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Review

Extracellular Vesicle-Mediated Mitochondrial Transfer in Myocardial Ischemia-Reperfusion Injury

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Abstract

Cardiovascular disease (CVD) remains the predominant cause of mortality worldwide. Reperfusion therapy is the cornerstone of the management of acute myocardial infarction. However, it can result in myocardial ischemia-reperfusion (I/R) injury through oxidative stress, calcium overload, and activation of the inflammatory cascade. Mitochondrial dysfunction is at the core of this altered pathophysiology. Therefore, therapeutic strategies targeting mitochondrial dysfunction have attracted considerable attention. Intercellular mitochondrial transfer has emerged as a rescue mechanism, with extracellular vesicles (EVs), particularly microvesicles, serving as effective delivery vehicles that result in protection from proteolytic degradation and oxidative injury and can be administered systemically without capillary occlusion. However, there is significant confusion regarding the classification of mitochondria-containing vesicles, the molecular mechanisms governing cargo selection, and the methodological standards required to distinguish intact organelles from contaminating fragments. This review summarizes underlying intercellular mitochondrial transfer, which is distinct from the cellular quality-control pathways that target damaged organelles for degradation. Whereas metabolic rescue through adenosine triphosphate (ATP) restoration and apoptosis inhibition represents a therapeutic mechanism validated across multiple species, emerging applications in ferroptosis regulation, immune modulation, and regenerative signaling still require rigorous independent validation. We further analyze engineering strategies for clinical translation, including super-donor engineering for enhanced mitochondrial yield, surface modification for targeted delivery, multimodal cargo engineering, and delivery system optimization. By integrating biological insights with critical analysis of methodological limitations, this review provides a comprehensive framework to guide the rational engineering of mitochondria-enriched extracellular vesicles as precision adjunctive therapies for ischemic heart disease.

Keywords: mitochondrial transfer; myocardial ischemia-reperfusion; extracellular vesicles; microvesicles; tunneling nanotubes; mesenchymal stem cells

1. Introduction

Cardiovascular disease remains the predominant cause of mortality worldwide. Reperfusion therapy remains the cornerstone of clinical management for acute myocardial infarction. Myocardial ischemia-reperfusion (I/R) injury involves interconnected mechanisms including oxidative stress, calcium dysregulation, inflammation, and mitochondrial dysfunction [1]. Mitochondrial dysfunction is a significant component of this pathophysiology, characterized by respiratory chain disruption, membrane potential collapse, and the release of pro-apoptotic factors. Therefore, repair of the injured mitochondria has become an important therapeutic target [1,2,3]. Intercellular mitochondrial transfer has emerged as a rescue mechanism that occurs through tunneling nanotubes, direct organelle transplantation, and extracellular vesicle-mediated transport. Among these approaches, extracellular vesicle (EV)-mediated mitochondrial delivery has been investigated as a means of improving myocardial bioenergetics [4]. However, there is significant confusion regarding the classification of mitochondria-containing vesicles and their molecular mechanisms.

Existing reviews have broadly addressed mitochondrial transfer in cardiovascular disease and extracellular vesicle biology in cardiac repair; yet, none have focused specifically on the unique mechanistic requirements for whole-mitochondria encapsulation within microvesicles, the critical distinction between quality control pathways and therapeutic transfer mechanisms, and the rigorous methodological standards necessary to distinguish intact organelles from contaminating fragments. This review examines mechanisms of intercellular mitochondrial transfer, methodological considerations for validating mitochondrial cargo, and the strength of evidence supporting proposed therapeutic effects. By integrating biological insights with a critical analysis of methodological limitations, we aim to guide the rational engineering of mitochondria-enriched extracellular vesicles as adjunctive therapies for ischemic heart disease.

