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# Stem cells-derived exosomes as cardiac regenerative agents

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ABSTRACT

Heart failure is a root cause of morbidity and mortality worldwide. Due to the limited regenerative capacity of the heart following myocardial injury, stem cell-based therapies have been considered a hopeful approach for improving cardiac regeneration. In recent years, different kinds of cell products have been investigated regarding their potential to treat patients with heart failure. Despite special attention to cell therapy and its products, therapeutic efficacy has been disappointing, and clinical application is not affordable. In the past few years, a subset of small extracellular vehicles (EVs), commonly known as "exosomes," was reported to grant regenerative and cardioprotective signals at a value similar to their donor cells. The conceptual advantage is that they may be ideally used without evoking a relevant recipient immune response or other adverse effects associated with viable cells. The evidence related to their beneficial effects in animal models of heart failure is rapidly growing. However, there is remarkable heterogeneity regarding source cells, isolation process, effective dosage, and delivery mode. This brief review will focus on the latest research and debates on regenerative potential and cardiac repair of exosomes from different sources, such as cardiac/non-cardiac stem, somatic cells, and progenitor cells.

Overall, the current state of research on exosomes as an experimental therapy for heart diseases will be discussed.

## 1. Introduction

Regenerative therapy aims to repair damaged tissue by stimulating the endogenous regenerative capacity of tissue and providing new cells as a replacement [1,2]. Although cardiac progenitor cells (CPCs) have been described to insist within the myocardium [3,4], the myocardium has no intrinsic regenerative capacity because of a lack of postnatal mitosis [5,6]. Transplantation of exogenous somatic cells into the damaged heart tissue to induce a significant improvement of heart function is considered in clinical trials [7]. Cell products derived from induced pluripotent or embryonic stem cells (iPSCs or ESCs) have not yet been investigated in clinical trials due to the complexity of the production process. Concerns about cellular immaturity, coupling with host cells, and genomic integrity are difficult to rule out in preclinical models.

With these explanations, kinds of lucrative effects following cell therapy in cardiac disease have been noticed without the durability of transplanted cells or stem cell differentiation. For example, studies related to the failure of transplanted mesenchymal stem cells (MSCs) show to some extent increase in cardiac function [8]. Furthermore, following research has reported that the conditioned medium of MSCs has cardioprotective effects, and their secreted cytokines alone were already able to increase cardiac function in an animal myocardial infarction (MI) model [9].

This review discusses exosome efficiency as cell-free therapeutic candidates that can improve cardiac repair. To provide this, we present current challenges and limitations regarding exosome-based therapy.

## 2. Exosomes as nanocarriers of biological messages

In organisms, cells can transfer information to their neighbors via extracellular vesicels (EVs), commonly referred to as "exosomes. EVs discovery is commonly attributed to the context of platelet maturation, and the first use of the term dates back to the 1970s [10]. EVs are

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surrounded by a phospholipid membrane containing up to 20,000 different protein molecules, such as enclosing cytosolic and transmembrane proteins with preserved catalytic binding activities [11]. Recently, EVs were also shown to contain microRNAs (miRNAs), mRNAs, and non-coding RNAs (ncRNAs), which play an important role in cell-to-cell information transfer [12]. Cells secret different kinds of EVs related to their subcellular origin. Current studies focused more on the endosomal vesicles and originated plasma membrane vesicles, called exosomes and micro-vesicles [13].

Exosomes, usually sizing from 30 to 100 nm, originate from the inward budding of the endosomal membrane vesicles and are exported out of the cell after the fusion of the endosomal membrane with the cell membrane [13]. Exosomes are differed from other EVs due to their specific membrane and cytosolic composition. In other words, exosomes have specific surface molecules that let them to exclusively targeted to other cells. Once attached to a recipient cell, vesicles can promote signaling through receptor-ligand interaction or endocytosis/phagocytosis to deliver their content into the cytosol [14].

EVs are secreted by different kinds of cells, such as smooth muscle cells, endothelial cells, tumor cells, neuronal cells, and stem cells. Also, they can be detected in most body fluids, including blood, cerebrospinal fluid, urine, and saliva. Cell- and body-fluid-derived EVs have recently gained more attention in biomedical applications such as heart tissue repair [15].

## 3. Myocardial reparative potential of different stem cell types

Different kinds of cell sources were investigated for a clinical approach with exosomes. Numerous studies have reported the effects of exosomes on cell survival, apoptosis, angiogenesis, and migration [16]. With the previous experience of the cells used in cardiac repair, some kinds of cells such as ESCs, iPSCs, multipotent/unipotent adult stem cell (ASCs) lineages such as mesenchymal stem cells (MSCs), CSCs, including cardio sphere-derived cells (CDCs), and EPCs are widely considered for regenerative exosome generation due to safeness and effectiveness (Fig. 1) [17]. A summary of the advantages, limitations, and potential efficiency of different source of stem cells in cardiac repair has been presented as Table 1.

### 3.1. Pluripotent stem cells

Since pluripotent stem cells (PSCs) can differentiate into different types of body cells, they have been eagerly investigated in cardiac repair therapy. Various studies have been done in this field. In one study, Wang et al. (2015) investigated that EVs-derived iPSCs, due to conveying signals to ischemic myocardium, can protect cardiomyocytes against MI injury *In-vivo*. These mechanisms are probably related to miRNA-mediated cytoprotection leading to apoptosis inhibition. These findings related to the therapeutic potential of iPSCs-derived exosomes in cardioprotection were highlighted for the first time [33]. In another study, Khan *et al.* (2015) reported that EVs secreted by ESCs can induce cardiac repair and keep cardiac function when injected intramyocardially in a murine infarction model. The authors concluded that these beneficial effects related to the transfer of exosomal miR-294 [34]. Following the previous studies, Adamiak *et al.* (2018) presented results showing that EVs derived from iPSCs admit *In-vitro* cytoprotective properties to cardiac cells and induce superior cardiac repair *In-vivo* [17]. These effects of iPSC-derived EVs could be related to their molecular content, which includes numerous miRNAs, proteins, and different growth factors such as teratocarcinoma-derived growth factor 1 (TDGF1), platelet-derived growth factor alpha [PDGFA], vascular endothelial growth factor C (VEGFC), and thrombospondin 1 (THBS1) [17].

