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# Exosome-Mediated Regulation of Macrophages in Myocardial Ischemia-Reperfusion Injury: Mechanisms and Therapeutic Perspectives

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## Abstract

Myocardial ischemia-reperfusion injury (MIRI) remains a major unresolved problem in the treatment of acute myocardial infarction and other clinical conditions requiring restoration of coronary blood flow. Although timely reperfusion is essential for salvaging ischemic myocardium, the abrupt reintroduction of oxygen and nutrients can paradoxically aggravate myocardial injury through oxidative stress, calcium overload, mitochondrial dysfunction, endothelial damage, and sterile inflammation. Among the immune cells involved in this process, macrophages play a central and dynamic role. In the early phase of reperfusion, pro-inflammatory macrophages amplify tissue injury by releasing cytokines, chemokines, reactive oxygen species, and inflammasome-related mediators. During the reparative phase, macrophages gradually acquire anti-inflammatory and tissue-repairing features, contributing to efferocytosis, angiogenesis, extracellular matrix remodeling, and functional recovery. Therefore, regulating macrophage phenotype and function has emerged as a promising strategy for the treatment of MIRI. Exosomes, more broadly referred to as small extracellular vesicles when their biogenesis has not been strictly demonstrated, are nanoscale membrane vesicles secreted by multiple cell types. They carry bioactive molecules, including microRNAs, long non-coding RNAs, proteins, lipids, and metabolites, and mediate intercellular communication in physiological and pathological settings. Accumulating evidence indicates that exosomes derived from mesenchymal stem/stromal cells, macrophages, cardiomyocytes, and other cell types can regulate macrophage

polarization and inflammatory responses in MIRI. Specific exosomal cargos, such as miR-148a, miR-182, miR-21-5p, miR-25-3p, and miR-125a-5p, have been implicated in the modulation of TXNIP, TLR4/NF-κB/NLRP3, PI3K/Akt, JAK/STAT, and related signaling pathways. These findings suggest that exosome-based therapy may provide a cell-free, immunomodulatory, and targeted approach for myocardial protection. This review summarizes the pathophysiological role of macrophages in MIRI, the biological characteristics of exosomes, the molecular mechanisms by which exosomes regulate macrophage function, and the translational challenges that must be addressed before clinical application.

## Keywords

Exosomes, Macrophage Polarization, Myocardial Ischemia-Reperfusion Injury, Inflammation, Cell-Free Therapy

## 1. Introduction

Acute myocardial infarction is one of the most serious manifestations of cardiovascular disease. In clinical practice, rapid restoration of coronary blood flow through percutaneous coronary intervention, thrombolysis, or surgical revascularization is the cornerstone of myocardial salvage [1] [2]. However, reperfusion itself may induce additional myocardial injury, a phenomenon known as myocardial ischemia-reperfusion injury (MIRI) [3]-[6]. This process contributes to infarct expansion, microvascular obstruction, arrhythmias, adverse ventricular remodeling, and subsequent heart failure [3]-[6].

The pathogenesis of MIRI is complex and involves interactions among cardiomyocytes, endothelial cells, fibroblasts, neutrophils, monocytes/macrophages, platelets, and extracellular matrix components [7]. During ischemia, oxygen deprivation rapidly impairs mitochondrial oxidative phosphorylation, decreases ATP production, and disrupts ionic homeostasis. Upon reperfusion, abrupt oxygen reentry promotes excessive generation of reactive oxygen species, mitochondrial permeability transition pore opening, calcium overload, and regulated cell death [3]-[6]. In parallel, damaged cardiomyocytes release damage-associated molecular patterns, which activate innate immune receptors and initiate sterile inflammatory responses [6]-[9].

Macrophages are indispensable regulators of this inflammatory and reparative process. They originate from both resident cardiac macrophage populations and circulating monocytes recruited after injury. In the early inflammatory phase, macrophages tend to exhibit pro-inflammatory features and release tumor necrosis factor-$\alpha$, interleukin-1$\beta$, interleukin-6, chemokines, and reactive oxygen species. These responses are important for debris clearance but may aggravate cardiomyocyte injury if excessive or prolonged. In the subsequent repair phase, macrophages gradually shift toward anti-inflammatory and pro-reparative states, promoting efferocytosis,

angiogenesis, and extracellular matrix remodeling [10]-[14]. Therefore, therapeutic strategies that suppress excessive early inflammation while preserving or enhancing reparative macrophage functions may provide a rational approach to MIRI treatment.

In recent years, exosomes and other small extracellular vesicles have attracted increasing attention as mediators of intercellular communication. Exosomes are nanoscale vesicles enriched in proteins, lipids, nucleic acids, and other bioactive cargos. Because they can transfer regulatory molecules to recipient cells, exosomes participate in immune regulation, angiogenesis, tissue repair, and pathological remodeling [15]-[20]. Importantly, several experimental studies have shown that exosomes can regulate macrophage polarization and inflammatory signaling in MIRI. Compared with direct cell transplantation, exosome-based therapy has potential advantages, including lower immunogenicity, reduced risk of uncontrolled differentiation, easier storage, and the possibility of cargo or surface engineering [21]-[24]. The overall pathological cascade of MIRI and the exosome-mediated macrophage regulatory network is summarized in **Figure 1**.

