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Beyond conventional biomarkers: Emerging importance of extracellular vesicles in osteoarthritis, metabolic disorders and cardiovascular disease

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ABSTRACT

Extracellular vesicles (EVs), initially recognized for their roles in intercellular communication, are being increasingly explored for applications in the diagnosis and therapy of various diseases, particularly those driven by chronic inflammation. This review provides insight into the defining characteristics and functions of EVs, focusing on their role in contributing to and acting as potential therapeutics for chronic inflammatory diseases, including osteoarthritis, metabolic disorders such as obesity and metabolic dysfunction associated fatty liver disease, and cardiovascular diseases such as atherosclerosis and ischaemic stroke. Finally, the issues limiting EV translation from bench to bedside, and the outlook of EV research are discussed.

1. Introduction

1.1. Extracellular Vesicles (EVs)

EVs are bilayered phospholipid nanoparticles secreted by cells under both homeostatic and pathological conditions. EVs can carry a diverse array of bioactive substances derived from the host cell, encompassing proteins, lipids, and nucleic acids including DNA and RNA such as mRNA, micro-RNA (miRNA), and long non-coding RNA (lncRNA), with the exact composition determined by the parent cell type and their physiological or microenvironmental conditions. EVs deliver their cargo to target cells *via* specific mechanisms including ligand-receptor interactions and endocytosis, through which they modulate tissue homeostasis and facilitate intercellular communication.[1] In the current literature, EVs are frequently classified based on size and biogenesis: small EVs (previously known as exosomes), large EVs (previously known as ectosomes), and apoptotic vesicles (apoVs).[2] Small EVs below 200 nm in diameter typically originate *via* an exosomal pathway, while large EVs above 200 nm in diameter can arise through direct outward budding or pinching of the cell membrane.[2] ApoVs are released by dying cells and vary considerably in size, spanning diameters from 50 to 5000 nm.[2]

EVs play important roles in biological processes including cell motility, differentiation, proliferation, apoptosis, reprogramming, and immunity.[3] For instance, stem cell-derived EVs can facilitate tissue regeneration, while those from dendritic cells and macrophages can regulate immune responses.[4] Conversely, EVs derived from cancer cells can contribute to tumour metastasis by priming new sites for invasion through promoting inflammatory factor release, hampering immunosurveillance, enhancing angiogenesis, and increasing vascular permeability.[5] Conversely, EVs from various cell types have shown promising therapeutic effects against infectious diseases, diabetes, tumours, neurodegenerative disorders, and cardiovascular diseases.[6] EVs are vital in many physiological processes and hold significant potential for enhancing our understanding of cellular communication and immunomodulation pathways, enabling biomarker discovery as well as harnessing their functions for use as new therapeutics.

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1.2. EV isolation and characterisation

Research into EVs is complicated by their molecular and structural heterogeneity. To facilitate the standardization of EV research, the International Society for Extracellular Vesicles (ISEV) recently published updated recommendations for the study of EVs (MISEV2023), including on EV isolation, characterization, application, and the reporting of EV research.[7] The isolation and purification of EVs remain a challenge owing to the heterogeneity of different EV populations. Various techniques are used for the enrichment and purification of EVs from different sources such as biological fluids and cell culture media, including ultracentrifugation (UC), gradient UC, co-precipitation, size-exclusion chromatography (SEC), field flow fractionation, and affinity capture.[7] There is no defined gold standard for EV isolation, since the most suitable isolation method may depend on the EV source or final application, with the most popular techniques being UC, SEC, or a combination of these.[8]

To determine the presence of EVs within samples, as well as the quality and quantity of EV isolation, MISEV recommends biophysical characterisation of EV isolates and identification of EV markers (Fig. 1). EV samples need to be positive for at least one transmembrane/lipid binding protein (*e.g.* CD63, CD81, and CD9) and one cytoplasmic protein (*e.g.* TSG101, Annexin V, or ALIX), as well as be negative for non-vesicular extracellular particles.[7] EVs can also express major histocompatibility complex class I and II molecules (MHCI and MHCII, respectively), as well as surface integrins and endosomal sorting complexes required for transport (ESCRT).[9] Various imaging methods can be used for EV visualisation, such as electron microscopy (EM) and atomic force microscopy (AFM), while EV quantity and concentration can be measured by techniques such as nanoparticle tracking analysis (NTA) and dynamic light scattering (DLS).[7] The precise identification and classification of EVs may also be complemented by protein analysis and single vesicle imaging platforms.

1.3. Sources of EVs

EVs can be produced by multiple sources[10] and be classified as conventional EVs (from humans or animals) or non-conventional EVs (from bacteria, fungi, parasites or plants).[11] Conventional EVs can be isolated from different bodily fluids (*e.g.* blood, saliva, urine, breast milk, amniotic fluid, ascitic fluid). Biological fluid-derived EVs are increasingly utilized as biomarkers for healthy and different disease states, and whilst an important topic, it is beyond the scope of this review and has been discussed in detail elsewhere.[12]

EVs can be isolated from conditioned media resulting from culturing different cell types (*e.g.* stem cells, dendritic cells, macrophages, epithelial cells, and tumour cells),[13] where the field is seeing increased interest in using these cell-derived EVs as therapeutics. Prior to considering the translation of such approaches, many practical issues need to be resolved, including the determination of optimal cell sources, cell culture methods, biological state of the cells (*e.g.* proliferating or differentiating), growth conditions (including 2D or 3D culture systems),[14] and scalability.

EVs from other sources, such as bacteria and microalgae, can be produced in large quantities with relative ease,[15] and as such, has driven recent interest in these non-conventional sources of EVs. In addition, plant-derived EVs can be isolated from fruits, vegetables, and plant cells,[16] making them cost effective and highlighting a potential to be used as therapeutic alternatives. As an example, ginger-derived EV-like particles were recently found to contain miRNA, which could suppress the expression of pro-inflammatory cytokines in the lung tissue of mice infected with SARS-CoV2.[17] Further research is needed to clarify the potential of using non-conventional EVs as therapeutic alternatives in a range of diseases and infections.[15] This review will focus on the biological roles and therapeutic uses of conventional EVs.

1.4. Extracellular vesicles and cellular signalling

The molecular composition of EVs includes proteins such as cell surface receptors, signalling proteins, transcription factors, enzymes, and extracellular matrix (ECM) proteins, as well as lipids and nucleic acids cargo (such as miRNA, mRNA, and DNA) that can be transferred from parent to recipient cells, mediating intercellular communication and molecular transfer. EV composition can vary depending on the parent cell type or specific conditions, and their composition determines the functions of EVs.[18] Generally, EVs function as conduits for transporting diverse cellular constituents, facilitating intricate cellular communication and mediating a plethora of biological processes. The

