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Concise Review: Endothelial Progenitor Cells in Regenerative Medicine: Applications and Challenges

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ABSTRACT

Endothelial progenitor cells (EPCs) are currently being studied as candidate cell sources for revascularization strategies. Significant advances have been made in understanding the biology of EPCs, and preclinical studies have demonstrated the vasculogenic, angiogenic, and beneficial paracrine effects of transplanted EPCs in the treatment of ischemic diseases. Despite these promising results, widespread clinical acceptance of EPCs for clinical therapies remains hampered by several challenges. The present study provides a concise summary of the different EPC populations being studied for ischemic therapies and their known roles in the healing of ischemic tissues. The challenges and issues surrounding the use of EPCs and the current strategies being developed to improve the harvest efficiency and functionality of EPCs for application in regenerative medicine are discussed. STEM CELLS TRANSLATIONAL MEDICINE *2016;5:530–538*

SIGNIFICANCE

Endothelial progenitor cells (EPCs) have immense clinical value for cardiovascular therapies. The present study provides a concise description of the EPC subpopulations being evaluated for clinical applications. The current major lines of investigation involving preclinical and clinical evaluations of EPCs are discussed, and significant gaps limiting the translation of EPCs are highlighted. The present report could be useful for clinicians and clinical researchers with interests in ischemic therapy and for basic scientists working in the related fields of tissue engineering and regenerative medicine.

INTRODUCTION

The term "endothelial progenitor cells" (EPCs) might be fundamentally used to refer to populations of cells that are capable of differentiation into mature endothelial cells (ECs), with purported physiological roles in angiogenesis (the sprouting of new blood vessels from existing ones) and vasculogenesis (de novo formation of vascular networks) [1]. These features make EPC populations valuable cellular candidates or therapeutic targets in regenerative medicine, with several strategies being developed to use them, including direct cellular transplantation and tissue engineering approaches. Efforts to translate these efforts to the clinic have, however, been hampered by several issues, including controversies over the identity and functions of EPCs, the limited numbers of EPCs, and their clinical potency. In the present report, we begin with a description of EPC populations, leading to an overview of clinical strategies that have been developed to use EPCs in regenerative medicine. The factors limiting the use of EPCs and the current research themes to resolve these issues are also discussed.

IDENTIFICATION AND CHARACTERIZATION OF EPC POPULATIONS

The discovery of endothelial progenitor cells has been credited to Asahara et al. for identifying a hematopoietic population in adult peripheral blood capable of eliciting postnatal vasculogenesis [2]. Subsequent studies suggested that EPC numbers could be used in clinics as a biomarker of cardiovascular disease [3], an important line of investigation that continues today [4]. In the context of regenerative medicine, it is the capacity for vascular regeneration and the potential for ischemic therapy for which EPCs are most valued. However, significant controversies exist over the identity and roles of EPCs in vascular repair. Thus, a brief discourse on the major EPC populations reported in published studies is necessary to facilitate further discussion. During the past two decades, the term "EPC" has been used to describe a burgeoning range of cell types defined by their isolation and culture methods, as well as the ontological sources, ranging from fetal trophoblastic tissue to adult bone marrow. A detailed discussion of the myriad EPCs used in

studies is beyond the scope of the present report, and the reader is directed to excellent articles on this topic [4, 5]. We briefly describe two major categories: hematopoietic EPCs and nonhematopoietic EPCs, which differ largely in their ontological origins and isolation methods. It is important to note, however, that it would not be possible to delineate a "superior" cell source for vascular regenerative therapies. Rather, the differences in the isolation and identification of these populations [6] and the potential different contributions to neovasculogenesis [7] should be recognized.

