Journal of
Clinical Medicine

MDPI

Review

Therapeutic Potential and Preclinical Trials of Mesenchymal Stem Cells and Their EVs for Atherosclerosis and Clinical Trials of MSC-Derived EVs for Cardiovascular Diseases

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Abstract

Atherosclerosis is a progressive disease driven by dyslipidemia, endothelial dysfunction, inflammation, oxidative stress, and vascular cell dysfunction. Extracellular vesicles (EVs) contribute to atherosclerotic pathogenesis by regulating intercellular communication, inflammatory responses, endothelial function, and vascular smooth muscle cell phenotypic switching. Premature mortality of the population and consequences in the form of cardiovascular diseases as well as early disability necessitate the search for new approaches to the treatment of atherosclerosis. Mesenchymal stem cells (MSCs) possess immunosuppressive, regenerative and atheroprotective effects, and MSCs that were preconditioned with cytokines and hypoxia are believed to have a more enhanced effect. Preclinical studies in animal models of atherosclerosis demonstrate that MSC-derived EVs can reduce lesion burden and inflammation in the early phase of atherosclerosis. However, small group sizes, heterogeneous methods of isolation of EVs and dosing, and the lack of tests in advanced lesions limit these findings. It is becoming evident that attempts are being made to utilize EVs for the treatment of cardiovascular diseases. However, the clinical studies reviewed here do not report specific results regarding the use of such EVs, which complicates a deeper understanding of this subject. It can be hypothesized that, perhaps due to a lack of sufficient clinical research, the use of EVs for human atherosclerosis has not yet been explored. Furthermore, several critical factors remain to be considered, including: the source of the MSCs from which EVs are isolated; the genetic profile and the presence of chronic conditions in the donor; the culture system of MSCs; the culture media (specifically the absence of components of animal origin); the parameters of the EVs' isolation method; and the route of administration of the EVs into the body, as well as their subsequent elimination. In this review, we considered the therapeutic perspective of MSCs and MSC-derived EVs, compiled the preclinical and clinical trials in accordance with disease category and mentioned the aspects of using EVs before clinical translation.

Keywords: mesenchymal stem cells; atherosclerosis; extracellular vesicles; preconditioning; preclinical trial; clinical trial; therapy

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Academic Editor: Adrian Covic

Received: 10 September 2026
Revised: 2 November 2026
Accepted: 5 November 2026
Published: 16 November 2026

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1. Introduction

Atherosclerosis (AS) is the vascular disease caused by the formation of lipid and cholesterol plaques on the inner side of blood vessels, leading to occlusion of arteries and insufficient blood supply to organs and tissues [1]. It was reported that this disease affected 17.8% of the population in the world, and 22.4% in Kazakhstan [2]. In this regard,

atherosclerosis underlies cardiovascular diseases leading to myocardial infarction, coronary heart disease, stroke and lower-limb ischemia [3]. The main risk factors for the development of atherosclerosis are diabetes mellitus, arterial hypertension, hyperlipidemic conditions, smoking, mental stress, poor nutrition and lack of physical activity [4].

According to the results of studies conducted experimentally and clinically, it was shown that, at the basis of the pathogenesis of atherosclerosis, there was a connection with inflammatory processes, as was found in [5]. It was assumed that immune dysregulation in the endothelium of the body's vessels contributed to the onset of the development of the stages of atherosclerosis. At the first stage, oxidized low-density lipoproteins (ox-LDLs) in the inner wall of the vessel activate endothelial cells to secrete specific factors and to express selectins and integrins, which contributes to the accumulation of monocytes and lymphocytes from the blood duct into the sub-endothelial space [4–6]. Monocytes transdifferentiate into macrophages and display the high expression of scavenger receptors (A, B1, CD36 and CD38) that bind native and ox-LDLs [7]. Then, the uptake of lipoproteins by macrophages and dendritic cells (DCs) leads to the formation of foam cells, which is a hallmark of atherosclerotic lesions. Moreover, macrophages have been shown to express Toll-like receptors (TLRs), mediating the activation of antigen-presenting cells (APCs) and a number of inflammatory cytokines such as interleukin-1 (IL-1) and tumor-necrosis factor alpha (TNF-α). The gradual accumulation of foam macrophages in the arterial intima leads to the formation of early atherosclerotic plaques [8]. Thus, chronic inflammation in the intima of the blood vessel wall leads to a constant recruitment of immune cells, which further aggravates the development of this disease. Despite the available treatments aimed at reducing the level of lipids and cholesterol in the blood of affected patients, atherosclerosis is the most common cause of disability and premature death in the population worldwide [1,9] (Figure 1).

By this time it is known that the therapy for AS includes statins; fibrates; gemfibrozil; lipid-lowering drugs; proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors; antiplatelet agents; methotrexate; prostaglandin inhibitors; antibodies blocking low-density lipoprotein receptors-1 (LOX-1); targeting CD80/86; and chronopharmacological drugs [10–12]. Limitations of the current AS therapy include statin intolerance, myalgia, the ineffectiveness of both fibrates and statins or prostaglandin inhibitors, and the evidence that approximately two-thirds of patients who were treated with statins continue to suffer from cardiovascular diseases [6,10]. In this regard, there is a need to search for new methods and approaches to the treatment of AS that will reduce the process of inflammation in the injured human endothelial wall and regulate the balance of the immune system.

To the present time, the interest in the use of MSCs by scientists and clinical researchers has increased, due to fact that MSCs possess pronounced immunoregulatory and immunomodulatory properties that can suppress the activity of pro-inflammatory cells of both innate and acquired immunity [13]. It has been found that the immunomodulatory abilities of MSCs occur both through cell–cell contacts and paracrine functions. It was reported that MSCs secrete bioactive molecules and factors that mobilize the anti-inflammatory reactions [14]. The therapeutic potential of MSCs in the treatment of atherosclerosis has been intensively studied in recent years in experimental animal models, mainly using Apolipoprotein E (ApoE) and low-density-lipoprotein receptor (LDLR) knockout mice [15,16]. A number of studies have shown that MSCs are capable of exerting immunomodulatory, regenerative and atheroprotective effects in animal models of atherosclerosis [17].

Based on these broad immunomodulatory properties, the therapeutic potential of MSCs and MSC-derived EVs in the treatment of atherosclerosis is continuing to be studied in preclinical research [18].

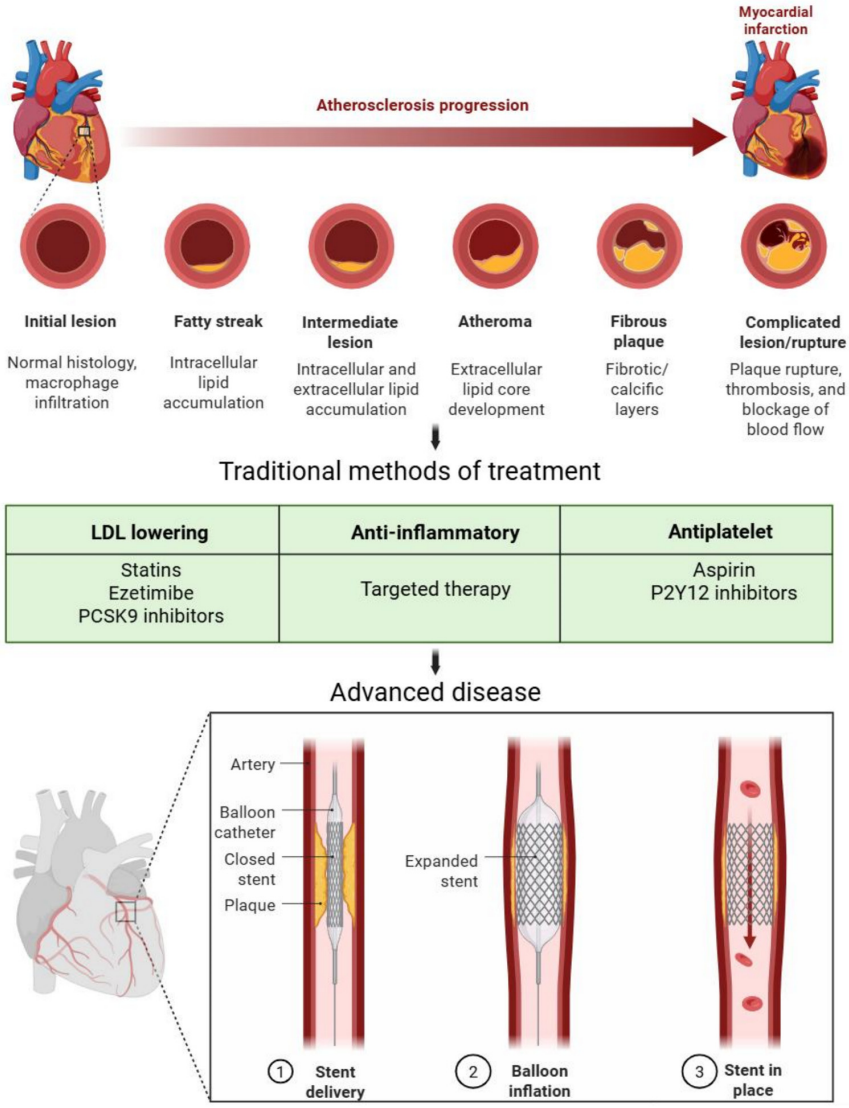


Figure 1. Development of atherosclerosis and methods of their treatment. Created with BioRender.com.

Despite certain achievements in the study of the therapeutic effect of MSCs and their EVs in atherosclerosis, there are questions about what methods can be used to improve the therapeutic efficacy of MSCs and MSC-derived EVs for application in clinical studies. To address this question, in this review, we considered the literature data on enhancing the effectiveness of MSCs and MSC-derived EVs, preclinical and clinical studies for past 5–6 years, and important aspects to consider before using EVs in clinical translation.

2. Pathophysiology of Atherosclerosis

Atherosclerosis is a chronic, progressive disease of the arterial wall and represents the pathological foundation of cardiovascular disease [3]. Rather than a passive process of lipid accumulation, it is now understood as a multifactorial disorder driven by complex interactions among dyslipidemia, endothelial dysfunction, inflammation, oxidative stress, vascular cell plasticity, and systemic metabolic influences [19,20]. L.M. Buja synthesized classical and contemporary evidence to emphasize that these mechanisms act in concert across disease stages, providing a unifying framework for modern atherosclerosis research [21]. Endothelial dysfunction constitutes the earliest detectable event in atherogenesis and facilitates the subendothelial retention of circulating low-density lipoproteins (LDLs). Thankam et al. demonstrated that minimally oxidized LDLs actively modulate

inflammatory signaling, extracellular matrix remodeling, and other biological processes within carotid atherosclerotic plaques [22]. Extending these findings, Li et al. applied spatial metabolomics to identify region-specific lipid signatures in human carotid lesions, demonstrating spatial heterogeneity in plaque lipid composition associated with structural vulnerability [23]. MSC-derived EVs intersect with this early vascular stage through their uptake by endothelial cells and delivery of regulatory cargo [24]. For example, MSC-derived exosomal miRNAs have been shown to regulate pathways associated with oxidized LDL-induced endothelial dysfunction, oxidative stress, inflammatory cytokine signaling, and endothelial senescence, indicating that EV-mediated endothelial–vascular communication is mechanistically connected to several initiating events in atherogenesis [25].