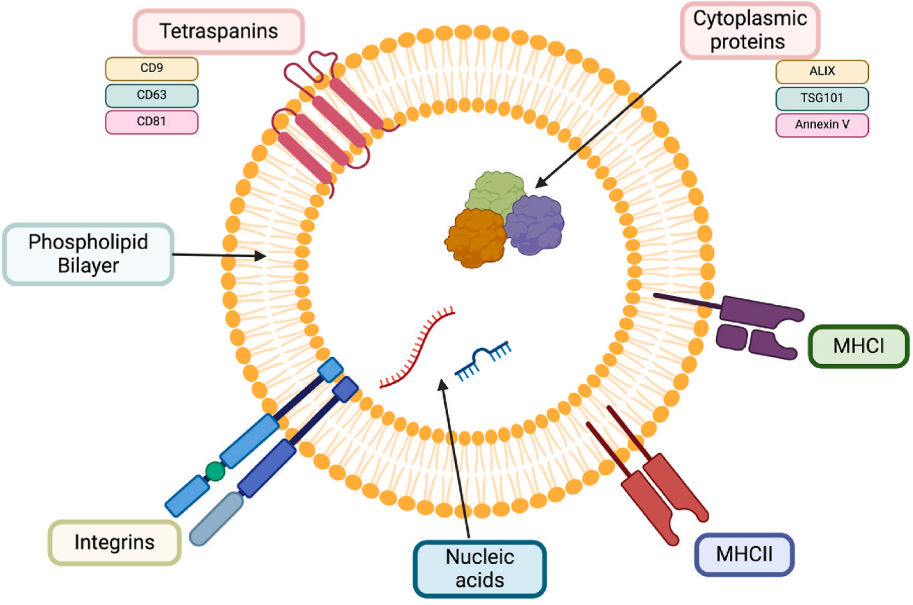

Fig. 1. Extracellular vesicle surface protein and cargo characterization
Apoptosis linked gene 2 interacting protein X (ALIX), tumour susceptibility gene 101 (TSG101), major histocompatibility complex (MHC). Created in BioRender. Zakarya, R. (2025) https://BioRender.com/i14f518.

Vesiclepedia online database provides a list of RNA, proteins, lipids and metabolites that are identified in EVs.[19]

EVs can serve as signaling complexes by transferring membrane receptors, delivering proteins to target cells, and modulating recipient cells through the horizontal transfer of genetic material.[20] Cellular communication mediated by EVs may involve cell targeting, EV fusion with the recipient cell, the release of EV cargo, and the transmission of molecular signals.[21] This intercellular communication is not only determined by EV composition and properties, but also by the cellular environment, such as ECM and other microenvironmental factors, as well as cell type, cell state and surface compounds of the parent and recipient cells. EV uptake by recipient cells may occur through multiple endocytic pathways, including clathrin-dependent endocytosis, caveolin-mediated uptake, macropinocytosis, phagocytosis, and lipid raft-mediated internalization.[22] Paracrine and autocrine signaling mediated by EVs may involve direct interactions between EV surface molecules and receptors on target cells, or indirect modulation of cellular pathways following EV cargo internalization.[23] The detailed regulatory mechanisms of EVs in cellular communication are reviewed in other papers.[20–24]

The physiological roles of EVs extend across a myriad of biological processes, including inflammation,[25] immune signaling, coagulation,[26] vascular reactivity,[27] angiogenesis,[28] and tissue repair.[29] Moreover, EVs have been implicated in the pathology of many diseases, such as cancer,[23] neurodegenerative disorders,[30] cardiovascular diseases,[31] and infections.[32] Their contributions often involve specific signalling pathways that are context-dependent. Comprehensive reviews on the role of EVs in these disease contexts are available in the cited literature.

2. EVs in models of chronic inflammatory diseases

Chronic inflammation induces pathological changes in tissues and organs, serving as a key driver of various diseases through inflammation-related pathways. Tissue EVs have been implicated in playing key roles in the pathogenesis of inflammatory diseases, by modulating the local immune response, participating in cellular communication by spreading inflammatory signals, transferring pathogenic factors between cells, and contributing to the persistence of inflammation within tissues.[26] Conversely, EVs have been seen to play crucial roles in physiological processes by suppressing inflammation, increasing anti-inflammatory factors, protecting target cells from apoptosis, and reducing the expression of disease related proteins.[18] Other EVs, particularly those derived from stem cells, have been posed as new therapeutics for chronic inflammatory diseases, benefitting from the innately anti-inflammatory and regenerative properties of stem cells. Examples of stem cell sources used to produce therapeutic EVs include mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs) that have both been shown to have regenerative functions in many human diseases such as cardiovascular disease, respiratory diseases, arthritis, and liver diseases.[33,34]

This review will explain the current knowledge on the role of EVs in disease pathogenesis relating to critical body systems including musculoskeletal, cardiovascular, and metabolic systems, and their potential to serve as new therapeutic opportunities.

2.1. Osteoarthritis

Osteoarthritis (OA) is a common chronic degenerative joint disease and the leading cause of disability worldwide.[35] This disease is characterized by synovial inflammation, progressive cartilage degradation, and subchondral bone remodelling, leading to joint pain, deformity, and dysfunction.[36] Many cellular factors and processes, such as transcription factors, epigenetic changes, cytokines, and proteases, play pivotal roles in regulating joint tissue homeostasis. Multiple pathophysiological conditions have been shown to induce and/or affect the progression of OA, including the presence of polyarticular disease, increasing age, obesity, joint instability and/or malalignment, muscle weakness, and peripheral neuropathy.[36] The disrupted joint tissue homeostasis is a key factor underlying the symptoms and structural damage observed in OA.[1] Notably, OA-associated immune cells, such as macrophages, within the synovium and infrapatellar fat pad have been shown to produce pro-inflammatory mediators, contributing to cartilage degradation.[37]

EVs play an important role in the pathogenesis of OA. The number and cargo content of EVs isolated from synovial fluid have been posed as an indicator of the severity of OA, with people with more severe forms of OA showing increased EV concentration containing a greater variety of peptides associated with the immune system.[38] EVs isolated from human synovial-like fibroblasts have been shown to induce OA-like changes in both *in vitro* and *ex vivo* models, such as significantly upregulating the expression of matrix metalloproteinase (MMP)-13 and a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS-5) in articular chondrocytes, while downregulating COL2A1 and ACAN genes coding for critical cartilage matrix components.[39] Similarly, subchondral bone osteoblasts have been shown to secrete small EVs containing miR-210-5p, which can increase the expression of MMP-13 and ADAMTS-5, as well as decrease SOX9 and COL2 chondrogenic factors in chondrocytes,[40] thereby contributing to the pathogenesis of OA.

2.1.1. EV therapies for OA

Traditional approaches for OA treatment predominantly focus on symptomatic relief. However, these have demonstrated limited efficacy in stopping or reversing the destruction of joint tissues including cartilage and subchondral bone.[41] Autologous conditioned serum therapy, which utilizes IL-1 receptor antagonist, takes advantage of the innate healing properties of blood serum to promote tissue repair, modulate inflammation, and bolster immune function, presenting a potential treatment avenue for OA.[42] However, despite promising results in animal models, the clinical efficacy of autologous conditioned serum and platelet-rich plasma remains controversial due to the nature of autologous blood-derived products.[43] Research has revealed the presence of abundant blood-derived EVs within these products,[44] suggesting the potential of EVs as vital components for OA treatment. Studies indicate that these blood-derived EVs, containing functional mitochondria and low levels of pathogenic cytokines, actively engage in substance exchange and cellular communication processes, exhibiting anti-inflammatory properties and inducing chondroprotective gene expression.[45] Additionally, advancements in regenerative medicine have led to the exploration of stem cell therapies for OA tissue repair, with MSCs showing promise due to their self-renewal, differentiation, and immune regulation capabilities.[46]

In contrast to transplanting exogenous MSCs (*i.e.* cell therapy), EVs represent an alternative regimen that is non-proliferative, non-immunogenic, and easier to store and transport than live cells. EVs also overcome the potential problems encountered with stem cells, such as low survival rate and uncontrollable or imprecise differentiation once delivered inside the body.[47] Numerous studies have demonstrated notable efficacy of MSC-derived EVs (MCS-EVs) in OA on pain relief, inhibition of inflammation, immunomodulation, and cartilage tissue regeneration.[48] A variety of MSC sources have been used to generate MSC-EVs, including bone marrow, adipose tissue, umbilical cord, synovial membrane/fluid, and MSCs derived from embryonic stem cells and iPSCs. Examples of these studies have been summarized in Table 1 and can be found illustrated in Fig. 2.

