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

Lazar E *et al.* Exosomes in 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 1010/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 angiotensin 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 cardio-protective 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, mi-RNA-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.

**REFERENCES**

1 **Kristensen SD**, Laut KG, Fajadet J, Kaifoszova Z, Kala P, Di Mario C, Wijns W, Clemmensen P, Agladze V, Antoniades L, Alhabib KF, De Boer MJ, Claeys MJ, Deleanu D, Dudek D, Erglis A, Gilard M, Goktekin O, Guagliumi G, Gudnason T, Hansen KW, Huber K, James S, Janota T, Jennings S, Kajander O, Kanakakis J, Karamfiloff KK, Kedev S, Kornowski R, Ludman PF, Merkely B, Milicic D, Najafov R, Nicolini FA, Noč M, Ostojic M, Pereira H, Radovanovic D, Sabaté M, Sobhy M, Sokolov M, Studencan M, Terzic I, Wahler S, Widimsky P; European Association for Percutaneous Cardiovascular Interventions. Reperfusion therapy for ST elevation acute myocardial infarction 2010/2011: current status in 37 ESC countries. *Eur Heart J* 2014; **35**: 1957-1970 [PMID: 24419804 DOI: 10.1093/eurheartj/eht529]

2 **Gyöngyösi M**, Lukovic D, Zlabinger K, Mandic L, Winkler J, Gugerell A. Cardiac Stem Cell-based Regenerative Therapy for the Ischemic Injured Heart - a Short Update 2017. *Journal of Cardiovascular Emergencies* 2017; **3**: 81-83 [DOI: 10.1515/jce-2017-0009]

3 **Wollert KC**, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, Fichtner S, Korte T, Hornig B, Messinger D, Arseniev L, Hertenstein B, Ganser A, Drexler H. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet* 2004; **364**: 141-148 [PMID: 15246726 DOI: 10.1016/S0140-6736(04)16626-9]

4 **Leistner DM**, Fischer-Rasokat U, Honold J, Seeger FH, Schächinger V, Lehmann R, Martin H, Burck I, Urbich C, Dimmeler S, Zeiher AM, Assmus B. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI): final 5-year results suggest long-term safety and efficacy. *Clin Res Cardiol* 2011; **100**: 925-934 [PMID: 21633921 DOI: 10.1007/s00392-011-0327-y]

5 **Gyöngyösi M**, Wojakowski W, Lemarchand P, Lunde K, Tendera M, Bartunek J, Marban E, Assmus B, Henry TD, Traverse JH, Moyé LA, Sürder D, Corti R, Huikuri H, Miettinen J, Wöhrle J, Obradovic S, Roncalli J, Malliaras K, Pokushalov E, Romanov A, Kastrup J, Bergmann MW, Atsma DE, Diederichsen A, Edes I, Benedek I, Benedek T, Pejkov H, Nyolczas N, Pavo N, Bergler-Klein J, Pavo IJ, Sylven C, Berti S, Navarese EP, Maurer G; ACCRUE Investigators. Meta-Analysis of Cell-based CaRdiac stUdiEs (ACCRUE) in patients with acute myocardial infarction based on individual patient data. *Circ Res* 2015; **116**: 1346-1360 [PMID: 25700037 DOI: 10.1161/CIRCRESAHA.116.304346]

6 **Pavo N**, Charwat S, Nyolczas N, Jakab A, Murlasits Z, Bergler-Klein J, Nikfardjam M, Benedek I, Benedek T, Pavo IJ, Gersh BJ, Huber K, Maurer G, Gyöngyösi M. Cell therapy for human ischemic heart diseases: critical review and summary of the clinical experiences. *J Mol Cell Cardiol* 2014; **75**: 12-24 [PMID: 24998410 DOI: 10.1016/j.yjmcc.2014.06.016]

