

A Powerful Tool in the Treatment of Myocardial Ischemia-Reperfusion Injury: Natural and Nanoscale Modified Small Extracellular Vesicles Derived from Mesenchymal Stem Cells

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Abstract: Myocardial ischemia-reperfusion injury (MI/RI) constitutes a pivotal determinant impacting the long-term prognosis of individuals afflicted by ischemic cardiomyopathy subsequent to reperfusion therapy. Stem cells have garnered extensive application within the realm of MI/RI investigation, yielding tangible outcomes. Stem cell therapy encounters certain challenges in its application owing to the complexities associated with stem cell acquisition, a diminished homing rate, and a brief in vivo lifespan. Small extracellular vesicles (sEV) originating from mesenchymal stem cells (MSCs) have been demonstrated to possess the benefits of abundant availability, reduced immunogenicity, and a diminished tumorigenic incidence. They can exert their effects on damaged organs, improving injuries by transporting a lot of constituents, including proteins, RNA, lipid droplets, and more. This phenomenon has garnered substantial attention in the context of MI/RI treatment. Simultaneously, MSC-derived sEV (MSC-sEV) can exhibit enhanced therapeutic advantages through bioengineering modifications, biomaterial incorporation, and natural drug interventions. Within this discourse, we shall appraise the utilization of MSC-sEV and their derivatives in the context of MI/RI treatment, aiming to offer valuable insights for future research endeavors related to MI/RI.

Keywords: myocardial ischemia-reperfusion injury, mesenchymal stem cells, small extracellular vesicles, nanoscale modification, natural drug

Introduction

As medical technology and management strategies continue to advance, the rate of reperfusion therapy for individuals afflicted by ischemic cardiomyopathy has seen perpetual enhancement. Consequently, the short-term mortality rate among patients has been significantly ameliorated.^{1,2} Nonetheless, the Global Burden of Disease Report for the year 2022 underscores that on a global scale, fatalities attributed to ischemic cardiomyopathy maintain their preeminent position as the foremost cause of death.³ The escalating mortality rate among individuals afflicted by ischemic cardiomyopathy is primarily associated with cardiac insufficiency stemming from myocardial infarction. Notably, myocardial ischemia-reperfusion injury (MI/RI), induced by the reperfusion therapy itself, assumes a pivotal role in precipitating the deterioration of cardiac function.^{4,5} Henceforth, the prevention of MI/RI, along with the revival of myocardial stunning and hibernation instigated by reperfusion injury, emerges as efficacious stratagems aimed at augmenting the enduring prognosis of individuals grappling with ischemic cardiomyopathy.^{6,7}

Since the advent of the 21st century, regenerative therapy involving stem cells has achieved remarkable milestones in the treatment of myocardial injury, particularly ischemic damage.^{8–10} Among these, mesenchymal stem cells (MSCs)

have distinguished themselves amidst the diverse array of stem cell types, owing to their innate attributes of self-renewal, versatile differentiation capabilities, and substantial autocrine and paracrine capacities.^{11,12} A notable instance of their clinical application can be found in the RIMECARD trial, wherein the intravenous administration of mesenchymal stem cells derived from umbilical cord tissue (UCMSCs) significantly ameliorated patients' cardiac function and overall quality of life.¹³ Furthermore, the HUC-HEART study demonstrated that patients who underwent coronary artery bypass grafting (CABG) in conjunction with UCMSC administration exhibited notably superior cardiac function one year post-surgery compared to those who received CABG alone.^{14,15} These outcomes inspired our faith in the potential of stem cell therapies. Nonetheless, the direct utilization of stem cells in the context of MI/RI therapy is not without challenges, including issues of low survival rates, abbreviated retention periods, tumorigenicity, and complexities surrounding standardized production.^{16–18} Furthermore, there remains a contentious debate regarding the potential for stem cell transplantation to precipitate ventricular arrhythmia.¹⁹ Small extracellular vesicles (sEV) represent lipid bilayered vesicles, secreted by cells, with a diameter less than 200nm, facilitating the transfer of cytoplasmic cargo and mediating intercellular transport.²⁰ Small extracellular vesicles, particularly those derived from Mesenchymal Stem Cells (MSC-sEV), are abundant in a plethora of functional proteins, RNA, and lipids, yielding myocardial protective effects akin to their parent cells.²¹ Simultaneously, cell-free therapeutic approaches relying on sEV circumvent issues such as immune responses, tumorigenicity, and infusion-related toxicity often associated with stem cell-based interventions, while enabling large-scale production.^{22,23}

Regarding the pathogenesis, MI/RI refers to the secondary damage to myocardial tissue following vascular reconstruction due to mechanisms like metabolic changes, mitochondrial dysfunction, inflammatory response, relaxation of autophagy regulation, and excessive production of reactive oxygen species.²⁴ These mechanisms lead to alterations in microvascular endothelial permeability, increased extravasation of fluids, immune cell infiltration, ultimately resulting in secondary damage to myocardial tissue. At present, clinical treatments for MI/RI primarily involve strengthening antiplatelet therapies, anticoagulation, and vasodilation of microvessels.^{25–27} However, the effectiveness of these strategies still requires further clarification, and they might also increase the risk of bleeding for patients.²⁸ Research indicates that MSC-sEV can mitigate myocardial damage induced by ischemia-reperfusion by modulating various pathological processes in cardiomyocytes, such as apoptosis, autophagy, and inflammatory responses.^{29,30} This cell-free therapy presents a new approach for treating MI/RI.

