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REVIEW

Stem cell-derived exosomes - an emerging tool for myocardial regeneration

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Abstract

Cardiovascular diseases (CVDs) continue to represent the number one cause of death and disability in industrialized countries. The most severe form of CVD is acute myocardial infarction (AMI), a devastating disease associated with high mortality and disability. In a substantial proportion of patients who survive AMI, loss of functional cardiomyocytes as a result of ischaemic injury leads to ventricular failure, resulting in significant alteration to quality of life and increased mortality. Therefore, many attempts have been made in recent years to identify new tools for the regeneration of functional cardiomyocytes. Regenerative therapy currently represents the ultimate goal for restoring the function of damaged myocardium by stimulating the regeneration of the infarcted tissue or by providing cells

that can generate new myocardial tissue to replace the damaged tissue. Stem cells (SCs) have been proposed as a viable therapy option in these cases. However, despite the great enthusiasm at the beginning of the SC era, justified by promising initial results, this therapy has failed to demonstrate a significant benefit in large clinical trials. One interesting finding of SC studies is that exosomes released by mesenchymal SCs (MSCs) are able to enhance the viability of cardiomyocytes after ischaemia/reperfusion injury, suggesting that the beneficial effects of MSCs in the recovery of functional myocardium could be related to their capacity to secrete exosomes. Ten years ago, it was discovered that exosomes have the unique property of transferring miRNA between cells, acting as miRNA nanocarriers. Therefore, exosome-based therapy has recently been proposed as an emerging tool for cardiac regeneration as an alternative to SC therapy in the post-infarction period. This review aims to discuss the emerging role of exosomes in developing innovative therapies for cardiac regeneration as well as their potential role as candidate biomarkers or for developing new diagnostic tools.

Key words: Acute myocardial infarction; Exosome; Stem cell; Cardiac regeneration; Cardiovascular diseases

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Core tip: Regenerative therapy represents the ultimate goal for restoring the function of damaged myocardium by stimulating the regeneration of infarcted tissue. Exosomes are small microvesicles released by living cells that act as miRNA nanocarriers, and exosomes can stimulate and modulate cellular proliferation and regeneration. Elevated exosome levels have been detected in human plasma in various cardiovascular diseases. Furthermore, myocardium-derived exosomes are potentially associated with myocardial healing. Given their paracrine properties, myocardium-derived exosomes have been proposed as a potential therapeutic option for myocardial regeneration. This review discusses the emerging roles of exosomes as candidate biomarkers and innovative therapies for cardiac regeneration.

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INTRODUCTION

Cardiovascular diseases (CVDs) continue to be the number one cause of death and disability in industrialized countries. Despite many efforts to increase the rate of early diagnosis for acute coronary syndromes (ACS) and decrease associated mortality, acute myocardial infarction (AMI) is associated with mortality as high as 12%[1].

In a substantial proportion of patients who survive AMI, loss of functional cardiomyocytes as a result of ischaemic injury leads to ventricular failure, resulting in significant alteration to quality of life and increased mortality. Therefore, many attempts have been made in recent years to identify new tools to regenerate functional cardiomyocytes.

Regenerative therapy represents the ultimate goal for restoring the function of damaged myocardium by stimulating the regeneration of the infarcted tissue or providing cells that can generate new myocardial tissue to replace the damaged tissue. Stem cells (SCs) have been proposed to represent a viable therapy option in these cases. However, despite the great enthusiasm at the beginning of the SC era, justified by promising initial results, this therapy has failed to demonstrate a significant benefit in large clinical trials[2]. This lack of significant clinical benefit was initially attributed to the different origins of the SCs and the different routes of delivery used in clinical trials[3-8].

One interesting finding of the SC studies was that exosomes released by mesenchymal SCs (MSCs) are able to enhance the viability of cardiomyocytes after ischaemia/reperfusion injury, suggesting that the beneficial effects of MSCs in the recovery of functional myocardium could be related to their capacity to secrete exosomes[9]. Therefore, exosome-based therapy has recently been proposed as an emerging tool for cardiac regeneration as an alternative to SC therapy in the post-infarction period.

The role of exosome vesicles in different cardiovascular applications was discovered several decades ago. However, major interest in exosomes began in 2007, when it was discovered that they have the unique property of transferring miRNA between cells and acting as miRNA nanocarriers[10].