### 3.2. Multipotent MSCs

MSCs have been demonstrated for cardiac repair in autologous and allogeneic settings in the past few years. Basically, MSCs can be found in different tissues, such as adipose tissue, bone marrow (BM), amniotic fluid, umbilical cord blood (UCB), and so on [35,36]. A distinctive feature is their differentiation capabilities to adipogenic, osteogenic, chondrogenic, neurogenic, etc. So far, differentiation into

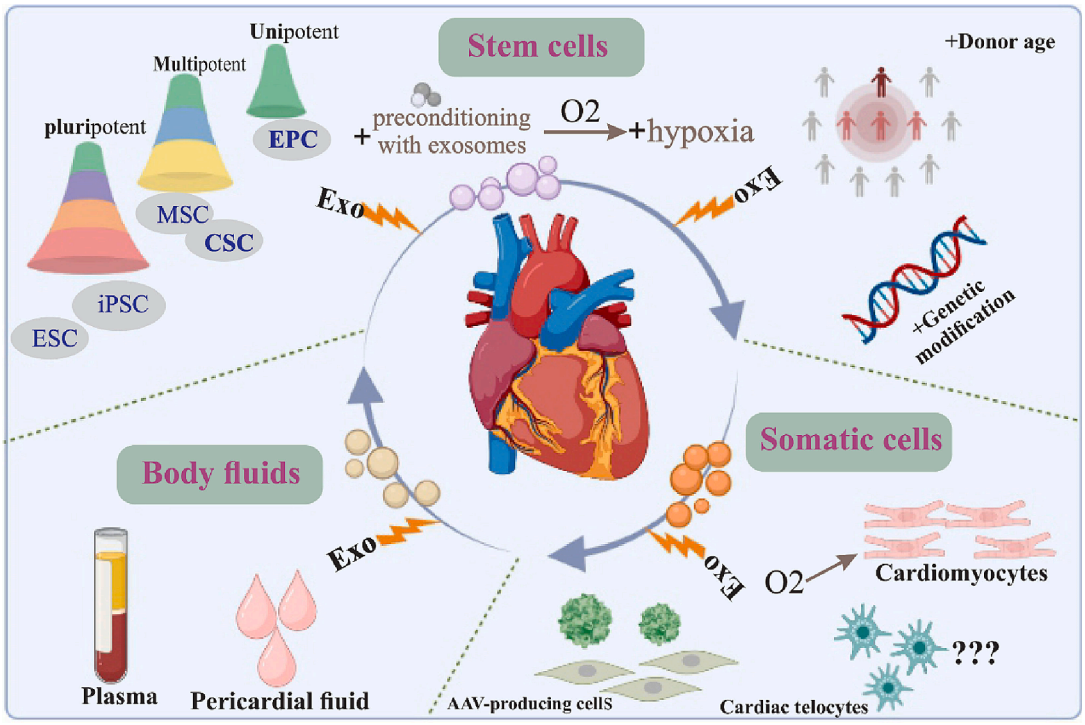


**Fig. 1.** A scheme depicting the discussed biological sources of exosomes for cardiac repair, along with different strategies for donor cell phenotype modification (This figure adapted from Fig. 1 of reference number 17) [17].

**Table 1**
**A** summary of the advantages, limitations, and potential efficiency of different source of stem cells in cardiac repair.

| Stem Cell Source | Advantages | Limitations | *Potential Efficacy in Cardiac Repair* | Ref. |
|---|---|---|---|---|
| MSCs | Easy isolation and amplification, and low immunogenicity | Low rate of migration to the ischemic myocardium, low tissue retention, and low survival rate after transplantation | Vascularization & Improvement in cardiac function | [18,19] |
| ESCs | Possess a high-proliferative capacity, providing a unique human model system to analyze mechanisms that control cardiomyocyte proliferation anddifferentiation | Ethical concerns, immunologicincompatibility and the formation of teratomas | Generate cardiac myocytes *in vitro*<br>One study reported that implanted ESC in slow heart-rate guinea pig model facilitated the repair of the MI and were able to achieve electrical integration | [20,2122] |
| iPSCs | Readily accessible, possibilities of large-scale production, stability after cryostorage without loss of function | Hard and exhausting isolation techniques like the isolation from other stem cells | Improved left ventricular (LV) systolic function and reducing infarct size | [23–25] |
| CSCs | Avoids ethical concerns, and lower risk of immune rejection<br>These cells can differentiate in cardiovascular cell types *in vivo* without the need of pre-implantation *in vitro* differentiation | Natural regeneration capacity of CSCs is too limited to be used in clinical therapy, and required *ex vivo*expansion<br>Major difficulties exist in the attainment and isolation of CSCs from myocardial samples, reducing available CSCs to be used for implantation | Injection of these cells appears to be safe and previous results reported an increase in viable myocardium, left ventricular ejection fraction, and other clinical parameters | [26,2728,29] |
| EPCs | EPCs are easy to harvest from different sources with minimal manipulations<br>They also secrete angiogenic factors *in vitro* and *in vivo* and play a crucial role in vascular repair *in vivo* | Extremely low numbers in peripheral blood and BM makes *ex vivo* expansion difficult | They participate in cardiac and vascular repair in coronary atherosclerosis, and ischemic cardiomyopathy. During tissue injury, circulating EPCs are recruited to injury sites, enabling the regenerate new blood vessels | [29–32] |

cardiomyocytes with *In-vitro* low efficiency has been seen in fetal MSCs. However, MSC-derived exosomes are being extensively investigated in clinical therapy to rebuild a damaged heart. In one study, Wang *et al.* (2017) confirmed the cardio-protection effects of endometrium-derived MSCs (En-MSC) in a rat model of MI. This study reported that miR-21 has a particular role in En-MSC therapy [37]. Ma *et al.* (2017) worked on UCB-MSCs-derived exosomes and reported that they promote cardiac regeneration and improve angiogenesis via platelet-derived growth factor delta (PDGFD) activation. In addition, it was also investigated that the ability of exosomes to repair MI tissue is enhanced in the hypoxia condition [38]. The cardioprotective effect of exosomes in this experiment was attributed to miR-210.

