Journal of Clinical and Translational Research 2022; 8(6): 476-487

Journal of Clinical and Translational Research

Journal homepage: http://www.jctres.com/en/home

REVIEW ARTILCE

Endothelial progenitor cell-derived small extracellular vesicles for myocardial angiogenesis and revascularization

Maher T. Al-Omar†, Mahmoud T. Alnajjar†, Ziyad T. Ahmed†, Faris M. I. Salaas, Tamim S. M. Alrefaei, Khawaja H. Haider*

Department of Basic Sciences, College of Medicine, Sulaiman Al Rajhi University, Al-Bukairyah 52726, Saudi Arabia
†These authors contributed equally to this work.

ARTICLE INFO

Article history:
Received: July 27, 2022
Revised: September 23, 2022
Accepted: October 20, 2022
Published online: October 31, 2022

Keywords:
angiogenesis
endothelial
endothelial progenitor cells
small extracellular vesicles
miRNA
stem cells

**Corresponding author:*
Khawaja H. Haider
College of Medicine, Sulaiman Al Rajhi University, Al-Bukairyah 52726, Saudi Arabia.
E-mail: kh.haider@sr.edu.sa

ABSTRACT

Background: Endothelial progenitor cells (EPCs) have been well-studied for their differentiation potential and paracrine activity *in vitro* and in experimental animal studies. EPCs are the precursors of endothelial cells (ECs) and a rich source of pro-angiogenic factors, and hence, possess enormous potential to treat ischemic heart through myocardial angiogenesis. Their proven safety and efficacy observed during the pre-clinical and clinical studies have portrayed them as a near ideal cell type for cell-based therapy of ischemic heart disease. In response to the chemical cues from the ischemic heart, EPCs from the bone marrow and peripheral circulation home-in to the ischemic myocardium and participate in the intrinsic repair process at the molecular and cellular levels through paracrine activity and EC differentiation. EPCs also release small extracellular vesicles (sEVs) loaded with bioactive molecules as part of their paracrine activity for intercellular communication to participate in the reparative process in the heart.
Aim: This literature review is based on the published data regarding the characteristic features of EPC-derived sEVs and their proteomic and genomic payload, besides facilitating safe and effective repair of the ischemic myocardium. In light of the encouraging published data, translational and clinical assessment of EPC-derived sEVs is warranted. We report the recent experimental animal studies and their findings using EPC-derived sEVs on cardiac angiogenesis and preservation of cardiac function.
Relevance for Patients: With the promising results from pre-clinical studies, clinical trials should be conducted to assess the clinical utility of EPC-derived sEVs in the treatment of the ischemic myocardium.

1. Introduction

Ischemic myocardial damage is characterized by irreversible loss of cardiomyocytes. With limited intrinsic repair mechanisms and a lack of interventional methods to compensate for the loss of functioning cardiomyocytes, a stem cell-based treatment strategy has emerged as a viable therapeutic option, such that it has progressed from its initial "hype to real hope" [1]. Endothelial progenitor cells (EPCs) are one of the few adult tissue-derived cell types that have advanced to the phase of clinical trials for myocardial reparability assessment, either by cell transplantation (naïve or genetically modified) or through intrinsic mobilization in patients (https://clinicaltrials.gov/ct2/home). These studies primarily focus on restoring regional blood flow through angiogenic repair. Diverging from EPCs-based therapy, this literature review provides the advancements made in the cell-free therapy approach using EPC-derived small extracellular vesicles (sEVs) for myocardial repair.

A literature search was performed using keywords to find research papers on PubMed, EMBASE, and SCOPUS. The found literature was then summarized to provide a

DOI: http://dx.doi.org/10.18053/jctres.08.202206.004

comprehensive overview of EPCs, their paracrine activities, the release of exosomes (hereafter referred to as sEVs, in line with the guidelines), and payload profile followed by reporting the recent experimental animal studies and their findings using EPCs-derived sEVs. For clinical studies, we searched Clinicaltrials.gov using the terms "exosomes" and "extracellular vesicles" to find the registered clinical studies.

2. EPCs

In 1997, Asahara *et al.* published an exciting paper with novel findings regarding the isolation of putative EPCs and their role in angiogenesis [2]. In their pioneering work, they successfully isolated $CD34^+$ mononuclear cells from peripheral blood and reported that the purified cells adopted endothelial cell (EC) phenotype under the optimal culture conditions with the right cues supporting their differentiation [2]. These data enhanced our understanding of EPCs' role in angiogenesis, providing a strong impetus for a promising future regarding their therapeutic potential in regenerative medicine. It has generally been considered that ECs originate from the hemangioblasts during the embryonic period and that the endothelial repair ensues only after the migration and proliferation of the pre-existing ECs [3]. These findings paved the way for enormous growth in the research about EPCs, especially their utility for cellular angiogenesis.

During the past few years, EPCs' presence has been reported in blood vessel walls for their essential role in postnatal vasculogenesis [4]. As summarized in Table 1, the early EPCs are characterized by CD133 (hematopoietic marker) and CD14 (monocyte marker); the endothelial colony-forming cells (ECFCs) lose these markers and express CD34 and vascular endothelial growth factor receptor 2 (VEGFR2) [5,6]. In addition to these markers, ECFCs display fundamental endothelial characteristics, such as proliferation, migration, and *de novo* vessel formation. On the other hand, early EPCs cannot form vessels, rather, they release various pro-angiogenic molecules, that is, VEGF, stromal cell-derived factor-1α (SDF1-α), and matrix metallopeptidase 9 [7]. Moreover, ECFCs can secrete pro-angiogenic factors but to a lesser extent and under special conditions. Exceptions to this limited release are endothelial nitric oxide synthase (eNOS) and caveolin-1, which are essential for their role in tubulogenesis [7].

Table 1. A direct comparison of the main characteristics of early EPCs and ECFCs.

Characteristic	Early EPCs	ECFCs
Culture isolation time	5 – 7 days	1 – 3 weeks
Colony shape	Spindle shaped	Cobblestone appearance
Tube formation	No tube formation	Tube formation on Matrigel
Paracrine angiogenesis	Better	Good
Support binding to UEA-1 and Ac-LDL uptake	Yes	Yes
eNOS and caveloin-1	Not found	Found
CD133, CD45 and CD14	+	-
CD34 and VEGFR-2	Low	High
Replating capacity	-	+

Ac-LDL: Acetylated low-density lipoprotein, ECFCs: Endothelial colony-forming cells, eNOS: Endothelial nitric oxide synthase, EPCs: Endothelial progenitor cells

EPCs are located in the specific zones between smooth muscle and the adventitial layers and can differentiate into mature ECs [8]. These vasculogenic zones are only found in large and mid-sized vessels. The cells from these niche-like zones provide progenitor cells for postnatal vasculogenesis [8]. A recently published systematic review of 125 studies has implied age-related dysfunctionality of these cells to adopt atherogenic phenotype, thus leading to altered vascular homeostasis. EPCs have been isolated from the bone marrow, adipose tissue stem cells, umbilical cord blood, induced pluripotent stem cells, and embryonic stem cells [9-14]. Umbilical cord blood is considered an ideal source of EPCs due to their acceptable immunological characteristics, long telomeres, and ease of availability without ethical issues [15].

Interestingly, EPCs derived from different tissue and cell sources have distinct transcriptomic and proteomic profiles [16]. For example, a direct comparison between umbilical cord blood and peripheral blood-derived EPCs revealed 1133 differentially expressed genes in the umbilical cord blood, of which 675 genes were upregulated and 458 genes were downregulated compared to peripheral blood-derived EPCs [17].