Hematopoietic EPCs

Asahara et al. postulated that EPCs could be isolated from a hematopoietic source and demonstrated that *CD34*$^+$ cells from peripheral blood can contribute to neovascularization and ischemic rescue after injection into an animal model of peripheral limb ischemia [2]. Similarly, *CD133*, another hematopoietic stem cell marker, can be targeted to derive less mature progenitor populations [8]. Cell sorting on *CD34* and/or *CD133* thus emerged as a strategy to derive populations enriched in circulating EPCs (cEPCs), and methods to characterize and derive endothelial cells from such populations have been extensively described [9, 10]. Numerous clinical trials have since been conducted to study the use of cEPC-enriched populations for the treatment of ischemic conditions, including acute myocardial infarction and critical limb ischemia [11]. However, questions remain regarding the precise definition of a bona fide cEPC. Initial studies suggested EPCs exhibited a *CD34*$^+$/*CD133*$^+$/*VEGFR2*$^+$ phenotype [12], a view supported by clinical observations of correlations between this phenotype and cardiovascular conditions [13]. This remains the most commonly recognized profile for cEPCs, despite other studies suggesting the use of other markers, including *CD45, CD105, CD106, CD117, CD144,* acetylated low-density lipoprotein uptake, and aldehyde dehydrogenase activity [5]. It was thus striking when clonal cultures of *CD34*$^+$/*CD133*$^+$/*VEGFR2*$^+$ cells were found to only be capable of differentiating into hematopoietic, and not endothelial, lineages, leading to suggestions that these cells were nonangioblastic hematopoietic progenitors, which support angiogenesis through paracrine effects [14]. In contrast, the nonhematopoietic *CD34*$^+$/*CD45*$^-$ fraction was found in the same study to generate adherent endothelial cells, which were capable of forming networked, vessel-like structures when cultured on Matrigel (BD Biosciences, Franklin Lakes, NJ, http://www.bdbiosciences.com), indicative of the presence of endothelial lineages in this population. Significant debate on the cEPC theory ensued, with proponents [15] arguing against the method used and the interpretation of results by Case et al. [14]. This has been, in large part, resolved by the development of highly defined assays to induce colony formation from cEPCs, with clonogenic assays performed to demonstrate the ability of *CD133*$^+$ cells to differentiate into both hematopoietic and endothelial lineages [9, 16]. Interestingly, it has been observed that *CD34*$^-$ cells are capable of augmenting in vitro vascular network formation and vascularization events in vivo [17], providing some basis for the argument that *CD133*$^+$/*CD34*$^+$ cells are bona fide EPCs but require the presence of auxiliary cells in the *CD34*$^-$ fraction to potentiate vasculogenesis.

In parallel with these efforts, EPCs were also observed to share many common characteristics with monocytic cells [18]. These cells were conventionally selected for their ability to adhere to tissue culture surfaces, leading to the term "early EPCs" (eEPCs). The attached cells demonstrate the ability to uptake lectin and acetylated low-density lipoproteins and express monocytic surface markers, including CD14 [19]. eEPCs have been suggested to derive from monocytes distinct from the CD34$^-$ cEPCs [20]. The exact lineage of these cells has been confounded by contaminant monocytes possibly imparting monocyte-like characteristics to the actual EPCs [21], or the EPCs acquiring endothelial-like characteristics secondary to culture in vascular endothelial growth factor (VEGF)-rich conditions [20]. Regardless of lineage, eEPCs play primarily supportive roles in angiogenesis vascular repair without differentiating themselves into functional endothelial cells [22]. Angiogenic factors secreted by eEPCs include CXCL12, CXCL1, and VEGF, with migration inhibitory factor, a potent cytokine known to induce endothelial and smooth muscle differentiation, the most prominent in the early and late stages of the ischemic event [23]. This has led to calls for a change in nomenclature to circulating angiogenic cells to better reflect their major capacity to induce angiogenesis and vascular sprouting, rather than in the direct formation of nascent blood vessels.

