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REVIEW

Mesenchymal Stem Cell Exosomes in the Treatment of Myocardial Infarction: a Systematic Review of Preclinical In Vivo Studies

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Abstract

Several prior studies have highlighted the promise of mesenchymal stem cells (MSCs) as tools for treating myocardial infarction (MI) patients. While MSCs were initially thought to mediate post-MI repair through differentiation and replacement of injured cells, they are now thought to function by releasing exosomes carrying important cargos which can prevent apoptosis and facilitate revascularization in the context of MI. Herein, we comprehensively survey prior preclinical studies examining MSC-derived exosomes (MSC-Exos) utility for the repair of MI-related tissue injury. In total, 24 relevant studies were identified in the PubMed, Web of Science, Embase, and Cochrane Library databases as per the PRISMA guidelines. In most studies, exosome-treated rodents exhibited improved cardiac function and angiogenesis together with decreased apoptotic cell death. MSC-Exos thus offer beneficial therapeutic efficacy when treating MI injury. However, further work will be necessary to standardize experimental preclinical models and to validate these results.

Keywords Mesenchymal stem cell · Exosomes · Angiogenesis · Apoptosis · Systematic review

Abbreviations

ATV	Atorvastatin
CSCs	Cardiac stem cells
EVs	Extracellular vesicles
HF	Heart failure
LAD	Left anterior descending artery
lncRNAs	Long non-coding RNAs
LV	Left ventricular
LVEF	Left ventricular ejection fraction
LVFS	Left ventricular fractional shortening
LVESD	Left ventricular end-systolic diameter
LVEDD	Left ventricular end-diastolic diameter
Mecp2	Methyl CpG binding protein 2
MI	Myocardial infarction
MIF	Macrophage migration inhibitory factor
miRNA	MicroRNA
mRNA	Messenger RNA
MSCs	Mesenchymal stem cells
MSC-Exos	MSC-derived exosomes
vWF	von Willebrand factor

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Introduction

Myocardial infarction (MI) is a leading cause of global morbidity and mortality, despite major advances in its prevention and treatment in recent years [1]. A primary goal of MI treatment is the salvaging and repair of infarcted myocardial tissue. However, even with rapid coronary intervention, a majority of patients nonetheless experience cardiomyocyte apoptosis, ventricular wall thinning, cavity dilatation, and eventual heart failure (HF) [2]. Many animal studies and clinical trials have explored the efficacy of bone marrow-derived mesenchymal cells (MSCs) as tools to facilitate MI repair [3–6], yet the efficacy of these cells and the mechanisms governing their purported efficacy remain controversial. These MSCs may function by undergoing differentiation into cell types that can replace those damaged in the context of MI. Other evidence, however, suggests that these MSCs primarily function in a paracrine manner [7, 8], secreting factors including proteins, lipids,

nucleic acids, and extracellular vesicles (EVs) that can modulate the biological activity of recipient cells [9–11]. EVs are clustered into four major classes based upon their size: exosomes (50–150 nm), microvesicles (100–1,000 nm), large oncosomes (1,000–10,000 nm), and apoptotic bodies (100–5,000 nm) [12], with exosomes being the most thoroughly studied of these particle types.

Exosomes are lipid bilayer-enclosed EVs produced by virtually all cells and are derived from multivesicular bodies that are generated via endosomal membrane invagination [13–15]. Exosomes can carry a range of functionally important cargos including proteins, RNAs, and DNA molecules. Multiple online databases including ExoCarta (http://exocarta.org) and Vesiclepedia (http://microvesicles.org) have sought to catalog exosomal cargos in a range of contexts. Exosomes have also been highlighted as important regulators of MI and related processes in recent years, with MSC-derived exosomes (MSC-Exos) offering great promise for treating MI, HF, and dilated cardiomyopathy [16–19]. Intramyocardial or intravenous exosome delivery has been shown to promote proliferation, suppress apoptosis, disrupt fibrosis, facilitate angiogenesis, and suppress oxidative stress, thereby exerting cardioprotective activity [20].

While there has been substantial variability with respect to the goals and models used in prior analyses of MSC-Exos as regulators of MI, studies generally follow four key steps: exosome isolation and characterization, coronary artery ligation, exosome delivery, and analyses of infarct size and cardiac function. In an effort to more broadly understand the therapeutic value of these stem cell–derived exosomes, the present systematic review was conducted to summarize the findings of prior in vivo analyses assessing the efficacy of MSC-Exos in the treatment of MI.

Materials and Methods

Search Strategy

The PubMed, Web of Science, Embase, and Cochrane Library databases were searched for all studies published as of 2 December 2020. Search terms were as follows: "Mesenchymal stem cell," "Exosome," and "Myocardial infarction."

Inclusion and Exclusion Criteria

Studies eligible for inclusion were (1) studies of the treatment of MI using bone marrow MSC-Exos in animal models, and (2) studies that were described in English. Articles were excluded if they were (1) reviews of meta-analyses, (2) conference abstracts, (3) non-experimental studies or studies lacking complete information, or (4) experimental studies using exosomes derived from cells other than bone marrow MSCs.

Study Quality Evaluation

The Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias assessment tool was used to examine the risk of bias associated with included studies [21], with the results of these quality analyses being shown in the Supplementary Material Table 1.

Data Extraction

Data were individually extracted from all studies, including information pertaining to both study design and outcomes. Recorded information included sample size, the sex and species of study model animals, the animal model system utilized, the comparison groups within the study, and the treatment details for individual groups. Treatment-related details included exosome or control sources, treatment concentrations, volume, delivery method, and frequency, and the timing of euthanasia for study animals. Both qualitative and quantitative details regarding study outcomes were noted where possible. The impact of MSC-Exos treatment on cellular apoptosis, proliferation, migration, and autophagy was also assessed. When details were not present within included studies, efforts were made to contact the authors of the original manuscript.

Data Analysis

Study outcomes were primarily evaluated in a qualitative manner owing to a lack of consistent quantitative data necessary to permit pooled analysis.

Results

Literature Search Results

Our initial search strategy initially identified 748 studies, of which 155 were duplicates. Following title and abstract screening, 536 of the remaining studies were excluded, while the remaining 57 were subjected to full-text review, with 24 being retained for inclusion in the final systematic review (Fig. 1).

Systematic Review

Overall, 24 studies were determined to be eligible for inclusion in this systematic review [22–45]. Details pertaining to the sample size, gender and speciesused in each study are shown in Table 1. Table 2 compiles the comparison groups

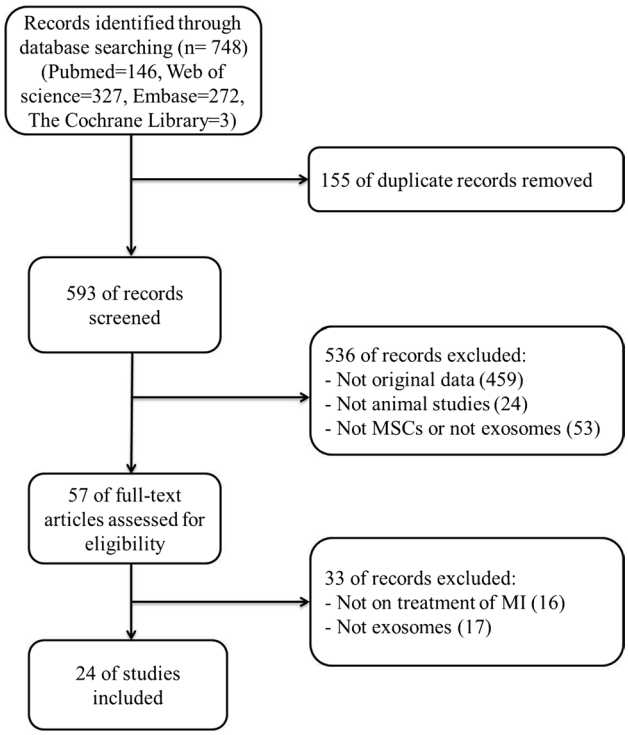

Fig. 1 Flow diagram of the literature search

used in different studies, while Tables 3 and 4 respectively detail the treatment volume, delivery, frequency of delivery, and timing of euthanasia for animals in the exosome and non-exosome treatment groups. A brief summary of the therapeutic outcomes in the different treatment groups is provided in Table 5. Details of the cells used in each study are shown in Supplementary Material Table 2.