7 **Gyöngyösi M**, Hemetsberger R, Posa A, Charwat S, Pavo N, Petnehazy O, Petrasi Z, Pavo IJ, Hemetsberger H, Benedek I, Benedek T, Benedek I Jr, Kovacs I, Kaun C, Maurer G. Hypoxia-inducible factor 1-alpha release after intracoronary versus intramyocardial stem cell therapy in myocardial infarction. *J Cardiovasc Transl Res* 2010; **3**: 114-121 [PMID: 20560024 DOI: 10.1007/s12265-009-9154-1]

8 **Gyöngyösi M**, Hemetsberger R, Wolbank S, Pichler V, Kaun C, Posa A, Petrasi Z, Petnehazy Ö, Hofer-Warbinek R, de Martin R, Gruber F, Benedek I, Benedek T, Kovacs I, Benedek I Jr, Plass CA, Charwat S, Maurer G. Delayed recovery of myocardial blood flow after intracoronary stem cell administration. *Stem Cell Rev* 2011; **7**: 616-623 [PMID: 21153508 DOI: 10.1007/s12015-010-9213-7]

9 **Feng Y**, Huang W, Wani M, Yu X, Ashraf M. Ischemic preconditioning potentiates the protective effect of stem cells through secretion of exosomes by targeting Mecp2 via miR-22. *PLoS One* 2014; **9**: e88685 [PMID: 24558412 DOI: 10.1371/journal.pone.0088685]

10 **Valadi H**, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* 2007; **9**: 654-659 [PMID: 17486113 DOI: 10.1038/ncb1596]

11 **Davidson SM**, Yellon DM. Exosomes and cardioprotection - A critical analysis. *Mol Aspects Med* 2018; **60**: 104-114 [PMID: 29122678 DOI: 10.1016/j.mam.2017.11.004]

12 **Dragovic RA**, Gardiner C, Brooks AS, Tannetta DS, Ferguson DJ, Hole P, Carr B, Redman CW, Harris AL, Dobson PJ, Harrison P, Sargent IL. Sizing and phenotyping of cellular vesicles using Nanoparticle Tracking Analysis. *Nanomedicine* 2011; **7**: 780-788 [PMID: 21601655 DOI: 10.1016/j.nano.2011.04.003]

13 **Vicencio JM**, Yellon DM, Sivaraman V, Das D, Boi-Doku C, Arjun S, Zheng Y, Riquelme JA, Kearney J, Sharma V, Multhoff G, Hall AR, Davidson SM. Plasma exosomes protect the myocardium from ischemia-reperfusion injury. *J Am Coll Cardiol* 2015; **65**: 1525-1536 [PMID: 25881934 DOI: 10.1016/j.jacc.2015.02.026]

14 **Barile L**, Moccetti T, Marbán E, Vassalli G. Roles of exosomes in cardioprotection. *Eur Heart J* 2017; **38**: 1372-1379 [PMID: 27443883 DOI: 10.1093/eurheartj/ehw304]

15 **Ottaviani L**, De Windt LJ, da Costa Martins PA. Exosomes: scytales in the damaged heart. *Ann Transl Med* 2016; **4**: 222 [PMID: 27384882 DOI: 10.21037/atm.2016.05.17]

16 **Sun HJ**, Zhu XX, Cai WW, Qiu LY. Functional roles of exosomes in cardiovascular disorders: a systematic review. *Eur Rev Med Pharmacol Sci* 2017; **21**: 5197-5206 [PMID: 29228434 DOI: 10.26355/eurrev\_201711\_13840]

17 **Bang C**, Batkai S, Dangwal S, Gupta SK, Foinquinos A, Holzmann A, Just A, Remke J, Zimmer K, Zeug A, Ponimaskin E, Schmiedl A, Yin X, Mayr M, Halder R, Fischer A, Engelhardt S, Wei Y, Schober A, Fiedler J, Thum T. Cardiac fibroblast-derived microRNA passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy. *J Clin Invest* 2014; **124**: 2136-2146 [PMID: 24743145 DOI: 10.1172/JCI70577]

18 **De Miguel MP**, Fuentes-Julián S, Blázquez-Martínez A, Pascual CY, Aller MA, Arias J, Arnalich-Montiel F. Immunosuppressive properties of mesenchymal stem cells: advances and applications. *Curr Mol Med* 2012; **12**: 574-591 [PMID: 22515979 DOI: 10.2174/156652412800619950]