### 3.3. Multipotent cardiac progenitor cells

Because of the successful application of MSC in cardiac repair therapies, exosomes derived from other ASCs have also become subjects of more attention. Particularly, the remarkable ability of CPC-derived exosomes causes intensive attention for the clinical treatment of heart failure. Due to the cardiac origins, these cells may display better candidates for cell therapy than stem cells derived from different sources like adipose tissue or BM. There are several populations of CPCs in the adult heart, including CDCs, with the differentiation potential into three major cell types: endothelial cells, cardiomyocytes, and smooth muscle [39]. CDCs are intrinsic to the heart, expressing specific profiles of antigens such as $CD45^+$ and $CD105^+$ and causing functional recovery in different kinds of heart failure [40]. In one previous work by Tseliou et al. (2015), CDC exosomes were reported to transform dermal fibroblasts into functional, active cells that could reduce scar size and promote cardiac function in a MI model [41]. In another study by Gamal-Eldin Ibrahim et al. (2014), the regenerative and functional effects of CDC-derived exosomes manifest themselves through cells exosomal miR-146a after direct transplantation into the injured heart in a murine model [42]. Exosomes have again been identified as key mediators for improving cardiac function in another study conducted following ischemia and reperfusion in pig animal models [43,43]. C-$kit^+$ stem cells, as one of the pioneer cells in research, have already been attracted in experimental *In-vitro* models. For example, the cardiac differentiation potential of BM-resident ($CD117^+$) C-$kit^+$ stem cells were investigated by Fathi et al. (2020) [44]. It was shown that L-carnitine could increase the telomere length as an effective factor in increasing the cell survival and maintenance of the cardiac differentiated bone marrow resident C-$kit^+$ stem cells via Wnt3/β-catenin and ERK1/2 signaling pathway components [44]. Another study by this group reported that the *In-vitro* effects of L-carnitine on cardiac differentiation of C-$kit^+$ cells could have resulted from the secreted cytokines IL-6, IGF-1, TGF-b, and VEGF [45]. Despite *In-vitro* evidence regarding the cardiac differentiation potential of C-$kit^+$ cells, their role in myocardial repair is still unclear [46]. Due to the poor results of transplanted cells in preclinical and clinical studies, the hypothesis related to paracrine mechanisms, cell-free approaches, and probable mechanisms involving the modulation of EVs must be investigated in more detail. One of the most important factors affecting the potency of CPCs may be the donor's age. Many studies have been conducted in this field between adults and infants, showing that C-$kit^+$ cells from neonates have more *In-vitro* proliferation rate than the adult type, leading to greater myocardial recovery after coronary artery ligation in rat animal models [47].

## 4. Advantages of EVs for therapeutic applications

Naturally occurring membranous vesicles have the potential to be used as therapeutic options in regenerative medicine because of their ability to effectively target recipient cells and distribute their bioactive contents. In fact, there are a number of distinct benefits to using bioactive, non-living exosomes rather than cells in heart repair. It is important to highlight that exosomes may be swiftly and selectively absorbed by target cells, providing a wealth of opportunities for tissue- and cell-specific targeting. In addition to the advantages already listed, exosomes are simpler to manipulate, produce, and store than parent cells since they are smaller, less complicated, and less brittle [48]. They are also less immunogenic and provide no danger for the growth of tumors.

## 5. Limitations of EVs for therapeutic applications

Significant measures must be done to solve a number of unanswered concerns in the area before exosomes may be used as pharmaceutical products in the clinic. Creating scalable and repeatable procedures for exosome storage and purification, as well as enhancing methods and standards for quality evaluations, are significant challenges. The right

isolation technique would produce sterile exosomes that are consistent in their potency and purity and would also need to adhere to good manufacturing practices [49]. Determining the dose (protein quantity or particle number) of exosomes, dosing regimen (single/multiple applications), and delivery method need the definition and establishment of suitable units. Exosomes' beneficial effects have also been shown to differ depending on the tissue of origin or the age of the donor. For instance, it has been shown that EVs formed from BM and umbilical cord MSCs inhibit the growth of tumor cells, but EVs obtained from ADSCs had the opposite effect [50]. Interestingly, distinct RNA, lipid, and protein compositions may be found in subpopulations of EVs from a single cell type that are differentiated depending on density. These subpopulations may also perform distinct biological activities on their target cells. The findings from earlier studies showed variation in the number and make-up of subpopulations dispersed at various densities. Therefore, more therapeutic effectiveness might possibly be achieved by dealing with purer isolates that only contain one particular subpopulation of EVs, in addition to carefully choosing the cells with desirable qualities to create EVs [51].

## 6. Therapeutic's role of exosomes in cardiovascular repair

Exosomes carry many metabolites and affect various aspects of cell biology. They are involved in many cellular processes, including immune modulation and differentiation [52]. Produced from suitable regenerative sources could be very effective in the regeneration processes after myocardial infarction, making them interesting new therapeutic agents. Since the cells investigated in post-MI cell therapy implement their effects through paracrine signaling pathways, studies have focused on investigating these sources' regenerative potential. Using exosomes from human mesenchymal stem cells was one of the first studies investigating the paracrine mechanism in cardiac repair (Fig. 2) [53]. They showed that the injection of MSC-derived exosomes into the tail vein area of mice about 5 min before cardiac reperfusion leads to a significant reduction in the size of the infarcted area 24 h after the operation. It was also shown that cardiac function improved in animals treated with exosomes compared to the control group in 28 days. The analysis of the results showed that during the first 24 h, the activation of cell survival signaling pathways, including pAkt and pGSK3 (glycogen synthase kinase 3), increases while the infiltration of immune cells decreases [54]. In another study, the protective effects of mesenchymal stem cells compared to exosomes derived from were compared after injection into the border zone. The results of the study revealed that both cell groups, i.e., mesenchymal stem cells and exosomes, to some extent

