Chapter 5

Endothelial Progenitor Cells in Cardiovascular Diseases

Somayyeh Rashidi[1], Sara Aghakhani Chegeni[2], Golbarg Roozbahani[3], Reza Rahbarghazi[4,5*], Fatima A. Karim[6]

[1]Department of Medical Biotechnology, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

[2]Department of Biochemistry and Clinical Laboratories, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

[3]Applied Cell Sciences Division, Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

[4]Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[5]Department of Applied Cell Sciences, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

[6]College of Dentistry, University of Al-Ameed, Kerbala, Iraq

Correspondence to Dr. Reza Rahbarghazi (DVM, Ph.D.); E-mails: rahbarghazir@tbzmed.ac.ir, rezarahbardvm@gmail.com; Address: Department of Applied Cell Sciences, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, 5166653431, Tabriz, Iran.

Abstract

The occurrence of ischemic diseases has increased in recent decades due to lifestyle changes. Along with the conventional therapeutic protocols, the development and emergence of novel modalities are at the center of debate to circumvent the pitfalls in the clinical setting. In recent years, the

discovery of various stem cell types has helped biologists and clinicians alleviate diverse pathologies with relatively satisfactory outcomes. Among them, EPCs[1] pave the way to accelerate the phenomenon of regeneration, especially in ischemic/hypoxic conditions. These cells exhibit prominent and bulk angiogenesis properties to re-establish the blood perfusion into the affected sites via direct differentiation into the mature ECs[2] or release of angiogenesis factors. In this chapter, recent progress in the application of EPCs in CVDs[3] was discussed in detail.

Keywords: Endothelial Progenitor Cells; Angiogenesis; Vasculogenesis; Ischemic Diseases

1. Introduction

It has been thought that adequate blood perfusion is an essential item in the control of diverse biological activities [1]. The process of blood formation has profound effects during embryonic development, morphogenesis, wound healing, progression of various pathologies, etc. [2]. To describe the vascularization and development of *de novo* blood units, two phenomena, including angiogenesis (also known as neo-angiogenesis) and vasculogenesis, are used in the biomedical fields [3]. However, researchers and clinicians should be aware of precise definitions and underlying differences between angiogenesis and vasculogenesis in different scientific disciplines such as vascular biology, oncology, and regenerative medicine [4]. The term vasculogenesis stands for the *in-situ* orientation of embryonic angioblasts to primitive ECs or the generation and extension of nascent vascular units in adults [1]. The differentiation of angioblasts in the embryo contributes to the formation of a vascular plexus,

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[1]. Endothelial progenitor cells
[2]. Endothelial cells
[3]. Cardiovascular diseases

which is followed by the formation of cardiac tissue and vascular units [1]. However, the phenomenon of vasculogenesis is rare in adults compared to angiogenesis, but is usually observed in the healing process and certain pathological conditions [1]. In contrast to vasculogenesis, angiogenesis is the formation of new blood vessels from pre-existing vascular networks [5]. Based on the great body of scientific data, angiogenesis occurs via different mechanisms as follows: sprouting, splitting, and coalescence [6]. The sprouting angiogenesis comes with the proliferation, polarization, and migration of ECs juxtaposed to the hypoxic zones along with the gradient density of pro-angiogenesis factors (**Figure 1**) [7, 8]. During the angiogenesis process, specialized ECs, namely tip cells, are exactly located at the head of sprouting vessels and appropriately sense the microenvironment signals and guidance cues [9]. In splitting[1] angiogenesis, the lumen of target blood vessels protrudes longitudinally, leading to the split of the single vessels into two vascular units [10]. Compared to splitting angiogenesis, in coalescent angiogenesis, blood vessels are merged to form large-sized vessels to afford the perfusion into the target sites [4]. This form of angiogenesis is commonly seen under specific pathological conditions related to tumor cell growth, metastasis, and wound healing [11]. In terms of biological effects, it is thought that angiogenesis acts like a blade with two edges in which it can be in volved in the progression of pathologies and acceleration of regeneration in the targeted tissues [12]. For example, the regulation and inhibition of angiogenesis is touted as a therapeutic strategy in certain conditions, such as cancer and retinopathies. On the other hand, the stimulation of angiogenesis-related signaling pathways is highly demanded in the regeneration of ischemic changes [12]. Therefore, anti-angiogenic

[1]. Known also as intussusceptive angiogenesis

strategies such as anti-VEGF[1] antibodies are used for the inhibition of tumor cell activity and treatment of ocular diseases such as macular degeneration or retinopathies [13, 14]. The increase of blood perfusion is highly demanded in pathological conditions such as coronary artery disease [15].

