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Endothelial Progenitor Cells in Regenerative Medicine: Applications and Challenges

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Abstract (Max 250 words)

Endothelial Progenitor Cells (EPCs) are currently being studied as candidate cell sources for revascularisation strategies. Significant findings have been made in understanding the biology of EPCs and pre-clinical studies have demonstrated the vasculogenic, angiogenic and beneficial paracrine effects of transplanted EPC in the treatment of ischaemic diseases. Despite these promising results, widespread clinical acceptance of EPC for clinical therapies remains hampered by several challenges. This review provides a concise summary of different EPC populations being studied for ischemic therapies, and their known roles in the healing of ischaemic tissues. Challenges and issues surrounding the use of EPCs will be discussed, as well as current strategies being developed to improve the harvest efficiency and functionality of EPCs for applications in regenerative medicine.

Table of Abbreviations

ACE2	Angiotensin Converting Enzyme 2
ADK	Adenosine Kinase
ALDH	Aldehyde Dehydrogenase
BMP	Bone Morphogenetic Protein
CAC	Circulating Angiogenic Cells
CD	Classification Determinant, Cluster of Differentiation
DES	Drug Eluting Stents
EC	Endothelial Cell
EPC	Endothelial Progenitor Cell
cEPC	Circulating Endothelial Progenitor Cell
eEPC	Early Endothelial Progenitor Cell
ECFC	Endothelial Colony Forming Cell
IL-10	Interleukin 10
LDL	Low-Density Lipoprotein
MMP2	Matrix Metalloproteinase 2

OEC/EOC	Outgrowth Endothelial Cells/ Endothelial Outgrowth Cells
SDF-1	Stromal Derived Factor 1
STEMI	ST-Elevation Myocardial Infarction
TGFβ	Transforming Growth Factor β
VEGF	Vascular Endothelial Growth Factor
VEGFR	Vascular Endothelial Growth Factor Receptor

Introduction

The term "Endothelial Progenitor Cells" (EPCs) may 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 utilise them, including direct cellular transplantations 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 EPC, limited numbers and their~~its~~ clinical potency. In this review, we will begin with a description of EPC populations, leading to an overview of clinical strategies that have been developed to utilise EPC in regenerative medicine. Factors limiting the use of EPC will be discussed, as well as current research themes to resolve these issues.

The Identification and Characterisation of EPC populations.

The discovery of endothelial progenitor cells has been credited to Asahara and co-workers, for identifying a haematopoietic population in adult peripheral blood that was capable of eliciting post-natal 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 which continues today [4]. In the context of regenerative medicine, however, it is the capacity for vascular regeneration and potential for ischaemic therapy that EPC are most valued. There exist significant controversies over the identity and roles of EPC in vascular repair, and a brief discourse on the ~~two~~ major EPC populations reported in literature is necessary here to facilitate further discussions. Over the past two decades, the term "EPC" has now been used to describe a burgeoning range of cell types defined by their isolation and culture methods, as well as ontological sources ranging from fetal

trophoblastic tissue to adult bone marrow. A detailed discussion of the myriad EPC in studies is beyond the scope of this concise review, and the reader is directed to excellent articles on this topic [4, 5]. Two major categories will be described briefly here: Haematopoietic EPC and non-haematopoietic EPC, which differ largely on their ontological origins and isolation methods. It is important to note at this point, however, that it would not be possible to delineate a "superior" cell source for vascular regenerative therapies. Rather, differences in the isolation and identification of these populations [6], as well as potential different contributions to neovasculogenesis [7] should be recognised.

Haematopoietic EPC

Asahara et al postulated that EPCs can be isolated from a haematopoietic source, and demonstrated that $CD34^+$ cells from peripheral blood can contribute to neovascularisation and ischaemic rescue, following injection into an animal model of peripheral limb ischaemia [2]. Similarly, CD133, another haematopoietic stem cell marker, may be targeted in order to derive more less ~~im~~mature progenitor populations [8]. Cell sorting on CD34 and / or CD133 thus emerged as a strategy to derive populations enriched in circulating EPCs (cEPC), and methods to characterise 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 ischaemic conditions, including acute myocardical infarction and critical limb ischaemia [11]. However, questions remain over the precise definition of a bona fide cEPC. Initial studies suggested EPC to exhibit 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 recognised profile for cEPC, despite other studies suggesting the use of other markers, including CD45, CD105, CD106, CD117, CD144, acetylated LDL uptake and ALDH activity [5]. It was thus striking when clonal cultures of $CD34^+$ / $CD133^+$ / $VEGFR2^+$ cells were found to only be capable of differentiating ~~ion~~ into haematopoietic, and not endothelial lineages, leading to suggestions that these cells were non-angioblastic haematopoietic progenitors which support angiogenesis through

