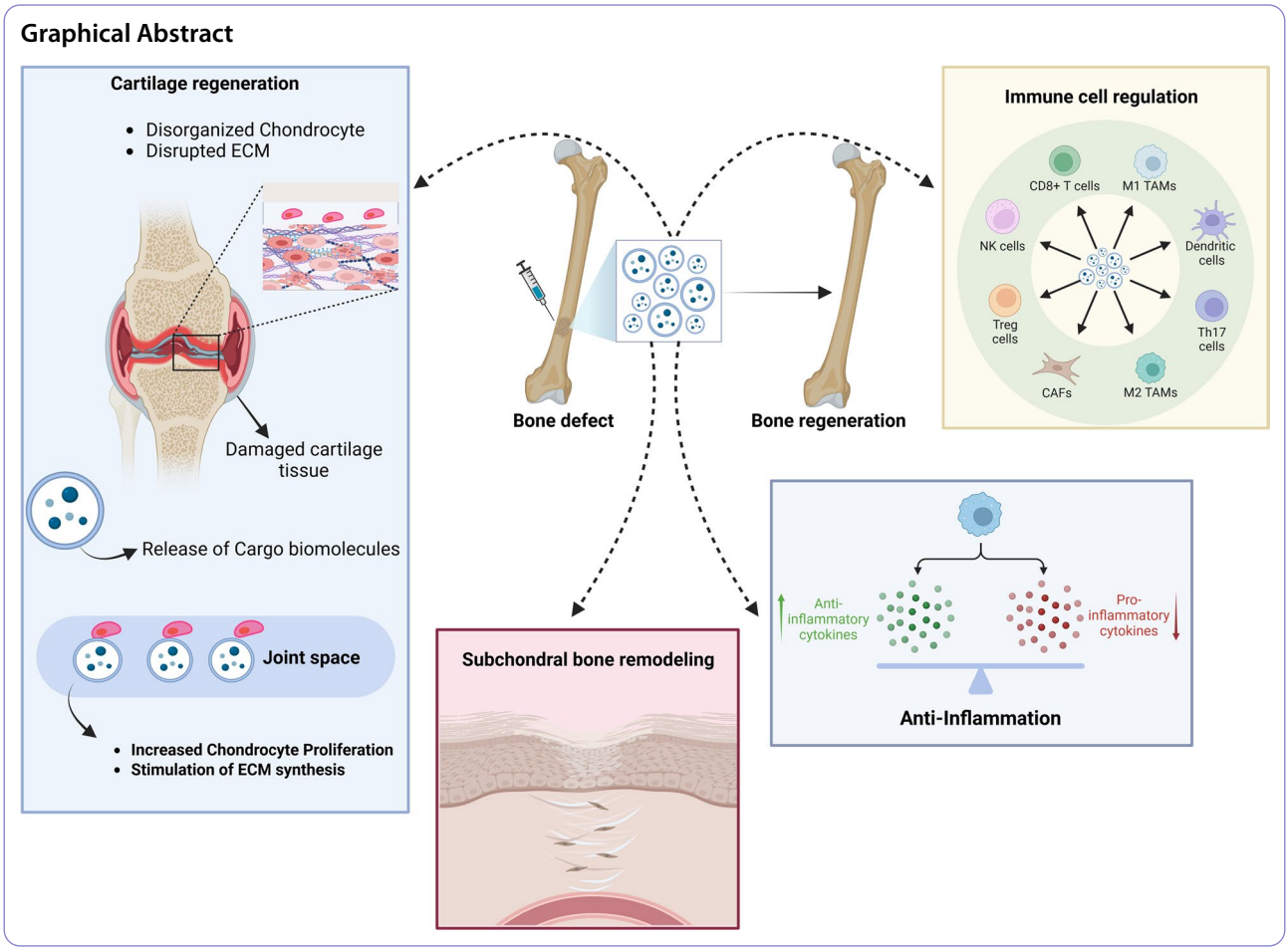
Keywords MSC-EVs, Musculoskeletal regeneration, Bioengineering, Therapeutic applications, Clinical translation

Introduction

The musculoskeletal system plays a critical role in maintaining bone and muscle homeostasis. Imbalances in this system can lead to a wide range of musculoskeletal disorders (MSDs) [1]. MSDs impair mobility due to injury or pain in musculoskeletal tissues such as muscles, bones, and joints. Common disorders include osteoarthritis (OA), rheumatoid arthritis (RA), neck pain, tendinitis, fibromyalgia, carpal tunnel syndrome, and bone fractures [2]. Major risk factors for MSDs include occupation, lifestyle, and family history, with age being a particularly significant contributor [3]. As the population ages and life expectancy increases, the prevalence of MSDs continues to rise. Current treatment options, such as mechanical implants and grafting procedures, have notable limitations, including postsurgical complications, limited availability of graft materials, risk of graft rejection, and long-term dependence on mechanical devices. These challenges underscore the urgent need for novel

*Correspondence:
Mohsen Sheykhhasan
msheykhhasan@muq.ac.ir

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



therapeutic strategies to promote musculoskeletal regeneration [4].

Regenerative medicine is a transdisciplinary strategy aimed at restoring complex tissues and organs affected by aging, disease, or injury. Mesenchymal stem cells (MSCs) and their derived extracellular vesicles (MSC-EVs) play a significant role in regenerative medicine approaches for musculoskeletal regeneration. MSCs demonstrate immunomodulatory activity, enhance tissue regeneration, and have high accessibility and multipotent differentiation ability [4, 5].

MSCs influence target cells through two primary mechanisms. Initially, it was assumed that MSCs induce regeneration by migrating to damaged sites, engrafting, and differentiating into specialized cell types, namely, direct action [6]. However, recent evidence suggests that the therapeutic properties of MSCs primarily arise from soluble factors and paracrine signaling mediated by extracellular vesicles (EVs) [7], which are lipid bilayer particles that carry a specific biological cargo [8]. EVs are nanosized EVs that originate from the endosomal pathway and are abundantly secreted by MSCs. Recent studies have indicated that EVs serve as the primary paracrine mediators of MSCs and efficiently reflect parent cell properties, including immunomodulatory, anti-inflammatory, angiogenic and tissue repair functions [4, 9–11].

MSC-EVs overcome the challenges of whole-cell transplantation by exhibiting lower immunogenicity and toxicity. They can also cross biological barriers and offer high circulation stability, more stable storage, and scalability [2, 10]. These advantages position MSC-EVs as promising cell-free alternatives for musculoskeletal regeneration [4].

MSC-EVs play crucial roles in bone, cartilage, tendon, and ligament repair through their regenerative properties [4, 12, 13]. They promote osteogenesis and angiogenesis and support osteoblast proliferation and differentiation, suggesting promising therapeutic effects in osteoporosis (OP) and bone fractures [14]. MSC-EVs aid in cartilage repair and alleviate osteoarthritic changes by enhancing matrix synthesis, exerting anti-inflammatory effects, providing chondroprotection, and promoting chondrocyte proliferation [15]. They contribute to tendon and ligament regeneration by promoting tenogenic differentiation, proliferation, and migration; remodeling the extracellular matrix (ECM); enhancing collagen production and organization; and modulating immune responses

[4, 13]. Together, this evidence supports the promising application of MSC-EVs in musculoskeletal regeneration [4].

It should also be considered that EVs are not always pro-regenerative; they can act as 'bad actors', promoting tissue degeneration and exacerbating disorders such as OA and RA. This dark side of EVs underscores their dual role. The pathogenic effects of EVs are mediated mainly through dysregulation of the local niche via intercellular signaling. For example, synovial fibroblast (SF)-derived EVs trigger the release of proinflammatory cytokines from macrophages while inhibiting the production of anti-inflammatory cytokines, ultimately leading to joint inflammation and cartilage destruction. These EVs also promote ECM degradation via the upregulation of matrix metalloproteinases (MMPs) and stimulate angiogenesis, processes closely associated with OA. Moreover, endothelial cell-derived EVs impair the antioxidative defense mechanisms of chondrocytes and increase intracellular ROS levels, leading to the activation of apoptotic pathways [16]. In addition, osteoblast-derived EVs likewise upregulate ECM catabolic enzymes in chondrocytes [17]. In addition, osteoarthritic chondrocytes produce Cx43 (connexin 43)-enriched EVs that promote senescence and inflammation in bone, cartilage, and synovial tissue [18]. Similarly, in RA, EVs promote inflammation, angiogenesis, and autoimmunity, thereby contributing to disease progression [19]. In addition to OA and RA, studies have shown that aged bone marrow–derived mesenchymal stem cell (BMSC)-derived EVs suppress osteogenesis (via the SATB2 and E2F2 pathways) and enhance osteoclastic differentiation (via the RhoA pathway) through their miR-31a-5p cargo [20]. Furthermore, ligament-derived EVs contribute to pathological new bone formation; IL-17A carried in ligament-derived EVs upregulates MMP14 expression in ankylosing spondylitis ligaments through activation of the JAK-STAT3 pathway, leading to cytoskeletal and ECM alterations and aberrant bone formation [21]. Taken together, EVs play a critical role in the pathogenesis of musculoskeletal disorders, a role that remains underexplored despite their importance for understanding disease mechanisms and improving diagnosis [17, 22].

Several recent reviews have summarized the therapeutic potential of MSC-EVs in musculoskeletal diseases and regeneration [23, 24]. While recent reviews have provided broad and valuable overviews of MSC-EVs for musculoskeletal applications, including EV biology, cargo characteristics, therapeutic potential, and clinical outlook [1, 4, 13], few offer an integrated assessment of the current state of the art, which spans EV mechanistic biology, engineering and cargo-modification strategies, advanced biomaterial-assisted delivery platforms, and translational progress. This review consolidates these domains to provide a comprehensive and engineering-oriented perspective. This review bridges mechanistic insights with engineering innovations and clinical translation, thereby providing a broader and more forward-looking framework than existing reviews do. Furthermore, this review provides a comprehensive and integrative perspective by addressing the signaling pathways through which MSC-EVs mediate musculoskeletal repair; discussing advances in engineering strategies to enhance therapeutic efficacy; evaluating translational progress alongside current challenges in large-scale production, standardization, and safety; and outlining future perspectives for clinical implementation.

Mechanisms of MSC-EVs in Musculoskeletal Regeneration

EVs, which are lipid bilayer particles secreted by all cell types, play crucial roles in cell–cell communication and biological regulation [25]. EVs are carriers of proteins, nucleic acids, and lipids and influence key biological functions, such as immunomodulation, inflammation, and angiogenesis [2]. Among various EV subtypes, MSC-EVs are a well-known class of EVs used in regenerative medicine and mirror the functional properties of their parent cells [5, 9]. MSC-EVs exhibit angiogenic, osteogenic, immunomodulatory, and cellular regulatory properties that support musculoskeletal regeneration [12, 13]. This section summarizes the underlying biological mechanisms, key regulatory pathways, and EV-mediated cell–cell communication involved.

Biological mechanisms

Cargo composition in tissue repair.

MSC-EVs regulate cellular processes and tissue repair through their bioactive regulatory factors, such as miRNAs, mRNAs, and mtDNA [5, 10, 26]. They also contain more than 41,860 types of proteins, 7540 RNAs, and 1116 lipid molecules, contributing to intercellular communication [27]. The miRNAs from MSC-EVs can be transferred to recipient cells and impact their function [10, 28]. The exosomal miRNA profile is critically regulated by microenvironmental conditions and stress, enabling the host cell to generate diverse miRNAs in response to tissue repair [10].