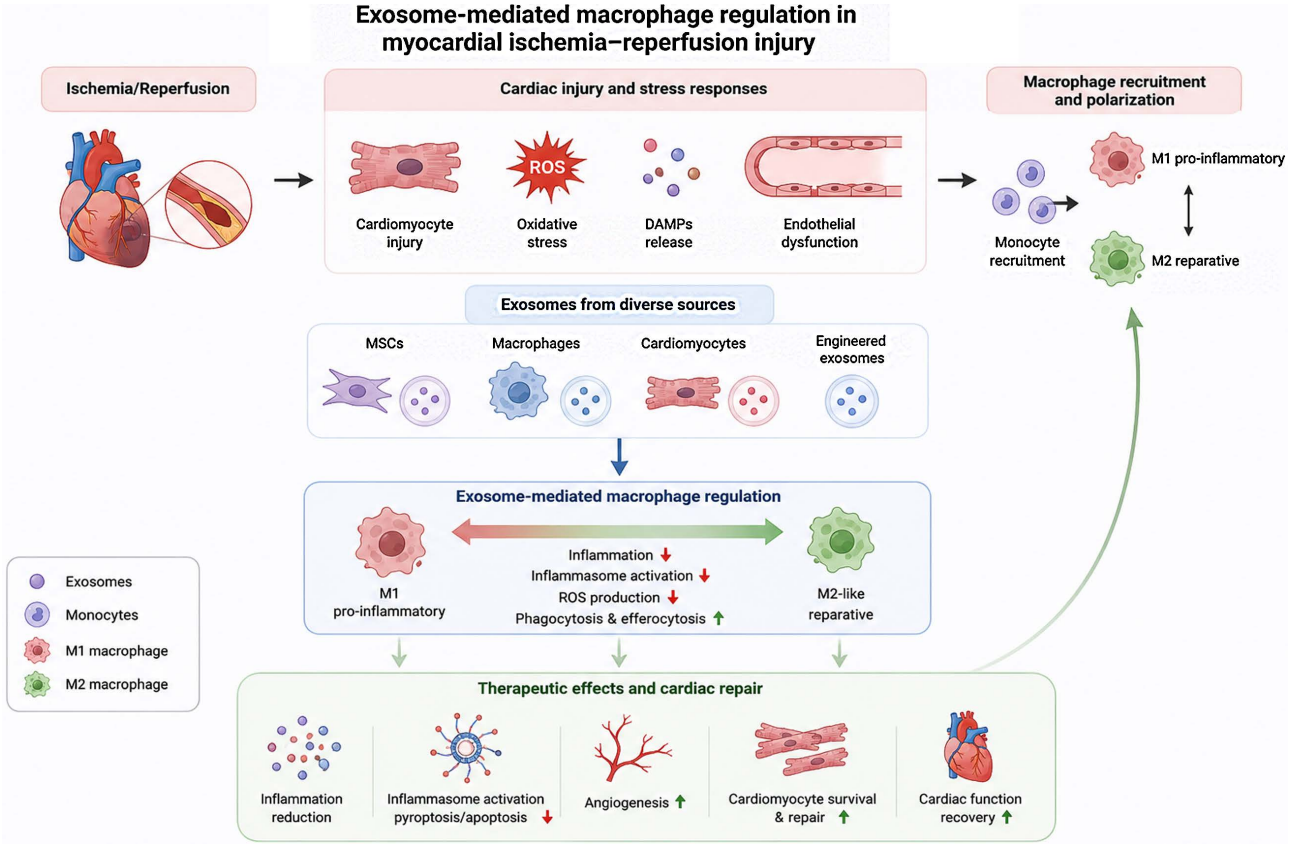



**Figure 1.** Overview of exosome-mediated macrophage regulation in myocardial ischemia-reperfusion injury.

Ischemia/reperfusion induces cardiomyocyte injury, ROS production, DAMP release, and endothelial dysfunction, leading to monocyte recruitment and macrophage polarization. Exosomes derived from MSCs, macrophages, cardiomyocytes,

and engineered vesicles regulate inflammatory activation, inflammasome signaling, M2-like repair, angiogenesis, and cardiac functional recovery. Abbreviations: DAMPs: damage-associated molecular patterns; MIRI: myocardial ischemia-reperfusion injury; MSCs: mesenchymal stromal cells; ROS: reactive oxygen species.

## 2. Methods

This narrative review was prepared by searching PubMed, Web of Science, Google Scholar, and major journal databases for studies related to myocardial ischemia-reperfusion injury, extracellular vesicles, exosomes, macrophage polarization, and inflammatory regulation. The following keywords were used alone or in combination: "myocardial ischemia reperfusion injury", "myocardial infarction", "exosomes", "small extracellular vesicles", "extracellular vesicles", "macrophage polarization", "M1 macrophage", "M2 macrophage", "NLRP3 inflammasome", "TLR4/NF-κB", "mesenchymal stem cell-derived exosomes", "microRNA" and "cell-free therapy". Original experimental studies, mechanistic studies, guidelines, position papers, and recent reviews were included when they were directly related to MIRI, macrophage regulation, or exosome-based therapy.

## 3. Pathophysiological Basis of Myocardial Ischemia-Reperfusion Injury

### 3.1. Ischemic Injury and the Paradox of Reperfusion

Myocardial ischemia occurs when coronary blood flow is insufficient to meet the metabolic demands of cardiomyocytes. During ischemia, oxygen deprivation impairs mitochondrial respiration and reduces ATP production. The failure of ATP-dependent ion pumps results in intracellular sodium and calcium accumulation, cellular edema, acidosis, and contractile dysfunction [3]-[5]. If ischemia persists, irreversible cell death occurs.

Reperfusion is essential for rescuing viable myocardium, but it also initiates a second wave of injury. The reintroduction of oxygen into previously ischemic tissue accelerates mitochondrial reactive oxygen species generation. Excessive reactive oxygen species damage lipids, proteins, and DNA, activate redox-sensitive inflammatory pathways, and destabilize mitochondrial membranes. Calcium overload further promotes mitochondrial permeability transition pore opening, leading to loss of mitochondrial membrane potential and cell death [3]-[6]. Reperfusion also activates endothelial cells and promotes leukocyte adhesion, platelet activation, and microvascular obstruction. These processes form a vicious cycle in which cardiomyocyte death, microvascular dysfunction, and inflammation reinforce each other [4]-[8].

