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Vascular Regeneration by Endothelial Progenitor Cells in Health and Diseases

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Abstract

Human endothelial progenitor cells (hEPCs) are adult stem cells, located in the bone marrow and peripheral blood. These cells can be differentiated into mature endothelial cells, which are involved in processes of angiogenesis and vessel regeneration. Different phenotypes and subtypes of endothelial progenitor cells (EPCs), such as early and late EPCs, have been described according to their functionality. Thus, it has been shown that early EPCs release cytokines that promote tissue regeneration and neovasculogenesis, whereas late EPC and endothelial colony forming cells (ECFCs) contribute to the formation of blood vessels and stimulate tube formation. It has been demonstrated that the number of circulating hEPC is decreased in individuals with hypercholesterolemia, hypertension, and/or diabetes. In addition, the number and the migratory activity of these cells are inversely correlated with risk factors such as hypertension, hypercholesterolemia, diabetes, and metabolic syndrome. On the other hand, the number of circulating hEPC is increased in hypoxia or acute myocardial infarction (AMI). hEPCs have been used for cell-based therapies due to their capacity to contribute in the re-endothelialization of injured blood vessels and neovascularization in ischemic tissues. This chapter provides an overview of the key role of hEPC in promoting angiogenesis and their potential use for cell therapy.

Keywords: stem cell, endothelial progenitor cells, angiogenesis, vascular regeneration, cell therapy

1. Introduction

Stem cells are characterized by their ability to proliferate and self-renew in response to signals or stimuli generated by the microenvironment. These signals can also induce the differentiation of stem cells into diverse cell types with specialized features and functions [1, 2]. According to their differentiation potential, stem cells can be classified as either embryonic or adult. The characteristics of both cell populations are summarized in **Table 1**. In this chapter, we will focus on adult stem cell. This subtype of stem cells is present in several tissues and is thought to be a part of the natural tissue repair system (**Figure 1**). Adult stem cells can be present not only in tissues with high regeneration potential, such as the skin, intestinal epithelium [3], and vascular tissue [3] but also in tissues with lower cell turnover like the brain [4]. They are responsible for tissue regeneration, and they can be classified as hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and endothelial progenitor cells (EPCs).

Characteristics	Embryonic stem cells	Adult stem cells
Proliferation capacity	+++	+
Potential differentiation	+++	++
Cellular availability	+++	+
Immunogenicity allogenic	++	+++
Teratogenicity	Yes	No
Ethical acceptability	No	Yes
Complexity of isolation	+++	++
Clinical practice	No	Yes

+++: high; ++: medium; +: low.
Adapted with permission from Smart and Riley [127] and Adams et al. [128].

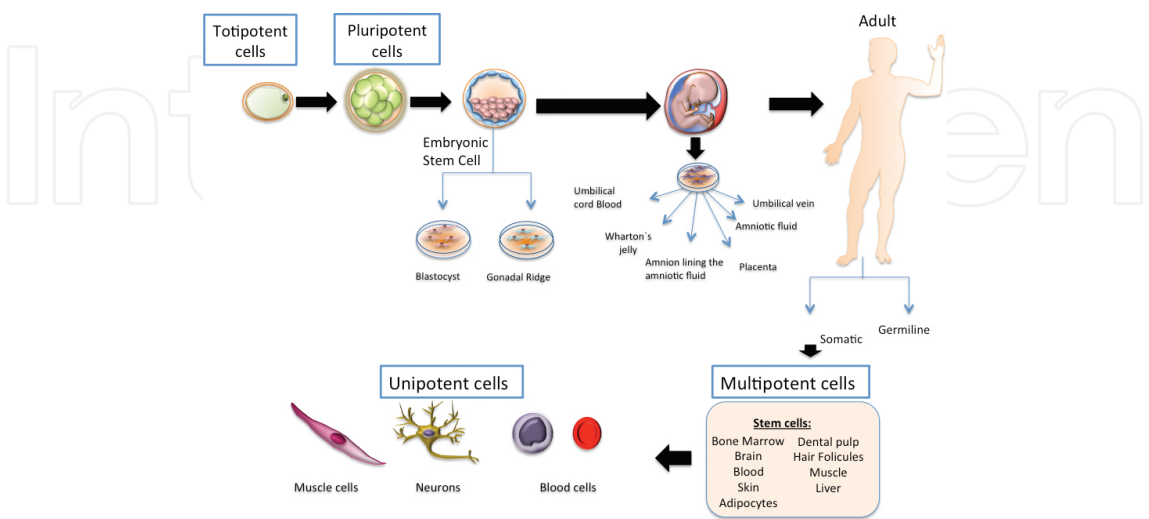**Table 1.** Main characteristics of human stem cells.

Figure 1. Types of stem cell and their potential differentiation.

2. Hematopoietic stem cells (HSCs)

HSCs are multipotent tissue-specific stem cells that give rise and maintain lifelong hematopoiesis [5]. HSCs only comprise approximately 0.001–0.01% of total bone marrow cells in mice and approximately 0.01–0.2% of total bone marrow mononuclear cells in humans [6]. Moreover, HSCs express cytokines receptors, allowing them to respond to signals from immune cells and to sense pathogens during inflammation or infection. This capacity allows them to adapt their cycling and differentiation behavior according to the requirements of the body [7].