Inflammation emerges as a dominant force throughout all stages of atherosclerosis (Figure 1) [26]. Modified lipoproteins activate endothelial cells and macrophages, triggering a sustained inflammatory cascade characterized by the release of pro-inflammatory cytokines and chemokines. The dynamic balance between pro- and anti-inflammatory cytokines plays a critical role in determining plaque growth and stability, with chronic dominance of pro-inflammatory signaling favoring lesion expansion and vulnerability [27–29]. Using mathematical modeling, Adak et al. characterized the dynamic behavior of pro- and anti-inflammatory cytokines and demonstrated that disruption of cytokine equilibrium sustains chronic inflammation and accelerates lesion development [30]. Bioinformatics analyses by Dong et al. further revealed shared immune and genetic pathways between atherosclerosis and periodontitis, supporting the concept that systemic inflammatory disorders can amplify vascular disease [31]. This inflammatory environment provides an important mechanistic context for MSC-derived EVs because their cargo can be transferred to macrophages and other immune cells and can influence transcriptional and post-transcriptional inflammatory programs [32]. In particular, MSC-derived EV-associated miRNAs have been linked to macrophage phenotype regulation [33]. At the same time, MSC-derived microvesicles carrying miR-223 have been shown to interact with the NLRP3 inflammasome pathway and pyroptosis-related signaling in macrophages [17]. Thus, macrophage activation, inflammasome signaling, and inflammatory cell–cell communication represent important pathological processes through which MSC-derived EV cargo can be mechanistically connected to atherosclerosis.

Oxidative stress represents a central mechanistic link between lipid accumulation and inflammation. Excessive generation of reactive oxygen species (ROS) promotes LDL oxidation, endothelial damage, and amplification of inflammatory signaling pathways. Ishida et al. demonstrated that oxidative stress-induced DNA damage promotes endothelial cell senescence and vascular smooth muscle cell (VSMC) dysfunction, thereby contributing directly to plaque progression and instability [34]. Complementing these findings, Alsalman et al. showed that impaired mitochondrial quality control—including defective mitophagy and altered mitochondrial dynamics—leads to excessive reactive oxygen species production, metabolic dysfunction, and heightened inflammatory signaling [35]. MSC-derived EVs are mechanistically relevant to this axis due to their bioactive cargo that can regulate redox-sensitive signaling and cellular responses to oxidative stress [36]. For instance, MSC-derived extracellular vesicles enriched in miR-124 have been reported to exert neuroprotective effects following spinal cord injury by modulating the p62–Keap1–Nrf2 signaling pathway, thereby mitigating oxidative stress and enhancing cellular antioxidant defenses [37]. More broadly, the capacity of EV cargo to influence mitochondrial homeostasis, ROS signaling, and stress-response pathways provides a mechanistic interface between MSC-derived EVs and the oxidative and metabolic abnormalities characteristic of atherosclerotic vascular cells.

As lesions progress, VSMCs assume a central role in shaping plaque structure and stability, undergoing phenotypic switching from a quiescent, contractile state to synthetic,

proliferative, osteogenic, or macrophage-like phenotypes (Figure 1). This phenotypic plasticity drives to extracellular matrix remodeling, fibrous cap formation, calcification, and, in advanced lesions, plaque destabilization. This plasticity drives extracellular matrix remodeling, fibrous cap formation, calcification, and, in advanced lesions, plaque destabilization. Ultrastructural, molecular, and proteomic studies further demonstrate coordinated changes in VSMC phenotype and networks regulating inflammation, lipid metabolism, oxidative stress, and extracellular matrix organization within human carotid plaques [38,39]. EV-mediated intercellular communication is closely integrated with this VSMC plasticity. MSC-derived EVs can deliver regulatory RNAs and proteins to VSMCs, providing a potential means of modifying signaling networks that control contractile-marker expression, proliferation, migration, extracellular matrix organization, and phenotypic transition [40]. This is particularly relevant because EVs constitute a natural mechanism of communication among endothelial cells, macrophages, and VSMCs within the plaque microenvironment, placing MSC-derived EVs within the broader EV-mediated network that regulates vascular cell phenotype.

Epigenetic regulation adds an additional layer of complexity to atherosclerotic pathophysiology. It has been shown that miRNAs regulate gene expression involved in lipid metabolism, inflammatory signaling, endothelial integrity, and VSMC phenotype [41]. Mahjoubin-Tehran et al. identified microRNAs with therapeutic potential through combined in silico and in vitro analyses, demonstrating their ability to modulate inflammatory and lipid-related pathways [42]. Clinically, Mazgutova et al. reported altered circulating microRNA profiles in young patients with obesity, even in the absence of overt cardiovascular disease, suggesting that epigenetic dysregulation may precede structural vascular pathology [43]. This epigenetic dimension is directly relevant to miRNAs and other noncoding RNAs that constitute important components of MSC-derived EV cargo. Following uptake by recipient vascular or immune cells, these molecules can regulate target transcripts and signaling pathways involved in inflammation, oxidative stress, lipid metabolism, endothelial function, and VSMC phenotype. Consequently, MSC-derived EVs can be viewed mechanistically as vehicles for intercellular transfer of epigenetic information within the atherosclerotic microenvironment.

Systemic metabolic conditions further modulate atherosclerotic risk and progression [44]. Van den Hoek et al. demonstrated that transcriptional changes associated with non-alcoholic steatohepatitis exacerbate inflammatory and lipid dysregulation pathways relevant to atherosclerosis, linking hepatic metabolic disease to vascular pathology [45]. In another study, Yang et al. showed that plaque instability in ApoE$^{-/-}$ mice is associated with altered plasma lipidomics, dysfunctional adipose tissue, and shifts in gut microbiota composition, reinforcing the concept that atherosclerosis is a multi-organ disorder [46]. The systemic nature of atherosclerosis also provides a broader context for MSC-derived EV biology, as the molecular composition of EVs reflects the metabolic and inflammatory state of their source cells, while circulating EVs can mediate communication among metabolically active tissues and the vascular wall [47].

Finally, plaque maturation involves necrotic core expansion, fibrous cap thinning, and persistent inflammation, increasing the risk of rupture and thrombosis. Early biomarkers, such as hyperhomocysteinemia in nutritionally deficient adolescents, underscore the translational relevance of early detection and targeted preventive intervention. Shirode et al. reported an association between hyperhomocysteinemia and subclinical atherosclerosis in vitamin B-deficient adolescents, highlighting nutritional and metabolic contributors to early vascular dysfunction [48]. At the therapeutic frontier, Choi and Rhee showed that VCAM-1-targeted plant-derived extracellular vesicles selectively accumulate in inflamed endothelium [49]. At advanced disease stages, MSC-derived EVs remain mechanistically relevant

as interconnected pathways converge within the vulnerable plaque microenvironment. Their regulatory RNA and protein cargo may modulate these processes, with cargo composition influencing the specific cellular pathways engaged. These mechanistic roles are distinct from the therapeutic effects of MSC-derived EVs discussed separately in this review.

Overall, these studies delineate atherosclerosis as a dynamic, multifactorial disease driven by coordinated disturbances in lipid metabolism, inflammation, oxidative stress, mitochondrial dysfunction, vascular cell plasticity, epigenetic regulation, and systemic metabolic states. Integrating these findings provides a mechanistic framework for understanding how MSC-derived EV cargo may modulate interconnected cellular and molecular processes underlying atherosclerosis, thereby informing the development of precise diagnostic approaches and targeted therapeutic strategies.

3. Preconditioned Mesenchymal Stem Cells in Atherosclerosis

Preconditioning refers to the ex vivo exposure of MSCs to defined stimuli, with the aim of enhancing their immunomodulatory, pro-survival or paracrine properties. Several types of preconditioning have been described: cytokines, hypoxia, pharmacological and chemical agents, trophic factors, physical factors, biomaterials and culture conditions [50,51]. Genetic modification of donor cells, post-isolation cargo loading and membrane engineering of extracellular vesicles are mechanistically distinct strategies; the first is addressed separately below, and the latter two in the following sections. Preclinical studies applying preconditioning to MSCs are summarized in Table 1. In the cardiovascular field, Matta et al. reviewed hypoxic, pharmacological and chemical preconditioning as well as other approaches, including genetically modified MSCs, with particular attention to ischemic heart disease [52]. Because atherosclerosis is a chronic inflammatory disease, preconditioning could strengthen the immunomodulatory functions of MSCs before administration [53]. MSC properties also vary with tissue source, which should be taken into account when assessing the effects of preconditioning.

According to Kirwin and colleagues, in contrast to bone marrow-derived mesenchymal stem cells (BM-MSCs), which have a low proliferation rate, and are difficult to isolate, and are limited in availability, adipose-derived MSCs (AD-MSCs) demonstrated high proliferative potential, accessibility, and reduced osteogenic differentiation potential. This may lower the risk of calcium accumulation in atherosclerotic plaques [54]. Also, in terms of regenerative capabilities, AD-MSCs have been shown to possess potent immunomodulatory properties and pro-angiogenic potential, which have been exploited in preclinical atherosclerosis models [53].

Despite the therapeutic potential of MSCs in normoglycemic models of atherosclerosis, their efficacy appears markedly limited in animal models of diabetes mellitus (DM). In a streptozotocin-induced hyperglycemia model (ApoE$^{-/-}$, C57BL/6), a single intravenous infusion of a clinically relevant ASC dose of MSCs (100,000 cells/mouse) did not produce any statistically significant reduction in atherosclerotic plaque area or overt changes in intraplaque inflammation, including macrophage content, CD45^{+} (CD45-positive) leukocyte infiltration, mast cell density, or endothelial intercellular adhesion molecule 1 (ICAM-1) expression. Only a borderline increase in intra-plaque fibrotic tissue and non-significant trends toward lower intimal CD45^{+} leukocyte and adventitial mast cell densities were observed, suggesting a modest improvement in plaque stability without an effect on plaque burden [55]. Similarly, a modified approach using Stem Bell technology (ASCs coupled to ultrasound-activated microbubbles), which had previously improved plaque stability in non-diabetic mice, failed to induce significant changes in plaque morphology or inflammation in the diabetic setting when applied at a four-fold lower dose (125,000 Stem Bells),

underscoring the limited efficacy of current MSC-based strategies in the hyperglycemic milieu and the likely need for higher and/or repeated dosing [56].

More broadly, in the study by Yan et al., conducted in HFD-fed ApoE^{-}/$^{-}$ mice with combined atherosclerosis and osteoporosis, microRNA let-7g (miR-let-7g) was identified as a shared regulator of mesenchymal stem cell fate and plaque pathology. The authors showed that the reduced degree of miR-let-7g in bone marrow MSCs has promoted High-mobility group AT-hook 2/CCAAT/enhancer-binding protein beta/peroxisome-proliferator activated receptor-gamma (HMGA2)/C/EBPβ/PPARγ)-driven adipogenic differentiation at the expense of osteogenesis and, in co-culture systems of BM-MSCs with Raw264.7 macrophages, has enhanced the foam cell formation. It was reported that paeonol treatment has restored miR-let-7g expression, reduced aortic plaque and foam cell burden, and improved bone mass, indicating that targeting the miR-let-7g axis in MSCs may provide a strategy to concurrently attenuate atherosclerosis and osteoporosis [57].

These findings collectively establish that the atherogenic microenvironment actively reprogrammed the identity, differentiation potential, and paracrine output of MSCs, providing the fundamental mechanistic rationale for ex vivo preconditioning strategies designed to stabilize and enhance MSC function before therapeutic application.