### 3.2. Sterile Inflammation in MIRI

Inflammation is a defining feature of MIRI. Unlike infection-induced inflammation, MIRI-associated inflammation is sterile and driven mainly by damage-associated molecular patterns released from injured cardiomyocytes and extracellular

matrix fragments [6]-[9]. These molecules activate pattern recognition receptors, including Toll-like receptors and receptor for advanced glycation end products, on immune cells and cardiac resident cells [7]-[9]. Downstream activation of NF-$\kappa$B promotes transcription of inflammatory mediators such as TNF-$\alpha$, IL-1$\beta$, IL-6, and chemokines [8]. These mediators recruit neutrophils and monocytes into the injured myocardium.

The NLRP3 inflammasome is another important inflammatory platform in MIRI. Reactive oxygen species, mitochondrial DNA, ionic imbalance, and lysosomal stress can activate NLRP3, leading to caspase-1 activation and maturation of IL-1$\beta$ and IL-18. Inflammasome activation contributes not only to inflammatory amplification but also to pyroptotic cell death [7] [9]. Macrophages are one of the major cell types involved in inflammasome signaling in the injured heart, making them a key target for limiting excessive inflammation [7] [9]-[14].

## 4. Macrophages in Myocardial Ischemia-Reperfusion Injury

### 4.1. Origin and Dynamic Changes of Cardiac Macrophages

Macrophages in the injured heart arise from at least two sources: tissue-resident cardiac macrophages and circulating monocytes that infiltrate the myocardium after injury. Resident macrophages contribute to immune surveillance, tissue homeostasis, and rapid local responses after injury [11]-[14]. Following ischemia and reperfusion, chemokines such as CCL2 recruit inflammatory monocytes from the circulation. These monocytes differentiate into macrophages within the infarcted or peri-infarct myocardium [12]-[14].

The macrophage response is highly time-dependent. Nahrendorf *et al.* demonstrated that the healing myocardium sequentially mobilizes monocyte subsets with divergent and complementary functions after myocardial infarction [10]. Subsequent studies further showed that monocytes and resident macrophages contribute differently to cardiac macrophage populations in steady state and after injury [12] [13]. In the early inflammatory phase, macrophages remove dead cells and extracellular debris but also release pro-inflammatory mediators. In the reparative phase, macrophages help terminate inflammation, promote efferocytosis, regulate fibroblast activation, and support angiogenesis [10]-[14]. A balanced macrophage response is therefore essential: insufficient inflammation may impair debris clearance, whereas excessive or prolonged inflammation may enlarge the infarct and worsen remodeling.

### 4.2. M1/M2 Polarization and Its Limitations

Macrophage activation is often simplified into M1 and M2 phenotypes. M1-like macrophages are generally induced by stimuli such as lipopolysaccharide and interferon-γ and are characterized by increased expression of inducible nitric oxide synthase, CD86, TNF-$\alpha$, IL-1$\beta$, and IL-6. They are associated with pathogen defense and inflammatory tissue injury. M2-like macrophages are induced by signals such as IL-4, IL-13, IL-10, and transforming growth factor-$\beta$ and often express

arginase-1, CD206, IL-10, and other reparative markers. They are associated with inflammation resolution, tissue repair, and extracellular matrix remodeling [10]-[14].

However, the M1/M2 classification is an oversimplified framework. *In vivo* macrophages exist along a continuum of phenotypes and may simultaneously express inflammatory, reparative, angiogenic, and metabolic programs [11]-[13]. In MIRI, macrophage behavior is shaped by local hypoxia, reactive oxygen species, dead cell debris, cytokines, metabolic stress, and extracellular vesicles. Therefore, rather than simply "converting M1 to M2", therapeutic modulation should aim to reduce harmful inflammatory signaling while preserving macrophage functions required for repair.

## 5. Biological Characteristics of Exosomes and Small Extracellular Vesicles

Extracellular vesicles are lipid bilayer-enclosed particles released by cells [15]-[20]. According to current extracellular vesicle research guidelines, the term "extracellular vesicles" is preferred when vesicle biogenesis has not been strictly demonstrated [16] [17]. Exosomes are commonly described as small vesicles of endosomal origin, typically generated through inward budding of multivesicular bodies and released after fusion with the plasma membrane [15] [18]-[20]. In many experimental studies, vesicles with a diameter of approximately 30 - 150 nm and expressing markers such as CD9, CD63, CD81, Alix, and TSG101 are referred to as exosomes [15]-[19]. However, because isolation methods often enrich a mixture of small extracellular vesicles rather than a pure exosome population, careful nomenclature and characterization are required [16] [17] [21]-[24].

Exosomes contain diverse cargos, including microRNAs, long non-coding RNAs, circular RNAs, mRNAs, proteins, lipids, and metabolites [15]-[20]. Their molecular composition depends on the parent cell type, physiological state, and microenvironmental stimuli. Hypoxia, oxidative stress, inflammation, and pharmacological pretreatment can alter both the quantity and cargo profile of released vesicles [15]-[20] [25]-[35]. After release, exosomes may be taken up by recipient cells through endocytosis, phagocytosis, macropinocytosis, membrane fusion, or receptor-ligand interactions [19] [20]. Through these mechanisms, exosomes can transfer functional cargos to macrophages and regulate their phenotype, metabolism, and inflammatory signaling.