Consequently, the development of biomedical agents associated with MSC-sEV has emerged as a pivotal breakthrough in the realm of MI/RI treatment. It is imperative to highlight that a consensus regarding the specific markers for sEV subtypes remains elusive. Therefore, in this discourse, we align ourselves with the standpoint set forth by the International Society for Extracellular Vesicles (MISEV2018) and refrain from employing nomenclature such as “exosomes”, “particles”, or “microvesicles”, as has been customary in numerous studies, opting instead for the term “small Extracellular vesicles (sEV)” as a precise specification.^{31,32} Concurrently, this review encompasses a diverse array of recent investigations into the application of MSC-sEV for the enhancement of MI/RI outcomes, delving into areas of nanoscale modification and natural drug intervention within the context of MSC-sEV. As research continues to advance, interdisciplinary collaboration is poised to exert a formidable influence in propelling the evolution of MI/RI therapeutics.

MSCs in MI/RI Regulation

Mesenchymal stem cells (MSCs) can be procured from a diverse array of tissues, encompassing bone marrow, adipose tissue, umbilical cord, placenta, synovial membrane, and dental pulp.^{33–38} These MSCs have garnered significant attention within the sphere of regenerative medicine research owing to their multifaceted abilities, encompassing the facilitation of wound healing, immune modulation, angiogenesis, anti-inflammatory effects, and antioxidant properties.³⁹ Presently, the MSCs employed in studies pertaining to MI/RI therapy predominantly originate from bone marrow, adipose tissue, umbilical cord, and heart-resident mesenchymal stem cells. While these MSCs share commonalities in surface marker expression, certain investigations have elucidated variances in marker profiles between them.^{40–47} Functionally, it has been posited that bone marrow mesenchymal stem cells (BMSCs) may exhibit a superior capacity for osteogenesis and angiogenesis in comparison to adipose-derived mesenchymal stem cells (ADMSCs) and UCMSCs.^{48,49} BMSCs, however, display limitations in terms of their extraction and proliferation capabilities, whereas

UCMSCs demonstrate a heightened propensity for proliferation relative to ADMSCs.^{50,51} Furthermore, UCMSCs and ADMSCs are characterized by their ample presence and robust anti-aging attributes, rendering them increasingly prominent candidates for therapeutic endeavors.^{52,53} While investigations comparing the functional attributes of cardiac mesenchymal stem cells (CMSCs) with those of other stem cell types are relatively scarce, prior studies have indicated that CMSCs exert a regulatory influence over numerous physiological and pathological processes within the cardiac domain.⁵⁴ The distinctive attributes of these aforementioned MSCs are summarized in Table 1.

The Regulation of Natural MSC-sEV on Multiple Pathological Pathways in MI/RI

Apoptosis

Apoptosis, being the classical form of programmed cell demise, represents the predominant mode of cellular attrition in response to ischemic or oxidative stimuli.⁵⁵ Under duress, the Protein kinase B/Nuclear factor kappa-B (AKT/NFκB) signaling cascade is activated, subsequently up-regulating the downstream classical apoptotic pathway encompassing Bcl-2/Bax/Caspase-3, thereby instigating the commencement of apoptosis.^{56,57} In the mouse model of MI/RI, wherein the left anterior descending branch was ligated and subsequently recanalized, sEV derived from ADMSCs, referred to as ADMSC-sEV, conveyed miR-221/222 to cardiomyocytes intracellularly. This internalization event mitigated the down-regulation of myocardial miR-221/222 induced by MI/RI, thereby inhibiting apoptosis.⁵⁸ A dual fluorescein reporter gene assay confirmed that the p53 up-regulated modulator of apoptosis (PUMA) and ETS proto-oncogene 1 (ETS-1) are the direct targets of miR-221/222, both of which can be silenced by miR-221/222. In vitro studies substantiated that ADMSC-sEV inhibited apoptosis in a hypoxia/reoxygenation (H/R) cardiomyocyte model by regulating the PUMA/AKT/NFκB pathway via miR-221/222 and curbed cardiomyocyte hypertrophy by restraining ETS-1 expression via miR-221/222. ETS-1 has been established as a pivotal gene in the provocation of cardiomyocyte hypertrophy. Although cell hypertrophy is commonly regarded as a compensatory response by surviving cells to systolic wall tension subsequent to cardiomyocyte depletion, hypertrophic cardiomyocytes also contribute significantly to abnormal wall motion, decreased compliance, and eventual heart failure.^{59,60} miR-486-5p, harbored within bone marrow-derived mesenchymal stem cell-secreted extracellular vesicles (BMSC-sEV), has been shown to down-regulate the expression of Phosphatase and tensin homolog (PTEN) in the myocardium of rat models afflicted with MI/RI. In vitro investigations have demonstrated that BMSC-sEV mitigate PTEN expression in cardiomyocytes via miR-486-5p, thereby activating the Phosphoinositide 3-kinase (PI3K)/AKT signaling pathway. This activation in turn regulates the downstream Bcl-2/Bax/CASP3 pathway, repressing the apoptotic cascade in cardiomyocytes under H/R conditions.⁶¹ Consequently, the modulation of cardiomyocyte apoptosis by MSC-sEV stands as a pivotal therapeutic approach for mitigating myocardial MI/RI injury.