This review aims to discuss the emerging role of exosomes in developing innovative therapies for cardiac regeneration, as well as their potential role as candidate biomarkers for developing new diagnostic tools.

EXOSOMES - DEFINITION AND ROLES

Exosomes are nanosized vesicles (30-150 nm diameter) of endosomal origin that are released by various cells and contain proteins, lipids, and genetic material[11]. Exosomes are present in enormous quantities in the blood, estimated to be 10^{10}/mL of plasma in healthy individuals[12,13].

It has been demonstrated that living cells are able to secrete vesicles of different sizes and intracellular origins. The main types of cell-generated vesicles are exosomes (diameter between 30 and 150 nm), microvesicles (diameter range 50-1000 nm) and apoptosomes (diameter range 50-5000 nm). The main differences between these populations of vesicles are

not only their diameter but also their mechanism of generation. While exosomes are generated by internal budding of plasma membranes, microvesicles arise from direct budding of injured cell plasma membranes, and apoptosomes originate as fragments of cells undergoing programmed death[14].

Exosomes result from inward budding of cell membrane ligands, a process associated with internalization of extracellular membrane ligands to the surface of the small vesicles generated by inward budding. This inward budding allows the internalization of small proteins, mRNAs, miRNA and DNA into the exosomes[15]. In the next stage, these small bodies are fused with the cell membrane and released through an exocytotic process, carrying various molecules, proteins, mRNAs, ncRNAs and enzymes[16]. After the exosomes are released into the circulation, they migrate to recipient cells. Once the exosomes are absorbed by the recipient, the molecules and RNA carried by the exosomes from the parent cells are transferred to the recipient cells. From the entire spectrum of microvesicles generated by living cells, exosomes are the category richest in miRNAs, thus representing an ideal nanocarrier for transferring miRNA molecules to target tissues.

Exosomes as intercellular communication messengers

Exosomes are able to transfer activated receptors to recipient cells and act as transfer molecules, generating signalling pathways[14]. They have the ability to transmit functional signals between cells (such as miRNAs), which are involved in various pathophysiological processes related to atheromatous plaque instability and ischaemic injury[10].

One fundamental property of exosomes is their ability to transfer non-coding RNA (including miRNA and lncRNA) from the parent cells to the recipient cells, thereby modulating the phenotype and protein expression of recipient cells[16]. As a result, exosome-mediated intercellular communication has been demonstrated to play a substantial role in two major mechanisms involved in acute cardiovascular events: (1) ensuring vascular integrity to prevent atheromatous plaque progression and rupture; and (2) ensuring a significant level of cardioprotection following AMI.

SOURCES OF EXOSOMES WITH POTENTIAL APPLICATIONS IN MYOCARDIAL REGENERATION

Cardiomyocytes are able to generate exosomes functionalized with heat shock protein 70 (HSP70) and heat shock protein 90 (HSP90) at their surface, while cardiac fibroblasts are able to secrete exosomes stimulating an giotensin Ⅱ production, thus promoting cardiomyocyte hypertrophy[17]. At the same time, exosomes obtained from healthy controls have been shown to exert a cardioprotective action on ischaemic myocardium from patients with coronary artery disease by releasing cardioprotective HSP70 and other protective signals. Therefore, there is a potential therapeutic role of these promising microparticles in clinical applications[13]. However, endothelial cells may be the most relevant source of exosomes under ischaemic conditions. It has been demonstrated that endothelial cell-derived exosomes express increased levels of intercellular adhesion molecules (ICAM-1 or VCAM-1), which are involved in the complex mechanisms of coronary atheromatous plaque vulnerabilisation[14].

SCs-derived exosomes

Different populations of SCs are able to generate exosomes that will serve as transfer mediators. The potential sources of SC-derived cardioprotective exosomes include MSCs, cardiac stem cells (CSCs), embryonic SCs, haematopoietic SCs, cardiosphere-derived SCs and plasma.

MSCs appear to have relevant immunosuppressive properties; therefore, MSC-generated exosomes may play a role in immune-mediated responses with immunosuppressive properties[18]. More than 700 proteins have been identified in the proteome of MSc exosomes[11,19], and these proteins are involved in the stimulation of vascular endothelial growth factor (VEGF) and hepatocyte-growth factor (HGF).