Several studies have investigated the function of human adipose-derived MSCs (hAD-MSCs) in OA therapy. Tofio-Vian et al.[49] found that hAD-MSC-derived EVs reduced the production of inflammatory mediators, tumor necrosis factor, interleukin 6 (IL-6), prostaglandin E2 and nitric oxide in IL-1 stimulated OA chondrocytes, while they increased the expression of anti-inflammatory cytokine IL-10 and cartilage matrix component type II collagen (COL II). Woo et al.[50] discovered that hAD-MSC-derived EVs (hAD-MSC-EV) not only increased human chondrocyte proliferation and migration, but also

Table 1
A summary of extracellular vesicle therapies in osteoarthritis.

Type of EV therapeutic	Effects in OA treatment	References
Adipose MSC EVs	Reduction in inflammatory mediators; reduction in proteins responsible for ECM degradation; increased type II collagen production; increased expression of anti-inflammatory cytokines	49
Adipose MSC EVs	Reduction in proteins responsible for ECM degradation; increased type II collagen production *in vitro*; reduction in disease progression and prevention of cartilage degeneration *in vivo*	50
MSC-EV miR-135b	Suppression of Sp1 and promotion of TGF-β1; increase chondrocyte proliferation	51
Bone marrow MSC EVs	Alleviation of pain in lumbar facet joint; attenuation of cartilage degeneration; facilitation of subchondral bone remodelling through inhibition of RANKL-RANK-TRAF6 signalling	52
Bone marrow MSC EVs	Inhibition of p38 and ERK1/2 phosphorylation; stimulation of Akt signalling pathway; maintaining chondrocyte proliferation during inflammatory stimulation	53
Bone marrow MSC EVs	Increased expression of chondrocyte markers and reduction of catabolic and inflammatory markers *in vitro*; protection of mice from joint damage *in vivo*	54
Umbilical cord MSC EVs miR-23a-3p	Enhanced chondrocyte and MSC migration, proliferation and differentiation; suppression of PTEN expression and increasing Akt pathway signalling	55
Primary chondrocyte EVs	Amelioration of mitochondrial dysfunction; polarisation of macrophages towards M2 phenotype	56
M2 macrophage EVs	Reduction of inflammatory response and articular damage in knee osteoarthritis; modulation of PI3K/Akt/MTOR signaling pathways	57
Fibroblast like synovium EVs lncRNA H19	Enhanced cell viability and migration; reduced ECM degradation in chondrocytes	58

reduced the expression of catabolic enzymes (MMP-1, MMP-3, MMP-13, and ADAMTS-5) involved in the degradation of cartilage ECM, as well as increased type II collagen production even in the presence of IL-1 inflammatory stimulation. Moreover, *in vivo* findings showed that intra-articular injection of hAD-MSC-EVs dramatically slowed the development of OA and prevented cartilage degeneration. Despite these results, there remains issues with large scale production of EVs to clinically relevant doses as well as the optimatisation of effective dosage and administration routes, highlighting the need for further research and the development of improved manufacturing processes to ensure consistent quality and efficacy.

EVs derived from bone marrow MSCs (BM-MSCs) have a broad influence on cellular behaviour, such as apoptosis, proliferation, invasion, and migration.[1] Additionally, BM-MSC-EVs regulate various physiological and pathological processes, including the immune response, osteogenesis, fibrosis, and angiogenesis. Numerous studies have indicated that BM-MSC-EVs promote the repair and regeneration of damaged joint tissues, notably cartilage and subchondral bone,[1] due to their specific cargo content and targets. Wang et al.[51] discovered that MSC-EV-derived miR-135b from rats, suppressed the expression of transcription factor Sp1 and promoted the expression of transforming growth factor beta 1 (TGF-β1), a growth factor with critical roles in facilitating chondrocyte proliferation, phenotype maintenance, and synthesis of ECM components, such as collagen and proteoglycans in a rat model of OA. Li et al.[52] found that BM-MSC-EVs from murine MSCs relieved OA-related pain through abrogation of aberrant CGRP-positive nerve and abnormal H-type vessel formation in the subchondral bone of the lumbar facet joint in mice. Additionally, BM-MSC-EVs attenuated cartilage degeneration and inhibited tartrate-resistant acid phosphatase expression and RANKL-RANK-TRAF6 signaling activation to facilitate subchondral bone remodelling in mice. Qi et al.[53] demonstrated that BM-MSC-EVs from rabbits can effectively maintain chondrocyte viability in an inflammatory environment and promote chondrocyte proliferation by inhibiting p38 and ERK1/2 phosphorylation and stimulating the protein kinase B (Akt) signaling pathway in culture. The above evidence indicates that BM-MSC-EVs could effectively maintain chondrocyte viability in an inflammatory environment. Cosenza et al.[54] reported that murine BM-MSC-EVs protected chondrocytes from apoptosis and

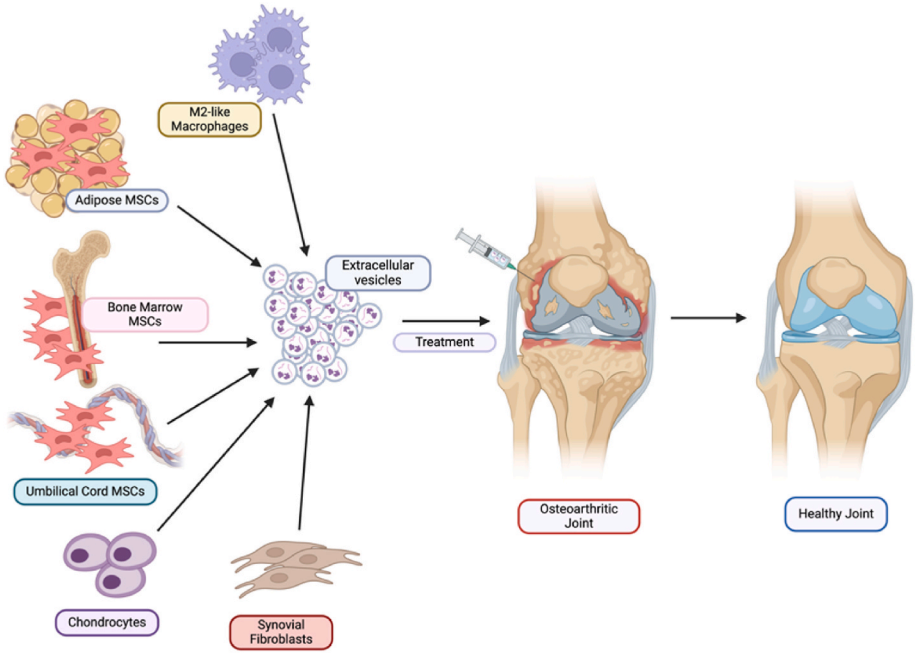

Fig. 2. Extracellular vesicles for the treatment of osteoarthritis *MSCs (Mesenchymal Stem Cells). Created in BioRender. Zakarya, R. (2025)* https://BioRender.com/c17e350.

suppressed macrophage activation *in vitro*. Meanwhile, *in vivo* experiments showed that these EVs could increase the expression of chondrocyte markers (COL II and ACAN) while reducing the levels of catabolic (MMP-13, ADAMTS-5) and inflammatory (iNOS) markers, suggesting the potential of BM-MSC-EVs in protecting against the development of OA.