Table 1 Characteristics of Each Mesenchymal Stem Cell

	BMSCs	ADMSCs	UCMSCs	CMSCs
Positive marker	CD13, CD44, CD73, CD90, CD105, CD166, CD271, STRO-1	CD9, CD13, CD29, CD44, CD54, CD73, CD90, CD105, CD106, CD146, CD166, HLA I, STRO-1	GD2, CD73, CD90, CD105	CD73, CD90, CD105, CD147
Negative marker	CD14, CD34, CD45	CD11b, CD14, CD19, CD31, CD34, CD45, CD79α, CD133, CD144, HLA-DR	CD271, CD34, CD45	CD34, CD45, CD133
Compared to other MSCs	Stronger osteogenic and angiogenic abilities	Stronger proliferation and anti-aging ability	Rich presence in the organization and easy to obtain	CD90 was expressed at lower frequency compared to other MSCs
Reference	[41,42,46]	[43,44,53]	[40,46,47,52]	[45]

Pyroptosis

The classical pathway of pyroptosis is mediated through the NLRP3 inflammasome/Caspase-1 (CASP1) pathway, with the NLRP3 inflammasome composed of Nod-like receptor protein 3 (NLRP3), apoptosis-associated speck-like protein containing a CARD (ASC), and CASP1. This pathway becomes activated under ischemia/reperfusion (I/R) conditions, resulting in the cleavage of the pyroptosis executive protein Gasdermin-D (GSDMD) by activated CASP1 within the NLRP3 inflammasome. Subsequently, the N-terminal fragment of GSDMD (GSDMD-N) undergoes oligomerization and integrates into the cell membrane, eliciting cardiomyocyte swelling, membrane rupture, and the release of inflammatory mediators, culminating in inflammatory cell death.⁶² Research has substantiated that NLRP3 is a direct target of miR-320b. In cell models subjected to H/R, BMSC-sEV delivered miR-320b, effectively suppressing the process of pyroptosis in cardiomyocytes by directly inhibiting the NLRP3/CASP1 pathway.⁶³ Furthermore, beyond its direct inhibition of NLRP3 inflammation, the regulation of the pyroptosis-effector protein GSDMD represents a promising anti-MI/RI strategy.⁶⁴ In both mouse MI/RI models and cell models exposed to H/R, miR-182-5p within BMSC-sEV attenuated the expression of GSDMD and inflammatory-associated proteins ASC and CASP1, thereby mitigating cell pyroptosis. Importantly, miR-182-5p exhibited no influence on NLRP3 expression.⁶⁵ This underscores the potential of BMSC-sEV in inhibiting MI/RI by concurrently targeting multiple facets of pyroptosis through the diverse constituents present in BMSC-sEV.

Ferroptosis

Ferroptosis stands as an oxidative cell demise pathway reliant upon iron ions, distinguishing itself from cell necrosis, apoptosis, and autophagy through its unique cellular death mechanisms.⁶⁶ Ferroptosis ensues when the intracellular glutathione (GSH)-dependent antioxidant system is disrupted, such as down-regulation of glutathione peroxidase GPX4, leading to the consequent up-regulation of lipid-activating enzymes, including Acyl-CoA synthetase (ACSL4). This results in the accumulation of lipid-reactive oxygen species and the induction of oxidative cell death.⁶⁷ Evidently, ferroptosis plays a significant role in myocardial I/R injury, where oxidative stress is a prominent pathological factor.⁶⁸ Zhang et al⁶⁹ harnessed BMSC-sEV to deliver the lncRNA miR9-3 host gene (miR9-3hg) to mouse cardiomyocytes subjected to H/R conditions, effectively suppressing the ferroptotic process. miR9-3hg inhibits ferroptosis by targeting Pumilio RNA binding family member 2 (PUM2) protein. Protein co-immunoprecipitation assays unveiled a protein interaction between PUM2 and Peroxiredoxin 6 (PRDX6), with PRDX6 emerging as a pivotal player in safeguarding cells against ferroptosis.⁷⁰ Thus, BMSC-sEV orchestrates the PUM2/PRDX6 signaling pathway within cardiomyocytes through miR9-3hg, averting ferroptosis induced by H/R and ameliorating reperfusion injury. Additionally, Song et al reported the substantial up-regulation of Divalent Metal Transporter 1 (DMT1) in mouse cardiomyocytes subjected to H/R conditions. DMT1 overexpression significantly promoted the ferroptotic process in cardiomyocytes. Luciferase gene reporter experiments elucidated that miR-23a-3p directly targeted DMT1. UCMSC-sEV intervention in H/R cardiomyocytes markedly repressed DMT1 expression in cardiomyocytes, thereby dampening the ferroptotic process. Mechanistic studies have suggested that miR-23a-3p, harbored within UCMSC-sEV, curtails DMT1 expression in cardiomyocytes.⁷¹