3. Mesenchymal stem cells (MSCs)

MSCs are bone marrow–derived stem cells that have the capacity to form plastic-adherent colony forming unit-fibroblasts (CFU-f) [8]. They exhibit a well-known phenotype ($CD73^+CD90^+CD105^+CD34^-CD45^-$), and they have the capacity to differentiate into osteoblasts, adipocytes, and chondroblasts [9]. Furthermore, they can be also differentiated into numerous cell types derived from all three embryonic layers, which include muscle, vascular, nervous, hematopoietic, and bone cells, among others. MSCs can be isolated from bone marrow, adipose tissue, synovium, skeletal muscle, dermis, pericytes, amniotic fluid, umbilical cord, and even human peripheral blood [10–13]. These cells are indeed promising candidates for tissue engineering and cell-based therapies not only because of their multipotent differentiation potential but also due to their low immunogenicity [14].

4. Human endothelial progenitor cells (hEPCs)

hEPCs are adult stem cells characterized by the capacity to proliferate [15], self-renew and repair endothelial tissue [16]. They have been successfully isolated from peripheral blood [16], placenta, and bone marrow [17]. Several cell surface markers have been described to identify hEPC, such as CD34 [18], vascular endothelial growth factor (VEGF) receptor-1 or Flt-1 [19, 20], CD133 or prominine-1 (surface glycoprotein), Tie-2 (endothelial receptor tyrosine kinase), Von Willebrand factor, Nanog, and Oct-4 (Octámer-4) [21].

The original description of hEPCs by Asahara et al. was based on (1) the ability of hEPC to adhere to fibronectin-coated surfaces and (2) the surface expression of both immature stem cells (CD34, CD45, VEGFR2, or Flk-1) and mature endothelial cell (EC) markers (CD31, E-selectin, and angiopoietin receptor Tie-2) [20]. In addition, the expression of endothelial nitric oxide synthase (eNOS), the synthesis of nitric oxide (NO), and the ability to incorporate low-density lipoproteins (LDL) have been also associated with differentiation of hEPC toward endothelial cells [22].

4.1. Origin of hEPCs

To date, at least four cell sources of circulating hEPCs have been described: (1) HCSs (hemangioblast and myeloid cell), (2) bone marrow–derived MSCs, (3) hEPC not derived from bone

marrow (fat and resident cells in tissues such as heart, liver, intestine, and nervous system), and (4) mature ECs migrating from the vascular wall [16, 23]. The best-characterized and most abundant hEPC are hematopoietic-derived hEPC, which can be isolated from peripheral blood mononuclear cells (PBMCs), umbilical cord, and placenta [16, 24]. Despite the fact that hematopoietic-derived hEPC are identified in different tissues, they have similar features, for example, hEPCs from umbilical cord exhibit the same surface markers (CD34, CD146, vWF, and VEGFR2) as hEPC from peripheral blood [25]. Other similarities between hematopoietic-derived hEPC include the ability to uptake modified LDL and the capacity to form capillary type structures in matrigel [26]. It has been shown that circulating monocytes have also the potential to differentiate into a variety of cell types (transdifferentiation), including EPCs [27]. Schmeisser et al. showed that CD14$^+$CD34$^-$ cells, isolated from PBMCs and cultured for 2–4 weeks on fibronectin-coated plates with VEGF supplemented medium, were able to express markers of ECs, such as von Willebrand factor (vWF) [20], vascular endothelial (VE)-cadherin, and eNOS [28]. In addition, these CD14$^+$ cells changed their phenotype toward endothelial morphology and were able to form capillary type structures on matrigel [29, 30]. The principal surface markers of hEPC are shown in **Table 2**.

Hemangioblast	Early hEPC	Late hEPC	Endothelial cell
CD 34$^+$	CD 34$^+$	CD 34$^+$	CD 34$^+$
CD 133$^\pm$	CD 133$^+$	CD 31$^+$	CD 31$^+$
VEGFR2$^+$	CD 31$^+$	VEGFR2$^+$	VEGFR2$^+$
	VEGFR2$^+$	VE-cad$^+$	VE-cad$^+$
		E-selectin$^+$	E-selectin$^+$
		e-NOS$^+$	e-NOS$^+$
		vWF$^+$	vWF$^+$

VEGFR2, vascular endothelial growth factor receptor; vWF, von Willebrand factor, eNOS, endothelial nitric oxide synthase; VE-Cad, vascular endothelial cadherin.
Adapted with permission from: Hur et al. [56].

Table 2. Surface markers of hEPC.

Hematopoietic-derived hEPCs are maintained in a particular niche in the bone marrow, and they can be released into circulation by cytokines such as VEGF or stromal-derived factor 1 (SDF-1), synthesized by ischemic tissues and hormonal stimuli. Once in circulation, hEPCs are recruited to repair damaged endothelium and/or induce blood vessel formation. In target tissues, they can be differentiated into mature ECs to lead re-endothelialization processes and neovascularization [16].