Cytokine-based preconditioning is particularly well-grounded physiologically, as it exposes MSCs to signals that replicate the inflammatory milieu that they will encounter in vivo at sites of vascular injury. Thus, MSCs that were preconditioned with pro-inflammatory cytokines (of mouse or human origin) demonstrate strong anti-angiogenic potential by decreasing tube formation in vitro [58].

Combined preconditioning with IFN-γ and TNF-α cytokines synergistically upregulated the immunoregulatory IDO and PGE2, and immunosuppressive ligands, producing more robust induction of Tregs and effector immune suppression than either cytokine priming alone. However, IFN-γ alone was generally avoided in the AS context due to its capacity to upregulate major histocompatibility complex class I (MHC class I) and major histocompatibility complex class II (MHC class II) molecules on MSCs, paradoxically increasing immunogenicity and potentially exacerbating the atherogenic immune response [53]. This finding underscores the necessity of tailoring preconditioning protocols to the specific immunological landscape of the target disease.

The rationale is further reinforced by the biology of endothelial-to-mesenchymal transition: in human umbilical vein endothelial cells (HUVECs), treatment with transforming growth factor-beta 2 (TGF-β2) and IL-1β, acting synergistically through a nuclear factor kappa B (NF-κB)-dependent mechanism, is sufficient to reprogram endothelial cell identity toward mesenchymal and osteochondrogenic fates in the atherosclerotic plaque [59]. The contrast between such sustained cytokine exposure and the brief, defined exposure used in ex vivo preconditioning underscores the importance of dose, duration, and cytokine combination in preconditioning protocols.

The in vivo efficacy of TNF-α preconditioning of MSCs was demonstrated by Sekenova et al. in the high-fat diet-treated ApoE^{-}/$^{-}$ mouse model and resulted in a 38% reduction in aortic lesion area compared to the control group, accompanied by decreased levels of circulating TNF-α and interferon-gamma (IFN-γ), increased interleukin-10 (IL-10), and a more than two-fold expansion of splenic forkhead box P3-positive (FOXP3^{+}) regulatory T cells (Tregs). These Tregs exert atheroprotective effects by suppressing macrophage foam cell formation and restraining effector T-cell activity [60].

Beyond cytokine priming, the remaining modalities have so far been tested outside atherosclerosis, and the available evidence is therefore extrapolated. Hypoxic preconditioning (1% O_2, 24 h) suppressed transforming growth factor-beta/Smad (TGF-β/Smad) signaling and increased VEGF, hepatocyte growth factor (HGF) and PGE2, reducing CD3^{+}

and CD68$^+$ cell infiltration and interstitial fibrosis in renal ischemia–reperfusion injury [61], and improved limb perfusion and CD31 expression in hindlimb ischemia [62]. Pharmacological priming with n-butylidenephthalide activated protein kinase B (Akt) and signal transducer and activator of transcription 3 (STAT3), promoted macrophage polarization towards the M2 phenotype and IL-10 production, and reduced cardiac fibrosis, although ejection fraction and infarct size were unchanged [63]. Sublethal oxidative stress [64], spheroid culture [65] and short-term glucose depletion [66] improved survival of the transplanted cells relative to conventionally cultured MSCs. Across these models the mechanisms converge on a small number of pathways—hypoxia-inducible factor-1 alpha (HIF-1α) and VEGF, phosphoinositide 3-kinase/Akt (PI3K/Akt) and STAT3, and macrophage polarization towards M2—the same processes that sustain plaque inflammation, which is what makes extrapolation to atherosclerosis reasonable. Ye et al. demonstrated that miR-126 overexpression in MSCs enhanced their vascular regenerative potential through the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway by increasing vascular endothelial growth factor/basic fibroblast growth factor (VEGF/bFGF) secretion, proliferation, migration, and endothelial differentiation, resulting in a significant reduction in neointimal hyperplasia in rat carotid atherosclerosis [67]. This approach, however, is a genetic modification of the donor cells rather than preconditioning, and the two strategies should be distinguished. From a translational perspective, several challenges remain. Preconditioning has been tested in an atherosclerosis model only for cytokine priming, and no two protocols have been compared within the same model; their relative potency therefore remains unknown, and the endpoints differ across models, precluding direct comparison. The dose-dependent manner of the MSCs' efficacy was illustrated by the contrast between the null result observed with 100,000 adipose-derived stem cells (ASCs) in an established hyperglycemic atherosclerosis model [55] and the positive outcomes reported with higher doses in non-diabetic models [68,69]. Timing of administration is equally critical: most studies demonstrating significant plaque reduction employed MSC delivery before or in an earlier stage of lesion development, whereas single-dose therapy in established disease may be insufficient. Also, source heterogeneity—bone marrow vs. adipose vs. hUC-MSCs—influences both baseline immunopotency and response to cytokine stimulation, as do passage number and donor metabolic status. Reported safety observations are limited but consistent: hypoxically preconditioned MSCs showed unchanged human leukocyte antigen (HLA)-A,B,C expression, and HLA-DR remained undetectable, indicating that preconditioning did not increase immunogenicity [61]; tri-lineage differentiation was preserved after oxidative preconditioning, although H_2O_2 concentrations above 150 μM induced dose-dependent apoptosis [64]. Cytokine preconditioning offers a rational strategy to partially overcome these variables by standardizing MSC functional output before administration, but the optimal cytokine combination, concentration, duration, safety margins, and timing relative to transplantation remain to be systematically established in the atherosclerotic context.

The preclinical evidence in Table 1 was identified between March 2026 and October 2026 by searching for papers from PubMed using combinations of the terms "mesenchymal stem cell" OR "mesenchymal stromal cell" AND precondition* OR priming OR primed AND atherosclerosis OR "atherosclerotic plaque" OR cardiovascular, supplemented by targeted searches for individual preconditioning modalities, by Google Scholar and by screening the reference lists of relevant articles. We included English-language original studies in which MSCs were exposed ex vivo to a defined stimulus before administration and compared with unconditioned MSCs. We excluded reviews, conference abstracts, studies without accessible full text, and genetic modification of donor cells. Eight studies met these criteria (Figure 2); their characteristics are extracted in Table 1, and in vitro experiments were treated only as mechanistic support. This part of the review is narrative in design.

Table 1. Preclinical studies on preconditioned MSCs. Studies performed in an established atherosclerosis model are marked as direct; those providing mechanistic information obtained in other disease models are marked as extrapolated.

Preconditioning Type	Cell Type	Animal Model	Stimulus & Concentration	Exposure Time	Disease Model	Dosage	Molecular Mechanism	Therapeutic/Functional Effect	Ref.
Cytokine	Mouse BM-MSC (C57BL/6)	ApoE^{-}/$^{-}$ mice; n = 5 per group	TNF-α, 20 ng/mL	24 h	High-fat western diet (0.15% cholesterol, 0.21% butter oil)—direct	1 × 10^6 cells i.v., two injections 1-week interval	↑ splenic FOXP3$^+$ Tregs; ↑ CD4$^+$ T cells; ↓ TNF-α, ↓ IFN-γ, ↑ IL-10, ↑ IL-6	↓ aortic lesion by 38% (TNF-α-MSCs) and by 41% (untreated MSCs); ↓ total cholesterol, ↓ LDL, ↑ HDL (TNF-α-MSCs and untreated MSCs)	[60]
Cytokine	Human BM-MSC	In vitro	IL-1β 25 ng/mL + IL-6 20 ng/mL + TNF-α 25 ng/mL	24 h	In vitro secretome profiling	n/a	↑ inflammation- and angiogenesis-related proteins; TIMP-1—key endothelium-targeting effector (anti-TIMP-1 antibody)	↓ HUVEC tube formation with primed CM, full restoration by TIMP-1 blockade	[58]
Hypoxia	Rat BM-MSC; human BM-MSC	Sprague-Dawley rats	1% O_2 vs. 21% O_2 (normoxia)	24 h	Renal IRI—extrapolated	5 × 10^5 cells in 0.2 mL PBS	↓ TGF-β/Smad (↓ p-Smad2, ↓ α-SMA); ↑ VEGF, ↑ HGF, ↑ PGE_2; VEGF-dependent HGF production confirmed by siRNA	Day 7: ↓ CD3$^+$ and ↓ CD68$^+$ cell infiltration, ↑ CD163$^+$ and ↑ CD68$^+$CD163$^+$ M2 macrophages. Day 21: ↓ interstitial fibrosis, ↓ collagen I and III; comparable for rat- and human-derived MSCs	[61]
Hypoxia	Human BM-MSC	Male C57BL/6J mice; n = 8 per group	1% O_2 vs. 21% O_2 (normoxia)	24 h	Hindlimb ischemia—extrapolated	1 × 10^5 cells total, 1 µL i.m. per site at four sites	↑ HIF-1α, ↑ VEGF-A, ↓ PGC-1α	↑ LDPI index, ↑ CD31	[62]
Pharmacological	Human AD-MSC	Male Wistar rats	n-Butylidenephthalide (BP) 7 µg/mL	16 h	MI (LAD ligation)—extrapolated	1 × 10^6 cells in 30 µL PBS	↑ p-Akt, ↑ p-STAT3, ↑ STAT3 DNA-binding; ↑ M2 macrophages, ↑ IL-10, ↑ IGF-1, ↑ HGF	↓ fibrosis, ↑ engraftment, ↓ LVESD, ↓ LVEDD, ↑ ±dp/dt vs. naive ADSCs; LVEF and infarct size unchanged	[63]
Oxidative stress	Mouse BM-MSC (BALB/c)	Male BALB/c mice, 21–23 g	H_2O_2, 50 µM	12 h	Skin wound healing—extrapolated	1 × 10^6 cells in 100 µL PBS, i.v.	↑ PI3K/Akt/mTOR; ↑ SDF-1, ↑ CXCR4, ↑ CXCR7; ↑ cyclin D1, ↓ p16; ↑ Bcl-2/Bax	Wound healing by day 10 (only preconditioned MSCs); ↑ cell retention, ↑ CD31$^+$ microvessel density, ↑ collagen deposition	[64]

Table 1. *Cont.*

Preconditioning Type	Cell Type	Animal Model	Stimulus & Concentration	Exposure Time	Disease Model	Dosage	Molecular Mechanism	Therapeutic/Functional Effect	Ref.
3D culture/spheroid	Human tonsil-derived MSC	Male BALB/c nude mice; $n = 5$ per group	3D spheroid on an ultra-low attachment plate, 500 cells/well	24 h	Hindlimb ischemia (femoral artery ligation and resection)—extrapolated	1×10^6 cells per mouse, i.m. into the gracilis muscle at four sites (20 µL each)	↑ ENA-78, ↑ VEGF, ↑ MCP-3, ↑ IL-6, ↑ PlGF	↑ LDPI index vs. monolayer MSCs; ↓ tissue necrosis vs. control	[65]
Metabolic (glucose depletion)	Mouse BM-MSC (C57BL/6)	Aged C57BL/6 mice (24-month-old)	Serum-free DMEM without glucose	1 h (1, 6 and 12 h tested)	MI (LAD ligation)—extrapolated	The paper states 20 µL containing 100,000 cells and a concentration of 1×10^6/mL, which are not reconciled	In aged MSCs: ↑ AKT, ↑ p-AKT, ↑ IGF-1, ↑ SIRT-1, ↑ VEGF, ↑ SDF-1, ↑ FGF-2; in myocardium at 7 d: ↑ PCNA, ↑ BCL-2, ↑ BCL-xL, ↓ BAX, ↓ BAK vs. untreated MSCs	↓ fibrosis at 30 d (14.47 vs. 22.38%) and ↓ $TUNEL^+$ nuclei at 7 d (11.07 vs. 15.15) vs. untreated MSCs	[66]

