

## Review Article

# Endothelial Progenitor Cells: Biology and Application in Ischemic Tissue Vascularization

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## Abstract

Endothelial progenitor cells (EPC) have rapidly emerged as a potential novel therapeutic approach in ischemic diseases. After the initial characterization of putative bone marrow-derived endothelial progenitor cells and their potential to promote neovascularization and to attenuate ischemic injury, a decade of intense preclinical research has led to the EPC-based clinical trials that have suggested their safety but with modest results. This review focuses on the role of endogenous and exogenous EPC therapy in promoting neovascularization in the ischemic settings to illustrate the future directions of EPC in therapeutic applications. We also reviewed clinical applications of EPC and potential strategies in improving EPC based therapeutics for ischemic tissue repair.

**Keywords:** Endothelial progenitor cells; Cell therapy; Ischemic diseases; Neovascularization

## Introduction

For more than a decade now, biology and therapeutic efficacy of Endothelial progenitor cells (EPCs) were largely driven by the first observations of Asahara et al. in identifying EPCs in adult peripheral blood (PB) [1] and were shown to derive from bone marrow (BM) further migrate and incorporate into foci of physiological or pathological neovascularization [2,3]. As a matter of fact, postnatal neovascularization was believed to be established by the mechanism of “angiogenesis,” by in situ proliferation and migration of pre-existing endothelial cells (ECs) [4]. However, the finding that EPCs can home to sites of neovascularization and differentiate into ECs in situ is consistent with “vasculogenesis,” a critical paradigm has been demonstrated in embryonic neovascularization [5] and also shown recently for the adult organism in which a pool of progenitor cells contributes to postnatal neovascular formation [6]. The discovery of EPCs has therefore considerably changed our understanding of adult blood vessel formation. Furthermore, we and other groups envisage the potential of EPC to improve the clinical applicability in the fight against ischemic diseases.

This review focuses on the potential value of EPCs (both endogenous and exogenous therapy) as a therapeutic regimen for the treatment of ischemic diseases.

## Characterization of EPC

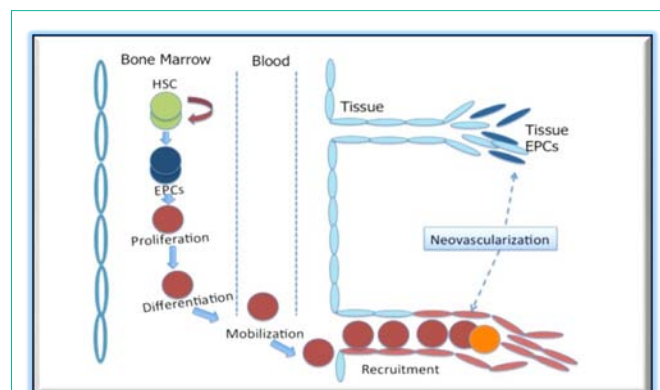
EPCs in circulation can be broadly sub-divided mainly into two categories, hematopoietic lineage EPCs and non-hematopoietic lineage EPCs (Figure 1). The hematopoietic EPCs originate from BM and represent a pro-vasculogenic subpopulation of hematopoietic stem cells (HSCs) ([7,9]. The non-hematopoietic EPCs are blood or tissue derived entities, exhibiting EC like phenotype [10] or ability to differentiate into EC like cells [11].

EPCs and HSCs have been shown to express variety of cell surface markers, including membrane receptors like CD31, CD133, (Fetal liver kinase-1) Flk-1, CXC chemokine receptor-4 (CXCR-4), CD105

(Endoglin), c-Kit for human samples [12-15] and receptors like c-Kit (CD117), stem cell antigen (Sca-1), and CD34 in combination with Flk-1 (vascular endothelial growth factor receptor-2 (VEGFR-2) in case of mouse samples [16-18]. Nevertheless, identifying a particular combination of markers for isolation of these cells has been a big challenge for the researchers in this field.

The origin of non-hematopoietic EPCs (NHEPCs) remains to be clarified, but they are generally thought to be derived from non-hematopoietic tissue resident lineage stem cells or organ blood vessels but not likely from HSC [19].