Exosome Sources

All exosomes in the included studies were isolated from bone marrow–derived MSCs. In three of these studies [32, 39, 41], MSCs were purchased from commercial sources, while in one study they were derived from human bone marrow [40]. One study did not report the source of MSCs [37].

Animal Model Establishment

Included studies established rodent models of MI in rats or mice via the ligation of the left anterior descending artery (LAD). Briefly, animals were anesthetized, connected to a ventilator with an orotracheal tube, and left thoracotomy was performed between the 3rd and 4th intercostal space under sterile conditions. The heart was exposed and the LAD was then permanently ligated with a suture [46, 47].

Treatment Delivery

Various studies have shown the intramyocardial or intravenous injection of exosomes to be linked to pro-angiogenic, anti-fibrotic, and/or anti-apoptotic effects in infarcted myocardial tissue. Of the included studies, 20 [22, 24–27, 30–41, 43–45] conducted intramyocardial MSC-Exos administration, although the exact sides of injection differed among studies, and included 3–4 sites around the infarcted region, the left ventricular wall, or a site close to the site of arterial ligation. Tail vein injection was the mode of MSC-Exos delivery in 3 studies [28, 29, 42]. In another study [23], exosomes and MSCs were prepared into monolayer cell sheets and then transferred to the infarcted myocardial region.

Echocardiographic Analysis Results

Of the 24 included studies, 21 assessed cardiac function following MSC-Exos treatment [23–32, 34, 35, 37–45]. Echocardiographic analysis indicated that administration of MSC-Exos and exosomes derived from microRNA (miRNA) overexpressed or drug-pretreated MSCs were associated with a reduction in MI-related left ventricular (LV) dilation. These treatments were also related to improvements in left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) relative to other treatments and were linked to lower left ventricular end-systolic diameter (LVESD) and left ventricular end-diastolic diameter (LVEDD) values, consistent with the ability of MSC-Exos to enhance cardiac function in animal models of MI.

Histological Analysis

TTC staining of myocardial tissue was performed in 2 studies after MSC-Exos treatment, and the results showed a reduction in myocardial infarct area in rats in the MSC-Exos group compared with the MI group [33, 39]. Histological analysis of infarcted cardiac tissue by HE staining was performed in detail in 8 studies after MSC-Exos treatment [25, 33, 34, 36, 38–40, 42]. Samples from MI model groups exhibited extensive cardiomyocyte necrosis, irregularly arranged myocardial cells, fractured myocardial fibers, and extensive inflammatory cell infiltration not present in sham control rodents. In contrast, these changes were ameliorated in the MSC-Exos treatment groups relative to the MI model group. Masson's trichrome staining of infarcted heart tissues was performed in 17 studies [22–26, 29, 31, 33–35, 38–43, 45], with collagenous fibers appearing in blue and vascular smooth muscle cells appearing in red. MI model rodents exhibited cardiomyocyte swelling, collagenous fiber disorder, a loss of nuclei, and coagulative necrosis within

Table 1 Details of the MI animal models within each study

Author	Year	Sample size (joints)	Gender	Animal
Feng [22]	2014	NR	NR	C57BL/6 J mice
Kang [23]	2015	NR	Female	Sprague–Dawley rats
Yu [24]	2015	NR	Female	Sprague–Dawley rats
Teng [25]	2015	NR	Male	Sprague–Dawley rats
Zhang [26]	2016	30	Male	Sprague–Dawley rats
Shao [27]	2017	30	Male	Sprague–Dawley rats
He [28]	2018	NR	Male	C57BL/6 J mice
Zhu [29]	2018	NR	NR	C57BL/6 J mice
Ma [30]	2018	NR	Female	C57BL/6 J mice
Zhu [31]	2018	NR	Male	C57BL/6 J mice
Xiao [32]	2018	NR	Male	C57BL/6 J mice
Zou [33]	2019	18	Male	Sprague–Dawley rats
Huang [34]	2019	NR	Female	Sprague–Dawley rats
Li [35]	2019	20	Male	Sprague–Dawley rats
Xu [36]	2019	NR	Male	C57BL/6 J mice
Zhang [37]	2019	24	Male	Sprague–Dawley rats
Huang [38]	2020	NR	Female	Sprague–Dawley rats
Li [39]	2020	72	Male	C57BL/6 J mice
Sun [40]	2020	NR	Male	Sprague–Dawley rats
Liu [41]	2020	32	NR	Sprague–Dawley rats
Sun [42]	2020	NR	Female	Sprague–Dawley rats
Cheng [43]	2020	NR	Male	Sprague–Dawley rats
Fu [44]	2020	40	Female	Sprague–Dawley rats
Wang [45]	2020	NR	NR	C57BL/6 J mice

NR, not reported

the infarcted zone that was not evident in sham-operated control animals. MSC-Exos treatment was associated with the abatement of these MI-related pathological changes. In some studies [22–26, 29, 31, 34, 38, 40–43, 45], quantitative analysis of the LV fibrotic area and the Masson's trichrome staining results were performed, revealing that MSC-Exos treatment was associated with significantly better outcomes. In analyses conducted by Huang et al. [38], Huang et al. [34], and Sun et al. [40], the collagen area was significantly reduced in therapy-treated groups as evidenced by Sirius-Red staining results.

Xu et al. [36] additionally analyzed macrophage subsets within ischemic cardiac tissues and examined the impact of exosomes derived from pro-inflammatory MSCs on MI-related injury. Through a series of immunohistochemical staining assays, they determined that exosome treatment was associated with reductions in M1 marker (CD11c) staining and with increases in M2 marker (CD206) staining, confirming that LPS-pretreated MSC-Exos significantly enhanced CD206 expression and suppressed CD11c expression relative to that observed in MSC-Exos and PBS control groups. These data suggested that LPS-pretreated MSC-Exos were able to suppress post-MI inflammation at least in part via driving macrophages towards an M2 phenotype in MI model mice.

Western Blot Analysis

Nine studies performed Western blot assays of myocardial tissues [22, 32, 33, 35–37, 39, 41, 43]. Zou et al. [33] detected the expressions of Apaf1 and autophagy-associated protein 13 (ATG13) in myocardial tissues of various groups of rats and further confirmed that MSC-Exos could effectively inhibit myocardial tissue damage caused by MI. Li et al. [35] and Xiao et al. [32] examined the expressions of LC3-I, LC3-II, and autophagic fluxes, respectively, and found that MSC-Exos could inhibit cardiomyocyte autophagy in MI rats through the delivery of miRNA. In three other studies [36, 37, 43], MSC-Exos treatment was found to reverse the expression of apoptosis-related proteins such as Bax and Cleaved-caspase 3 in the myocardial tissue of MI rats. Li et al. [39] evaluated the effect of MSC-Exos on myocardial tissue fibrosis, and Western blot results showed that compared with the MI group, the expression levels of collagen-I, collagen-III, and fibronectin were reduced in the MSC-Exos group. Liu et al. [41] found that MSC-Exos