Autophagy

Autophagy, a highly conserved intracellular program, encompasses three primary forms, namely macroautophagy, microautophagy, and chaperone-mediated autophagy. Among these, macroautophagy has been most extensively and comprehensively studied in the context of disease.⁷² Autophagy permits cells to eliminate aged and damaged cellular components, including proteins, lipids, and dysfunctional organelles, thereby promoting cellular longevity. Nevertheless, under certain unmitigated lethal stimuli, autophagy can precipitate cell death, referred to as autophagic death.^{73–75} The equilibrium of autophagy is pivotal in determining the ultimate fate of cardiomyocytes in ischemic cardiomyopathy. Chen et al revealed the heightened expression of miR-143-3p within BMSC-sEV. BMSC-sEV effectively curtailed apoptosis and autophagy in H9C2 cardiomyocytes exposed to H/R conditions. miR-143-3p played a key role in inhibiting the cardiomyocyte Checkpoint Kinase 2 (CHK2) through targeted repression.⁷⁶ CHK2 is instrumental in activating the CHK2/Beclin1 signaling cascade, which in turn promotes autophagy. Thus, miR-143-3p inhibition of CHK2/Beclin1

pathway safeguards cardiomyocytes from excessive autophagy-induced damage during reperfusion.⁷⁷ In the realm of MI/RI treatment, beyond safeguarding cardiomyocytes to enhance cardiac function, the preservation of cardiac microvascular endothelial cells (CMECs) to ensure adequate blood supply to ischemic regions has emerged as a crucial therapeutic strategy.⁷⁸ Research has delineated that heightened miR-143-3p expression under MI/RI conditions hinders the Bcl-2/Beclin1 signaling axis, fostering apoptosis and autophagy in CMECs. Within this context, Urothelial carcinoma-associated 1 (UCA1) contained within UCMSC-sEV competes for miR-143-3p binding in CMEC cells, enhancing cell viability.⁷⁹ This underscores the significance of maintaining autophagic balance for cell survival, and MSC-sEV emerges as a potent regulator of autophagic equilibrium within diverse cardiac microenvironmental cell types, promising substantial applicability in the prevention and management of MI/RI.

Macrophage Polarization

The MI/RI process is invariably accompanied by an inflammatory cascade within the cardiac microenvironment, with Macrophages (M ϕ) playing a pivotal role in orchestrating the inflammatory environment.⁸⁰ In the initial stages of injury, M ϕ predominantly undertake the task of debris clearance, manifesting the pro-inflammatory M1 phenotype. Subsequently, M ϕ gradually polarize towards the anti-inflammatory M2 phenotype, secreting not only anti-inflammatory mediators but also a multitude of growth factors, crucial for wound healing and scar tissue formation.^{81,82} Shen et al demonstrated that myocardial infusion of BMSCs ameliorated cardiac function in MI/RI mouse models by modulating M2 polarization of macrophages, and this effect was compromised upon the inhibition of miR-21-5p expression within BMSCs. This underscores the pivotal role of miR-21-5p in regulating the M1/M2 equilibrium within myocardial macrophages orchestrated by BMSC-sEV.⁸³ An additional study observed that BMSC-sEV, apart from significantly enhancing cardiac function and reducing myocardial infarction size in MI/RI mice, effectively mitigated inflammatory infiltration in myocardial tissue. BMSC-sEV were internalized by macrophages, promoting M2 polarization contingent on miR-182.⁸⁴ Mechanistic studies unveiled that miR-182 within BMSC-sEV inhibited Toll-like receptor 4 (TLR4) expression, resulting in the down-regulation of the Myeloid differentiation primary response protein MyD88 (MyD88)/NF- κ B signaling pathway and the concurrent up-regulation of the PI3K/Akt signaling pathway, thus fostering M2 macrophage polarization. This underscores the multifaceted regulation of BMSC-sEV on the M1/M2 balance within macrophages, thereby modulating the extent of MI/RI. Therefore, a comprehensive perspective is imperative for exploiting BMSC-sEV in regulating macrophages and ameliorating MI/RI pathology. The collective regulation of MSC-sEV on various pathological facets of MI/RI is illustrated in Figure 1.

The Regulation of Nanoscale Modified MSC-sEV in MI/RI

As a therapeutic modality, sEV also encounters impediments, such as the deficiency in its targeting prowess towards specific organs and its swift clearance by mononuclear macrophages. These limitations constrict its broader application. Unaltered sEV tends to amass predominantly within the liver, kidney, spleen, and other organs subsequent to intravenous administration, a disposition unfavorable for its utilization in cardiovascular maladies.⁸⁵ Concurrently, the propensity of phagocytic cells to efficiently engulf sEV surpasses that of non-phagocytic cells, thus impeding the effective delivery of sEV to afflicted target organs.^{86,87} Other investigations have additionally revealed that intramuscular administration of myospherogenic sEV can mitigate scar formation and enhance myocardial ejection fraction in myocardial ischemia model pigs, while intracoronary administration of the same sEV yields negligible therapeutic impact.⁸⁸ This phenomenon may be attributed to the innate deficiency in homing ability exhibited by most naturally occurring sEV. Consequently, the modification of MSC-sEV to attain immune evasion, precise organ targeting, and prolonged organ residence emerges as a viable strategy to augment the efficacy of MSC-sEV in the context of MI/RI.