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2. Biological Foundations of Mitochondrial Packaging and Transfer

2.1 Classification, Sources, and Biogenesis of Mitochondria-Containing Vesicles

The biogenesis of mitochondria-enriched extracellular vesicles requires careful discrimination between distinct vesicular populations based on size, origin, and cargo composition. Plasma membrane-derived extracellular vesicles (EVs) form through outward budding. Mitochondria-containing EV preparations have been investigated in cardiac repair [4], but neither vesicle biogenesis nor intact mitochondrial encapsulation should be inferred from size alone [5]. This mechanism fundamentally differs from exosome formation, which involves the inward budding of endosomal membranes to create multivesicular bodies that fuse with the plasma membrane, yielding vesicles typically 50–150 nm in diameter—too diminutive to accommodate whole organelles. Mitochondria-derived vesicles constitute a third distinct class. These vesicles, typically 50–200 nm in diameter, bud directly from the outer mitochondrial membrane to sequester oxidized proteins and damaged mitochondrial DNA for lysosomal degradation or extracellular disposal as part of cellular quality control mechanisms [6]. Recent evidence has identified engineered cellular sources that overcome the limitation of low mitochondrial yield in conventional preparations. Specifically, genetic engineering of mesenchymal stem cells (MSCs) via activation of the cluster of differentiation 38 (CD38)/inositol 1,4,5-trisphosphate receptor (IP_3R)/Ca^{2+} pathway generates "super-donor" cells that produce Super-extracellular vesicles (EV)-Mito with threefold increase in EV-Mito yield compared to naive MSCs [7]. This non-viral gene delivery approach upregulates CD38 expression, catalyzing cyclic ADP-ribose synthesis to elevate cytoplasmic and mitochondrial calcium levels and thereby trigger actin-dependent plasma membrane budding that captures intact mitochondria [7]. Separate assays evaluated basal respiratory stability over 48 hours and resistance to calcium-associated membrane-potential loss after 24 hours [7].

EVs from regenerating neonatal cardiac tissue following apical resection showed Wdr75-associated effects on cardiomyocyte proliferation, apoptosis, and angiogenesis [8]. These findings concern regenerative EV cargo and do not establish intact mitochondrial transfer. These observations underscore that the cellular microenvironment and specific engineering strategies profoundly influence vesicle cargo composition and therapeutic potency [9].

2.2 Molecular Mechanisms of Mitochondrial Packaging and Transfer

The encapsulation of intact, respiratory-competent mitochondria within microvesicles involves specific molecular machinery distinct from quality control pathways that target damaged organelles for degradation. In MSCs, CD38/IP_3R/Ca^{2+} signaling has been implicated in the release of mitochondria-containing EVs [7]. Whether this process selectively enriches functional mitochondria, captures cytoplasmic contents more broadly, or also participates in mitochondrial disposal remains to be established in the relevant donor-cell system.

Previous studies have suggested that the PTEN-induced kinase 1 (PINK1)/Parkin pathway governs mitochondrial segregation into extracellular vesicles; however, current evidence indicates that this pathway primarily regulates mitophagy and the formation of mitochondria-derived vesicles containing oxidized protein cargo and fragmented DNA destined for degradation—rather than the packaging of functional, respiratory-competent organelles into microvesicles [6]. PINK1 accumulates on the outer mitochondrial membrane of damaged, depolarized organelles, recruiting the E3 ubiquitin ligase Parkin to mediate outer membrane protein ubiquitination and target the mitochondrion for autophagic degradation [10,11]. PINK1 and Parkin participate in a mitochondrial-derived vesicle pathway that delivers selected mitochondrial cargo to lysosomes [12]. Separately, Parkin-independent secretory mitophagy has been reported in a tumour model [6]. These findings describe distinct quality-control processes and do not establish therapeutic transfer of intact mitochondria.

In alveolar epithelial cells, the G protein-coupled receptor class C group 5 member A (GPRC5A)–Mitochondrial Rho GTPase 2 (MIRO2) pathway facilitates mitochondrial-fragment trafficking into late endosomes and contributes to mitochondrial quality control [13]. This finding should not be interpreted as evidence for therapeutic packaging of intact mitochondria into plasma membrane-derived microvesicles. Improved cardiomyocyte bioenergetics has been reported following EV-mediated mitochondrial cargo transfer [4] and isolated mitochondrial transfer [14]. These findings support the potential metabolic benefits of mitochondrial transfer but do not establish how donor cells selectively package functional mitochondria into EVs.