Table 2 Details of the comparison groups within each study

Author	Year	Groups
Feng [22]	2014	1. Scramble | 2. Mecp2 siRNA | 3. miR-22 mimic | 4. $Exo^{non-IPC}$ | 5. Exo^{IPC} | 6. $Exo^{IPC+miR-22\ inhibitor}$
Kang [23]	2015	1. Sham | 2. MI | 3. MI + PBS | 4. MI + MSC | 5. MI + $MSC^{ExoCtrl}$ | 6. MI + MSC^{ExoCR4} | 7. MI + $MSC^{ExosiCR4}$
Yu [24]	2015	1. Sham | 2. Control | 3. Exo^{Null} | 4. Exo^{GATA-4}
Teng [25]	2015	1. PBS | 2. Exo-depleted CM | 3. MSC-Exos
Zhang [26]	2016	1. Control | 2. CSCs | 3. $CSCs^{Exo}$
Shao [27]	2017	1. PBS | 2. MSC | 3. MSC-Exos
He [28]	2018	1. Control | 2. MI | 3. MSC-Exos | 4. NC-MSC-Exos | 5. GATA-4-MSC-Exos
Zhu [29]	2018	1. Sham | 2. PBS | 3. Nor-Exo | 4. Hypo-Exo | 5. NC-Hypo-Exo | 6. $miR125b^{KD}$-Hypo-Exo | 7. Cy5.5-labeled Scr-Exo | 8. Cy5.5-IMT-Exo
Ma [30]	2018	1. PBS | 2. miR-132 | 3. Exo-null | 4. Exo-miR-132
Zhu [31]	2018	1. Sham | 2. PBS | 3. Exo^{N} | 4. Exo^{H}
Xiao [32]	2018	1. MI | 2. MSC-Exo | 3. $MSC\text{-}Exo^{NC}$ | 4. $MSC\text{-}Exo^{anti-miR125b}$
Zou [33]	2019	1. Sham | 2. MI | 3. MI + Exo
Huang [34]	2019	1. Sham | 2. MI | 3. Exo | 4. MSC^{d1} | 5. MSC^{d3} | 6. MSC^{d7} | 7. Exo + MSC^{d1} | 8. Exo + MSC^{d3} | 9. Exo + MSC^{d7}
Li [35]	2019	1. Sham | 2. MI | 3. MSC-Exos | 4. MSC-301-Exos
Xu [36]	2019	1. Sham | 2. PBS | 3. Exo | 4. L-Exo
Zhang [37]	2019	1. Sham | 2. MI | 3. AMI + N-exo | 4. AMI + H-exo
Huang [38]	2020	1. Sham | 2. MI | 3. MSC-Exos | 4. MSC^{ATV}-Exos | 5. MSC^{ATV}(Si)-Exos | 6. MSC(H19)-Exos
Li [39]	2020	1. Sham | 2. MI | 3. MI + MSCs-Exo | 4. Exo $^{agomiR\ NC}$ | 5. Exo $^{miR-185\ agomiR}$ | 6. Exo $^{antagomiR\ NC}$ | 7. Exo $^{miR-185\ antagomiR}$ | 8. Exo $^{miR-185\ agomiR}$ + overexpressed (oe)-NC | 9. Exo $^{miR-185\ agomiR}$ + oe-SOCS2
Sun [40]	2020	1. Sham | 2. MI | 3. Aged-Exo | 4. Young-Exo | 5. Ctrl-Exo | 6. miR-221-Exo
Liu [41]	2020	1. Control | 2. MI | 3. MSC-Exos | 4. MIF-MSC-Exos
Sun [42]	2020	1. PBS | 2. Exo | 3. Exo-HIF-1α
Cheng [43]	2020	1. Sham | 2. MI | 3. MI + exosome | 4.GW4869 | 5. MI + exo^{miR210} | 6. MI + $exo^{anti-miR210}$ | 7. MI + $exo^{Vehicle}$
Fu [44]	2020	1. Sham | 2. PBS | 3. Exo-control | 4. Exo-338 mimic
Wang [45]	2020	1. Sham | 2. Control | 3. MSCs + Exo/miR-Ctrl | 4. Exo/miR-19a/19b 5. MSCs + Exo/miR-19a/19b

ATV, atorvastatin; CM, culture medium; CSCs, cardiac stem cells; Exo, exosome; ExoN, exosomes from normoxia-treated MSCs; ExoH, exosomes from hypoxia-treated MSCs; HIF-1α, hypoxia-inducible factor-1α; H-exo, hypoxic preconditioning MSCs-exosomes; Hypo, hypoxia; IMT, ischemic myocardium-targeted; IMT-Exo, IMT-conjugated Hypo-Exo; PC, ischemic preconditioning; KD, knockdown; L-Exo, LPS-primed MSC-derived exosomes; Mecp 2, methyl CpG binding protein 2; MI, myocardial infarction; MIF, macrophage migration inhibitory factor; MSCs, mesenchymal stem cells; N-exo, normoxic preconditioning MSCs-exosomes; NC, negative control; Nor, normoxia; PBS, phosphate-buffered saline; SOCS2, suppressor of cytokine signaling 2

treatment significantly reversed the expressions of fission 1 and mitofusin 2, indicating that MSC-Exos treatment was able to inhibit mitochondrial fragmentation. In addition, the expression of Mecp2 gene which was targeted by miR-22 was only examined in the study of Feng et al. [22] to confirm the ability of MSC-Exos to exert cardioprotective effects through miR-22 targeting Mecp2.

Angiogenesis

Following MI-induced injury, cardiomyocyte apoptosis and death occur, and the cardiac tissue remains in a reparative state that can lead to ventricular remodeling and scar formation in the infarcted region, with neovascularization in the infarcted region being particularly important [48–50]. In some studies [31, 34, 38, 40], immunofluorescent staining for arterioles and capillaries was performed with antibodies specific for α-SMA and CD31 in order to assess the impact of MSC-Exos treatment on angiogenesis and associated tissue repair. Huang et al. compared the relative effects of exosomes derived from MSCs that were or were not pretreated with atorvastatin (ATV) and found that MI model rats treated with MSC-Exos derived from ATV-pretreated cells exhibited increased arteriole density [38]. Huang et al. also found that arteriole density was significantly higher in animals treated with MSCs and MSC-Exos relative to in animals treated with MSCs alone [34]. Sun et al. observed increased border zone capillary density in ischemic heart tissues from animals treated with exosomes derived from young donors relative to those of animals treated with exosomes from older donors [40]. Zhu et al. also found that arteriole density was enhanced following treatment with exosomes from hypoxia-treated MSCs relative to exosomes isolated from MSCs under normoxic conditions [31]. Kang

Table 3 Summary of parameters for the animals receiving exosome treatment

Author	Year	Group number	Treatment	Concentration (protein/particle concentration)	Volume	Delivery	Frequency	Euthanasia
Feng [22]	2014	G4	Exosomes from MSCs without IPC	1 μg	NR	Along the border between infarct zone and normal myocardium injection	After LAD ligation	4 weeks
		G5	Exosomes from MSCs following IPC	1 μg	NR	Along the border between infarct zone and normal myocardium injection	After LAD ligation	4 weeks
		G6	Exosomes from MSCs after inhibition of miR-22 prior to IPC	1 μg	NR	Along the border between infarct zone and normal myocardium injection	After LAD ligation	4 weeks
Kang [23]	2015	G5	Exosomes from MSCs transfected with null lentivirus vector	NR	NR	Onto the infarcted area of the myocardium injection	NR	4 weeks
		G6	Exosomes from MSCs transfected with lentivirus vector overexpress CXCR4	NR	NR	Onto the infarcted area of the myocardium injection	NR	4 weeks
		G7	Exosomes from MSCs transfected with siRNA against CXCR4	NR	NR	Onto the infarcted area of the myocardium injection	NR	4 weeks
Yu [24]	2015	G3	Exosomes from MSCs transduced with null lentivirus vector	Exosomes harvested from 4×10^6 MSCs	50 μL	At the perimeter of the infarct region injection	After LAD ligation	4 weeks
		G4	Exosomes from MSCs transduced with recombinant GATA-4	Exosomes harvested from 4×10^6 MSCs	50 μL	At the perimeter of the infarct region injection	After LAD ligation	4 weeks
Teng [25]	2015	G3	Exosome-depleted MSCs culture medium	80 μg	100 μL	At 4 different sites of the viable myocardium bordering the infarcted zone injection	60 min after LAD ligation	4 weeks
			Exosomes from MSCs	80 μg	100 μL	At 4 different sites of the viable myocardium bordering the infarcted zone injection	60 min after LAD ligation	4 weeks
Zhang [26]	2016	G3	CSCs that were preconditioned with MSC-Exos	400 μg/mL	NR	Surrounding the infarct zones at 4 sites injection	48 h after MI	At 28 days
Shao [27]	2017	G3	Exosomes from MSCs	20 μg /20 μL	20 μL	Along the infarct border region at two different sites injection	After LAD ligation	1 week

Table 3 (continued)