Nonhematopoietic EPCs

In contrast to the hematopoietic EPC, EPCs have been demonstrated to derive from nonhematopoietic tissue, presumably from vessel walls [24, 25]. Termed "endothelial colony forming cells" (ECFCs) or "outgrowth endothelial cells" (OECs/EOCs) for their ability to form colonies of endothelial outgrowths under permissive conditions, ECFCs are most commonly isolated by plating blood-derived mononuclear cells on collagen-coated substrates in endothelial-supportive media [26]. Endothelial outgrowths can be observed to emerge following extended culture, and these cells are capable of rapid amplification, stably generating endothelial progeny with potent vasculogenic properties [27]. It is of interest that the cells derived from such long-term cultures more readily generate mature endothelial progeny in vitro and have also been observed to physically contribute to vasculogenesis [28]. In contrast, it has generally been recognized that hematopoietic EPCs, and eEPCs, in particular, potentiate angiogenesis through the secretion of cytokines [18, 29].

Thus, isolated ECFCs actually represent a heterogeneous mix of progenitors and terminally differentiated endothelial cells with varying proliferative potential, and the lack of surface markers to definitively isolate vasculogenic progenitor populations have contributed to the lack of enthusiasm for translating the use of these cells to the clinic. Proposed profiles for the identification of ECFC-initiating cells include *CD146*$^+$/*CD45*$^-$/*CD133*$^-$, which would be in line with the hypothesis that these EPCs originate from vessel walls rather than the bone marrow [30]. More recently, a *CD45*$^-$/*CD34*$^+$/*CD31*^{low} profile was used to prospectively isolate cells from term placental tissues, which generated pure endothelial populations in culture [25]. Selection on such stringent profiles, however, has been known to yield extremely low yields and, thus, be nonviable for therapeutic use [4].

Clinical Application of EPCs in Regenerative Medicine

In spite of the ongoing controversy over EPC identity, the clinical potential of EPCs in vascular regenerative applications cannot be overlooked [5, 6], with currently more than 150 interventional studies registered at ClinicalTrials.gov. The disease conditions being investigated include ischemic diseases, such as myocardial infarction and peripheral vascular disease (Tables 1, 2). From the completed and ongoing trials, three major applications targeting

Table 1. Completed interventional trials involving endothelial progenitor cells listed on ClinicalTrials.gov

Treatment	Disease therapy	ClinicalTrials.gov no.	Comments	Conclusions
Cellular injections	Lymphedema	NCT01112189	Unsorted mononuclear cells from bone marrow	Potentially effective; reduction of arm volume, pain, and sensitivity [84].
	Advanced liver cirrhosis	NCT01333228	Unsorted mononuclear cells from bone marrow	No published results found
	Leg ulcer/gangrene	NCT00221143	Circulating EPCs ($CD34^+$) from G-CSF mobilized blood	Safe, potentially effective; findings from long-term follow-up (208 weeks) suggest long-term efficacy [82]
	Dilated cardiomyopathy	NCT00629096	Unsorted mononuclear cells from bone marrow	Phase II study; no published results found
	Idiopathic pulmonary arterial hypertension	NCT00641836, NCT00257413	Early EPCs from venous blood	Safe, potentially effective; improved exercise capacity and pulmonary hemodynamics [37]
	Coronary artery disease, refractory angina	NCT00694642	Early EPCs	No published results found
	Critical limb ischemia	NCT01595776	Circulating EPCs ($CD133^+$) from G-CSF mobilized blood	Six of 8 patients demonstrated complete healing of wounds, pain cessation at rest, and walking recovery; suggested highly purified autologous $CD133^+$ cells can stimulate neoangiogenesis [83]
	Pulmonary arterial hypertension	NCT00469027	eNOS-transfected early EPCs	Improved pulmonary hemodynamics; 2 severe adverse reactions reported but not proved to be directly linked to cell therapy; findings suggest safety and efficacy of augmented EPC approach [54]
Stent	Percutaneous coronary intervention (Genous stent)	NCT00494247	Circulating EPCs ($CD34^+$)	Phase IV randomized study of 60 patients; less neointimal hyperplasia and restenosis versus bare metal stents [84]
	Percutaneous coronary intervention (COMBO stent)	NCT01756807	Circulating EPCs ($CD34^+$)	Clinical study to establish healing profile of COMBO stent comprising EPC capture surface and sirolimus-eluting properties; 100% coverage at 150 days; no neoatherosclerosis or late-stent thrombosis observed in 39 patients who completed follow-up; findings not peer-reviewed [85]
	Percutaneous coronary intervention (Genous stent)	NCT01272895; NCT01274234	Circulating EPCs ($CD34^+$)	Early healing profile established by OCT; 100% strut coverage after 42 days; further validation required; findings not peer-reviewed [86]
	Percutaneous coronary intervention (Genous stent)	NCT00349895	Circulating EPCs ($CD34^+$)	Statin therapy combined with stent did not contribute to reduction of in-stent restenosis; concomitant statin therapy stimulated EPC recruitment but did not improve angiographic outcome of stent; angiographic late loss significantly reduced at 6 to 18 months [87]
	Percutaneous coronary intervention (Genous stent)	NCT00732953	Circulating EPCs ($CD34^+$)	Reduced restenosis rates with paclitaxel-coated stent [88]