Human umbilical cord MSCs (hUC-MSCs) exhibit many beneficial biological characteristics, especially robust growth capacity compared to other MSC sources.[1] The therapeutic potential of hUC-MSC EVs for treating OA has been investigated in several studies. For example, Hu et al.[55] found that hUC-MSC EVs enhanced chondrocyte and BM-MSC migration, proliferation, and differentiation. This was mediated by the transfer of miR-23a-3p, the most abundant miRNA expressed in hUC-MSC EVs, which suppressed phosphatase and tensin homolog (PTEN) expression while increasing Akt expression, thereby enhancing cartilage regeneration. Yan et al.[59] discovered that hUC-MSC EVs transferred the lncRNA H19 to chondrocytes. Within chondrocytes, lncRNA H19 functioned as an endogenous sponge for miR-29b-3p and directly interacted with proteins, including Forkhead box O3 (FoxO3), thereby contributing to the regulation of cellular processes critical for cartilage repair and regeneration, as well as modulation of chromatin structure. Additionally, Yan et al.[60] discovered that EVs derived from hUC-MSCs through 3D culture (using a hyper-fiber bioreactor) exerted a more potent effect in stimulating chondrocyte proliferation, migration, and matrix synthesis, while inhibiting apoptosis, compared to EVs obtained through conventional 2D culture methods. This enhanced efficacy may be attributed to the activation of TGF-β1 and Smad2/3 signaling pathways and may represent a new avenue of EV production and therapeutic benefit.

The therapeutic potential of EVs derived from other cell sources has also been studied. Zheng et al.[56] reported that EVs isolated from primary chondrocytes exhibited the ability to ameliorate mitochondrial dysfunction and polarize macrophages towards an M2 phenotype. Furthermore, Song et al.[61] encapsulated primary chondrocyte-derived EVs in a hydrogel matrix, and *in vivo* intra-articular injection demonstrated that sustained local release of these EVs could alleviate OA by promoting the phenotypic transformation of macrophages from M1 to M2. Zha et al.[57] studied M2 macrophage-derived EVs and found that they markedly reduced the inflammatory response and pathological damage to articular cartilage in OA rats. This protective effect was primarily mediated through modulation of the PI3K/Akt/mTOR signaling pathway, a crucial intracellular signaling pathway involved in regulating various processes such as cell growth, proliferation, survival, metabolism, and protein synthesis. Tan et al.[58] studied fibroblast-like synovium derived EVs and found that these could enhance chondrocyte cell viability and migration as well as alleviate matrix degradation. This beneficial effect was attributed to cellular signaling regulation mediated by lncRNA H19 carried by EVs, which modulated the miR-106b-5p/TIMP2 axis. This collection of recent studies highlights the therapeutic potential of EVs from multiple cellular sources in the treatment and alleviation of OA, through the modulation of inflammation and promotion of tissue repair through key signaling pathways.

In patients with OA, associations have been found between the severity of cartilage lesions and aberrant expression of miRNAs.[62] Therefore, researchers have directed efforts toward customizing (engineering) EVs to modulate miRNA expression. The goal of this is to be able to more specifically modify diseases features including mitigating inflammation and apoptosis, modulating chondrocyte proliferation and migration and promoting chondrocyte matrix secretion.[63] Engineered EVs also offer advantages over naturally-derived EVs as they can be made to target specific cell types or contain desired molecules to target cellular pathways. This solves issues associated with off-target effects and allows therapeutic molecules to be delivered in significantly smaller doses, overcoming the drawbacks of potential adverse effects arising from high-dose systemic administration as well as high therapeutic cost. For example, Liang et al.[64] adopted a new approach by incorporating a chondrocyte-affinity peptide (CAP) with the lysosome-associated membrane glycoprotein 2b onto the surface of chondrocyte-targeting EVs. These engineered CAP-EVs exhibited efficient encapsulation of miR-140 and transport into the deep cartilage region, navigating through the dense mesochondrium. This targeted delivery strategy effectively suppressed cartilage-degrading proteases, thereby contributing to the alleviation of OA progression. In another study, Liu et al.[65] alleviated OA symptoms in rats by loading exogenous miR-223 into hUC-MSC EVs by electroporation and modifying their surface protein corona with a collagen II-targeting peptide (WYRGRL), which achieves more targeted and efficient RNA delivery to cartilage. This dual-engineered EV showed an enhanced effect on inhibiting NLRP3 inflammasome activation and chondrocyte pyroptosis.[66] Xu et al.[67] engineered small EVs to deliver kartogenin (a molecule that drives cartilage repair and regeneration) into synovial fluid-derived MSCs before transplanting the modified cells into the joints of OA rats, which showed superior therapeutic effects in cartilage regeneration and OA treatment compared to pure kartogenin controls. These studies highlight the potential of engineering EVs to complement EV-based therapies,[67] endowing the capability to more specifically target key pathological processes involved in OA.

To reverse the surface charge of MSC-EVs to enhance chondrogenic absorption, cartilage penetration and joint retention, Feng et al.[68] modified MSC-sEVs with a new cationic amphiphilic polymer ε-polylysine-polyethylene-stearyl phosphatidylethanolamine (PPD). The resulting new PPD-sEVs showed more effective cellular uptake and homeostasis modulation ability in chondrocytes than unmodified MSC-sEVs. To address the issues of rapid joint clearance of drugs (*i.e.*, short half-life) and therapeutic targets deep in the cartilage that drugs can hardly reach, Wei et al.[69] loaded EVs with phospholipase a2 inhibitors to penetrate deep into the cartilage matrix and reduce inflammation and prolong joint space retention. Additionally, some studies found that EVs isolated from stimulated or pre-conditioned stem cells may lead to enhanced outcomes in OA treatment. For example, Rong et al.[70] showed that EVs obtained through hypoxic stimulation of MSCs enhanced the proliferation, migration, and apoptosis suppression of chondrocytes through the regulation of miR-216a 5p/JAK2/STAT3 signaling pathway compared to EVs derived from MSCs cultured in normoxia. Meanwhile, Sun et al.[71] discovered that EVs derived from TGF-β3 pre-conditioned BM-MSCs presented enriched miR-455 that could alleviate OA development and promote cartilage regeneration by activating the SOX11/FOXO signalling pathway. Liu et al.[72] also discovered that EVs derived from kartogenin-preconditioned BM-MSCs were more effective in promoting cartilage matrix formation and reducing degradation in rats with critical cartilage injuries compared to EVs derived from unconditioned BM-MSCs.

The above studies indicate significant promise of using EVs from various cell sources as new therapeutic agents for OA. However, the translation of EVs in OA treatment still requires overcoming some challenges. As OA is a whole-joint disease, large amounts and repeated administration of EVs may be necessary to produce long-term effects at modulating disease, for which the large-scale production of EVs is currently a challenge. Furthermore, uncertainties remain regarding the biological distribution, pharmacokinetics, and specificity of EVs delivered into OA joints, which may undermine the effectiveness of EV therapy and hinder its clinical translation. Continuous technological innovations and the broader adoption of pre-clinical models that better mimic human-like disease states will help to overcome these limitations and unlock the full therapeutic potential of EVs in OA treatment.