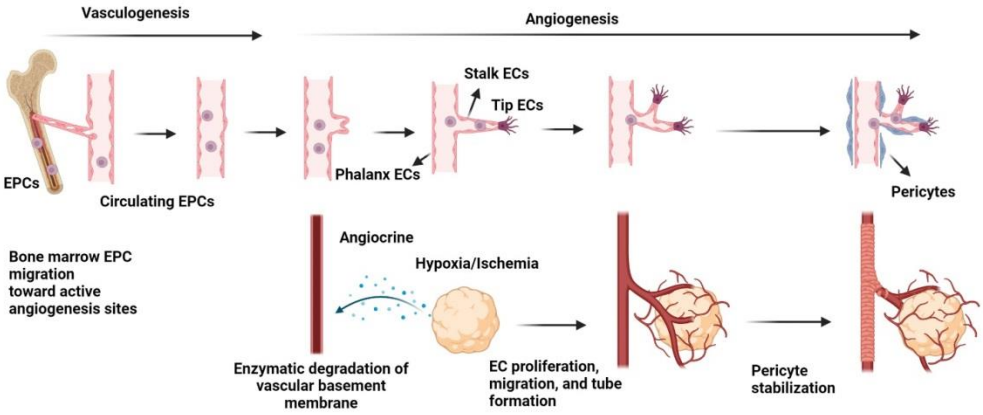

Figure 1. An illustration of blood vessel formation by angiogenesis and vasculogenesis. In angiogenesis, the release and attachment of numerous factors to close vessels under hypoxic conditions contribute to the loosening of the pericyte-EC and EC-EC connection. The released ECs exhibit specific morphology and phenotype ($CD34^+$/$VEGFR\text{-}2^+$ tip cells) and migrate toward the hypoxic site in a gradient density of cytokines to guide the formation of vessels. The ECs behind tip cells are called stalk cells and have the potential to generate a preliminary vascular lumen and become phalanx ECs after being quiescent. In the next step, the addition of pericytes can strengthen the vascular units. In vasculogenesis, BM EPCs are recruited into the site of blood vessel formation, where they can further mature into functional ECs and/or release several angiogenesis-related factors. Copyright. [1]. 2024. Cell Proliferation.

2. Angiogenesis properties of stem cells

In recent years, several experiments have confirmed both angiogenesis and antiangiogenetic properties of stem cells [16, 17]. Stem cells may

[1]. Vascular endothelial growth factor

produce and release nano-sized EVs[1], including Exos[2], and MV[3] with multiple angiogenesis-regulating factors. These factors can promote cell adhesion, migration, and angiogenesis potential [18]. It was suggested that exosomal cytokines such as VEGF increase the survival and proliferation of ECs at the site of injury [19]. Direct differentiation of stem cells to the endothelial lineage has also been documented in several studies [20]. For instance, it was shown that rat NSCs[4], mouse CPCs[5], human MSCs[6], and EPCs can be directly converted into vascular cells under different experimental settings [20]. Due to the incidence of ischemic diseases, the application of stem cells has been extended in the therapy of MI[7], PAD[8], and wound healing [21]. In response to hypoxic/ischemic conditions, stem cells release diverse angiogenesis-related cytokines such as VEGF, FGF[9], PDGF[10], HGF[11], etc. The attachment of these cytokines to endothelial receptors leads to the activation and angiogenesis potential. To be specific, the stimulated ECs lose their physical connection with the neighboring ECs and non-ECs via the production of several degrading enzymes, such as MMPs[12] , mainly MMP-2 and -9 [22]. These enzymes digest the underlying basal membrane and allow the ECs to undergo proliferation, leading to the migration of the leading tip ECs toward the hypoxic/ischemic zones in a gradient density of cytokines [23]. Other ECs acquire certain phenotypes, which are named stalk and phalanx cells

1. Extracellular vesicles
2. Exosomes
3. Microvesicles
4 Neural stem cells
5. Cardiac progenitor cells
6. Mesenchymal stem cells
7. Myocardial infarction
8. Peripheral arterial disease
9. Fibroblast growth factor
10. Platelet-derived growth factor
11. Hepatocyte growth factor
12. Matrix metalloproteinases

based on the exact location in the vascular unit and reciprocal interaction with multiple cytokines [24]. It is thought that stalk ECs provide tight EC-to-EC junctions to ascertain the stability of new sprouts and lumen formation [25]. As the new vessels are elongated, the stalk ECs lose activity and transform into quiescent phalanx ECs [26]. In the next step, the phenomenon of arteriogenesis stabilizes the formation of blood vessels via wrapping by pericytes [27]. In contrast to angiogenesis, in vasculogenesis, EPCs are recruited to the site of injury, where they can directly differentiate into mature ECs to replace the injured ECs or release several angiogenesis factors to promote the activity of ECs [1]. Upon the entry of EPCs into the blood, these cells are called CECs[1], followed by the gradual loss of stemness [28].

3. EPC phenotypes and behavior

As above-mentioned, ECs and EPCs are leading cell lineages required in angiogenesis and vascularization [29, 30]. These cells support the generation and progression of vascular sprouts into the hypoxic tissues under physiological and pathological conditions [4]. EPCs mainly reside in bone marrow and with less content in systemic circulation, with an inevitable role in vasculogenesis and vascular homeostasis. A small fraction of EPCs is found in vascular beds, placenta, spleen, adipose, cardiac, and neural tissues, etc. [31]. Thus, in recent years, these cells have been used as an alternative therapeutic option for the alleviation of different human diseases [32]. The application of EPCs has been extended for vascular regenerative medicine, especially in ischemic conditions, because of paracrine activity (angiocrine panel and differentiation properties). Therefore, EPC-based modalities are at the center of attention

[1]. Circulating EPCs

in the alleviation of pathological conditions via the promotion of angiogenesis [33, 34]. Currently, BM[1], PB[2], and UCB[3] are the main sites for the isolation and purification of EPCs [35]. In adults, BM is the preferred tissue for EPC isolation; however, UCB contains higher EPC contents with prominent stemness and proliferation properties, especially for biobanking and clinical use [36]. Besides, ESCs[4] and iPSCs[5] are interesting cell sources for obtaining EPCs with the potential to acquire venous and arterial EC phenotypes [37, 38]. Despite the possibility of EPC isolation from different sources, no standard protocols and tissues have been provided yet for the large-scale production of these cells.