Table 1. (*Cont'd*)

Treatment	Disease therapy	ClinicalTrials.gov no.	Comments	Conclusions
Mobilization	Diabetic microvasculopathy	NCT02056210	Circulating EPCs ($CD34^+/KDR^+$)	G-CSF plus CXCR4 antagonist plerixafor treatment effective for EPC mobilization in diabetic patients [89]
	Refractory angina pectoris	NCT00272571	Circulating EPCs ($CD34^+/KDR^+$); early EPCs	Mechanical manipulation of blood flow is associated with increased EPC number and function; concomitant improvement in cardiovascular parameters [90]
	Peripheral occlusive artery disease	NCT01952756	Circulating EPCs ($CD34^+/KDR^+$); early EPCs	Cilostazol treatment significantly increased circulating EPC levels and function and proliferation of early EPCs and improved collateral vessel formation and distal runoff in patients [91]
	Hypertension	NCT01041287	Circulating EPCs (CD34, CD133, KDR, CXCR4)	Both nebivolol and metoprolol increased circulating levels of CD34/CD133; improved arterial stiffness and oxidative stress parameters observed in parallel [92]
	ST-segment elevation myocardial infarction	NCT00378352	Circulating EPCs ($CD34^+/CD45^+$)	Randomized, double-blind, placebo-controlled multicenter trial to evaluate erythropoietin on reduction of infarct expansion and remodeling; no beneficial effect of erythropoietin found; EPC levels not reported; associated with adverse cardiovascular events [93]
	Coronary heart disease	NCT01096875	Circulating EPCs ($CD34^+/VEGFR2^+/CD133^+/CD45^-$)	Short-term atorvastatin use increased circulating EPCs pre- and postoperatively; associated with better preservation of sinus rhythm and reduced hsCRP levels [94]
	Metabolic disease	NCT00166036	Not described	Study to evaluate effect of statins on oxidative stress and EPC levels in metabolic disease patients; no results on EPC levels published
	Cardiovascular disease in HIV patients	NCT01552694	Circulating EPCs ($CD34^+/CD133^+/VEGFR2^+$)	Use of sitagliptin to reduce cardiovascular risk in HIV patients; EPC enumeration as secondary outcome measure; no results on EPC levels published
	Diabetes mellitus type 2, insulin resistance	NCT00094796	Not described	Study to evaluate rosiglitazone in diabetes, circulating EPC levels to be measured as secondary outcome; no results on EPC levels published
	Type 1 diabetes mellitus (adolescent)	NCT02019186	Circulating nonhematopoietic EPCs ($CD34^+/CD133^+/CD45^-$)	Negative results showing no effects of vitamins C and E on function or quantity of EPCs [95]
	Coronary artery disease	NCT00641758	Not described	Study to evaluate effect of Pycnogenol on endothelial function in patients with CAD; EPC enumeration as secondary outcome measure; no results on EPC levels published

Abbreviations: CAD, coronary artery disease; eNOS, endothelial nitric oxide synthase; EPCs, endothelial progenitor cells; G-CSF, granulocyte colony-stimulating factor; hsCRP, high-sensitivity C-reactive protein; VEGF, vascular endothelial growth factor.