In addition to miRNAs, MSC-EVs can transport significant quantities of proteins to target cells. Over 1000 proteins, including enzyme complexes (such as glycolysis-associated enzymes) and signaling molecules (cytokines, interleukins, chemokines, and growth factors), have been characterized in MSC-EVs. This proteome plays an essential role in cellular communication and tissue repair [10, 11, 29].

MSC-EVs also transport various functional lipids and lipid metabolism-related enzymes, which are crucial

for EV biogenesis, stability, uptake, and fate. They modify their lipid composition to modulate their function. Although the biological effects of MSC exosomal lipids are unclear, their lipid content can regulate target cell lipid metabolism and activate molecular pathways [10, 30]. However, exosomal lipids and associated enzymes have been implicated in modulating inflammation and cellular signaling, processes central to tissue repair [31].

Anti-inflammatory, proangiogenic, and immunomodulatory effects.

As previously mentioned, MSC-EVs carry a specific cargo, which endows them with anti-inflammatory, immunomodulatory, and proangiogenic properties [2, 28]. The immune system influences bone homeostasis and the pathogenesis of MSDs, leading to a new paradigm known as "osteoimmunology" [32]. MSC-EVs influence the immune system through diverse mechanisms, as illustrated in Fig. 1. They suppress immune system overactivity by subverting M1 macrophage activity and shifting them toward regulatory M2 macrophages, lowering inflammatory $CD4^+$ Th1 and Th17 lymphocyte production, decreasing the antigen-presenting capacity of dendritic cells (DCs), and inducing immunosuppressive Treg cells and tolerogenic DCs [6, 33–35]. This capacity to modulate immune cells, particularly macrophages, remains a central focus of therapeutic development, as confirmed by recent investigations [36]. They also reduce the expression of inflammatory cytokines such as TNF-a, IL-1b, and IL-6 and increase the expression of anti-inflammatory cytokines and Arginase-1, which are macrophage regulators [4, 5]. Under inflammatory conditions, MSC-EVs carry specific miRNAs, such as miR-21 [37], miR-223 [38], miR-181c [39], and miRNA-34a-5p [40], which regulate inflammation and induce tissue repair [41]. Moreover, studies have demonstrated that immunomodulatory lipids and metabolites in MSC-EVs enhance their anti-inflammatory effects [30].

Proangiogenic approaches play crucial roles in musculoskeletal regeneration, with both myogenesis and

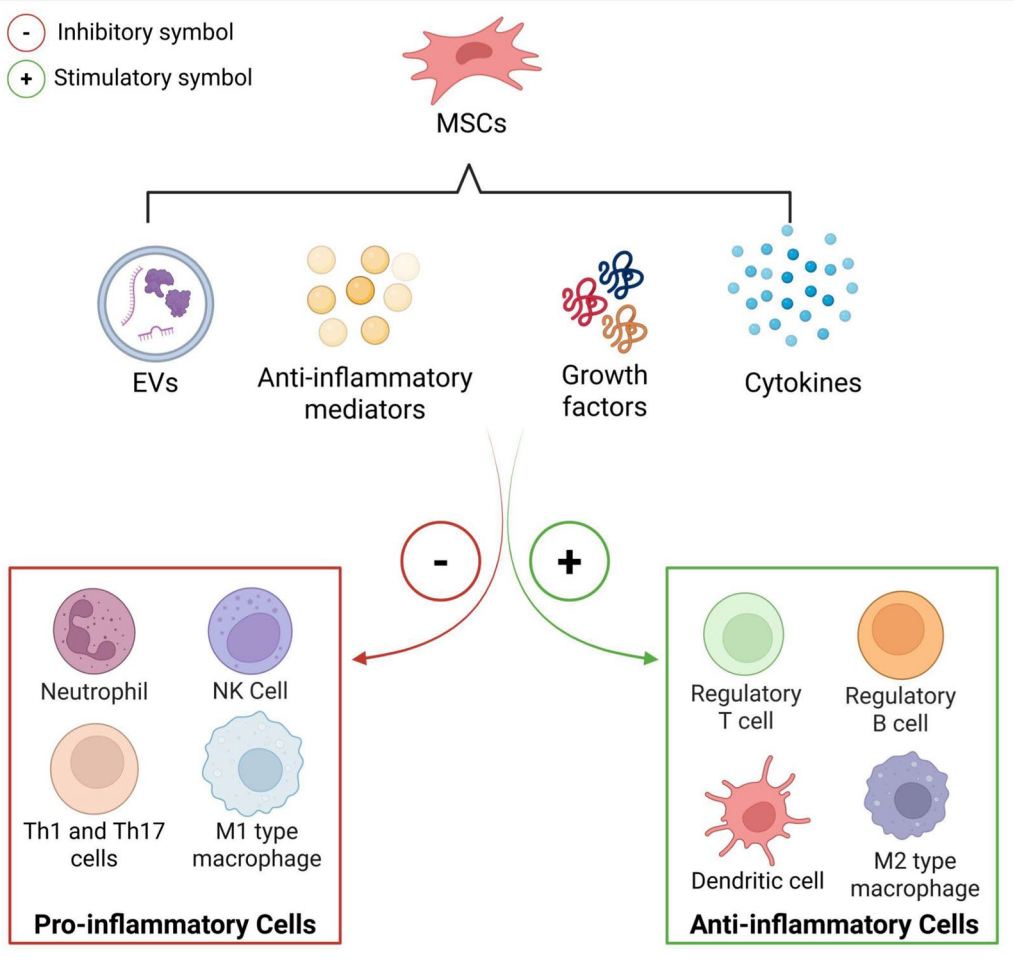


Fig. 1 MSCs regulate the immune system and suppress inflammation at injury sites through their secretome, which includes soluble factors and EVs. They inhibit the activity of proinflammatory cells while promoting the function of anti-inflammatory cells [1]. Created in BioRender. Aziziyan, F. (2025) https://BioRender.com/yuyep0h

angiogenesis. Fibrosis formation and insufficient myofiber repair render musculoskeletal regeneration incomplete, establishing angiogenesis as a critical prerequisite. Many studies have explored the angiogenic potential of MSC-EVs and their miRNAs for the treatment of MSDs and other diseases. These particles induce angiogenesis-associated tissue regeneration, activate endothelial cells, and stimulate signaling pathways (PI3K, p44/42^{mapk}) via miRNAs (miRNA-126, miRNA-214, miRNA-296, miRNA-125a, miRNA-31, and miRNA-150), proteins (VEGF, FGF-2, PDGF, IL-8, TGF-β1, and transcription factors), and lipids (sphingosine-1-phosphate) [42]. Bone marrow-derived MSC-derived EVs (BMSC-EVs) promote angiogenesis by activating the PI3K/Akt signaling pathway via miRNA-126 [43]. Adipose-derived stem cell-derived EVs (ADSC-EVs) have also demonstrated angiogenic potential via miR-125a and miR-29b-3p [44–46], serving as a potential strategy to increase angiogenesis in musculoskeletal regeneration [28].

Key Signaling Pathways influenced by EVs

The effects of EV therapy are established through the activation and regulation of key signaling pathways that are strongly interconnected [47]. MSC-EVs modulate several signaling pathways, especially via their RNA cargo. Figure 2 illustrates several key signaling pathways regulated by MSC-EVs in musculoskeletal regeneration. Wnt growth factors serve as short- or long-range signaling molecules and act by regulating the transcriptional coactivator β-catenin. The Wnt/β-catenin pathway is essential for embryonic development, adult homeostasis, and proliferation during wound healing [48, 49]. Yu et al. demonstrated that BMSC-derived exosomal miRNA-136–5p indirectly promotes the Wnt/β-catenin pathway by suppressing low-density lipoprotein receptor-related protein 4 (LRP4), leading to osteoblast proliferation and fracture healing [50]. Conversely, exosomal miR-92a-3p downregulates Wnt5A expression and mitigates cartilage destruction [49].

The TGF-β superfamily affects many cell types in various aspects of cellular and tissue physiology, regulating cellular activities such as proliferation, apoptosis, autophagy, senescence, and dormancy [51]. BMSC-EVs influence the TGF-β pathway by competitively activating SMAD signaling via the BMPR2 and ACVR2B receptors, whereas ADSC-EVs activate the SMAD1/5/9 and SMAD2/3 pathways, contributing to musculoskeletal regeneration [52, 53]. Moreover, ADSC-EVs enhance collagen remodeling by promoting the expression of collagen-associated proteins, including type I and III collagen, bFGF, and TGF-β1 [54].

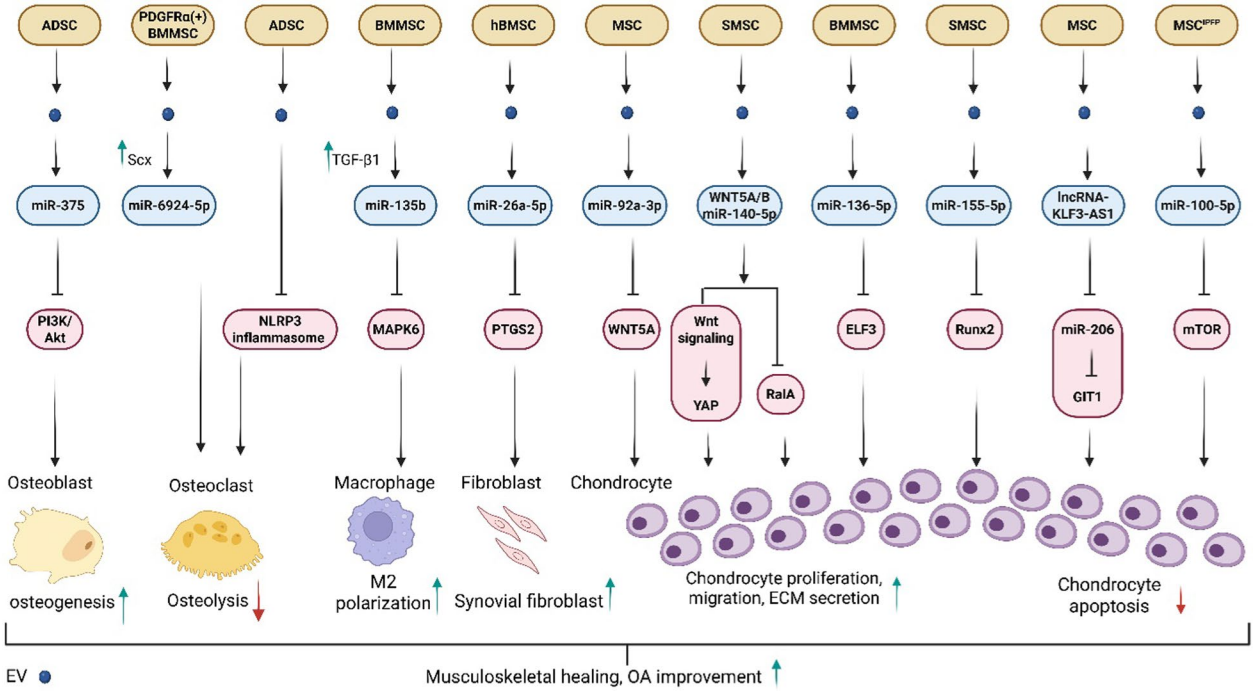


Fig. 2 MSC-EVs contribute to musculoskeletal regeneration through immunomodulation, chondroprotection, the regulation of cell behavior, and the modulation of the ECM. PDGFRa^{+}: Platelet-Derived Growth Factor Receptor alpha-Positive; hBMSC: Human Bone Marrow–Derived Mesenchymal Stem Cell; SMSC: Synovial Mesenchymal Stem Cell; MSCIPFP: Mesenchymal Stem Cell Derived from Infrapatellar Fat Pad; Scx: Scleraxis; RalA: Ras-Related Protein Ral–A; YAP: Yes-Associated Protein; NLRP3 inflammasome: NOD-Like Receptor Family Pyrin Domain–Containing 3 Inflammasome; PTGS2: Prostaglandin-Endoperoxide Synthase 2; ELF3: E74-Like Factor 3; Runx2: Runt-Related Transcription Factor 2; GIT1: G-Protein–Coupled Receptor Kinase–Interacting Protein 1. Created in BioRender. Aziziyan, F. (2025) https://BioRender.com/4wq1i0m

MSC-EVs also regulate additional signaling pathways. Studies have shown that BMSC-EVs influence the BMP-2/Smad1/RUNX2 [55], SMURF1/RUNX2 [56], and angiopoietin-1/Tie2-NO [57] signaling pathways in the treatment of fractures and other musculoskeletal diseases [47].