The surfaces of nanovesicles originating from different cells often bear membrane proteins specific to their parent cells. By merging sEV with nanovesicles or fusing nanovesicles among themselves, nanoparticles possessing multiple cellular characteristics can be acquired. Such nanoparticles are widely employed in drug delivery.⁸⁹ Platelets and monocytes conspicuously accumulate within injured blood vessels and myocardial tissue subsequent to MI/RI. Platelets adhere to damaged vasculature via binding interactions with von Willebrand factor (vWF) secreted by endothelial cells, facilitated by platelet glycoprotein Ib alpha chain (GPIb α) on their surface. This adhesion is further

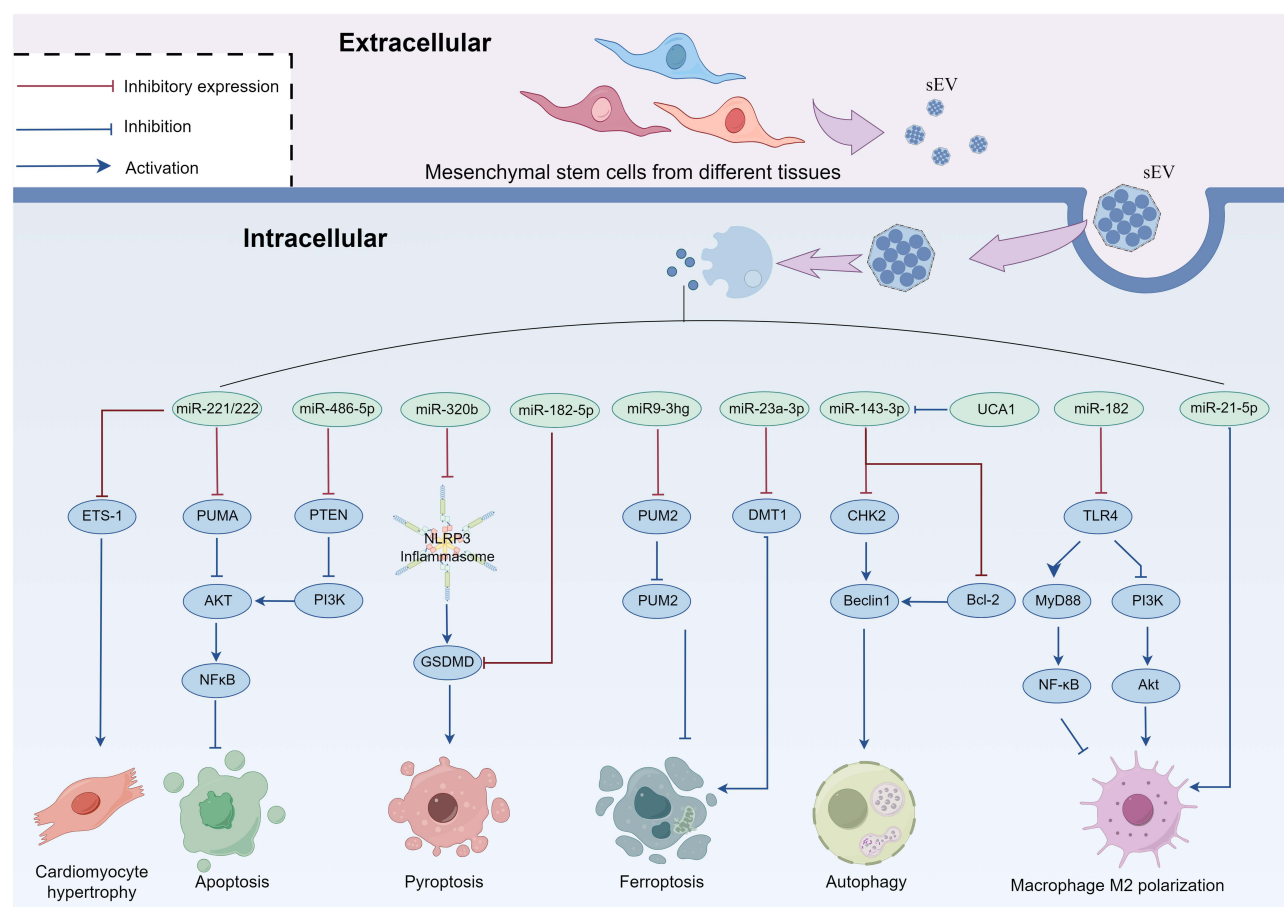


Figure I The regulation of natural MSC-sEV on multiple pathological pathways in MI/RI (By FigDraw).