2.3 Recipient Cell Uptake and Functional Integration

Once released, mitochondria-enriched microvesicles must be internalized by recipient cardiac cells and the transferred organelles integrated into the host metabolic network to achieve the therapeutic effect. Cardiomyocytes internalize these vesicles through multiple routes, including clathrin-mediated endocytosis, caveolin-dependent uptake, macropinocytosis, and direct membrane fusion, with the specific internalization mechanism influencing the functional fate of the transferred mitochondria [15]. Following internalization, the vesicle membrane must be degraded or fused with the endosomal membrane to release the encapsulated mitochondria into the cytoplasm. The transferred organelles may persist within recipient cells for periods ranging from several hours to several days, during which they contribute to adenosine triphosphate (ATP) syn-

thesis through oxidative phosphorylation, buffer calcium overload, and restore mitochondrial membrane potential [4,14,16].

Functional integration requires the preservation of the integrity of mitochondrial DNA and the retention of oxidative phosphorylation complexes. However, the long-term fate of transferred organelles remains incompletely characterized, with evidence supporting both temporary functional complementation without genomic integration and rare instances of longer-term survival [15]. The efficiency of uptake and functional integration varies significantly depending on the metabolic state of the recipient cell, with ischemic cardiomyocytes demonstrating enhanced internalization capacity, potentially due to upregulation of specific scavenger receptors or increased membrane fluidity under stress conditions [4].

3. Methodological Standards for Isolation and Characterization

3.1 Isolation Techniques and Their Limitations

The translation of mitochondria-enriched extracellular vesicles from experimental models to clinical applications requires rigorous standardization of isolation protocols. Current methodologies exhibit significant heterogeneity, which compromises reproducibility and complicates comparison amongst studies [17]. Differential (ultra)centrifugation uses sequentially increasing centrifugal forces. A final step at approximately 100,000–200,000 ×g generally enriches a smaller EV fraction, whereas intermediate steps at approximately 10,000–20,000 ×g may enrich larger or denser EVs; the recovered populations can overlap [5]. Centrifugal force and size alone do not establish microvesicle biogenesis or the presence of intact mitochondrial cargo, which require experimental characterization [5].

Density gradient centrifugation utilizing iodixanol or sucrose gradients provides superior purification by exploiting differences in buoyant density between vesicle-encapsulated mitochondria, free organelles, and protein aggregates [18]. This method can effectively distinguish intact microvesicles from mitochondrial fragments and soluble proteins, though the extended centrifugation times required may impair the functionality of sensitive mitochondrial cargo. Size exclusion chromatography offers a gentler separation mechanism that preserves vesicle integrity but may inadequately remove microvesicles from apoptotic bodies or cellular debris within the 100–1000 nm range, necessitating careful pre-filtration and subsequent validation [17,19].

A conceptual workflow is presented for obtaining mitochondria-containing EVs from bone marrow-derived mesenchymal stem cells and illustrating their potential transfer to recipient cells (Fig. 1, Ref. [5]). The suitability of individual separation and characterization steps requires validation for the target EV population.

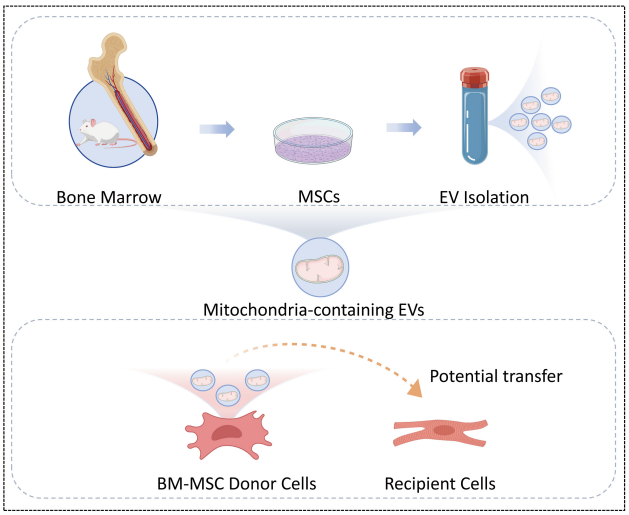


Fig. 1. Conceptual workflow for obtaining mitochondria-containing extracellular vesicles from bone marrow-derived mesenchymal stem cells. The diagram illustrates donor-cell culture, EV isolation, and potential transfer to recipient cells. CD9, CD63, and CD81 are commonly examined EV-associated tetraspanins rather than MSC-specific markers [5]. This schematic does not represent a single experimentally validated isolation protocol.