Uptake by target cells and functional modulation

The uptake and cargo delivery of EVs are not completely understood. However, they are recognized as cargo carriers, and their uptake occurs in three steps, as summarized in Fig. 3. Initially, EVs are selectively targeted to specific cells, and their uptake depends on specific surface markers expressed by recipient cells [2]. The source of the cell or tissue, as well as its physiological or pathological condition, can influence the abundance, construction, and functional properties of EVs [58]. However, some EVs undergo nonspecific uptake. In the next step, EVs enter recipient cells through either nonspecific (micro- and macropinocytosis) or specific (receptor-dependent) mechanisms via endocytosis through both clathrin-dependent and clathrin-independent pathways [59]. Certain surface proteins, such as proteoglycans, integrins, and lectins, may play a role in EV uptake. Some EVs may bypass this step and influence the target cell through their surface molecules without being internalized [2, 60]. For example, tetraspanin markers (CD9, CD63, and CD81), along with other markers such as HSP90, HSP70, and Flot-1, are present on the surface of BMSC-derived EVs and may play essential roles in cargo selection, EV biogenesis, cellular uptake, and cell targeting [61, 62]. Additionally, miR-21, miR-4532, miR125b-5p, and miR-338-3p, together with other small noncoding RNAs carried by EVs, play vital roles in regulating the expression of bone-related genes and may contribute to bone regeneration [61].

In the final step, EVs can follow multiple intracellular pathways. They can enter endosomes and release their contents in response to the acidic conditions of multivesicular bodies (MVBs) [63]. Some EVs are transferred to lysosomes, where they degrade, whereas others may be resecreted into the extracellular space. After uptake, EVs or EVs contribute to cell–cell communication by modulating the previously mentioned signaling pathways [64]. As mentioned, MSC-EVs promote bone regeneration by regulating downstream signaling pathways, including the BMP/Smad, Wnt/β-catenin, and PI3K/AKT pathways [58].

Engineering strategies for increased therapeutic potential

3.1. MSC-EVs as drug delivery vehicles

MSC-EVs, which express specific markers such as CD44, CD73, CD29, and CD90, retain the therapeutic functions

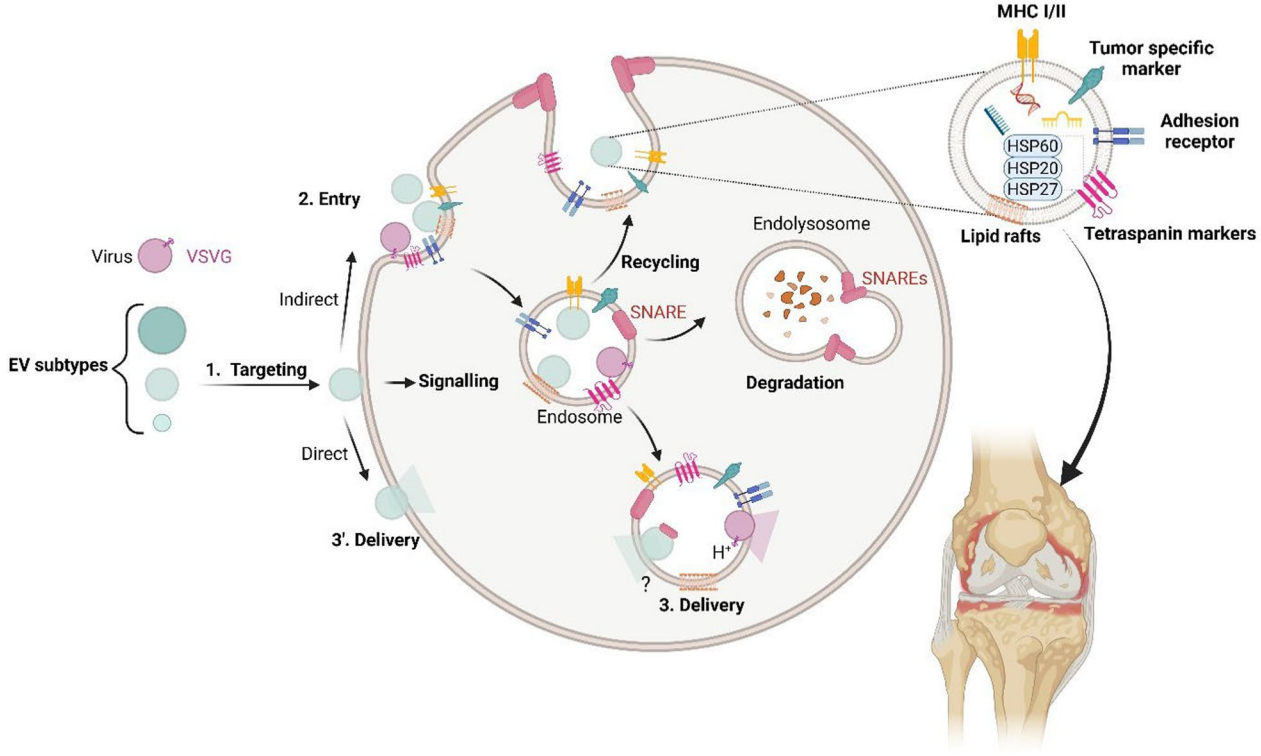


Fig. 3 Schematic representation of the three steps in EV uptake by recipient cells: targeting, entry, and delivery [65]. Created in BioRender. Aziziyan, F. (2025) https://BioRender.com/pxyl0wg

of their parent MSCs (Fig. 4). These markers make MSC-EVs promising vehicles for drug delivery in disease therapy. However, several challenges remain in their clinical application [66].

Loading techniques and therapeutic cargo examples

The unique structure of EVs enables them to transport pharmaceutical ingredients, including nucleic acids, proteins, and small molecules. Drug loading into EVs can be achieved through endogenous and exogenous approaches. The endogenous method involves modifying the biogenesis process in donor cells to directly produce drug-loaded EVs. Exogenous methods represent another widely used approach for loading drugs into EVs because of their simplicity and well-established techniques. Despite the promising advantages of EVs in clinical drug delivery, several challenges hinder their widespread application. For instance, electroporation is unsuitable for large-scale industrial production due to safety concerns. Additionally, the transfection method poses limitations due to its potential toxicity and reliance on chemical reagents, which may interfere with gene expression within EVs. Therefore, further research is necessary to optimize drug-loading technologies and ensure the safe, efficient, and scalable use of EVs as drug carriers [68].

Engineering approaches

Surface engineering and cargo engineering are two strategies that increase the therapeutic efficacy of EVs as natural drug carriers. Through bioengineering, EVs can be optimized for use in targeted and personalized therapeutics (Fig. 5) [69].

Today, the engineering of EVs has made them promising therapeutic candidates for a variety of diseases, particularly musculoskeletal disorders. The main challenge in this therapeutic approach lies in selecting the most suitable technique for manipulating EVs and loading therapeutic molecules to increase their functionality and efficacy [70].

Cargo customization

Loading exogenous components, such as drugs, small molecules, and proteins, into engineered EVs or their parental cells can be achieved through two primary approaches. The exogenous (direct) loading method involves introducing external cargo directly into isolated EVs. In contrast, the endogenous (indirect) loading method involves integrating cargo into parental cells, allowing the materials to be incorporated into EVs during their natural biogenesis process [69].

Exogenous cargo loading The encapsulation of therapeutic drugs can be performed directly after EV extraction through various exogenous loading techniques. One com-

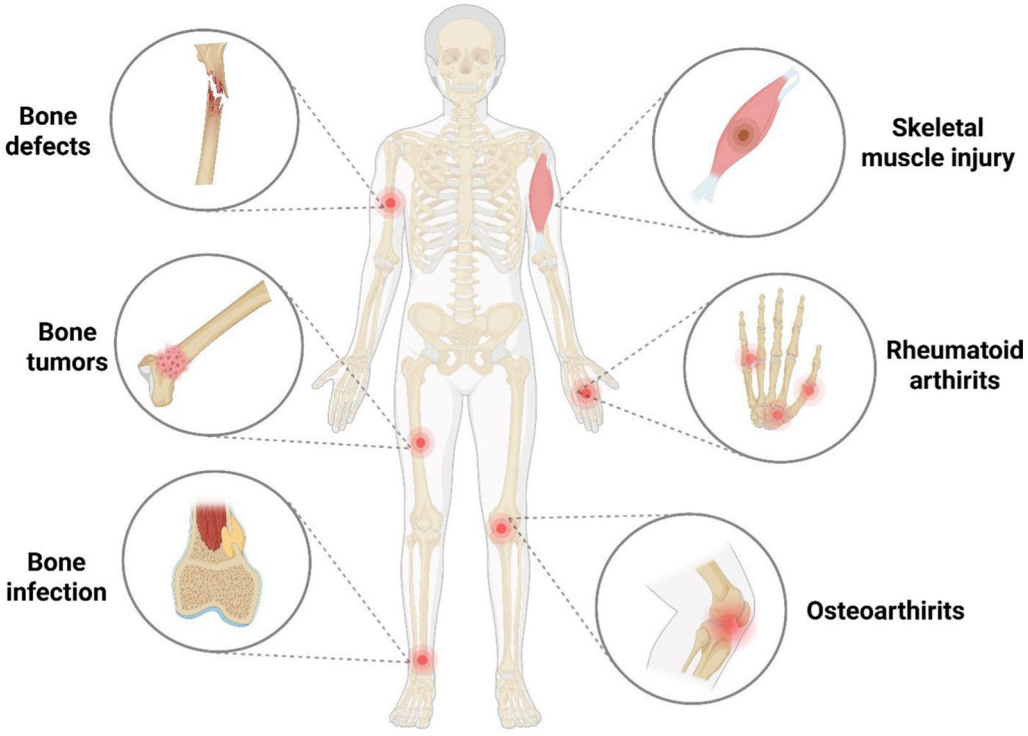


Fig. 4 MSC-EVs can serve as drug delivery vehicles for treating a wide range of diseases across various tissues [67]. Created in BioRender. Aziziyan, F. (2025) https://BioRender.com/58skw9u