Arslan *et al*[20] demonstrated that injection of MSc-derived exosomes can decrease infarct size by 45% and reduce systemic inflammation. At the same time, intramyocardial infusion of MSc-derived exosomes improved contractility of cardiomyocytes and reduced infarct size in a rat model of AMI[21], demonstrating that exosomes definitely play a cardioprotective role by preventing cardiac remodelling during the post-AMI period.

CSC-derived exosomes have been isolated from the right atrial appendage of patients undergoing bypass surgery and have shown an increased capacity to stimulate endothelial tube formation[22]. Under hypoxic conditions, antifibrotic miRNA-enriched exosomes are transferred from cardiac progenitor cells to fibroblasts, thereby decreasing cardiac fibrosis and apoptosis and increasing angiogenesis[22,23]. Embryonic SC-derived exosomes have also been demonstrated to induce neovascularization, increase cardiomyocyte survival, and reduce fibrosis during the post-infarction period[24]. Haematopoietic SC-derived exosomes were demonstrated to increase tube formation as well as endothelial cell viability and proliferation[11,25].

Cardiosphere-derived exosomes can have a delayed protective effect on cardiomyocytes as a result of their action on cardiac macrophages. This induces a specific cardioprotective phenotype at this level[26] and stimulates both angiogenesis and proliferation of cardiomyocytes[27]. Interestingly, human cardiosphere-derived exosomes have been shown to reduce infarct size after intramyocardial administration, but without any

significant benefit following intracoronary administration.

Isolation and purification of exosomes

Exosomes can be isolated from various cell cultures, such as cells from haematologic origin (B-, T-lymphocytes, mast cells, dendritic cells and platelets), colorectal cells, tumour cells, neurons and body fluids (blood, urine, bronchial lavage, breast milk, sperm, ascites and synovial fluid). The challenge of obtaining high yields of pure exosomes arises from the fact that the cultures are frequently contaminated by shedding microvesicles (SMVs) and apoptotic blebs (ABs). A comparative analysis of studies that have investigated exosomes has proven to be difficult due to the various purification techniques that were implemented. Contamination can be avoided by proper isolation and purification procedures. Exosomes exhibit smaller sizes (30-150 nm diameter *vs* 100-1000 nm for SMV and 50-500 nm for AB), different densities (1.10-1.21 g/mL *vs* 1.16-1.28 g/mL) and cell type-specific proteins. Based on these biophysical properties, pure exosomes can be obtained using differential centrifugation with membrane filters, rate zonal centrifugation and immunoaffinity capture with magnetic beads using specific antibodies/proteins[28,29].

EMERGING ROLE OF EXOSOMES IN CVD

It has been demonstrated that exosomes have beneficial effects on injured hearts, protecting cardiomyocytes in both acute and chronic models of ischaemia or in acute ischaemia/reperfusion injury[11]. Their beneficial effects have been related to a significant decrease in infarct size, reduction of fibrosis and associated remodelling, stimulation of angiogenesis and alteration of immune function[11].

Exosomes as a source of biomarkers in CVD

The potential of exosomes to serve as reliable biomarkers for CV diseases relies on their ability to incorporate miRNAs, RNAs, proteins and lipids for various clinical conditions. Bioinformatics tools are currently able to differentiate the composition of a large number of miRNAs. As a result, specific mRNAs/miRNAs have been discovered in exosomes isolated from patients with AMI or with atheromatous plaques. Patients with CAD exhibit increased levels of circulating exosomes, especially a subpopulation rich in miR-199a and miR-126, thus showing a great potential to serve as biomarkers for CAD[30]. At the same time, elevated levels of miR-1 and miR-133 have been identified in the serum of patients with acute coronary syndromes and have been shown to correlate well with troponin values[31]. Several studies have demonstrated increased levels of miR-1 and miR-133 in the peripheral circulation of patients with various types of ACS, including unstable angina, AMI or Takotsubo cardiomyopathy[31], while patients with troponin-positive ACS exhibited increased levels of miR-133a and miR-499[32]. However, very few studies have attempted to validate the role of exosomes as biomarkers in coronary artery disease (CAD).

Cardiomyocytes produce a large number of miRNAs. From these, four types are specifically related to AMI - miRNA-1, miRNA-133a and b, miRNA-208a and miRNA-499. During AMI, these miRNAs rapidly increase in the peripheral blood up to 3000-fold compared to healthy individuals, indicating myocardial damage. Therefore, such a panel of miRNA biomarkers can serve as reliable markers of myocardial necrosis with a higher specificity than traditional biomarkers. Furthermore, their elevation occurs much earlier than the increase in troponin, thus representing a promising tool for an immediate and accurate diagnosis of AMI.