2.1. EPCs and paracrine activity

Akin to the other stem and progenitor cells, EPCs release many bioactive molecules as a part of their paracrine activity to exert cytoprotective and reparative effects [18]. EPCs primarily contribute their pro-angiogenic activity by undergoing endothelial differentiation and integration within the neovasculature. Moreover, EPCs release soluble pro-angiogenic factors, which directly or indirectly support the angiogenesis process by influencing migration, differentiation, mesenchymal to endothelial transition (MET), and integration of the differentiated cells as part of the intrinsic repair process [19]. EPCs also release sEVs with a specific payload of pro-angiogenic miRNAs that regulate the molecular signaling pathways involved in neovascularization. In a recently published transcriptome analysis using the published data from PubMed and Gene Ontology databases, Abdelgawad *et al.* have grouped 61 EPCs secreted paracrine factors in five major groups relevant to their primary function, that is, angiogenesis and vascular development group; proliferation, migration, and survival group; immunomodulation group; small molecules such as mRNA, lncRNA, miRNA, etc., with regulatory activities; and molecules involved in ligand internalization and endocytosis, etc. [19]. The more commonly reported EPC-derived angiogenic factors in their conditioned medium are summarized in Table 2.

It is pertinent to mention that the composition of EPCs' secretome is influenced by many factors, that is, cells' physical, chemical, and genetic manipulation, making it amenable to changes in its soluble factors composition [26]. For example, modifying culture conditions significantly enhance the quality and composition of paracrine factors. In a recent study, the culture of EPCs under simulated microgravity 3D culture conditions induced HIF-1α-mediated eNOS and nitric oxide release in the secretome [27].

DOI: http://dx.doi.org/10.18053/jctres.08.202206.004

Table 2. Paracrine soluble factors released by EPCs.

Author/year	Commonly reported growth factors in the conditioned medium
Rehman *et al*., 2003 [20]	VEGF, HGF, G-CSF, and GM-CSF.
Urbich *et al*., 2005 [21]	VEGF-A, VEGF-B, SDF-1, and IGF-1
Quevedo *et al*. (2009) [22]	IGF, VEGF, and PDGF
Mirotsou *et al*., 2010 [23]	VEGF, SDF-1, TGF-β1, tissue factor 1, and MCP-1
Di Santo *et al*., 2014 [24]	Angiogenin, EGF, bFGF, leptin, thrombopoietin, PDGF-BB, VEGF, and VEGF-D
Maki *et al*., 2018 [25]	Angiogenin, SDF-1, PDGFAA/AB/BB, VEGF-B, several MPPs, thrombospondin-2; IGF-1, EGF, and interleukin-1β.

Ang: Angiopoietin, bFGF: Basic fibroblast growth factor, EGF: Epidermal growth factor, G-CSF: Granulocyte colony-stimulating factor, GM-CSF: Granulocyte-macrophage colony-stimulating factor, HGF-1: Hepatocyte growth factor, IGF-1: Insulin-like growth factor 1, PDGF: Placenta-derived growth factor, SDF-1: Stromal cell-derived factor, VEGF: Vascular endothelial growth factor.

The paracrine secretions of the cultured cells were rich in pro-angiogenic factors that caused EPCs' enhanced proliferation and migration activity to support tubulogenesis and accelerated bone fracture healing in an experimental animal model [27].

Similarly, BM-derived EPC-seeded sheets significantly improved the paracrine activity of the cells compared to the monolayer cell culture [28]. The authors observed copious secretions of VEGF, EGF, and SDF1a, with the possible involvement of the PI3k/Akt/eNOS signaling pathways. Moreover, EPCs from the cell sheets showed better migration and tubulogenesis compared to single-cell suspensions [28]. The authors attributed this functional improvement to the expression of pro-angiogenic factors. Similar data have also been reported by Kawamura *et al.* who developed tissue-engineered bilevel sheets of smooth muscle cells and EPCs in temperature-responsive cell culture dishes [29]. Transplantation of bilayered cell sheets significantly preserved cardiac function in a rodent heart model of diabetes-induced cardiomyopathy. A concomitant reduction in cardiac fibrosis and attenuated left ventricular remodeling was also observed [29]. Fate tracking of the transplanted cells revealed their integration in the recipient heart neovasculature. Molecular studies showed significantly upregulated VEGF protein expression in the cell sheet transplanted hearts as compared to the untreated controls. Other physical and environmental factors impacting the secretome profile of EPCs have been detailed by Yan *et al.* [18].

3. EPCs and Release of sEVs

EPCs' paracrine secretions are rich in exosomes with a specific payload of bioactive molecules. Exosomes are nano-sized sEVs (<150 nm in diameter) produced intracellularly by the fusion of the multivesicular bodies with the cell membrane [26]. Following MISEV guidelines, we have used sEVs to replace exosomes throughout the manuscript [30]. Initially considered as "garbage bags" of the cells to eliminate metabolic waste, these vesicles are integral to the process of intercellular communication. The sEVs payload primarily contains DNA, miRNA, cytokines, growth factors, lipids, metabolites, and proteins for transfer to the neighboring cells [31,32]. Zeng *et al.* have provided a head-to-head comparison of EPCs and their derived sEVs, showing the superiority of sEVs for their safety profile, that is, lack of immunogenicity and immunological rejection, tumorigenicity, metastasis, etc., however, with the same therapeutic potential as that of their EPCs precursors [33]. They are gaining popularity as an important candidate for cell-free therapy due to the possibility of off-the-shelf availability, high-level biocompatibility, low immunogenicity, and lack of tumorigenicity compared to their parent cells of origin with comparable reparability [30].

Given their pro-angiogenic payload, EPCs-derived sEVs have been considered a potential therapeutic tool for treating vascular dysfunction pathologies [34]. Interestingly, besides the expression of sEVs membrane surface protein markers, that is, CD63, CD81, and CD9, they are rich in endothelial cell-specific marker proteins, that is, CD31, VEGFR2, CD146, endoglin, and VE-cadherin, the expression level of which may fluctuate with the health of the donor sourced to isolate the EPCs [35]. The cell origin-specific markers, that is, CD105/CD144 and CD34/KDR, have been used to identify sEVs derived from ECs and EPCs, respectively [36].

4. EPC-Derived sEVs Payload Profiling

Despite their extremely small size, EVs are the carriers of a heterogeneous mix of biomolecules. The outer bilayer lipid membrane primarily protects sEVs payload until they fuse with the recipient cell membrane to deliver their payload (Figure 1).

The underlying mechanism of payload assortment and loading into sEVs and the process behind determining the nature and proportions of the payload remain less well-understood. Nevertheless, it appears that the nature, composition, and proportion of payload in sEVs depend on the tissue and cell of origin and the types of diverging cues received by the parent cells [16]. The payload is horizontally transferred to the recipient cells during treatment with sEVs to initiate the desired functionality. It remains the primary information transfer mechanism from sEVs to the recipient cells [37]. For example, EPC-derived sEVs have been shown to activate the angiogenic program by transferring relevant mRNA in the recipient ECs [37]. Interestingly, pre-treatment of EVs with RNAse abrogated the pro-angiogenic and pro-proliferative effects of sEVs treatment on ECs [38]. It is generally considered that the diversity of genetic material in the payload forms the basis of various functional outcomes after sEVs treatment. Different research groups have published transcriptome profiling of EPCs with or without manipulation of the cells. Salybekov and colleagues have reported a direct comparison of miRs profiles of EPCs and their derivative sEVs and showed that the majority of the upregulated miRs were shared between them [30]. The commonality of miRs strongly suggests a similarity of transcriptome profiles between EPCs and their derivative sEVs.