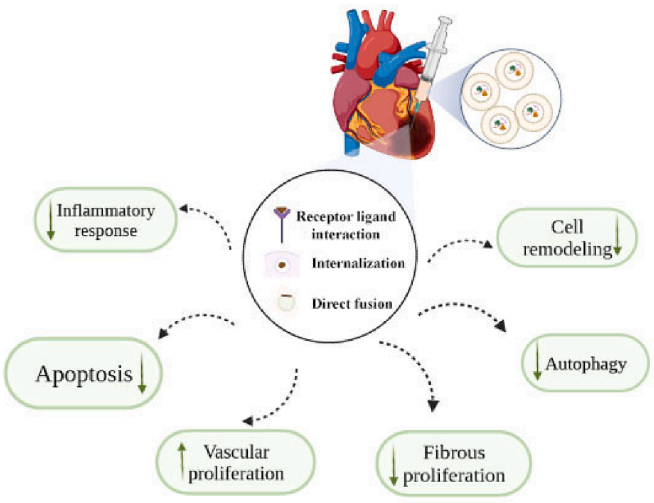


**Fig. 2.** Possible mechanism of exosome treatment of AM (This figure adapted from figure 17 of reference number 37) [53].

reduced the infarcted size within 28 days and improved cardiac function. Also, in the group treated with exosomes, it was shown that vascular density was increased [55]. Several other *In-vitro* studies have confirmed the angiogenic effects of exosomes derived from mesenchymal stem cells. Significant induction of tubule formation, proliferation, and migration of different cells has been shown [55,56]. However, pro-angiogenic proteins in exosomes need to explain these effects in detail, including VEGF, bFGF, and some metalloproteinases [56]. *In-vitro* studies have proven that exosomes derived from rat MSCs cause increased proliferation, migration, and tubule formation of cardiac stem cells. Also, upon exosome treatment, some miRNAs were changed into these cells, such as up-regulation and/or down-regulation of miR-147, miR-503-3p, miR-207, and miR-326-5p [57]. Before injection into the cardiac tissue, when cardiac stem cells were treated with exosomes derived from rat MSCs, vascular density at the infarcted site and cardiac function improvement was seen after 28 days [57]. Some endothelial and cardiomyocyte gene expression was up-regulated in CPCs after stimulation with exosomes derived from ES. Also, exosomes derived from iPS or ES cells increased proliferation and tubule formation in CPCs, while in *In-vitro*, apoptosis was reduced in CPCs and cardiomyocytes [34]. Injection of exosomes derived from ES-derived after MI caused reduced apoptosis after 4 weeks and 48 h [34]. It also increased vessel density and proliferation and caused cardiac function improvement, while injection of CPCs pre-stimulated with exosomes-derived ES reduced infarct size and improved cardiac function. The observed effects could be due to several known types of miRNAs present in exosomes, which are even capable of re-inducing and proliferation of CPCs *In-vitro* [34]. Some reports have been documented in the literature regarding the regenerative potential of exosomes derived from CPC. For example, exosomes derived from Mice and rat CPC reduce cardiomyocyte apoptosis and increase endothelial cells' tubule formation in different conditions [58]. Rat CPC-derived exosomes can improve cardiac function and reduce fibrosis [58]. Human CPC-derived exosomes could also reduce the infarcted size within 7 and 30 days after MI, increase vascular density, improve cardiac function, and significantly decrease fibrosis [42]. Therefore, the results obtained from the studies show that miRNAs are considered a very important mediator in exosomes. However, further evaluation needs to be done regarding other mediators of miRNAs, such as miR-146a. In this regard, one study showed that injection of miR-146a in a mice model of MI causes to a reduction in infarcted size and improves the cardiac function, while without miR-146a, no improvement is achieved [59]. According to the studies, exosomes derived from CPC in *In-vitro* could be considered for cell function improvement through proangiogenic effects. It has been shown that various angiogenic factors are present in the exosomes derived from human CPC, among which extracellular matrix metalloproteinase inducer, VEGF, and MMP-9 are very important factors. These factors cause significant proliferation, tubule formation, and cell migration. Removing some factors, such as extracellular matrix metalloproteinase inducer led to a significant reduction in angiogenesis in different conditions, i.e., *In-vitro* or *In- vivo* [56]. It is essential to note that different types of cell-derived exosomes play an important role in repairing cell damage with several programmed death as describing bellow.

## 7. Exosomes reduced apoptosis

Exosomes produced from BMSCs are essential for healing myocardial damage brought on by tissue reperfusion, and exosomal miR-486-5p inhibits myocardial apoptosis by blocking the PTEN de-activation pathway [60]. Wherein hypoxic pretreatment is the most often used technique. Research has shown that pretreating BMMSCs with hypoxia dramatically increases the amount of miR-214 in released exosomes [61]. It has also been shown that exosomal miR-214 is transported to cardiomyocytes and inhibits the expression of CaMKII. An additional study revealed that pretreating mouse BMMSCs under hypoxia

enhanced the expression of miR-125b-5p inside their exosomes. Cardiomyocytes' capacity to fend off apoptosis was markedly increased after injection into the infarcted region by blocking p53 and BAK1 [62]. Moreover, the upregulation of miR-24 and miR-210 in the exosomes of hypoxic bone marrow-derived mesenchymal stem cells (BMMSCs) might enhance the ability of cardiomyocytes to resist apoptosis. The precise method by which apoptosis is prevented is not yet fully understood, however the production of exosomal miR-210 is contingent upon the level of neutral sphingomyelinase 2 (nSMase2). Gene or microRNA modified exosomes are thought to enhance the exosomes' resistance to cardiomyocyte death. Research has shown that GATA binding protein-4 (GATA-4) controls the production of miR-15 family members in bone marrow-derived mesenchymal stem cells (BMMSCs) and enhances their ability to survive in a low oxygen environment.

Exosomes generated from alternative stem cells have also undergone thorough investigation. Research has shown that exosomes obtained from adipose-derived stem cells (ADSCs) have the ability to control the signals of S1P/SK1/S1PR1. Due to its high expression in exosomes produced from ADMSCs, miR-214 has the ability to suppress the expression of Bcl2L11 and SLC8a1 when transported to the site of myocardial infarction. The protein encoded by Bcl2L11 may trigger apoptosis by activating Bax or neutralizing anti-apoptotic proteins [63]. Under cardiac stress, the sodium/calcium exchange protein encoded by SLC8A1 induces cardiomyocyte calcium overload-related death. Exosomes generated from ADMSCs have been used in studies to demonstrate the hypoxic cardiomyocytes' capacity to inhibit apoptosis by overexpressing miR-146a. The primary mechanism behind miR-146a's anti-apoptotic impact in I/R damaged tissue is the suppression of early growth response factor 1 (EGR1). Through the suppression of Bax and pro-caspase expression, exosomes produced from umbilical cord MSCs overexpressing TIMP2 dramatically improve the anti-apoptotic abilities of hypoxic cardiomyocytes. Exosomes produced from iPSCs are also essential in preventing miR-21 from inhibiting apoptosis in infarcted cardiomyocytes by targeting PDCD4/AP-1 [64].