paracrine effects [14]. In contrast, the non-haematopoietic $CD34^+$ / $CD45^-$ 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, indicative of the presence of endothelial lineages in this population. Significant debate on the cEPC theory ensued, with proponents arguing against the methodology employed and interpretation of results by Case et al [15]. This has been, in large part, resolved by the development of highly defined assays to induce colony formation from cEPC~~.~~, with clonogenic assays performed to demonstrate the ability of CD133+ cells to differentiate into both haematopoietic and endothelial lineages [9, 16]~~.~~, Interestingly, it has been observed that CD34- cells are capable of augmenting in vitro vascular network formation, and vascularisation events in vivo [17], providing some basis for the argument that CD133+/CD34+ cells are, indeed, *bona fide* EPC, but require the presence of auxiliary cells in the CD34- fraction to potentiate vasculogenesis.~~and the demonstration of distinct roles played by the different sub-populations in vascular regeneration [17].~~

In parallel to these efforts, EPC were also observed to share many common characteristics with monocytic cells [18]. These cells were conventionally selected on their ability to adhere to tissue culture surfaces, leading to the term early EPC (eEPC). The attached cells demonstrate ability to uptake lectin and acetylated low-density lipoproteins, and express monocytic surface markers including CD14 [19]. eEPC have been suggested to derive from monocytes distinct from the CD34-negative cEPC [20]. The exact lineage of these cells has been confounded by contaminant monocytes possibly imparting monocyte-like characteristics to the actual EPC [21], or acquiring endothelial-like characteristics secondary to culture in VEGF-rich conditions [20]. Regardless of lineage, eEPC play primarily supportive roles in angiogenesis vascular repair without differentiating themselves into functional endothelial cells ~~eEPC play a primarily supportive role in vascular repair~~ [22]. Angiogenic factors secreted by eEPCs include CXCL12, CXCL1 and VEGF, with migration inhibitory factor (MIF), a potent cytokine known to induce endothelial and smooth muscle differentiation, being the most prominent in early and late stages of the ischemic event [23]. ~~,~~leading to calls for a change in nomenclature to Circulating Angiogenic Cells (CAC) instead, ~~reflecting~~ to better reflect their major

capacity to induce angiogenesis and vascular sprouting, rather than in the direct formation of nascent blood vessels.

Non-haematopoietic EPC

In contrast to the haematopoietic EPC, EPC have been demonstrated to derive from non-haematopoietic tissue, presumably from vessel walls [24, 25]. Termed "Endothelial Colony Forming Cells" (ECFC) or "Outgrowth Endothelial Cells" (OEC / EOC) for their ability to form colonies of endothelial outgrowths under permissive conditions, ECFC are most commonly isolated by plating blood-derived mononuclear cells on collagen-coated substrates in endothelial-supportive media [26]. Endothelial outgrowths may 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 to note that cells derived from such long term cultures more readily generate mature endothelial progeny in vitro, and have also been observed to physically contribute towards vasculogenesis [28]. In contrast, it is generally recognised that haematopoietic EPC , and eEPC, in particular, potentiate angiogenesis through the secretion of cytokines [29, 30].

Thus isolated, ECFC actually represent a heterogeneous mix of progenitors and terminally differentiated endothelial cells with varying proliferative potentials and the lack of surface markers to definitively isolate vasculogenic progenitor populations have contributed towards the lack of enthusiasm for translating these cells to the clinics. Proposed profiles for the identification of ECFC-initiating cells include $CD146^{+}$ / $CD45^{-}$ / $CD133^{-}$, which would be in line with the hypothesis that these EPC originate from vessel walls rather than the bone marrow [31]. 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 [32]. Selection on such stringent profiles, however, has been known to yield extremely low yields, and thus, non-viable for therapeutic use [4].