In the cardiovascular system, exosomes participate in crosstalk among cardiomyocytes, endothelial cells, fibroblasts, immune cells, and progenitor cells [25] [26]. In MIRI, exosomes may exert either protective or detrimental effects depending on their source, timing, and cargo composition. For example, exosomes derived from mesenchymal stem/stromal cells or reparative macrophages often show anti-inflammatory and cardioprotective effects, whereas vesicles released from severely injured or stressed cells may propagate inflammatory signals [25]-[35]. This source-dependent functional diversity is a central issue in the development of exosome-

based therapy. The biogenesis, cargo composition, and major macrophage uptake mechanisms of exosomes are illustrated in **Figure 2**.

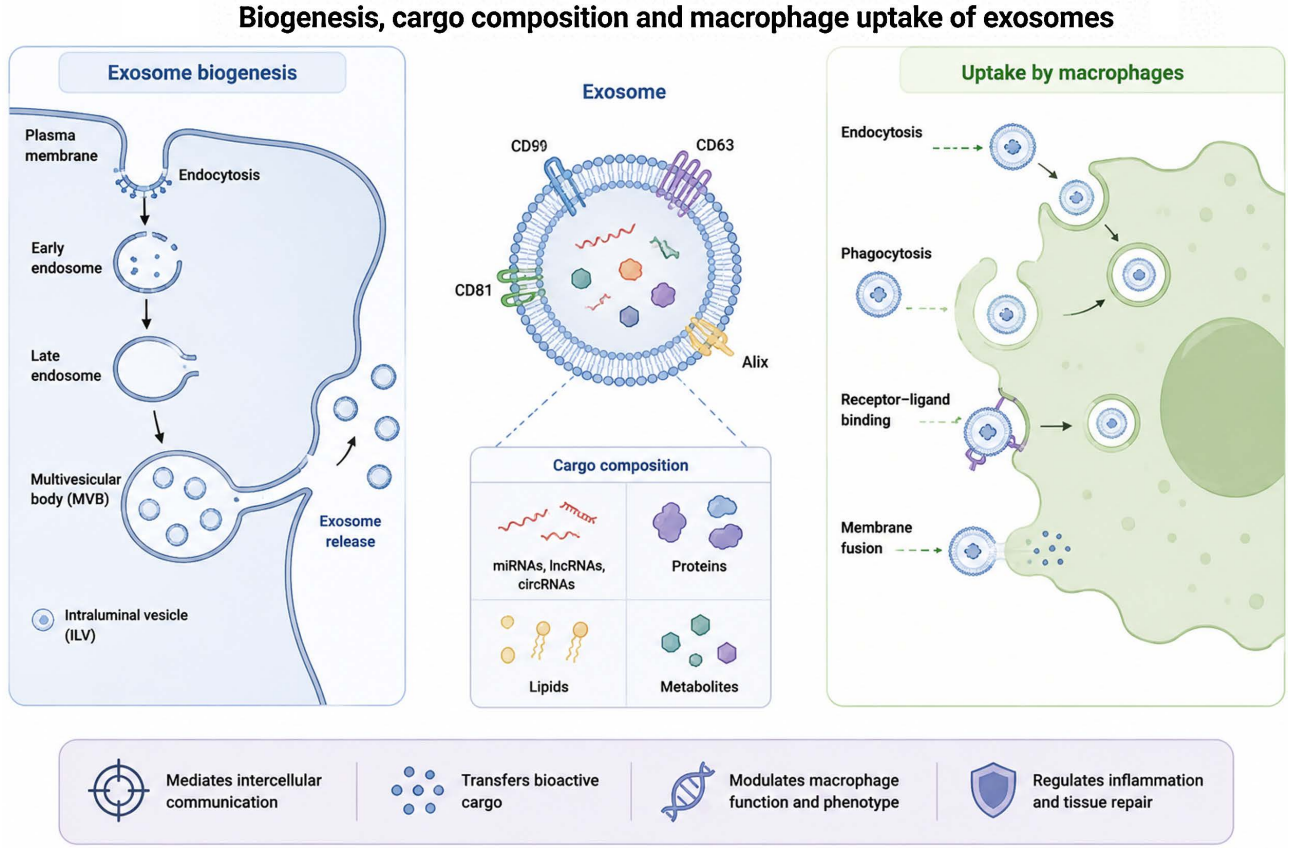



**Figure 2.** Biogenesis, cargo composition, and macrophage uptake of exosomes.

Exosomes are generated through endocytosis, endosomal maturation, multivesicular body formation, and fusion with the plasma membrane. They carry miRNAs, proteins, lipids, and metabolites, and can be internalized by macrophages through endocytosis, phagocytosis, receptor-ligand binding, and membrane fusion. Abbreviations: ILVs: intraluminal vesicles; MVBs: multivesicular bodies; miRNAs: microRNAs.

## 6. Exosome-Mediated Regulation of Macrophages in MIRI

### 6.1. MicroRNA Transfer as a Core Mechanism

MicroRNAs are among the most extensively studied exosomal cargos in MIRI. By binding to target mRNAs and suppressing their translation or promoting degradation, microRNAs can reshape macrophage signaling networks. Several exosomal microRNAs have been shown to regulate macrophage polarization, inflammasome activation, and inflammatory cytokine production in MIRI [27]-[32].

One representative study demonstrated that M2 macrophage-derived exosomes carry miR-148a and alleviate myocardial ischemia-reperfusion injury by targeting thioredoxin-interacting protein and suppressing the TLR4/NF-κB/NLRP3

inflammasome pathway [28]. Thioredoxin-interacting protein is closely associated with oxidative stress and NLRP3 inflammasome activation [9] [28]. By reducing thioredoxin-interacting protein expression, miR-148a-containing exosomes attenuate inflammatory amplification, decrease cardiomyocyte apoptosis, and improve myocardial function [28]. This study provides direct evidence that macrophage-derived exosomes can transmit anti-inflammatory signals back to the injured myocardium.