## 8. Exosomes regulate autophagy-dependent cell death

Exosomes have been proven in studies to minimize myocardial autophagy and death following a MI or I/R. Exosomes produced from human MSCs have been shown in prior studies to minimize I/R damage by suppressing cardiomyocyte autophagy, although the particular mechanism has yet to be established [65]. MiR-125b-5p is substantially expressed in exosomes produced from BMMSCs, according to experiments. When exosomal miR-125b-5p is administered to the MI heart, it decreases autophagy of myocardial cells in the infarcted location and suppresses the production of P53 [66]. In the mouse myocardial I/R damage model, miR-29c expression decreased while autophagy flow of myocardial cells increased. High miR-29c expression may minimize excessive autophagy in hypoxic myocardium by directly decreasing PTEN expression, hence limiting I/R-induced excessive autophagy via the PTEN/AKT/mTOR signaling pathway [67]. Similarly, overexpressing SDF1 in BMMSCs greatly boosted SDF1 expression in exosomes, which in turn raised Bcl-2 expression in hypoxic cardiomyocytes. In contrast, Bax, Beclin-1, LC3, and the LC3II/LC3I ratio were considerably lowered, eliminating excessive autophagy in cardiomyocytes [68].

## 9. The required dose of exosomes

It is essential to find a way to enhance EVs' short plasma half-life and poor targeting efficiency if EV-based treatments are to be successfully transferred from bench to clinic [69]. The majority of animal trials used intramyocardial or intracoronary injections to deliver EVs [70]. These are certainly effective ways to give EV, but since they involve invasive, complicated, and risky procedures, they are not appropriate for use in clinical settings. Conversely, intravenous distribution is more recommended since it is technically simpler and repeatable, especially considering that patients with MI may not be able to withstand repeated invasive procedures. Unfortunately, inadequate EV accumulation in the heart tissue and off-target binding pose challenges to this strategy. Following intravenous delivery, it has been shown that EVs are mostly transported to the liver, lungs, kidneys, and spleen [71,72].

An additional dosage of EVs is required to offset non-specific delivery, since fewer than 10 % of EVs given by intravenous injection are eventually absorbed by an undamaged heart; Because the myocardial capillary endothelium is continuous with tight connections and has a diameter of about 4 nm, EVs given by a systemic route are seldom absorbed by the interstitial myocardium. This restricts the mobility of EVs but enables the transfer of blood-borne chemicals [73].

Lai et al. were the first to demonstrate the acute cardioprotective effects of exosomes derived from MSCs [74]. Exosomes released by MSCs in culture were purified using HPLC before being injected into the tail capillaries of rodents undergoing 30 min of myocardial ischaemia via coronary artery ligation [75]. After 24 h, the extent of the infarct in the injected animals was dramatically decreased, and after 28 days, the heart function was restored. Notably, dose–response research was conducted, necessitating the use of at least 4 μg/kg exosomes to show a discernible advantage. Moreover, the isolated, perfused heart was also shielded by the MSC exosomes, demonstrating that protection was provided without the aid of circulating immune cells. Numerous variables, including as the manner of administration, the size, dosage, and membrane components of EVs, as well as the kind of donor and EV receptor cells, might affect the dispersion of EVs [54].

The distribution of EVs can be influenced by many factors, including delivery mode, EVs' size, injected dose, membrane components of EVs, as well as the type of EV receptor cells and donor cells [76].

## 10. The potential of modified exosomes

Considering that the content of exosomes depends on the cellular content, there are different strategies to increase the efficiency of exosomes in heart tissue repair. $CD34^{+}$ cells were genetically modified to overexpress sonic hedgehog (SHH), which resulted in an increased cardiac function and vessel density after MI. It was partly related to the reason that the $CD34^{+}$ cells exosomes contain SHH and can be transferred to the heart recipient cells, and cause to increase in SHH signaling [77]. Overexpression of GATA binding protein 4 (GATA4) in BM-derived MSCs in the treatment condition with exosomes causes to reduce hypoxia-induced apoptosis of cardiac cells. Since miR-19 is considered a key effector in survival pathways, GATA4-overexpressing exosomes increase miR-19 content and finally restore mitochondrial integrity. *In-vivo* experimental studies showed that following injection of GATA4 overexpressing exosomes, an increase in miR-19 content and cardiac function and finally a decrease in infarct size were observed [78].

In addition to genetically altering the donor cell to modify the protein expressions, exosomes can also be changed by altering the conditions of the donor cell at the time of exosome generation. As mentioned before, hypoxia is famous for having deep effects on cells, and ischemic preconditioning has been reported to have positive effects in the clinic [79]. After isolating exosomes from MSCs or hearts that have undergone ischemic preconditioning, these exosomes cause reduced apoptosis in cardiomyocytes, infarct size, and fibrosis after MI.

With all these interpretations, most of the preparation methods of modified exosomes can lead to membrane damage or alteration of surface proteins. These alterations are considered as challenges which encountered with [80].

## 11. Future studies

Combining these targeted tactics will be a viable area for future research. For instance, surface modification of cardiac homing peptides in conjunction with "passive targeting" may significantly increase the

targeting efficacy. Additionally, it has been extensively reported that a medicine encapsulated in a hydrogel may release a large dosage in a short amount of time upon exposure to ultrasonic irradiation [81]. It follows that combining hydrogel with ultrasonic stimulation makes sense as a way to regulate when EVs are delivered to the heart and improve absorption efficiency. Wang et al. created an ultrasound-responsive nanodrug and targeting peptide in a different work for cancer treatment; it would be worthwhile to attempt this strategy for EV targeting as well [82]. Finally, it should be mentioned that the techniques used for EV heart targeting are also applicable to other domains, such tumor and brain targeting [83].