Clinical Application of EPC in Regenerative Medicine

In spite of the ongoing controversy over EPC identity, the clinical potential of EPC towards vascular regenerative applications cannot be overlooked [5, 6], with currently over 150 interventional studies registered on ClinicalTrials.gov. Disease conditions being investigated include ischemic diseases, such as myocardial infarction and peripheral vascular disease (Table 1 and Table 2). Of the completed and ongoing trials, three major applications targeting EPC may be identified (i) cellular injections for ischemic conditions (ii) EPC-capture stents and (iii) EPC mobilisation therapies.

Cellular injections

EPCs as a candidate cell source for therapy offer many attractive characteristics, including (i) ready accessibility from peripheral blood (ii) potent angiogenic and vasculogenic effects (iii) stability of lineage / reduced risk of tumorigenicity. These features led to many studies on their possible utility for therapeutic neovascularisation, of which the haematopoietic EPC have been largely favoured in such applicaions for reasons of ease of harvest, with minimal manipulations and culture periods [33].

In a murine model of peripheral limb ischaemia 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 cEPC group versus 7.1% in control group) [34]. Data from this and other similarly themed studies led to significant optimism for the use of cEPC for the treatment of ischaemic conditions, and initiation of Phase I/II clinical trials involving cEPC injections into ischaemic myocardia [35]. Results from the primary endpoints in this study suggest the safety of cellular injections, and were borne out by further evidence from randomised, placebo-controlled trials [36, 37]. Administration of unfractionated bone marrow, however, is unable to rescue ischaemia in critical limb ischaemia studies, suggesting the EPC fraction to be responsible for the therapeutic effects [38]. Aside from cEPC, eEPC have also been evaluated in the clinics. In a randomised, controlled study on idiopathic pulmonary arterial hypertension, intravenous infusion of autologous eEPC resulted in improved pulmonary hemodynamics, without severe adverse effects [39]. Taken

together these results provide cautious optimism for EPC as a cellular candidate for regenerative therapies and more data from ongoing trials will be useful in establishing the safety profile of EPC therapy. It should be noted at this point that questions remain over the best route of administration for safety and efficacy. In treatments of peripheral arterial disease (PAD), a meta-analysis conducted on 108 studies involving cellular therapies for the treatment of PAD suggest intra-muscular and intra-arterial injections to be equally well-tolerated, with the former presenting improved clinical outcomes [40]. Thus, while intra-arterial delivery provides the advantage of improved distribution, particularly to "occult" and inaccessible sites, the inefficiency of homing curtails 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 intra-muscular cell injections were supplemented with intra-arterial cellular injections to improve distribution to distal vasculature [41]. Other approaches include strategies to improve stem cell homing, through gene therapy or local injections of homing factors (reviewed by Hermann et al) [42].

Safety and efficacy notwithstanding, ~~however,~~ a major limitation on the feasibility of approach lies in the insufficiency of cellular numbers for therapy; Extrapolating from animal studies, an estimated 12 litres of blood would be required to generate sufficient EPC for the effective treatment of ischaemia in an average adult patient ~~it is estimated that 12 litres of blood is required from each patient to generate the 20,000 cells per kilogram body weight recommended for effective treatment of ischaemia in adults~~ [11]. This inadequacy is exacerbated by compromised EPC quantity and quality in patients suffering from cardiovascular and metabolic disorders [11]. In their clinical study, Losordo et al addressed this issue by supplementing patients with cytokines to mobilise EPC from the bone marrow into circulation, prior to harvesting of $CD34^+$ progenitors. However, this protocol may be associated with mobilising committed haematopoietic precursors and not EPC *per se* [15]. Additionally, some concern exists over cardiac enzyme elevations arising from the cell mobilisation regime [36].

