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

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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
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-1 (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].

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 neovascularature. 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].
The paracrine secretions of the cultured cells were rich in proangiogenic 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 bilayer 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 neovascularature. 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, tumorogenicity, 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 tumorogenicity 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
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 reduced fibrosis and scar size, the STG-sEVs treated animals
Treatment with sEVs significantly increased endothelial cell function, 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 sEV 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 VEGFR2, 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...
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 anti-apoptotic 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.

### 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 | EVSEVSI/4ILK 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, promoting 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

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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 |
|----------------|-------------|---------|------------------------|--------------------------------------|
| ClinicalTrials.gov Identifier: NCT04327635 | Safety evaluation of intracoronary infusion of sEVs in patients with AMI | Heart attack | Drug: PEP in Acute Myocardial Infarction Drug: Ticagrelor Drug: Clopidogrel | Mayo Clinic Rochester, Minnesota, United States |
| ClinicalTrials.gov Identifier: NCT05109364 | Antiplatelet therapy effect on sEVs in acute myocardial infarction | Myocardial infarction | | 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 | Ercyes 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 tear (lateral) (medial), meniscus lesion (and 6 more...) | Drug: SF-MSC-EX Drug: SF-MSC | Eskisehir Osmangazi University, Eskisehir, Turkey |
| ClinicalTrials.gov Identifier: NCT04388982 | The safety and 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 Jiao tong 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 Jiao tong University. Shanghai, Shanghai, China |

sEVs as diagnostic tools

| NCT Identifier | Study title | Disease | Method of intervention | Institution and location of the trial |
|----------------|-------------|---------|------------------------|--------------------------------------|
| 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...)

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Table 4. (Continued).

| NCT identifier | Study title | Disease | Method of intervention | Institution and location of the trial |
|----------------|-------------|---------|------------------------|---------------------------------------|
| (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 (EXOSARC) | Type 1 Diabetes | 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 1b/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.

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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].

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Conflicts of Interest

The authors declare no conflicts of interest.

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