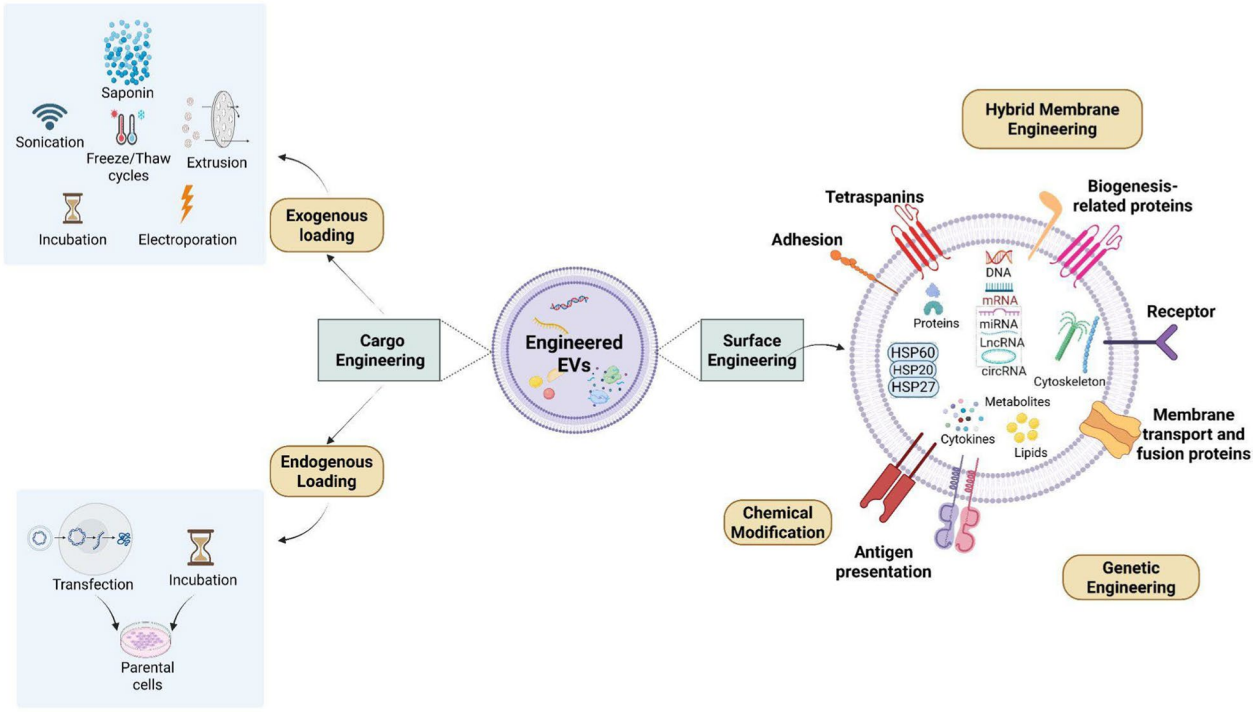


Fig. 5 Techniques to optimize the therapeutic effectiveness of EVs. (Left) *EV* cargo engineering methods include endogenous loading, where parental cells are equipped to naturally incorporate the desired cargo during EV biogenesis, and exogenous loading, where cargo is introduced into already isolated EVs. (Right) *EV surface engineering*: Genetic modification of parental cells enables the production of EVs expressing transmembrane proteins fused with targeting ligands. Additionally, isolated EVs can be chemically modified by attaching targeting moieties to their surface through covalent bonding, lipid self-assembly, or other noncovalent interactions. Hybrid membrane engineering also allows the fusion of synthetic liposomal nanoparticles with natural EVs. Created in BioRender. Aziziyan, F. (2025) https://BioRender.com/uotagmi

monly used passive method is incubation, which is suitable for encapsulating hydrophobic drugs because of their natural affinity for the EV membrane. In contrast, active loading techniques are typically employed for the encapsulation of hydrophilic molecules, which require physical or chemical methods to diffuse across the hydrophobic membrane into the intraluminal space of EVs [69]. Feng et al. employed a strategy to induce surface charge reversal to increase the therapeutic potential of MSC-EVs for OA. To achieve this, they incubated sEVs with the amphiphilic cationic polymer PPD, which effectively reversed their surface charge. These findings demonstrated that this modification not only preserved the physicochemical properties of the EVs but also significantly improved cartilage uptake and joint retention [71].

One such technique is electroporation, which uses high-intensity electrical pulses to temporarily permeabilize the EV membrane, facilitating drug loading [69]. This method was explored by Cui and colleagues, who constructed engineered EVs capable of delivering siShn3 to osteoblasts. These findings demonstrated that this engineered platform, BT-Exo-siShn3, which employs electroporation for siRNA delivery, has significant antiosteoporotic therapeutic potential [70].

Sonication is another effective method for enhancing the active loading of various biomolecules into EVs, which generates transient pores in the EV membrane, allowing small hydrophilic molecules to enter [68]. Yerneni et al. investigated the exogenous loading of bone morphogenetic protein-2 (BMP-2) into the lumen of EVs via sonication and electroporation. They demonstrated that incorporating BMP-2 into EVs in this way increased their efficiency and biological activity, enabling them to stimulate osteogenic differentiation [72].

The freeze–thawing technique involves mixing EVs with the therapeutic cargo at room temperature, followed by repeated cycles of freezing and thawing. This process disrupts the membrane structure, making it more receptive to cargo incorporation [69]. Lee and colleagues synthesized a complex via a freeze–thaw method to fuse EVs with miR-140 for cytoplasmic miRNA delivery. They demonstrated that this approach enhanced cell differentiation and promoted cartilage regeneration [73].

Other exogenous loading techniques include transfection, microwave-assisted methods, extrusion, and saponin-assisted loading. In the transfection method, specific reagents are used to encapsulate drugs in EVs. The microwave-assisted method uses a nonionizing

electromagnetic field to increase cell permeability through both thermal and nonthermal effects [68].

Permeation using the compound saponin is a chemically based exogenous loading method that employs detergent-like action to facilitate cargo entry into EVs. In this technique, cholesterol is selectively removed from the EV membrane, leading to pore formation without compromising membrane integrity [69].

Park et al. developed saponin-based nanoparticles (Ad-SNPs) containing alpha-lipoic acid (ALA) and dexamethasone (Dex). This approach was designed to promote the osteogenic differentiation of human MSCs and facilitate bone regeneration. These findings demonstrated that this complex enabled rapid and complete bone regeneration and repair in a rat femoral bone defect model [74].

Extrusion, on the other hand, is a technique used to artificially generate vesicles known as EV mimetics. This method involves the mechanical disruption of cells followed by the reassembly of cellular contents into vesicle-like structures that mimic natural EVs, preserving their biological and physical characteristics [75]. Extrusion involves passing a mixture of EVs and drugs through a porous membrane via a lipid extruder to facilitate loading [68]. Hu et al. employed the extrusion method to design C-X-C motif chemokine receptor 4 (CXCR4)-EVs fused with antagomir-188 liposomes for targeted delivery to the bone marrow. These findings demonstrated enhanced osteogenesis and improvement in age-related bone loss in mice [76]. Furthermore, studies have highlighted the potential of this method to address the challenge of the low yield of EVs in therapeutic drug delivery. Zha and colleagues constructed EV mimics (EMs) capable of rapid and large-scale production. They demonstrated that engineered EMs used to design a gene-activated matrix (GAM) could sustainably transfer the VEGF gene, thereby promoting vascularized osteogenesis [77].

Overall, each of these loading techniques presents both advantages and limitations. A key challenge involves maintaining the structural integrity and stability of both the EV membrane and the encapsulated cargo while minimizing undesirable effects such as aggregation. For example, while the incubation method preserves membrane integrity, it is generally less efficient for cargo loading than are active loading strategies such as electroporation or sonication [69].

Endogenous cargo loading Endogenous loading depends on the presence of cargo within producer cells and the functional activity of the cellular machinery responsible for incorporating that cargo into EVs. External cargo can be introduced into producer cells through either active or passive loading mechanisms. Small drug molecules can be incorporated into EVs through simple incubation, while genetic bioengineering of EVs enables them to carry RNAs and proteins [69].

Numerous studies have investigated the therapeutic potential of EVs that transfer endogenous miRNAs. Liu and colleagues demonstrated that engineered miR-181b exosomes promote M2 polarization and enhance osteogenesis through the secretion of BMP2 and VEGF [78]. Additionally, studies have shown that EVs derived from BMP-2-overexpressing rats can promote regeneration and induce osteogenesis in rats with calvarial defects [58].

Proteins and mRNAs can also be endogenously loaded into EVs by transfecting or transducing parental cells with genes encoding the desired cargo [69]. For example, H Li et al. demonstrated that modifying BMSCs with mutant HIF-1α enhances the differentiation of osteogenic cells. They also reported that EVs derived from mutant HIF-1α–modified BMSCs accelerate angiogenesis and osteogenesis, thereby facilitating the repair of early steroid-induced avascular necrosis of the femoral head (SANFH) [79]. Similarly, the transfection of parental cells facilitates the incorporation of synthetic therapeutic agents into EVs. While endogenous loading strategies can generally produce stable, bioengineered EVs that preserve membrane integrity and functional cargo, they are often more time-consuming, costly, and less efficient than exogenous methods and may also negatively affect the viability of parental cells [69].

Surface Functionalization

One of the key challenges in administering EVs in vivo is their rapid clearance from the body, primarily due to their uptake by cells in organs such as the liver, gastrointestinal tract, spleen, and lungs. The surface of EVs plays a crucial role in determining their biological fate. By modifying the surface of EVs, their ability to cross biological barriers, target specific cell types, and extend their circulation time can be improved, thereby enhancing delivery to intended sites. Strategies for surface functionalization include genetic manipulation, chemical modification, and hybrid membrane engineering, each of which is discussed in the following Sects. [69].

Genetic manipulation Engineered EVs with specific surface features can be produced through the genetic manipulation of parental cells. This process involves modifying the transmembrane proteins of native EVs with exogenous ligands, such as proteins/peptides, antibodies, and lipid raft-associated components. These ligands are recognized by receptors on target cells [69]. For example, Luo and colleagues developed a strategy to deliver bone marrow stromal cell-derived exosomes (STExos) to bone by conjugating them with a high-affinity BMSC-targeting aptamer. The aptamer facilitates the uptake of the STExos by the

BMSCs in vitro and promotes their accumulation in bone in vivo. This approach reduces the metabolism and clearance of STExos, thereby enhancing bone regeneration in mice with femoral fractures and postmenopausal OP[80].