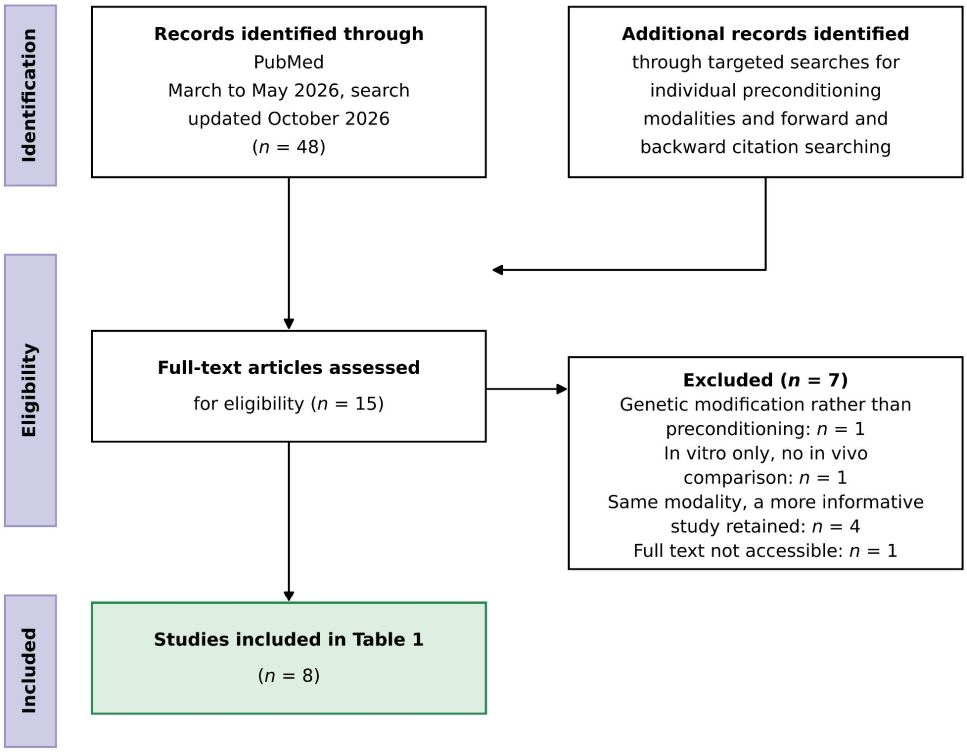


Figure 2. Flow diagram of preclinical evidence screening.

4. Therapeutic Capabilities of MSC-Derived EVs

EVs isolated from hUC-MSCs can be examined as a favorable cell-free therapeutic approach. They are nanosized EVs with a size of 30–150 nm, and enveloped by a lipid membrane and dispensed from multivesicular bodies, and their cargo includes proteins, lipids, messenger RNA (mRNA), microRNA (miRNA) and other biologically active molecules. According to MISEV2023 [70], minimal EV characterization should report particle size and concentration (e.g., NTA), morphology (electron microscopy), at least three positive markers (including CD9/CD63/CD81 and TSG101 or ALIX) and one negative marker (e.g., calnexin), purity as the particle-to-protein ratio, a reproducible isolation protocol, and a dose standardized by particle number and protein mass, normalized to body weight in animal studies.

EVs from hUC-MSCs have been reported to carry paracrine, immunomodulatory, anti-inflammatory, antifibrotic, and regenerative effects of their original cells. As a cell-free approach exosomes are promising, while avoiding the risks associated with the use of cells themselves [56,71].

Atherosclerotic plaque is characterized by the chronic inflammatory microenvironment in the endothelium, sometimes complicated by hyperlipidemia—reflected in markedly elevated triglycerides (TGs), total cholesterol (TC), and LDL levels in experimental animal models—that constitutes the hostile host environment into which the transplanted MSCs execute their effect. TNF-α cytokine is abundantly present within atherosclerotic plaques and in the systemic circulation of patients with atherosclerosis, and its effect is mediated by MSCs primarily through the NF-κB pathway—the same pathway in which were upregulated in atherosclerotic vascular cells alongside NF-κBp50/p65, activating transcription factor (ATF-2) and mothers against decapentaplegic homolog 2/3 (Smad2/3) [71].

Comarita et al. confirmed the therapeutic potential of MSC-derived EVs as standalone agents in the hypertensive–hyperlipidemic hamster model: both ADSC-derived and BM-MSC-derived EVs reduced circulating lipid parameters, downregulated key VCAM-1,

matrix metalloproteinase-2 (MMP-2), collagen type I alpha chain (COL1A), alpha-smooth muscle actin (α-SMA), connexin 43 (Cx43), decreased M1 macrophage and T-cell infiltration, and improved structural and functional arterial parameters, including the wall thickness, distensibility, and contractile response to noradrenaline. Notably, adipose-derived stem cell (ADSC)-derived EVs demonstrated greater efficacy than BM-MSC-derived EVs across outcome measures [71]. This is consistent with the broader principle that MSC-derived EVs serve as key effector vehicles: in the streptozotocin-induced ApoE$^{-/-}$ model, BMSC-derived EVs demonstrated anti-atherosclerotic effects—reducing plaque area, increasing collagen content, decreasing blood glucose and LDL [72]. It can be proposed that MSC-derived EVs can antagonize this process through multiple miRNA-mediated pathways. The authors reported that adipose MSC-derived EVs suppressed VSMC proliferation and migration through inhibition of mitogen-activated protein kinase/protein kinase B (MAPK/Akt) phosphorylation, with concurrent decreases in interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) expression. It was found that exosomes of MSCs loaded with microRNA-221 (miR-221) agomir reduced the aortic plaque formation in ApoE$^{-/-}$ mice via the miR-221/N-acetyltransferase 1 (NAT1)/insulin-like growth factor 2 (IGF2)/insulin-like growth factor 2 receptor (IGF2R) pathway; miR-221 was reduced in both patient AS plaques and in experimental models, and its restoration via MSC-derived EVs attenuated the plaque inflammation and promoted stability [40].

Fracchia et al. determined 364 genes that were expressed in stimulated T cells after co-culturing with exosomes from bone marrow-isolated MSCs. Importantly, in this study, the Ras homology family member A (RhoA) pathway was featured; however, a different aspect of this pathway was observed by the expression of genes responsible for "cellular development, movement, growth and proliferation, cell-to-cell interaction and inflammatory response". Moreover, the application of exosomes affected the pathway responsible for apoptosis, and, in addition, enhanced the down-modulated genes associated with the output of the TNF-α and IFN-γ cytokines, and the overexpressing genes of apoptosis, genes related to the migration process of lymphocytes and T-cell differentiation [73]. Bone marrow MSCs after preconditioning with the IL-1β factor and oxygen (low concentrations) had enhanced levels of IL-6, interleukin-8 (IL-8), C-X-C motif chemokine ligand 1 (CXCL1), monocyte chemotactic protein 1 (MCP-1), monocyte chemotactic protein 3α (MCP-3α), growth-related oncogenes alpha, beta, gamma (GROα/β/γ), and declining levels of IFN-γ, IL-10, interleukin-4 (IL-4), interleukin-5 (IL-5), and TNF-α [74].

Li et al. revealed that TNF-α-preconditioned MSCs contributed to the polarizing transition of M1 macrophages to M2 macrophages. The authors found that exosomes from TNF-α-preconditioned MSCs contributed to the elevated level of Galectin-1 expression. Importantly, the authors showed that the signaling Janus kinase–signal transducer and activator (JAK-STAT) pathway maintained the polarizing transition to M2 macrophages with Galectin-1 participation [75]. Souza et al. revealed that exosomes from adipose MSCs improved the propagation of HaCaT and NHDF-1 cell types, and the secretion of inflammatory cytokines by THP-1 macrophages, such as transcription growth factor-β (TGF-β) and IL-1β, under in vitro conditions. Thus, activation of angiogenesis was noted for these exosomes [76].

Wang et al. reported on feature mechanisms of EVs from MSCs known as immunomodulatory, paracrine, anti-inflammatory and regenerative. To date, the properties of exosomes from MSCs have been studied in cardiovascular, neurological, autoimmune, musculoskeletal and systemic diseases. The authors also considered the engineered exosomes [69]. Therefore, Malik et al. examined exosomes from three sources and found that exosomes from human induced pluripotent stem cells showed their regeneration capacities in the treatment of wound and nerve diseases; exosomes from embryonic stem cells may possess

the ability to restore fibrosis; and exosomes from MSCs can contribute to angiogenesis [77]. Lenero et al. found that CD146-positive endometrial MSCs were improved in the next four miRNAs—homo sapiens microRNA 320e (hsa-miR-320e, homo sapiens microRNA 182p (hsa-miR-182-3p), homo sapiens microRNA 378g (hsa-miR-378g), and hsa-let7e-5p—which are responsible for T-cell activation, macrophage polarization, and the regulation of inflammatory cytokine production, as reported. Also, the authors detected that IL-10 and interleukin-13 (IL-13) proteins, which are responsible for the inhibition of inflammation, were elevated in CD146+ endometrial MSCs [78]. Buschman et al. revealed that bioactive molecules of the cell secretome promote cell–cell interchange, which is important for the treatment of inflammation-based diseases. The authors noted that next parameters are vital for the obtainment of the standardized secretome: cell proliferation, number of cell passages, confluent monolayer, the period of conditioning, the composition of culture medium and microenvironmental stimuli [79]. Deshmukh et al. indicated the cell-free approach that uses exosomes for cardiovascular disease treatment such as myocardial infarction, atherosclerosis, heart failure, myocarditis and pulmonary hypertension. The authors remarked on the anti-inflammatory, restorative and vascular-contributory capabilities of exosomes. The authors mentioned that the main importance for application in clinical studies consists of conducting the standard operational protocols, Good Manufacturing Practice (GMP) measures in the laboratory, testing for immunogenicity, procedures for avoiding animal components and tumorigenicity [80].