EPCs can be identified: (a) cellular injections for ischemic conditions; (b) EPC-capture stents; and (c) EPC mobilization therapies.

Cellular Injections

EPCs as a candidate cell source for therapy offer many attractive characteristics, including (a) ready accessibility from peripheral blood; (b) potent angiogenic and vasculogenic effects; and (c) the stability of the lineage and a reduced risk of tumorigenicity. These features led to many studies on their possible utility for therapeutic neovascularization, for which hematopoietic EPCs have been largely favored in such applications because of their ease of harvest, with minimal manipulations and culture periods [31].

Table 2. Ongoing interventional trials involving endothelial progenitor cells listed on ClinicalTrials.gov

Treatment	Disease therapy	ClinicalTrials.gov no.
Stent	Coronary artery lesions	NCT00967902
	Stable coronary artery disease	NCT00911339
Mobilization	Type 2 diabetes	NCT01822548
	Diabetes mellitus	NCT02042339
	Type 2 diabetes	NCT02301806
	Diabetic ulcer	NCT01353937
	Cardiovascular diseases	NCT02194686
	Diabetic retinopathy	NCT02353923

In a murine model of peripheral limb ischemia induced by femoral artery ligation, cEPC injections were shown to significantly improve tissue perfusion and were associated with increased limb salvage rates (58.8% in the cEPC group vs. 7.1% in the control group) [32]. Data from that and other similarly themed studies led to significant optimism for the use of cEPCs for the treatment of ischemic conditions and the initiation of phase I/II clinical trials involving cEPC injections into ischemic myocardia [33]. The results from the primary endpoints suggested the safety of cellular injections, which was borne out by further evidence from randomized, placebo-controlled trials [34, 35]. The administration of unfractionated bone marrow, however, was unable to rescue ischemia in critical limb ischemia studies, suggesting the EPC fraction is responsible for the therapeutic effects [36]. Aside from cEPCs, eEPCs have also been evaluated in the clinic. In a randomized, controlled study of idiopathic pulmonary arterial hypertension, intravenous infusion of autologous eEPCs resulted in improved pulmonary hemodynamics, without severe adverse effects [37]. Taken together, these results provide cautious optimism for EPCs as a cellular candidate for regenerative therapies, and more data from ongoing trials will be useful in establishing the safety profile of EPC therapy. However, questions remain regarding the best route of administration for safety and efficacy. In treatment of peripheral arterial disease (PAD), a meta-analysis conducted of 108 studies involving cellular therapies for the treatment of PAD suggested intramuscular and intra-arterial injections were equally well-tolerated, with the former presenting improved clinical outcomes [39]. Thus, although intra-arterial delivery provides the advantage of improved distribution, in particular, to "occult" and inaccessible sites, the inefficiency of homing has curtailed such approaches, and direct injections into the injured tissue remain preferred [11]. In their study, Franz et al. demonstrated the safety and efficacy of a "dual-administration" approach, in which intramuscular cell injections were supplemented with intra-arterial cellular injections to improve distribution to the distal vasculature [39]. Other approaches include strategies to improve stem cell homing through gene therapy or local injections of homing factors (reviewed by Herrmann et al. [40]).

The safety and efficacy notwithstanding, a major limitation on the feasibility of the approach lies in the insufficiency of the cellular numbers for therapy. Extrapolating from animal studies, an estimated 12 liters of blood would be required to generate sufficient EPCs for the effective treatment of ischemia in an average adult patient [11]. This inadequacy is exacerbated by the compromised EPC quantity and quality in patients with cardiovascular and metabolic disorders [11]. In their clinical study, Losordo et al. addressed this issue by supplementing patients with cytokines to mobilize EPCs from the bone marrow into circulation before harvesting the $CD34^+$ progenitors. However, this protocol might be associated with mobilizing committed hematopoietic precursors and not EPCs per se [15]. Additionally, some concern exists over cardiac enzyme elevations arising from the cell mobilization regimen [34].