Circulating hEPCs can be isolated and cultured from PBMCs by three different methods:

a.   Cell-culture on fibronectin matrix in the presence of VEGF [20]. Under these conditions, hEPCs are selected by their ability to bind fibronectin. After removing PBMCs in suspen-

sion, early hEPC can be identified after 3 days of culture, whereas late hEPCs are observed after 2 weeks of culture.

b. Successive cell-cultures on fibronectin matrix [31]. This method takes into consideration a preliminary cell-culture of PBMCs in fibronectin-coated plates with medium without VEGF for 48 h. After that, cells in suspension are cultured for 5 days in a second fibronectin-coated plate to induce the adherence of hEPCs to the matrix and the generation of colony forming units of endothelial cells (CFU-ECs). Recent studies indicate that this technique selects a mixture of hematopoietic cells, including monocytes, lymphocytes, and progenitor cells [29].

c. Cell-culture on collagen matrix. For this method, PBMCs are cultured in basal medium for 24 h in collagen-coated plates to induce the adherence of hEPCs to the collagen. hEPCs are then recovered and cultured again for 14 days to obtain mature ECs with high proliferative capacity called CFU-ECs [31]. These cells were initially considered as "real" hEPCs because they do not express myeloid or hematopoietic markers and have the ability to form capillary-type structures similar to mature ECs. CFU-ECs express high levels of CD34 and KDR and low levels of CD45 on their cell membrane. The origin of the ECFCs has not been described yet, but it has been suggested that these cells could be derived from the vascular wall [30].

4.2. Quantification of circulating EPCs

Since EPCs can be identified from peripheral blood samples, their detection, quantification, and characterization may be considered as potential diagnostic and prognostic biomarkers and as a novel therapeutic option for cardiovascular disorders. The main methods to quantify EPCs in human studies can be divided into two approaches: flow cytometry and CFU assays; these are also the two most widely used methods for EPCs quantification. Flow cytometry offers the advantage of a multiparameter approach that allows the identification of both endothelial and stem cell markers. However, the gating strategies used to interpret the flow cytometric events are still highly variable and dependent on the criteria of each research group; therefore, a well-defined and uniform gating strategy to identify these cells has not been fully established yet.

The quantification of EPCs by flow cytometry requires a combination of antibodies that recognize antigens of both progenitor and endothelial cells. This technique has allowed to identify that *in vitro* cultured $CD34^+/KDR^+$ cells home to sites of neovascularization. Based on a review of studies using EPC phenotypes as biomarker in different diseases, the $CD34^+/KDR^+/CD45^{dim}$ phenotype appears to be the best option to identify these cells in terms of sensitivity, specificity, and reliability to quantify EPC in the clinical settings [32].

In terms of absolute quantification, it has been shown that peripheral blood samples from healthy donors ($n = 10$) have a median value of 1.88 $CD45^{dim}CD34^+VEGFR2^+$ EPCs per microliter. Similar data reported by Van Craenenbroeck et al. showed that the median value of $CD34^+VEGFR-2^+CD133^+$ EPCs was 1.95 per microliter [33]. Other authors have reported similar values of peripheral blood EPCs [34–36].

The different absolute numbers obtained for circulating EPC quantification could be explained by the use of different gating strategies and phenotypes to identify EPC subpopulation.

4.3. Migration, recruitment, and differentiation toward EPCs

In healthy individuals, hEPC correspond to the 0.0001–0.01% of the total cells in blood circulation [37]. The majority of these cells are located in the bone marrow as stem cells in a quiescent state. In this tissue, hEPCs are surrounded by stromal cells in a microenvironment characterized by low oxygen tension and high levels of chemoattractant molecules [29, 38]. Different factors such as hypoxia, trauma, physical exercise, estrogen, or cytokines can access to the bone marrow from circulation and induce the release of stem cells with the potential to differentiate toward hEPCs. Once released, stem cells migrate via circulatory system to the injury zone. How these cells reach the site of injury is not totally understood; however, it has been described that cells can be guided by the concentration gradient of different chemoattractant molecules [39].
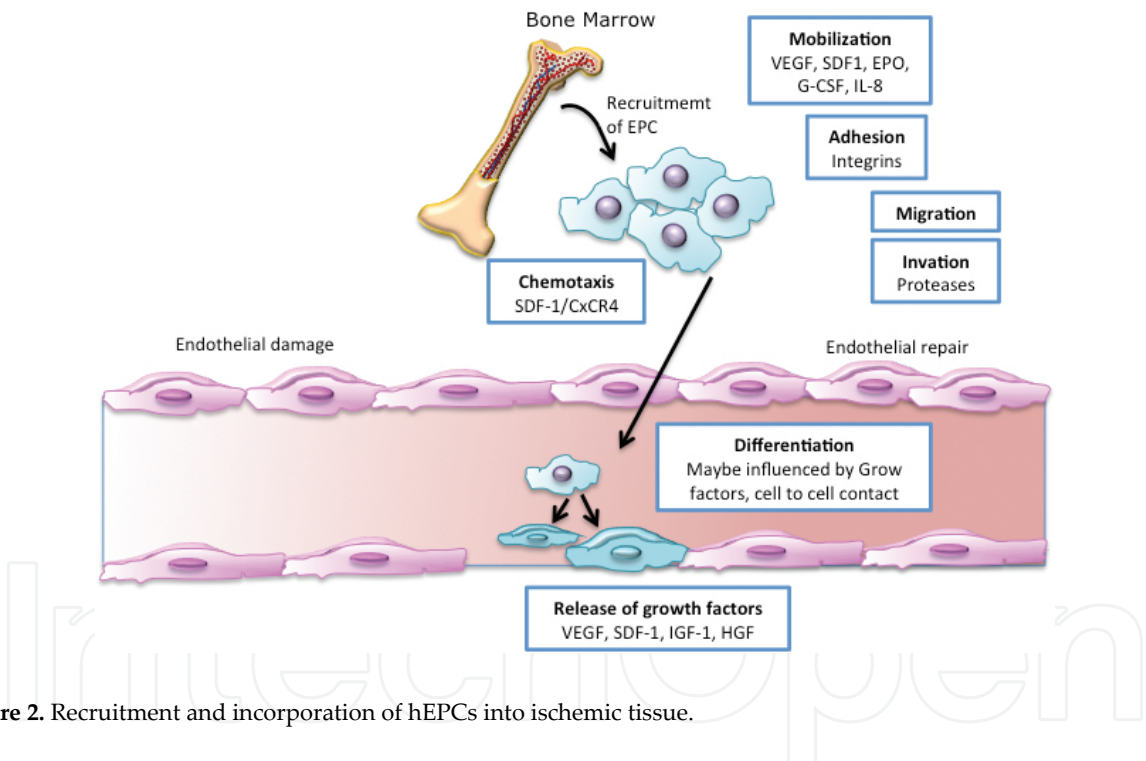