NHEPCs have been shown to be derived from non-hematopoietic tissues. A novel cell type endothelial out growth cell (EOC) has been shown to be a type of NHEPC, these EOCs are derived from endothelial colony formation assay system developed and reported by Yoder's group and others [20,21]. Unlike hematopoietic EPC, EOC readily form tubes in vitro. However, origin and role of EOC is still a debatable but appears to be a potential therapeutic regimen for neovascularization [22,23]. Recently, Aicher et al. [24], has



**Figure 1:** Mechanism of EPC mediated postnatal neovascularization. Circulating EPCs mobilized from BM are recruited into foci of neovascularization and contribute to new blood vessel formation.

shown circulating cells from BM and non-BM help in vasculature, demonstrated by the mobilization of c-kit+/CD45- progenitor by a parabiosis model from liver and small intestine. In spite of the above findings, it is difficult to define EPCs because of the ambiguity in the phenotype and characteristics that are fundamental for EPC identity.

### **EPCs contribute to neovascularization**

In the perspective of EPC biology, vasculogenesis mainly comprises the de novo blood vessel formation via in situ migration, proliferation, differentiation, and/or incorporation of bone marrow (BM)-derived EPCs into regenerating vasculature [3]. This fact of BM-derived EPCs incorporation into site of physiological or pathological neovascularization has been thoroughly validated in various animal models. Nevertheless, one well-established model, allowing the detection of BM-derived EPCs, uses transplantation of BM cells from transgenic mice in which LacZ is expressed under the regulation of flk-1 or Tie-2(Flk-1/LacZ/BMT, Tie-2/LacZ/BMT) lineage-specific promoter, into wild-type control mice, using the same in various ischemic injury models. It has been shown by using the above model that BM-derived Flk-1- and/or Tie-2- expressing endothelial lineage cells can localize to vascular structures wound healing [25,3]and cardiac ischemia [16,17], Regardless of the origin of EPCs, they undoubtedly play a significant role contributing to neovascularization via vasculogenesis in ischemic tissues.

The tissue resident EPCs secrete a variety of pro-angiogenic cytokines and growth factors in a paracrine fashion there by actively supporting proliferation and migration of pre-existing ECs thus promoting angiogenesis and contributing in an indirect mode to neovascularization to restore tissue homeostasis[4].

In fact observations of the preclinical studies remind us the evidence of enhanced intrinsic recipient angiogenesis by extrinsic factors derived from transplanted EPCs in myocardial ischemia models [26,27].

This paracrine factor secretion of EPC activity show their indirect contribution to neovascularization was established by our and several other groups, identifying EPC secretome with presence of various cytokines and other proangiogenic factors such as viz., vascular endothelial growth factor (VEGF), hepatic growth factor (HGF), angiopoietin (Ang-1, stroma derived factor (SDF)-1a, insulin-like growth factor (IGF)-1, and endothelial nitric oxide synthase (eNOS)[28-30]. Therefore, EPCs can also mediate tissue-protective effects and contribute to neovascularization in ischemic tissues via production of several important indirect support factors such as exosomes [31].

### **EPC Therapy:Pre-clinical animal models**

Since EPCs were first described, more than a decade ago, our group focused especially on the regenerative potential of these enigmatic cells trying to understand the biology and functions with the ultimate goal to translate to bedside. As this cell type has got enormous potential to treat ischemic diseases [5,11,32,33]. The preclinical transplantation of blood/BM-derived EPCs, opened new avenue for the treatment of ischemic diseases.

It was shown that therapeutic efficacy of EPCs to be safe and enhanced neovascularization and regeneration of ischemic tissues [5,11,32,33]. Coronary artery disease can be attenuated by the

collateral circulation development; after recognizing the role of EPCs in neovascularization, investigators started evaluating potential therapeutic efficacy of EPCs. This was validated by recovery of blood flow s in mice with hindlimb ischemia treated with EPCs compared to control mice that received mature ECs [34]. It was also suggested a similar benefits with administration of cord blood derived EPCs isolated in a nude rat model of hind limb ischemia [35]. Lately, human CD34+ cells transplanted into a nude rat model of myocardial ischemia and observed preservation of (Left ventricular) LV function and inhibition of cardiac apoptosis. These findings were in corroboration with the observed preservation of LV function and a reduction in infarction size [17,27,36]. Our study demonstrates that EPC therapy after diabetic myocardial infarction (MI) effectively reduces cardiac fibrosis and preserves cardiac function through release of HGF, which effectively inhibits cardiac microRNA-155-mediated pro-fibrosis signaling and attenuates left ventricular dysfunction in diabetic MI mice [37].