It has also been demonstrated that in patients with ACS, injured cardiomyocyte-released exosomes are rich in cardiac-specific miRNAs, such as miRNA-1, miRNA-208 and miRNA-133. At the same time, miRNA-133 present in exosomes can serve as a reliable biomarker for myocardial damage in AMI[16]. Elevated serum levels of exosome-derived miR-208a were correlated with deterioration of the hemodynamic status, as expressed by an increase in the Killip class (class Ⅰ: no evidence of heart failure, class Ⅱ: mild to moderate heart failure, with rales less half way up the lung fields, class Ⅲ: pulmonary oedema, and class Ⅳ: cardiogenic shock) and reduced survival in AMI patients[33]. Interestingly, in patients with AMI, various miRNAs inside exosomes have been associated with the occurrence of heart failure (HF) during the post-infarction period. Matsumoto *et al*[34] showed that exosomal-derived miRNA-192, miRNA-194 and miRNA-34a were significantly increased in patients with AMI who developed HF and ventricular remodelling.

Exosomes as therapeutic tools in CVD

The use of exosomes as therapeutic tools is based on the premise that the use of paracrine mediators of SCs could be more effective than the use of whole SCs. It has been demonstrated that only a small proportion of injected SCs are retained at the site of infusion and that cell engraftment is rare. This observation raises serious doubts about the capability of the SCs to act as a reliable regeneration tool and led to a hypothesis about the paracrine-mediated effects of the SCs. However, reliable *in vivo* tracing of exosomes is not currently feasible, and it is difficult to explain why the paracrine factor (exosome) would be more effective than the parent cell[11]. Therefore, a new hypothesis could rely on the capacity of exosomes to reprogram immune cells to confer a cardioprotective effect.

MSC-derived exosomes recapitulate the properties of their parent cells in terms of immunomodulation and cardioprotection[35,36]. The advantages of using exosomes instead of SC therapy for myocardial regeneration are several. First, this new therapy can provide active molecules, such as mRNA, miRNA and proteins, to target cells, and these molecules can be modified by source cell manipulation or by external means. Second, this source of therapy is associated with very low immunogenicity.

However, the disadvantages of this approach are the very labourious and inefficient isolation techniques as well as the exosomes' short-term use and inability to regenerate[37].

A promising application of exosomes is represented by their potential to act as vehicles for the delivery of specific miRNAs to target tissues. The therapeutic effect of SC-derived exosomes has been attributed to the delivery of specific microRNAs, such as miR-146a, miR-22, miR-21, miR-126 or miR-210, to the ischaemic myocardium[38]. It has been shown that treatment with MSC-derived exosomes significantly changed the miRNA expression profile in CSCs, suggesting that the miRNAs play a major role in mediating the beneficial effects of MSC-derived exosomes[39]. The fact that MSC-derived exosomes have a therapeutic effect that is superior to that of MSCs can also be explained by the increased expression of several miRNAs, such as miR-15 and miR-21, in MSC-derived exosomes compared to their expression in MSCs[40]. Similarly, a significant enrichment of mi-294 in ESC-derived exosomes compared to the level in ESCs was recorded, suggesting that the beneficial effects of exosomes can be attributed to the increased delivery of miR-294 to cardiac cells[41].

Another use for exosomes in cardiovascular applications is related to the treatment of SCs with exosomes. In a recent study, miR-133 transfection of MSCs improved cardiac function in a rat model of myocardial infarction[42]. CSC pretreatment with exosomes showed upregulation of miR-147 and miR-503-3p and downregulation of miR-207, miR-326-5p and miR-702-5p, leading to improved cardiac function and increased vessel density at the site of infarction[43,44]. Additionally, Zhang *et al*[39] demonstrated that pretreatment of CSCs with MSC-derived exosomes stimulated proliferation, migration and tube formation of CSCs in a rat model of myocardial infarction. This pretreatment was also associated with improved survival, enhanced capillary density and reduced cardiac fibrosis[39].