Figure 2. Recruitment and incorporation of hEPCs into ischemic tissue.

It has been shown that hEPC migration and mobilization is related to the secretion of angiogenic growth factors such as VEGF-A, VEGF-B, stromal cell-derived factor 1 (SDF-1), and insulin-like growth factor-I (IGF-1) that attract cells to the site of injury [40]. SDF-1 is a potent chemoattractant molecule released by platelets during endothelial damage [41], and its effects are dependent on the activation of the CXCR4 receptor. VEGF exerts its effect via tyrosine kinase receptors, VEGFR1 or VEGFR2, VEGFR3, which are mainly expressed in ECs from blood and lymph vessels. VEGF is produced by different cell types, such as ECs and smooth muscle cells, and is a potent angiogenic agent that regulates key steps in the process of angiogenesis, including proliferation and migration of ECs [42] and hEPC [43]. Cytokines, such

as tumor necrosis factor alpha (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), interleukin (IL)-6, and IL-3, trigger the mobilization and recruitment of hEPC. *In vivo* studies by Jin et al. in animal models subjected to ischemia demonstrated that the release of soluble proteins such as thrombopoietin (TPO), sKitL hematopoietic cytokines (soluble ligand kit), erythropoietin (EPO), and GM-CSF induced the release of SDF-1 from platelets, enhancing neovascularization via mobilization of CXCR4$^+$VEGFR1$^+$ hemangiocytes [44]. Another study observed that there is an early vascular response involving platelet adhesion to exposed subendothelium, which represents a critical step in the homing of hEPCs to the site of endothelial disruption [45] (**Figure 2**).

As mentioned, hEPCs migrate and home to specific sites following ischemic via growth factor and cytokine gradients. Some growth factors are unstable under acidic conditions of tissue ischemia; therefore, synthetic analogues stable at low pH may provide a more effective therapeutic approach for inducing hEPC mobilization and cerebral neovascularization after an ischemic stroke [46, 47].
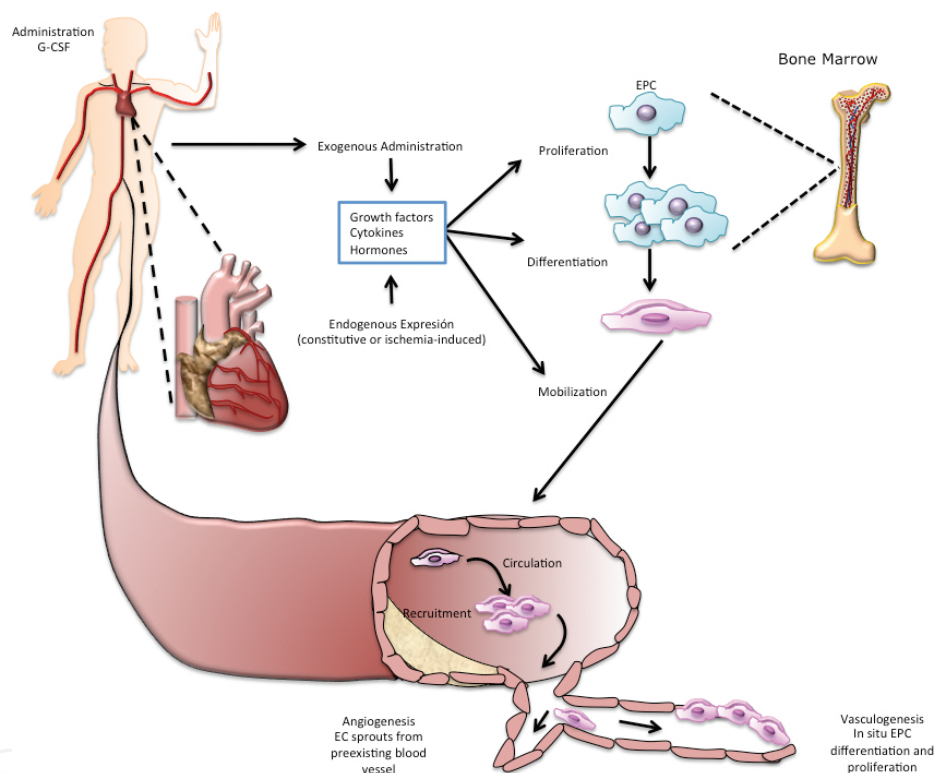
Also, the release of hEPC from the stem cell niche in the bone marrow has been associated with the activation of proteinases such as elastase, cathepsin G, and matrix metalloproteinases (MMP) [48]. It has been shown that stromal cells can maintain precursor stem cells or hEPCs in the bone marrow via the interaction of c-Kit ligand (cKitL), expressed on stromal cells and their receptors expressed on precursor hEPCs. The mechanism of this interaction is under investigation; however, it is known that stromal cells induce the synthesis of nitric oxide (NO) and MMP-9 in response to VEGF, SDF-1, and GM-CSF. The production of these two proteins has been associated with the cleavage of cKitL in stromal cells, allowing the release of hEPCs toward circulation [49–51].