In silico analysis also showed the involvement of the highly expressed miRs in supporting angiogenesis. In a recent study, miR profiling of EPC-derived sEVs revealed 385 differentially expressed miRs compared to EPCs, out of which 100 were upregulated and 285 were downregulated [39]. It is pertinent to

DOI: http://dx.doi.org/10.18053/jctres.08.202206.004

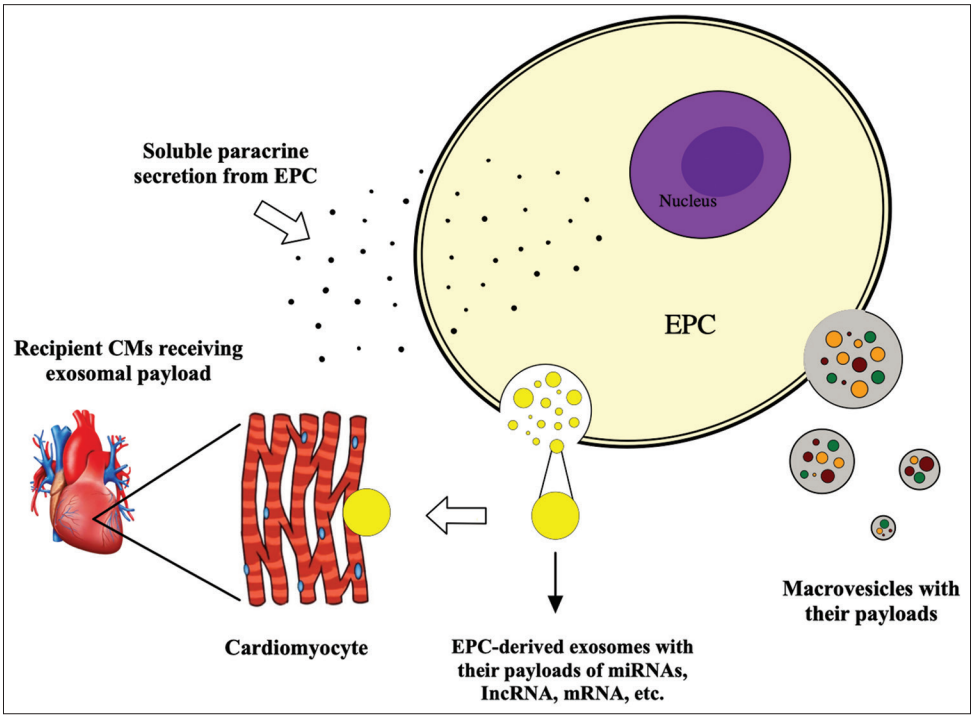

Figure 1. Schematic representation of sEVs delivery of payload to the cardiomyocytes after EPC-based cell therapy.

mention that the miRs profile of EPCs and their derivative sEVs get altered in response to many factors [33].

5. EPC-Derived sEVs for Myocardial Angiogenesis and Repair in Experimental Animal Models

The release of sEVs as part of the EPCs' paracrine activity is central to the tissue repair mechanism rather than their actual engraftment in the host tissue [18]. EPC-derived sEVs deliver their payload to the recipient cells in the heart to contribute to the myocardial repair process, that is, reduction in cardiomyocyte apoptosis, reduction in cardiac fibrosis, attenuated remodeling, increased angiogenesis, and favorable changes in the cardiac hemodynamic parameters [39-42]. Sahoo *et al.* reported that the treatment of human umbilical vein endothelial cells (HUVEC) with $CD34^+$ cell-derived sEVs significantly induced their angiogenic activity *in vitro*, which was equivalent to the angiogenic induction with $CD34^+$ cell coculture or treatment with their conditioned medium. On the other hand, sEVs' depletion in the conditioned medium resulted in the abrogation of angiogenic activity in HUVEC [43]. A comparable angiogenic response was observed during Matrigel plug and corneal angiogenesis assays in mice in response to the treatment with $CD34^+$ cell-derived sEVs. The authors attributed the pro-angiogenic activity of sEVs to the pro-angiogenic miRNAs transferred to the recipient cells [43]. Using a rat model of balloon-induced vascular injury, Li *et al.* reported that sEVs from human umbilical cord blood-derived EPCs supported successful re-endothelialization in the rat carotid artery [44]. These data were supported by *in vitro* experiments, which showed that treatment with conditioned medium rich in sEVs enhanced ECs proliferation and migration, in addition to the increased expression of pro-angiogenic factors. The protective role of EPC-derived sEVs has also been observed on ECs and smooth muscle cells (SMCs) in an experimental rat model [27]. The authors used a carotid balloon injury model to determine the protective effects of EPC-derived sEVs. Treatment with sEVs significantly reduced the intimal to medial ratio compared to the control group on days 14 and 28 of observation. However, no re-endothelialization was observed in rats sacrificed on earlier time points of days 2 and 4. The authors attributed these effects to improved ECs function and repair rather than suppressing SMCs proliferation and migration. Moreover, sEV-treated ECs showed increased expression of pro-angiogenic growth factors [27].

Chen *et al.* used a sustained-release approach for EPC-derived sEVs delivery using shear-thinning hydrogel (STG) technology to treat infarcted myocardium [40]. sEVs were isolated from allogeneic rat bone marrow-derived EPCs' conditioned medium and incorporated in STG. *In vitro* characterization by nanoparticle tracking analysis showed that sEVs were uniform in size distribution between 50 and 200 nm and were rich in several pro-angiogenic factors [40]. The rat heart model of acute myocardial infarction (AMI) was developed by coronary left anterior descending (LAD) artery ligation and sEVs suspended in saline or shear-thinning hydrogel were delivered by intramyocardial injections under direct vision in the peri-infarct zone. STG enhanced injectate retention with a cumulative sEVs release for 21 days of observation. Four weeks later, animal hearts treated with STG + sEVs or STG+ EPCs showed significantly increased capillary density and improved hemodynamic functions compared with the saline-treated control group [40]. Besides significant reduction in fibrosis and scar size, the STG-sEVs treated animals

DOI: http://dx.doi.org/10.18053/jctres.08.202206.004

had preserved LV geometry and improved LVEF. These data show the therapeutic benefits of sEVs, especially when manipulated, so their washout from the delivery site might be delayed. The same group has previously used STG technology to deliver EPCs and reported the sustained effects of cell-based therapy in enhanced vasculogenesis, preserved myocardial geometry, reduced fibrosis, and improved ischemic myocardial function, as observed by echocardiography and Doppler flow analysis [45,46]. Put together, prolonged cell retention, slow release from STG, and incorporation in the vascular structures at the site of the cell graft were critical to limiting adverse myocardial remodeling and preserving global contractile function.

Chung *et al.* used a similar approach of STG-based delivery of EPC-derived sEVs. However, the intramyocardial injection of STG-sEVs in the rat model of AMI was performed at different time points, that is, 0 and 3 h to 4 days and 2 weeks, after LAD coronary artery ligation [47]. Moreover, the authors observed significant preservation of the left ventricular function and hemodynamic parameters and reduced scar thickness in the animal groups receiving treatment on day 4 after AMI. They also noted the effect of prolonged hypoxia in culture on the quality of EPC-derived sEVs, which showed a higher rate of uptake and more tubulogenesis than the normoxia cultured EPC-derived sEVs [47]. Besides showing the therapeutic significance of EPC-derived sEVs, the data also show the importance of optimized treatment timing with sEVs, which may differ from the optimal time of cell-based therapy. Therefore, future studies are warranted to determine the optimal timing for sEV-based myocardial treatment.

Various research groups have elucidated the mechanism of sEVs' contribution to improved cardiac function. They have also reported different strategies to engineer sEVs for their payload and modulate their stability, biodistribution, site-specific targeting, and uptake by the target cells. For example, EPCs have been genetically and epigenetically modulated to obtain sEVs with the desired payload [48-50]. EPCs were genetically modified for osteocalcin overexpression such that their derivative sEVs had osteocalcin-rich cargo [48]. Treatment of rat aorta ECs with osteocalcin-rich sEVs *in vitro* significantly enhanced endothelial cell function, proliferation, migration, and angiogenic potential [48]. These are clinically relevant data because osteocalcin overexpressing EPCs have been observed in patients with cardiovascular pathologies and have a significant role in coronary atherosclerosis [51].