19 **Kim HS**, Choi DY, Yun SJ, Choi SM, Kang JW, Jung JW, Hwang D, Kim KP, Kim DW. Proteomic analysis of microvesicles derived from human mesenchymal stem cells. *J Proteome Res* 2012; **11**: 839-849 [PMID: 22148876 DOI: 10.1021/pr200682z]

20 **Arslan F**, Lai RC, Smeets MB, Akeroyd L, Choo A, Aguor EN, Timmers L, van Rijen HV, Doevendans PA, Pasterkamp G, Lim SK, de Kleijn DP. Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. *Stem Cell Res* 2013; **10**: 301-312 [PMID: 23399448 DOI: 10.1016/j.scr.2013.01.002]

21 **Yu B**, Kim HW, Gong M, Wang J, Millard RW, Wang Y, Ashraf M, Xu M. Exosomes secreted from GATA-4 overexpressing mesenchymal stem cells serve as a reservoir of anti-apoptotic microRNAs for cardioprotection. *Int J Cardiol* 2015; **182**: 349-360 [PMID: 25590961 DOI: 10.1016/j.ijcard.2014.12.043]

22 **Barile L**, Lionetti V, Cervio E, Matteucci M, Gherghiceanu M, Popescu LM, Torre T, Siclari F, Moccetti T, Vassalli G. Extracellular vesicles from human cardiac progenitor cells inhibit cardiomyocyte apoptosis and improve cardiac function after myocardial infarction. *Cardiovasc Res* 2014; **103**: 530-541 [PMID: 25016614 DOI: 10.1093/cvr/cvu167]

23 **Gray WD**, French KM, Ghosh-Choudhary S, Maxwell JT, Brown ME, Platt MO, Searles CD, Davis ME. Identification of therapeutic covariant microRNA clusters in hypoxia-treated cardiac progenitor cell exosomes using systems biology. *Circ Res* 2015; **116**: 255-263 [PMID: 25344555 DOI: 10.1161/CIRCRESAHA.116.304360]

24 **Khan M**, Nickoloff E, Abramova T, Johnson J, Verma SK, Krishnamurthy P, Mackie AR, Vaughan E, Garikipati VN, Benedict C, Ramirez V, Lambers E, Ito A, Gao E, Misener S, Luongo T, Elrod J, Qin G, Houser SR, Koch WJ, Kishore R. Embryonic stem cell-derived exosomes promote endogenous repair mechanisms and enhance cardiac function following myocardial infarction. *Circ Res* 2015; **117**: 52-64 [PMID: 25904597 DOI: 10.1161/CIRCRESAHA.117.305990]

25 **Sahoo S**, Klychko E, Thorne T, Misener S, Schultz KM, Millay M, Ito A, Liu T, Kamide C, Agrawal H, Perlman H, Qin G, Kishore R, Losordo DW. Exosomes from human CD34(+) stem cells mediate their proangiogenic paracrine activity. *Circ Res* 2011; **109**: 724-728 [PMID: 21835908 DOI: 10.1161/CIRCRESAHA.111.253286]

26 **Kanazawa H**, Tseliou E, Malliaras K, Yee K, Dawkins JF, De Couto G, Smith RR, Kreke M, Seinfeld J, Middleton RC, Gallet R, Cheng K, Luthringer D, Valle I, Chowdhury S, Fukuda K, Makkar RR, Marbán L, Marbán E. Cellular postconditioning: allogeneic cardiosphere-derived cells reduce infarct size and attenuate microvascular obstruction when administered after reperfusion in pigs with acute myocardial infarction. *Circ Heart Fail* 2015; **8**: 322-332 [PMID: 25587096 DOI: 10.1161/CIRCHEARTFAILURE.114.001484]

27 **Ibrahim AG**, Cheng K, Marbán E. Exosomes as critical agents of cardiac regeneration triggered by cell therapy. *Stem Cell Reports* 2014; **2**: 606-619 [PMID: 24936449 DOI: 10.1016/j.stemcr.2014.04.006]