Mesenchymal stromal cell-derived exosomes have also been shown to regulate macrophage polarization. Zhao *et al*. reported that MSC-derived exosomes attenuated myocardial ischemia-reperfusion injury through miR-182-mediated modulation of macrophage polarization. Mechanistically, exosomal miR-182 was linked to the regulation of the TLR4/NF-$\kappa$B/PI3K/Akt signaling cascade. By suppressing excessive inflammatory activation and promoting a reparative macrophage phenotype, MSC-derived exosomes reduced inflammatory injury and improved cardiac outcomes in experimental MIRI [27].

MSC-derived exosomal miR-21-5p has similarly been implicated in macrophage regulation after myocardial reperfusion injury. Shen and He showed that MSC exosomes promoted M2-like macrophage polarization via miR-21-5p, reduced inflammatory responses, and facilitated cardiac repair [29]. In another context, exosomal miR-21a-5p was also reported to mediate cardioprotection by mesenchymal stem cells [30]. More recently, BMSC-derived exosomal miR-25-3p was shown to protect against MIRI by constraining M1-like macrophage polarization [31]. These studies collectively support the view that exosomes can function as endogenous or therapeutic delivery systems for microRNAs that reprogram macrophage behavior.

### 6.2. Regulation of TLR4/NF-$\kappa$B/NLRP3 Signaling

The TLR4/NF-$\kappa$B/NLRP3 pathway is one of the most important inflammatory pathways in MIRI [7]-[9] [28]. Damage-associated molecular patterns released from injured myocardium activate TLR4 on macrophages, leading to MyD88-dependent NF-$\kappa$B activation and transcription of pro-inflammatory cytokines [8]. NF-$\kappa$B also primes the NLRP3 inflammasome by upregulating NLRP3 and pro-IL-1$\beta$. Subsequent activation signals, including reactive oxygen species, mitochondrial dysfunction, and ionic disturbance, promote inflammasome assembly and caspase-1 activation [7]-[9].

Exosomes can inhibit this pathway at multiple levels. M2 macrophage-derived exosomal miR-148a inhibits thioredoxin-interacting protein and reduces NLRP3 inflammasome activation [28]. MSC-derived exosomal miR-182 modulates TLR4-related inflammatory signaling [27]. Other exosomal cargos may suppress NF-$\kappa$B activation, decrease IL-1$\beta$ and IL-6 production, and reduce macrophage-driven inflammatory injury [27]-[29] [32]. By targeting upstream innate immune receptors, transcriptional inflammatory programs, and inflammasome activation, exosomes may provide a multi-layered anti-inflammatory effect.

### 6.3. JAK/STAT, PI3K/Akt and Pro-Reparative Signaling

In addition to suppressing inflammatory pathways, exosomes may activate signaling pathways associated with survival and repair. The PI3K/Akt pathway is involved in cell survival, endothelial function, angiogenesis, and macrophage phenotype regulation [27]. Exosome-mediated activation of PI3K/Akt signaling may reduce apoptosis, enhance endothelial repair, and promote anti-inflammatory macrophage responses [27] [35] [36].

The JAK/STAT pathway also participates in macrophage regulation. Depending on the upstream cytokine environment and STAT subtype involved, JAK/STAT signaling may promote either inflammatory or anti-inflammatory macrophage programs. In exosome-mediated therapy, specific microRNA cargos and parental cell pretreatments may alter JAK/STAT activity and thereby shape macrophage phenotype [27]-[29] [31]-[33]. However, the precise direction and cell-specific role of this pathway in MIRI require further clarification.

### 6.4. Exosomes from Different Cellular Sources

The biological effects of exosomes depend strongly on their cellular source.

Mesenchymal stem/stromal cell-derived exosomes are the most widely studied in MIRI. The early study by Lai *et al.* showed that exosomes secreted by MSCs reduced myocardial ischemia-reperfusion injury [25]. Later studies further showed that MSC-derived exosomes could regulate macrophage polarization, reduce inflammatory injury, and improve cardiac outcomes [27] [29]-[32]. Xu *et al.* reported that exosomes derived from LPS-primed BMSCs reduced inflammation and myocardial injury by mediating macrophage polarization after myocardial infarction [32]. These findings suggest that MSC-derived exosomes reproduce many of the paracrine benefits of their parent cells, including anti-apoptotic, pro-angiogenic, anti-fibrotic, and immunomodulatory effects [25] [27] [29] [31]-[36].

Macrophage-derived exosomes are also highly relevant because macrophages are both producers and recipients of extracellular vesicles. M2 macrophage-derived exosomes may carry anti-inflammatory microRNAs and proteins that promote tissue repair [28]. By contrast, exosomes derived from M1-like macrophages may propagate inflammatory signals. This duality suggests that macrophage-derived exosomes may serve as both therapeutic agents and biomarkers of inflammatory status.

Cardiomyocyte-derived exosomes may transmit stress signals under ischemic or hypoxic conditions. They can influence macrophages, endothelial cells, and fibroblasts, thereby affecting inflammation and remodeling [37]. Depending on the degree of injury and cargo composition, cardiomyocyte-derived vesicles may either promote repair or amplify injury [37].

Exercise-induced circulating exosomes represent another promising area. Long-term exercise-derived exosomal miR-342-5p has been reported to mediate cardioprotection [34]. Although this mechanism is not limited to macrophage regulation, it supports the broader concept that physiological interventions can modify

exosomal cargo and improve myocardial resistance to ischemic injury. The major exosomal miRNAs and signaling pathways involved in macrophage regulation during MIRI are summarized in **Figure 3**.