Molecular studies have indicated that EPCs have some common surface markers with ECs and HSCs[6] (**Figure 2**). However, no definite molecular pattern has been described for EPCs for precise immunophenotyping. Thus, a panel of markers is used for EPC characterization [1]. Soon after EPC entry into the blood and homing to target sites, EPCs lose the stemness marker. In blood, EPCs exhibit positivity for CD45, CD34, and CD133, VEGFR-2[7], and Tie-2 [39]. By time and during differentiation into mature ECs, the expression of CD133 is diminished, which coincides with the expression of EC factors such as CD31[8], CD144 (VE-cadherin)[9], and vWF[10] [40]. In laboratory settings, the culture of EPCs contributes to the stemness factor removal over time and EC phenotype acquisition (VE-cadherin↑, vWF↑, and CD146↑) [41, 42].

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1. Bone marrow
2. Peripheral blood
3. Umbilical cord blood
4. Embryonic stem cells
5. Induced pluripotent stem
6. Hematopoietic stem cells
7. Vascular endothelial growth factor receptor-2
8. Cluster of differentiation 31 (PECAM-1
9. Vascular endothelial cadherin
10. von Willebrand factor

About 4-10 days of *in vitro* culture, eEPCs[1] ($CD31^+/CD146^-$/ $CD34^-/CD14^+/CD45^+$ are spindle-shaped, while by increasing the culture time, these cells transform into cobblestone-shaped lEPCs[2] ($CD31^+/CD105^+/CD146^+/VEGFR2^+/vWF^+/CD45^-/CD14^-/CD133^-$) (**Figure 2**) [43, 44]. The isolation of MNCs[3] by gradient density and expansion on fibronectin-coated surface in the presence of endothelial growth factors is the conventional method for increasing EPC number. eEPCs are usually generated after 2–4 weeks of *in vitro* culture with low potential to differentiate into functional ECs. However, eEPCs can stimulate the blood vessel formation via activating quiescent ECs in a paracrine manner. In physiological conditions, the number of eEPCs is very low in the circulation system. The application of certain exogenous and endogenous factors and the occurrence of pathological conditions can alter the number of eEPCs [11]. Compared to EPCs, lEPCs (ECFCs[4]) have a high potential to generate mature ECs *in vitro* and *de novo* blood vessels [45]. Similar to eEPCs, the number of ECFCs is also low in the blood. In response to vascular injuries and ischemic conditions, their number increases. Based on one hypothesis, lEPCs' origin is possibly BM ECs [11]. Hypoxic/ischemic conditions increase the entry and release of lEPCs into circulation. It is thought that these cells exert their therapeutic potential via multiple mechanisms. The direct integration into the injured vascular bed and maturation into ECs can influence blood perfusion. Meanwhile, the release of several cytokines via paracrine activity, and providing a milieu for stem cell maturation, helps the vascular tissue regeneration [11]. Although both eEPCs and lEPCs are actively involved in vascular tissue

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1. Early EPCs
2. Late EPCs
3. Mononuclear cells
4. Endothelial Colony-Forming Cells

regeneration, eEPCs are first recruited into the injured site in a vascular bed, and in the next step, the homing of lEPCs improves the regeneration potential [11]. Despite the great regenerative potential of EPCs, EPC-based modalities have not been extended into the clinical setting because of low retention time and viability at the site of injection. Thus, attempts should be directed toward the development and increase of EPC-related efficiency [33].