Yue *et al.* used interleukin-10 (IL-10) knockout mice to investigate how IL-10 abrogation affects the payload of EPC-derived sEVs and their reparative potential [49] in comparison with wild-type EPC-derived sEVs. Using a mouse model of AMI, treatment with wild-type EPC-derived EVs significantly reduced cell apoptosis, infarct size, and promoted neovascularization compared to the IL-10 knockout mice EPC-derived sEVs. A comparative analysis of the sEVs payload from the two cell types revealed a distinct payload profile for each sEVs type. The data provided direct evidence of how systemic inflammation can affect the reparability of EPCs and their derived sEVs with a possible role of IL-10 and integrin-linked kinase (ILK), which was enriched in the IL-10 knockout EPCs and their derived sEVs. Although IL-10 abrogation did not alter sEVs secretion, the secreted sEVs were found to be functionally inert post-delivery in a rat model of MI [49]. Unlike the animals treated with wild-type sEVs, there was no significant improvement in cardiac function until 31 days after treatment with IL-10 knockout EPC-derived sEVs. Another research group has shown that IL-10 deficiency impaired the reparative potential of EPC-derived sEVs with negative regulation of miR-375 by IL-10, which significantly contributed to the reparability of bone marrow-derived EPCs [52].

Given that fibrogenic activity post-MI is the primary intrinsic repair mechanism to replace dead myocytes, various research groups have explored the possibility of using stem/progenitor cell-derived sEVs to interfere with fibroblasts' activation and regulation of fibrosis as an alternative to cell-based therapy [53]. Ke *et al.* used EPC-derived sEV-based treatment strategy to alleviate fibrogenic activity in the ischemic myocardium [41]. The authors used late-stage EPCs from peripheral blood to isolate sEVs to assess their role in determining cardiac fibroblasts' (CFs) differentiation to adopt EC phenotype, promoting their proliferation and participation in angiogenesis through supporting MET *in vitro*. Treatment with sEVs significantly increased endothelial-specific markers, that is, CD31 and VEGR2, on CFs and reduced the intrinsic expression of alpha-smooth muscle actin, vimentin, collagen, and TGF-beta. Besides promoting MET, there was a significant reduction in the myocardial fibrosis regulated high mobility group box 1 protein B1 (HMGB1) [41]. With this encouraging data *in vitro*, the authors suggested future studies to assess the EPC-derived sEVs to promote myocardial angiogenesis.

Based on their previously published data that treatment with sEV-derived from EPCs increased cell proliferation and angiogenesis in CFs [41], the authors investigated the therapeutic efficacy of EPCs sEV-derived miRs, especially the two differentially expressed miR-363-3p and miR-218-5p in a rat model of MI [39]. A total of 385 differentially expressed miRs were detected in EPC-derived sEVs as compared to EPCs. CFs' treatment with sEVs miR-218-5p or miR-363-3p upregulated p53, downregulated the junction-mediating and regulatory protein (JYM), and promoted their MET. Infarcted animal hearts treated with sEVs or the two miRs mimic restored myocardial tissue integrity through p53/JYM signaling. The authors also observed improved left ventricular ejection fraction and left ventricular fractional shortening. While von Willebrand factor expression was elevated, smooth muscle actin expression was reduced dramatically [39].

As a part of the novel developments to enhance the therapeutic and functional benefits of EPC-derived sEVs, attempts are also underway to modify sEVs derived from EPCs by loading sEVs with the payload of interest. In this regard, researchers have focused on genetically modifying the payload of the EPC-derived sEVs to enhance their anti-apoptotic and pro-survival effects, as apoptosis remains integral to cardiovascular diseases, that is, atherosclerosis, ventricular remodeling, heart failure, myocardial infarction, and other peripheral arterial disorders [54,55]. Numerous recent studies have suggested that EPC-derived sEVs exhibit antiapoptotic properties that protect the cells through altering miRNAs and

DOI: http://dx.doi.org/10.18053/jctres.08.202206.004

several downstream signaling cascades. For example, published data show that hypoxamir-210 has a cytoprotective effect against hypoxic injury *in vitro* and post-engraftment of stem cells [56,57]. Ma *et al.* loaded EPC-derived sEVs with miR-210 mimetics to enhance their cytoprotective effects [58]. The authors genetically modified human EPCs with miR-210 mimetics and collected their sEVs for use in functional analysis. Treatment with sEVs carrying miR-210 payload successfully protected ECs subjected to hypoxia-reoxygenation injury by protecting their mitochondrial function. The authors also observed enhanced migrational activity and tubulogenesis *in vitro* in ECs treated with sEVs carrying miR-210 payload. On the same note, Zhang *et al.* adopted a pre-conditioning approach to prime EPCs by angiotensin-converting enzyme 2 (ACE2) overexpression, which showed upregulated miR-18a [59]. Treatment with sEVs from ACE2-primed EPCs significantly protected ECs against hypoxia-reoxygenation injury. Mechanistically, the authors proposed that miR-18a downregulated Nox2/reactive oxygen species (ROS) pathway. In another study, endothelial colony-forming cell-derived sEVs were rich in miR-486-5p, acting on the PTEN/Akt pathway [60]. Results of this study showed decreased PTEN and increased Akt phosphorylation, which ameliorated apoptosis. Similarly, the upregulation of miRNA 375-3p enhanced cardiac function, reduced apoptosis, inflammation, and oxidative stress, in cardiomyocytes in septic rats [61]. As the underlying mechanism of antiapoptotic effects of EPCs-derived sEVs remains unclear, further research is warranted to support their pre-clinical and clinical use. Some of these studies are summarized in Table 3.

6. Clinical Use of EPC-Derived sEVs

Despite promising data from the experimental animal studies regarding the safety and efficacy of sEVs from different stem/progenitor cells, including EPCs for myocardial angiogenesis and repair, their use in patients remains a less explored area in research, especially in the field of regenerative medicine. Today, when this narrative review is being written (September 16, 2022), there are 282 clinical trials listed on www.clinicaltrials.gov, using exosomes as a keyword. On the other hand, using the term sEVs fetched 123 clinical trials. Most of these clinical studies primarily focus on the therapeutic use in non-cardiac conditions, including

Table 3. EPC-derived payload in experimental animal studies.

Author/year	Mode of delivery	Model type	Animal	Main findings
Chen *et al*., 2018 [40]	I/M injection	AMI	Rat	EVS. Robust increase in capillary density was observed in the EPC-sEVs treatment group with significantly preserved cardiac function and improved hemodynamic parameters of the heart. These pro-angiogenic effects in the heart were supported by *in vitro* experiments. Of the 2000 unique proteins analyzed in EPCs and sEVs, 1327 were unique for EPCs, 47 for sEVs, and 662 were common between the two, with several of these molecules being pro-angiogenic.
Yue *et al*., 2020 [49]	I/M injection	MI	Mice	EVSEVSILK was highly enriched in IL-10 knockout EPC-derived sEVs abrogation of which restored reparative function of sEVs, restored cardiac function, and reduced infarct size with significant increase in capillary density in the infarcted heart.
Chung *et al*., 2020 [47]	I/M injection	MI	Rat	sEVs from hypoxia-treated EPCs showed higher uptake during *in vitro* experiments and higher rate of tubulogenesis. EPC-derived sEVs delivered I/M in the peri-infarct area in experimental heart model on day 4 after MI were therapeutically most effective than sEVs delivered on other time points post-MI, that is, 0 h, 3 h, 4 days, and 2 weeks post-MI. Besides improved cardiac functions, there was significant increase in blood vessel density in the infarct region.
Huang *et al*., 2021 [42]	I/M injection	AMI	Rat	In vitro experiments showed that sEVs with the two miRs 1246 and 1290 promoted phenotypic changes in cardiac fibroblasts to ECs and angiogenesis in human CFs. Abrogation of the two miRs significantly reduced the angiogenic potential of EPC-derived sEVs. I/M injection of EPC-derived sEVs or sEVs overexpressing mimics of miR-1246 and miR-1290 significantly reduced myocardial injury in rat model of AMI. There was increase in ELF5, SP1, and CD31 expression but a concomitant reduction in alpha smooth muscle actin in the exosome-treated animal hearts. Besides histological evidence of attenuated infarct size, LVEF and LVFS were significantly better in the exosome-treated animals compared to control.
Ke *et al*., 2021 [39]		AMI	Rat	A total of 385 differentially expressed miRs were detected in EPC-derived sEVs as compared EPCs, of which 100 were upregulated and 285 were downregulated. Treatment of cardiac fibroblasts *in vitro* with sEVs miR-218-5p or miR-363-3p upregulated p53 and downregulated JYM, promoted mesenchymal to endothelial transition, and prevented cardiac fibrosis. Infarcted animal hearts treated with sEVs or the two miR mimics led to restored myocardial tissue integrity through p53/JYM signaling. Improved LVEF and LVFS were observed. While vWF expression was elevated, the expression of smooth muscle actin was reduced dramatically.
Chung *et al*., 2021 [62]		AMI	Rat	The study was intended to determine the feasibility of off-the-shelf allogenic EPC-derived sEVs cryopreserved before use in an experimental rat model of AMI. STG-suspended sEVs delivered intramyocardially successfully improved myocardial contractile function, enhanced angiogenesis, and attenuated scar thickness. More importantly, there was minimal immune activity observed at 4 weeks after treatment. The therapeutic activity of sEVs was retained until 2 months of cryopreservation.