Liu et al. examined that preconditioning with IL-1β, IFN-γ and TNF-α was influenced on the phenotype and properties of MSCs, affecting anti-inflammatory abilities, immunogenicity, antimicrobial abilities and immunomodulation of T cells and macrophages [81]. Kim et al. investigated that MSC exosomes decreased lipid aggregation and improved cholesterol efflux via up-coordination of the PPARγ/liver X receptor alpha (LXRα)/member 1 of human transporter sub-family ABCA (ABCA1)/adenosine triphosphate (ATP)-binding cassette sub-family G member 1 (ABCgG1) axis. Significantly, MSC exosomes decreased the aortic plaque size, inflammation and hepatic steatosis [82]. Cipriano et al. investigated EVs for spinal cord injury in a rat experimental model. The authors found that EV treatment significantly improved the 30-day survival compared with untreated SCI (log-rank test), with untreated animals showing progressive deterioration. Also, blood–brain barrier (BBB) scores improved in EV-treated animals from the day 7 onward, and were sustained to day 30, while untreated SCI animals remained near-paralyzed. In inflammation state, the levels of TNF-α and interleukin-1β (IL-1β) were reduced; however, the levels of IL-10 and brain-derived neurotrophic factor (BDNF) were increased, at both 24 h and 7 d; ionized calcium-binding adapter molecule 1 (IBA1) + microglial reactivity was significantly reduced at day 7. The authors observed the reduction of necrosis, histiocytic infiltration, glial fibrillary acidic protein (GFAP)+ astrogliosis, and chondroitin sulphate proteoglycan (CSPG) deposition in EV-treated tissue—consistent with attenuated glial scarring [83]. Therefore, Park et al. compared the proteome content of EVs obtained from different donors of adipose MSCs. The authors found a comparable size (150–170 nm), morphology, and purity across donors; CD63/CD9 were stable, but CD81 showed significant batch-to-batch variation—flagged explicitly as a caveat. Proteomic consistency: 84–94% of the 2615 identified proteins were statistically unchanged across any given pairwise donor comparison; 73.2% (1909 proteins) were unchanged across all three pairwise comparisons simultaneously—a "core proteome." Membrane proteome: 361 membrane-associated proteins (transporters, adhesion molecules, transmembrane signaling receptors) were conserved across all donors, were enriched for integrin signaling, cadherin adhesion, inflammation, and growth-factor pathways, and were proposed as the functional backbone of EV bioactivity (referencing the group's earlier findings on evidence that EVs

retain therapeutic activity after cytosolic content was stripped out osmotically). The functional reproducibility: all three donor batches suppressed lipopolysaccharide (LPS)/outer membrane vesicle (OMV)-induced TNF-α and IL-6 in RAW264.7 macrophages in a dose-dependent, statistically indistinguishable manner (>50% inhibition at the top dose); i.e., proteomic conservation translated into functional (anti-inflammatory) consistency. Batch 1 was proteomically more distinct from batches 2 and 3, which clustered closer together—an unexplained but transparently reported donor-effect gradient [84].

5. Preclinical Studies of Using MSC-Derived EVs in Atherosclerosis

In this chapter the preclinical studies are analyzed, and the literature data are constituted according to Figure 3. Preclinical studies in mouse and hamster models have reported the therapeutic potential of MSC-derived EVs (in studies they were written as exosomes) in attenuating atherosclerosis progression across multiple animal models (Table 2). Li et al. [85] reported that intravenous MSC-derived exosomes reduced plaque area and macrophage infiltration and shifted plaque macrophages towards an M2 phenotype in HFD-fed ApoE^{-}/$^{-}$ mice, and attributed this to exosomal miR-let-7 (HMGA2/NF-κB, IGF2BP1/PTEN) and miR-21a-5p (KLF6, ERK1/2), respectively; the miRNA-dependent steps were demonstrated mainly in cultured macrophages. Yang et al. [86] delivered miR-145-enriched exosomes from lentivirally modified human umbilical cord MSCs (80 μg weekly for 8 weeks) to C57BL/6J mice with a carotid silicone ring and HFD; plaques were smaller and JAM-A expression lower, but the comparison concerns miR-145-overexpressing rather than native exosomes, and this is not the ApoE^{-}/$^{-}$ model. Zhang et al. [87] linked exosomal lncRNA FENDRR to the miR-28/TEAD1 axis and, unlike most reports, included knockdown and inhibitor arms in vivo. Bai et al. [88] gave BM-MSC-derived exosomes (400 μg/kg weekly for 8 weeks) to female ApoE^{-}/$^{-}$ mice (n = 4 per group) and found lower serum TC, TG, IL-1β and IL-18 and lower aortic NLRP3, caspase-1 and GSDMD expression, with qualitatively more collagen and a thicker fibrous cap; plaque area was not quantified, and the pyroptosis pathway was not manipulated in vivo.

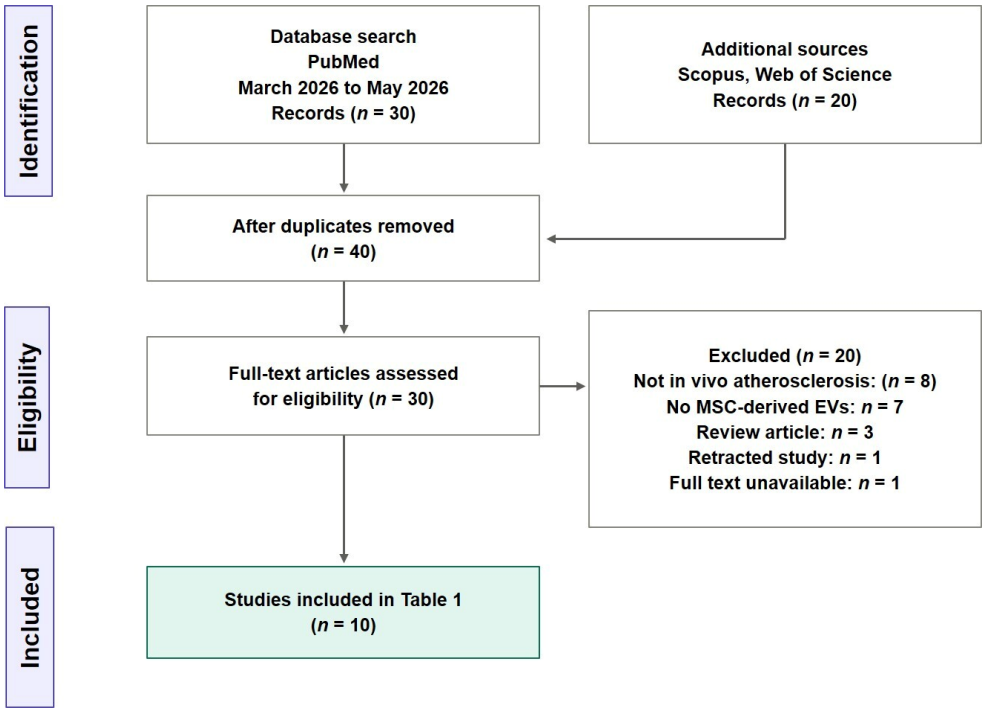


Figure 3. Flow diagram of the study exclusion and inclusion.

Table 2. Preclinical studies using exosomes from mesenchymal stem cells for the treatment of atherosclerosis (BM-MSCs—Bone marrow mesenchymal stem cells, HFD—High-fat diet, *i.v.*—Intravenous, TC—Total cholesterol, TG = Triglyceride). An upward arrow means increasing, a downward arrow means decreasing, and an arrow pointing to the right or left means "follow" or "next".

Animal Model (Strain, Sex, *n* per Group, Diet)	Vesicle Source and Type	Isolation and Characterization	Dose (Unit), Route, Frequency, Duration; Timing vs. Disease Induction	Comparator(s)	Main Outcomes (Quantitative Where Reported)	Mechanism Claimed; Level of Evidence	Ref.
Male ApoE^{-}/$^{-}$ mice on high-fat diet (15% lard, 20% sugar, and 1.2% cholesterol) 2 groups: (1) high-fat diet group (HFD), (2) MSC exosome treatment group (MSC-exo)	Mouse BM-MSC exosomes.	HPLC, centrifugation and filtration methods. TEM	100 µg exosomes dissolved in 200 µL saline treatment IV (tail vein) for 12 weeks alongside HFD.	HFD PBS/HFD exosomes	↓ Plaque area; ↓ macrophage infiltration and ↑ M2 macrophages in plaque; exosomes homed to plaque and localized near macrophages after IV injection.	miR-let-7 (enriched in MSC exosomes) → HMGA2/NF-κB (M2 polarization) and IGF2BP1/PTEN (migration/infiltration). Pathway tested in vitro (U937 cells); in vivo: miR-let-7 ↑ in aortic root (correlative).	[85]
C57BL/6J female mice (not ApoE^{-}/$^{-}$): right common carotid silicone-ring placement at 8 weeks + HFD (15% fat, 0.25% cholesterol). *n*/group not stated in text. Patient serum exosomal miR-145 (16 vs. 16 sequencing; 60 vs. 60 qPCR).	**Human** umbilical cord MSC (xenogeneic to mouse). Exosomes from miR-145-overexpressing MSCs (lentivirus; ≈6.4-fold miR-145) vs. non-transduced MSCs.	Precipitation kit (Total Exosome Isolation reagent, Thermo Fisher), 10,000× *g* 1 h. TEM, NTA (50–150 nm), WB CD9/CD63. No purity or negative-marker data.	80 µg per mouse (protein), IV tail vein, once weekly, weeks 9–16 of age (eight doses; started ≈1 week after ring placement, i.e., early/preventive).	Normal MSC exosomes and no-HFD blank used in vitro/in vivo.	Smaller plaques (H&E); ↑ lumen-area ratio; ↓ JAM-A in plaque (IHC/WB); body weight unchanged. Numerical values not given in text.	miR-145 → JAM-A 3′UTR (luciferase, RIP); ↓ HUVEC migration, rescued by JAM-A plasmid (in vitro). In vivo: association only. Effect refers to miR-145-enriched, not native, exosomes.	[86]
ApoE^{-}/$^{-}$ mice on HFD 21% fat and 0.25% cholesterol; HUV-EC-C endothelial cells (ox-LDL) in vitro. Sex not reported. *n* = 6/group, 10 groups (60 mice).	Human BM-MSC-derived exosomes.	Differential centrifugation (300× *g* for 5 min, 2000× *g* for 10 min, 10,000× *g* for 35 min), then 0.22 µm filtration. Followed by ultracentrifugation at 100,000× *g* for 2 h, a PBS wash, and a second 100,000× *g* spin for 2 h. TEM, WB CD63/CD90/TSG101	In vivo: 1.2 µg/g EXO (or DMSO vehicle), tail vein, twice weekly for 2 weeks. Dosing began after 8 weeks of HFD, and HFD continued throughout. Lentivirus arms: 2×10^{9} pfu, tail vein, twice weekly for 2 weeks, after EXO dosing. In vitro: cells were pre-treated with oxLDL for 24 h, then given 20 µg EXO (dissolved in DMSO) for 48 h.	FENDRR knockdown; miR-28 inhibitor; TEAD1 loss (in vivo loss-of-function arms).	MSC exosomes restored endothelial cell viability in vitro and reduced arterial injury; FENDRR knockdown aggravated injury; miR-28 inhibitor reduced plaque formation.	lncRNA FENDRR (delivered by exosomes, microarray) sponges miR-28 → ↑ TEAD1. In vivo perturbation of the axis performed (knockdown/inhibitor).	[87]