3.2 Criteria for Functional Mitochondrial Validation

Establishing definitive criteria for intact mitochondrial cargo remains essential, albeit methodologically challenging. Morphological confirmation through transmission electron microscopy with immunogold labeling can demonstrate the presence of double-membrane structures with preserved cristae architecture localized within the vesicle lumen, though this technique cannot assess functional respiratory capacity. Functional evaluation should distinguish direct respiratory measurements of isolated EV preparations from bioenergetic responses in recipient cells. Seahorse XF analysis was used to measure basal, ATP-linked, and maximal respiration in hypoxia-injured iPSC-derived cardiomyocytes after treatment with mitochondria-rich EVs, rather than in isolated EVs [4]. Direct basal respiratory activity of isolated EV-Mito preparations was assessed separately according to the previous research [7].

Recent studies emphasize the necessity of calcium resistance assays, as intact mitochondria within EVs maintain membrane potential in high-calcium environments (1.25–2.5 mM) that rapidly depolarize free mitochondria [7,20]. Molecular characterization via quantitative PCR to determine mitochondrial DNA copy number and Western blot analysis to detect oxidative phosphorylation complexes provides complementary metrics. However, these approaches cannot distinguish intact organelles from fragmented mitochondrial components containing residual DNA and proteins [7].

The emergence of single-vesicle analysis technologies offers promise for resolving these limitations. Imaging flow cytometry and nano-flow cytometry enable simultaneous visualization and fluorescence quantification of individual vesicles, allowing determination of the percentage of the vesicle population containing intact, membrane potential–retaining mitochondria versus those carrying only mitochondrial proteins or fragmented DNA [5].

3.3 Standardization Under Minimal Information for Studies of Extracellular Vesicles (MISEV) 2023 Guidelines

MISEV2023 provides a framework for characterizing EV preparations using complementary methods and appropriately selected markers, including assessment of EV-associated components and potential non-vesicular contaminants; it does not mandate a fixed panel of CD9, CD63, CD81, ALIX, and TSG101 [5]. However, specific standards for mitochondria-enriched populations remain undefined, creating significant barriers to translational development. Critical gaps include a lack of consensus regarding minimum thresholds for mitochondrial enrichment, standardized functional assays that measure bioenergetic rescue in recipient cells rather than mere morphological presence, and quality control parameters to ensure batch-to-batch consistency in mitochondrial cargo. Addressing these limitations requires developing potency assays that correlate mitochondrial transfer efficiency with functional outcomes, such as ATP restoration or improved cell viability in target cells, alongside rigorous protocols to exclude free mitochondrial contaminants that may co-purify with vesicle fractions due to similar sedimentation properties [5].

Beyond isolation and characterization, the clinical translation of mitochondria-enriched EVs necessitates consideration of optimal delivery routes to maximize myocardial retention and therapeutic efficacy. Three principal administration strategies have been explored in preclinical models of ischemic heart disease: intravenous infusion, intracoronary injection, and intramyocardial injection. Each modality presents distinct pharmacokinetic profiles and biodistribution patterns that influence the efficiency of mitochondrial transfer to injured cardiomyocytes. A comparative overview of these delivery approaches is provided in Fig. 2.