fortified through binding between platelet surface glycoproteins GPIa/IIa and GPVI to sub-endothelial collagen, and GPIIb/IIIa to fibrin.^{90,91} Chemokine receptors present on monocyte surfaces become activated in response to upregulated chemokines induced by MI/RI injury, thereby altering the configuration of cell surface adhesion proteins, generating binding sites for endothelial ligands, and ultimately culminating in adhesion, migration, differentiation into macrophages, and localization within ischemic and peripheral regions.^{92,93} These macrophages play a pivotal role in the clearance of deceased cell debris and the facilitation of cardiac restoration. However, research has indicated that the recruitment of certain monocyte subtypes within cardiac tissue can exacerbate myocardial damage, casting uncertainty on whether monocytes foster cardiac function recovery or exacerbate injury following cardiac trauma.^{94,95} Nevertheless, this tendency of accumulation within damaged tissues remains a subject of considerable interest. Additionally, platelet membrane surface protein P-selectin can engage with monocyte surface protein PSGL1, while platelet membrane protein GPIb binds to Macrophage receptor 1 (MAC1), thus stabilizing intercellular adhesion. Subsequently, monocytes, as carrier cells, transport platelets into the myocardium via their own chemotactic attributes, facilitating the delivery of platelets to the injured myocardium.⁹⁶ Therefore, employing nano modification to endow MSC-sEV with adhesion and chemotactic capabilities from platelets and monocytes may prove to be an efficacious approach in facilitating targeted therapy of injured tissues.

Nanovesicles modified with platelet membrane proteins (P-sEV), obtained by co-squeezing BMSC-sEV with nanoscale platelet membrane vesicles of platelet origin through fusion, has endothelium tropism reliant on platelet membrane proteins. P-sEV exhibit heightened internalization by endothelial cells distressed by hypoxia. Furthermore, P-sEV enhances endothelial cell vitality and angiogenesis capacity, and inhibiting endothelial cell apoptosis. Moreover, the potential of P-sEV to target endothelial cell injury, inhibit endothelial cell apoptosis, and stimulate angiogenesis has

been corroborated in mouse models of MI/RI.⁹⁷ Notably, platelet membrane proteins GPIb α and P-selectin adorn the surface of P-sEV, and these P-sEV enter injured tissues in concert with monocytes through adhesion. M1-polarized macrophages (of circulating monocyte origin) absorb P-sEV, avoiding lysosomal degradation via an endosomal escape mechanism, and subsequently release their contents into the cytoplasm of M1 macrophages. This process modulates the immune milieu within the damaged myocardium, fostering the transition of macrophages from a pro-inflammatory phenotype (M1) to an anti-inflammatory phenotype (M2).⁹⁸ In addition to harnessing platelet membrane properties, the modification of MSC-sEV with monocytes membranes holds promising applications. Zhang et al employed an extrusion technique to fuse isolated MSC-sEV with monocytes membrane (Mons), resulting in monocyte-mimicking bioinspired MSC-sEV (Mon-sEV). Mon-sEV conserves the characteristics of MSC-sEV in terms of characterization and biological functionality, with monocytes membrane-derived adhesion proteins gracing its surface. Mon-sEV demonstrates a pronounced ability to chemotax endothelial cells and cardiomyocytes. Moreover, Mon-sEV exhibits significantly superior anterior ventricular wall enrichment and mitigation of myocardial injury in comparison to MSC-sEV in mouse models of myocardial MI/RI injury. This effect stems from the heightened expression of ICAM1 on the surfaces of cardiac endothelial cells and cardiomyocytes under MI/RI injury conditions, inducing homing of Mon-sEV containing adhesion proteins on their surfaces.⁹⁹ These investigations validate that nano modification aimed at enhancing targeting proficiency of MSC-sEV towards damaged tissues holds considerable promise.

In addition to bolstering the capacity to target damaged tissues, the extension of MSC-sEV's residence time within the organism constitutes a pivotal strategy to ensure *in vivo* efficacy.¹⁰⁰ *In vivo*, sEV clearance primarily occurs via macrophage-mediated processes.⁸⁷ However, certain tumor cells can robustly express the surface protein CD47, which belongs to the immunoglobulin superfamily, thereby achieving immune evasion through CD47 binding to signal-regulatory protein alpha (SIRRP α) on macrophages, releasing a "do not eat me" signal and inhibiting phagocytosis.¹⁰¹ Wei et al harnessed CD47 functionality to fabricate sEV (CD47-sEV) derived from CD47-overexpressing MSCs, effectively prolonging sEV retention time *in vivo*. Simultaneously, electrotransfection was employed to "load" miR-21 into the sEV, resulting in stable miR-21-enriched anti-phagocytic sEV (miR21-CD47-sEV).¹⁰² Further research indicates that these nanoscale modified sEV can target and downregulate the expression of PTEN in MI/RI mouse myocardial cells via miR-21. This suppression inhibits cell apoptosis, promotes the release of anti-inflammatory factors in myocardial tissue, and fosters angiogenesis, thereby ameliorating the cardiac functional damage induced by MI/RI.