28 **Simpson RJ**, Jensen SS, Lim JW. Proteomic profiling of exosomes: current perspectives. *Proteomics* 2008; **8**: 4083-4099 [PMID: 18780348 DOI: 10.1002/pmic.200800109]

29 **Mathivanan S**, Ji H, Simpson RJ. Exosomes: extracellular organelles important in intercellular communication. *J Proteomics* 2010; **73**: 1907-1920 [PMID: 20601276 DOI: 10.1016/j.jprot.2010.06.006]

30 **Boulanger CM**, Loyer X, Rautou PE, Amabile N. Extracellular vesicles in coronary artery disease. *Nat Rev Cardiol* 2017; **14**: 259-272 [PMID: 28150804 DOI: 10.1038/nrcardio.2017.7]

31 **Kuwabara Y**, Ono K, Horie T, Nishi H, Nagao K, Kinoshita M, Watanabe S, Baba O, Kojima Y, Shizuta S, Imai M, Tamura T, Kita T, Kimura T. Increased microRNA-1 and microRNA-133a levels in serum of patients with cardiovascular disease indicate myocardial damage. *Circ Cardiovasc Genet* 2011; **4**: 446-454 [PMID: 21642241 DOI: 10.1161/CIRCGENETICS.110.958975]

32 **De Rosa S**, Fichtlscherer S, Lehmann R, Assmus B, Dimmeler S, Zeiher AM. Transcoronary concentration gradients of circulating microRNAs. *Circulation* 2011; **124**: 1936-1944 [PMID: 21969012 DOI: 10.1161/CIRCULATIONAHA.111.037572]

33 **Bei Y**, Das S, Rodosthenous RS, Holvoet P, Vanhaverbeke M, Monteiro MC, Monteiro VVS, Radosinska J, Bartekova M, Jansen F, Li Q, Rajasingh J, Xiao J. Extracellular Vesicles in Cardiovascular Theranostics. *Theranostics* 2017; **7**: 4168-4182 [PMID: 29158817 DOI: 10.7150/thno.21274]

34 **Matsumoto S**, Sakata Y, Suna S, Nakatani D, Usami M, Hara M, Kitamura T, Hamasaki T, Nanto S, Kawahara Y, Komuro I. Circulating p53-responsive microRNAs are predictive indicators of heart failure after acute myocardial infarction. *Circ Res* 2013; **113**: 322-326 [PMID: 23743335 DOI: 10.1161/CIRCRESAHA.113.301209]

35 **Baglio SR**, Pegtel DM, Baldini N. Mesenchymal stem cell secreted vesicles provide novel opportunities in (stem) cell-free therapy. *Front Physiol* 2012; **3**: 359 [PMID: 22973239 DOI: 10.3389/fphys.2012.00359]

36 **Chen TS**, Yeo RWY, Arslan F, Yin Y, Tan SS, Lai RC, Choo A, Padmanabhan J, Lee CN, de Kleijn DPV, Tan KH, Lim SK. Efficiency of exosome production correlates inversely with the development al maturity of MSC donor. *J Stem Cell Res Ther* 2013; **3**: 145 [DOI: 10.4172/2157-7633.1000145]

37 **Dougherty JA**, Mergaye M, Kumar N, Chen CA, Angelos MG, Khan M. Potential Role of Exosomes in Mending a Broken Heart: Nanoshuttles Propelling Future Clinical Therapeutics Forward. *Stem Cells Int* 2017; **2017**: 5785436 [PMID: 29163642 DOI: 10.1155/2017/5785436]

38 **Huang P**, Tian X, Li Q, Yang Y. New strategies for improving stem cell therapy in ischemic heart disease. *Heart Fail Rev* 2016; **21**: 737-752 [PMID: 27459850 DOI: 10.1007/s10741-016-9576-1]