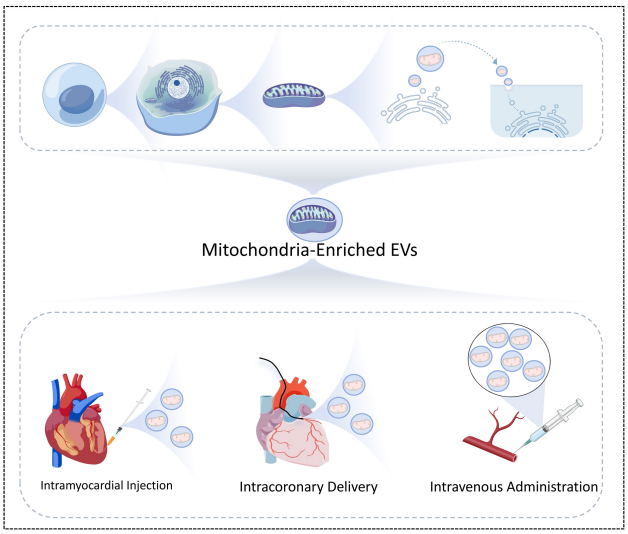


Fig. 2. Schematic of EV formation, mitochondrial enrichment, and administration routes for cardiac applications. The upper panel conceptually depicts donor cells, mitochondrial cargo, and vesicular packaging and release, leading to the mitochondria-enriched extracellular vesicles (EVs) illustrated in the centre. The lower panel shows three potential administration routes: intramyocardial injection directly into myocardial tissue, intracoronary delivery through the coronary circulation, and intravenous administration into the systemic circulation. The illustration summarizes proposed preparation and delivery concepts; it does not establish a specific intracellular biogenesis pathway or indicate that all depicted processes and routes have been validated for a single EV preparation.

4. Therapeutic Efficacy in Myocardial Ischemia-Reperfusion Injury

A preclinical study of induced pluripotent stem cell (iPSC)-derived cardiomyocyte EVs demonstrated improved bioenergetics in hypoxia-injured cardiomyocytes and improved cardiac function following intramyocardial administration in a mouse myocardial infarction model [4]. This evidence should be distinguished from studies of free mitochondrial transplantation and from myocardial I/R models. The cardioprotective mechanisms underlying these effects are multifaceted and well-characterized. Restoration of mitochondrial membrane potential in recipient cardiomyocytes prevents the release of cytochrome c and subsequent activation of caspase-dependent apoptotic cascades [21,22]. Enhanced resistance to calcium-associated loss of mitochondrial membrane potential has been reported for an engineered EV-Mito preparation compared with isolated mitochondria [7]. In addition, transferred mitochondria attenuate arrhythmogenic substrate formation by stabilizing cellular electrophysiology and reducing the heterogeneity of repolarization in damaged tissue [21]. These established effects have been corroborated across species ranging from murine to porcine models, with consistent findings regarding metabolic recovery and improvements in cardiac function [15,23].

Recent investigations have suggested additional therapeutic modalities that extend beyond straightforward bioenergetic rescue, although these have limited experimental data and require further validation. Ferroptosis is an iron-dependent form of regulated cell death associated with lipid peroxidation. Arachidonate 15-lipoxygenase (Alox15)/15-hydroperoxyeicosatetraenoic acid (15-HpETE)-mediated cardiomyocyte ferroptosis contributes to myocardial ischemia–reperfusion injury

[24]. Whether EV-mediated mitochondrial transfer inhibits ferroptosis in myocardial I/R injury, and whether any effect depends on glutathione peroxidase activity or reduced lipid peroxidation, remains to be established through direct mechanistic experiments.

In experimental lung-injury models, human mesenchymal stromal cell (MSC)-derived EVs transferred mitochondria to macrophages, enhanced macrophage oxidative phosphorylation, and promoted an anti-inflammatory, highly phagocytic phenotype [25]. These findings arise from lung-injury models and do not establish the same mechanism in myocardial I/R injury. Whether MSC-derived mitochondria-containing EVs similarly reprogram resident cardiac macrophages and reduce myocardial fibrosis remains to be determined. Determining whether macrophage responses are attributable to mitochondrial cargo rather than other EV components requires appropriate functional perturbation and control experiments [5].