Exosomes as drug delivery carriers

Exosomes can be modified to become an effective delivery tool for transferring bioactive molecules to specific cells[45]. They have been demonstrated to represent effective targeted drug delivery systems. Personalized exosome-mimetic nanovesicles could represent a promising emerging application in the future as a novel drug delivery system.

An emerging therapeutic field of exosome-based therapy is nanotherapy. This new field of exosome-related treatment is based on the incorporation of miRNA into exosomes to deliver miRNAs to recipient tissues for their cardioprotective effect or for the reduction of inflammation and atheromatous plaque formation.

Exosomes can be used as nanoparticles for targeted delivery of miRNAs to promote angiogenesis and myocardial regeneration. Interestingly, exosomes have been proposed to serve as an efficient nanocarrier for transporting protein regulators such as Shh protein regulators, morphogenic proteins involved in cardioprotection and in promoting neovascularization in the post-MI heart, with significant anti-apoptotic and vasculoprotective properties[46].

The main approaches proposed thus far for using exosomes as nanocarriers include loading exosomes isolated from parental cells with different drugs, loading parental cells with drugs that will be released into the exosomes, or transfecting parental cells with active compounds to be released into the exosomes[47]. However, none of these approaches has so far been validated in clinical trials.

EXOSOMES AND ATHEROSCLEROSIS

Exosomes, inflammation and atheromatous plaque progression

Atherosclerosis is associated with augmented systemic inflammation, the release of inflammatory cytokines, increased oxidative stress and endothelial cell activation. It is well known that cardiomyocytes and endothelial cells interact with each other *via* exosome-mediated transfers. MiR-223 secreted by activated macrophages and included in the exosomes released by these macrophages is involved in the inflammatory response associated with atherosclerosis development. Some recent data suggest that exosomes containing the HSP70 protein may be involved in the migration of monocytes in the subendothelial space[16]. At the same time, exosomes released by cells associated with atheromatous plaques stimulate the expression of adhesion molecules (ICAM and VCAM) and trigger local inflammation[48].

The role of exosomes in CAD is related to their effect on inflammation, thrombosis, neoangiogenesis and cell survival. They can also promote the adhesion of monocytes to the endothelium, increase the endothelial expression of adhesion molecules and increase the expression of adhesion molecule receptors in monocytes[30]. miRNA-222, which is present in exosomes, can also regulate ICAM-1 expression[49]. Interestingly, exosomes from atheromatous plaques can also transfer ICAM-1 directly to recipient cells[48], favouring early atherosclerotic processes.

Shear stress has been shown to represent a vulnerability factor associated with atheromatous plaque progression. Some reports shown the role of increased sub-endothelial stress in determining particular types of acute coronary syndromes[50]. It has been demonstrated that exosomes containing miR-143/145 are increased in cells exposed to increased shear stress[51-53]. Activation of Kruppel-like factor 2, which is largely dependent on the level of shear stress, can lead to the release of exosomes containing miR-143 and miR-145, which inhibit smooth muscle cell de-differentiation and thus support a potential cardioprotective effect[30,54].

At the same time, platelet-derived exosomes may make a substantial contribution to the atherosclerotic

process by prompting pro-inflammatory activation of endothelial smooth muscle cells[55]. Alternately, exosomes released by monocytes activate macrophages, and endothelial cells favour the progression of atherogenesis[56]. Interestingly, platelet-derived exosomes have both protective and detrimental effects. The protective effects result from their capacity to stimulate angiogenesis, while their detrimental effects are related to their pro-thrombotic activity.

Exosomes are also involved in the development of arterial calcification. It has been demonstrated that vascular smooth muscle releases exosomes that promote vascular calcification. Additionally, the injection of exosomes into apoE- mice was associated with a reduction in atherosclerotic lesion development in the aorta[57].

Exosomes and atheromatous plaque vulnerability

In the case of atheromatous plaque rupture, the contents of the vascular wall are exposed to procoagulant components of the blood, thereby leading to thrombotic occlusion of the vessels. The mechanisms which exosomes act in vulnerable plaques (VP) are various. First, VPs contain a large number of microvesicles with advanced procoagulant properties, especially at the level of the necrotic core. VPs are characterized by a large amount of low density cholesterol and a thin fibrous cap[58-60]. When the fibrous cap of the VP ruptures, this procoagulant content is exposed to the components of the blood, favouring immediate thrombus formation. At the same time, microvesicles promote local inflammation at the site of the VP, which favours plaque rupture[61].