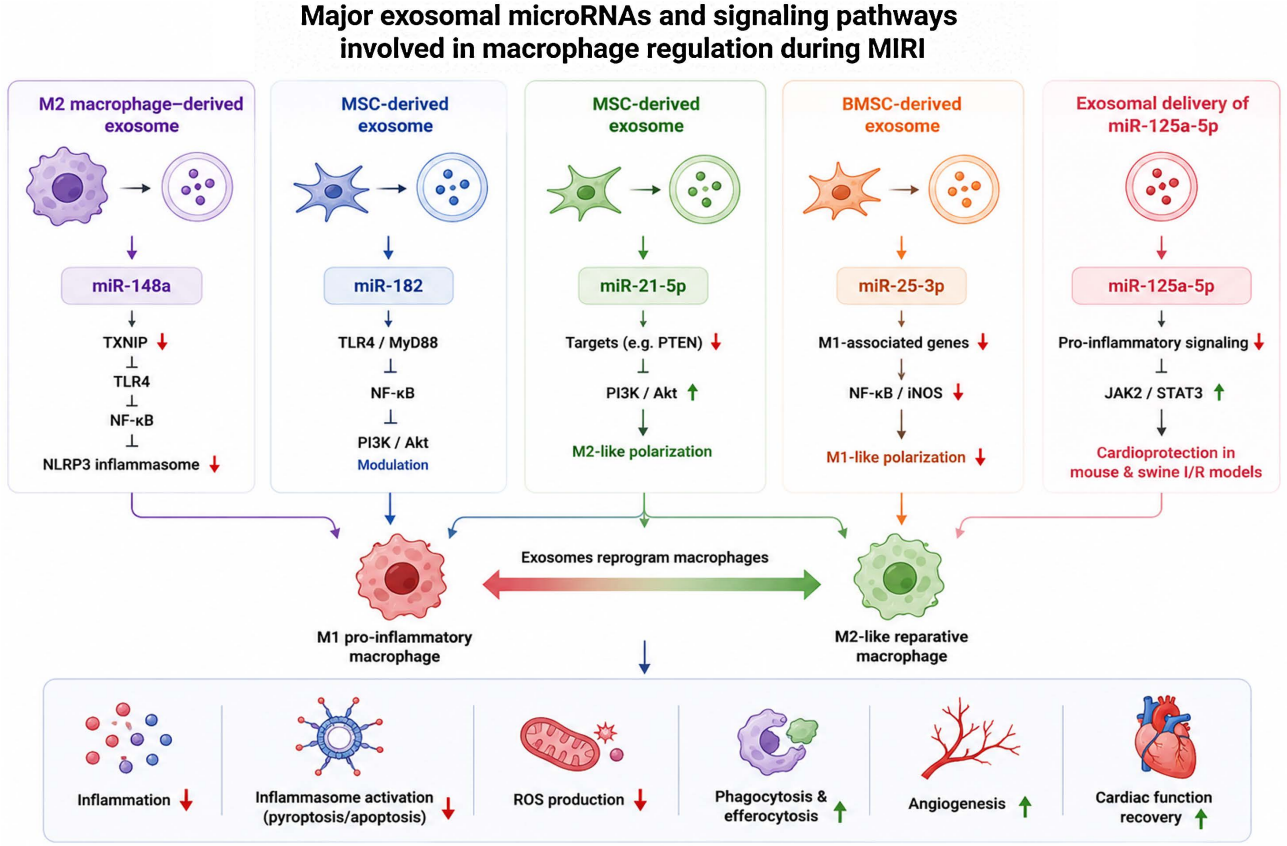



**Figure 3.** Major exosomal microRNAs and signaling pathways involved in macrophage regulation during MIRI.

M2 macrophage-derived exosomal miR-148a suppresses TXNIP and inhibits the TLR4/NF-κB/NLRP3 pathway. MSC-derived exosomal miR-182 modulates TLR4/NF-κB/PI3K/Akt signaling, while miR-21-5p promotes M2-like polarization and BMSC-derived miR-25-3p suppresses M1-like activation. miR-125a-5p delivery confers cardioprotection in mouse and swine I/R models. Abbreviations: BMSC: bone marrow mesenchymal stromal cell; I/R: ischemia/reperfusion; MIRI: myocardial ischemia-reperfusion injury; MSC: mesenchymal stromal cell; NF-κB: nuclear factor-κB; NLRP3: NOD-like receptor family pyrin domain containing 3; TXNIP: thioredoxin-interacting protein.

## 7. Therapeutic Potential of Exosome-Based Strategies

### 7.1. Native Exosomes as Cell-Free Therapeutics

Native exosomes derived from MSCs, M2 macrophages, or other reparative cells may be used as cell-free therapeutics. Their advantages include nanoscale size, natural membrane structure, intrinsic biocompatibility, and the ability to carry multiple bioactive cargos simultaneously. In MIRI, native exosomes may protect

the heart through several mechanisms: reducing cardiomyocyte apoptosis, suppressing oxidative stress, inhibiting macrophage-driven inflammation, promoting angiogenesis, and limiting adverse remodeling [25]-[29] [31]-[36] [38]-[40].

The earliest evidence for MSC-exosome cardioprotection showed that exosomes secreted by MSCs reduced infarct size in a mouse model of myocardial ischemia-reperfusion injury [25]. Plasma exosomes from healthy donors were also reported to protect the myocardium from ischemia-reperfusion injury [26]. Subsequent studies identified specific microRNAs and signaling pathways involved in the protective effects, including miR-182, miR-148a, miR-21-5p, miR-25-3p, and miR-125a-5p [27]-[33]. These findings provide a foundation for developing exosomes as therapeutic agents rather than merely biomarkers.