Novel therapeutic concepts have emerged from limited experimental studies and, while promising, require extensive validation before clinical consideration. Extracellular vesicles derived from regenerating neonatal mouse heart tissue following apical resection have been reported to contain significantly elevated levels of Wdr75 protein and demonstrate superior capacity to promote cardiomyocyte proliferation and angiogenesis compared to adult cardiac vesicles [8]. EVs from regenerating neonatal heart tissue promote cardiomyocyte cell-cycle re-entry through Wdr75-associated suppression of the p53-p21 pathway [8]. However, these findings are currently derived from single-study observations with limited characterization of the involved mechanisms. Furthermore, the use of neonatal cardiac tissue for clinical vesicle production presents significant practical constraints.

Innovative delivery strategies, including transpulmonary inhalation of stem cell-derived vesicles, have shown efficacy in porcine myocardial infarction models, achieving cardiac targeting through pulmonary capillary transit and subsequent myocardial uptake [26,27]. While conceptually elegant and clinically appealing due to its non-invasive nature, this approach currently rests upon limited experimental validation, and the mechanisms underlying lung-to-heart vesicle trafficking remain speculative. An injectable alginate–collagen hydrogel supported sustained EV release for at least 7 days in a rat myocardial infarction model [28]. This formulation should be distinguished from sodium alginate microsphere delivery of neonatal cardiac tissue-derived EVs [8].

The studies previously mentioned reveal a clear disparity in the strength of evidence for different cardioprotective mechanisms attributed to EV-mediated mitochondrial transfer. The most validated effects, supported by consistent findings across multiple species and independent laboratories, are metabolic rescue via restoration of ATP and inhibition of apoptosis [23]. In contrast, the emerging roles in ferroptosis regulation [29] and immune modulation via macrophage polarization [30] remain mechanistically plausible but await independent verification. Other promising concepts, including Wdr75-mediated regenerative signaling from neonatal cardiac tissue-derived EVs [8] and novel delivery strategies such as transpulmonary inhalation [26] or alginate microsphere encapsulation [28], are currently based on single-study observations with limited data on the involved mechanisms. Representative preclinical studies comparing different EV donor sources, animal models, delivery routes, research outcomes and existing limitations are summarized in Table 1 (Ref. [4,7,8,26,28]).

5. Engineering Strategies for Clinical Translation

5.1 Enhancing Mitochondrial Yield and Quality Through Super-Donor Engineering

Realizing the clinical potential of mitochondria-enriched extracellular vesicles requires sophisticated engineering approaches that address the critical limitations of yield enhancement and target specificity. The clinical translation of mitochondrial transplantation faces challenges concerning mitochondrial sourcing and isolation [31]. For mitochondria-enriched EVs, reproducible production and adequate yields of functional mitochondrial cargo remain important development objectives. In adipose-derived MSCs, non-viral activation of CD38/IP_3R/Ca^{2+} signaling increased the yield of mitochondria-containing EVs (Super-EV-Mito) approximately threefold compared with control MSCs [7]. Non-viral CD38 gene delivery to rat adipose-derived MSCs increased the yield of Super-EV-Mito to approximately three times that obtained from control MSCs [7]. Isolated Super-EV-Mito preparations also showed higher basal oxygen consumption normalized to mitochondrial protein [7]. The study evaluated LHON and rotenone-induced mitochondrial dysfunction models, including intravitreal administration in mice; it did not investigate myocardial ischemia–reperfusion injury [7].

Manipulation of mitochondrial dynamics through careful modulation of Dynamin-related protein 1 (DRP1)-mediated fission or promotion of mitofusin-2 (MFN2)-dependent fusion increases the mitochondrial mass within donor cells [2].

5.2 Surface Modification and Targeting

Surface modification strategies aim to improve myocardial targeting and reduce off-target clearance by the reticuloendothelial system. Distinct EV-targeting strategies include selectin-binding peptide modification for injured endothelial cells [32] and cardiomyocyte-binding peptide engineering for cardiac delivery [33]. Valine-Histidine-Proline-Lysine (VHPK)-mediated vascular cell adhesion molecule-1 (VCAM-1) targeting has also been investigated using plant-derived EVs in atherosclerosis [34], representing a different disease and delivery context. However, op-

Table 1. Representative preclinical studies relevant to mitochondrial cargo transfer and EV-based cardiac repair.