Table 2. *Cont.*

Animal Model (Strain, Sex, *n* per Group, Diet)	Vesicle Source and Type	Isolation and Characterization	Dose (Unit), Route, Frequency, Duration; Timing vs. Disease Induction	Comparator(s)	Main Outcomes (Quantitative Where Reported)	Mechanism Claimed; Level of Evidence	Ref.
Female ApoE^{-}/$^{-}$ mice, 4–6 wk; HFD (10% sucrose, 10% lard, 2% cholesterol, 0.5% bile acids) for 4 wk before treatment. ***n* = 4/group** (+4 C57BL/6 normal-diet controls).	Mouse BM-MSC (commercial, Procell CP-M131); exosomes.	Differential centrifugation + UC 120,000× *g* 70 min ×2; BCA; TEM, NTA (mean ≈ 148 nm), WB CD9/CD63/Alix.	**400 µg/kg** once weekly, IV tail vein, 8 consecutive weeks (≈3.2 mg/kg cumulative; ≈10 µg per dose in a 25 g mouse); started after 4 wk HFD, diet continued.	Untreated ApoE^{-}/$^{-}$ model (no vehicle injection described); normal-diet C57BL/6.	vs. model: TC and TG ↓ ($p < 0.05$); IL-1β and IL-18 ↓ ($p < 0.05$); NLRP3, caspase-1, GSDMD mRNA/protein ↓; lesions qualitatively smaller with ↑ collagen and thicker fibrous cap (Masson/ORO). **Plaque area not quantified.** Aortic RNA-seq: 3852 DEGs.	Inhibition of NLRP3/caspase-1/GSDMD pyroptosis. Correlative (no in vivo pathway perturbation, no cargo identified).	[88]
ApoE^{-}/$^{-}$ mice; western diet D12108C (40 kcal% fat, 1.25% cholesterol) 12 wk, then 4 wk treatment on diet. ***n* = 6/group**; sex not stated.	Mouse BM-MSC (commercial). **Exosome-mimetic nanovesicles (ENVs) made by serial extrusion** (1 µm → 400 → 200 nm), not naturally secreted exosomes; ENVs fused with human platelet membrane (P-ENVs).	Extrusion + iodixanol gradient, UC 100,000× *g*; NTA (ENV 142.5 nm, P-ENV 150.9 nm), TEM, WB CD81/TSG101/Alix/CD73 (+GPVI, GPIbα, CD62P on P-ENV).	**150 µg** per injection, IV tail vein, twice weekly × 4 weeks (≈1.2 mg cumulative), after 12 wk diet (established lesions in mice; diet continued).	PBS; unmodified ENVs. No native-exosome arm in vivo.	Whole-aorta ORO plaque area: P-ENV **8.3 ± 0.9%** vs. ENV 15.9 ± 1.0% vs. PBS **29.6 ± 3.5%** ($p < 0.001$). Necrotic core 6.7 ± 1.0% vs. 13.5 ± 2.2% vs. 28.2 ± 3.4%. Serum TG/TC/LDL/HDL unchanged. No organ toxicity on H&E.	Platelet-membrane targeting (IVIS: ↑ aortic accumulation vs. ENV); ↑ ABCA1/ABCG1 (WB; VSMC in vitro and aorta) → cholesterol efflux. miRNA profiles of ENV and P-ENV similar (association only). Same group and protocol as [13].	[89]
Male ApoE^{-}/$^{-}$ mice, ≈8 wk; HFD (21% fat, 0.15% cholesterol); ***n* = 15/group** (+10 C57BL/6J on chow).	**Human** umbilical cord MSC exosomes (xenogeneic); miR-100-5p mimic/inhibitor-transfected MSCs for in vitro work.	Differential centrifugation, UC 100,000× *g* 1 h ×2; TEM, NTA (≈163 nm), WB CD63/CD9/CD81/CD82.	200 µL exosomes per injection, IV tail vein. **Protein dose per mouse, frequency and duration are not reported** (80 µg/mL used in vitro).	PBS 200 µL; exosomes + miR-100-5p inhibitor; wild-type control.	↓ Plaque area (H&E; values only in figure); ↓ serum ECP, TNF-α, IL-1β, IL-6, IL-8; effects reversed by miR-100-5p inhibitor ($p < 0.05$).	miR-100-5p → FZD5 → Wnt/β-catenin in ox-LDL-treated bone-marrow eosinophils (in vitro; luciferase, rescue). In vivo: inhibitor arm supports miR-100-5p dependence.	[90]

Table 2. *Cont.*

Animal Model (Strain, Sex, *n* per Group, Diet)	Vesicle Source and Type	Isolation and Characterization	Dose (Unit), Route, Frequency, Duration; Timing vs. Disease Induction	Comparator(s)	Main Outcomes (Quantitative Where Reported)	Mechanism Claimed; Level of Evidence	Ref.
Male ApoE^{-}/$^{-}$ mice, 8 wk; standard diet 2 wk → HFD 4 wk → streptozotocin 50 mg/kg/day i.p. ×3 d (glucose ≥ 8.6 mmol/L = diabetic). n = 10 at start, **six analyzed/group**.	Primary mouse BM-MSC (male C57BL/6J, 3–4 wk). Authors term the particles "EVs" (ISEV recommendation).	Ultrafiltration + ultracentrifugation; TEM, NTA (134.4 ± 75.1 nm), WB TSG101/CD9/CD63, calnexin-negative.	**100 µg** (200 µL) IV tail vein, twice weekly (≈2.4 mg cumulative); ≈12 weeks (duration stated only in the liver-histology section); started after diabetes was established.	Diabetic (DA) + saline; non-diabetic HFD; chow; rapamycin 1 mg/kg/day i.p.; PCSK9 inhibitor SBC-115076 4 mg/kg/day s.c.; BM-MSC cells IV.	vs. DA: glucose, TC, TG, LDL ↓; insulin ↑; HDL unchanged; aortic plaque area ↓ (ORO/H&E); plaque collagen ↑ (all $p < 0.05$; values in figures). TC/TG/LDL not different from PCSK9-inhibitor group.	↑ autophagy (LC3B ↑, p62 ↓) and ↓ iNOS^{+} M1 macrophages in plaque (correlative in vivo). AMPK/mTOR dependence shown only in RAW264.7 cells (Compound C, bafilomycin A1). Glucose lowering is a confounder for the plaque effect.	[72]
Male Golden Syrian hamsters, 3 mo; "HH" model: chow + 3% cholesterol + 15% butter + daily 8% NaCl gavage, 16 wk. n: control 11; HH 18; ADSC-EV 10; MSC-EV 9; +siRNA 5 and 6; siRNA alone 8 (total 67).	Allogeneic hamster ADSC- and BM-MSC-derived EVs: **mixed fraction ≈30% exosomes/≈70% microvesicles**; Smad2/3-siRNA-loaded EVs (Lipofectamine).	UC 100,000× g 20 h; Zetasizer (bimodal 50–100/100–1000 nm), flow cytometry CD63/CD9/CD81 and Annexin V, TEM.	100 µg/mL in 300 µL PBS (≈30 µg per injection), **retro-orbital**, **once monthly ×4 months**, from week 2 of the diet (concurrent/preventive).	Healthy control; untreated HH; Smad2/3 siRNA alone (s.c.).	HH vs. EV-treated: cholesterol 641.7 → 244.5 (ADSC-EV)/300.8 (MSC-EV) mg/dL; TG 1073 → 212/175; LDL 635 → 307/303 (all remained above control). Aortic wall thickness ↓ in all EV groups; **inner diameter unchanged**; distensibility ↑ with ADSC-EV only. MSC-EV did not lower plasma Ang II or carotid macrophage/M1 infiltration. **No plaque morphometry.**	TGF-β1/Ang II → Smad2/3, ATF-2, NF-κB; miR-21/29a/192/200b/210/146a ↓ (tissue expression; correlative). Vascular function endpoints (echo, myography), not lesion size.	[71]
Male C57BL/6 mice (not ApoE^{-}/$^{-}$), 6–8 wk; HFD 60 kcal% fat, 16 wk total; treatment from week 12. ***n* = 5/group** (control, HFD model, +MSC-exo, +Ba-exo).	Mouse adipose-derived MSC (commercial, Pricella CP-M138); baicalin-preconditioned (1 µM, 48 h; Ba-exos) vs. unconditioned exosomes.	Differential centrifugation to 12,000× g 70 min ×2 (0.22 µm filtration); TEM, NTA (70–200 nm), WB CD9/CD63/TSG101.	**150 µg** twice weekly × 4 weeks (weeks 12–16; ≈1.2 mg cumulative).	Saline control; HFD model; unconditioned MSC-exos.	MSC-exos and Ba-exos ↓ aortic-arch lipid-containing lesions/plaque area (ORO, Masson) and serum MCP-1, IL-6, VCAM-1, ICAM-1. **Ba-exos vs. MSC-exos: difference in vivo not significant.** Values in figures.	Ba-exos ↑ SIRT1 → ↓ p65 acetylation/NF-κB in ox-LDL-treated VSMCs (si-SIRT1 rescue in vitro). In vivo: WB correlation only. C57BL/6 mice are relatively atherosclerosis-resistant; lesions are early.	[91]

Table 2. *Cont.*

Animal Model (Strain, Sex, *n* per Group, Diet)	Vesicle Source and Type	Isolation and Characterization	Dose (Unit), Route, Frequency, Duration; Timing vs. Disease Induction	Comparator(s)	Main Outcomes (Quantitative Where Reported)	Mechanism Claimed; Level of Evidence	Ref.
ApoE$^{-/-}$ mice; western diet D12108C 12 wk, then 4 wk treatment on diet. ***n* = 6/group**; sex not stated.	Mouse BM-MSC exosomes fused with human platelet membrane (MSC-ExoP).	UC 120,000× *g* 70 min ×2 after 13,500× *g*; TEM, NTA (exosome 111.5 nm → MSC-ExoP 143.0 nm), WB CD81/TSG101/Alix/CD73 (+GPVI, GPIbα, CD62P).	**150 µg** per injection, IV tail vein, twice weekly × 4 weeks (≈1.2 mg cumulative), after 12 wk diet. 3-MA 3 mg/100 g i.p. every other day (autophagy-inhibition arm).	PBS; unmodified MSC-exo; MSC-ExoP + 3-MA.	Whole-aorta ORO plaque area: MSC-ExoP **5.62 ± 0.83%** vs. MSC-Exo 13.19 ± 1.42% vs. PBS **22.32 ± 2.29%** ($p < 0.001$). Necrotic core 7.28 ± 3.71% vs. 19.00 ± 4.18% vs. 33.59 ± 4.54%. Collagen 1.61-fold vs. MSC-exo. Serum lipids unchanged.	Platelet-membrane targeting; ↑ VSMC autophagy (LC3-II/I ↑, p62 ↓) → ↓ proliferation, migration, foam-cell formation; effect abolished by 3-MA in vitro and in vivo (non-selective inhibitor). Same group and protocol as [6].	[92]

In diabetic ApoE^{-}/$^{-}$ mice (HFD plus streptozotocin), Liu et al. [72] reported that BM-MSC-derived EVs (100 µg twice weekly for about 12 weeks) lowered glucose, TC, TG and LDL-cholesterol, reduced plaque area and increased plaque collagen, with lipid effects not different from a PCSK9-inhibitor comparator. Because glycemia and lipids improved together, the plaque effect cannot be ascribed to a direct vascular action, and the AMPK/mTOR-autophagy mechanism was tested only in RAW264.7 macrophages. Comarita et al. [71] used hypertensive–hyperlipidemic Golden Syrian hamsters, in which allogeneic ADSC- or BM-MSC-derived EVs (a mixed fraction of about 30% exosomes and 70% microvesicles; roughly 30 µg per retro-orbital injection, once monthly for 4 months, starting in week 2 of the diet) lowered plasma cholesterol (from 642 to 244–301 mg/dL), triglycerides and LDL-cholesterol, reduced aortic wall thickness and vascular inflammatory markers, and improved myographic responses. The effects were not uniform: MSC-derived EVs did not improve aortic distensibility, plasma angiotensin II or carotid macrophage infiltration, inner diameter did not change significantly, and lesion size was not measured.