Chemical modification Chemical modification enables the incorporation of targeting ligands onto the surface of EVs through various mechanisms, including hydrophobic insertion, lipid self-assembly, covalent bonding, and noncovalent interactions. The simplest approach involves directly inserting hydrophobic or amphiphilic molecules into the hydrophobic EV membrane. For example, phospholipid derivatives can be used to immobilize targeting ligands by integrating into the EV membrane. Phospholipid-PEG derivatives enhance EV stability and prolong their circulation time, leading to increased accumulation in specific tissues and more effective cargo delivery. Another strategy involves conjugating nanoparticles to transferrin receptors on blood-derived EVs via noncovalent interactions, which facilitates efficient EV extraction from the blood [69]. Wu et al. constructed a targeted delivery system to increase the retention of EVs. They synthesized DMPE-PEG-CREKA through hydrophobic interactions and incorporated it into the EV membrane to generate CREKA-EVs. These results demonstrated that CREKA-EVs promote the osteogenic differentiation of BMSCs and enhance the angiogenic capacity of human umbilical vein endothelial cells, suggesting that CREKA-EVs represent a promising strategy for bone repair [81].

Hybrid membrane engineering Another approach to functionalizing the surface of EVs is hybrid membrane engineering. This method leverages the lipid bilayer of EVs to facilitate fusion with other membrane structures. As a result, natural EVs can be combined with synthetic liposomes to form hybrid nanoparticles without compromising their inherent biological properties [69]. Numerous studies have investigated the functional potential of hybrid membrane-engineered EVs. For example, Hu and colleagues engineered NIH3T3 cells to produce EVs displaying the chemokine receptor CXCR4 on their surface. These EVs were then fused with liposomes carrying antagomir-188, generating hybrid nanoparticles. The accumulation of these nanoparticles in the bone marrow promoted osteogenesis [76]. Overall, despite some challenges, these studies highlight the promising therapeutic potential of EVs and suggest an optimistic outlook for their future clinical application [69].

Therapeutic applications in musculoskeletal tissues

As summarized in Table 1, many studies have explored the therapeutic applications of MSCs and their derived EVs in musculoskeletal regeneration [4, 12, 13]. The major therapeutic areas include bone regeneration [14], cartilage repair, and tendon and ligament healing. MSC-EVs have shown promising potential in promoting angiogenesis [82], modulating immune responses [83], remodeling the ECM, and facilitating mineralization [84, 85]. They also mitigate apoptosis, enhance proliferation and cell integration, and promote cell growth and viability through signaling pathways such as the ERK1/2 and MAPK pathways [86]. Additionally, various engineering strategies, including overexpression and surface modification, have been employed to increase the regenerative potential of these materials [58].

Bone regeneration

Bone possesses an intrinsic regenerative capacity; however, this ability is limited when defects exceed a critical size [96, 97]. The incidence of bone disorders is increasing due to the aging population, although these conditions are not confined to the elderly [98]. Common skeletal disorders, such as fractures resulting from trauma, infection, tumor excision, or congenital malformations, often require advanced therapeutic interventions [97]. Conventional treatments are typically invasive and may be associated with pain and secondary complications. If left untreated, these conditions can result in chronic pain, fragility fractures, and skeletal deformities [14, 58, 99]. Increasing evidence suggests that MSC-EVs play a role in bone regeneration by promoting osteogenesis and angiogenesis [100–102].

Role in osteogenesis, fracture healing, and OP

Effective induction of both osteogenesis and angiogenesis is essential for optimal bone regeneration and fracture healing [55, 103]. Exosomal miR-136–5p derived from BMSCs promotes fracture healing by inhibiting LRP4 expression and enhancing osteoblast proliferation and differentiation through activation of the Wnt/β-catenin signaling pathway [50]. As an engineering strategy, BMSC-EVs combined with a low dose of Fe_3O_4 nanoparticles and applied under a static magnetic field are enriched with miR-1260a. This formulation enhances osteogenesis via HDAC7 inhibition and promotes angiogenesis via COL4A2 suppression [82]. Liu et al. demonstrated that MSC-derived EVs generated under hypoxic conditions (Hypo-EVs) exhibit superior fracture healing properties compared with those derived under normoxia, primarily through the upregulation of miR-126 [101]. Additionally, neural innervation plays a crucial role in bone regeneration. MSC-EVs stimulated with nerve growth factor (NGF) acquire neuro-promotive cargo, enhancing innervated bone regeneration via activation of the MAPK and PI3K-Akt signaling pathways [104].

EVs can also enhance the bioactivity and osteogenic performance of scaffolds. For example, a tricalcium

Table 1 Summary of therapeutic studies utilizing MSC-EVs for musculoskeletal regeneration

Application	Source	Isolation method	Administration protocol	Preconditioning/engineering strategies	Findings	Mechanistic insights	References
Bone fracture healing	Mouse BMSCs	Ultracentrifugation	Tail vein injection once daily for three consecutive days	-	Exosomal miR-136-5p elevated osteoblast proliferation and differentiation, enhancing fracture healing in mouse models	Activation of the Wnt/β-catenin signaling pathway via LRP4 inhibition	[50]
Nonunion fracture healing	Rat BMSCs		Weekly local injections of exosomes (10^{10} particles/100 µL) at the fracture site	-	BMSC-EVs promoted osteogenesis, angiogenesis, and bone healing	Activation of HIF-1α/VEGF and the BMP-2/Smad1/RUNX2 signaling pathways	[87]
Bone regeneration	Human BMSCs		EVs loaded into porous absorbable gelfoam and implanted at the fracture site	Cells preconditioned with Fe_3O_4 nanoparticles (with or without static magnetic field)	BMSC-Fe_3O_4-SMF-EVs promoted osteogenesis and compared to BMSC-EVs	Exosomal miR-1260a targeted HDAC7 and COL4A2	[88]
Bone repair	hiPS-MSCs		Implantation of EV/β-TCP scaffolds in defect area	-	EV/β -TCP scaffolds promoted osteogenesis compared with β -TCP scaffolds alone	Activation of PI3K/Akt signaling pathway in hBMSCs	[89]
Osteoarthritis	Human BMSCs		Weekly intra-articular injections of MSC-EVs or MSC-miR-92a-3p-EVs (500 µg/mL, 15 µL)	Transfection of MSCs with miR-92a-3p mimic or inhibitor	MSC-miR-92a-3p-EVs enhanced cartilage proliferation and matrix synthesis	miR-92a-3p inhibited WNT5A, contributing to cartilage homeostasis	[90]
Cartilage regeneration	hUC-MSCs	Centrifugation	Injection of gel precursors (100 µL) into the defect area	KGN/TGF-β1-loaded MSC-EVs (CEKT) crosslinked into Gel-CEKT hydrogel	Gel-CEKT promoted BMSC recruitment and enhanced cartilage regeneration and integration	CEKT's positive charge facilitated deep cartilage penetration	[91]
Osteoarthritis	Rat BMSCs	Ultracentrifugation	Intra-articular injection of EVs (100 µL, 400 µg/mL) into the right knee twice weekly for 4 weeks	EVs treated with low-intensity pulsed ultrasound (LIPUS)	Promoted cartilage regeneration, increased chondrocyte proliferation and matrix synthesis, and suppressed inflammation	Inhibition of IL-1β-induced NF-κB signaling pathway	[92]
Tendon healing	Rat ADSCs		Local application of ADSC-EVs (200 µg in 30 µL GelMA) to patellar tendon defects, crosslinked in situ via UV light	EVs loaded on gelatin methacryloyl (GelMA)	ADSC-EVs enhanced tenogenic differentiation, proliferation, and migration, while reducing inflammation	Activation of SMAD2/3 and SMAD1/5/9 signaling pathways in tendon stem cells	[93]
	Rat BMSCs		Local implantation of Fibrin–DiR–EVs into rat knees; assessed via in vivo imaging system at days 3 and 14	EVs labeled with DiR dye and embedded in fibrin	Enhanced tenogenic differentiation and proliferation, improved histological scores and mechanical strength	EVs activated endogenous tendon stem cells	[94]
Bone–tendon healing	Mouse ADSCs and BMSCs		Local injection of 20 µg EVs (between tendon and tuberosity) once weekly for 4 weeks	-	EVs enhanced BMSC proliferation, migration, and differentiation, accelerating bone–tendon healing	ADSC-EVs and BMSC-EVs exerted comparable regenerative effects	[95]

hiPS-MSCs: human induced pluripotent stem cell-derived mesenchymal stem cells; hUC-MSCs: human umbilical cord-derived mesenchymal stem cells; GelMA: gelatin methacryloyl; β-TCP: beta-tricalcium phosphate; CEKT: cartilage extracellular matrix–loaded therapeutic hydrogel; KGN: kartogenin; SMF: static magnetic field; DiR: 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide

phosphate (β-TCP) scaffold combined with EVs derived from human-induced pluripotent stem cell-derived MSCs (hiPS-MSC-EVs) exhibited greater osteogenic potential than pure β-TCP scaffolds alone [105]. This pioneering work on integrating EVs with scaffolds has laid the foundation for the development of more advanced biomaterial-based delivery systems in recent years [106]. Numerous studies have investigated hydrogel scaffolds loaded with BMSC-EVs or exosome-encapsulated hydrogels, demonstrating improved potential for bone tissue repair and regeneration [107–112]. OP, a prevalent multifactorial skeletal disorder, is characterized by reduced bone mineral density and increased bone fragility. Addressing OP effectively requires the development of novel therapeutic strategies. MSC-EVs influence the differentiation and activity of bone cells and help maintain osteohomeostasis, making them promising candidates for OP treatment [12, 113, 114]. For example, BMSC-derived exosomal MALAT1, a long noncoding RNA, regulates SATB2 expression and enhances osteogenesis and osteoblast activity under osteoporotic conditions [102] (Fig. 6).

Preclinical and clinical evidence

Zhang et al. applied BMSC-EVs in a rat model of femoral nonunion and reported that BMSC-EVs promoted osteogenesis by regulating the BMP-2/Smad1/RUNX2 signaling pathway. In addition, they enhance angiogenesis, as well as cell proliferation and migration, thereby improving the treatment of nonunion [55]. Similarly, BMSC-derived exosomal miR-25 has been shown to induce angiogenesis during fracture healing in mice [56].

To date, no clinical evidence has been reported for the use of MSC-EVs in the treatment of OP, with current studies limited to animal models [113]. For example, BMSC-derived exosomal miR-935 significantly enhanced osteoblast proliferation and differentiation through STAT1 regulation in OP rats. In diabetic OP, which is closely associated with chronic inflammation, adipocyte-derived MSC-EVs have been shown to suppress inflammatory cytokine secretion and reduce NLRP3 inflammasome activation in osteoclasts in streptozotocin-induced diabetic OP rats [115].

Despite promising preclinical data, only a few clinical trials evaluating the use of MSC-EVs for bone regeneration have been initiated. Current research is largely focused on animal models, and further preclinical evidence is needed. Addressing key challenges in EV-based therapies, such as delivery efficiency, dosing, and scalability, is essential for the successful clinical translation of MSC-EVs in bone regeneration [116, 117].