### 7.2. Engineered Exosomes and Cargo Optimization

Although native exosomes have therapeutic potential, their cargo composition is heterogeneous and may vary with cell source, passage number, culture conditions, and pretreatment [16] [17] [21]-[24]. Engineering strategies can enhance their efficacy and specificity. Parent cells may be genetically modified or preconditioned with hypoxia, inflammatory stimuli, pharmacological agents, or mechanical stress to enrich protective cargos. Alternatively, exosomes can be loaded after isolation with microRNAs, siRNAs, proteins, or small molecules [21]-[24] [33].

For MIRI, engineered exosomes could be designed to enrich anti-inflammatory microRNAs, such as miR-148a, miR-182, miR-21-5p, miR-25-3p, or miR-125a-5p [27]-[33]. Surface modification may also improve myocardial targeting or macrophage-specific delivery. For example, conjugation with peptides or antibodies that recognize injured endothelium, inflammatory macrophages, or ischemic myocardium may increase local retention and reduce off-target distribution. However, engineering steps must be carefully evaluated because they may alter vesicle stability, biodistribution, immunogenicity, and safety.

### 7.3. Delivery Route, Timing and Dosage

The therapeutic effect of exosomes depends not only on cargo composition but also on delivery route, timing, and dosage [21]-[24]. Common routes in preclinical studies include intravenous injection, intramyocardial injection, intracoronary delivery, and local biomaterial-assisted retention systems. Intravenous injection is simple and clinically feasible but may result in uptake by the liver, spleen, and lung [22]. Intramyocardial injection provides local delivery but is invasive and may not be ideal in acute clinical settings. Intracoronary delivery is attractive for myocardial infarction because it can be integrated with reperfusion procedures, but safety issues such as microvascular obstruction must be considered.

Timing is equally important. Early administration during reperfusion may suppress inflammatory amplification and reduce acute cell death, whereas later administration may enhance repair and remodeling. Because macrophage phenotype changes over time, the same exosome therapy may have different effects depending on the phase of injury [10]-[14]. Future studies should define optimal

therapeutic windows rather than applying a single fixed time point.

## 8. Challenges in Translation

Despite encouraging preclinical evidence, several challenges remain before exosome-based therapy can be translated into clinical treatment for MIRI.

First, nomenclature and characterization require standardization. Many studies use the term "exosomes" based mainly on particle size and marker expression, but commonly used isolation methods often produce mixed populations of small extracellular vesicles [16]. According to MISEV2018 and MISEV2023, studies should report particle size distribution, morphology, protein markers, purity indicators, isolation methods, and storage conditions [17]. Without standardized characterization, results across studies are difficult to compare.

Second, exosome heterogeneity remains a major issue. Vesicles derived from different cell sources, culture conditions, or stimulation protocols may have distinct cargos and biological effects [15]-[24]. Even vesicles from the same cell type may vary between donors or passages. Therefore, therapeutic development requires reproducible production and strict quality control [21]-[24].

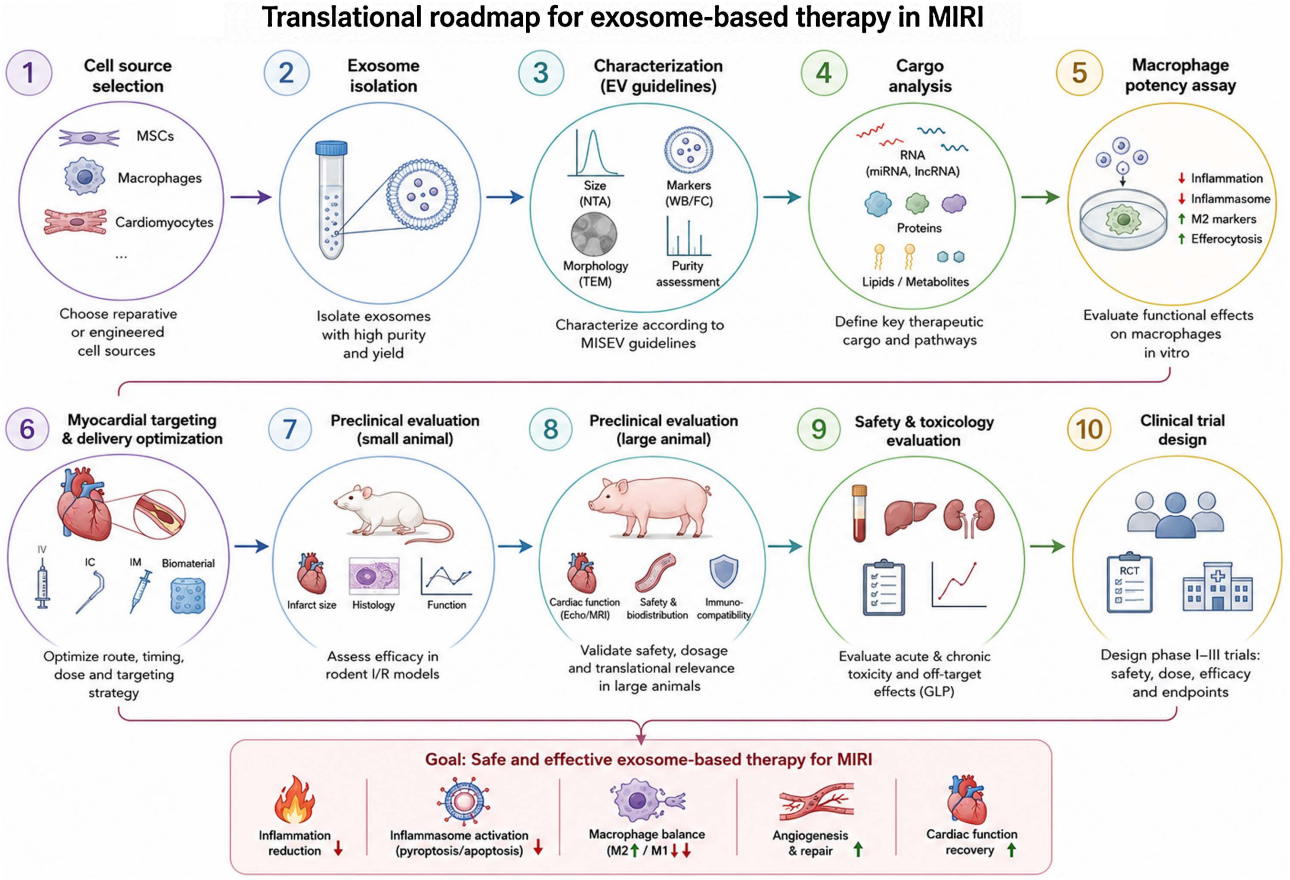
Third, potency assays are not yet fully established. For macrophage-targeted therapy, potential potency assays may include inhibition of TNF-$\alpha$, IL-1$\beta$, and IL-6 release; reduction of NLRP3 inflammasome activation; enhancement of efferocytosis; induction of reparative macrophage markers; and improvement of cardiomyocyte survival in co-culture systems [7]-[14] [27]-[29] [31]-[33]. However, these assays must be linked to *in vivo* efficacy.