One of the great mysteries of clinical medicine is still the restoration of broken hearts. Heart failure outcomes have been markedly improved by developments in early revascularization after myocardial infarction and the creation of devices to support the failing myocardium. At this time, there is no medication or treatment that can prevent cardiac injury or stimulate its regeneration. There are currently no efficacious interventions that can induce the formation of new cardiac blood vessels. All of the current medical treatments for heart failure and myocardial infarction are based on the pharmacological combination of small molecules and fundamentally seek to maintain the function of the myocardium that has survived. The utilization of cells, exosomes, and RNAs for heart regeneration is one technique that has gained increasing recognition over the last several decades. Noncoding RNAs (ncRNAs) and protein-coding mRNAs are two distinct types of RNAs that are involved in a wide variety of cellular functions, including those of all cardiovascular system cells. Successful RNA therapeutics for the heart depend on both the identification of useful RNA molecules and the resolution of a number of technical issues. The fact that no therapeutic angiogenesis-related clinical study has been successful so far serves as a good example of this [84].

## 12. Conclusion

Over the last five decades, exosomes have been identified in the studies of many researchers, but their therapeutic approach in preclinical trials is back to the last decade. According to numerous basic studies and clinical trials, the beneficial effects of these organelles have been demonstrated in various models of cardiac diseases. Many current activities and approaches regarding the need to use exosomes in regenerative medicine, including cardiac repair, are considered along with cell therapy strategies. The question that arises now is whether using these organelles could be considered an innovation and new therapeutic approach in cardiac diseases.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References


[1] E. Fathi, et al., A general view of CD33+ leukemic stem cells and CAR-T cells as interesting targets in acute myeloblatsic leukemia therapy, Blood Res. 55 (1) (2020) 10.

[2] E. Fathi, R. Farahzadi, Mesenchymal stem cells as a cell-based therapeutic strategy targeting the telomerase activity of KG1 acute myeloid leukemia cells, Acta Med. Iran. (2022).

[3] R.R. Smith, et al., Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens, Circulation 115 (7) (2007) 896–908.

[4] E. Fathi, et al., An overview of the myocardial regeneration potential of cardiac c-Kit(+) progenitor cells via PI3K and MAPK signaling pathways, Fut. Cardiol. 16 (3) (2020) 199–209.

[5] S.D. Prabhu, N.G. Frangogiannis, The biological basis for cardiac repair after myocardial infarction: from inflammation to fibrosis, Circ. Res. 119 (1) (2016) 91–112.

[6] J.A. Maring, et al., Myocardial regeneration via progenitor cell-derived exosomes, Stem Cells Int. 2017 (2017) 7849851.

[7] P. Nigro, et al., Cell therapy for heart disease after 15 years: Unmet expectations, Pharmacol. Res. 127 (2018) 77–91.

[8] B. Tao, et al., Percutaneous intramyocardial delivery of mesenchymal stem cells induces superior improvement in regional left ventricular function compared with bone marrow mononuclear cells in porcine myocardial infarcted heart, Theranostics 5 (2) (2015) 196–205.

[9] N.S. Mabotuwana, et al., Paracrine factors released by stem cells of mesenchymal origin and their effects in cardiovascular disease: a systematic review of pre-clinical studies, Stem Cell Rev. Rep. 18 (8) (2022) 2606–2628.

[10] N. Crawford, The presence of contractile proteins in platelet microparticles isolated from human and animal platelet-free plasma, Br. J. Haematol. 21 (1) (1971) 53–69.

[11] M.A. Di Bella, Overview and update on extracellular vesicles: considerations on exosomes and their application in modern medicine, Biology (Basel) 11(6) (2022).

[12] M. Tkach, C. Théry, Communication by extracellular vesicles: where we are and where we need to go, Cell 164 (6) (2016) 1226–1232.

[13] S.L.N. Maas, X.O. Breakefield, A.M. Weaver, Extracellular vesicles: unique intercellular delivery vehicles, Trends Cell Biol. 27 (3) (2017) 172–188.

[14] L.A. Mulcahy, R.C. Pink, D.R.F. Carter, Routes and mechanisms of extracellular vesicle uptake, J. Extracell. Vesicl. 3 (1) (2014) 24641.

[15] S. Sahoo, et al., Therapeutic and diagnostic translation of extracellular vesicles in cardiovascular diseases: roadmap to the clinic, Circulation 143 (14) (2021) 1426–1449.

[16] R. Yue, et al., Mesenchymal stem cell-derived exosomal microRNA-182-5p alleviates myocardial ischemia/reperfusion injury by targeting GSDMD in mice, Cell Death Discov. 8 (1) (2022) 202.

[17] M. Adamiak, S. Sahoo, Exosomes in myocardial repair: advances and challenges in the development of next-generation therapeutics, Mol. Ther. 26 (7) (2018) 1635–1643.

[18] D. Simpson, et al., A tissue engineering approach to progenitor cell delivery results in significant cell engraftment and improved myocardial remodeling, Stem Cells 25 (9) (2007) 2350–2357.

[19] Y. Guo, et al., The therapeutic potential of mesenchymal stem cells for cardiovascular diseases, Cell Death Dis 11 (5) (2020) 349.

[20] R. Gulati, R.D. Simari, Cell therapy for acute myocardial infarction, Med. Clin. N. Am. 91 (4) (2007) 769–785.

[21] C. Xu, et al., Characterization and enrichment of cardiomyocytes derived from human embryonic stem cells, Circ. Res. 91 (6) (2002) 501–508.

[22] Y. Shiba, et al., Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts, Nature 489 (7415) (2012) 322–325.

[23] P. Menasché, Stem cell–derived exosomes and the failing heart: Small cause, big effect, J. Thorac. Cardiovasc. Surg. 156 (3) (2018) 1089–1092.

[24] C. Fan, et al., Utilization of human induced pluripotent stem cells for cardiac repair, Front. Cell Dev. Biol. 8 (2020) 36.

[25] K. Miki, et al., Bioengineered myocardium derived from induced pluripotent stem cells improves cardiac function and attenuates cardiac remodeling following chronic myocardial infarction in rats, Stem Cells Transl. Med. 1 (5) (2012) 430–437.