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 EPC on microparticles that may be scaled up with bioreactor cultures [43]. Such platforms with minimised manipulations facilitate upscaling and ease of transition into the clinics. Additionally, the immuno-selection methodology may be applied to other sources of EPC; white adipose tissue, for example, has been shown to be an accessible source of EPC [44]. Alternatively, EPC may be retrieved from cryogenically preserved cord blood for autologous use [45]. Fetal tissues demonstrate significant advantages over their adult counterparts, including faster proliferation rates and expansion capacity [26]. Aside from cord blood, other perinatal tissue such as the placenta, may be exploited as a source of primitive EPC. Postulating a perivascular niche for EPCs, Patel et al performed cell selection on a $CD34^{+}$ / $CD45^{-}$ / $CD31^{Lo}$ profile [25]. Placental tissue is highly vascularised and angiogenically-dynamic, and a single term-placenta was shown to yield 27 times as much ECFC as a single unit of cord blood. Following isolation, culture expansion protocols have been developed to expand harvested populations. These include extended culture in defined cytokine-rich environments, which were shown to induce up to 1468-fold cEPC expansion [16, 46]. Results from these and other similarly themed studies suggest expanded cells to retain their potency in the rescue of murine hindlimb ischaemia [9, 34]. On this note, protocols to effectively derive and expand ECFC under xeno-free conditions have also been developed, which may provide a cost-effective method to prepare ECFC for clinical applications [47]. Excessive expansion, however, is associated with replicative senescence and impaired capacities of ECFC for vascular repair [48] and, in light of the lack of adequate markers, the potency of injected cells remains impossible to predict. This uncertainty is compounded by the potentially impaired functionality of EPC in diseased patients [49]. EPC function, for example, is known to be compromised by impaired glucose metabolism at multiple stages [50].

Aside from quantity, enhancement of efficacy and bioactivity presents another possibility to improve EPC therapies. Strategies for ex vivo priming include the use of SDF-1 to elicit surface expression of

Integrin alpha-4 and alpha-M, as well as MMP-2 secretion, leading to improved homing to ischemic sites [51]. More recently, Bouchentouf et al described the addition of cytokines to suspension blood bags, which served to prime the mononuclear cells towards an angiogenic phenotype [52]. When these primied 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 cells following injection, and the main mechanism for repair may not have been revascularisation, but other paracrine effects. Additionally, the blood was obtained from healthy volunteers, and it remains unclear if EPC-compromised patients would respond similarly. Other possible strategies to improve EPC functionality includes augmentation of angiogenic genes, such as ACE2 [53] and IL-10 [54]. EPC modified with VEGF, for example, were shown to restore erectile function in diabetic rats, following intra-penile injection. Another related application is the use of EPC as a delivery vehicle for ex vivo gene transfer applications. Due to their ability to incorporate into host vaculature, and the resultant constant proximity to circulatory blood, genetically-engineered EPC may be used to deliver therapeutic factors directly into circulation [55]. Additionally, the therapeutic genes may 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 eEPC were systemically administered to Pulmonary Arterial Hypertension (PAH) patients [56]. EPC injections have previously been shown to stimulate endothelial repair and ameliorate PAH conditions [57], and in the PHACeT trial, the use of eNOS-augmented eEPC was expected to have increased vasodilatory and vaso-regenerative 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 two of seven patients (one death and one case of sepsis), causal links to the therapy were deemed unlikely, and hemodynamic parameters throughout the cell

administration and follow-up periods suggest the feasibility and safety of gene-augmented EPC injection therapies.

Capture stents

Another major research topic centred on EPC in regenerative medicine revolves around the use of "capture" stents for cardiovascular applications, which sequester EPC from the circulation to promote endothelialisation of the denuded luminal surface. Capture is effected by immobilised antibodies on the stent surface, typically against CD34. The regenerated endothelia is then suggested to reduce risk of restenosis, stent thrombosis and to eliminate the need to prolonged anti-coagulative regimes , problems that continue to plague existing stent designs [63]. Additionally, antibody-conjugation has been shown to passivate the surface, reducing platelet adhesion and coagulative effects to improve haemocompatibility measures [64].

Randomised clinical trials conducted on the EPC capture (namely, OrbusNeich Genous) stents have shown them to be safe and post-marketing surveillance has yielded no evidence to suggest increased risks of adverse cardiac events from use. Compared to drug-eluting stents (DES), they are associated with higher instent late loss and target vessel failure, but reduced incidences of late-stent thrombosis [65]. Results from the endothelial progenitor cells capture (EPC) stent in the treatment of acute ST-elevation myocardial infarction (STEMI) trial does suggest increased risk of stent thrombosis [65], although this finding is currently under dispute. More recently, OrbusNeich has produced "COMBO", a new-generation EPC capture stent which elutes sirolimus over 180 days, thus combining the efficacy of DES with the longer-term improved safety profile of "bioengineered stents" [66]; a prospective, multicentre, randomised clinical trial is underway to compare the COMBO stent against current DES (Clinicaltrials.gov identifier: NCT00967902).