4.4. EPCs and angiogenesis *in vivo*

Angiogenesis and re-endothelialization are required for the maintenance of vascular homeostasis. Initially, it was thought that these processes occurred exclusively by the migration and proliferation of mature ECs surrounding the endothelial injury. Nowadays, new vascular repair mechanisms involving precursor cells from bone marrow, such as hEPCs have been proposed [52–54]. *In vitro* studies conducted in matrigel angiogenesis have shown that hEPCs have the ability to form capillary structures, depending on their maturation stage [55, 56]. For example, early hEPCs can migrate into a tubular network already formed and secrete IL-8 and VEGF, but they cannot form new capillary structures [57]. On the other hand, late hEPC lose their secretory capacity, but they can form capillary structures *in vitro* [56]. The ability of hEPC to form capillary structures *in vitro* and *in vivo* allowed the development of new treatments for vascular diseases. It has been demonstrated that cell therapy performed with *in vitro*-cultured EPCs, successfully promote neovascularization in ischemic tissues without the coadministration of angiogenic growth factors [58]. Several studies have shown that hEPCs from peripheral blood can induce endothelial cells turnover, via differentiation into functional mature ECs [59–62]. Kalka et al. performed this therapeutic strategy of neovascularization for the first time in 2000 [63]. They showed improved neovascularization and functional recovery when hEPCs were injected intravenously in immunodeficient mice suffering from ischemia in the lower

limbs. In rat models of myocardial ischemia, the treatment with hEPC improved the migration of cells into the neovascularization area, as well as their ability to differentiate into mature ECs, which in turn was associated with the recovery of ventricular function and reduction of the ischemic area size [64, 65]. In another study, Cui et al. injected green fluorescent protein-tagged EPCs (GFP-EPCs) in murine models exhibiting damaged endothelium by ligation of the left carotid artery. In these animals, GFP-EPCs were detected at the site of injury contributing to the process of re-endothelialization [59]. The presence of GFP-EPCs in the injury enhances re-endothelialization associated with decreased neointimal formation, demonstrating that EPCs have an active role in tissue repair [59] (**Figure 3**). Other research groups have also shown that EPCs have been associated with improvements in the re-endothelialization and neointimal formation in animal models [60, 62, 65].

Figure 3. Mobilization of hEPCs from the bone marrow.

All these studies have shown that hEPC are crucial for vascular repair, and it has been observed that the number and migratory activity of these cells in blood are inversely correlated with the presence of risk factors for coronary artery disease [66]. Therefore, an adequate number and a correct functional state of hEPCs are required for the maintenance of the endothelium and vascular remodeling.

4.5. Mobilization mechanisms of EPCs in ischemia

One of the main transcription factors induced during acute and chronic ischemia in response to hypoxia is the hypoxia inducible factor 1 (HIF-1). In general, the activation of the HIF-1

pathway has been associated with protective responses during ischemia. The mechanism of activation of HIF-1 has been extensively described by Agani and Jiang [67]. HIF-1 is a transcription complex formed by two subunits, alpha (Hif-1α) and beta (Hif-1β). While Hif-1β is constitutively expressed, Hif-1α levels are highly regulated by cellular oxygen partial pressure, thus Hif-1α-mediated cellular responses depend on oxygen levels [68]. After Hif-1α induction, in response to low oxygen partial pressure, the ECs undergo prosurvival signals, which include the increased expression of VEGF and angiogenesis. HIF-1α is the main direct regulator of EC function and its upregulation in EPCs promoted differentiation, proliferation, and migration in a model of hindlimb ischemia [69].

HIF-1α-transfected EPCs exhibited higher revascularization potential, as increased capillary density was observed at the site of injury. This study suggests that siRNA-mediated downregulation of the HIF-1 α gene can effectively sensitize EPCs to hypoxic conditions. It can also significantly blunt early EPC growth and differentiation into ECs [70]. The underlying mechanisms of the effect of HIF-1α in EPC have been well described [69–72].