Unlike microvesicles, platelet-derived exosomes have been shown to play a major antithrombotic role and act rather as anticoagulants, inhibiting platelet aggregation in a murine model of carotid artery injury[62]. Platelet-derived exosomal miR-320 demonstrated a clear atheroprotective effect by reducing endothelial expression of adhesion molecules such as ICAM-1, reducing inflammation, and inhibiting thrombus formation[63]. This is consistent with the conclusions of a pilot study suggesting that SCs could play a protective role in the vascular endothelium by reducing atherosclerosis progression and calcium accumulation in coronary arteries[64].

EXOSOMES AS EMERGING TOOLS FOR POST-MYOCARDIAL INFARCTION REGENERATION

Exosomes and cardioprotection

In the case of AMI, reperfusion of an occluded coronary vessel can lead to reperfusion injury, which adds to the initial injury caused by the abrupt occlusion. Therefore, the reducing reperfusion injury is crucial for improving the long-term evolution of AMI survivors.

The acute cardioprotective effects of exosomes were demonstrated in 2010, following the observation that exosomes injected into mice suffering a 30 min ischaemia led to a significant reduction of infarct size within 20 h[37]. However, exosomes have been demonstrated to play a significant cardioprotective role in models of continuous ischaemia without reperfusion. In a study by Zhao *et al*[65], after ligation of the left anterior descending artery in rats, injection of exosomes was associated with a significant improvement in systolic function at 4 wk, concomitant with a significant reduction in cardiac fibrosis and apoptosis.

Plasma exosomes originating from various cells demonstrated significant cardioprotective effects in the post-AMI period, reducing infarct size after intravenous administration. At the same time, the release of cytoprotective HSP70 and HSP90 from exosomes has been identified in mouse cardiomyocytes[66]. HSP70 present at the surface of plasma exosomes stimulates the activation of several cardioprotective pathways[13].

The effects of exosomes on ischaemic hearts can be mediated through various types of receptor cells. In macrophages and other cells, exosomes are involved in immunosuppression mechanisms. Alternately, they stimulate angiogenesis at the level of endothelial cells, inhibition of fibrosis at the level of fibroblasts, and cardioprotection at the level of cardiomyocytes[11].

Post-myocardial infarction release of exosomes containing cardiac-specific miRNA is essential to ensure an adequate level of cardioprotection, as cardiac-specific miRNA exhibits significant protective effects: miRNA-133 has anti-apoptotic and anti-fibrotic effects; miRNA-1 has a specific anti-oxidant role; and miRNA-499 has anti-apoptotic properties[67]. In another study, microRNA analysis of CPC-derived exosomes indicated the presence of increased levels of miR-210, miR-132 and miR-146a-3p in a myocardial infarction model, inducing a sustained anti-apoptotic and pro-angiogenic response[68].

Hypoxic exosomes contain higher amounts of pro-angiogenic miRNAs, showing a more pronounced angiogenic potential[23]. Interestingly, exosomes from the pericardial fluid during the post-infarction period also exhibited cardioprotective effects by decreasing apoptosis and enhancing arteriogenesis[69].

An interesting finding was the role of exercise in further increasing the number of circulating exosomes in healthy individuals but not in patients with CAD[70]. At the same time, cardiomyocyte-derived exosomes from exercised mice expressed higher levels of miR-29b and miR-455 compared to sedentary ones, and these miRNAs had the capacity to downregulate matrix-metalloprotease 9 and reduce cardiac fibrosis[71]. Thus, we can conclude that in the post-MI period, cardiac cells release exosomes with augmented cardioprotective effects to promote myocardial regeneration.

Exosomes as myocardial regenerative tools

The efforts to regenerate myocardium *via* injecting various types of SCs into the myocardium or into the infarct-

affected coronary arteries did not lead to significant evidence of their potential to generate new myocardium. However, the benefits of SCs have been attributed to their paracrine effects, which could be mediated by exosomes[64,72]. Following the observation that the SCs remain at the site of injection release factors mediating this paracrine effect, exosomes have been proposed as important potential paracrine mediators for myocardial regeneration. Given their carrier capacity, exosomes exhibit the potential for delivering biologics containing proteins or small interfering RNA (siRNA). Experimental studies have demonstrated that engineered $CD34^+$ SCs were able to excrete manipulated exosomes containing a proangiogenic factor, which was delivered to infarcted mouse myocardium and led to decreased infarct size, increased angiogenesis and improved long-term regeneration[11,73].