Tied to the controversy on EPC identity, questions remain on the choice of CD34 as an appropriate capture target. To meet the need for rapid endothelialisation, it has been argued that late EPC or even circulating EC should be specifically targeted instead, and surfaces coated with antibodies

against CD309 [67] or CD144 [68] are associated with improved endothelialisation outcomes. Extending this theme further, Chen et al modified the capture surface further to facilitate transfection of the captured cell [69]. In their study, they demonstrated the local transfection of captured CD133-expressing cells with siRNA against Adenosine Kinase (ADK). This resulted in upregulation of adenosine, and, hence, improved EPC functionality. In this light, a clearer definition of the surface antigens for capture, and elucidation of major signalling networks in the differentiation and functionality of EPC may provide for more rational design of EPC capture and post-capture modification. In the context of this review, the discussion on EPC capture surfaces for in vivo endothelialisation may be further extended to vascular tissue engineering [70]. Regeneration of the luminal surface remains a critical issue in vascular tissue engineering, with most efforts centred on "pre-seeding" the luminal surfaces with endothelial cells prior to implantation [71]; such in vitro endothelialisation methods are, however, labour- and cost- intensive and, thus, impractical. Much research activity is now centred on adopting, developing and improving EPC capture technologies for vascular grafts, and is discussed in detail in a recent review [72].

Mobilisation treatments

Exogenous mobilisation of circulating EPC was first proposed using cytokine therapy as a means to mirror endogenous mobilisation by ischemic tissue [73]. The elevated EPC numbers in circulation is then thought to increased EPC homing to and augmentation of neovascularisation in ischaemic sites. In contrast to cellular injections, this process is more readily translatable, as the need for external manipulations is eliminated, and 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 mobilisation is being studied for use in treating deep vein thromobosis, where EPC are thought to home to thrombotic sites, resolve clots, form new vasculature and exert protective effects in the prevention of clot recurrence [74]. Similar to the above-mentioned studies on cellular infusions for orthopaedic applications, mobilisation of EPC has

also been shown to have beneficial effects for fracture healing [75]. Common mobilising agents those used for HSC manipulation in oncology, including chemokines, growth factors and cytokines [76]. These typically operate on the basis that EPC reside in the haematopoietic fraction, and that haematopoietic mobilisation would, in turn, release EPC into circulation. For example, 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 MMPs and other enzymes from neutrophils, resulting in modification of the haematopoietic niche and subsequent release of haematopoietic precursors. This process has been shown to increase circulating $CD34^+$ cell numbers, and associated with increased arteriogenesis in coronary artery disease patients. [77] The identity of these CD34+ cells and their roles in the remodelling process, however, remain unclear. In their meta-analysis, Fadini et al suggest G-CSF monotherapy may limited effects on peripheral arterial disease patients, and thus failed to improve similar endpoints against cellular injection therapies [40]. As compared to drugs that target the haematopoietic 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 elevated $CD34^+/CD133^+/KDR^+$ levels in heart failure patients[78]. The extent of EPC-mediated vascular repair via statin activation remains unclear, however, although in vitro studies indicate increased viability and delayed senescence of EPC with atorvastatin supplementation [79].

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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 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 EPC in health and disease. These efforts will provide valuable data to guide our efforts towards rational design and engineering of cellular therapeutics.