It has been shown that hypoxia-induced HIF-1 is reduced in patients with chronic heart failure (CHF) [73]; however, it has been also observed that exercise transiently increases circulating hEPCs in CHF patients. This transient effect can be sustained for approximately 4 weeks when exercise is combined with statins and/or VEGF treatment [43, 63, 74, 75].
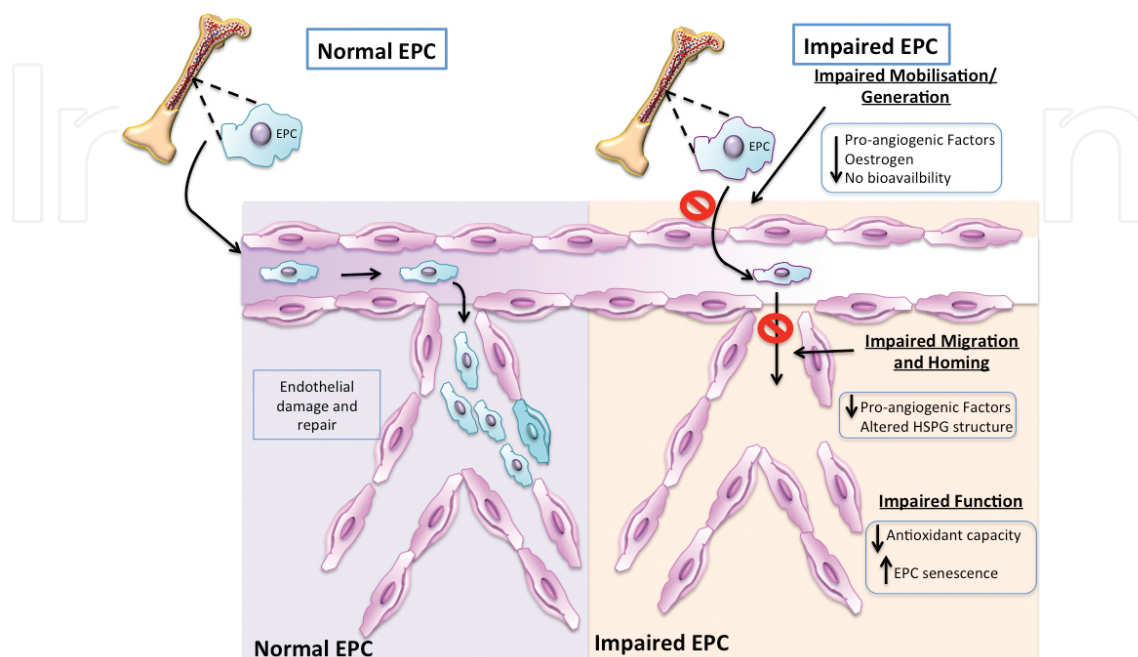
This evidence suggests that EPCs mobilization and recruitment could also be mediated by hypoxic conditions via HIF-1-induced expression of VEGF.

4.6. Cardiovascular risk factors and hEPC function

hEPCs number and functional status are important for their repair capacity; however, these parameters are greatly influenced by clinical condition and risk factors. Indeed, several studies have shown that patients with cardiovascular risk factors such as age, gender, smoking habits, hypertension, diabetes mellitus (DM), and dyslipidemia have reduced number and function of hEPCs in peripheral blood. In contrast, some cytokines, hormones, drugs, and physical activity can increase not only the circulating number of hEPC but also their function [30, 49, 74, 76] (**Figure 4**).

Vasa et al. showed that the number of hEPC inversely correlates with cardiovascular risk factors (age and LDL cholesterol levels). According to these results, patients with higher cardiovascular risk factors have lower number of circulating hEPC compared with the control group [66]. Studies by Hill et al. showed a positive correlation between hEPC colony numbers in culture and endothelium-dependent vasodilatation and a negative correlation between hEPC colony number and the Framingham index [31]. Moreover, a negative correlation between the severity of atherosclerosis and hEPC levels has been described, showing decreased circulating hEPCs levels as an early risk factor of subclinical atherosclerosis [77]. Furthermore, reduced number of circulating hEPCs has been found in patients with hypercholesterolemia, which correlates with the fact that increased plasma cholesterol levels have been linked with endothelial damage. In the same study, the number of hEPCs was negatively correlated with total cholesterol and low-density lipoprotein (LDL) cholesterol level [78]. On the other hand,

it has been also observed that the number of circulating hEPCs increases significantly after exercise [79] and in response to statins [80], antidiabetic (Pioglitazone, Sitagliptin) [81], and antihypertensive drugs (Ramipril and Enalapril) [82, 83].

Figure 4. Mechanism of contributes EPC to the repair of injured vessels.

4.7. Correlation of EPC and clinical conditions

In addition, lower numbers of circulating hEPCs have been observed in individuals with stable and unstable angina [84], erectile dysfunction [85], and atherosclerosis [86] compared with healthy volunteers. Patients with type 1 and 2 diabetes also show lower number and functionality of hEPC than healthy individuals [87]. For instance, poor glycemic control, determined by HbA1c levels, appears to be associated with a reduction in the number of circulating EPCs, whereas an adequate control of glycemia seems to increase their numbers [88]. Several mechanisms seem to be involved in that, including advanced glycation end products formation [89], reduced activity of silent information regulator 1 (SIRT1), and increased synthesis of platelet-activating factor (PAF) [90].

Patients with familial hypercholesterolemia and hypertension [91, 92] also showed lower number and function of circulating hEPC. However, this last effect was reversed when angiotensin-converting-enzyme inhibitor (ACE-inhibitor) was used, a phenomenon associated with reduction in the progress of vascular damage [93]. Imanishi et al. have reported that hEPCs senescence is accelerated in both experimental hypertensive rats and in patients with essential hypertension, which may be related to telomerase inactivation [94, 95]. They also found that the hypertension-induced EPC senescence decreases vascular remodeling process [95].

Other conditions affecting the functionality of hEPC are ischemic heart disease and nonalcoholic fatty liver disease (NAFLD) [96]. Also, in patients with stable coronary artery disease (CAD [66, 97]), heart failure deterioration has been correlated with low number of circulating hEPC.