2.2. Cardiovascular diseases (CVD)

CVD encompasses a variety of diseases affecting the heart and vascular system, and are the leading cause of death globally.[73] These include coronary heart disease, hypertension, and cerebrovascular

disease, resulting in serious adverse outcomes such as stroke and myocardial infarction (MI), which can lead to death. The development of CVD is often multifactorial including both genetic factors such as sex and age, and modifiable risk factors such as obesity and smoking. In some patients, clinical presentation is completely asymptomatic, often making diagnosis challenging, such as for those with silent ischemia and hypertension.[74] Despite currently available treatments for the primary and secondary prevention of CVD, such as beta blockers, statins and other lipid lowering medications, there is still significant mortality from CVD worldwide. EVs play important roles in the pathogenesis of CVD, and recent evidence suggests that they may provide new therapeutic opportunities. Examples of these studies are summarized in Table 2 and Fig. 3A for myocardial infarction, 3B for Ischemic Stroke and 3C for Atherosclerosis.

2.2.1. EVs and myocardial infarction

EVs play important roles in maintaining cellular communication within the heart, and their production is significantly increased following tissue damage or injury.[80] During MI, the heart is subjected to hypoxic stress, which leads to significant cardiomyocyte damage. Cardiomyocyte apoptosis induces proliferation in cardiac fibroblasts, contributing to cardiac remodelling and impaired cardiac function. Following coronary artery ligation in mice, the release of EVs from the left ventricle was significantly increased compared to sham mice at 15 and 24 h post-ligation, with cardiomyocytes and endothelial cell-derived EVs contributing significantly to this pool.[81] Circulating miRNAs are often used as biomarkers for cardiac disease and are transported within the systemic circulation encased in EVs.[82] Circulating miR-21 has been linked with the development of cardiac fibrosis and hypertrophy, and proposed as an effective biomarker of heart failure mortality and re-hospitalisation.[83] miR-21 was also found to be significantly increased in EVs derived from cardiac fibroblasts compared to expression levels in the parent cardiac fibroblasts,[84] and levels in EVs were further increased when cardiac fibroblasts were subjected to the hypertensive agent angiotensin II.[84] Furthermore, when cardiomyocytes were exposed to EV-depleted media from cardiac fibroblasts, the hypertrophic effects were reduced compared to non-EV-depleted conditioned media.[84] Following infarction, cardiomyocytes also release EVs, which have been shown to contain miR-328-3p, a miRNA involved in cellular apoptosis.[85] When EVs isolated from infarcted cells were applied to healthy cardiomyocytes, a significant increase in cellular apoptosis was observed, most likely through the activation of caspase-3 pathways.[85] These studies highlight the important roles of EVs in potentiating death responses in cardiomyocytes following MI, as well as the development of cardiac remodelling and subsequent fibrosis.

Contrary to the above, EVs isolated from stem cells have been shown to promote cardiac recovery following MI. When EVs isolated from cardiac progenitor cells were delivered by intramyocardial administration to pigs that had undergone experimental MI followed by reperfusion, treated animals showed significant improvements in left ventricular function and reduction in infarct size.[75] Furthermore, at one month post infarct, pigs treated with EVs showed significantly reduced scar size and mass, and reduced fibrotic area both at the injection site and across the whole heart. The results of this study are supported by earlier findings that EVs from MSCs carry paracrine factors such as vascular endothelial growth factor, fibroblast growth factor 2 and hepatocyte growth factor, contributing to the success of MSC therapy in models of MI.[86]

Table 2
A summary of EV therapies for cardiovascular diseases.

Type of EV therapeutic	Disease model	Treatment effects	References
Cardiac progenitor cell EVs	Experimental myocardial infarction	Improvements in left ventricle function; reduction in infarct size; reduction in cardiac fibrosis	75
Bone marrow MSC EVs miRNA-138-5p	Ischemic stroke	Promotion of astrocyte proliferation; reduction in inflammation	76
Cardiac progenitor cell EVs mir-322	Experimental myocardial infarction	Reduction in infarct size; promotion of angiogenesis;	77
MSC EVs miR-145	Atherosclerosis	Reduction in atherosclerotic development; promotion of tight gap junctions; reduction of monocyte attachment; reduction of endothelial cell migration	78
Cardiac progenitor EVs overexpressing CXCR4	Experimental myocardial infarction	Improvement of ejection fractions; reduction in infarct size and scar size	79

2.2.2. EVs and atherosclerosis

Atherosclerosis is defined as a chronic, low grade inflammatory disease with endothelial damage leading to the gradual development of lipid-rich plaques in arteries. In some cases, these plaques can rupture and lead to ischemic heart attack or stroke, or occlude blood flow. The number of circulating endothelial cell (EC) EVs has been shown to increase with cardiovascular risk factors, such as metabolic syndrome and dyslipidaemia,[87] possibly as a result of direct endothelial injury and EC apoptosis. Stimulated ECs and their derived EVs have been shown to significantly impair vascular function, particularly the function of the endothelium, through their capacity to carry superoxide.[88] Moreover, EVs from human coronary ECs exposed to high glucose induced significant impairments in endothelial function when administered to atherosclerotic mice.[89] These mice also showed increased macrophage recruitment into lesions and increased production of reactive oxygen species (ROS), suggesting that EVs generated under diabetic conditions might promote the future development of atherosclerosis.[89]

Endothelial progenitor cells (EPCs) are circulating cells with similar surface markers as those in the endothelium of vessels.[90] Upon endothelial damage, they adhere to blood vessels and participate in new vessel formation.[90] EVs from EPCs have been shown to reduce vascular oxidative stress, inflammation and atherosclerosis when injected into diabetic atherosclerotic mice.[91] Moreover, EPC-derived EVs contain multiple miRNAs that can promote angiogenesis and regulate inflammation. For example, Li et al.[92] identified that miR-199a-3p serum levels were greatly reduced in atherosclerotic mice, which correlated with increased serum levels of pro-inflammatory IL-6 and greater lesion coverage. Meanwhile, miR-199a-3p expression was significantly increased in EPC-derived EVs and could be delivered directly to ECs. The effects of these EVs included reducing smooth muscle cell proliferation and migration, EC ferroptosis, and endothelial injury, which in turn prevent or slow atherogenesis.