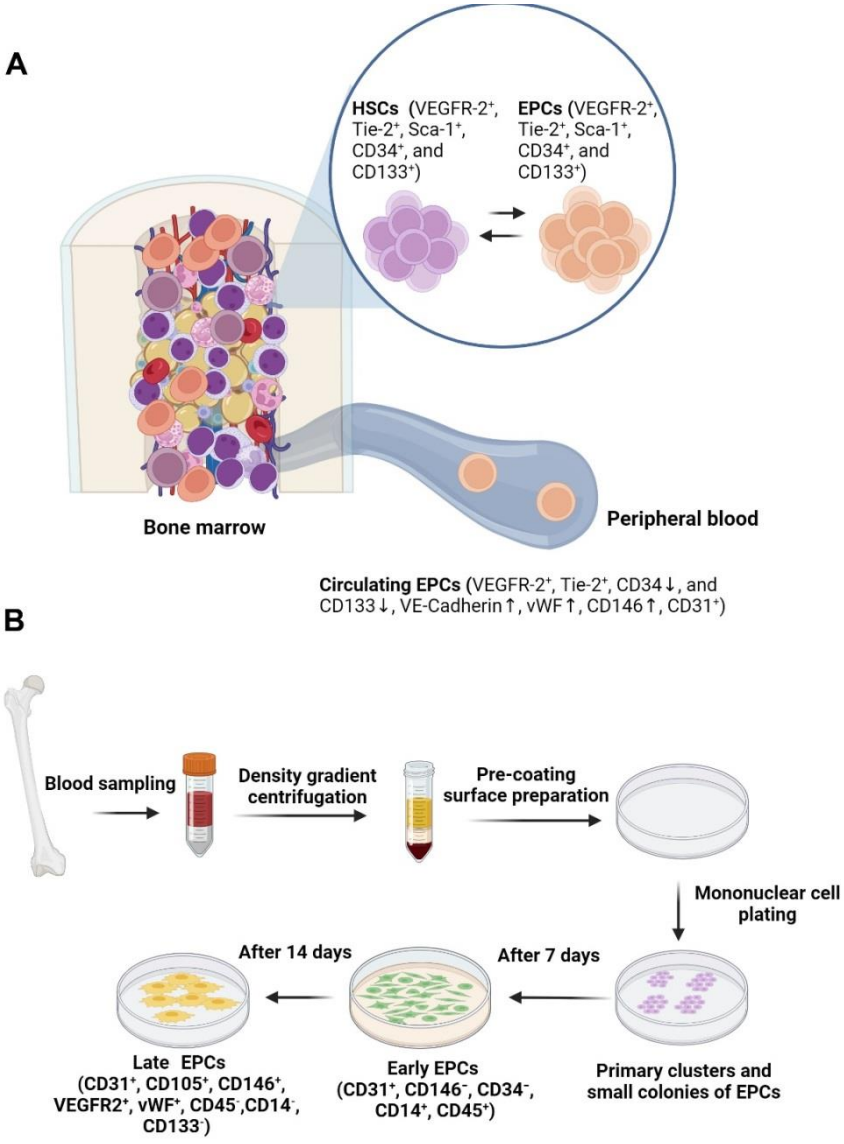

Figure 2. Different surface markers used for the characterization of BM EPCs in in vivo (A) and in vitro conditions (B). It is thought that EPCs share common markers with the HSCs. Copyright. [1]. 2024. Cell Proliferation.

4. Application of EPCs in preclinical studies

EPCs are promising progenitors for therapeutic purposes with the potential to repair vascular injuries and generate new blood vessels inside the body [46]. Notably, these cells are valid cell sources for the stimulation of improving angiovasculogenic properties following ischemic injury in the heart, brain, and hindlimbs, and cutaneous tissue [47]. To this end, numerous preclinical studies have been conducted so far. Based on the data from preclinical experiments, EPCs open new options for the alleviation of hindlimb ischemic changes in patients with poor wound healing after pharmaceutical treatments and/or surgical operations [48]. In animals and rodents, HLI[1] is commonly used for the induction of PAD[2] via the ligation of the femoral artery to diminish or interrupt blood perfusion to the lower regions, resulting in ischemia [41, 49]. Asahara et al. previously showed a successful vascularization rate after the recruitment and localization of autologous EPCs into the ischemic region in HLI rabbits [50]. Additionally, the administration of exogenous EPCs in various species, i.e., rats, mice, and rabbits, effectively improves neovascularization following ischemia [51-54]. Likewise, EPC transplantation in stroke animal models has led to neuroangiogenesis and restoration of neuronal function [55, 56]. To achieve neurovascular repair, endogenous EPCs are recalled to the ischemic region with the brain parenchyma, especially the BBB[3]. Even though the concurrent exogenous EPCs use via systemic injection or direct intracerebral transplantation administration can accelerate the healing properties [57]. To induce the experimental stroke, the MCAO[4] model is induced in different animal species to recapitulate similar ischemic conditions in human counterparts. Data have confirmed that the combination of EPCs with other therapeutic agents can increase the regenerative potential, in which

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1. Hindlimb ischemia model
2. Peripheral arterial disease
3. Blood-brain barrier
4. Middle cerebral artery occlusion

simultaneous application of EPCs with an MMP inhibition, namely BT-94, blunts the IS[1] pathologies in mice via the regulation of MMPs, apoptotic TUNEL$^+$ cells, and reduction of neurological deficit scores [55]. To increase the on-target delivery of EPCs into the injured sites, novel release strategies have been applied. For instance, data indicated that the delivery efficiency of SiO4@SPIONs[2]-labeled EPCs into ischemic brain increases in MCAO-treated mice under magnetic field treatment. The labeled EPCs can stimulate angiogenesis by enhancing migration and angiocrine in the ischemic cerebral cortex [47]. Both angiogenesis and vasculogenesis mechanisms have been indicated in the restoration of the BBB[3] after IS [57]. It has been indicated that EPCs can restore BBB integrity in different stroke animals. Of note, BBB permeability is induced following the WBI[4] of brain tumor patients. This effect would be possibly due to direct vascular cell injury or the reduction of EPCs following the irradiation. Under such conditions, EPC transplantation can in part reduce RBI[5]. In mice with IS subjected to WBI, EPC transplantation can restore selective BBB function via up-regulation of tight junction proteins such as claudin-5 and ZO-1[6]. Along with these changes, the local density of vWF$^+$ ECs increased in the ischemic regions, reflecting enhanced vascular recovery [58]. Interestingly, intra-arterial injection of PB EPCs in IS nude mice alleviates the neurological deficits via the reduction of ischemic size, promotion of angiogenesis, and apoptosis inhibition [46]. In rats with chronic brain ischemia, intramuscular injection of EPCs along with a concurrent indirect revascularization method, EMS,[7] can reverse brain hypoperfusion (**Figure 3**) [59]. Taken together, data have confirmed that EPCs are interesting