Furthermore, EPCs play an important role in the development and regulation of vascularization in pregnancy. Luppi et al. reported a progressive increase of circulating CD133$^+$/VEGFR-2$^+$ cells from the first trimester onwards, with a significant rise of CD34$^+$/VEGFR$^+$ cells near-term [98]. In preeclampsia for example, a pregnancy condition associated with hypertension, Matsubara et al. reported no difference in the number of circulating EPCs [99]. In contrast, studies from Sugawara et al. and Lin et al. showed lower cell numbers of circulating hEPCs in this condition compared with normal pregnancies [100, 101].

Conditions	Number hEPC	Function hEPC	Reference
Rheumatoid arthritis *in vitro*	Increased CD34$^+$/CD133$^+$/KDR$^+$	Decreased migration	[129, 130]
Atherosclerosis/heart attacks	Increased colony forming units	Increased migration in patients	[131]
Gestational diabetes 3rd trimester	Decrease in CD34$^+$/CD133$^+$	ND	[132]
Diabetes mellitus type II	Increased in CD34$^+$	Decreased vasculogenesis and adhesion capacity	[133, 134]
Erectile dysfunction	Decreased number of CD34$^+$/KDR$^+$	ND	[135]
Exercising	Increased in CD34$^+$ cells in vitro	Increased migration, proliferation	[136]
		Angiogenic capacity	[137, 138]
Erythropoietin	Increased CD34+	Increased migration	[139, 140]
		Angiogenesis	[140]
Statins	Increase proliferation	Increased migration	[66, 74]
Estradiol	Increased CD34+	Increased mobilization	[141]
Hyperhomocysteinemia	Increased CD34+ in vitro	Decreased proliferation, migration, adhesion and vasculogenic capacity	[76, 92, 142]
Acute myocardial infarct	Increased colony forming units	Increased migration and proliferation	[43, 75]
Low-density lipoprotein	CD34$^+$/KDR$^+$	Decreased migration	[31, 66]
Hypertension			[31, 143]
Prostaglandin E	Increased CD34$^+$	Increased migration and improves the function	[144]
C-reactive protein	Decreased CD34$^+$ in vitro	Decreased angiogenic capacity	[145]

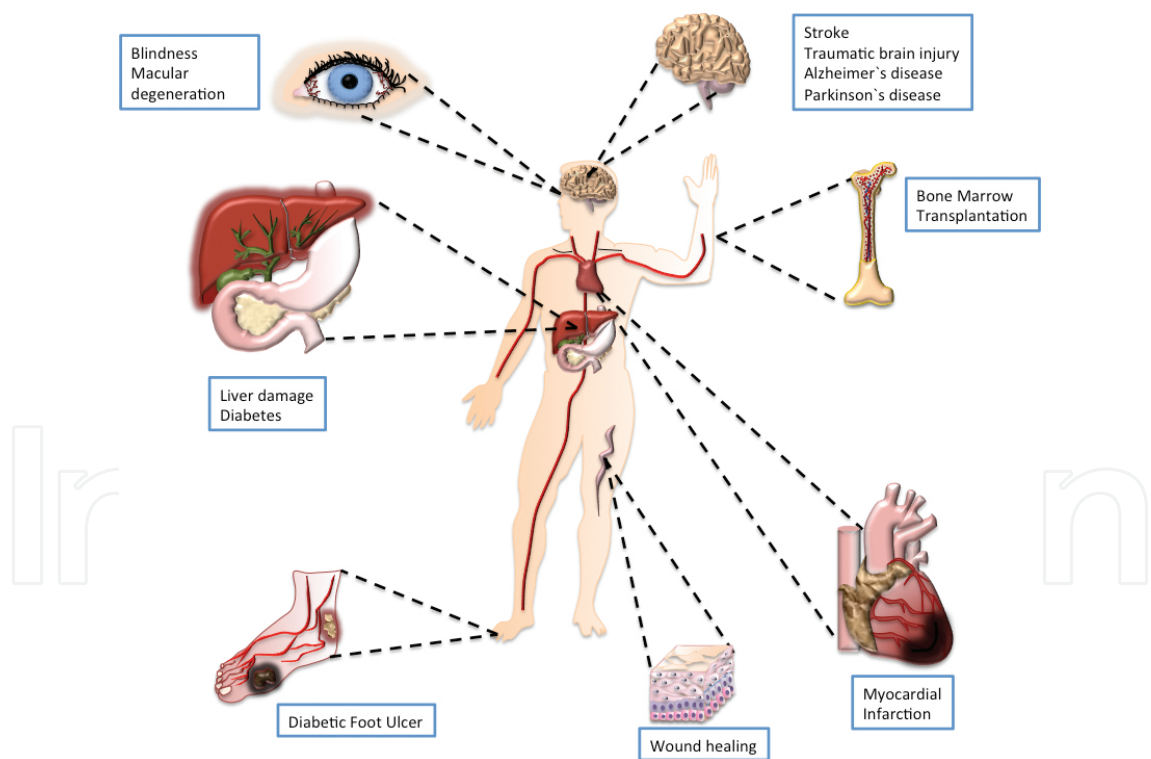**Table 3.** Physiological and pathological conditions and their effect on hEPC.