Beyond the modulation of the heart's intrinsic process of remodeling and repair, the therapeutic approach of implanting cardiac tissue engineering materials into the afflicted region through surgical or minimally invasive techniques has garnered significant attention.^{103,104} The biological hydrogel is a hydrophilic polymer nanostructure with high water content and rapid diffusion rates. When in its liquid state and injected into the injured area, it undergoes gelation and integrates with host tissues induced by factors such as light, chemicals, ions, temperature, and pH. This aims to provide mechanical support for the infarcted heart, reduce ventricular wall stress, compensate for contractile function, and inhibit ventricular remodeling.^{103,105} AT-EHBPE and HA-SH form a pair of nano-hydrogel precursors with excellent conductivity and low cytotoxicity. Zou et al designed a gel system, AT-EHBPE/HA-SH, capable of anchoring UCMSC-sEV (Gel@sEV). This composite system possesses attributes such as controllable gel kinetics, shear-thinning injectability, electrical conductivity harmonizing with native myocardium, pliability, dynamic stability congruent with heart-beat, and commendable cellular compatibility. Importantly, Gel@sEV administration, in comparison to sole UCMSC-sEV injection, significantly prolongs sEV retention time in rats.¹⁰⁶ Furthermore, Gel@sEV exhibits enhanced efficacy in terms of mitigating myocardial fibrosis and promoting angiogenesis compared to either sEV or hydrogel administered individually. The distinct characteristics of various nanomodified MSC-sEV are showcased in Table 2.

MSC-sEV Pretreated with Natural Drugs

While there exists a substantial body of evidence supporting the effectiveness of natural remedies in direct disease treatment, this approach has encountered challenges such as the limited oral bioavailability of certain constituents, a low cellular uptake rate, and the instability of active ingredients over time.^{107–109} These hurdles constrain the standardized application and exploration of natural pharmaceutical preparations.¹¹⁰ A promising solution to surmount these issues

Table 2 Nanoscale Modified MSC-sEV

	Source Cells of sEV	Engineering Methods	Characteristic	Reference
P-sEV	BMSCs	Incubation-extrusion method	Targeted regulation of endothelial injury through endothelial tropism Enriched to damaged myocardium through adhesion to monocytes	[97,98]
Mon-sEV	BMSCs	Incubation-extrusion method	Tendency to damage myocardium and endothelial cells	[99]
miR21-CD47-sEV	BMSCs	Lentiviral transfection and electroporation	Immune escape ability, inhibit cardiomyocyte apoptosis, promote anti-inflammatory factor release and promote angiogenesis	[102]
Gel@sEV	UCMSCs	CP05 peptide was embedded within the designed hydrogel to anchor sEV	Extended dwell time and fill weak heart muscle	[106]

involves harnessing nanocarriers, including small extracellular vesicles, for the extraction and conveyance of the active elements found in natural medicines.¹¹¹

In China, the utilization of Suxiao Jiuxin pills (SJP) to ameliorate postoperative symptoms in patients suffering from acute coronary syndromes (ACS) following percutaneous coronary intervention (PCI) has garnered clinical validation.¹¹² Optimizing the administration route of SJP could unearth additional pharmacological benefits. Ruan et al conducted an experiment in which mouse CMSCs were pretreated with SJP, and the secretion of sEV from CMSCs was assessed. The findings demonstrated that SJP could augment sEV release from CMSCs via a GTP-dependent pathway, a more pronounced effect compared to the individual treatment of CMSCs with the core constituents of SJP (tetramethylpyrazine, borneol, etc.). This underscores the harmonizing influence inherent to Chinese herbal medicine.¹¹³ In addition to augmenting sEV secretion, the intervention of natural remedies can also enhance the efficacy of sEV in the context of MI/RI. In another investigation, Ruan et al employed sEV (SJP-sEV) obtained from SJP-pretreated CMSCs to intervene in mouse cardiomyocytes. The results exhibited that SJP could inhibit the expression of Histone demethylase UTX (UTX) and elevate the levels of histone H3K27 trimethylated protein (H3K27me3) within the cells.¹¹⁴ Histone-dependent chromatin remodeling mediated by H3K27me3 plays an indispensable role in cell regeneration, survival, and proliferation.¹¹⁵ SJP-sEV stimulates cardiomyocyte proliferation under conditions of oxidative stress by modulating the UTX/H3K27me3 pathway. This research introduces a novel avenue for Traditional Chinese Medicine (TCM) treatment of MI/RI.

Oridonin, an isolated compound derived from oridonin leaves, belongs to the ent-kaurane tetracyclic diterpene class and possesses anti-inflammatory, antibacterial, and cell-regulatory properties. Nevertheless, the clinical development of Oridonin is hampered by its limited aqueous solubility and poor bioavailability.^{116,117} Studies have demonstrated that BMSC-sEV can upregulate the expression of autophagy-related proteins (Beclin-1, ATG13, etc.) and downregulate the expression of apoptosis-related proteins (Apaf1, Bax, etc.) in rat cardiomyocytes under MI/RI conditions, thereby enhancing cardiac function in MI/RI-afflicted rats. Oridonin-sEV, derived from BMSCs pretreated with oridonin, effectively encapsulates oridonin and exhibits more pronounced myocardial protective capabilities compared to BMSC-sEV alone.¹¹⁸ Irisin, a glycosyloxyisoflavone extract from iris rhizomes, not only ameliorates myocardial injury induced by ischemia but also augments the protective effects of ADMSCs in the context of ischemic heart injury by promoting myocardial homing of ADMSCs.^{119–121} Simultaneously, sEV can serve as carriers for transporting Irisin to injured myocardial tissue. Research indicates that irisin-sEV, obtained through irisin pretreatment of BMSCs, can inhibit pyroptosis of cardiomyocytes under conditions of H/R and protect cardiomyocyte vitality by suppressing the NLRP3 inflammasome pathway. This protective effect surpasses that achieved by BMSC-sEV intervention alone.¹²² Myocardial resident macrophages encompass various subtypes, with C-C chemokine receptor 2-positive macrophages (Mφ^{CCR2+})