Further, Schatteman et al. [38] reported transplantation of human CD34+ cells into diabetic nude mice with hind limb ischemia resulted in significant blood flow recovery in ischemic limbs. Interestingly, a recent study in porcine models of MI, EPC administration along with AKT inhibitor (LY294002) significantly reduced their neovascularization ability suggesting EPC mediated neovascularization and preserved cardiac function were Akt-dependent[39]. All these above studies indicate that EPC to be a potential stem cell type for the treatment of ischemic diseases in the pre-clinical settings and encouraged to move on to EPC based clinical trials.

The functional benefits observed with EPC-based therapy pre-clinical studies might have been from several aspects. At the ischemic zone, both transplanted as well as endogenous EPCs could protect ECs and ischemic tissue resident cells from ischemia-induced cell death by secreting various growth factors such as VEGF, SDF-1 and IGF-1. Thus EPCs secreted factors enhance neovascularization and functionally and structurally rebuild the injured tissue contributing to the recovery.

### **Novel therapeutic strategies for improving EPC based therapeutics for ischemic tissue repair**

A central recognized challenge that precludes full functional benefits of EPC based therapies is the poor survival and retention of transplanted cells thereby greatly compromising their therapeutic efficacy. Emerging evidence from preclinical studies suggests that hostile microenvironment in the infarcted myocardium, including inflammation and oxidative damage, has adverse effects on transplanted stem cell survival and function [40,41].

Previous reports from our and other labs has established a significant cardio-protective role of anti-inflammatory cytokine, interleukin-10 (IL-10) therapy in mice models of AMI and pressure-overload, which in turn is mediated by IL-10-mediated modulation in a number of signal transduction pathways including p38 mitogen-activated protein kinases (MAPK), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and signal transducer and activator of transcription-3 (STAT-3) pathway [42-45]. Our recently published work [17] demonstrated that combined therapy with EPC+IL-10 is significantly more effective in the improvement of

**Table 1:** Abbreviations: AMI, acute myocardial infarction; AP, angina pectoris; BM, bone marrow; CLI, critical limb ischemia; EPCs, endothelial progenitor cells; G-CSF (granulocyte colony-stimulating factor; NUF, nonunion fracture; OMI, old myocardial infarction; PB, peripheral blood; RMI, recent myocardial infarction; RT, randomized trial; T/C, treatment/control, ICM- ischemic cardiomyopathy, NICM, non-ischemic cardiomyopathy, CICM, chronic ischemic cardiomyopathy. ↑ - Increased, ↓ - decreased.

EPC type	Trail Name	Disease type	Patients (T/C)	Outcome	Reference
G-CSF-PB-CD34+	Fujita et al. 2014	CLI	11	Rest pain scale Physiological parameters ↑	[55]
G-CSF-PB-CD34+	Povsic et al, 2013	AP	200/100	Improvement of functional capacity in patients with refractory angina. LVEF increased	[56]
CD133+	Forcillo et al 2013	CICM			[57]
G-CSF-PB-CD34+/ CD34+	Pogljajen et al, 2014	ICM	33	LVEF ↑	(58)
	Vrtovec et al, 2013	NICM	20/20	LVEF ↑ Transendocardial injections associated with better clinical outcome	[59]
G-CSF-PB-CD34+/CD133+	Burt et al. 2010	CLI	9/0	Physical component score ↑ Leg amputation ↓	[60]
PB/BM Derived EPCs	TOPCARE-AMI.2009	AMI	30/29	LVEF ↑ Perfusion ↑ Infarct size ↓	[61]
G-CSF-PB-CD34+	Losordo et al. 2007	AP	18/6	LVEF ↑ CCS class ↑	[48]
CD133+	Li et al. 2007	AMI	35/35	LVEF ↑	[62]
CD133+	Stamm et al 2007	CICM	20/20	LVEF ↑	[63]
G-CSF-PB-CD34+	Boyle et al. 2006	OMI	5/0	Myocardial neovascularization ↑	[64]
G-CSF-PB-CD34+	Bartunek et al. 2005	AMI	19/16	LVEF ↑ Perfusion ↑	[65]
G-CSF-PB-CD34+	Stamm et al. 2003	RMI	46/9	LVEF ↑ Perfusion ↑	[66]

post-acute myocardial infarction (AMI) left ventricular function and ischemic angiogenesis than EPC alone, and that this effect of IL-10 is mediated in part by its effect on enhanced EPC survival and function in the ischemic myocardium.