Cartilage repair

Cartilage possesses limited intrinsic regenerative capacity because of its avascular nature [118], and currently, there are no effective treatments for most cartilage disorders [119]. Conventional strategies, such as autologous chondrocyte implantation, often result in the formation of unstable fibrocartilage, which can lead to the loss of the chondrocyte phenotype and, in some cases, tumor formation [120, 121]. From a pathophysiological perspective, cartilage injuries are generally classified into two major types: focal cartilage defects and degenerative cartilage damage associated with OA. OA is a progressive

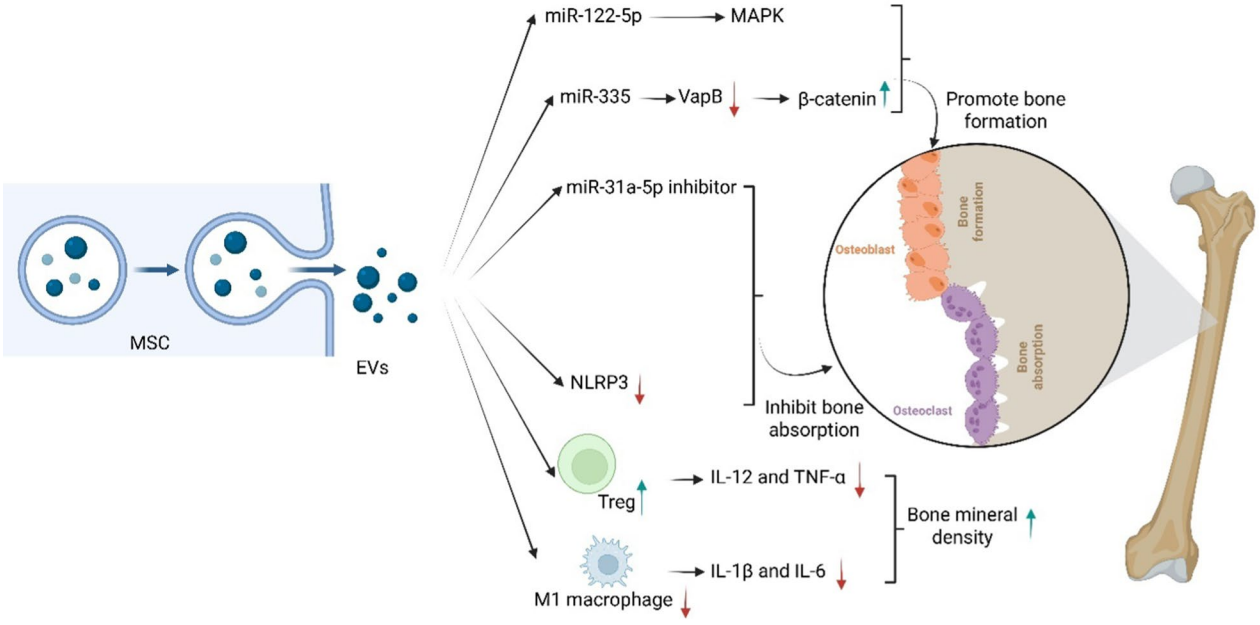


Fig. 6 MSC-EVs influence bone formation, absorption, and mineral density in OP. Created in BioRender. Aziziyan, F. (2025) https://BioRender.com/v90lnl9

joint disorder characterized by cartilage degradation, structural alterations, and chronic pain. It is one of the leading causes of disability and healthcare burden among elderly individuals. As OA progresses, it affects all joint tissues, including subchondral bone, ligaments, and synovium [12, 13, 122]. Emerging studies have demonstrated that MSC-EVs play a critical role in cartilage repair because of their multifaceted therapeutic properties. These include promoting cell proliferation and ECM synthesis, enhancing anti-inflammatory M2 macrophage infiltration, restoring mitochondrial function, and reducing chondrocyte apoptosis, senescence, and inflammation [15, 123, 124].

Chondroprotection, ECM modulation, and OA treatment. MSC-EVs have shown promising results in both in vitro and animal studies by promoting osteochondral repair, alleviating OA degeneration, enhancing cellular proliferation, and supporting matrix deposition [14]. The major mechanisms involved are summarized in Fig. 7. Mitochondrial dysfunction and reduced mitochondrial numbers are key features of age-related OA. These deficits result in impaired ATP production, decreased cellular activity, and disrupted tissue homeostasis. ATP-generating enzymes present in MSC-EVs significantly increase ATP levels in chondrocytes, thereby facilitating chondroprotection, an essential aspect of effective OA treatment [125]. The chondroprotective potential of MSC-EVs remains a consistent finding, with subsequent studies further elucidating the underlying molecular mechanisms [126]. BMSC-EVs have been shown to stimulate cartilage regeneration by enhancing chondrocyte proliferation and regulating ECM synthesis. Moreover, they suppress inflammation and inhibit IL-1β-induced activation of the NF-κB pathway [127]. WNT5A, which is associated with the activation of MMPs, contributes to ECM degradation in cartilage [128]. MSC-exosomal miR-92a-3p has been found to inhibit WNT5A expression and prevent cartilage degradation in OA mouse models [129]. Further studies have demonstrated that MSC-EVs can suppress cartilage-degrading enzymes, such as MMP13, ADAMTS5, COX2, and microsomal prostaglandin E synthase-1. In addition, their miRNA cargo plays a key role in regulating the TGF-β/SMAD signaling pathway, highlighting the therapeutic potential of MSC-EVs in OA treatment [130].

Studies have also explored the use of MSC-EV-loaded scaffolds for cartilage repair. Tu et al. developed an injectable and adhesive hydrogel incorporating MSC-EVs enriched with kartogenin and TGF-β1. To enhance the interaction with the scaffold, the EV surface was coated with positively charged succinylated chitosan. This engineered hydrogel effectively promoted cartilage repair and chondrocyte production [131]. Similarly, Zheng et al. preconditioned synovial MSCs (SMSCs) with growth differentiation factor 5, a key chondrogenic factor, to generate chondrogenic exosomes (G-Exos). These exosomes were then incorporated into a glycyrrhizic acid/methacrylate-acylated hyaluronic acid (GA/HA) scaffold, forming GA/HA/G-EV constructs. The resulting scaffolds

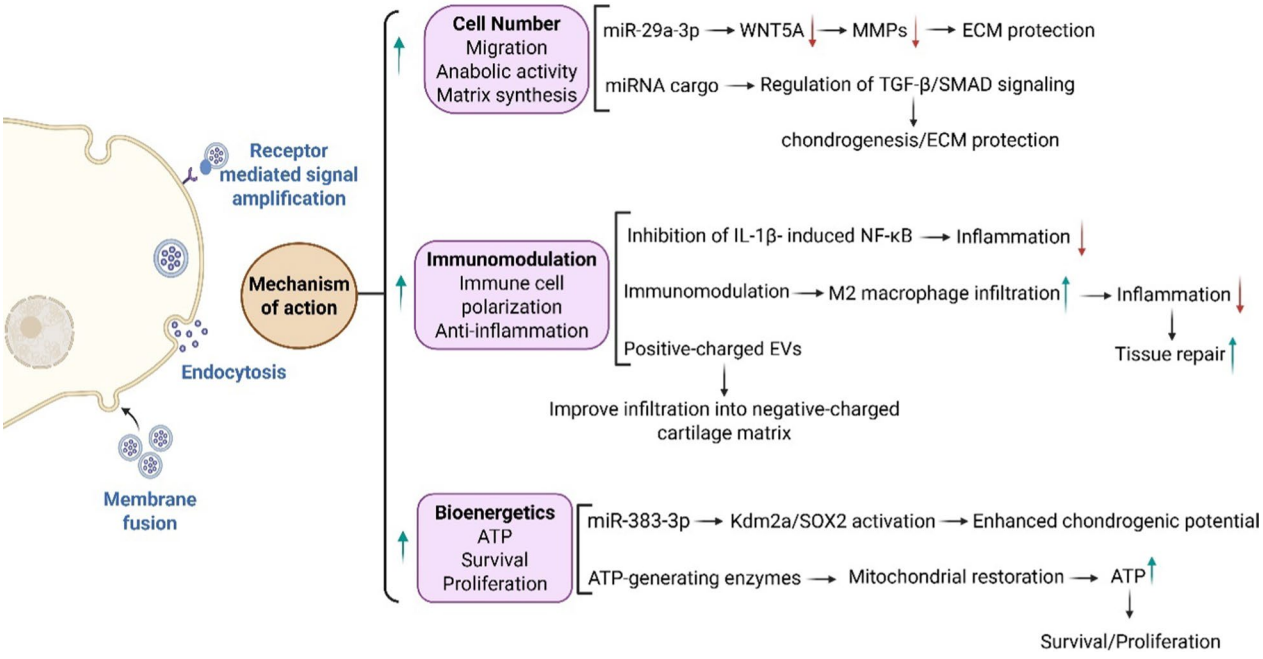


Fig. 7 Main mechanisms involved in MSC-EV-mediated cartilage regeneration. Created in BioRender. Aziziyan, F. (2025) https://BioRender.com/v1m0w2w

significantly enhanced articular cartilage regeneration. Mechanistically, G-exosomal miR-383-3p activated the Kdm2a/SOX2 signaling pathway, thereby enhancing the chondrogenic potential of SMSCs [132].

Challenges in cartilage avascularity and EV delivery

The cartilage matrix is dense and highly negatively charged, making it impermeable to many drugs and therapeutic agents. To address this challenge, Tu et al. engineered positively charged EVs, which significantly enhanced infiltration into cartilage tissue [131]. In addition, several studies have utilized MSC-EV-loaded hydrogels to improve the degree of integration between scaffolds and native cartilage. This combined approach leverages the advantages of both hydrogels and MSC-EVs, resulting in enhanced repair of osteochondral defects [123, 133]. Despite these advances, most studies to date have employed intra-articular injections as the delivery method for MSC-EVs [14].

Tendon and ligament healing

Tendons and ligaments are collagen-rich fibrous connective tissues essential for musculoskeletal function. However, they are particularly susceptible to damage due to collagen disorganization, aging, excessive mechanical loading, and previous injuries, which are key risk factors that compromise their integrity [134]. These tissues have limited intrinsic regenerative capacity, and current treatment options often fail to achieve full functional recovery. Consequently, increasing interest in regenerative therapies has led to the application of MSC-EVs as a promising strategy for tendon and ligament healing [4, 13, 135].

Tenogenic differentiation, collagen remodeling, and injury recovery.

MSC-EVs promote tendon and ligament regeneration by inducing tenogenic differentiation, enhancing cell proliferation and migration, remodeling the ECM, increasing collagen production and organization, and exerting anti-inflammatory effects [4, 13]. For example, the injection of BMSC-EVs loaded into a fibrin matrix has been shown to stimulate the differentiation and proliferation of tendon stem/progenitor cells (TSPCs), increase the expression of type I collagen, tenomodulin, and mohawk, and improve the mechanical strength of regenerated tendon tissue [94]. Moreover, MSC-EVs have been demonstrated to reduce the M1/M2 macrophage ratio and suppress inflammation at ligament injury sites, thereby facilitating recovery through collagen remodeling [136]. ADSC-EVs have also been shown to influence TSPCs by activating the SMAD2/3 and SMAD1/5/9 signaling pathways while concurrently suppressing inflammation [53]. Notably, a study by Tan et al. demonstrated that both BMSC-EVs and ADSC-EVs are equally effective at promoting bone–tendon junction healing [137]. Collectively, these findings support the use of MSC-EVs as a promising therapeutic strategy for tendon and ligament repair [138]. As discussed throughout this review, orthopedic disorders require effective and targeted therapeutic approaches. Owing to their regenerative properties and minimally invasive delivery potential, MSC-EVs have gained considerable attention in musculoskeletal regeneration. While extensive in vitro and in vivo studies support their efficacy, further clinical trials are essential to validate therapeutic outcomes and overcome existing translational challenges [4].