39 **Zhang Z**, Yang J, Yan W, Li Y, Shen Z, Asahara T. Pretreatment of Cardiac Stem Cells With Exosomes Derived From Mesenchymal Stem Cells Enhances Myocardial Repair. *J Am Heart Assoc* 2016; **5**: pii: e002856 [PMID: 26811168 DOI: 10.1161/JAHA.115.002856]

40 **Shao L**, Zhang Y, Lan B, Wang J, Zhang Z, Zhang L, Xiao P, Meng Q, Geng YJ, Yu XY, Li Y. MiRNA-Sequence Indicates That Mesenchymal Stem Cells and Exosomes Have Similar Mechanism to Enhance Cardiac Repair. *Biomed Res Int* 2017; **2017**: 4150705 [PMID: 28203568 DOI: 10.1155/2017/4150705]

41 **Blin G**, Nury D, Stefanovic S, Neri T, Guillevic O, Brinon B, Bellamy V, Rücker-Martin C, Barbry P, Bel A, Bruneval P, Cowan C, Pouly J, Mitalipov S, Gouadon E, Binder P, Hagège A, Desnos M, Renaud JF, Menasché P, Pucéat M. A purified population of multipotent cardiovascular progenitors derived from primate pluripotent stem cells engrafts in postmyocardial infarcted nonhuman primates. *J Clin Invest* 2010; **120**: 1125-1139 [PMID: 20335662 DOI: 10.1172/JCI40120]

42 **Chen Y**, Zhao Y, Chen W, Xie L, Zhao ZA, Yang J, Chen Y, Lei W, Shen Z. MicroRNA-133 overexpression promotes the therapeutic efficacy of mesenchymal stem cells on acute myocardial infarction. *Stem Cell Res Ther* 2017; **8**: 268 [PMID: 29178928 DOI: 10.1186/s13287-017-0722-z]

43 **Maring JA**, Beez CM, Falk V, Seifert M, Stamm C. Myocardial Regeneration via Progenitor Cell-Derived Exosomes. *Stem Cells Int* 2017; **2017**: 7849851 [PMID: 29333167 DOI: 10.1155/2017/7849851]

44 **Chong JJ**, Yang X, Don CW, Minami E, Liu YW, Weyers JJ, Mahoney WM, Van Biber B, Cook SM, Palpant NJ, Gantz JA, Fugate JA, Muskheli V, Gough GM, Vogel KW, Astley CA, Hotchkiss CE, Baldessari A, Pabon L, Reinecke H, Gill EA, Nelson V, Kiem HP, Laflamme MA, Murry CE. Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. *Nature* 2014; **510**: 273-277 [PMID: 24776797 DOI: 10.1038/nature13233]

45 **Loyer X**, Vion AC, Tedgui A, Boulanger CM. Microvesicles as cell-cell messengers in cardiovascular diseases. *Circ Res* 2014; **114**: 345-353 [PMID: 24436430 DOI: 10.1161/CIRCRESAHA.113.300858]

46 **Soleti R**, Martinez MC. Sonic Hedgehog on microparticles and neovascularization. *Vitam Horm* 2012; **88**: 395-438 [PMID: 22391314 DOI: 10.1016/B978-0-12-394622-5.00018-3]

47 **Batrakova EV**, Kim MS. Using exosomes, naturally-equipped nanocarriers, for drug delivery. *J Control Release* 2015; **219**: 396-405 [PMID: 26241750 DOI: 10.1016/j.jconrel.2015.07.030]

48 **Rautou PE**, Leroyer AS, Ramkhelawon B, Devue C, Duflaut D, Vion AC, Nalbone G, Castier Y, Leseche G, Lehoux S, Tedgui A, Boulanger CM. Microparticles from human atherosclerotic plaques promote endothelial ICAM-1-dependent monocyte adhesion and transendothelial migration. *Circ Res* 2011; **108**: 335-343 [PMID: 21164106 DOI: 10.1161/CIRCRESAHA.110.237420]

49 **Jansen F**, Yang X, Baumann K, Przybilla D, Schmitz T, Flender A, Paul K, Alhusseiny A, Nickenig G, Werner N. Endothelial microparticles reduce ICAM-1 expression in a microRNA-222-dependent mechanism. *J Cell Mol Med* 2015; **19**: 2202-2214 [PMID: 26081516 DOI: 10.1111/jcmm.12607]