Our recent report suggests that small molecules such as valproic acid and/or 5-azacytidine based epigenetic reprogramming of EPCs, both murine and human, results in an open epigenome conducive to enhanced global gene expression including the induction of cardiomyocyte specific gene expression. We have shown that, modifying human CD34+ EPCs with small molecules induce cardiomyogenic differentiation and histological fibrosis area, and echocardiographic functional parameters were better persevered in immune deficient mice model of MI [16]. Further, intramyocardial transplantation of reprogrammed EPCs improved paracrine capacity of reprogrammed cells. Our approach to use small molecules is an innovative way to induce multipotency and cardiomyogenic differentiation potential in EPCs, without the undesired complications involved with induced pluripotent cells, is novel.

We recently also reported Arg-Gly-Asp-Ser peptide (RGDS-PA) nanofibers presenting integrin-binding epitope facilitate enhanced retention of bone marrow derived angiogenic progenitor cells and improved efficacy upon their therapy in a murine model of hind limb ischemia. This represents a novel application of PA nanofiber technology to augment cell retention after cell-based therapy [46].

### EPC Therapy: Clinical trials

Numerous clinical trials have been initiated and are currently in progress to understand the safety issues and therapeutic efficacy of EPCs observed in animal models on ischemic diseases [47](Table 1).

Our group performed a phase I/II, randomized, placebo-controlled, dose-ranging, clinical trial to evaluate the intramyocardial

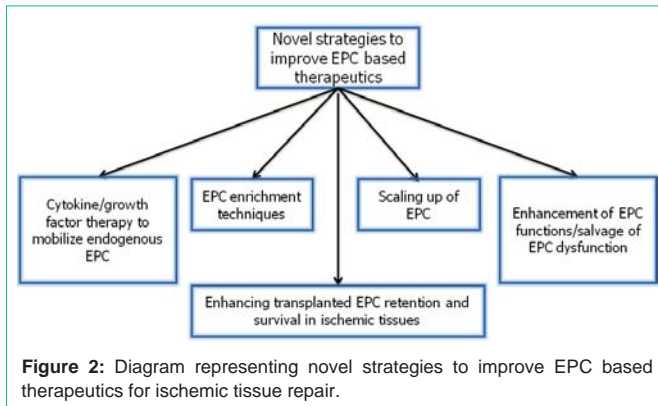
transplantation of G-CSF-mobilized CD34+ cells in 24 patients with intractable angina pectoris [48]. Favorable trends in angina frequency, exercise tolerance, and perfusion defect were observed in patients administered CD34+ cells compared with patients who received placebo.

As for the evaluation of safety issues, neither death nor life-threatening adverse events were observed in therapy group, the results from this phase II study support the safety and efficacy of intramyocardially injected autologous CD34+ cells for symptom reduction and improved exercise capacity in “no-option” patients with refractory angina. Larger-scale studies are warranted to verify these effects and to refine the methods for collecting and administering CD34+ cells to patients with disabling angina symptoms.

### Clinical EPC Therapies: Challenges and Limitations

In most patients undergoing EPC therapy for ischemic diseases have background diseases such as aging [49], diabetes [50] hypercholesterolemia [51], hypertension [52] and smoking [53,54] which affect the number and function of both circulating and BM EPCs. Which might be a potential cause for the modest clinical outcome of EPC transplantation for ischemic diseases.

Therefore, considering autologous EPC therapy, resolving certain issues may help to overcome the shortcomings of EPCs should include: [1] EPCs delivery at the site of injury [2], enhancing strategies to mobilize endogenous EPC (appropriate cytokine/growth factor therapy) [3], EPC enrichment techniques (such as apheresis or BM aspiration) [4], enhancement of EPC functions/ salvage of EPC dysfunction by gene therapy or small molecule treatment [5] Improving strategies to scale-up EPCs culture and expansion for clinical use [6]. Enhancing transplanted EPC retention and survival in ischemic tissues by anti-inflammatory cytokine IL-10 or biomaterials.



The above strategies might compensate the current disadvantages of transplanting dysfunctional EPCs for autologous therapy in ischemic diseases by increasing the number and also the quality of transplanted EPCs.

## Conclusions

Taken together, EPCs serve as a highly promising and novel therapeutic option for ischemic tissue repair. It is important to clarify if EPC-based therapy is superior over other types of stem/progenitor cells. Thus additional investigations related to optimize techniques for EPC isolation, expansion, mobilization, recruitment, and EPC retention and survival strategies post transplantation in a ischemic tissue are needed to continue the advancement of this novel therapeutic modality (Figure 2). Thus resolving the above issues will achieve full-benefits of EPC based therapies for ischemic tissue repair representing a future direction.

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