Exosome-mimetic nanovesicles were produced by serial extrusion, not secreted exosomes; en face plaque area was 8.3 ± 0.9% with P-ENVs, 15.9 ± 1.0% with unmodified ENVs and 29.6 ± 3.5% with PBS [89]. Gao et al. [90] showed that human umbilical cord MSC-derived exosomes reduced plaque area and serum eosinophil cationic protein and cytokines in ApoE^{-}/$^{-}$ mice ($n = 15$ per group), and that co-administration of the miR-100-5p inhibitor reversed the effect; the FZD5/Wnt/β-catenin mechanism itself was established in cultured eosinophils. Also, Yang et al. [91] found that baicalin-preconditioned adipose MSC-derived exosomes suppressed NF-κB signaling in ox-LDL-treated VSMCs more strongly than unconditioned exosomes (SIRT1-dependent deacetylation of p65), whereas in C57BL/6 mice on HFD ($n = 5$ per group) the additional benefit over unconditioned exosomes was not statistically significant; C57BL/6 mice are relatively resistant to atherosclerosis, and their lesions are early. Jiang et al. [89,92] coupled MSC-derived vesicles to platelet membranes to enhance plaque targeting. In [92], platelet-membrane-fused exosomes gave 5.6 ± 0.8%, 13.2 ± 1.4% (unmodified exosomes) and 22.3 ± 2.3% (PBS), and the benefit was attenuated by the autophagy inhibitor 3-MA. Both studies come from the same group, used the same protocol (12 weeks of western diet, then 150 µg twice weekly for 4 weeks, $n = 6$ per group) and reported unchanged serum lipids; they should be regarded as related rather than independent confirmations.

MSC sources include mouse bone marrow (commercial or primary), mouse adipose tissue, human umbilical cord (xenogeneic in mice) and hamster bone marrow and adipose tissue. EVs were obtained by ultracentrifugation in most of the studies, but also by a precipitation kit [86], ultrafiltration plus ultracentrifugation [72], extrusion of whole cells [89] and, in one case, a mixed exosome/microvesicle pellet [71]. Characterization generally comprised electron microscopy, NTA and two to four positive markers; a negative (non-vesicular) marker was reported in only one study [72] and particle-to-protein purity ratios in none of the full texts we reviewed, so conformity with current MISEV2023 recommendations cannot be assumed. Dose was expressed per mouse [70,72,86,89,90,92], per kilogram [8], as an injected volume without protein mass [90] or as a concentration [86], and in the full texts we reviewed always as protein mass rather than particle number. Assuming a 25 g mouse for [88], the reported regimens correspond to cumulative doses from about 0.08 mg to 2.4 mg per mouse, an approximately 30-fold range, with treatment lasting from 4 weeks to 4 months and frequency from twice weekly to once monthly. Animal sex is inconsistent (female [86,88]; male [71,72,90,91]; not stated [89,92]), group sizes were small (4–15), and in the texts available to us randomization was mentioned only occasionally, while blinded outcome assessment and sample-size justification were not described. Background pathology also differs (diet-induced hyperlipidemia, streptozotocin diabetes, hypertension plus

hyperlipidemia, surgical carotid injury), and outcomes range from en face plaque area to serum cytokines, aortic gene expression and vascular reactivity, so that "plaque reduction" is not a uniform end point. Plasma lipids fell in some studies [71,72,88] and remained unchanged in others [89,92], which means that part of the observed lesion effect may be indirect in some models and direct in others.

Chen et al. reported that BM-MSC-derived EVs were developed as a nanoconstructed drug shipper that possessed the objective form and active protein form. In objected form, for the definitive addressing of vascular cells, the authors made a combination of cholesterol-modified aptamers of VEGF (vascular endothelial growth factor) with EVs. For the active protein form, EVs with VEGF were affected by SIRT1 (silent information regulator 2-related enzyme 1 of the adenovirus). The modified exosomes were studied in in vitro and in vivo conditions. The authors found that injection of these exosomes to mice *i.v.* demonstrated the decrease in the development of AS and abolished the process of endothelial–mesenchymal transition in vascular endothelial cells of the aortic branch in atherosclerosis. The results of the histology analysis demonstrated that EVs with SIRT1 and VEGF reduced the diameter of atherosclerotic lesions [93].

It was reported that EVs had supported cardio-defensive responses: decreasing apoptosis; promoting angiogenesis and immunomodulation; reducing fibrosis and oxidative stress; and regenerative properties [94]. Ding et al. noted that microRNAs such as miRNA-29b (miR-29b), miRNA-133a-3p (miR-133a-3p), and miRNA-24-3p (miR-24-3p) were involved in addressing the pathophysiology of myocardial infarction. However, the authors noted that for clinical transition, there is a need to take into account these parameters: suitability to standard procedures, manufacturing conditions, and regulatory compliance [94].

All studies delivered EVs while the atherogenic diet continued, and treatment began either together with the diet [71,85] or after 4–12 weeks of diet, when murine lesions are still fatty streaks or early fibro-fatty plaques [72,88,89,91,92]. In the full texts we could examine, no study included a baseline group devastated at the start of treatment, so their results demonstrate attenuation of progression rather than regression, and none tested advanced, calcified or ruptured lesions, or vesicle treatment after diet normalization. The results therefore relate to the prevention or slowing of early experimental plaque formation and cannot be extrapolated to the treatment of established, complex human atherosclerotic disease that develops over decades. Illustratively, 150 µg per 25 g mouse equals 6 mg protein/kg; body-weight-based allometric scaling to a 70 kg adult gives about 34 mg per dose (and simple linear scaling about 420 mg), figures that depend on vesicle yield and characterization and that no study has related to the dose–response curve.

The preclinical literature supports a limited conclusion: intravenous MSC-derived EVs, with or without engineering, reduced lesion burden and inflammatory markers in mouse models of diet-induced atherosclerosis, and platelet-membrane coating increased plaque targeting in this setting. The evidence is nevertheless narrow. First, the direction of effect is uniform, which is compatible with publication bias toward positive preclinical findings and with the small group sizes. Second, most mechanistic claims rest on cell culture; in vivo loss-of-function tests exist only for a few axes [87,90,92]. Third, the models have limited external validity: ApoE^{-}/$^{-}$ mice develop lesions through markedly elevated remnant lipoproteins, rarely show plaque rupture with thrombosis, and are studied over weeks rather than decades; LDLR^{-}/$^{-}$ mice and large animals have not been used; human-derived EVs were injected into immunocompetent mice [86,90]; and one study [91] used a strain that hardly develops atherosclerosis. Fourth, independence is limited (two studies from one group with an identical protocol [89,92]): only one study included an active comparator [59], and none compared doses. Finally, safety data are confined to histology and blood chemistry over 4–12 weeks [71,89,92]; bio-distribution shows substantial hepatic and splenic uptake [89].

These findings justify further work, but they do not establish therapeutic efficacy in humans. Priorities before clinical development are independent replication under standardized EV reporting, dose–response designs with baseline groups, testing in advanced lesions and in LDLR^{-}/$^{-}$ and large-animal models, and direct comparison with statins or PCSK9 inhibitors.

The preclinical evidence in Table 1 was identified between March 2026 and May 2026 by searching for papers from PubMed/MEDLINE, Scopus, Web of Science, published between 2019 and 2025, using combinations of the terms "mesenchymal stem cell" OR "mesenchymal stromal cell" AND exosome* OR "extracellular vesicle*" AND atherosclerosis OR "atherosclerotic plaque", supplemented by screening the reference lists of relevant articles. We included English-language original in vivo studies in which MSC-derived exosomes or EVs were administered to animals with experimental atherosclerosis. We excluded reviews, conference abstracts, studies without accessible full text, and a study retracted by the journal. Ten studies met these criteria; their characteristics are extracted in Table 1, and in vitro experiments were treated only as mechanistic support.

6. Clinical Studies of Using MSCs and MSC-Derived EVs in Cardiovascular Diseases

In the previous chapter, we considered studies on the application of MSCs and MSC-derived EVs for atherosclerosis based on literature data from the last 5 years (Figures 4 and 5). When considering clinical studies on literature data of the period of last 7 years on the application of MSCs and MSC-derived EVs for atherosclerosis, we found only studies on cardiovascular diseases. This chapter considered clinical studies using MSC-derived EVs for cardiovascular diseases to date of search-year 2026, august 30 (Table 3). Within the cardiovascular domain specifically, the number of registered human trials using EV-based therapeutics remains strikingly limited. The terms were focused on how EVs were applied to diseases connected with cardiovascular conditions over the past 7 years from 2019. We aimed to consider, in this section, the therapeutic potential of MSC-derived EVs on alleviation of cardiovascular-connected conditions, in clinical trials.

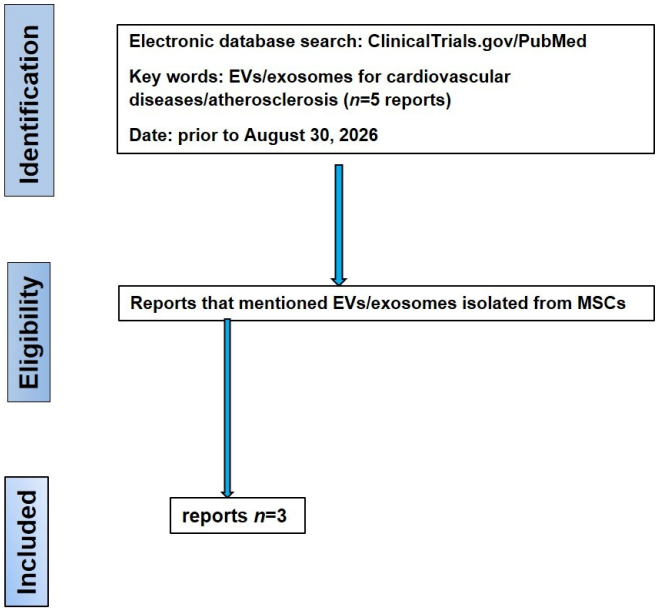


Figure 4. Diagram of the study of involvement and exception.

As of early 2025, only six studies related to EVs derived not only from MSCs in patients with cardiovascular diseases were identifiable in the ClinicalTrials.gov database [95].

Humbert et al. reported that this paucity of clinical trials reflects both the technical barriers to GMP-compliant EV manufacturing at the clinical scale and the absence of standardized regulatory frameworks for EV-based investigational medicinal products [96].

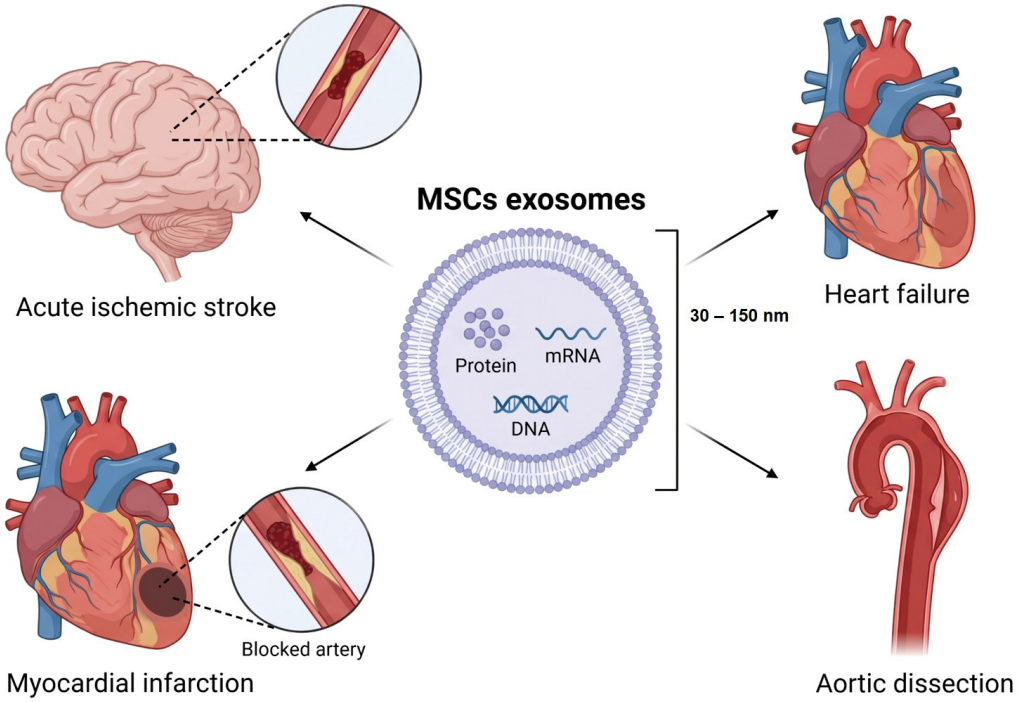


Figure 5. Clinical trials of MSC-derived EVs' therapy. Outline figure of clinical trials by disease type. Created with BioRender.com.