[26] B.C. du Pré, P.A. Doevendans, L.W. van Laake, Stem cells for cardiac repair: an introduction, J. Geriatr. Cardiol.: JGC 10 (2) (2013) 186.

[27] R.R. Makkar, et al., Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial, Lancet 379 (9819) (2012) 895–904.

[28] A. Leri, et al., Myocardial regeneration and stem cell repair, Curr. Probl. Cardiol. 33 (3) (2008) 91–153.

[29] Q. Sun, Z. Zhang, Z. Sun, The potential and challenges of using stem cells for cardiovascular repair and regeneration, Genes Diseases 1 (1) (2014) 113–119.

[30] H. Huang, W. Huang, Regulation of endothelial progenitor cell functions in ischemic heart disease: new therapeutic targets for cardiac remodeling and repair, Front. Cardiovasc. Med. 9 (2022) 896782.

[31] Y. Zhu, et al., MALAT1/miR-15b-5p/MAPK1 mediates endothelial progenitor cells autophagy and affects coronary atherosclerotic heart disease via mTOR signaling pathway, Aging (Albany NY) 11 (4) (2019) 1089.

[32] Y. Cheng, et al., Transplantation of bone marrow-derived endothelial progenitor cells attenuates myocardial interstitial fibrosis and cardiac dysfunction in streptozotocin-induced diabetic rats, Int. J. Mol. Med. 30 (4) (2012) 870–876.

[33] Y. Wang, et al., Exosomes/microvesicles from induced pluripotent stem cells deliver cardioprotective miRNAs and prevent cardiomyocyte apoptosis in the ischemic myocardium, Int. J. Cardiol. 192 (2015) 61–69.

[34] M. Khan, et al., Embryonic stem cell-derived exosomes promote endogenous repair mechanisms and enhance cardiac function following myocardial infarction, Circ. Res. 117 (1) (2015) 52–64.

[35] E. Fathi, R. Farahzadi, Isolation, culturing, characterization and aging of adipose tissue-derived mesenchymal stem cells: a brief overview, Braz. Arch. Biol. Technol. 59 (2016).

[36] E. Fathi, R. Farahzadi, N. Sheikhzadeh, Immunophenotypic characterization, multi-lineage differentiation and aging of zebrafish heart and liver tissue-derived mesenchymal stem cells as a novel approach in stem cell-based therapy, Tissue Cell 57 (2019) 15–21.

[37] K. Wang, et al., Enhanced cardioprotection by human endometrium mesenchymal stem cells driven by exosomal microRNA-21, Stem Cells Transl. Med. 6 (1) (2017) 209–222.

[38] J. Ma, et al., Exosomes derived from Akt-modified human umbilical cord mesenchymal stem cells improve cardiac regeneration and promote angiogenesis via activating platelet-derived growth factor D, Stem Cells Transl. Med. 6 (1) (2017) 51–59.