Author	Year	Group number	Treatment	Concentration (protein/particle concentration)	Volume	Delivery	Frequency	Euthanasia
He [28]	2018	G3	Exosomes from MSCs	20 μg/mL	1 mL	Tail vein injection	At 48 h after induction of MI	At 48 h, 72 h, and 96 h
		G4	Exosomes from MSCs without GATA-4 transduction	20 μg/mL	1 mL	Tail vein injection	At 48 h after induction of MI	At 48 h, 72 h, and 96 h
		G5	Exosomes from GATA-4-expressing MSCs	20 μg/mL	1 mL	Tail vein injection	At 48 h after induction of MI	At 48 h, 72 h, and 96 h
Zhu [29]	2018	G3	Exosomes from MSCs in normoxia condition	200 μg per 20 g body wt	NR	Ischemic heart region injection	Immediately after MI surgery	4 weeks
		G4	Exosomes from MSCs in hypoxia condition	200 μg per 20 g body wt	NR	Ischemic heart region injection	Immediately after MI surgery	4 weeks
		G5	Exosome from negative control-hypoxia-conditioned MSCs	200 μg per 20 g body wt	NR	Ischemic heart region injection	Immediately after MI surgery	4 weeks
		G6	Exosome from miR125b KD hypoxia-conditioned MSCs	200 μg per 20 g body wt	NR	Ischemic heart region injection	Immediately after MI surgery	4 weeks
		G7	Cy5.5-labeled Scramble-Hypoxia-conditioned MSCs-derived exosomes	200 μg per 20 g body wt	NR	Tail vein injection	At 0 h, 4 h, 24 h, 48 h, and 7 d	4 weeks
		G8	Cy5.5 labeled IMT peptide conjugated Hypo-exosomes	200 μg per 20 g body wt	NR	Tail vein injection	At 0 h, 4 h, 24 h, 48 h, and 7 d	4 weeks
Ma [30]	2018	G3	Exosomes from MSCs	600 μg	20 μL	Near the ligation site in the free wall of the left ventricle injection	After LAD ligation	At 28 days
		G4	Exosomes from MSCs with miR-132-electroporated loaded	600 μg	20 μL	Near the ligation site in the free wall of the left ventricle injection	After LAD ligation	At 28 days
Zhu [31]	2018	G3	Exosomes from MSCs under normoxia	Exosomes derived from 2×10^7 MSCs	30 μL	Around the border zone of infarcted heart at five sites injection	NR	At 28 days
		G4	Exosomes from MSCs under hypoxia	Exosomes derived from 2×10^7 MSCs	30 μL	Around the border zone of infarcted heart at five sites injection	NR	At 28 days

Table 3 (continued)

Author	Year	Group number	Treatment	Concentration (protein/particle concentration)	Volume	Delivery	Frequency	Euthanasia
Xiao [32]	2018	G2	Exosomes from MSCs	5 μg in 25 μL	25 μL	At the border zone of infarcted heart at five sites injection	NR	At 28 days
		G3	Exosomes from MSCs transfected with control oligonucleotides	5 μg in 25 μL	25 μL	At the border zone of infarcted heart at five sites injection	NR	At 28 days
		G4	Exosomes from MSCs transfected with anti-miR-125b oligonucleotides	5 μg in 25 μL	25 μL	At the border zone of infarcted heart at five sites injection	NR	At 28 days
Zou [33]	2019	G3	Exosomes from rabbit bone marrow MSCs	10 μg	NR	Left ventricular wall injections	NR	NR
Huang [34]	2019	G3	Exosomes from MSCs	10 μg in 100 μL	100 μL	Around the border zone of infarcted heart injections to three sites	At 30 min after LAD ligation	5 weeks
		G7	Exosomes from MSCs combined with MSCs transplantation at d1 post MI	10 μg in 100 μL	100 μL	Around the border zone of infarcted heart injections to three sites	At 30 min after LAD ligation	5 weeks
		G8	Exosomes from MSCs combined with MSCs transplantation at d3 post MI	10 μg in 100 μL	100 μL	Around the border zone of infarcted heart injections to three sites	At 30 min after LAD ligation	5 weeks
		G9	Exosomes from MSCs combined with MSCs transplantation at d7 post MI	10 μg in 100 μL	100 μL	Around the border zone of infarcted heart injections to three sites	At 30 min after LAD ligation	5 weeks
Li [35]	2019	G3	Exosomes from MSCs	NR	NR	Around the border zone of infarcted heart injections at five points	At 30 min after LAD ligation	4 weeks
		G4	Exosomes from MSCs transfected with miR-301 mimics	NR	NR	Around the border zone of infarcted heart injections at five points	At 30 min after LAD ligation	4 weeks
Xu [36]	2019	G3	Exosomes from MSCs	50 μg in 50 μL	50 μL	Around the infarct border zone injections at four sites	Immediately after LAD ligation	At days 3 and 7
		G4	Exosomes from LPS-primed MSCs	50 μg in 50 μL	50 μL	Around the infarct border zone injections at four sites	Immediately after LAD ligation	At days 3 and 7

Table 3 (continued)

Author	Year	Group number	Treatment	Concentration (protein/particle concentration)	Volume	Delivery	Frequency	Euthanasia
Zhang [37]	2019	G3	Exosome from normoxic preconditioning MSCs	NR	NR	MI margin area at the left ventricular anterior wall below the ligation site injection	NR	At 28 days
		G4	Exosome from hypoxic preconditioning MSCs	NR	NR	MI margin area at the left ventricular anterior wall below the ligation site injection	NR	At 28 days
Huang [38]	2020	G3	Exosomes from MSCs non-treated with ATV	10 μg in 100 μL	100 μL	Around the border zone of infarcted heart injections to three sites	At 30 min after LAD ligation	At days 3, 7, and 28
		G4	Exosomes from MSCs treated with ATV	10 μg in 100 μL	100 μL	Around the border zone of infarcted heart injections to three sites	At 30 min after LAD ligation	At days 3, 7, and 28
		G5	Exosomes from MSCs transfected with lncRNA H19 siRNA in ATV pretreated	10 μg in 100 μL	100 μL	Around the border zone of infarcted heart injections to three sites	At 30 min after LAD ligation	At days 3, 7, and 28
		G6	Exosomes from MSCs transfected with lncRNA H19	10 μg in 100 μL	100 μL	Around the border zone of infarcted heart injections to three sites	At 30 min after LAD ligation	At days 3, 7, and 28

Table 3 (continued)

Author	Year	Group number	Treatment	Concentration (protein/particle concentration)	Volume	Delivery	Frequency	Euthanasia
Li [39]	2020	G3	Exosomes from MSCs	5 μg in 25 μL	25 μL	Around the border zone of infarcted heart injections at five points	At 30 min after LAD ligation	28 days
		G4	Exosomes from MSCs transfected with ago-miR- NC	5 μg in 25 μL	25 μL	Around the border zone of infarcted heart injections at five points	At 30 min after LAD ligation	28 days
		G5	Exosomes from MSCs transfected with miR-185 agomiR	5 μg in 25 μL	25 μL	Around the border zone of infarcted heart injections at five points	At 30 min after LAD ligation	28 days
		G6	Exosomes from MSCs transfected with antagomiR NC	5 μg in 25 μL	25 μL	Around the border zone of infarcted heart injections at five points	At 30 min after LAD ligation	28 days
		G7	Exosomes from MSCs transfected with miR-185 antagomiR	5 μg in 25 μL	25 μL	Around the border zone of infarcted heart injections at five points	At 30 min after LAD ligation	28 days
		G8	Exosomes from MSCs transfected with miR-185 antagomiR combined with NC of overexpressed SOCS2 plasmid	5 μg in 25 μL	25 μL	Around the border zone of infarcted heart injections at five points	At 30 min after LAD ligation	28 days
		G9	Exosomes from MSCs transfected with miR-185 antago-miR combined with overexpressed SOCS2 plasmid	5 μg in 25 μL	25 μL	Around the border zone of infarcted heart injections at five points	At 30 min after LAD ligation	28 days

Table 3 (continued)