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1. Ischemic stroke
2. Superparamagnetic iron oxide nanoparticles
3. Blood-brain-barrier
4. Whole-brain irradiation
5. Radiation-induced brain damage
6. Zonula occludens
7. Encephalomyosynangiosis

alternative therapeutics for the alleviation of cerebral ischemia via direct integration into the vascular trunk or angiocrine capacity [60-62]

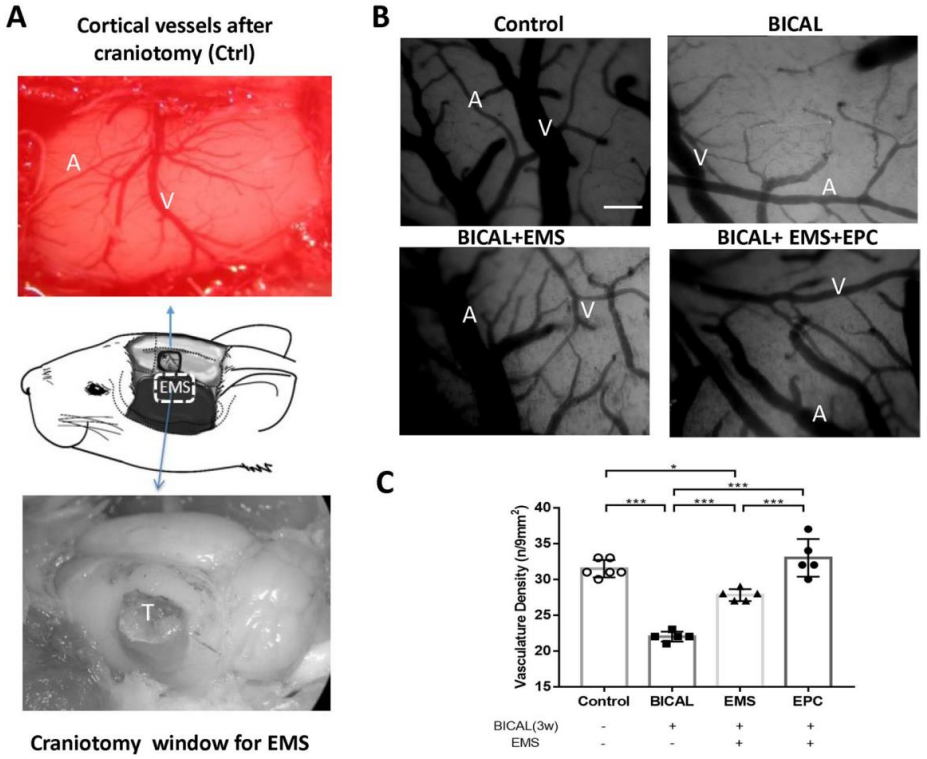

Figure 3. Monitoring the therapeutic effects of EPC transplantation with simultaneous indirect revascularization (EMS) in rats subjected to chronic cerebral ischemia (A-C). The craniotomy was done via the induction of a 3 x 3 mm orifice for measuring microvascular density and blood perfusion (**A**). Two weeks following the EMS surgery (bottom panel, white dotted box), the temporalis muscle (labeled as T) attaches to the external surface of the brain, indicating a successful EMS process. The vascular units of the brain surface, either artery (A) or vein (V), can be detected using a video microscope (**B;** scale bar =100 µm). Three weeks after the BICAL[1] procedure, the cortex's surface lacks microcirculation. Based on the obtained data, microcirculation was significantly diminished following BICAL, while EMS alone and EMS plus EPC transplantation improved the vascularization properties. One-way ANOVA followed by Tukey analysis. *p<0.05, ***p<0.001, n = 5–6. Copyright. [59]. 2022. Stem Cell Research & Therapy.

Like brain pathologies, EPCs had the potential to restore the function of ischemic myocardium in different rodent models. For example, the

[1]. Bilateral internal carotid artery ligation

injection of EPCs in MI[1] rats by expression of gap junction protein like connexin-43, inflammation and apoptosis reduction, and VEGF, leading to enhanced microvascular density, and normal ECG[2] [63].

Chen et al. monitored the angiogenesis potential of EPC EVs loaded into STG[3] hydrogel in MI rats. Data confirmed that the vascular density was enhanced using STG + EPCs and STG + EVs; however, these effects were superior in the STG + EVs group (**Figure 4**) [64]. The administered EPCs to MI nude mice led to significant neovascularization via differentiation toward mature ECs [65]. Hong et al. found that PB EPCs delay the development and progression of rats with CKD[4] by increasing angiogenesis, regulation of oxidative stress (antioxidant production↑), inflammation, fibrotic changes, and apoptosis [66]. Commensurate with these data, EPCs are effective angiogenesis activators in cardiac tissue ischemia with potential translation into clinics [67].