[39] T. Le, J. Chong, Cardiac progenitor cells for heart repair, Cell Death Discov. 2 (2016) 16052.
[40] K. Cheng, et al., Human cardiosphere-derived cells from advanced heart failure patients exhibit augmented functional potency in myocardial repair, JACC Heart Fail 2 (1) (2014) 49–61.
[41] E. Tseliou, et al., Fibroblasts rendered antifibrotic, antiapoptotic, and angiogenic by priming with cardiosphere-derived extracellular membrane vesicles, J. Am. Coll. Cardiol. 66 (6) (2015) 599–611.
[42] A.G. Ibrahim, K. Cheng, E. Marbán, Exosomes as critical agents of cardiac regeneration triggered by cell therapy, Stem Cell Rep. 2 (5) (2014) 606–619.
[43] R. Gallet, et al., Exosomes secreted by cardiosphere-derived cells reduce scarring, attenuate adverse remodelling, and improve function in acute and chronic porcine myocardial infarction, Eur. Heart J. 38 (3) (2017) 201–211.
[44] E. Fathi, et al., L-carnitine extends the telomere length of the cardiac differentiated CD117(+)- expressing stem cells, Tissue Cell 67 (2020) 101429.
[45] E. Fathi, et al., Cardiac differentiation of bone-marrow-resident c-kit(+) stem cells by L-carnitine increases through secretion of VEGF, IL6, IGF-1, and TGF- β as clinical agents in cardiac regeneration, J. Biosci. 45 (2020).
[46] N. Sultana, et al., Resident c-kit(+) cells in the heart are not cardiac stem cells, Nat. Commun. 6 (2015) 8701.
[47] S. Sharma, et al., A deep proteome analysis identifies the complete secretome as the functional unit of human cardiac progenitor cells, Circ. Res. 120 (5) (2017) 816–834.
[48] P. Mathiyalagan, et al., Angiogenic mechanisms of human CD34+ stem cell exosomes in the repair of ischemic hindlimb, Circ. Res. 120 (9) (2017) 1466–1476.
[49] J.P.G. Sluijter, et al., Extracellular vesicles in diagnostics and therapy of the ischaemic heart: position paper from the working group on cellular biology of the heart of the european society of cardiology, Cardiovasc. Res. 114 (1) (2018) 19–34.
[50] U. Agarwal, et al., Experimental, systems, and computational approaches to understanding the microRNA-mediated reparative potential of cardiac progenitor cell–derived exosomes from pediatric patients, Circ. Res. 120 (4) (2017) 701–712.
[51] C. Lässer, S.C. Jang, J. Lötvall, Subpopulations of extracellular vesicles and their therapeutic potential, Mol. Aspects Med. 60 (2018) 1–14.
[52] S. Yu, et al., Tumor-derived exosomes in cancer progression and treatment failure, Oncotarget 6 (35) (2015) 37151–37168.
[53] Y.L. Zheng, et al., Stem cell-derived exosomes in the treatment of acute myocardial infarction in preclinical animal models: a meta-analysis of randomized controlled trials, Stem Cell Res. Ther. 13 (1) (2022) 151.
[54] F. Arslan, et al., 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. 10 (3) (2013) 301–312.
[55] S. Bian, et al., Extracellular vesicles derived from human bone marrow mesenchymal stem cells promote angiogenesis in a rat myocardial infarction model, J. Mol. Med. (Berl.) 92 (4) (2014) 387–397.
[56] K.R. Vrijsen, et al., Exosomes from cardiomyocyte progenitor cells and mesenchymal stem cells stimulate angiogenesis via EMMPRIN, Adv. Healthc. Mater. 5 (19) (2016) 2555–2565.
[57] P. Huang, et al., Combinatorial treatment of acute myocardial infarction using stem cells and their derived exosomes resulted in improved heart performance, Stem Cell Res. Ther. 10 (1) (2019) 300.
[58] W.D. Gray, et al., Identification of therapeutic covariant microRNA clusters in hypoxia-treated cardiac progenitor cell exosomes using systems biology, Circ. Res. 116 (2) (2015) 255–263.
[59] V. Jeevanantham, et al., Adult bone marrow cell therapy improves survival and induces long-term improvement in cardiac parameters: a systematic review and meta-analysis, Circulation 126 (5) (2012) 551–568.
[60] X.-H. Sun, et al., Exosomes of bone-marrow stromal cells inhibit cardiomyocyte apoptosis under ischemic and hypoxic conditions via miR-486-5p targeting the PTEN/PI3K/AKT signaling pathway, Thromb. Res. 177 (2019) 23–32.
[61] Y. Wang, et al., Exosomes derived from miR-214-enriched bone marrow-derived mesenchymal stem cells regulate oxidative damage in cardiac stem cells by targeting CaMKII, Oxid. Med. Cell. Longev. 2018 (2018).
[62] L.-P. Zhu, et al., Hypoxia-elicited mesenchymal stem cell-derived exosomes facilitates cardiac repair through miR-125b-mediated prevention of cell death in myocardial infarction, Theranostics 8 (22) (2018) 6163.
[63] S. Shukla, et al., BH3-only protein BIM: an emerging target in chemotherapy, Eur. J. Cell Biol. 96 (8) (2017) 728–738.
[64] J. Xiao, et al., Cardiac progenitor cell-derived exosomes prevent cardiomyocytes apoptosis through exosomal miR-21 by targeting PDCD4, Cell Death Dis. 7 (6) (2016) e2277–e.
[65] X. Jiang, et al., Human mesenchymal stem cell-derived exosomes reduce ischemia/reperfusion injury by the inhibitions of apoptosis and autophagy, Curr. Pharm. Des. 24 (44) (2018) 5334–5341.
[66] C. Xiao, et al., Transplanted mesenchymal stem cells reduce autophagic flux in infarcted hearts via the exosomal transfer of miR-125b, Circ. Res. 123 (5) (2018) 564–578.
[67] T. Li, et al., Bone marrow mesenchymal stem cell-derived exosomal miRNA-29c decreases cardiac ischemia/reperfusion injury through inhibition of excessive autophagy via the PTEN/Akt/mTOR signaling pathway, Circ. J. 84 (8) (2020) 1304–1311.
[68] Y. Li, et al., Exosomal miR-301 derived from mesenchymal stem cells protects myocardial infarction by inhibiting myocardial autophagy, Biochem. Biophys. Res. Commun. 514 (1) (2019) 323–328.
[69] X. Yin, L.-H. Jiang, Extracellular vesicles: targeting the heart, Front. Cardiovasc. Med. 9 (2023) 1041481.
[70] Z. Wei, et al., miRNA-181a over-expression in mesenchymal stem cell-derived exosomes influenced inflammatory response after myocardial ischemia-reperfusion injury, Life Sci. 232 (2019) 116632.
[71] M. Kang, et al., Biodistribution of extracellular vesicles following administration into animals: a systematic review, J. Extracell. Vesicl. 10 (8) (2021) e12085.
[72] D. Gupta, et al., Quantification of extracellular vesicles in vitro and in vivo using sensitive bioluminescence imaging, J. Extracell. Ves. 9 (1) (2020) 1800222.
[73] E. Hausner, S.A. Elmore, X. Yang, Overview of the components of postnatal cardiac metabolism: implications for pediatric drug development? Drug Metab. Dispos. (2019).
[74] R.C. Lai, et al., Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury, Stem Cell Res. 4 (3) (2010) 214–222.
[75] S.M. Davidson, D.M. Yellon, Exosomes and cardioprotection–a critical analysis, Mol. Aspects Med. 60 (2018) 104–114.
[76] P. Duan, et al., Potential role of exosomes in the pathophysiology, diagnosis, and treatment of hypoxic diseases, Am. J. Transl. Res. 11 (3) (2019) 1184.
[77] A.R. Mackie, et al., Sonic hedgehog-modified human CD34+ cells preserve cardiac function after acute myocardial infarction, Circ. Res. 111 (3) (2012) 312–321.
[78] B. Yu, et al., Exosomes secreted from GATA-4 overexpressing mesenchymal stem cells serve as a reservoir of anti-apoptotic microRNAs for cardioprotection, Int. J. Cardiol. 182 (2015) 349–360.
[79] Y.J. Cho, et al., Long-term clinical outcomes of Remote Ischemic Preconditioning and Postconditioning Outcome (RISPO) trial in patients undergoing cardiac surgery, Int. J. Cardiol. 231 (2017) 84–89.
[80] N. Kučuk, et al., Exosomes engineering and their roles as therapy delivery tools, therapeutic targets, and biomarkers, Int. J. Mol. Sci. 22 (17) (2021) 9543.
[81] C.-H. Wu, et al., One injection for one-week controlled release: In vitro and in vivo assessment of ultrasound-triggered drug release from injectable thermoresponsive biocompatible hydrogels, Ultrason. Sonochem. 62 (2020) 104875.
[82] Z. Wang, et al., Tumor-homing, pH-and ultrasound-responsive polypeptide-doxorubicin nanoconjugates overcome doxorubicin resistance in cancer therapy, J. Control. Release 264 (2017) 66–75.
[83] S. Bashyal, C. Thapa, S. Lee, Recent progresses in exosome-based systems for targeted drug delivery to the brain, J. Control. Release 348 (2022) 723–744.
[84] M. Giacca, Fulfilling the promise of RNA therapies for cardiac repair and regeneration, Stem Cells Transl. Med. 12 (8) (2023) 527–535.