Author	Year	Group number	Treatment	Concentration (protein/particle concentration)	Volume	Delivery	Frequency	Euthanasia
Sun [40]	2020	G3	Exosomes from MSCs harvested from aged donors	1 μg/μL	50 μL	Around the infarcted region injection	30 min after establishing a model of MI	4 weeks
		G4	Exosomes from MSCs harvested from young donors	1 μg/μL	50 μL	Around the infarcted region injection	30 min after establishing a model of MI	4 weeks
		G5	Exosomes from MSCs transfected with lentiviruses containing miR-221 NC	1 μg/μL	50 μL	At the border area injection	30 min post MI	4 weeks
		G6	Exosomes from MSCs transfected with lentiviruses containing miR-221	1 μg/μL	50 μL	At the border area injection	30 min post MI	4 weeks
Liu [41]	2020	G3	Exosomes from MSCs	30 μg in 30 μL	30 μL	Around the border zone of infarcted heart injections at four sites	Following ligation	4 weeks
		G4	Exosomes from MSCs transfected with MIF plasmid	30 μg in 30 μL	30 μL	Around the border zone of infarcted heart injections at four sites	Following ligation	4 weeks
Sun [42]	2020	G2	Exosomes from control MSCs	2×10^{10} particles	500 μL	Tail vein injection	After MI surgery	At days 7 and 28
		G3	Exosomes from HIF-1α overexpressed MSCs	2×10^{10} particles	500 μL	Tail vein injection	After MI surgery	At days 7 and 28
Cheng [43]	2020	G3	Exosomes from MSCs	Exosomes harvested from 1×10^{6} MSCs	20 μL	Cardiac muscle close to the area of arterial ligation injection	NR	7 days and 4 weeks
		G4	Exosomes from MSCs cultured in 10 μM GW4869	Exosomes harvested from 1×10^{6} MSCs	20 μL	Cardiac muscle close to the area of arterial ligation injection	NR	7 days and 4 weeks
		G5	Exosomes from MSCs infected with miR-210	Exosomes harvested from 1×10^{6} MSCs	20 μL	Cardiac muscle close to the area of arterial ligation injection	NR	7 days and 4 weeks
		G6	Exosomes from MSCs infected with anti-miR-210	Exosomes harvested from 1×10^{6} MSCs	20 μL	Cardiac muscle close to the area of arterial ligation injection	NR	7 days and 4 weeks
		G7	Exosomes from MSCs infected with empty lentivirus	Exosomes harvested from 1×10^{6} MSCs	20 μL	Cardiac muscle close to the area of arterial ligation injection	NR	7 days and 4 weeks

Table 3 (continued)

Author	Year	Group number	Treatment	Concentration (protein/particle concentration)	Volume	Delivery	Frequency	Euthanasia
Fu [44]	2020	G3	Exosome from MSCs transfected with NC	NR	50 µL	Ischemic myocardium injection	Before the chest was closed	At 7 days
		G4	Exosome from MSCs transfected with miR-338 mimic	NR	50 µL	Ischemic myocardium injection	Before the chest was closed	At 7 days
Wang [45]	2020	G3	Exosome from MSC loaded miR-NC combined with MSCs transplantation	NR	MSC suspension at 2×10^6 cells/mouse, and with 0.5 µmol of miR-NC-loaded exosomes	Ischemic myocardium injection	NR	3 weeks
		G4	Exosome from MSC loaded miR-19a/19b	NR	0.25 µmol of each	Ischemic myocardium injection	NR	3 weeks
		G5	Exosome from MSC loaded miR-19a/19b combined with MSCs transplantation	NR	MSC suspension at 2×10^6 cells/mouse, and with 0.5 µmol of miR-19a/19b-loaded exosomes	Ischemic myocardium injection	NR	3 weeks

ATV, atorvastatin; CM, culture medium; CSCs, cardiac stem cells; Exo, exosome; G, group; H, hypoxia; HIF-1α, hypoxia inducible factor-1α; Hypo, hypoxia; IMT, ischemic myocardium-targeted; IPC, ischemic preconditioning; KD, knockdown; LAD, left anterior descending artery; lncRNA H19, long non-coding RNA H19; LPS, lipopolysaccharide; MI, myocardial infarction; MIF, macrophage migration inhibitory factor; MSCs, mesenchymal stem cells; N, normoxia; NC, negative control; NR, not reported; SOCS2, suppressor of cytokine signaling 2

Table 4 Summary of parameters for the animals receiving control and non-exosome treatment

Author	Year	Group number	Treatment	Concentration (particle/cell number)	Volume	Delivery	Frequency	Euthanasia
Feng [22]	2014	G2	Mecp2 siRNA	1 nmol	NR	Along the border between infarct zone and normal myocardium injection	After LAD ligation	4 weeks
		G3	miR-22 mimic	80 ng	NR	Along the border between infarct zone and normal myocardium injection	After LAD ligation	4 weeks
Kang [23]	2015	G3	PBS	NA	NR	NR	NR	4 weeks
Yu [24]	2015	G2	PBS	NA	50 μL	At the perimeter of the infarct region injection	After LAD ligation	4 weeks
Teng [25]	2015	G1	PBS	NA	100 μL	At 4 different sites of the viable myocardium bordering the infarcted zone injection	60 min after LAD ligation	4 weeks
		G2	Exosome-depleted MSC culture medium	NA	100 μL	At 4 different sites of the viable myocardium bordering the infarcted zoneinjection	60 min after LAD ligation	4 weeks
Zhang [26]	2016	G1	Saline	NA	NR	Surrounding the infarct zones at 4 sites injection	48 h after MI	At 28 days
Shao [27]	2017	G1	PBS	NA	20 μL	Along the infarct border region at two different sites injection	After LAD ligation	1 week
He [28]	2018	G2	Saline	NA	1 mL	Tail vein injection	At 48 h after induction of MI	At 48 h, 72 h, and 96 h
Zhu [29]	2018	G2	PBS	NA	200 μL	Ischemic heart region injection	Immediately after MI surgery	4 weeks
Ma [30]	2018	G1	PBS	NA	20 μL	Near the ligation site in the free wall of the left ventricle injection	After LAD ligation	At 28 days
Zhu [31]	2018	G2	PBS	NA	30 μL	Around the border zone of infarcted heart at five sites injection	NR	At 28 days
Xiao [32]	2018	G1	PBS	NA	25 μL	At the border zone of infarcted heart at five sites injection	NR	At 28 days
Zou [33]	2019	G2	NR	NA	NR	NR	NR	NR

Table 4 (continued)

Author	Year	Group number	Treatment	Concentration (particle/cell number)	Volume	Delivery	Frequency	Euthanasia
Huang [34]	2019	G2	PBS	NA	100 μL	Around the border zone of infarcted heart injections to three sites	At 30 min after LAD ligation	5 weeks
		G4	MSCs	2×10^6 MSCs	NR	Tail vein injection	At 1 day, 3 days, or 7 days	5 weeks
		G5	MSCs	2×10^6 MSCs	NR	Tail vein injection	At 1 day, 3 days, or 7 days	5 weeks
		G6	MSCs	2×10^6 MSCs	NR	Tail vein injection	At 1 day, 3 days, or 7 days	5 weeks
Li [35]	2019	G2	PBS	NA	200 μL	Around the border zone of infarcted heart injections at five points	At 30 min after LAD ligation	4 weeks
Xu [36]	2019	G2	PBS	NA	50 μL	Around the infarct border zone injections at four sites	Immediately after LAD ligation	At days 3 and 7
Zhang [37]	2019	G2	NR	NA	NR	NR	NR	At 28 days
Huang [38]	2020	G2	PBS	NA	100 μL	Around the border zone of infarcted heart injections to three sites	At 30 min after LAD ligation	At days 3, 7, and 28
Li [39]	2020	G2	NR	NA	NR	NR	NR	NR
Sun [40]	2020	G2	PBS	NA	50 μL	Around the infarcted region injection	NR	4 weeks
Liu [41]	2020	G2	PBS	NA	30 μL	Around the border zone of infarcted heart injections at four sites	Following ligation	4 weeks
Sun [42]	2020	G1	PBS	NA	500 μL	Tail vein injection	After MI surgery	At 28 days
Cheng [43]	2020	G2	PBS	NA	20 μL	Cardiac muscle close to the area of arterial ligation injection	NR	7 days or 4 weeks
Fu [44]	2020	G2	PBS	NA	50 μL	Ischemic myocardium injection	NR	At 7 days
Wang [45]	2020	G2	PBS	NA	NA	NR	NR	3 weeks