AMI: Acute myocardial infarction, vWF: von Willebrand factor, JYM: Junction-mediating and regulatory protein, I/M: Intramyocardial, LVEF: Left ventricular ejection fraction, LVFS: Left ventricular fractional shortening

DOI: http://dx.doi.org/10.18053/jctres.08.202206.004

diabetes type-I and type-II, stroke, chronic kidney disease, macular degeneration, cancer, and cancer-associated conditions, COVID-19, and ARDS. Besides, some of these studies assess the diagnostic potential of sEVs (Table 4). The term EPC-derived sEVs and heart diseases or cardiovascular diseases did not fetch any clinical trials. Using EPC-derived sEVs with a well-defined cargo may provide a safer therapeutic option in the future than EPCs.

Table 4. Therapeutic and diagnostic use of sEVs for different pathological conditions (data from Clinicaltrials.gov).

NCT identifier	Study title	Disease	Method of intervention	Institution and location of the trial
Therapeutic applications of sEVs in clinical trials				
ClinicalTrials.gov Identifier: NCT04327635	Safety evaluation of intracoronary infusion of sEVs in patients with AMI	Heart attack	Drug: PEP in Acute Myocardial Infarction	Mayo Clinic Rochester, Minnesota, United States
(ClinicalTrials.gov Identifier: NCT05109364	Antiplatelet therapy effect on sEVs in acute myocardial infarction	Myocardial infarction	Drug: Ticagrelor Drug: Clopidogrel	Laboratory of Experimental Clinical Chemistry, Academic Medical Centre of the University of Amsterdam Amsterdam, Netherlands
(ClinicalTrials.gov Identifier: NCT03478410)	Role of sEVs derived from epicardial fat in atrial fibrillation	Atrial Fibrillation	Procedure: Epicardial fat biopsy	Sheba medical center Ramat Gan, Israel
(ClinicalTrials.gov Identifier: NCT03384433)	Allogenic MSC-derived sEVs in patients with acute ischemic stroke	Cerebrovascular disorders	Biological: sEVs	Shahid Beheshti University of Medical Sciences, Tehran, Iran
(ClinicalTrials.gov Identifier: NCT04798716)	A pilot clinical study on inhalation of MSCs sEVs treating severe novel coronavirus pneumonia	Coronavirus	Biological: MSCs-derived sEVs	Ruijin Hospital Shanghai Jiao Tong University School of Medicine, Shanghai, Shanghai, China
(ClinicalTrials.gov Identifier: NCT05413148)	The effect of stem cells and stem cell sEVs on visual functions in patients with retinitis pigmentosa	Retinitis pigmentosa	Biological: Subtenon injection of Wharton jelly derived MSCs Biological: Subtenon injection of Wharton jelly derived MSCs sEVs. Other: Placebo	Erciyes University, Faculty of Medicine. Kayseri, Turkey
(ClinicalTrials.gov Identifier: NCT02138331)	Effect of microvesicles and sEVs therapy on β-cell mass in T1DM.	T1DM	Biological: MSC sEVs	Sahel Teaching Hospital Sahel, Cairo, Egypt
(ClinicalTrials.gov Identifier: NCT05261360	Clinical efficacy of sEVs in degenerative meniscal injury	Knee; injury, meniscus (lateral) (medial), meniscus tear, meniscus lesion (and 6 more...)	Drug: SF-MSC-EX Drug: SF-MSC	Eskisehir Osmangazi University, Eskisehir, Turkey
(ClinicalTrials.gov Identifier: NCT04388982)	The safety and the efficacy evaluation of allogenic adipose MSC-sEVs in patients with Alzheimer's disease	Alzheimer Disease	Biological: Low, mild, and high dosage MSCs-sEVs administrated for nasal drip	Ruijin Hospital Affiliated to Shanghai Jiaotong University School of Medicine Shanghai, Shanghai, China
(ClinicalTrials.gov Identifier: NCT04602442)	Safety and efficiency of method of sEVs inhalation in COVID-19-associated pneumonia.	Covid19 SARS-CoV-2 PNEUMONIA COVID-19	Drug: sEVs 1 Inhalation Drug: sEVs2 inhalation Drug: Placebo inhalation	Medical Centre Dynasty Samara, Russian Federation
(ClinicalTrials.gov Identifier: NCT04602104	Clinical study of mesenchymal stem cell sEVs nebulizer for the treatment of ARDS acute respiratory distress syndrome	Acute respiratory distress syndrome	Biological: Low-dose hMSC-sEVs. Biological: Medium-dose hMSC-sEVs. Biological: High-dose hMSC-sEVs (and 3 more.)	Ruijin Hospital, Medical School of Shanghai Jiaotong University. Shanghai, Shanghai, China
sEVs as diagnostic tools				
(ClinicalTrials.gov Identifier: NCT04127591	Differential expression and analysis of peripheral plasma sEVs miRNA in patients with myocardial infarction	Myocardial infarction	Other: sEVs	Ethics Committee of Xinhua Hospital Shanghai, Shanghai, China
(ClinicalTrials.gov Identifier: NCT05424029	sEVs and particles (EVP) as biomarkers of recurrence in non-small cell lung cancer	Non-small cell lung cancer	Exosome	Memorial Sloan Kettering Cancer Center. New York, New York, United States
(ClinicalTrials.gov Identifier: NCT05370105)	sEVs as stroke biomarkers	Stroke Rehabilitation	Other: blood withdrawal	IRCCS Don Gnocchi, Fondazione Don Carlo Gnocchi ONLUS. Florence, Italy. IRCCS S. Maria Nascente, Fondazione Don Carlo Gnocchi ONLUS Milano, Italy

(Contd...)

DOI: http://dx.doi.org/10.18053/jctres.08.202206.004

Table 4. *(Continued)*.

NCT identifier	Study title	Disease	Method of intervention	Institution and location of the trial
sEVs as diagnostic tools				
(ClinicalTrials.gov Identifier: NCT04127591)	Differential expression and analysis of peripheral plasma sEVs miRNA in patients with MI	MI	Other: sEVs	Ethics Committee of Xinhua Hospital, Shanghai, China
(ClinicalTrials.gov Identifier: NCT04164966	Development of novel biomarkers for the early diagnosis of type 1 diabetes	Type 1 Diabetes (EXOSARC)	sEVs	AdventHealth Translational Research Institute Orlando, Florida, USA
(ClinicalTrials.gov Identifier: NCT03830619)	Serum sEV-derived long non-coding RNAs as potential biomarkers for lung cancer diagnosis	Lung cancer	Diagnostic test: Collect samples	Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hub
(ClinicalTrials.gov Identifier: NCT03874559)	sEVs in rectal cancer	Rectal cancer	Diagnostic test: Blood draw	University of Kansas Medical Center/Cancer Center, Kansas City, Kansas, USA

sEVs: Small extracellular vesicles, miRNA: microRNA, MSC: Mesenchymal stem cell

Table 5. Some recent studies reporting EPC sEV-derived miRs, their respective target genes, and the outcome of the downstream signaling activation.