[1]. Myocardial infarction
[2]. Electrocardiogram
[3]. Injectable shear-thinning
[4]. Chronic kidney disease

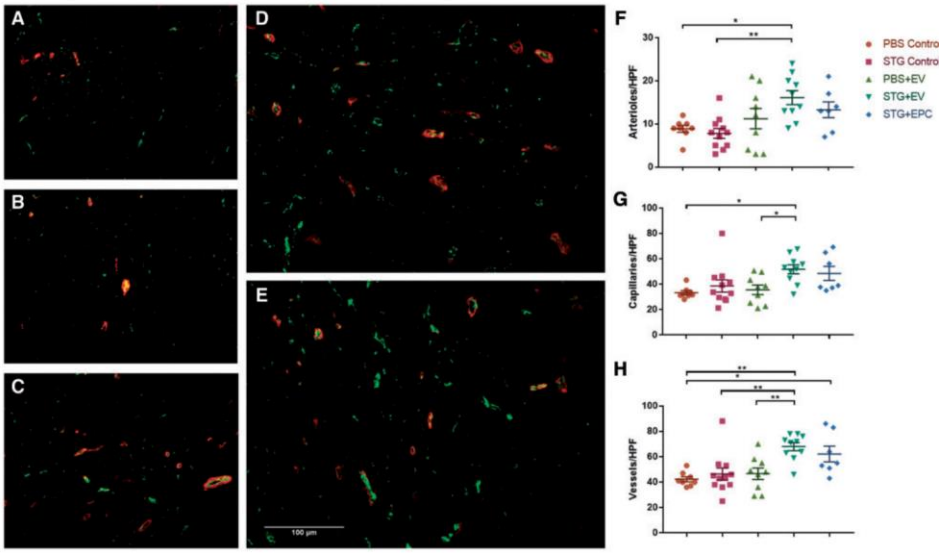

Figure 4. Monitoring the angiogenesis properties of EPC + STG, and EPC EVs + STG in a rat model of MI (A-H). Data confirmed that STG + EV enhances microvascular density in the periphery of the infarct area. Immunohistochemistry of peri-infarct myocardium was used to measure vascular density in different groups as follows; PBS[1] Control (A; n = 8), STG (B; n = 11); PBS + EV (C; n = 9), STG + EV (D; n = 10), and STG + EPC (E; n = 7) (Magnification 20X). Blue-colored DAPI^{+} nuclei; Green-fluorescent vWF^{+} capillaries; red-fluorescent α-SMA arterioles. Mean number of arterioles (F), capillaries (G), and total vessels (H) per high-power field. *p<0.05, **p<0.01. Copyright [64]. 2018. Cardiovascular Research

In recent years, the application of EPCs has been extended to other pathological conditions. For example, the osteo-angiogenesis properties of EPCs have also been investigated using several experiments [68-70]. To achieve appropriate bone healing, coordinated angiogenesis and osteogenesis behavior is mandatory. In this regard, in a rat FOM[2], local administration of pre-cultured EPCs contributes to callus formation and filling of osteotomy gap with simultaneous vascularization in the target site [71, 72]. It seems that the co-administration of EPCs with other stem cell types can intensify the regenerative potential. In an experiment, co-

[1]. Phosphate buffered saline
[2]. Femur osteotomy model

transplantation of EPCs and BM MSCs in ONFH[1] rabbits via osteo-angiogenesis induction and inhibition of adipogenesis (**Figure 5**) [73]. In different small animal models, systemic administration of EPCs is touted as a safe model for improving pulmonary hemodynamics [74, 75]. The intravenous injection of BM EPCs in PAH[2] rats can restore the hemodynamic function of ventricles via up-regulation of connexin43, eNOS[3], and apoptosis inhibition (Bcl-2↓) with simultaneous improvement of alveolar tissue and pulmonary arteriole function [76]. In the canine model of PAH, EPCs increase heart function, lung vascular stability, and regulate arterial pressure [77]. The administration of EPCs in lipopolysaccharide-induced ALI[4] rats regulates pulmonary edema, interstitial hemorrhage, exudate formation, and inflammation (IL-10↑, iNOS[5]↓, and endothelin-1↓) [78]. Likewise, systemic EPC administration in COPD[6] mice reduces senile changes (β-galactosidase activity↓, p16↓, and USP7/p300 axis↓) [79].

1. Steroid-induced osteonecrosis of the femoral
2. Pulmonary arterial hypertension
3. Endothelial nitric oxide synthase
4. Acute lung injury
5. Inducible nitric oxide synthetase
6. Chronic obstructive pulmonary disease