Fourth, biodistribution and targeting efficiency require improvement. Systemically administered exosomes are often rapidly taken up by the mononuclear phagocyte system, especially in the liver and spleen [22]. Although this may be useful for some immunomodulatory applications, myocardial delivery after infarction remains inefficient. Engineering strategies, biomaterial-assisted delivery, and local intracoronary administration may help overcome this limitation [21]-[24].

Fifth, safety must be carefully evaluated. Exosomes carry complex biological cargos, and their long-term effects are not fully understood. Potential risks include unwanted immune suppression, pro-fibrotic effects, pro-coagulant activity, arrhythmogenic effects, off-target gene regulation, and batch-dependent variability. In addition, the functional transfer of extracellular RNA in mammals remains technically challenging to prove rigorously, and experimental interpretation requires careful controls [24]. Large animal studies and well-designed clinical trials are needed to evaluate these risks. A stepwise translational roadmap for exosome-based therapy in MIRI is proposed in **Figure 4**.

## 9. Future Directions

Future research should move beyond descriptive observation and focus on mechanistic precision, standardized production, and clinically relevant models.




EV characterization, cargo analysis, macrophage potency assays, myocardial targeting, small and large animal validation, safety evaluation, and clinical trial design. This roadmap highlights the key steps needed to move exosome-based therapy from mechanistic discovery to clinical application. Abbreviations: EV: extracellular vesicle; GMP: good manufacturing practice; MIRI: myocardial ischemia-reperfusion injury; MSC: mesenchymal stromal cell.

**Figure 4.** Translational roadmap for exosome-based therapy in MIRI.

First, single-vesicle analysis and multi-omics technologies should be used to define the active subpopulations of therapeutic vesicles. Bulk exosome preparations contain heterogeneous vesicle populations. Identifying which vesicle subtypes carry protective cargos will help improve therapeutic reproducibility.

Second, macrophage heterogeneity in MIRI should be investigated more deeply. The traditional M1/M2 framework is useful but insufficient. Single-cell RNA sequencing, spatial transcriptomics, and lineage tracing can reveal distinct macrophage subsets in different regions and phases of the injured heart. These approaches may identify more precise macrophage targets for exosome therapy.

Third, engineered exosomes should be developed with defined cargos and targeting properties. For example, exosomes enriched with miR-148a, miR-182, miR-21-5p, or miR-25-3p may be compared directly in standardized MIRI models. Combining anti-inflammatory cargos with pro-angiogenic or mitochondrial protective molecules may further improve therapeutic efficacy.

Fourth, clinically relevant delivery strategies are needed. Because MIRI occurs in the setting of reperfusion therapy, exosome administration should be designed

to match clinical workflows. Intracoronary delivery during PCI, intravenous delivery immediately after reperfusion, or biomaterial-assisted local release may represent feasible strategies, but each requires careful safety testing.

Finally, large animal studies are essential. Many exosome studies have been performed in rodents, but rodent hearts differ from human hearts in size, immune response, coronary anatomy, and remodeling patterns. Studies in pigs or other large animals will be important for evaluating dosage, delivery route, biodistribution, safety, and functional outcomes.

## 10. Conclusions

MIRI is a complex pathological process involving oxidative stress, mitochondrial dysfunction, calcium overload, endothelial injury, and sterile inflammation. Macrophages are central regulators of both injury and repair. Their excessive pro-inflammatory activation aggravates myocardial damage, whereas timely transition toward reparative phenotypes promotes inflammation resolution and tissue healing. Exosomes and small extracellular vesicles provide a promising means of regulating macrophage function through the transfer of microRNAs, proteins, and other bioactive cargos.

Current evidence indicates that exosomes derived from MSCs, M2 macrophages, and other protective cell sources can attenuate MIRI by modulating macrophage polarization and inflammatory signaling pathways, particularly TXNIP, TLR4/NF-$\kappa$B/NLRP3, PI3K/Akt, and related networks. Exosomal cargos such as miR-148a, miR-182, miR-21-5p, miR-25-3p, and miR-125a-5p have shown important cardioprotective potential in preclinical studies. Nevertheless, clinical translation remains limited by vesicle heterogeneity, insufficient targeting, unclear dosing, lack of standardized potency assays, and incomplete safety data. Future studies should integrate rigorous extracellular vesicle characterization, precise macrophage phenotyping, engineered vesicle design, and clinically relevant large animal models. With these advances, exosome-based macrophage regulation may become an important cell-free therapeutic strategy for myocardial ischemia-reperfusion injury.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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