Recent research efforts have thus turned to cell isolation and expansion methods. Wadajkar et al. described the use of growth factor-loaded, antibody-conjugated magnetic microparticles for the one-step capture and in situ culture of EPCs on microparticles that can be scaled up with bioreactor cultures [41]. Such platforms, with minimized manipulations, facilitate upscaling and the ease of transition into the clinic. Additionally, the immunoselection method can be applied to other sources of EPCs; white adipose tissue, for example, has been shown to be an accessible source of EPCs [42]. Alternatively, EPCs can be retrieved from cryogenically preserved cord blood for autologous use [43]. Fetal tissues have demonstrated significant advantages over their adult counterparts, including faster proliferation rates and expansion capacities [26]. Aside from cord blood, other perinatal tissue, such as the placenta, can be exploited as a source of primitive EPCs. Postulating a perivascular niche for EPCs, Patel et al. performed cell selection on a $CD34^+/CD45^-/CD31^{low}$ profile [25]. Placental tissue is highly vascularized and angiogenically dynamic and a single term-placenta was shown to yield 27 times as much ECFCs as a single unit of cord blood. Culture expansion protocols have been developed to expand harvested populations after isolation. These have included extended culture in defined cytokine-rich environments, which were shown to induce up to 1,468-fold cEPC expansion [16, 44]. The results from these and other similarly themed studies suggest that expanded cells retain their potency in the rescue of murine hind limb ischemia [9, 32]. Thus, protocols to effectively derive and expand ECFCs under xeno-free conditions have also been developed, which might provide a cost-effective method to prepare ECFCs for clinical applications [45]. Excessive expansion, however, has been associated with replicative senescence and impaired capacities of ECFCs for vascular repair [46]. Also, in light of the lack of adequate markers, the potency of the injected cells remains impossible to predict. This uncertainty has been compounded by the potentially impaired functionality of EPCs in diseased patients [47]. EPC function, for example, is known to be compromised by impaired glucose metabolism at multiple stages [48].

Aside from the quantity, the enhancement of efficacy and bioactivity presents another possibility to improve EPC therapies. Strategies for ex vivo priming include the use of stromal-derived

factor-1 to elicit surface expression of integrin α-4 and α-M, as well as matrix metalloproteinase-2 secretion, leading to improved homing to ischemic sites [49]. More recently, Bouchentouf et al. described the addition of cytokines to suspension blood bags, which served to prime the mononuclear cells toward an angiogenic phenotype [50]. When these primed cells were injected into murine models of myocardial infarction, cardiac function was improved and angiogenesis enhanced, suggesting the efficacy of this approach. The study, however, did not detail the fate of the cells after injection, and the main mechanism for repair might not have been revascularization but other paracrine effects. Additionally, the blood was obtained from healthy volunteers, and it remains unclear whether EPC-compromised patients would respond similarly. Other possible strategies to improve EPC functionality include augmentation of angiogenic genes, such as *ACE2* [51] and *IL10* [52]. EPCs modified with VEGF, for example, were shown to restore erectile function in diabetic rats after intrapenile injection. Another related application is the use of EPCs as a delivery vehicle for ex vivo gene transfer applications. With their ability to incorporate into host vasculature, and the resultant constant proximity to circulatory blood, genetically engineered EPCs could be used to deliver therapeutic factors directly into the circulation [53]. Additionally, the therapeutic genes can be placed under the control of inducible promoters, such that the factors can be released on demand. In the recently concluded pulmonary hypertension and angiogenic cell therapy (PHACeT) trial, endothelial nitric oxide synthase (eNOS)-transfected eEPCs were systemically administered to patients with pulmonary arterial hypertension (PAH) [54]. EPC injections have previously been shown to stimulate endothelial repair and ameliorate PAH conditions [55], and in the PHACeT trial, the use of eNOS-augmented eEPCs was expected to have increased vasodilatory and vasoregenerative effects. Modest improvements in quality of life measures were observed in patients following treatment, although these could not be sustained, and the group was unable to ascertain the safety or efficacy of this approach. Although severe adverse reactions were observed in 2 of 7 patients (one death and one case of sepsis), causal links to the therapy were deemed unlikely, and the hemodynamic parameters throughout the cell administration and follow-up periods suggested the feasibility and safety of gene-augmented EPC injection therapies. In the context of cellular injections, it is perhaps interesting to note that EPC injections might have applications beyond revascularization. As a case in point, there is currently an increasing trend to study the use of EPCs for orthopedic applications [42]. Peripheral *CD34*$^+$ cells have been shown to home to fracture sites in a rat model of nonunion fracture [56]. On engraftment, these cells induced significant revascularization and contributed significantly to osteogenic repair of the fracture. These observations likely resulted in part from the osteopotentiating effects of endothelial cells, which secrete bone-inductive factors such as bone morphogenetic protein and transforming growth factor-β1 [57]. Ongoing research being undertaken by our group and others include optimizing of in vitro culture conditions to improve the vasculogenic and osteogenic properties of EPCs [58]. Aside from bone, such mutually synergistic effects are being studied for the engineering of a wide variety of tissues [59].