In AMI, myocardial tissue is exposed to increased ischaemic stress signals. As a result, cardiomyocytes respond by increasing the secretion of exosomes, which has been identified in different amounts in peri-infarcted areas and in healthy myocardium. Exosomes released by the damaged myocardium transfer proteins and miRNAs that send ischaemic signals to distant tissues or organs, such as bone marrow (BM), and stimulate the production of SC from the BM. In turn, BM releases SCs and exosomes that travel back to the ischaemic myocardium to stimulate the repair process and trigger myocardial regeneration[38]. Injured myocardium exhibits a multitude of responses to injury, including necrosis, inflammation, apoptosis, remodelling and fibrosis. Paracrine effects of exosomes released by non-injured myocardium from peri-infarcted areas can reprogram cardiomyocytes and rescue the peri-infarcted region from these deleterious mechanisms. This is mediated by the specific transfer of RNAs, peptides and small molecules[74].

Several preclinical studies have demonstrated the beneficial role of SC-derived exosomes in the repair of ischaemic tissues and myocardial regeneration[27,75-77]. Therefore, exosomes can represent a new line of cell-free therapy for myocardial regeneration in AMI. However, their translation into clinical application is still far away.

Arslan *et al*[20] showed that exosome treatment in the post-MI period enhanced myocardial viability and reduced adverse ventricular remodelling by decreasing oxidative stress and activating the PI3K/Akt pathway. Lai *et al*[77] also demonstrated that the administration of MSC-derived exosomes significantly reduced infarct size in mice. Intramyocardial injection of CSC-derived exosomes in mice undergoing ischaemia-reperfusion injury led to a 53% reduction in cardiomyocyte-related apoptosis[78]. Furthermore, Barile *et al*[22] found that intramyocardial injection of CSC-derived exosomes reduced the amount of scar tissue, increased vessel density *via* angiogenic effects and significantly decreased apoptosis of cardiomyocytes.

In a study on acute myocardial ischaemic injury, Luo *et al*[79] demonstrated that exosomes derived from adipose-derived stem cells (ADSCs) overexpressing miR-126 decreased myocardial injury by reducing the expression of inflammation factors. This suggests that ADSC-derived exosomes can also protect myocardial cells from apoptosis, inflammation and fibrosis, thus preventing myocardial damage and favouring angiogenesis and myocardial repair[79]. These findings were demonstrated in both *in vitro* and in *vivo* environments; thus, the administration of miR-126–enriched exosome treatment may serve as a potential therapeutic alternative where SC therapy fails to reduce myocardial injury or promote the regeneration process after myocardial infarction.

Exosomes may also play a role in vascular regeneration. Endothelial cells, monocytes and vascular smooth muscle cells also possess the ability to secrete exosomes, which stimulate and mediate angiogenesis, vascular healing, and remodelling by promoting cell migration, adhesion, and proliferation[80]. Experimental studies also suggest that due to their autocrine and paracrine effects, exosomes are implicated in the modulation of physiological processes such as thrombus formation by binding coagulation factors. In contrast to the protective effects, vascular smooth muscle cell exosomes can play a detrimental role in vascular calcification and atherogenesis[81]. These findings open the way for therapeutic approaches targeting inhibition of exosome secretion, thus preventing excessive coagulation and vascular calcification. Inhibiting exosome secretion may be extremely challenging considering the fine line between the physiological role of exosomes in healing processes and the harmful effect in pathological conditions.

It is interesting to note that different cells release exosomes that can exhibit a dual role in CVD: On the one hand, a protective role, especially with respect to their cardioprotective properties, and on the other hand, a destructive role, with respect to their role in mediating inflammatory responses.

CONCLUSION

Exosomes offer unique opportunities for the development of new therapies, representing promising cell-free therapeutic options for myocardial repair. However, because of their ubiquitous presence and effect on both physiological and pathological processes, the role in cardiac regeneration needs further investigation to validate them as both biomarkers and as a therapeutic option. The results of recent experimental studies suggest that exosomes possess great therapeutic potential that might overcome the shortcomings of SC therapy and could open new frontiers in regenerative cardiovascular medicine; however, this hope needs to be validated by further clinical studies.

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