Author	EPC-derived sEVs miRNAs	Target genes	Signaling pathway activation
Zhang *et al*., 2016 [65]	miR-21	Erk1/2 signaling	EPC-sEVs stimulated angiogenic activities of ECs by activating Erk1/2 signaling, which finally facilitates cutaneous wound repair and regeneration
Zhou *et al*., 2018 [66]	miR-126-5p and 3p	HMGB1 and VCAM1	Suppression of LPS-induced HMGB1 and VCAM1 levels in human microvascular ECs
Wu *et al*., 2018 [67] and Jia *et al*., 2019 [68]	miR-126	SPRED1	SPRED1 and implicated RAF/ERK signaling pathways
Huang *et al*., 2021 [42]	miR-1246 and miR-1290	ELF5 and SP1	ELF5 and SP1 played important roles in HCFs transforming in to ECs and subsequent angiogenesis
Ke *et al*., 2021 [39]	miR-218-5p/miR-363-3p	P53 and JMY	Mesenchymal to endothelial transition of CFs and proliferation, angiogenesis, and tube formation of CFs through p53/JMY signaling pathway
Ke *et al*., 2022 [69]	miR-21-5p	SIPA1L2	Regulates autophagy flux to promote vascular endothelial repair by inhibiting SIPL1A2
Halurkar *et al*., 2022 [70]	miR-126	PI3/AKT	PI3K/Akt pathway to protect cells against hypoxia-reoxygenation injury and apoptosis

CFs: Cardiac fibroblasts, ECs: Endothelial cells, HMGB1: High mobility group box 1, miR: microRNA, VCAM1: Vascular cell adhesion molecule 1

Given EPC-derived sEVs can be easily isolated from various body fluids and characterized for their payload, there is a broader scope for their detection and characterization for use as diagnostic and prognostic markers in various cardiovascular pathologies. For use as a therapeutic modality, optimizing protocols for large-scale clinical grade EPC-sEVs production, their delivery strategy, biodistribution, and elimination and other kinetic features are essential to ensure optimal therapeutic outcomes. Moreover, a hybrid approach based on a mixture of EPCs and their derivative sEVs may enhance their therapeutic efficiency.

FDA has recently approved three Investigational New Drugs (IND) applications using Purified sEVs for three different conditions, including wound healing (Phase 1b/2a study; ClinicalTrials.gov identifier: NCT04664738), fistulizing Crohn's disease, and AMI. For AMI, a Phase Ib/2a entitled "Safety Evaluation of Intracoronary Infusion of Extracellular Vesicles in Patients With AMI" has been initiated (ClinicalTrials.gov identifier: NCT04327635). Dr. Guy Reeder at Mayo Clinic is leading an interventional study recruiting patients (21 – 85 years of age) to assess dose-limiting toxicities and the maximum tolerated dose of a single dose of PEP™ (during PCI or AMI) at escalating concentrations of EVs.

7. Conclusion and Future Perspective

EPCs, the precursors of ECs, are considered potential candidates for biological bypass surgery of the ischemic myocardium through myocardial angiogenesis, besides their potential to serve as non-invasive diagnostic and prognostic biomarkers. They are also a rich source of pro-angiogenic factors. The release of sEVs is an integral part of the paracrine mechanism underlying EPC-based therapy for myocardial repair and regeneration; hence, cell-free therapy using sEVs is gaining more acceptance due to biocompatibility, low immunogenicity, and ease of large-scale production. Despite being nanosized, the molecular payload of sEVs is vast and diverse, ranging from DNA, mRNA, and miRNA to growth factors, metabolites, and proteins. The payload composition, its paracrine release, and uptake by the recipient cells are influenced

DOI: http://dx.doi.org/10.18053/jctres.08.202206.004

by physical, genetic, and environmental factors. Table 5 summarizes some recently published studies reporting the role of EPCs sEV-derived miRNAs in diverse cellular processes. Besides their natural payload, they can be engineered for desired results by carrying a payload of interest, which can be loaded by various techniques, that is, transfection or electroporation of the EPCs with the gene of interest, genetic, physical, or pharmacological preconditioning of EPCs, etc. However, the protocols for these payload manipulations must be further developed and optimized. In addition, they can be surface modified for targeted delivery to the recipient cells. As discussed, EPC-derived sEVs are rich in pro-angiogenic growth factors and miRs. Therefore, they are considered ideal candidates for biological bypass surgery of the ischemic myocardium through angiogenesis as a part of the reparative processes (Table 5).

Regarding EPCs' participation in angiogenesis, the miRNA component of their derivative sEVs-payload remains the most important pro-angiogenic molecule regulating pathways involved in neovascularization. Experimental animal studies have demonstrated that these miRNAs functionally preserve the ischemic heart through different mechanisms, including enhanced epithelial cells migration, proliferation, MET, prevention of hypoxia-reoxygenation injury, mitochondrial protection, and reduction in fibrosis and attenuation of ventricular remodeling [63]. The future of sEV-based cell-free therapeutic modality is promising, as protocols to genetically modify the sEVs payload and their surface modification for targeted delivery is expected to enhance their angiogenic potential, tissue healing, and integrity [64].

Acknowledgments

None.

Funding

None.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Haider KH, Aziz S. Stem Cells-from Hype to Real Hope. Germany: De Gruyter; 2019.

[2] Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, *et al*. Isolation of Putative Progenitor Endothelial Cells for Angiogenesis. Science 1997;275:964-6.

[3] Schatteman GC, Awad O. Hemangioblasts, Angioblasts, and Adult Endothelial Cell Progenitors. Anat Rec A Discov Mol Cell Evol Biol 2004;276:13-21.

[4] Zengin E, Chalajour F, Gehling UM, Ito WD, Treede H, Lauke H, *et al*. Vascular Wall Resident Progenitor Cells: A Source for Postnatal Vasculogenesis. Development 2006;133:1543-51.

[5] Haider KH, Aziz S, Al-Reshidi MA. Endothelial Progenitor Cells for Cellular Angiogenesis and Repair: Lessons Learned from Experimental Animal Models. Regen Med 2017;12:969-82.

[6] Rose JA, Erzurum S, Asosingh K. Biology and Flow Cytometry of Proangiogenic Hematopoietic Progenitors Cells. Cytometry A 2015;87:5-19.

[7] Yoon CH, Hur J, Park KW, Kim JH, Lee CS, Oh IY, *et al*. Synergistic Neovascularization by Mixed Transplantation of Early Endothelial Progenitor Cells and Late Outgrowth Endothelial Cells: The Role of Angiogenic Cytokines and Matrix Metalloproteinases. Circulation 2005;112:1618-27.

[8] Pacilli A, Pasquinelli G. Vascular Wall Resident Progenitor Cells: A Review. Exp Cell Res 2009;315:901-14.

[9] Ii M. Bone Marrow-derived Endothelial Progenitor Cells: Isolation and Characterization for Myocardial Repair. Methods Mol Biol 2010;660:9-27.

[10] Nguyen HT, Van Pham P. Conversion of Human Adipose Derived Stem Cells into Endothelial Progenitor Cells. Progress Stem Cell 2017;4:229-40.

[11] Qin M, Guan X, Wang H, Zhang Y, Shen B, Zhang Q, *et al*. An Effective *ex-vivo* Approach for Inducing Endothelial Progenitor Cells from Umbilical Cord blood Cd34+ Cells. Stem Cell Res Ther 2017;8:25.

[12] Shen WC, Chou YH, Huang HP, Sheen JF, Hung SC, Chen HF. Induced Pluripotent Stem Cell-Derived Endothelial Progenitor Cells Attenuate Ischemic Acute Kidney Injury and Cardiac Dysfunction. Stem Cell Res Ther 2018;9:344.

[13] Rufaihah AJ, Haider HK, Heng BC, Ye L, Toh WS, Tian XF, *et al*. Directing Endothelial Differentiation of Human Embryonic Stem Cells via Transduction With an Adenoviral Vector Expressing the Vegf165 Gene. J Gene Med 2007;9:452-61.