Clinical translation and trials

Current clinical trials

The earliest in vitro research on MSC-EVs was conducted less than ten years ago, making them relatively recent [139]. Table 2 summarizes the results of clinical and preclinical trials investigating the musculoskeletal applications of MSC-EVs. For example, clinical-grade UC-MSC-derived sEVs were successfully standardized, extensively characterized, and shown to exert potent anti-inflammatory and cartilage-regenerating effects by promoting M2-like macrophage polarization and repairing OA lesions in preclinical models. First-in-human intra-articular administration has demonstrated excellent safety over 12 months, supporting its advancement toward early-phase clinical translation for knee OA [140]. Despite strong preclinical evidence [141] and early indications of clinical benefit, the use of MSC-EVs for bone repair has not yet advanced beyond initial safety evaluations. Future efforts should focus on standardizing EV production, optimizing dosing strategies, and expanding patient-centered clinical trials in areas such as fracture healing and OP. These steps are essential to fully realize their potential as effective cell-free therapies [142].

Delivery strategies

Local injections

Intravenous administration remains the most common route for delivering MSC-EVs [141]. While it enables systemic distribution, it can also result in rapid clearance, potentially limiting sustained therapeutic effects [156]. To increase the local concentration, researchers have explored direct injections into target tissues, such as joints, for bone or tendon repair [157]. This localized approach can improve EV retention at injury sites but often requires multiple doses to compensate for rapid washout [156].

Biomaterial-based carriers

Early studies in neural tissue engineering demonstrated that hydrogels and biomaterial scaffolds, which are

Table 2 Summary of key clinical and preclinical studies utilizing MSC-EVs for MSDs. WOMAC: Western Ontario and McMaster Universities Arthritis Index; RCTs: randomized controlled trials

Clinical trials

Condition	Source of EVs	Route of Administration	Findings	Phase	Clinical trial number	References
Osteoarthritis	PMSC	Single intra-articular injection	Safe; no improvement in pain, WOMAC, Lequesne, or MRI compared with placebo	I/II	IRCT20210423051054N1	[143]
	PMSC	Intra-articular injection	Recruiting	I	NCT06937528	[144, 145]
	MSC	Two intra-articular injections (Day 1 and Day 90)	Recruiting	not labeled	NCT06466850	[146, 147]
	MSC	Single intra-articular injection	Unknown status	I	NCT05060107	[148, 149]
	MSC	Single intra-articular injection	Safe	-	-	[140]
Degenerative meniscal injury	Synovial fluid MSC	Intra-articular injection	Recruiting	II	NCT05261360	[150, 151]

Preclinical

Condition	Source of EVs	Isolation method	Preconditioning or engineering strategies	Administration protocol	Findings	Mechanistic insight	References
Osteoarthritis	AD-MSC-EVs + hyaluronic acid	Standard EV isolation (ultracentrifugation, filtration)	Combined with a hyaluronic acid	Intra-articular injection into osteoarthritic joint [1–3 injections over several weeks]	Delayed OA progression, reduced synovial inflammation, improved cartilage histology, increased expression of ECM markers (GAG and collagen type II) compared to control	Immunomodulation, downregulation of pro-inflammatory cytokines and MMPs, upregulation of ECM synthesis pathways	[152]
Cartilage defect	AD-MSC- EVs + hydrogel		EV-loaded multilayer cryogel/hydrogel scaffold (e.g., gelatin-chitosan with or without nano- hyaluronic acid)	Implantation of EV-loaded scaffold into defect site; follow-up 4–12 weeks via histology and imaging	Enhanced chondrocyte proliferation, upregulated GAG, restored cartilage	EV cargo activates pro-chondrogenic pathways (AKT/ERK), reduces MMPs, promotes ECM synthesis	[153]
Bone fracture	MSC-EVs	Ultracentrifugation, SEC	3D-printed porous scaffolds (PCL/β-TCP/hydroxyapatite) functionalized with EV; sometimes conjugated with chemokines/aptamers for retention	Implantation of EV-loaded scaffold into defect (single surgery); follow-up 4–12 weeks via micro-CT, BV/TV, histology	Enhanced bone regeneration and mineralization	EVs promote osteogenic differentiation, angiogenesis, and modulate local inflammation to favor bone formation	[154]
Tendon injury		Ultracentrifugation, SEC	hypoxia/miRNA loading	Local tendon injection	Improved biomechanical strength and collagen alignment	EV cargo promotes tenogenic differentiation, collagen synthesis, modulates inflammation, and matrix remodeling	[155]

Table 2 (continued)

Clinical trials						
Osteoporosis	STExo-aptamer	Standard EV isolation (ultracentrifugation)	Chemical functionalization of EV surface with bone-homing aptamer (ST-aptamer) to increase bone uptake after delivery	Intravenous injections (systemic); repeated over weeks in ovariectomized mice	Increased bone mass, reduced porosity	Bone targeting enhances EV uptake by osteogenic progenitors, activates osteogenic signaling, reduces bone resorption [80]
Ligament injury	BMSC-EVs and EEMs	Ultracentrifugation	Education of macrophages with MSC EVs (for EEMs)	Single injection postinjury; follow-up at 3, 7, and 14 days with histology, immunohistochemistry, and biomechanical testing	EEMs improved mechanical strength, EVs enhanced collagen alignment and reduced scarring	Immunomodulation, ECM remodeling, differential effects on inflammation and biomechanics [136]

SEC: size-exclusion chromatography; PCL: polycaprolactone; β-TCP: beta-tricalcium phosphate; micro-CT: microcomputed tomography; BV/TV: bone volume/total volume; EEMs: engineered extracellular matrix mimics

designed for optimal porosity, elasticity, and biodegradability, can guide axonal regrowth and enable the sustained release of EVs to injured areas of the central nervous system (CNS) [158]. Studies have investigated the use of exosome-loaded hydrogels for skin injury repair, demonstrating their contribution to collagen accumulation and wound closure [159]. These principles now inform emerging strategies in bone regeneration. For example, an injectable hydrogel composed of hyaluronic acid and alginate, which codelivered MSC-EVs and hydroxyapatite, stimulated both osteogenesis and angiogenesis in a rat skull defect model. The system increased alkaline phosphatase (ALP), osteocalcin (OCN), and collagen type I alpha 1 (COL1A1) levels in vitro and enhanced mineralization in vivo [112]. These hydrogels integrate osteoconductive cues with controlled EV release, addressing the challenges of rapid vesicle clearance and synchronized release with the early phases of bone repair [160]. Figure 8 shows a schematic representation of biomaterial-based carriers designed for the delivery of MSC-EVs.

Targeted delivery

Surface modifications initially developed in nonmusculoskeletal models, such as RAGE-peptide tagging to increase the lung retention of curcumin-loaded EVs [161] or IL-3 fusion strategies for targeting leukemia cells [162], have demonstrated how ligand display can improve EV targeting and therapeutic efficacy. When applied to bone, STexos conjugated with aptamers (STExo aptamers) have been shown to increase bone mass in osteoporotic mice and accelerate femoral fracture healing following intravenous administration [80]. Similarly, CXCR4-functionalized EVs combined with antagomir-188-loaded liposomes effectively targeted the bone marrow, reducing adipogenesis and restoring trabecular bone structure in aged mice [76]. These surface modifications not only increase the expression of osteogenic and angiogenic genes but also improve the localization of MSC-EVs to sites where regeneration is most needed, maximizing their healing potential [163]. Moreover, surface modification of MSC-EVs with chondrocyte-binding peptide (CAP) improves cargo delivery to chondrocytes and deep articular tissues, resulting in improved therapeutic efficacy in OA mice [164]. Chen et al. genetically fused a chondrocyte-homing peptide (CHP) at the N-terminus of LAMP2B, which is expressed on the surface of ADSC-EVs. These CHP-engineered EVs successfully delivered functional miRNAs into auricular chondrocytes and promoted cartilage formation [165]. Similarly, tendon stem cell-derived EVs tagged with a collagen-binding domain (CBD) coupled with collagen-based scaffolds enhanced regenerative efficacy in tendon injury models by promoting collagen deposition and biomechanical restoration

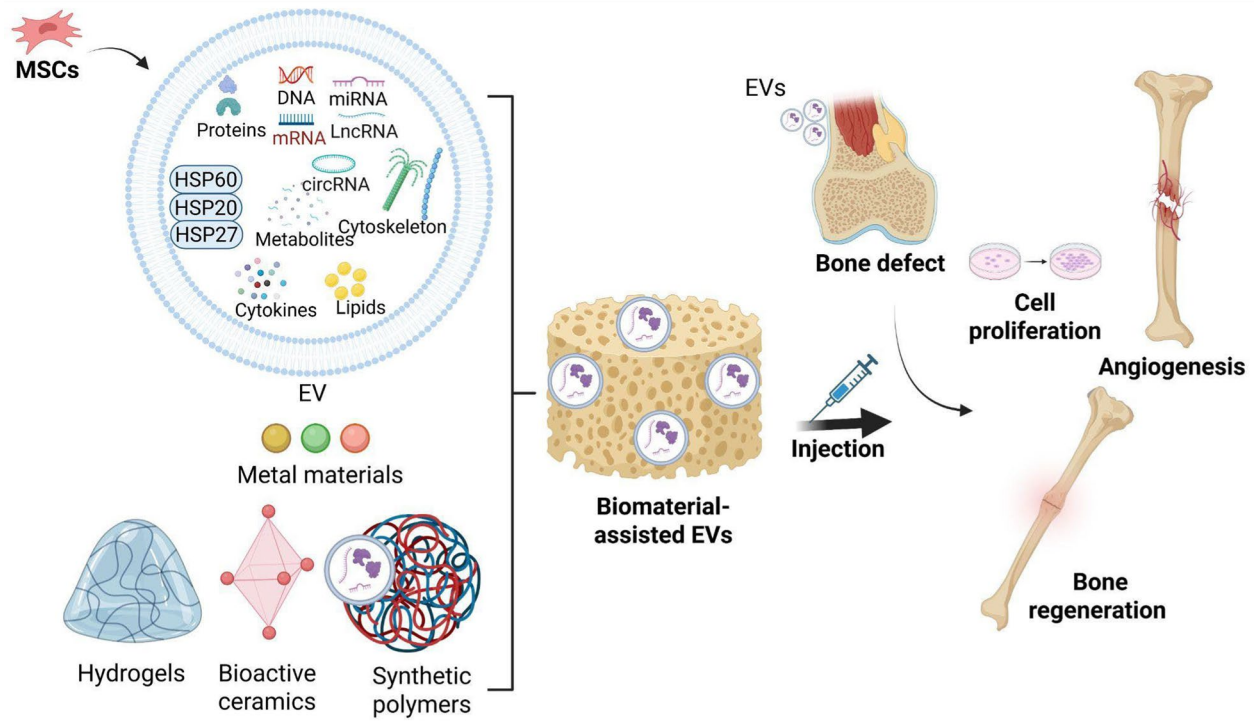


Fig. 8 Schematic illustration of an injectable hydrogel-based biomaterial carrier for the delivery of MSC-EVs. The system features a porous hydrogel matrix composed of hyaluronic acid and alginate, which encapsulate EVs and hydroxyapatite (HA) nanoparticles. Additional bioactive components and growth factors contribute to enhanced bone tissue regeneration, osteogenesis, and angiogenesis. This platform enables the sustained, controlled release of EVs and provides a supportive microenvironment for cellular growth and mineralization at the defect site. Created in BioRender. Aziziyan, F. (2025) https://BioRender.com/0o4p257

[166, 167]. However, engineering strategies specifically tailored for tendon and ligament regeneration remain underexplored, and further research is needed to optimize their translational potential.