50 **Benedek I**. Atherosclerosis-Triggered Hypertension or Hypertension-Triggered Atherosclerosis? A Challenging Hypothesis. *Journal of Cardiovascular Emergencies* 2017; **3**: 5-8 [DOI: 10.1515/jce-2017-0001]

51 **Vengrenyuk Y**, Carlier S, Xanthos S, Cardoso L, Ganatos P, Virmani R, Einav S, Gilchrist L, Weinbaum S. A hypothesis for vulnerable plaque rupture due to stress-induced debonding around cellular microcalcifications in thin fibrous caps. *Proc Natl Acad Sci USA* 2006; **103**: 14678-14683 [PMID: 17003118 DOI: 10.1073/pnas.0606310103]

52 **Criqui MH**, Denenberg JO, Ix JH, McClelland RL, Wassel CL, Rifkin DE, Carr JJ, Budoff MJ, Allison MA. Calcium density of coronary artery plaque and risk of incident cardiovascular events. *JAMA* 2014; **311**: 271-278 [PMID: 24247483 DOI: 10.1001/jama.2013.282535]

53 **Martin SS**, Blaha MJ, Blankstein R, Agatston A, Rivera JJ, Virani SS, Ouyang P, Jones SR, Blumenthal RS, Budoff MJ, Nasir K. Dyslipidemia, coronary artery calcium, and incident atherosclerotic cardiovascular disease: implications for statin therapy from the multi-ethnic study of atherosclerosis. *Circulation* 2014; **129**: 77-86 [PMID: 24141324 DOI: 10.1161/CIRCULATIONAHA.113.003625]

54 **Pfeifer P**, Werner N, Jansen F. Role and Function of MicroRNAs in Extracellular Vesicles in Cardiovascular Biology. *Biomed Res Int* 2015; **2015**: 161393 [PMID: 26558258 DOI: 10.1155/2015/161393]

55 **Liu ML**, Scalia R, Mehta JL, Williams KJ. Cholesterol-induced membrane microvesicles as novel carriers of damage-associated molecular patterns: mechanisms of formation, action, and detoxification. *Arterioscler Thromb Vasc Biol* 2012; **32**: 2113-2121 [PMID: 22814745 DOI: 10.1161/ATVBAHA.112.255471]

56 **Hoyer FF**, Giesen MK, Nunes França C, Lütjohann D, Nickenig G, Werner N. Monocytic microparticles promote atherogenesis by modulating inflammatory cells in mice. *J Cell Mol Med* 2012; **16**: 2777-2788 [PMID: 22697268 DOI: 10.1111/j.1582-4934.2012.01595.x]

57 **Hergenreider E**, Heydt S, Tréguer K, Boettger T, Horrevoets AJ, Zeiher AM, Scheffer MP, Frangakis AS, Yin X, Mayr M, Braun T, Urbich C, Boon RA, Dimmeler S. Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. *Nat Cell Biol* 2012; **14**: 249-256 [PMID: 22327366 DOI: 10.1038/ncb2441]

58 **Benedek T**, Gyöngyösi M, Benedek I. Multislice computed tomographic coronary angiography for quantitative assessment of culprit lesions in acute coronary syndromes. *Can J Cardiol* 2013; **29**: 364-371 [PMID: 23333164 DOI: 10.1016/j.cjca.2012.11.004]

59 **Benedek T,** Bucur O, Pascanu I, Benedek I. Analysis of coronary plaque morphology by 64-multislice computed tomography coronary angiography and calcium scoring in patients with type 2 diabetes mellitus. *Acta Endocrinologica* 2011; **7**: 59-68 [DOI: 10.4183/aeb.2011.59]

60 **Benedek T**, Jako B, Benedek I. Plaque quantification by coronary CT and intravascular ultrasound identifies a low CT density core as a marker of plaque instability in acute coronary syndromes. *Int Heart J* 2014; **55**: 22-28 [PMID: 24463925 DOI: 10.1536/ihj.13-213]