Patients with obesity were reported to have reduced numbers of circulating hEPCs, and this was inversely associated with an increased intima-media thickness [102]. Obesity was a more prominent predictor of the number of hEPC than any other cardiovascular risk factors, and

weight loss was associated with an increased hEPC count and an improved brachial artery flow-mediated dilation. Similar evidence suggests that overweight is associated with reduced capacity to produce colony-forming units [103].

Altogether these studies support the idea that hEPCs play an important role in the maintenance of vasculature homeostasis. Thus, new therapeutic strategies should aim to increase their number and functionality in circulation. A summary of the main physiological and pathological conditions associated with functionality of hEPC is shown in **Table 3**.

4.8. Clinical translation of EPC therapy

Stem cell therapy holds great promise to restore damaged vessels. Researchers have made significant progress in cell transplantation in preclinical and clinical settings. For example, initial preclinical studies have reported favorable improvements in left ventricular function in a rat model of acute myocardial infarction (AMI) after intravenous injection of *ex vivo* expanded human CD34+ cells [104]. In another study, the intramyocardial injection of EPC in a swine model of AMI reduced the scar formation and prevented the left ventricular dysfunction after AMI, providing encouraging outcomes in favoring the application of EPCs as a potential therapy in clinical trials [105, 106] (**Figure 5**).

Figure 5. Potential therapeutic features and the sources of their extraction of EPC.

In the human studies performed by Li et al. [108] and Lasala et al. [107] it has been shown that intracoronary infusion of hEPC in patients with AMI were associated with the migration and incorporation of hEPCs in the infarcted tissue, a reduction of infarct size, and secretion of

angiogenic growth factors including VEGF, SDF-1, and IGF-1, which produced more capillarity and higher transdifferentiation of cells to cardiac progenitor cardiomyocytes [107, 108]. Moreover, these hEPCs also reduced apoptosis of endothelial cells and increased myocardial viability in the infarcted area [109, 110]. Studies from Dobert et al. described increased myocardial viability in patients receiving intracoronary infusion of peripheral blood bone marrow-derived hEPCs 4 days after myocardial infarction [111]. In addition, other studies [112, 113] suggest that adhesion and differentiation of hEPC into mature ECs in infarcted tissue is partially modulated by fibrin, which in turn promotes angiogenesis. Similar studies have been conducted in patients with chronic critical limb ischemia of the lower extremities. In a Phase II clinical study, patients who received CD133+ cells, obtained from peripheral blood and mobilized with G-CSF, experienced limb salvage, symptomatic relief, appearance of blood flow, and significant functional improvement at the site of injury [114–116]. Similarly, treatment with autologous G-CSF-mobilized peripheral blood CD34+ cells in nonhealing diabetic foot patients have been promising [117].

Bone marrow-derived EPCs may be mobilized to stimulate angiogenesis and may attenuate tissue ischemia CAD and peripheral arterial disease (PAD). For instance, intramyocardial transplantation of autologous CD34+ cells improved survival in patients with cardiovascular diseases [118]. In another study, patients with refractory angina who received autologous CD34+ cells showed a reduction of angina frequency and improvement of exercise tolerance [119].

In addition, hEPCs may contribute to liver repair and regeneration by promoting the secretion of supportive factors to induce host's endogenous repair mechanisms [120]. EPC treatment has been shown to halt the progression of liver fibrosis in rats by suppressing hepatic cell activation by increasing the MMP activity and regulating hepatocyte [121].

Similar evidence has suggested that hEPCs are involved in the recovery after deep vein thrombosis (DVT). DVT is characterized by a fibrotic vein injury with loss of venous compliance and subsequent venous hypertension [122]. In this disease, hEPCs were involved in blood vessel recanalization in organized venous thrombi [123]. Human studies suggest that children with idiopathic pulmonary arterial hypertension (IPAH) had no severe adverse events after hEPCs infusion and improved pulmonary functions [124, 125]. In animal models, Baker et al. (2013) described the use of autologous bone marrow-derived EPCs in a rat model of pulmonary arterial hypertension (PAH) [126]. They found that EPCs reduced the hemodynamics and ventricular weight, at the same time that they increased connexin, eNOS expression and activity, Bcl-2 expression, and the number of alveolar sacs and small lung arterioles.

5. Concluding remarks

Vascular regeneration is a dynamic area of research showing remarkable medical advances, both in basic science and in the clinical application field. The preclinical and clinical studies reviewed here strongly support a therapeutic potential use of EPCs in the treatment of cardiovascular diseases; however, the very low number of these cells limits their use for cell-

based therapies. The number of EPCs needed for therapy in human adults is relatively large, that is, about 3×10^8 to 6×10^8 cells, which means that 8.5–120 L of peripheral blood are required to isolate an adequate number of EPCs. Therefore, protocols aimed to expand EPCs will be needed for future therapies. However, EPCs can be used in the present as a biomarker to identify the state of diverse diseases.

The mechanisms by which EPCs mediate vessel growth and repair could potentially be ascribed to a variety of angiogenic factors produced by EPCs. However, optimal quality/ quantity of EPCs is essential to set up a successful therapeutic EPC-based approach. In order to get this, it is important to improve the isolation, characterization, and expansion methods to obtain the optimal numbers and functionality of EPCs. In addition, it is also relevant to improve the administration of these cells and the cellular application techniques such as quantification of EPC. Finally, a positive clinical outcome will be the main indicative to demonstrate whether these are able to repair the disease-based dysfunction by the different mechanism already mentioned in this chapter.

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