G, group; *LAD*, left anterior descending artery; *Mecp2*, methyl CpG–binding protein 2; *MI*, myocardial infarction; *MSCs*, mesenchymal stem cells; *NA*, not applicable; *NR*, not reported; *PBS*, phosphate-buffered saline

et al. assessed neovascularization by examining the expression of von Willebrand factor (vWF) and found that CR4-overexpressing MSC-Exos treatment significantly increased numbers of vWF-positive vessels relative to control treatment [23]. Three additional studies assessed angiogenesis in the ischemic border zone using lectin as an immunofluorescent tool for marking endothelial cells [24–26]. Sun et al. observed enhanced angiogenesis in animals treated with exosomes derived from HIF-1α-overexpressing MSCs relative to those from control MSCs, suggesting that HIF-1α was able to promote angiogenesis and thereby exert cardioprotective activity [24]. Teng et al. also analyzed rats treated with MSC-Exos and observed significant increases in the numbers of new capillaries relative to those in animals treated with PBS or exosome-depleted culture media [25]. Zhang et al. also showed that animals treated with cardiac stem cells (CSCs) that had been MSC-Exos preconditioned exhibited increased numbers of arterioles and capillaries,

Table 5 Summary of therapeutic outcomes

Author	Year	Key Outcomes	
		In vitro	In vivo
Feng [22]	2014	NA	Exosomes isolated from MSCs subjected to ischemic preconditioning suppressed cardiac fibrosis through the miR-22-mediated suppression of Mecp2
Kang [23]	2015	Exo^{CR4} significantly increased IGF-1α and pAkt levels and suppressed caspase-3 activation within cardiomyocytes, in addition to enhancing the expression of VEGF and the formation of vessels	Exo^{CR4}-treated MSC-sheet implantation improved the restoration of cardiac function via increasing angiogenesis, reducing infarct size, and augmenting cardiac remodeling
Yu [24]	2015	MSC-Exos increased cardiomyocyte resistance to hypoxic injury. miR-19a transferred by $Exo^{GATA\text{-}4}$ suppressed BIM and PTEN expression in cardiomyocytes and thereby inactivated the Erk and Akt pathways	MSC-Exos enhanced cardiac function and decreased infarct size. Exo^{GATA-4} enhanced cardioprotection
Teng [25]	2015	MSC-Exos treatment enhanced HUVEC tube formation and suppressed T cell proliferation and function	MSC-Exos treatment decreased infarct size and improved cardiac systolic and diastolic performance relative to PBS treatment in a rat MI model, in addition to enhancing new capillary density and blood flow recovery
Zhang [26]	2016	MSC-Exos treatment enhanced CSCsproliferation, migration, and tube formation in a dose-dependent fashion	MSC-Exos–preconditioned CSCs exhibited enhanced survival, better capillary density, decreased cardiac fibrosis, and better long-term restoration of cardiac function
Shao [27]	2017	MSC-Exos treatment enhanced the proliferation of H9c2 cells while suppressing their H_2O_2-induced apoptotic death and inhibiting TGF-β induced fibroblast transformation into myofibroblasts	MSC-Exos treatment improved myocardial function, suppressed fibrosis, and inhibited inflammation better than MSCs
He [28]	2018	Exosomes from MSCs and MSCs transduced with GATA-4 exhibited anti-apoptotic activity, also induced cardiomyocyte proliferation	Exosomes derived from MSCs overexpressing GATA-4 enhanced cardiac function owing to increases in cardiac blood vessel density and numbers of c-kit-positive cells, and also reduced numbers of apoptotic cardiomyocytes
Zhu [29]	2018	Hypo-Exos-derived miR-125b-5p exhibited enhanced anti-apoptotic effects when used to treat ischemic cardiomyocytes owing to its ability to suppress p53 and BAK1	Hypo-Exos treatment following MI was linked to reductions in infarct size and improved LV function. Hypo-Exos with a covalently attached IMT peptide significantly enhanced Exo targeting to ischemic cardiac tissues and better suppressed cardiomyocyte apoptosis following MI
Ma [30]	2018	miR-132-loaded MSC-Exos significantly enhanced endothelial cell tube formation	miR-132-loaded MSC-Exos administration in the myocardium of MI model mice enhanced peri-infarct neovascularization and preserved cardiac function
Zhu [31]	2018	Exo^{H} enhanced the pro-angiogenesis and anti-apoptosis in HUVECs and cardiomyocytes	Exo^{H} treatment was associated with improved vascular density, reduced cardiomyocyte apoptosis, decreased fibrosis, and improved cardiac progenitor cell recruitment to infarcted heart tissues relative to Exo^{N}
Xiao [32]	2018	Exosomes derived from MSCs overexpressing miR-125b-5p decreased cardiomyocyte autophagic flux via the regulation of p53/Bnip3 autophagic signaling	MSC-Exos treatment decreased autophagic flux following injection into infarcted cardiac tissues in a miR125b-5p-dependent manner
Zou [33]	2019	MSC-Exos inhibited migration, proliferation, and cardiomyocyte apoptosis during H/R by altering the expression of apoptosis and autophagy-associated genes including Apaf1 and ATG13	MSC-Exos treatment inhibited MI-related myocardial injury, potentially via regulating autophagy
Huang [34]	2019	MSC-Exos treatment significantly enhanced MSC survival under conditions of hypoxia	Relative to control animals injected with only exosomes or MSCs, the sequential injection of MSC-Exos into the myocardium followed by MSC transplantation better improved cardiac function, reduced infarct size, and increased neovascularization
Li [35]	2019	NA	MSC-Exos from MSCs overexpressing miR-301 markedly enhanced cardiac function and reduced the infarcted area in MI model rats through the regulation of myocardial autophagy

Table 5 (continued)

Author	Year	Key Outcomes	
		In vitro	In vivo
Xu [36]	2019	L-Exo increased the level of anti-inflammatory cytokines, enhanced M2 macrophage polarization, inhibited LPS-dependent NF-κB signaling pathway, and promoted AKT1/AKT2 pathway activation under LPS-stimulated conditions	LPS pre-conditioned MSC-Exos were more effective in the attenuation of post-MI inflammation and cardiomyocyte apoptosis owing to their ability to modulate macrophage polarization in a murine MI model
Zhang [37]	2019	Hypoxia-preconditioned MSC-Exos suppressed H9c2 cell apoptosis via the upregulation of miR-24	Hypoxia-preconditioned MSC-Exos decreased the infarcted area, enhanced myocardial function, and suppressed apoptotic cardiomyocyte death after MI
Huang [38]	2020	ATV-pretreated MSC-derived exosomes accelerated endothelial but not cardiomyocyte migration, survival, and tube formation. MSC^{ATV}-Exo-treated endothelial cells protected cardiomyocytes from H/SD-induced apoptotic death	ATV-pretreated MSC-derived exosomes improved cardiac functional recovery, reduced infarct size, and prevented cardiomyocyte apoptosis. MSC^{ATV}-Exo treatment was linked to increased angiogenesis, and to reductions in peri-infarct IL-6 and TNF-α levels. From a mechanistic perspective, MSC^{ATV}-Exo-derived lncRNA H19 was shown to regulate miR-675 and to thereby activate VEGF and ICAM-1 to drive angiogenesis
Li [39]	2020	NA	Exosomes derived from MSCs overexpressing miR-185 enhanced myocardial function, suppressed myocardial injury in MI model mice, and prevented apoptosis in cardiomyocytes by suppressing the expression of SOCS2
Sun [40]	2020	MSC-Exos from young donors were better able to suppress fibrosis, enhance tube formation, and prevent in vitro cardiomyocyte apoptosis relative to those from older donors. Levels of miR-221-3p produced by MSCs from older donors suppressed cardiomyocyte angiogenesis and enhanced cardiomyocyte survival. Exosomal miR-221-3p secreted from older MSCs enhanced HUVEC migration, proliferation, and angiogenesis and suppressed H9c2 cell apoptosis through the PTEN/Akt pathway	MSC-Exos from young donors were better able to enhance cardiac structure and function in MI model rats relative to those from older donors. Exosomal miR-221-3p from older MSCs suppressed angiogenesis and enhanced cardiomyocyte survival in this model
Liu [41]	2020	MIF-MSC-Exos were superior to MSC-Exos for the attenuation of H/SD-induced cardiomyocyte injury through AMPK regulation	Relative to MSC-Exos, MIF-MSC-Exos were linked to improved cardiac function, reduced remodeling, the suppression of cardiomyocyte mitochondrial fragmentation, and decreases in both reactive oxygen species generation and apoptosis
Sun [42]	2020	HIF-1α-overexpressing exosomes enhanced the proliferation, migration, and angiogenic activity of hypoxia-injured HUVECs	HIF-1α-overexpressing exosomes preserved cardiac function via the promotion of neovascularization and the inhibition of fibrosis in a rat MI model
Cheng [43]	2020	miR-210 overexpression in MSC-Exos enhanced their ability to protect cardiomyocytes via targeting AIFM3 and regulating PI3K/AKT and p53 signaling	Exosomes derived from MSCs overexpressing miR-210 suppressed apoptotic death while enhancing cardiac function and decreasing post-MI infarct size
Fu [44]	2020	MSC-Exos overexpressing miR-338 suppressed apoptotic death induced by H_2O_2 in H9c2 via the regulation of the MAP3K2/JNK pathway	MSC-Exos overexpressing miR-338 enhanced cardiac functionality in a rat MI model system
Wang [45]	2020	MSC-Exos loaded with miR-19a/19b improved the ability of cardiac cells to survive under hypoxia	Exo/miR-19a/19b administration, together with MSC transplantation, markedly improved cardiac functional recovery and decreased cardiac fibrosis in an MI model significantly enhanced the recovery of cardiac function and reduced cardiac fibrosis in the MI model