Safety and efficacy

MSC–derived EVs face major hurdles before clinical deployment [168]. First, no unified protocol exists for EV isolation or purification, leading to wide variability in vesicle yield, purity, and integrity; ultracentrifugation can deform membranes and co-pellet contaminants, whereas precipitation kits sacrifice purity for simplicity [169–173]. Second, the lack of unique surface markers permits co-isolation of microvesicles and apoptotic bodies, and high-speed pelleting introduces membrane damage, protein aggregation, and shifts in marker expression (e.g., calnexin loss versus stable CD63/CD9) [174]. Third, quantifying EV dose relies on disparate metrics, such as particle counts, protein content, and cell equivalents (referring to the EV yield derived from a specific number of source cells), and measurements, such as nanoparticle tracking analysis (NTA) or tunable resistive pulse sensing (TRPS), hinder direct comparisons of biological effects [175–179]. Fourth, EVs exhibit short half-lives, poor zeta potential, and rapid clearance (0–6% retention at two weeks in large animals), raising critical questions about optimal dosing regimens and routes of administration [180–182]. Finally, large-scale, GMP-compliant production remains elusive: EV cargo, potency, and stability can shift with minor process changes, and no consensus exists on automation, storage (–80 °C vs. lyophilization), or release criteria [183]. These initial challenges in EV characterization and standardization are now being systematically addressed through updated international guidelines, such as MISEV2023, which provide a refined framework for the field [184].

To advance the field, it is crucial to define exosome potency units (EPUs) and standardize MSC culture, harvest, and storage practices under GMP guidelines for scalable pharmaceutical use. The development of advanced, single-vesicle analytical tools and closed-system manufacturing with built-in quality controls will ensure consistent EV identity and potency. Compared with MSCs, EV-based therapies are expected to have safer profiles, eliminating concerns such as mutagenicity and tumorigenicity [178].

Challenges and Limitations

Despite the promising results of EV-based therapies, several challenges and limitations must be addressed for clinical application, including large-scale production, lack of standardization, and biological safety. Overcoming these challenges through continued research will enhance MSC-EV-based strategies for musculoskeletal regeneration [185–188].

Production Issues: Scalability, heterogeneity, and isolation methods

Some limitations in EV utilization arise from production issues. Cells secrete low amounts of EVs, which are difficult to scale up, but isolation methods are not optimal, which makes clinical use expensive [189, 190]. However, scale-up strategies are needed for the clinical application of MSC-EVs. Studies have demonstrated that modified cell culture conditions, physicochemical changes, bioreactors, and genetic alterations increase the quantity of secreted EVs. A bioreactor can increase the EV yield up to tenfold compared with that of conventional culture flasks [191]. Additionally, hypoxic conditions lead to a 1.9-fold increase in daily EV secretion. Recent efforts have focused on developing scalable and GMP-compliant manufacturing processes to address these challenges [192]. Importantly, evaluating strategies may influence EV cargo, therapeutic efficacy, and function, limiting their usage in clinical applications [193, 194].

In addition to scalability, heterogeneity is another major production challenge. Studies have demonstrated that the composition and therapeutic properties of EVs from different mesenchymal tissues, even from one donor, are heterogeneous. Cell culture conditions, enrichment methods, and downstream process parameters also contribute to EV heterogeneity [5, 195, 196].

Production challenges persist during the isolation and characterization stage, which is considered the most difficult phase [197]. Although none of these isolation techniques are broadly approved, a wide range of conventional isolation methods are available, such as size exclusion [198], ultracentrifugation [199], and immunoaffinity capture [200]. These methods are not highly effective, and new strategies, including microfluidics [201] and membrane-based methods, have been developed. Each method offers its own advantages and disadvantages and is useful for a specific source [5, 202, 203]. Moreover, the size and density overlap between EVs and biological fluid components, such as lipoproteins, chylomicrons, and other EVs, complicate the isolation process [11, 27, 204]. Consequently, the choice of isolation strategy must be carefully aligned with the intended therapeutic application, given the inherent trade-off between scalability, purity, and functional EV yield [205]. Considering these findings, several investigations are needed for technical standardization, clinical preparation of EVs, and appropriate isolation methods for each condition [186, 206, 207].

Regulatory hurdles: lack of standardized protocols for EV therapies

The isolation method can affect EV cargo, purity, and efficiency, highlighting the importance of technical standardization [185, 208]. Nevertheless, there are no approved good manufacturing practices (GMPs) or global standards for clinical-grade EV production and application [5, 197]. The cell origin of EVs, in contrast to conventional pharmaceutical agents, leads to heterogeneity, biological activity, and various production methods, making this topic more challenging for the formulation of standardized procedures and regulatory approval [209]. Variability in EV isolation methods is particularly challenging for controlling and documenting GMP during EV production [210], and long-term immune reactions and biological side effects should be investigated via preclinical studies and clinical trials [211]. Establishing frameworks and adapted guidelines between researchers and regulators will be essential for progress in this field and ensuring safe and effective clinical translation in musculoskeletal regeneration [212].

Biological constraints: short half-life, immune clearance, and off-target effects

In terms of the therapeutic applications of MSC-EVs, researchers face biological constraints, including clearance, limited absorption, and off-target effects [213]. There is an inadequate understanding of EV interactions with specific cells or tissues and their uptake mechanisms, which are important for the approval of EV-based therapies [208, 214]. The aggregation of EVs in tissues such as the liver and spleen leads to a short plasma half-life and off-target effects [186]. This also disrupts the utilization rate and dosage [215], especially for systemic injections [186, 212]. Immune clearance also reduces the half-life of EVs because EV cargo is immunogenic and elicits immune responses. However, MSC-EVs have lower immune clearance [41, 216]. Unknown EV cargo molecules and their effects, alongside the modulation of numerous genes by a single miRNA, increase off-target effects [217]. On the other hand, EVs may induce apoptosis via caspase-3 and inhibit the accumulation of chemotherapy drugs, which enhances tumor progression [190, 218].

To overcome these challenges, many studies have investigated engineered EVs. Engineering technologies, especially surface modifications, can significantly increase their uptake rate and targeting efficiency and reduce EV clearance and off-target effects [219]. For example, studies have demonstrated that CD47 and signal regulatory

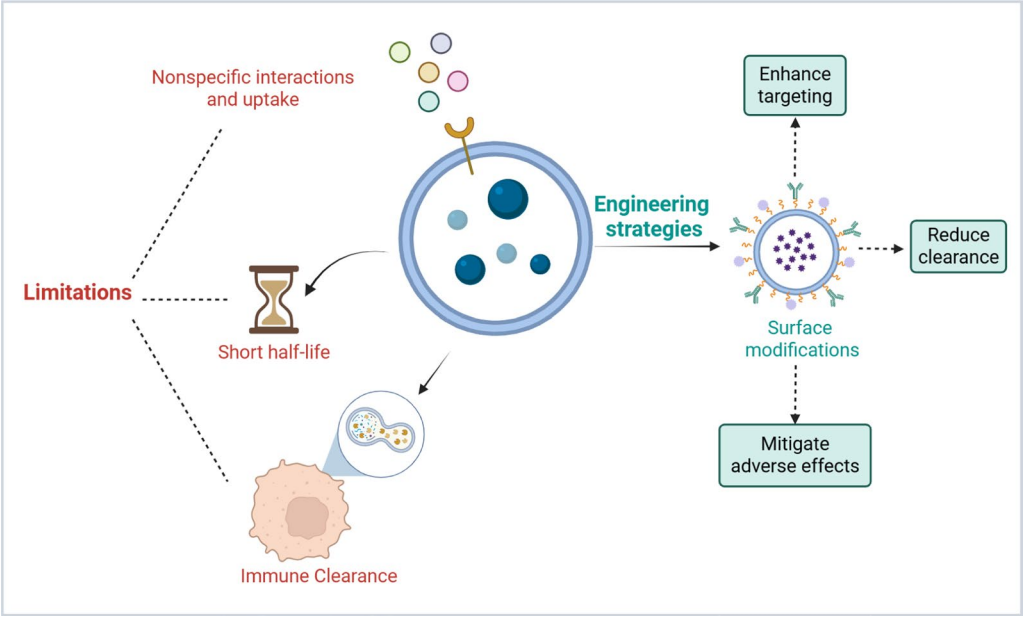


Fig. 9 Biological Constraints of MSC-EVs in Musculoskeletal Regeneration and Engineering Strategies to Enhance Therapeutic Efficacy They face several limitations, including immune clearance, a short half-life owing to hepatic and splenic accumulation, and off-target effects resulting from unknown cargo interactions and nonspecific uptake. Engineering strategies can enhance targeting, reduce clearance, and mitigate adverse effects. Created in BioRender. Aziziyan, F. (2025) https://BioRender.com/bzlosf0

protein alpha (SIRPα) in the exosomal membrane can reduce macrophage clearance [219, 220]. Additionally, dextran sulfate blocks scavenger receptor class A and decreases hepatic clearance [191]. These constraints and engineering approaches are summarized in Fig. 9.

Fundamental research is essential to elucidate the therapeutic mechanisms of MSC-EVs and enhance their efficacy and stability. Numerous studies are needed to establish standardized protocols for isolation, culture, preservation, and clinical application. Collaborative efforts between the scientific community and industrial sectors are needed to address technical, logistical, and commercial challenges [207, 216].

Conclusions

In musculoskeletal tissues, EVs are emerging as important mediators of cellular communication, regulating both homeostasis and disease processes. In addition to promoting regeneration, they can also contribute to inflammation and matrix degradation, thereby influencing disease progression. Owing to these dual roles, EVs are considered both attractive therapeutic agents and potential biomarkers for musculoskeletal conditions. The standardization of EV extraction and characterization, the determination of effective therapeutic dosages, and the use of appropriate preclinical models remain major challenges that must be addressed before translation into clinical applications can be achieved. For drug and gene delivery in particular, advances in EV engineering and preconditioning techniques offer promising opportunities to increase therapeutic efficacy. EV-based interventions hold strong potential to evolve into novel, targeted, and more effective regenerative strategies, driven by the increasing prevalence of musculoskeletal disorders and the limitations of current cell-based therapies.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13287-025-04879-1.

Additional file 1

Additional file 2

Author contribution

F.A. and S.S.A. conducted the literature search and data collection. M.M. and R.N.F. performed data analysis and interpretation. M.S. conceptualized the study, supervised the project, and was responsible for the overall manuscript preparation. F.A. and M.S. wrote the main manuscript text, and S.S.A. prepared the figures and tables. All authors reviewed, edited, and approved the final version of the manuscript.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern, Freiestrasse 3, 3012 Bern, Switzerland
[2]Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

[3]Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran
[4]Cellular and Molecular Research Center, Qom University of Medical Sciences, Qom, Iran

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