Capture Stents

Another major research topic centered on EPCs in regenerative medicine revolves around the use of "capture" stents for cardiovascular applications, which sequester EPCs from the circulation to promote endothelialization of the denuded luminal surface. Capture is effected by immobilized antibodies on the stent surface, typically against CD34. The regenerated endothelia are then suggested to reduce risk of restenosis and stent thrombosis and to eliminate the need for prolonged anticoagulative regimens, problems that continue to plague existing stent designs [60]. Additionally, antibody conjugation has been shown to passivate the surface, reducing platelet adhesion and the coagulative effects to improve hemocompatibility measures [61].

Randomized clinical trials conducted on the EPC capture stents have shown them to be safe, and postmarketing surveillance has yielded no evidence to suggest increased risks of adverse cardiac events from use. Compared with drug-eluting stents (DESs), they have been associated with higher in-stent late loss and target vessel failure but a reduced incidence of late-stent thrombosis [62]. The results from the endothelial progenitor cells capture stent in the treatment of acute ST-segment elevation myocardial infarction trial does suggest an increased risk of stent thrombosis [62], although this finding is currently under dispute. More recently, OrbusNeich has produced the "COMBO," a new-generation EPC capture stent, which elutes sirolimus for 180 days, thus combining the efficacy of DESs with the longer term improved safety profile of "bioengineered stents" [63]. A prospective, multicenter, randomized clinical trial is underway to compare the COMBO stent against current DESs (Clinicaltrials.gov identifier, NCT00967902).

Tied to the controversy on EPC identity, questions remain regarding the choice of CD34 as an appropriate capture target. To meet the need for rapid endothelialization, it has been argued that late EPCs or even circulating ECs should be specifically targeted instead, and surfaces coated with antibodies against *CD309* [64] or CD144 [65] have been associated with improved endothelialization outcomes. Extending this theme further, Chen et al. modified the capture surface further to facilitate transfection of the captured cells [66]. In their study, they demonstrated the local transfection of captured *CD133*-expressing cells with small interfering RNA against adenosine kinase. This resulted in upregulation of adenosine and, hence, improved EPC functionality. Thus, a clearer definition of the surface antigens for capture and elucidation of major signaling networks in the differentiation and functionality of EPCs might provide for more rational designs of EPC capture and postcapture modifications. In the context of the present report, the discussion on EPC capture surfaces for in vivo endothelialization can be further extended to vascular tissue engineering [67]. Regeneration of the luminal surface remains a critical issue in vascular tissue engineering, with most efforts centered on "preseeding" the luminal surfaces with endothelial cells before implantation [68]; such in vitro endothelialization methods are, however, labor- and cost-intensive and, thus, impractical. Much research activity is now centered on adopting, developing, and improving EPC capture technologies for vascular grafts and has been discussed in detail in a recent review [69].