[14] Rufaihah AJ, Haider HK, Heng BC, Ye L, Tan RS, Toh WS, *et al*. Therapeutic Angiogenesis by Transplantation of Human Embryonic Stem Cell-Derived cd133+ Endothelial Progenitor Cells for Cardiac Repair. Regen Med 2010;5:231-44.

[15] Janic B, Arbab AS. Cord Blood Endothelial Progenitor Cells as Therapeutic and Imaging Probes. Imaging Med 2012;4:477-90.

[16] Garcia-Martin R, Brandao BB, Thomou T, Altindis E, Kahn CR. Tissue Differences in the Exosomal/Small Extracellular Vesicle Proteome and their Potential as Indicators of Altered Tissue Metabolism. Cell Rep 2022;38:110277.

[17] Gao X, Yourick JJ, Sprando RL. Comparative Transcriptomic Analysis of Endothelial Progenitor Cells Derived from Umbilical Cord Blood and Adult Peripheral Blood: Implications for the Generation of Induced Pluripotent Stem Cells. Stem Cell Res 2017;25:202-12.

[18] Yan F, Liu X, Ding H, Zhang W. Paracrine Mechanisms

of Endothelial Progenitor Cells in Vascular Repair. Acta Histochem 2022;124:151833.

[19] Abdelgawad ME, Desterke C, Uzan G, Naserian S. Single-cell Transcriptomic Profiling and Characterization of Endothelial Progenitor Cells: New Approach for Finding Novel Markers. Stem Cell Res Ther 2021;12:145.

[20] Rehman J, Li J, Orschell CM, March KL. Peripheral Blood "Endothelial Progenitor Cells" are Derived from Monocyte/Macrophages and Secrete Angiogenic Growth Factors. Circulation 2003;107:1164-9.

[21] Urbich C, Aicher A, Heeschen C, Dernbach E, Hofmann WK, Zeiher AM, *et al*. Soluble Factors Released by Endothelial Progenitor Cells Promote Migration of Endothelial Cells and Cardiac Resident Progenitor Cells. J Mol Cell Cardiol 2005;39:733-42.

[22] Quevedo HC, Hatzistergos KE, Oskouei BN, Feigenbaum GS, Rodriguez JE, Valdes D, *et al*. Allogeneic Mesenchymal Stem Cells Restore Cardiac Function in Chronic Ischemic Cardiomyopathy Via Trilineage Differentiating Capacity. Proc Natl Acad Sci 2009;106:14022-7.

[23] Mirotsou M, Jayawardena TM, Schmeckpeper J, Gnecchi M, Dzau VJ. Paracrine Mechanisms of Stem Cell Reparative and Regenerative Actions in the Heart. J Mol Cell Cardiol 2011;50:280-9.

[24] Di Santo S, Seiler S, Fuchs AL, Staudigl J, Widmer HR. The Secretome of Endothelial Progenitor Cells Promotes Brain Endothelial Cell Activity through pi3-kinase and Map-kinase. PLoS One 2014;9:e95731.

[25] Maki T, Morancho A, Segundo PM, Hayakawa K, Takase H, Liang AC, *et al*. Endothelial Progenitor Cell Secretome and Oligovascular Repair in a Mouse Model of Prolonged Cerebral Hypoperfusion. Stroke 2018;49:1003-10.

[26] Haider KH, Aramini B. Mircrining the Injured Heart with Stem Cell-Derived Exosomes: An Emerging Strategy of Cell-Free Therapy. Stem Cell Res Ther 2020;11:23.

[27] Kong J, Wang F, Zhang J, Cui Y, Pan L, Zhang W, *et al*. Exosomes of Endothelial Progenitor Cells Inhibit Neointima Formation After Carotid Artery Injury. J Surg Res 2018;232:398-407.

[28] Xue F, Bai Y, Jiang Y, Liu J, Jian K. Construction and a Preliminary Study of Paracrine Effect of Bone Marrow-Derived Endothelial Progenitor Cell Sheet. Cell Tissue Banking 2022;23:185-97.

[29] Kawamura M, Paulsen MJ, Goldstone AB, Shudo Y, Wang H, Steele AN, *et al*. Tissue-engineered Smooth Muscle Cell And Endothelial Progenitor Cell Bi-Level Cell Sheets Prevent Progression of Cardiac Dysfunction, Microvascular Dysfunction, and Interstitial Fibrosis in a Rodent Model of Type 1 Diabetes-Induced Cardiomyopathy. Cardiovasc Diabetol 2017;16:142.

[30] Salybekov AA, Kunikeyev AD, Kobayashi S, Asahara T. Latest Advances in Endothelial Progenitor Cell-Derived Extracellular Vesicles Translation to the Clinic. Front Cardiovasc Med 2021;8:734562

[31] Edgar JR. Q&a: What are Exosomes, Exactly? BMC Biol 2016;14:46.

[32] Hessvik NP, Llorente A. Current Knowledge on Exosome Biogenesis and Release. Cell Mol Life Sci 2018;75:193-208.

[33] Zeng CY, Xu J, Liu X, Lu YQ. Cardioprotective Roles of Endothelial Progenitor Cell-Derived Exosomes. Front Cardiovasc Med 2021;8:717536.

[34] Xing Z, Zhao C, Liu H, Fan Y. Endothelial Progenitor Cell-derived Extracellular Vesicles: A Novel Candidate for Regenerative Medicine and Disease Treatment. Adv Healthc Mater 2020;9:e2000255.

[35] Harper R, Cockshell M, Bonder C, Reynolds P. Characteristics and Utility of Exosomes Obtained from Peripheral Blood Derived Endothelial Progenitor Cells of Pah Patients. In: ERS International Congress 2017 Abstracts; 2017.

[36] Wang J, Guo R, Yang Y, Jacobs B, Chen S, Iwuchukwu I, *et al*. The novel Methods for Analysis of Exosomes Released from Endothelial Cells and Endothelial Progenitor Cells. Stem Cells Int 2016;2016:2639728.

[37] 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;9:654-9.

[38] Deregibus MC, Cantaluppi V, Calogero R, Lo Iacono M, Tetta C, Biancone L, *et al*. Endothelial Progenitor Cell-Derived Microvesicles Activate an Angiogenic Program in Endothelial Cells by a Horizontal Transfer of MRNA. Blood 2007;110:2440-8.

[39] Ke X, Yang R, Wu F, Wang X, Liang J, Hu X, *et al*. Exosomal mir-218-5p/mir-363-3p from Endothelial Progenitor Cells Ameliorate Myocardial Infarction by Targeting the p53/jmy Signaling Pathway. Oxid Med Cell Longev 2021;2021:5529430.

[40] Chen CW, Wang LL, Zaman S, Gordon J, Arisi MF, Venkataraman CM, *et al*. Sustained Release of Endothelial Progenitor Cell-Derived Extracellular Vesicles from Shear-Thinning Hydrogels Improves Angiogenesis and Promotes Function after Myocardial Infarction. Cardiovasc Res 2018;114:1029-40.

[41] Ke X, Yang D, Liang J, Wang X, Wu S, Wang X, *et al*. Human Endothelial Progenitor Cell-Derived Exosomes Increase Proliferation and Angiogenesis in Cardiac Fibroblasts by Promoting the Mesenchymal-Endothelial Transition and Reducing High Mobility Group Box 1 Protein B1 Expression. DNA Cell Biol 2017;36:1018-28.

[42] Huang Y, Chen L, Feng Z, Chen W, Yan S, Yang R, *et al*. Epc-derived Exosomal Mir-1246 and Mir-1290 Regulate Phenotypic Changes of Fibroblasts to Endothelial Cells to Exert Protective Effects on Myocardial Infarction by Targeting elf5 and sp1. Front Cell Dev Biol 2021;9:647763.

DOI: http://dx.doi.org/10.18053/jctres.08.202206.004

[43] Sahoo S, Klychko E, Thorne T, Misener S, Schultz KM, Millay M, *et al*. Exosomes from Human cd34(+) Stem Cells Mediate their Proangiogenic Paracrine Activity. Circ Res 2011;109:724-8.