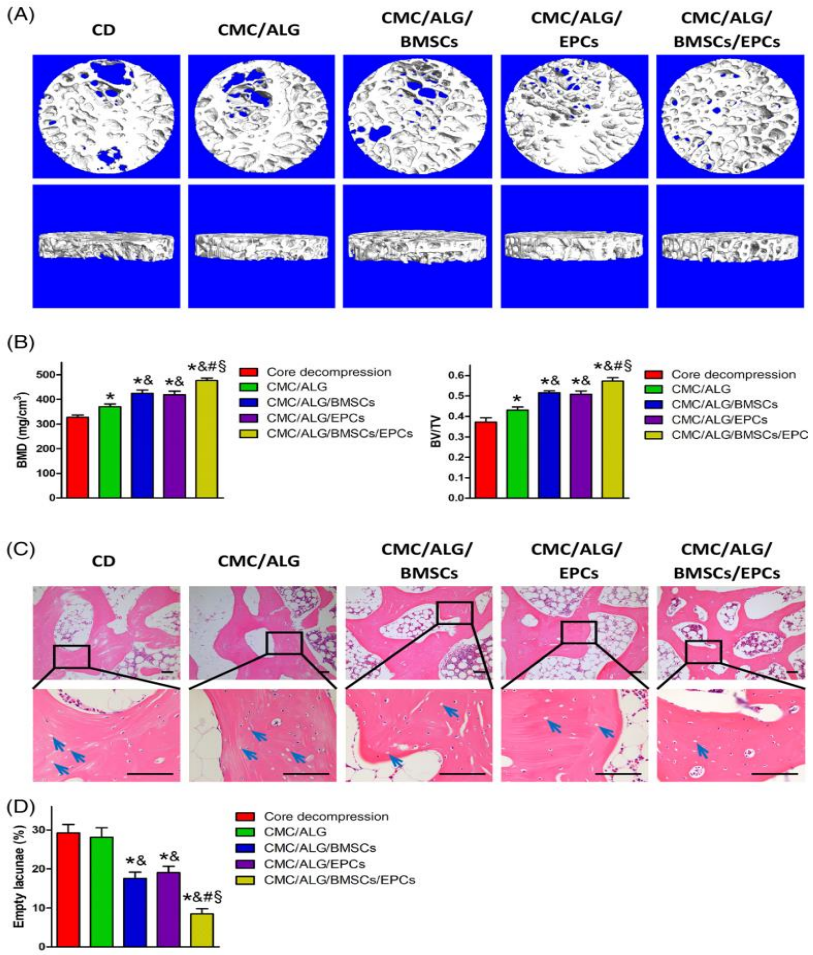

Figure 5. Monitoring therapeutic effects of BM MSCs- and EPCs-loaded CMC/ALG in a rabbit model of ONFH (**A-D**). 3D images of the subchondral femoral region were imaged using micro-CT (**A**). Trabecular bone parameters such as BMD[1] and BV/TV[2] were calculated (**B**). H & E[3] staining indicated an empty zone (blue arrows) in the necrotic zone located at the head of the femurs (**C**; scale bar = 100 μm). The percentage of empty lacuna was also measured (**D**). Significance in comparison with the CD[4] group (*), CMC/ALG group (&), CMC/ALG/BMSCs group (#), and CMC/ALG/ EPCs group (§). Copyright. [73]. 2020. STEM CELLS Translational Medicine.

1. Bone mineral density
2. Bone volume/total volume
3. Hematoxylin and eosin
4. Core decompression

The regeneration of cutaneous tissue is another application of EPCs in which these cells can foster a healing process via the stimulation of vascularization and an increase in microvascular density [80-82]. The transplantation of EPC-loaded Gel[1]/Sr^{2+}@POSS[2] hydrogels into rats led to the healing of deep cutaneous tissue injuries via re-epithelization, reduction of fibrotic changes (Col[3]↓), and angiogenesis [83]. As above-mentioned, co-transplantation of EPCs with other stem cells can yield better regenerative outcomes. In support of this notion, the co-administration of EPCs and ASCs[4] enhanced the angiogenesis potential via improved migration properties of EPCs in rats with severe cutaneous tissue defects [81]. Taken together, several preclinical studies have confirmed the eligibility of EPCs in the acceleration of ischemic changes in different pathologies such as MI, PAH, and bone injuries, indicating the possible efficiency of these cells in human counterparts [84-87].

5. Clinical trials

In recent years, the application of EPCs has been extended to human medicine. In this regard, several clinical trials have been performed. Based on a search conducted on https://clinicaltrials.gov/ on March 15, 2025, the status of studies was classified in terms of study status, condition, interventions, and study type (**Figure 6**).