AIFM3, apoptosis-inducing factor, mitochondrion-associated 3; AMPK, adenosine 5'-monophosphate-activated protein kinase; Apaf1, apoptotic protease activating factor-1; ATG13, autophagy-related protein 13; ATV, atorvastatin; Bnip3, Bcl-2 19-kDa interacting protein 3; CSCs, cardiac stem cells; Erk, extracellular signal-regulated kinase; Exo, exosome; ExoH, exosomes from hypoxia-treated MSCs; ExoN, exosomes from normoxia-treated MSCs; H2O2, hydrogen peroxide; HIF-1α, hypoxia-inducible factor 1-alpha; H/R, hypoxia-reoxygenation; H/SD, hypoxia and serum deprivation; HUVEC, human umbilical vein endothelial cells; Hypo, hypoxia; ICAM-1, intercellular adhesion molecule-1; IGF-1α, insulin-like growth factor-1α; IL-6, interleukin 6; IMT, ischemic myocardium-targeted; IPC, ischemic preconditioning; JNK, c-Jun N-terminal kinase; L-Exo, LPS-primed BMSC-derived exosomes; lncRNA H19, long non-coding RNA H19; LPS, lipopolysaccharide; LV, left ventricle; MAP3K2, mitogen-activated protein kinase kinase kinase 2; Mecp2, methyl CpG binding protein 2; MI, myocardial infarction; MIF, macrophage migration inhibitory factor; MSCs, mesenchymal stem cells; NA, not applicable; NC, negative control; NF-κB, nuclear factor-κB; nSMase2, neutral sphingomyelinase 2; PBS, phosphate-buffered saline; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog deleted on chromosome ten; SOCS2, suppressor of cytokine signaling 2; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor

suggesting enhanced neovascularization within ischemic heart tissues [26].

These in vivo results were consistent with in vitro data demonstrating that MSC-Exos had positive effects on cell proliferation, migration, and tube formation. In total, seven studies detailed positive impacts of MSC-Exos on cell proliferation, migration, and endothelial cell vessel formation [23–25, 30, 31, 38, 40]. Zhang et al. explored the impact of MSC-Exos treatment on CSC angiogenesis in vitro [26]. Together, these data indicate that MSC-Exos can enhance cell proliferation, migration, and tube formation, thereby enhancing neovascularization.

The Anti-apoptotic Activity of MSC-Exos

Suppressing the apoptotic death of myocardial cells after MI is vital to preventing widespread HF [51–53]. In total, 17 of the included studies described the effects of MSC-Exos treatment on MI-related cardiomyocyte apoptosis [22, 24, 28, 29, 31–34, 36–41, 43–45]. Of these, some studies favored TUNEL staining [22, 28, 29, 31–34, 36–41, 43], revealing a significant reduction in the number of apoptotic cells in the myocardial border zone following MSC-Exos treatment relative to PBS treatment.

In 13 studies, the messenger RNA (mRNA) or miRNAs present within MSC-Exos were evaluated as regulators of post-MI apoptosis [22, 24, 28, 29, 31, 32, 37, 39–41, 43–45]. Yu et al. [24] found that exosomes derived from MSCs overexpressing GATA-4 were able to effectively enhance myocardial contractile function while reducing infarct size in an MI model, with miR-19a being present at much higher levels in these exosomes. This miRNA was able to function by inhibiting multiple phosphatases. He et al. [28] also determined that GATA-4-MSC-Exos treatment suppressed the apoptotic death of hypoxic cardiomyocytes more effectively than control exosomes, with similar efficacy being observed in vivo. These results were consistent with those of Yu et al. [24], suggesting a similar mechanism of action. Feng et al. [22] also determined that ischemia-pretreated and miR-22-enriched MSC exosomes were able to suppress apoptosis in the ischemic myocardium, with the anti-apoptotic effects of miR-22 being mediated by its ability to target Mecp2 such that in a murine MI model, the administration of these MSC-Exos suppresses infarct size and enhanced myocardial fibrosis.

Consistent with these in vivo results, 11 articles [23, 24, 28, 29, 31, 33, 37, 40, 41, 43, 45] found that MSC-Exos had a positive impact on hypoxia-induced apoptotic death in cardiomyocytes. Additionally, two studies [31, 44] demonstrated the ability of MSC-Exos to inhibit H_2O_2-induced cardiomyocyte apoptosis. In these reports, MSC-Exos were able to suppress apoptosis-related gene expression, thereby protecting against cardiomyocyte injury.

The Anti-inflammatory, Anti-fibrotic, and Anti-autophagic Properties of MSC-Exos

Inflammation is a key mechanism that governs myocardial damage following MI, with secreted cytokines and chemokines additionally serving to recruit multiple immune cell types to the infarcted region [54–56]. Xu et al. [36] found that exosomes derived from MSCs treated with low levels of LPS were better able to promote M2 macrophage polarization and to attenuate post-MI inflammation and cardiomyocyte apoptosis in vitro and in vivo relative to control MSC-Exos. Consistent with these findings, Teng et al. [25] determined that MSC-Exos were able to suppress T cell proliferation in vitro and to inhibit ventricular infiltration by inflammatory cells in vivo.

Three studies explored the impact of MSC-Exos treatment on autophagic activity in the context of MI [32, 33, 35], revealing that exosome treatment was associated with increases in the expression of autophagy-related genes such as ATG13 in H9c2 cells and rats after MI modeling, with these changes coinciding with a reduction in the expression of apoptosis-related genes such as Apaf1 [33]. In other reports [32, 35], the overexpression of miR-301 or miR-125b within MSC-Exos was found to protect against MI by inhibiting autophagy.

In vitro, Shao et al. reported the capacity of MSC-Exos to inhibit the transformation of fibroblast to myofibroblast, which explains why MSC-Exos injection could reduce cardiac fibrosis after MI [27].

Discussion

MI is the most common cardiovascular disease-related cause of death [57]. As cardiomyocytes largely lack the capacity for self-renewal, many researchers have sought to leverage stems cells for cardiac tissue repair. However, stem cell transplantation efforts have exhibited only limited efficacy to date and have been hampered by the relatively limited survival of transplanted cells as well as by immune responses to transplantation [58–60]. Cell-free regenerative medicine approaches have evolved rapidly over the last decade, in part owing to the discovery that MSC-Exos are primary mediators of the beneficial paracrine signaling activity of MSCs in the context of regenerative treatment for MI patients, suggesting that these MSC-Exos may represent a viable alternative to direct MSC transplantation [61]. Importantly, these exosomes can carry substantial quantities of proteins and nucleic acid cargos that are internalized by recipient cells, thus allowing for efficient macromolecule delivery. The

present systematic review clearly demonstrates that MSC-Exos treatment holds promise as a therapeutic strategy in the context of MI, as evidenced by preclinical findings indicating that these exosomes can enhance cardiac function, suppress myocardial apoptotic cell death, inhibit inflammation, and augment neovascularization following MI. In analyzed studies, these treatment-related effects were shown to be superior to those associated with control treatments with saline solution or MSCs. Overall, these findings thus offer robust evidence for the potency of cell-free regenerative medicine as an approach to MI patient treatment.