2.2.3. EVs and ischaemic stroke

Stroke is a significant outcome of CVD associated with high mortality, with up to 20 % of ischaemic strokes occurring due to carotid plaque rupture.[93] The disruption of blood flow to the brain causes the development of hypoxia and local shortage of glucose supply, resulting in extensive neuronal damage and apoptosis,[94] and can result in significant physical disabilities or death.[95] The number of EC-EVs increases significantly following ischaemic stroke, likely as a result of EC apoptosis and inflammation within the penumbra,[96] and this increase has been proposed as a potential biomarker for the severity of stroke. In a mouse model of ischaemic stroke,[97] MSC-EVs administered through tail vein injection were observed to home to the ischaemic lesion site within the brain, and treated mice showed reductions in inflammatory markers accompanied by improved neuroregeneration and angiogenesis following stroke. In patients with transient ischaemic attack,[98] the

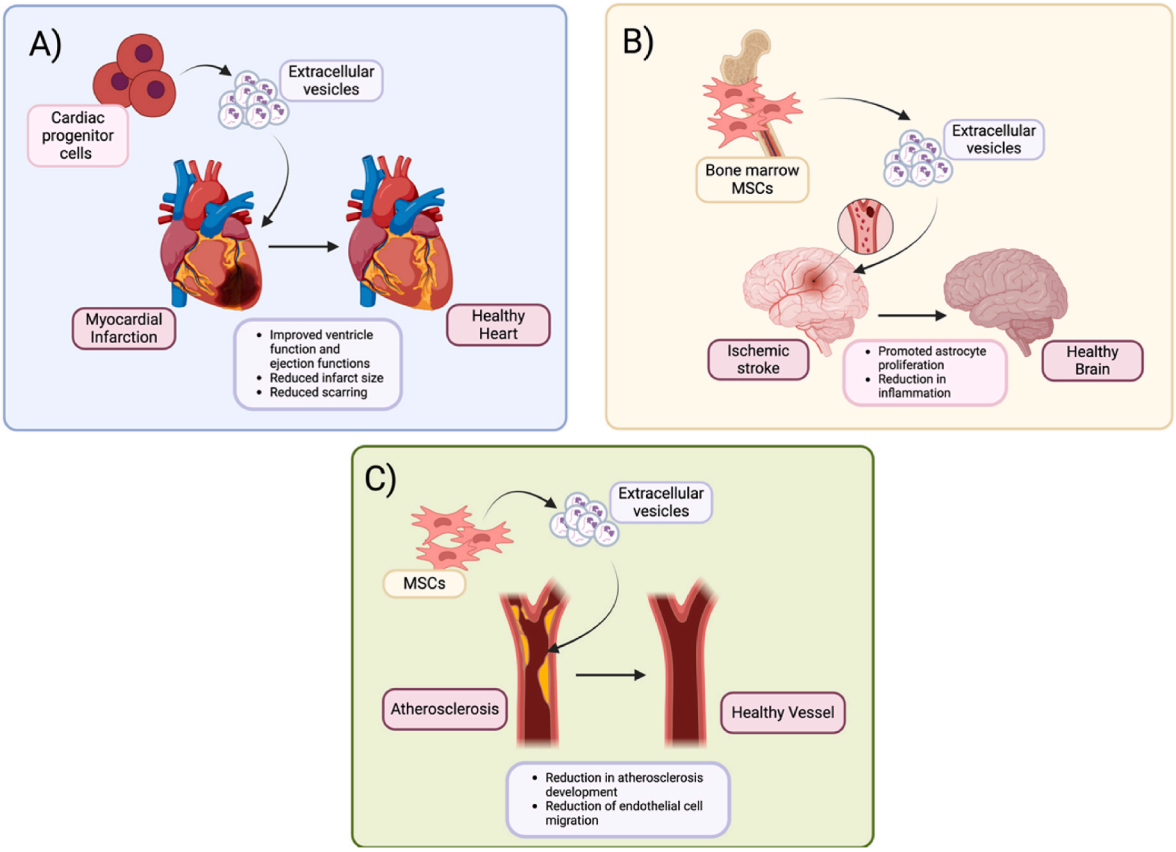

Fig. 3. Extracellular vesicles for the treatment of cardiovascular disease
MSCs (Mesenchymal Stem Cells). Created in BioRender. Zakarya, R. (2025) https://BioRender.com/q64g93.

number of circulating EVs is increased compared to healthy controls, with a significant number of these EVs derived from platelets as a key component in the pro-thrombotic cascade. The EVs also presented specific antigens which might be useful as biomarkers for predicting the probability that the patient had, or was likely to have, a real ischemic stroke as well as stroke severity. This study highlights the potential utility of EV profiling in biomarker discovery and disease prediction for CVD.

2.2.4. *EVs as therapeutics for cardiovascular diseases*

The advantages of using EVs as therapeutics for CVD compared to live cells arise from their improved retention in the body and low immunogenicity, allowing treatments to be administered over longer periods between doses or with lower dose levels, and with less immune clearance, overall leading to reduced burden on patients. As a new approach to developing CVD therapeutics, EVs may be engineered to serve as targeted drug delivery systems by encapsulating specific molecules or drugs. A systematic review analysing 28 pre-clinical studies containing over 1760 animals[99] showed that engineered EVs performed better than unmodified stem cell-derived EVs when used to treat ischaemic stroke. Overall, engineered EVs produced better effects in reducing infarct size within the brain and promoting behavioural and neurological recovery and function in treated animals. As an example, Deng et al.[76] engineered EVs using BM-MSCs overexpressing miRNA-138-5p. These EVs were found to promote astrogliosis and reduce inflammatory responses after ischaemic stroke in mice.

EV cargo can be modified to enhance their therapeutic function in treating CVD, which can be achieved through various techniques such as electroporation, sonication, extrusion, and repeated freeze-thaw cycles. For instance, EVs derived from cardiac progenitor cells (CPCs) and transfected with pro-angiogenic miR-322 showed significant cardioprotective functions in a mouse model of MI following systemic administration.[77] Mice treated with CPC-miR-322 EVs showed reduced infarct size and enhanced angiogenesis through the production of Nox2 dependant H_2O_2. Modified EVs have also been effective in treating models of atherosclerosis. For example, EVs from MSCs and adipose tissue transfected with small interfering RNA (siRNA) targeting Smad 2/3 showed significant improvements in vascular function and a reduction in vascular wall thickness when administered to atherosclerotic mice.[100] In another study, EVs from human MSCs transfected with miR-145 significantly reduced the development of atherosclerosis in mice, by promoting tight gap junctions to inhibit monocyte attachment and reduce endothelial cell migration.[78]

EVs can also be engineered to change their surface proteins, which may enhance their affinity for specific cell types. Ciullo et al.[79] engineered CPC-EVs to overexpress CXCR4, which increased their binding to stromal derived factor 1α (SDF-1α). Following MI in mice, increased tissue expression of SDF-1α enhanced the delivery of CPC-EVs overexpressing CXCR4 to the infarct area, resulting in significant reductions in infarct size and improvements in ejection fraction at 1 month post infarction compared to mice treated with unmodified CPC-EVs.

Studies assessing engineered EVs in CVD treatment are currently limited, but the potential of this new therapeutic approach is promising. Future research may benefit from engineering the surface protein corona of EVs to improve homing to target organs, as well as the optimization of drug loading protocols to enhance loading efficiency and minimize off-target effects.

2.3. *EV therapies for metabolic disorders*

Disturbances in metabolism can negatively impact cellular nutrient processing, which can lead to insulin resistance, hyperglycaemia,

dyslipidaemia, and adiposity.[101] Metabolic disorders are commonly associated with obesity and can result in a range of conditions, such as CVD, type 2 diabetes, and metabolic disease associated fatty liver disease (MAFLD).[101] Obesity is caused by an imbalance between energy intake and expenditure, leading to fat over accumulation and is a significant risk factor for type 2 diabetes and CVD.[101] Insulin resistance during type 2 diabetes can impair cellular uptake of blood glucose for ATP synthesis, which in turn mobilizes lipids in the adipocytes as an alternative fuel. This process can lead to hyperlipidemia and ectopic lipid accumulation in the liver, resulting in accelerated atherosclerosis and MAFLD.[102] As a result, CVD complications are the leading cause of death in patients with type 2 diabetes.