EV source	Mouse and porcine MI models	Delivery route	Major findings	Key limitations
iPSC-CMs	Mouse MI	Intramyocardial	Restored myocardial energetics; improved LVEF; enhanced mitochondrial biogenesis [4]	Long-term immunogenicity unknown; iPSC scalability
Rat adipose-derived MSCs engineered through non-viral CD38 gene delivery (Super-EV-Mito)	LHON and rotenone-induced mitochondrial dysfunction models, including cell and mouse experiments	Intravitreal administration in the ocular mouse experiments	EV-Mito yield approximately three times that obtained from control MSCs; higher basal oxygen consumption normalized to mitochondrial protein. Basal respiratory activity remained relatively stable for up to 48 h. A separate calcium-resistance assay measured MitoTracker Red fluorescence after 24 h of Ca^{2+} exposure [7]	Non-cardiac engineering proof of concept; efficacy in myocardial ischemia–reperfusion injury was not evaluated
Neonatal cardiac tissue	Mouse MI	Intramyocardial	Wdr75-mediated inhibition of p53-p21 pathway; regenerative effects associated with Wdr75 signaling [8]	Single study; not clinically scalable
Lung spheroid cell-derived exosomes	Mouse and porcine myocardial infarction models	Inhalation/nebulization	Inhaled exosomes improved cardiac outcomes in mouse and porcine MI models; endothelial CD36-related metabolic regulation was investigated in mouse experiments [26]	Mitochondrial cargo transfer was not established
EV-loaded injectable alginate–collagen hydrogel	Rat myocardial infarction	Injectable hydrogel	Sustained EV release for at least 7 days [28]	Do not establish preservation or delivery of intact, functional mitochondrial cargo

Abbreviations: EV, extracellular vesicle; iPSC-CMs, induced pluripotent stem cell-derived cardiomyocytes; I/R, ischemia-reperfusion; LVEF, left ventricular ejection fraction; MI, myocardial infarction; MSCs, mesenchymal stem cells; CD36, cluster of differentiation 36; LHON, Leber hereditary optic neuropathy.

timizing targeting ligand density is critical: ligand density should be optimized experimentally, with parallel assessment of targeting efficiency, biodistribution, and immune interactions.

5.3 Multimodal Cargo Engineering

Multimodal cargo engineering is a proposed strategy for combining complementary therapeutic functions. EV-mediated Vascular endothelial growth factor A (VEGF-A) mRNA delivery has shown therapeutic effects in ischemic injury models [35], but this study does not establish co-loading of intact mitochondria and VEGF-A mRNA within the same vesicles. Mitochondrial transplantation has been investigated as a cardioprotective strategy in myocardial I/R injury [21]. Building on this background, combining mitochondrial cargo with anti-apoptotic miRNAs or ferroptosis inhibitors represents a potential engineering direction, but any synergistic benefit requires direct experimental validation and appropriate single-cargo controls.

5.4 Delivery System Optimization

Delivery system innovations must address the practical constraints of clinical administration. While intracoronary injection remains the most feasible route for immediate post-reperfusion therapy, achieving global myocardial distribution without procedural complications requires optimization of infusion parameters [22]. Sodium alginate hydrogel microspheres have been investigated as injectable carriers for sustained delivery of neonatal cardiac tissue-derived EVs [8]. The development of inhalable formulations offers the potential for non-invasive administration, although standardizing nebulization parameters to preserve vesicle integrity remains challenging [26,27].

6. Conclusions

Extracellular vesicle-mediated mitochondrial transfer represents a paradigm shift in the therapeutic approach to myocardial ischemia-reperfusion injury. It provides distinct biological advantages over direct organelle transplantation, including enhanced cargo protection, immune compatibility, and the potential for multimodal therapeutics. This review has provided a mechanistic synthesis that resolves prevailing ambiguity regarding the PINK1/Parkin pathway. CD38/IP_3R/Ca^{2+} signaling has been implicated in the release of mitochondria-containing EVs from MSCs [7]. By contrast, the GPRC5A–MIRO2 pathway described in alveolar epithelial cells facilitates mitochondrial-fragment trafficking into late endosomes as part of mitochondrial quality control [13]. These findings should not be combined as evidence for a general mechanism of intact mitochondrial packaging for cardiac therapy.