61 **Falk E**, Nakano M, Bentzon JF, Finn AV, Virmani R. Update on acute coronary syndromes: the pathologists' view. *Eur Heart J* 2013; **34**: 719-728 [PMID: 23242196 DOI: 10.1093/eurheartj/ehs411]

62 **Srikanthan S**, Li W, Silverstein RL, McIntyre TM. Exosome poly-ubiquitin inhibits platelet activation, downregulates CD36 and inhibits pro-atherothombotic cellular functions. *J Thromb Haemost* 2014; **12**: 1906-1917 [PMID: 25163645 DOI: 10.1111/jth.12712]

63 **Gidlöf O**, van der Brug M, Ohman J, Gilje P, Olde B, Wahlestedt C, Erlinge D. Platelets activated during myocardial infarction release functional miRNA, which can be taken up by endothelial cells and regulate ICAM1 expression. *Blood* 2013; **121**: 3908-3917, S1-26 [PMID: 23493781 DOI: 10.1182/blood-2012-10-461798]

64 **Benedek I**, Bucur O, Benedek T. Intracoronary infusion of mononuclear bone marrow-derived stem cells is associated with a lower plaque burden after four years. *J Atheroscler Thromb* 2014; **21**: 217-229 [PMID: 24126180 DOI: 10.5551/jat.19745]

65 **Zhao Y**, Sun X, Cao W, Ma J, Sun L, Qian H, Zhu W, Xu W. Exosomes Derived from Human Umbilical Cord Mesenchymal Stem Cells Relieve Acute Myocardial Ischemic Injury. *Stem Cells Int* 2015; **2015**: 761643 [PMID: 26106430 DOI: 10.1155/2015/761643]

66 **Gupta S**, Knowlton AA. HSP60 trafficking in adult cardiac myocytes: role of the exosomal pathway. *Am J Physiol Heart Circ Physiol* 2007; **292**: H3052-H3056 [PMID: 17307989 DOI: 10.1152/ajpheart.01355.2006]

67 **Chistiakov DA**, Orekhov AN, Bobryshev YV. Cardiac Extracellular Vesicles in Normal and Infarcted Heart. *Int J Mol Sci* 2016; **17**: pii: E63 [PMID: 26742038 DOI: 10.3390/ijms17010063]

68 **Kishore R**, Khan M. More Than Tiny Sacks: Stem Cell Exosomes as Cell-Free Modality for Cardiac Repair. *Circ Res* 2016; **118**: 330-343 [PMID: 26838317 DOI: 10.1161/CIRCRESAHA.115.307654]

69 **Foglio E**, Puddighinu G, Fasanaro P, D'Arcangelo D, Perrone GA, Mocini D, Campanella C, Coppola L, Logozzi M, Azzarito T, Marzoli F, Fais S, Pieroni L, Marzano V, Germani A, Capogrossi MC, Russo MA, Limana F. Exosomal clusterin, identified in the pericardial fluid, improves myocardial performance following MI through epicardial activation, enhanced arteriogenesis and reduced apoptosis. *Int J Cardiol* 2015; **197**: 333-347 [PMID: 26159041 DOI: 10.1016/j.ijcard.2015.06.008]

70 **Bei Y**, Xu T, Lv D, Yu P, Xu J, Che L, Das A, Tigges J, Toxavidis V, Ghiran I, Shah R, Li Y, Zhang Y, Das S, Xiao J. Exercise-induced circulating extracellular vesicles protect against cardiac ischemia-reperfusion injury. *Basic Res Cardiol* 2017; **112**: 38 [PMID: 28534118 DOI: 10.1007/s00395-017-0628-z]

71 **Chaturvedi P**, Kalani A, Medina I, Familtseva A, Tyagi SC. Cardiosome mediated regulation of MMP9 in diabetic heart: role of mir29b and mir455 in exercise. *J Cell Mol Med* 2015; **19**: 2153-2161 [PMID: 25824442 DOI: 10.1111/jcmm.12589]