MAFLD encompasses a range of pathological changes, including excessive fat accumulation in the liver (>5 %, simple steatosis), metabolic disease associated steatohepatitis (MASH), and severe fibrotic changes, *i.e.* cirrhosis.[103] It has been estimated that the risk of MAFLD among overweight/obese individuals can be as high as >50 %, which will be the leading cause of liver transplant in the next 20 years.[104] Despite the significant disease and economic burden imposed by MAFLD, to date, Resmetirom is the only FDA-approved drug for MAFLD, a thyroid hormone receptor B agonist effective in 30 % of patients in a Phase 3 clinical trial.[105] Although there is active research to turn the antidiabetic and antiobesity drug glucagon-like peptide-1 (GLP-1) agonists into an MAFLD treatment, large-scale and long-term clinical trials are needed to provide strong evidence to re-purpose this group of drugs. Also, the high cost and global supply shortage make this drug not accessible to many diabetic patients. Therefore, new treatments are still urgently needed for metabolic disorders, given their global rising prevalence.

MSCs, which are capable of self-renewal and differentiation, have been gradually applied to the MAFLD treatment in clinics. However, the safety concern about tumorigenicity and immune rejection is still challenging. Compared to MSCs, the EVs from MSCs address the many risks and limitations associated with direct MSC administration. For example, EVs lack cellular components and thus cannot differentiate into tumor cells, which eliminates the risk of tumorigenesis. Additionally, EVs are less likely to provoke immune reactions compared to MSCs because of the absence of immunogenic surface markers. Furthermore, the ease of storage, handling and scalability makes EVs a better candidate for future clinical applications.

A limited number of studies have investigated the therapeutic potential of EVs, including their miRNA content, in metabolic disorders, which are summarized in Table 3 and illustrated in Fig. 4. Persistent inflammatory response is the main cause of liver fibrosis, which could activate hepatic satellite cells (HSCs) and transform HSCs into cell types linked to fibrosis. Furthermore, activated HSCs can release collagen, fibronectin and other substances that form collagen fibres and finally lead to fibrosis. Numerous studies have demonstrated that EVs from MSCs can inhibit the activation of HSCs and further alleviate the progression of fibrosis. In mouse models of high fat diet induced obesity, treatment with adipose stem cell-derived EVs improved glucose tolerance and insulin sensitivity, lowered blood levels of triglyceride and total cholesterol, and ameliorated liver steatosis.[106] In mouse models of fibrosis induced by CCL4, increasing miR-486-5p in human tonsil-derived MSCs suppresses hedgehog signaling amnd ameliorates hepatic fibrosis.[107] Additionally, exosomes derived from hUC-MSCs and human embryonic stem cell (hESC) reduced collagen deposition by inactivating the TGF-beta/Smad signaling pathway.[108,109] As described above, continuous inflammatory responses are primary drivers of liver fibrosis. Therefore, various studies have explored the therapeutic effects of EVs from MSCs in suppressing the accumulation and activation of inflammatory cells. For example, in rat fibrosis models induced by CCL4, EVs from amnion-derived MSCs (AMSCs) reduced Kupffer cell numbers through suppressing the TLR4 signaling pathway.[110] Additionally, EVs derived from human bone marrow MSCs (hBM-MSCs) reduced inflammation by inhibiting the Wnt/β-catenin signaling pathway.[111] In a mouse model of liver fibrosis and inflammation using a methionine- and choline-deprived diet, EVs derived from human liver stem cells were shown to reduce liver fibrosis and inflammation.[112] In addition, EVs from human adipose-derived stem cells can promote energy expenditure by increasing the expression of uncoupling protein 1, thus reducing weight gain induced by high fat diet consumption, as well as improving glucose tolerance and mitigating liver steatosis.[113] miR-193b, miR-328, miR-378a and miR-196a within the EVs were suggested to be

Table 3
Studies using EVs to treat metabolic disorders.

EV source	Disease model	Responsible molecules	Actions	References
C57BL/6J mice bone marrow	STZ induced Type 1 diabetes in C57BL/6 mice	miR-106b-5p and miR-222-3p	Promote pancreatic β-cell proliferation; down-regulate Cip/Kip pathway	119
C57BL/6J mice Adipose derived stem cell	High fat diet induced obesity in C57BL/6 mice	STAT3 protein	Improve glucose tolerance, insulin sensitivity and hyperlipidaemia; reduce liver steatosis; turn macrophage to anti-inflammatory M2 type.	106
Human tonsil-derived stem cells	CCL4 induced fibrosis model in male C57BL/6 mice	miR-486-5P	Target the hedgehog receptor to suppress hedgehog signaling	107
Human embryonic stem cells	CCL4 induced fibrosis model in male ICR mice	miR-6766-3p	Suppress LX2 cell activation through TGFβRII/Smad pathway	109
Amnion-derived stem cells	CCL4 induced fibrosis model in male Sprague-Dawley rats	Toll-like receptor 4 (TLR4)	Decrease fibre accumulation, KC number, and hepatic stellate cell (HSC) activation	110
Human bone marrow derived stem cells	CCL4 induced fibrosis model in female Sprague-Dawley rats	Wnt/β-catenin signaling pathway	Reduce serum levels of liver enzymes, including ALT, AST, TBIL, ALP, and γ-GT	111
Human adipose derived stem cells	High fat diet induced obesity in BALB/c mice	miR-193b, miR-328, miR-378a and miR-196a	Increase UCP1 and Dio2 expression in brown fat; reduce weight gain and liver steatosis; improve glucose tolerance.	113
Human umbilical cord MSC	High fat diet and STZ induced type 2 diabetes in Sprague Dawley rat		Reduce blood glucose levels; partially reverse insulin resistance; increase liver glycogen storage. Restore IRS1 and PKB phosphorylation; promote muscle Glut4 expression; prevent β-cell apoptosis.	120
Human liver stem cells	Methionine- and choline-deprived diet induced NASH in NOD/SCID mice		Prevent liver cell injuries; reduce liver fibrosis; downregulate liver pro-fibrotic and pro-inflammatory genes.	112
Plasma from healthy C57BL/6J mice; EV loaded with antagomiR-122, antagomiR-192 or miR-133b mimics	High fat diet induced obesity in C57BL/6 mice	miR-133b mimics	Reduce hepatic steatosis; increase insulin sensitivity; decrease blood glucose level and triglyceridemia. Inhibit liver fatty acid and cholesterol biosynthesis pathways.	117

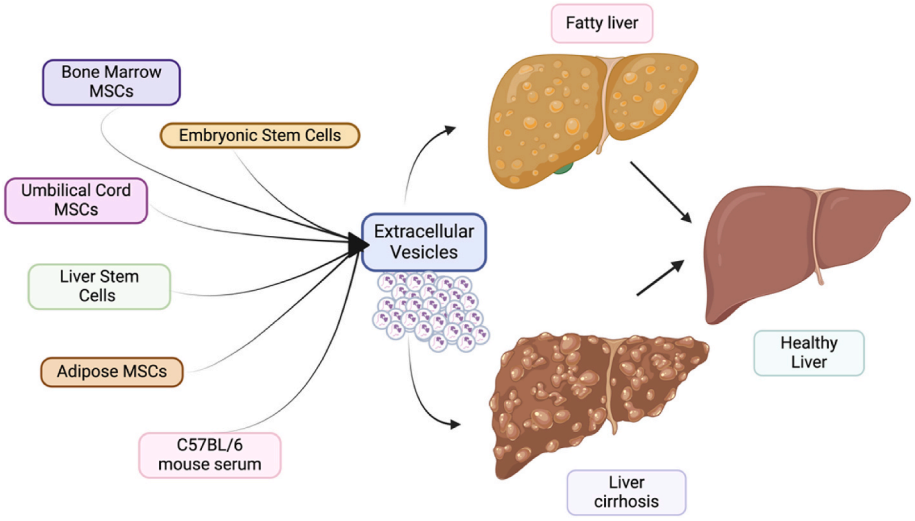

Fig. 4. Extracellular vesicles for the treatment of metabolic disorders
MSCs (Mesenchymal Stem Cells). Created in BioRender. Zakarya, R. (2025) https://BioRender.com/z73w247.