demonstrating a significant increase under MI/RI conditions. Myocardial-resident $M\phi^{CCR2+}$ serve as inducers for monocyte homing from the circulatory system to the myocardium, thereby exacerbating myocardial inflammatory damage. Conversely, myocardial-resident $M\phi^{CCR2-}$ exhibit an inhibitory effect on inflammation.¹²³ The expression level of miR-223-5p in tanshinone IIA (TSC)-pretreated UCMSC-sEV (TSC-sEV) was significantly elevated, and miR-223-5 targeted and suppressed the expression of CCR2. TSC-sEV mitigated the accumulation of $M\phi^{CCR2+}$ within the myocardium through the miR-223-5/CCR2 pathway, thereby ameliorating the inflammatory milieu in the myocardial microenvironment. Moreover, TSC-sEV exhibited a stronger pro-angiogenesis effect compared to umbilical cord mesenchymal stem cell-derived sEV alone.¹²⁴ Thus, the encapsulation of natural compound extracts within sEV not only facilitates drug delivery but also amplifies the efficacy of sEV, meriting further investigation.

Conclusion and Prospect

Drawing from the aforementioned investigations, it becomes evident that MSC-sEV wield a plethora of merits in the realm of mitigating MI/RI. These advantages encompass the following: (1) Given that MSC-sEV are excreted by MSCs, which are plentiful in origin and amenable to substantial *in vitro* cultivation, the scalable production of MSC-sEV is an attainable endeavor. (2) MSC-sEV therapy hinges upon the conveyance of signaling moieties such as proteins, RNA, and assorted molecules. Consequently, MSC-sEV therapy boasts the distinct advantage of eliciting minimal immunogenicity. (3) MSC-sEV encapsulate a diverse array of signaling molecules, engendering an expansive signal network that orchestrates MI/RI modulation, thereby imparting multi-target effects. (4) An additional facet of note pertains to the exploration of sEV freeze-drying methodologies, wherein the incorporation of a cryoprotective excipient bestows the convenience of prolonged storage and streamlined transportation.¹²⁵ Concomitantly, the augmentation of MSC-sEV via the integration of membrane proteins from monocytes and platelets or the inclusion of immune evasion factors onto their membrane surface serves to significantly enhance tissue tropism within damaged areas. This augmentation extends their residence duration and safeguards them from monocyte phagocytosis. Furthermore, the prospect of coupling MSC-sEV with biomaterials for the surgical restoration of injured myocardium harbors considerable potential.

It is imperative to acknowledge that MSC-sEV therapy confronts a host of challenges: (1) Presently, researchers have proposed a plethora of divergent methodologies for MSC-sEV production and purification, a majority of which lack standardized specifications and quality control. (2) MSC-sEV assimilate disparate signal factors contingent upon their source, necessitating the meticulous classification and functional discernment of MSC-sEV as prerequisites for the standardized production and clinical deployment of these vesicles. (3) MSC-sEV offer a range of administration routes, including intravenous, intranasal, and local injection, among others. While evidence underscores the efficacy of these routes, instances dictate that intramyocardial injection is indispensable for certain MSC-sEV to ameliorate MI/RI. However, this requirement poses practical challenges within the clinical domain.

Of course, not only MSC-sEV but sEV from other sources have also been applied in research related to MI/RI. For instance, studies have reported that sEV isolated from serum of myocardial infarction patients can reduce myocardial damage area in MI/RI rat models and improve cardiac function.¹²⁶ However, using sEV isolated from patient serum for MI/RI treatment is challenging due to difficulties in mass production and ethical concerns. Obtaining MSC-sEV through the cultivation of MSCs helps overcome these issues, even though research on MSC-sEV is still at an early stage. In summation, MSC-sEV therapy is a strategy teeming with advantages for MI/RI treatment, yet it contends with an array of challenges. Therefore, we aspire to see more comparative studies, standardization of the MSC-sEV preparation process, and clarification of the administration routes for MSC-sEV. Concurrently, conducting clinical research will facilitate the early application of MSC-sEV in the clinical treatment of MI/RI.

Abbreviations

MI/RI, Myocardial ischemia-reperfusion injury; sEV, small Extracellular vesicles; MSCs, Mesenchymal stem cells; CABG, Coronary artery bypass grafting; PMVs, Platelet membrane vesicles; Mons, Mononuclear cell membrane; SJP, Suxiao Jiuxin pills; ACS, Acute coronary syndromes; PCI, Percutaneous coronary intervention; TCM, Traditional Chinese Medicine.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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