The attempts of application of EVs for cardiovascular diseases from the period of the last 7 years are considered in this chapter. The search at the ClinicalTrials.gov website had shown the registered clinical trials with "completed" status; however, therapeutic outcome was reported only for one. In the interventional study NCT03384433, the effects of EVs derived from allogenic MSCs that were combined with microRNA 124 (miR-124) were explored on patients who suffered acute ischemic stroke, aged 40–80 years, with a period of observation of 12 months (Table 2). The study was "randomized, single-blind, placebo-controlled, phase 1 and 2 trial", and both male and female acute ischemic patients were enrolled. As reported, results were evaluated by the MRS (modified ranking scale); however, if the completion data was from the year 2021, no data was found for any of the obtained results [97].

In the second clinical trial #NCT05669144, the authors investigated the impact of MSC-derived EVs on patients aged 35–80 years that were suffering from heart failure and acute myocardial infarction. The study was started in 2022 and ended in 2024, and was described as an interventional, phase 1 and 2 trial, placebo-controlled, randomized study. There were three groups presented EV treatment. The first group was intra-myocardial and intracoronary, which was administered with 1 mL of exosomes (100 μg); the second group, intra-myocardial and intracoronary, was administered with 1 mL of exosomes and 10×10^6 of mitochondria; the third group had intra-myocardial and intracoronary co-administration of 1 mL of exosomes (100 μg) combined with 10×10^6 of mitochondria. The methods of monitoring patients at the first month and 3 months after surgery were: measuring of blood indicators, SPECT (single-photon emission computed tomography), cardiac MRI (magnetic resonance imaging) and Dobutamine stress Speckle Echocardiography. On the "Clinical Trials. Gov" website final results were not shown [98].

Table 3. Clinical studies using MSC-derived EVs for cardiovascular diseases.

Type of Disease	Cell Source	Dose/Route of Delivery	Course of Treatment	Aim of the Study	Therapeutic Outcome	Database and Trial Registries	References
Acuteischemic stroke aged 40–80 years	Exosomes from bone-marrow MSCs delivered using mir-124	200 mg	12 months	To assess the therapeutic potential of miR-124-enriched MSC-derived exosomes in patients with acute ischemic stroke	Ongoing	ClinicalTrials.gov, 2021. ID NCT03384433	[97]
Heart failure and acute myocardial infarction, aged 35–80 years	Exosomes (Ex.) from MSCs and mitochondria (Mt.)	Three groups: intra-myocardial and intracoronary of Ex.; intra-myocardial and intracoronary administration of Mt.; intra-myocardial and intracoronary administration of Ex. and Mt.	3 months	To assess the effects of MSC-derived exosomes and autologous mitochondria, alone or in combination, on cardiac function in patients with severe heart failure undergoing CABG	No information	ClinicalTrials.gov, 2022. ID NCT05669144	[98]
Acute type A aortic dissection, female or male patients	Exosomes from MSCs	150 mg quantity, intravenous	Once a day for 14 times, 12-month period of time	To assess the therapeutic effects of MSC-derived exosomes in patients with MODS (Multiple organ dysfunction syndrome) after surgical repair of acute type A aortic dissection.	Ongoing	ClinicalTrials.gov, 2019. ID NCT03552848	[99]

In the third cardiovascular clinical trial—#NCT03552848—the authors studied the use of MSC treatment for acute type A aortic dissection (ATAAD). The study was started in 2018, and supposed termination was 2019; it was a randomized and interventional trial. The treatment was conducted on a cohort of 60 female or male patients diagnosed with ATTAD aged 20–60 years. Based on the eligibility criteria, patients were chosen according to the presence of the next conditions: ATAAD, having ascending aortic replacement, surgery, loss of two organs, sequential organ failure with the assessment rate ≥10. Patients were administered with 1×10^6 cells/kg, once every 4 days for four times, intravenously. The control group did not receive the MSCs. The methods of examination were observation of patients after treatment till 6 months of the survival rate, observation of unfortunate accidents during 2 years, sequential organ failure assessment score (SOFA) till 6 months and evaluation of IL-6 degree. The additional examination measures were adjusting the parameters of kidney, liver, lung, coagulation and central nervous system. However, the final results were not displayed. Taken together, three clinical trials were found that related to MSCs, with different concentrations of applied EVs, and in two of them as the treatment agent were used the EVs combined with microRNA 124 or with mitochondria [99].

In another study MSC-derived EVs were considered on clinical translation in different diseases. A comprehensive systematic review by Mizenko et al. catalogued 471 EV-related clinical trials registered in public repositories, spanning indications across more than 200 diseases. Of these, therapeutic trials—as opposed to diagnostic or companion diagnostic studies—predominantly utilized MSC-derived EVs, with respiratory conditions such as acute respiratory distress syndrome (ARDS) and COVID-19-associated pneumonia representing the most frequent indications. Cancer diagnostics constituted the largest category overall. Critically, the authors identified profound heterogeneity in isolation and characterization methodologies: ultracentrifugation and RNA sequencing were the most commonly reported techniques, yet neither was consistently described in study records. Only 11% of trials incorporated EV subpopulation analysis into their experimental design, underscoring the fundamental gap between preclinical rigor and clinical methodological reporting that threatens reproducibility and limits cross-study comparability [100].

Before considering the use of MSC-derived EVs for clinical applications, it is necessary to examine several aspects. The choice of the source of the MSCs as Almeria et al. reported (bone marrow, adipose tissue, umbilical cord blood, and umbilical cord) is important and should be considered when isolating EVs from each of them. Also, factors associated with the source of the MSCs, namely the age of the donor, the genetic state and predisposition to definite diseases, burdened state by chronic diseases (such as diabetes), and quality of well-being, all of them adjust the functional efficacy and output of the secretion process of EVs [101]. The authors found that the functional activity of secreted EVs is imperative and can depend on the propagation state of cells because long-term passaging can lead to the senescence of MSCs. Therefore, at high passage numbers, the meaningful abilities of EVs of MSCs, such as capability for regeneration, angiogenesis and anti-apoptosis, can decrease. Additionally, the culture system, such as choosing between 2D culturing or 3D culturing, may impact functional efficacy because, for example, in 3D conditions when culturing MSCs, the emerging dynamic bioreactor force may create some mechanical tension and can impact the amount of EV secretion [101]. Another aspect is that the composition of the medium must also be taken into account when deriving a sufficient quantity of EVs, as culture medium components of animal origin—such as fetal bovine serum—cannot be used for culturing MSCs and harvesting EVs from them [102]. Certain cautions are associated with proteins prone to solubilization and complement factors, which may also be co-isolated when EVs are extracted from serum or plasma. This affects the purity and reproducibility from one batch to another, as well as safety; therefore, the use of serum-free culture medium

components or specific purification methods, such as gel chromatography, can improve EV yield [102]. Notably, delivering microRNAs (miR-21, miR-126, and miR-210) and proteins to the target site, EVs can reduce apoptosis of cardiomyocytes, suppress ischemia, decrease fibrosis, and promote angiogenesis [103]. However, when considering EVs for use as a cell-free therapy in clinical practice, concerns are raised regarding off-target effects connected from their variegated composition. It is hypothesized that, as a result, these EVs could trigger undesirable biological processes in non-target tissues [104].

With the ongoing refinement of methods, it was indicated that the aspect of choosing the EV isolation technique that yields a sufficient quantity of vesicles is important. Key considerations include speed of the EV isolation process, its scalability, and the ability to perform the isolation in a manner that ensures the absence of protein aggregates and cellular debris [105]. The next aspect is the manner of dissemination and removal in the host organism after administration of EVs, which may vary from minutes to a period of hours. The authors state that, following systemic administration, less than 2% of the vesicles are likely to be transported to the target site—the damaged myocardium [106]. Regarding the aspect of biological compatibility, it is known that MSCs have low immunogenicity; however, the xenogenic components—such as those found in culture media containing serum of animal origin—can be problematic and may trigger an undesirable immune response [107]. It was reported that rather than triggering the active immune response, EVs of MSCs can actively modulate the local and systemic immune microenvironment. It was reported that EVs from MSCs can suppress excessive inflammation, promote anti-inflammatory M2-type macrophage polarization, and reduce pro-inflammatory cytokine expression in damaged myocardial tissue [108]. The other aspect to consider is that studies using human whole-blood samples demonstrated that standard EVs from MSC preparations did not result in any measurable enhancement of blood coagulation. The authors found that maintaining the safety and the absence of thrombogenicity depends on the adherence to GMP standards during the process of MSC cultivation and handling and EV isolation, purification, storage and bringing to the final product [109]. The final aspect as to whether MSC-derived EVs can be oncogenic or possess such properties remains an open question. It is hypothesized that this might be related to the fact that EVs contain microRNAs and growth factors, and whether they are delivered locally or systemically [110].

7. Conclusions

Atherosclerosis, as a multifactorial disease characterized by inflammation in the subendothelial space of the endothelium, was previously explored for reduction of its development by applying MSCs, which had demonstrated remarkable immunoregulatory and immunosuppression properties. Based on this, EVs isolated from MSCs were examined for cardiovascular diseases, including also atherosclerosis. Preclinical studies had demonstrated that MSC-derived EVs, through M1–M2 macrophage polarization, autophagy and miRNA reprogramming, can reduce the development of atherosclerosis, indicating immunosuppressive, atheroprotective and regenerative properties. Preconditioning of MSCs can contribute to advanced immunosuppressive properties of isolated EVs. Studies of clinical trials were completed; however, the authors of each clinical trial did not represent the final results on the effect of MSC-derived EVs for each investigated type of the cardiovascular condition. MSC-derived EVs can be supposed to be the safe in comparison with cellular therapy; however, there are concerns regarding toxicity, immunogenicity, the source of MSCs, target place, the method of delivery, quantity per ml, purity of the product, and instability with storage. Thus, because of insufficient data, the evidence for the safe application of MSC-derived EVs for cardiovascular disease was not constituted. Meanwhile, maintaining and conducting good clinical practices for the isolation of EVs from MSCs and

manufacturing, disease- and patient-specific approaches and elaboration on the shipment to the target place are required for a prosperous MSC exosome therapy.

Author Contributions: Conceptualization, A.S. and V.O.; methodology, A.S. and M.Z.; validation, A.S. and M.S.; formal analysis, A.S. and M.S.; investigation, A.S. and M.Z.; resources, A.S., M.Z., M.I. and M.Z.; data curation, A.S., M.Z. and M.I.; writing—original draft preparation, A.S., M.Z. and M.I.; writing—review and editing, A.S. and V.O.; visualization, A.S.; supervision, A.S.; project administration, A.S.; funding acquisition, A.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by a grant from the Ministry of Science and Higher Education of the Republic of Kazakhstan (AP27509399).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data uncovered in this article will be shared upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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