MSC-Exos-based Therapeutic Cardiac Regeneration

Many different stem cell types have previously been explored in clinical contexts pertaining to regenerative medicine, including bone marrow mononuclear cells, bone marrow MSCs, embryonic stem cells, cardiac stem cells, and induced pluripotent stem cells [62, 63]. Bone marrow MSCs offer great promise for the treatment of myocardial ischemia owing to the ease with which they can be isolated, cultured, and expanded while maintaining important stem cell properties [64]. Researchers initially hypothesized that transplanted MSCs would be able to differentiate into cardiomyocytes and vascular endothelial cells, but more recent evidence suggests that the majority of transplanted cells die within a month under hypoxic conditions within the ischemic microenvironment, while their differentiation cannot be effectively controlled [65].

Paracrine mechanisms are now thought to be the primary mechanism whereby MSCs exert beneficial effects on target cells, with exosomes being among the most important secreted paracrine factors derived from these cells. Exosomes can interact with nearby cells or can enter into the systemic circulation and can be internalized via endocytosis or fusion with the plasma membrane of target cells, leading to the release of exosomal contents into the cytoplasm [66]. Exosomes are believed to be important mediators of cell–cell communication, and the miRNAs present within many exosomes represent a key form of information that is readily transmitted via this mechanism. Exosomes and miRNAs derived therefrom are important regulators of the function of cardiomyocytes, endothelial cells, vascular smooth muscle cells, and inflammatory cells, thus contributing to the development and progression of MI [67].

The Pro-angiogenic Effects of MSC-Exos Treatment

Exosomes can carry proteins, miRNAs, and other molecules capable of promoting neovascularization after MI. For example, miR-132, miR-221, and miR-130 have been shown to drive vascular smooth muscle cell proliferation, differentiation, migration, and angiogenesis [68–70]. These miRNAs primarily impact angiogenesis via the regulation of related signaling molecules and pathways including VEGF, HIF, PTEN, and Akt/eNOS [71]. Ma et al. found that exosomes derived from MSCs overexpressing miR-132 were better able to promote angiogenesis than were exosomes from control MSCs [30]. In contrast, miR-221-3p levels were elevated in MI patients and correlated with troponin and left ventricular systolic function [72]. Recent clinical trials of antisense drugs targeting miR-132 have been conducted in HF patients and have shown promising results, and clinical trials using miRNAs in patients with MI are expected in the near future [73].

There have been relatively few studies to date examining changes in the properties of MSC-Exos following pharmacological intervention. In one report, Huang et al. found that atorvastatin-pretreated MSC-Exos were better able to drive endothelial cell migration, tube formation, and survival relative to control MSC-Exos [38]. Studies of the relative therapeutic impact of administering both MSCs and MSC-Exos or MSCs alone have shown that the combination approach is more efficacious than MSC transplantation in isolation [34]. While the therapeutic benefits of MSC-Exos have been detected in multiple studies, the results may be specific to the particular exosome source used in a given analysis, and all of these findings remain to be replicated in independent reports.

While the exact mechanisms whereby MSC-Exos treatment enhances cardiac outcomes following MI remain to be determined, it is important to note that several of the studies in the present systematic review found that these exosomes functioned through a multi-faceted mechanism associated with the enhancement of proliferation, migration, and angiogenesis coinciding with reductions in autophagic and apoptotic activity.

The Anti-apoptotic Effects of MSC-Exos Treatment

As cardiomyocytes exhibit a very limited capacity for regeneration, MI typically results in extensive cardiomyocyte death, ventricular remodeling, HF, and even mortality in severe cases. In some reports, exosomes derived from genetically modified MSCs were shown to exhibit therapeutic efficacy in the treatment of MI [22, 24, 28, 29, 31, 32, 37, 39–41, 43–45]. Macrophage migration inhibitory factor (MIF) is an inflammatory cytokine that has been shown to activate the AMPK pathway, leading to the upregulation of SIRT1 and the downregulation of pro-apoptotic p53, thereby suppressing the apoptotic death of exposed cells [74]. For example, Liu et al. found that exosomes derived from MSCs overexpressing MIF were better able to promote post-MI myocardial repair relative to control MSC-Exos [41]. Yu et al. also found that exosomes derived from

GATA-4 overexpressing MSCs were able to enhance survival and maintain mitochondrial membrane potential values in hypoxia treated cardiomyocytes in vitro, in addition to enhancing myocardial contractile function and decreasing the infarct size in a murine MI model [24]. They further determined that these GATA-4 overexpressing MSC-Exos contained elevated levels of miR-19a, which was able to suppress many phosphatases and thereby preserve MI.

MSC-Exos containing miR-185, miR-210, miR-22, miR-338, miR-19a/19b, and miR-125b have been reported to inhibit cardiomyocyte apoptosis after MI [22, 29, 39, 43–45]. In an animal model of MI, MSC-Exos overexpressing miR-185 inhibited cardiomyocyte apoptosis by suppressing SOCS2 expression [39]; MiR-210 exerted cardioprotective effects by targeting PI3K/AKT and p53 signaling [43]. In clinical trials, miR-22 and miR-185 were reported to be increased in the supernatant after platelet aggregation and disappeared in the thrombus of patients with MI, thus demonstrating their association with thrombosis during MI [75]. In contrast, miR-210 was reported to have contributed to the growth of atherosclerosis and plaque instability [76]. MiR-19b expression was upregulated in plasma from patients with ST-segment elevation MI, while miR-19b expression peaked earlier than troponin in a study of 280 patients, suggesting a possible use as a diagnostic biomarker for MI with ST-segment elevation [77].

Potential Confounders and Limitations

While the above studies highlight the therapeutic promise of MSC-Exos treatment for MI-related tissue injuries, much of this research is in its early stages, and its clinical relevance remains to be defined. All included studies were conducted using rat or mouse models, consistent with a need for further progress focused on large animal studies and subsequent clinical trials.

Many exosome-based phase I/II clinical trials are currently underway, particularly in antitumor therapy [78]. However, clinical trials of exosomes for the treatment of MI have not yet started. Many challenges remain before its clinical application: (1) the isolation of exosomes is complicated and standardized procedures are lacking; (2) the duration of exosome exertion in the infarcted myocardium cannot be assessed; (3) assessment of MSC- Exos in a clinical setting, such as their biodistribution, metabolism, excretion, etc., cannot be performed; (4) lack of guidelines for large-scale production and quality control; and (5) optimal dose/concentration and mode of administration of MSC-Exos remain to be determined. In addition, the safety, tolerability, and efficacy of exosomes for the treatment of MI still need to be elucidated through preclinical studies before entering clinical trials [79, 80].

Despite the formulation of guidelines aimed at standardizing the improving overall reporting pertaining to experimental animal use, such as the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines, our analysis underscores the need for improvements in the methodological reporting for many studies [81, 82]. In addition, while the included studies appeared to be methodologically sound, the SYRCLE risk of bias tool suggested all of these studies have an unclear risk of bias for most analyzed domains owing to a lack of clear documentation pertaining to many relevant parameters. As such, there is a clear need for better methodological documentation in future studies in an effort to improve the reliability and credibility of the resultant publications [81, 82].

Owing to a lack of uniformity with respect to the reporting of quantitative results, we were unable to conduct aggregate meta-analyses of the findings of these prior studies. As such, future research efforts will be essential to improve and standardize the statistical analysis and reporting approaches in related studies in order to permit more robust meta-analyses of the underlying data.

Conclusion

In summary, the results of the present systematic review suggest that MSC-Exos offer therapeutic value as a tool for the treatment or inhibition of MI-associated cardiac tissue damage. Animals in the majority of identified studies exhibited exosome treatment-related improvements in cardiac function, together with increased angiogenic activity and suppression of cardiomyocyte apoptosis. This review thus serves as a comprehensive analysis of recent preclinical animal model data on this topic and underscores the therapeutic benefit of MSC-Exos for the treatment of MI injury.

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Declarations

Ethics Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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