Mobilization Treatments

Exogenous mobilization of circulating EPCs was first proposed using cytokine therapy as a means to mirror endogenous mobilization by ischemic tissue [70]. The elevated EPC numbers in circulation were then thought to increased EPC homing to,

and augmentation of, neovascularization in ischemic sites. In contrast to cellular injections, this process is more readily translatable, because the need for external manipulations is eliminated, and the drugs used in the process typically have well-established safety protocols. Additionally, it is particularly useful for the treatment of systemic conditions or conditions involving inaccessible tissue sites. For example, EPC mobilization is being studied for use in treating deep vein thrombosis, in which EPCs are thought to home to thrombotic sites, resolve clots, form new vasculature, and exert protective effects in the prevention of clot recurrence [71]. Similar to the above-mentioned studies on cellular infusions for orthopedic applications, mobilization of EPCs has also been shown to have beneficial effects for fracture healing [72]. Common mobilizing agents include those used for hematopoietic stem cell manipulation in oncology, including chemokines, growth factors, and cytokines [73]. These typically operate on the basis that EPCs reside in the hematopoietic fraction and that hematopoietic mobilization would, in turn, release EPCs into circulation. For example, granulocyte colony-stimulating factor (G-CSF) is currently used clinically to stimulate bone marrow production of granulocytes for the treatment of neutropenia. In vivo, G-CSF elicits release of matrix metalloproteinases and other enzymes from neutrophils, resulting in modification of the hematopoietic niche and subsequent release of hematopoietic precursors. This process has been shown to increase circulating $CD34^+$ cell numbers and has been associated with increased arteriogenesis in patients with coronary artery disease [74]. The identity of these $CD34^+$ cells and their roles in the remodeling process, however, remain unclear. In their meta-analysis, Fadini et al. suggested G-CSF monotherapy might have limited effects in patients with peripheral arterial disease and would thus fail to improve similar endpoints against cellular injection therapies [38]. Compared with drugs that target the hematopoietic fraction, vasomodulatory drugs have been explored in more targeted efforts. Statins, for example, are commonly prescribed to reduce the risk of cardiovascular events, and atorvastatin has recently been found to elevate $CD34^+/CD133^+/KDR^+$ levels in patients with heart failure [75]. The extent of EPC-mediated vascular repair via statin activation remains unclear, however, although in vitro studies have indicated increased viability and delayed senescence of EPCs with atorvastatin supplementation [76]. Other agents being studied to increase circulating EPC numbers include nonpharmacological interventions, such as physical activity [77] and diet [78]. In all such applications, however, it should be noted that the precise role of EPCs in the resolution of disease and/or achieving clinical outcomes is difficult to delineate, because they are typically measured as a biomarker and evaluated in correlational studies. Thus, no clinical data exist to conclusively demonstrate mobilization of EPCs as the primary mechanism of cure. Another limitation of the approach is the nonspecificity of the treatment, in that mobilized EPCs might not traffic adequately to the disease site. Additionally, patients with chronic vascular diseases are unlikely to benefit from such therapies owing to underlying EPC dysfunction and senescence. As previously alluded to, glucose metabolism influences EPC function on multiple levels, including homing and differentiation into functional vessels. Thus, EPC mobilization therapies for diabetic patients might be well-served by concurrent or pretreatment with antidiabetic agents [79] and homing factors [80].

Conclusion

Endothelial progenitor cells are important therapeutic targets in the field of regenerative medicine, with potential utility, not just in cardiology and cardiovascular therapies, but also in other tissue engineering applications. Significant gaps lie in our understanding of EPC biology, however, and continued research is required to understand the identity and roles of EPCs in health and disease. These efforts will provide valuable data to guide our efforts toward the rational design and engineering of cellular therapeutic agents.

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Author Contributions

M.S.K.C., W.K.N., and J.K.Y.C.: manuscript writing, final approval of manuscript.

Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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