responsible for these effects.[113] In another study, plasma EVs from obese mice were shown to have increased levels of miR-122 and miR-192,[114] while exercise can increase muscle-specific miR-133b in these EVs.[115] Similarly, inhibiting miR-122 has been shown to activate the LKB1/AMPK pathway, which can prevent lipid overproduction in the liver.[116] EVs can also serve as drug carriers for treating metabolic disorders. For example, engineered plasma EVs containing antagomiR-122, antagomiR-192 or a miR-133b mimic can effectively inhibit fatty acid and cholesterol biosynthesis in the liver, reduce blood and hepatic lipid levels, and improve insulin sensitivity and glycaemic control in obese mice.[117]

Pancreatic β-cells are decreased in patients with long-term type 2 diabetes. Therefore, protecting β-cells can preserve insulin production and secretion to improve hyperglycaemia.[118] Streptozotocin (STZ) can induce β-cell toxicity, which is commonly used to model diabetes. BM-MSC-EVs containing miR-106b-5p and miR-222-3p were shown to promote β-cell proliferation to restore insulin-producing function after STZ injection.[119] In addition, EVs from hUC-MSCs were also shown to inhibit STZ-induced β-cell apoptosis and partially reverse insulin resistance induced by high fat diet consumption.[120] Additionally, EVs derived from hUC-MSCs have been shown to increase glucose uptake by increasing muscle Glut4 expression, and prevent glucose release and increase glycogen storage in the liver,[120] demonstrating their potential as new antidiabetic drugs.

3. Clinical trials involving the use of EVs

There are emerging clinical trials using EVs for the treatment of OA, cardiovascular and metabolic diseases. A current pilot clinical trial using MSC-EVs to treat knee OA (Clinical trial ID: NCT06431152) requires that EVs be delivered to patients within 6 h of product manufacture to meet Good Manufacturing Practice (GMP) guidelines. In real-world application, this short time frame may impose logistical issues relating to the manufacturing, storage, and transportation of MSC-EVs for upholding their therapeutic function, particularly in areas where access to EV isolation technology may be limited. Another trial (Clinical trial ID: NCT05881668) involved the use of MSC-EVs for acute chronic liver failure but was withdrawn due to issues with EV supply, highlighting the challenges of achieving scale-up EV manufacturing and the ability to source sufficiently high, therapeutic doses of EVs from parent cells. There are also ongoing clinical trials involving the use of EVs for treating cardiovascular diseases, including ischaemic stroke and non-ischaemic cardiomyopathies. However, there are significant differences in the doses and delivery routes across the different trials and there is a lack of proper reporting of isolation methodology. In fact, a recent systematic review found that only 12.1 % of the 471 EV-related clinical trials included in the review reported the EV isolation protocol.[121] The chosen isolation method can affect the purity of the EV fraction, which may generate unexpected detrimental or beneficial effects that are induced independently of the biological actions of EVs. Furthermore, different isolation methods can concomitantly enrich for different EV fractions, highlighting the need for standardisation and to carefully consider the methodology chosen such that only the intended EV fraction is isolated for therapeutic use. To ensure that outputs from EV treatment-related clinical trials are reproducible, able to be compared, and attributable to the effects of specific EV fraction(s), it is critical for collection methods to be disclosed. Addressing these challenges will accelerate advances in the development of clinically viable and effective EV-based therapies for a range of chronic diseases.

4. Challenges and outlook

EV research in human diseases has rapidly evolved, unveiling exciting therapeutic possibilities. However, several challenges hinder the successful translation of EV-based therapeutics into clinical applications. A significant obstacle is the inherent heterogeneity of EV populations, as their composition and function are heavily influenced by the parent cell type and changes in environmental factors. This variability poses substantial challenges for quality control, particularly in large-scale manufacturing. Furthermore, despite the availability of various isolation methods, the yield of EVs remains low using current techniques, making scalable production of EVs resource-intensive and economically unfeasible for achieving clinically relevant doses. Additionally, the reliance on specific parent cells for EV production can further complicate scalability. For instance, MSC-EVs as therapeutics in general are limited by the slow proliferation, high cost, and limited expansion potential of MSCs. Moreover, insufficient standardisation in protocols for EV isolation, characterization, and storage complicates reproducibility and quality assurance across studies. Another critical challenge, similar to that of currently existing drugs, is optimizing therapeutic dosage and ensuring effective delivery to target tissues. The wide biodistribution of EVs increases the risk of off-target effects, which can compromise safety and efficacy. Addressing these obstacles is essential for realizing the full therapeutic potential of EVs.

Ongoing technological advancements and continued research are needed to address these obstacles, paving the way for EVs to become

powerful tools in disease diagnosis and therapeutic delivery. Exploring alternative cell sources, such as inducible pluripotent stem cells, with robust proliferation and scalability is essential for producing therapeutic EVs at clinically relevant scales. Bioreactors optimized for high-density culture of parent cells can further enhance EV production efficiency. Additionally, the development of cost-effective EV isolation methods that enable high yields and large-scale production is highly desirable. The recently published MISEV2023 recommendations serve as a critical foundation for standardizing processes across the field of EV research. Implementation of these recommendations by global research groups, coupled with advances in isolation techniques such as tangential flow filtration and size exclusion chromatography will improve reproducibility and consistency in EV research and production.

Synthetic EV mimetics, which replicate or improve the biological properties of natural EVs, offer a promising alternative for large-scale applications. These mimetics may circumvent the limitations of natural EV heterogeneity and dependence on specific cell sources, while also providing a valuable platform for studying the functions and molecular mechanisms of natural EVs. Furthermore, engineering EVs for targeted delivery presents an effective solution for enhancing therapeutic specificity. Modifying EV surfaces with targeting ligands, such as peptides, antibodies, or aptamers, can significantly improve EV homing ability to specific tissues, thereby minimizing off-target effects. Collectively, these strategies hold promise for overcoming current challenges in harnessing EVs as effective therapies for inflammatory diseases.

CRediT authorship contribution statement

Madison Coward-Smith: Writing – review & editing, Writing – original draft, Conceptualization. **Ye Zhang:** Writing – review & editing, Writing – original draft, Conceptualization. **Chantal Donovan:** Writing – review & editing, Supervision, Funding acquisition. **Richard Y. Kim:** Writing – review & editing, Supervision, Funding acquisition. **Baoming Wang:** Writing – review & editing, Writing – original draft. **Razia Zakarya:** Writing – review & editing, Supervision, Funding acquisition. **Hui Chen:** Writing – review & editing, Supervision, Funding acquisition. **Jiao Jiao Li:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Brian G. Oliver:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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