1. Gelatin
2. Polyhedral oligomeric silsesquioxane
3. Collagen
4. Adipose-derived stem cells

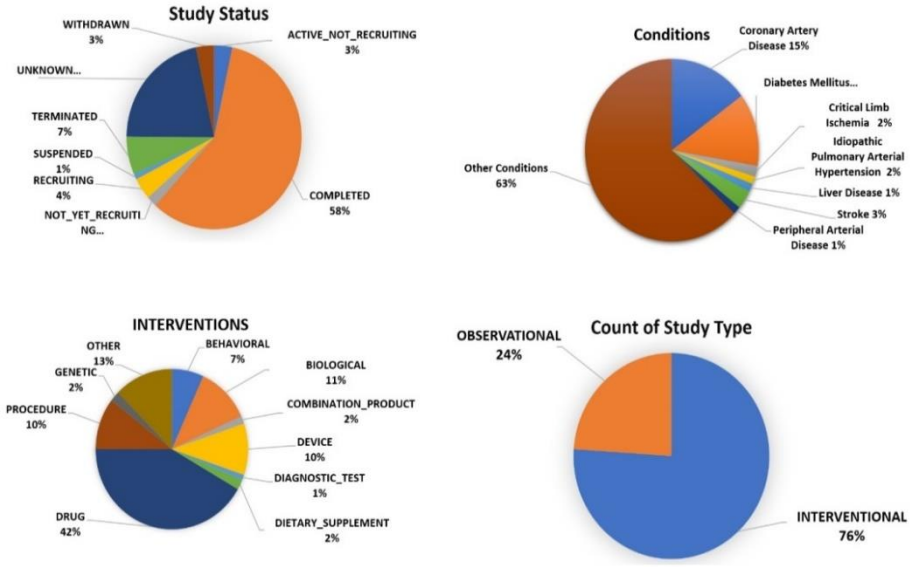

Figure 6. The application of EPCs in clinical trials. Data were obtained from https://clinicaltrials.gov/

Based on our evaluation, nearly 209 clinical trials were listed on ClinicalTrials.gov for the treatment of various pathological conditions. The study status analysis indicates that most clinical trials (58%) have been finished. It is thought that these data may offer a firm groundwork for further research in terms of the safety and efficacy of EPC-based therapies. About 22% of clinical trials were marked as UNKNOWN status, and this would be because of in-time reporting, follow-up, etc. However, the lack of supporting data makes it problematic to conclude these studies. The remaining studies were about 7% of the total trials and marked as terminated status. It is possible that the lack of enough participants and therapeutic effect, safety issues, and other reasons would lead to the termination of studies. Of note, the ongoing studies were classified into RECRUITING (4%) and ACTIVE_NOT_RECRUITING (3%) status. About

3% of enlisted trials were related to WITHDRAWN (3%) or NOT_YET_RECRUITING (2%), indicating further careful research strategies.

Based on the online analysis, EPCs were used in different pathological conditions, especially CVDs. Among various CVD conditions, CAD[1] is the most explored condition in trials, reaching 15%, and type 2 DM[2] is about 13% of total trials. These findings are consistent with the regenerative potential of EPCs in the restoration of vascular cell function. Data confirmed the application of EPCs for other pathologies such as critical limb ischemia (2%), hepatic tissue disease (1%), idiopathic PAH (2%), and PAD (1%). About 3% of total EPC-related clinical trials investigated the relationship of EPCs with stroke, indicating the growing interest in EPCs for the repair of neurovascular units. The remaining trials (63%) are associated with other conditions, indicating the potential and general uses of EPCs in other conditions, like renal failure, wound healing, etc. Calling attention, most of the interventions in clinical trials on EPCs (42%) are drug-based modalities. This implies that most of the studies have utilized drugs to enhance the motility, function, or survival of EPCs inside the body. It is of paramount concern to evaluate the effectiveness and safety of active treatments. Hence, most of the designed trials (76%) are interventional. The remaining (24%) are observational studies that play an important role in the study of the natural behavior of EPCs in various diseases and the identification of possible biomarkers or therapeutic targets.

It has been indicated that several factors can trigger EPC motility and entrance into the blood. For example, HGF can increase the migration of

[1]. Coronary artery disease
[2]. Diabetes mellitus

EPCs in CVD conditions. Intracoronary administration of HGF-expressing adenoviral particles in CAD patients led to an increase of HG, c-met, and simultaneous $CD34^+/CD117^+$ PB CECs [88]. Other cytokines and factors, such as EPO[1], can influence the NO levels and EPC migration. Clinical manifestations have shown that the administration of engineered EPO, Darbepoetin, in CAD patients can increase endothelial integrity. Compared to the placebo group, Darbepoetin-treated patients revealed an FMD[2] value of 7.5±1.64% coincident with the increase of $CD34^+/CD117^+$ PB CECs, leading to enhanced perfusion rate [89].

In a randomized SORT OUT XI study, CD34 antibody-coated stents were used to provoke CECs in patients with vasculopathy. It is suggested that the application of combined therapeutic strategies can yield better regenerative outcomes [90]. To reduce restenosis of implanted stents, paclitaxel-coated balloons plus EPC stents were used in CAD patients, leading to preservation of the lumen and reduction of restenosis [91].

It was suggested that EPCs can exert therapeutic properties in diabetic vasculopathies. In diabetic patients who received alogliptin or long-release gliclazide, the levels of HbA1c[3] were decreased while the number of $CD45^-/CD34^+/KDR^+$ CECs increased [92]. Taken together, data from several clinical trials indicate simultaneous application of drugs, tissue engineering modalities, and advanced technologies can increase the angiogenesis potential of EPCs, resulting in the development of valid therapeutic approaches in individuals with vascular disease.

6. Conclusion

[1]. Erythropoietin
[2]. Flow-mediated vasodilation
[3]. Hemoglobin A1c

EPCs are one of the valid cell sources in repairing endothelial dysfunction and promoting angiogenesis, making them a promising cell-based therapy option. Though animal studies of EPC transplantation are feasible in several ischemic conditions in humans and small animals. Although these cells have benefits for vascular therapeutics, the clinical use of EPCs depends on the development of standard protocols to yield high-rate pure cells with biological function. Even though individual variations and route of administration should be clearly defined.

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