Vesicle nomenclature should reflect demonstrated biogenesis or operational characteristics [5]. Neither vesicle size nor detection of mitochondrial proteins alone establishes the presence of an intact mitochondrion. Characterization should follow the general principles of MISEV2023 [5]. For mitochondria-containing EV preparations, additional evidence is needed to establish cargo localization, mitochondrial integrity and function, and the contribution of co-isolated free mitochondria.

A number of important insights have emerged from these studies that extend beyond existing reviews. Different mitochondrial cargo pathways should be distinguished by their experimental context. The MSC Super-EV-Mito study concerns CD38/IP_3R/Ca^{2+} signaling [7], whereas brown adipose tissue (BAT)-derived cardioprotective vesicles involve Beclin 1 (BECN1)–vacuolar protein sorting 35 homolog (VPS35)-associated cargo packaging and transfer of mitochondrial membrane-associated bioenergetic components to cardiac macrophages [36]. The validation of this framework, emphasizing calcium resistance assays and single-vesicle analysis, offers a practical approach to distinguishing intact, functional mitochondria from co-purifying fragments or free organelles under MISEV2023 guidelines [5]. The studies reviewed report bioenergetic and anti-apoptotic outcomes in specific experimental models. The proposed roles of EV-mediated mitochondrial transfer in ferroptosis regulation, cardiac immune modulation, and regenerative signaling require more direct mechanistic evidence and independent validation.

While metabolic rescue through ATP restoration and apoptosis inhibition represents a robustly supported therapeutic mechanism validated across multiple species and laboratories [4,14,20,37], emerging applications in ferroptosis regulation [29], immune modulation [30], and regenerative signaling [8] require rigorous independent validation before clinical consideration. The translational gap between preclinical optimization and clinical implementation remains significant, particularly regarding the translation of experimental super-donor approaches into reproducible manufacturing processes [7], the standardization of quality control parameters [5], and the long-term safety of repeated mitochondrial transplantation [2,4].

Future investigations must prioritize elucidating selective packaging signals that distinguish functional from damaged mitochondria, characterizing receptor-mediated uptake mechanisms specific to cardiac cells, and optimizing targeted delivery systems, including inhalable formulations and surface-engineered homing peptides [26,27]. By addressing these biological and methodological challenges using the rigorous analytical framework established in this review, mitochondria-enriched extracellular vesicles may ultimately transition from an experimental concept to a clinical reality as precision adjunctive therapies for ischemic heart disease—complementing rather than replacing existing reperfusion strategies [38,39].

Abbreviations

EV, Extracellular Vesicle; MSC, Mesenchymal Stem Cell; I/R, Ischemia-Reperfusion; MIRO2, Mitochondrial

Rho GTPase 2; MFN2, Mitofusin-2; ROS, Reactive Oxygen Species; MISEV, Minimal Information for Studies of Extracellular Vesicles.

Author Contributions

JX proposed the research topic, formulated the review framework, and drafted the manuscript. ZJ contributed to the conception of the review and the development of its framework, conducted literature retrieval and screening, and verified the references. ZJ also contributed to drafting and critically revising Fig. 2 and the corresponding text, particularly the descriptions of mitochondrial delivery routes and administration procedures, and critically reviewed the manuscript as a whole. CL analyzed and interpreted published evidence concerning EV sources and isolation methods, identified scientific issues in the manuscript, and critically revised the relevant sections for important intellectual content. YL and ZR contributed to the analysis and interpretation of the published methods concerning MSC isolation and centrifugation, identified scientific inaccuracies in the manuscript's methodological descriptions, and participated in substantive revision and critical review of the relevant content. YL also performed proofreading and formatting. WL, HL, and ZZ (corresponding author) participated in the discussion of research ideas, supplemented literature analysis, and reviewed the final version. All authors have read and approved the final manuscript. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

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Conflicts of Interest

The authors declare no conflicts of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used Kimi to check grammar and polish language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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