72 **Madonna R**, Van Laake LW, Davidson SM, Engel FB, Hausenloy DJ, Lecour S, Leor J, Perrino C, Schulz R, Ytrehus K, Landmesser U, Mummery CL, Janssens S, Willerson J, Eschenhagen T, Ferdinandy P, Sluijter JP. Position Paper of the European Society of Cardiology Working Group Cellular Biology of the Heart: cell-based therapies for myocardial repair and regeneration in ischemic heart disease and heart failure. *Eur Heart J* 2016; **37**: 1789-1798 [PMID: 27055812 DOI: 10.1093/eurheartj/ehw113]

73 **Yellon DM**, Davidson SM. Exosomes: nanoparticles involved in cardioprotection? *Circ Res* 2014; **114**: 325-332 [PMID: 24436428 DOI: 10.1161/CIRCRESAHA.113.300636]

74 **Walsh DG**, Kaplan LR, Burney RE, Topol EJ, O'Neill WW. Use of tissue plasminogen activator in the emergency department for acute myocardial infarction. *Ann Emerg Med* 1987; **16**: 243-247 [PMID: 2949679 DOI: 10.1161/CIRCRESAHA.118.312657]

75 **Ong SG**, Lee WH, Huang M, Dey D, Kodo K, Sanchez-Freire V, Gold JD, Wu JC. Cross talk of combined gene and cell therapy in ischemic heart disease: role of exosomal microRNA transfer. *Circulation* 2014; **130**: S60-S69 [PMID: 25200057 DOI: 10.1161/CIRCULATIONAHA.113.007917]

76 **Mackie AR**, Klyachko E, Thorne T, Schultz KM, Millay M, Ito A, Kamide CE, Liu T, Gupta R, Sahoo S, Misener S, Kishore R, Losordo DW. Sonic hedgehog-modified human CD34+ cells preserve cardiac function after acute myocardial infarction. *Circ Res* 2012; **111**: 312-321 [PMID: 22581926 DOI: 10.1161/CIRCRESAHA.112.266015]

77 **Lai RC**, Arslan F, Lee MM, Sze NS, Choo A, Chen TS, Salto-Tellez M, Timmers L, Lee CN, El Oakley RM, Pasterkamp G, de Kleijn DP, Lim SK. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. *Stem Cell Res* 2010; **4**: 214-222 [PMID: 20138817 DOI: 10.1016/j.scr.2009.12.003]

78 **Chen L**, Wang Y, Pan Y, Zhang L, Shen C, Qin G, Ashraf M, Weintraub N, Ma G, Tang Y. Cardiac progenitor-derived exosomes protect ischemic myocardium from acute ischemia/reperfusion injury. *Biochem Biophys Res Commun* 2013; **431**: 566-571 [PMID: 23318173 DOI: 10.1016/j.bbrc.2013.01.015]

79 **Luo Q**, Guo D, Liu G, Chen G, Hang M, Jin M. Exosomes from MiR-126-Overexpressing Adscs Are Therapeutic in Relieving Acute Myocardial Ischaemic Injury. *Cell Physiol Biochem* 2017; **44**: 2105-2116 [PMID: 29241208 DOI: 10.1159/000485949]

80 **Deng L**, Blanco FJ, Stevens H, Lu R, Caudrillier A, McBride M, McClure JD, Grant J, Thomas M, Frid M, Stenmark K, White K, Seto AG, Morrell NW, Bradshaw AC, MacLean MR, Baker AH. MicroRNA-143 Activation Regulates Smooth Muscle and Endothelial Cell Crosstalk in Pulmonary Arterial Hypertension. *Circ Res* 2015; **117**: 870-883 [PMID: 26311719 DOI: 10.1161/CIRCRESAHA.115.306806]

81 **Kapustin AN**, Shanahan CM. Emerging roles for vascular smooth muscle cell exosomes in calcification and coagulation. *J Physiol* 2016; **594**: 2905-2914 [PMID: 26864864 DOI: 10.1113/JP271340]

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