[44] Li X, Chen C, Wei L, Li Q, Niu X, Xu Y, *et al*. Exosomes Derived from Endothelial Progenitor Cells Attenuate Vascular Repair and Accelerate Reendothelialization by Enhancing Endothelial Function. Cytotherapy 2016;18:253-62.

[45] Gaffey AC, Chen MH, Venkataraman CM, Trubelja A, Rodell CB, Dinh PV, *et al*. Injectable Shear-Thinning Hydrogels used to Deliver Endothelial Progenitor Cells, Enhance Cell Engraftment, and Improve Ischemic Myocardium. J Thorac Cardiovasc Surg 2015;150:1268-76.

[46] Gaffey AC, Chen MH, Trubelja A, Venkataraman CM, Chen CW, Chung JJ, *et al*. Delivery of Progenitor Cells with Injectable Shear-Thinning Hydrogel Maintains Geometry and Normalizes Strain to Stabilize Cardiac Function after Ischemia. J Thorac Cardiovasc Surg 2019;157:1479-90.

[47] Chung JJ, Han J, Wang LL, Arisi MF, Zaman S, Gordon J, *et al*. Delayed Delivery of Endothelial Progenitor Cell-Derived Extracellular Vesicles Via Shear Thinning Gel Improves Postinfarct Hemodynamics. J Thorac Cardiovasc Surg 2020;159:1825-35.e22.

[48] Yi M, Wu Y, Long J, Liu F, Liu Z, Zhang YH, *et al*. Exosomes Secreted from Osteocalcin-Overexpressing Endothelial Progenitor Cells Promote Endothelial Cell Angiogenesis. Am J Physiol Cell Physiol 2019;317:C932-41.

[49] Yue Y, Wang C, Benedict C, Huang G, Truongcao M, Roy R, *et al*. Interleukin-10 Deficiency Alters Endothelial Progenitor Cell-Derived Exosome Reparative Effect on Myocardial Repair Via Integrin-Linked Kinase Enrichment. Cir Res 2020;126:315-29.

[50] Man K, Brunet MY, Jones MC, Cox SC. Engineered extracellular vesicles: Tailored-made nanomaterials for medical applications. Nanomaterials (Basel) 2020;10:1838.

[51] Yang SW, Hennessy RR, Khosla S, Lennon R, Loeffler D, Sun T, *et al*. Circulating Osteogenic Endothelial Progenitor Cell Counts: New Biomarker for the Severity of Coronary Artery Disease. Int J Cardiol 2017;227:833-9.

[52] Garikipati VN, Krishnamurthy P, Verma SK, Khan M, Abramova T, Mackie AR, *et al*. Negative regulation of mir-375 by interleukin-10 Enhances Bone Marrow-Derived Progenitor Cell-Mediated Myocardial Repair and Function after Myocardial Infarction. Stem Cells 2015;33:3519-29.

[53] Hohn J, Tan W, Carver A, Barrett H, Carver W. Roles of Exosomes in Cardiac Fibroblast Activation and Fibrosis. Cells 2021;10:2933.

[54] Teringova E, Tousek P. Apoptosis in Ischemic Heart Disease. J Transl Med 2017;15:87.

[55] Mitchell RG, Duscha BD, Robbins JL, Redfern SI, Chung J, Bensimhon DR, *et al*. Increased Levels of Apoptosis in Gastrocnemius Skeletal Muscle in Patients with Peripheral Arterial Disease. Vasc Med 2007;12:285-90.

[56] Kim HW, Haider HK, Jiang S, Ashraf M. Ischemic Preconditioning Augments Survival of Stem Cells via Mir-210 Expression by Targeting Caspase-8-Associated Protein 2. J Biol Chem 2009;284:33161-8.

[57] Kim HW, Jiang S, Ashraf M, Haider KH. Stem Cell-Based Delivery of Hypoxamir-210 to the Infarcted Heart: Implications on Stem Cell Survival and Preservation of Infarcted Heart Function. J Mol Med (Berl) 2012;90:997-1010.

[58] Ma X, Wang J, Li J, Ma C, Chen S, Lei W, *et al*. Loading mir-210 in Endothelial Progenitor Cells Derived Exosomes Boosts their Beneficial Effects on Hypoxia/Reoxygeneation-Injured Human Endothelial Cells Via Protecting Mitochondrial Function. Cell Physiol Biochem 2018;46:664-75.

[59] Zhang C, Wang J, Ma X, Wang W, Zhao B, Chen Y, *et al*. Ace2-epc-exs Protect Ageing Ecs Against Hypoxia/Reoxygenation-Induced Injury through the mir-18a/nox2/ros Pathway. J Cell Mol Med 2018;22:1873-82.

[60] Viñas JL, Burger D, Zimpelmann J, Haneef R, Knoll W, Campbell P, *et al*. Transfer of Microrna-486-5p from Human Endothelial Colony Forming Cell-Derived Exosomes Reduces Ischemic Kidney Injury. Kidney Int 2016;90:1238-50.

[61] Hong X, Wang J, Li S, Zhao Z, Feng Z. Retracted: Microrna-375-3p in Endothelial Progenitor Cells-Derived Extracellular Vesicles Relieves Myocardial Injury in Septic Rats Via Brd4-Mediated pi3k/akt Signaling Pathway. Int Immunopharmacol 2021;96:107740.

[62] Chung JJ, Kim ST, Zaman S, Helmers MR, Arisi MF, Li EC, *et al*. Therapeutic Efficacy of Cryopreserved, Allogeneic Extracellular Vesicles for Treatment of Acute Myocardial Infarction. Int Heart J 2021;62:381-9.

[63] Haider KH, Khan M, Sen C. Micrornas with Mega Functions in Cardiac Remodeling and Repair: The Micromanagement of Matters of the Heart. Cambridge: Academic Press; 2015.

[64] Haider KH, Najimi M. Exosome-based Cell-Free Therapy in Regenerative Medicine for Myocardial Repair. Handbook of Stem Cell Therapy. Singapore: Springer Nature Singapore; 2022. p. 1-29.

[65] Zhang J, Chen C, Hu B, Niu X, Liu X, Zhang G, *et al*. Exosomes Derived from Human Endothelial Progenitor Cells Accelerate Cutaneous Wound Healing by Promoting Angiogenesis through erk1/2 Signaling. Int J Biol Sci 2016;12:1472-87.

[66] Zhou Y, Li P, Goodwin AJ, Cook JA, Halushka PV, Chang E, *et al*. Exosomes from Endothelial Progenitor Cells Improve the Outcome of a Murine Model of Sepsis. Mol Ther 2018;26:1375-84.

[67] Wu X, Liu Z, Hu L, Gu W, Zhu L. Exosomes Derived from Endothelial Progenitor Cells Ameliorate Acute Lung Injury

DOI: http://dx.doi.org/10.18053/jctres.08.202206.004

by Transferring mir-126. Exp Cell Res 2018;370:13-23.

[68] Jia Y, Zhu Y, Qiu S, Xu J, Chai Y. Exosomes Secreted by Endothelial Progenitor Cells Accelerate Bone Regeneration During Distraction Osteogenesis by Stimulating Angiogenesis. Stem Cell Res Ther 2019;10:12.

[69] Ke X, Liao Z, Luo X, Chen JQ, Deng M, Huang Y, *et al*. Endothelial Colony-Forming Cell-Derived Exosomal Mir-21-5p Regulates Autophagic Flux to Promote Vascular Endothelial Repair by Inhibiting sipl1a2 in Atherosclerosis. Cell Commun Signal 2022;20:30.

[70] Halurkar MS, Wang J, Chen S, Bihl JC. Epc-exs Improve Astrocyte Survival and Oxidative Stress through different Uptaking Pathways in Diabetic Hypoxia Condition. Stem Cell Res Ther 2022;13:91.

DOI: http://dx.doi.org/10.18053/jctres.08.202206.004