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Treatment	Disease Therapy	ClinicalTrials No.	Comments	Conclusion
Cellular injections	Lymphedema	NCT01112189	Unsorted mononuclear cells from bone marrow	Potentially effective. Reduction of arm volume, pain and sensitivity. [85]
	Advanced Liver Cirrhosis	NCT01333228	Unsorted mononuclear cells from bone marrow	No published results found.
	Leg Ulcer/Gangrene	NCT00221143	Circulating EPC (CD34+) from G-CSF mobilised blood	Safe, potentially effective. Long term follow-up (208 weeks) suggest long-term efficacy. [86]
	Dilated Cardiomyopathy	NCT00629096	Unsorted mononuclear cells from bone marrow	Phase 2 study; No published results found.
	Idiopathic Pulmonary Arterial Hypertension	NCT00641836, NCT00257413	Early EPC from venous blood	Safe, potentially effective. Improved exercise capacity and pulmonary hemodynamics. [39]
	Coronary Artery Disease, Refractory Angina	NCT00694642	Early EPC	No published results found.
	Critical Limb Ischaemia	NCT01595776	Circulating EPC (CD133+) from G-CSF mobilised blood	Pilot study on three patients; 100% limb salvage. Findings not peer-reviewed.
	Pulmonary Arterial Hypertension	NCT00469027	Endothelial NO Synthase (eNOS) Transfected early EPC	Improved pulmonary hemodynamics. 2 severe adverse reactions reported, but not proven to be directly linked to cell therapy. Suggests safety and efficacy of augmented EPC approach. [56]

Stent	Acute Coronary Syndromes	NCT00494247	Circulating EPC	No paper linked on Clinicaltrials.gov
	Coronary Disease	NCT01756807	Circulating EPC	OCT provides unique insights, can be used to optimise results. OCT and IVUS have complimentary diagnostic values
	Coronary Artery Disease	NCT01272895	Circulating EPC	Stent is safe and feasible, further developments warranted to evaluate efficiacy
	Coronary Disease/Stenosis	NCT00349895	Circulating EPC	Statin therapy in combination with stent does not contribute to reduction of in-stent restenosis. Concomitant statin therapy stimulates EPC recruitment but does not improve angiographic outcome of stent. Angiographic late loss significantly reduced between 6 to 18 months.
	Coronary Restenosis/ Thrombosis	NCT01274234	Circulating EPC	Stent is safe and feasible, further developments warranted to evaluate efficiacy
	Coronary Artery Disease	NCT00732953	Circulating EPC	Paclitaxel-coated plus EPC stent implantation is superior
Mobilization	Diabetes	NCT02056210	EPC type not mentioned	Mobilization after G-CSF with or without chemotherapy affected by diabetes, but no effect on mobilization by G-CSF with plerixafor
	Coronary Artery Disease	NCT00272571	Circulating EPC	No paper linked on Clinicaltrials.gov
	Peripheral Occlusive Artery Disease	NCT01952756	Circulating EPC	Cilostazol treatment significantly increased colony formation from early human EPCs
	Hypertension	NCT01041287	Circulating EPC	Both nebivolol and metaprolol increased circulating levels of CD34/CD133
	Myocardial Infarction	NCT00378352	Circulating EPC	Single intravenous bolus of epoetin alfa within 4 hours of PCI in patients with STEMI who had successful reperfusion with primary or rescue PCI does not reduce infarct size. Also associated with adverse cardiovascular events

Coronary Artery Bypass Surgery	NCT01096875	Circulating EPC	Short term atovastatin use increases circulating EPCs pre and post-operation. Associated with better preservation of sinus rhythm and reduced hsCRP levels
Diabetes Mellitus, Metabolic Syndrome X, Hypercholesterolemia	NCT00166036	EPC type not mentioned	Atorvastin associated with greater reduction of lipid markers compared to pravastatin. Further investigation required to identify if the agents are responsible for outcome differences in clinical trials
Inflammation, Macrophage Infiltration, Cardiovascular Disease	NCT01552694	Circulating EPC	Sitagliptin has beneficial systemic and adipose anti-inflammatory effects
Diabetes Mellitus Type II, Insulin Resistance	NCT00094796	EPC type not mentioned	No paper linked on Clinicaltrials.gov
Type 1 Diabetes Mellitus (Adolescent)	NCT02019186	EPC type not mentioned	No paper linked on Clinicaltrials.gov
Coronary Artery Disease	NCT00641758	EPC type not mentioned	Pycnogenol improves endothelial function in patients with CAD by reducing oxidative stress

Table 1: Completed interventional trials involving Endothelial Progenitor Cells listed on ClinicalTrials.org.

Treatment	Disease Therapy	Clinicaltrials 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

Table 2: Ongoing interventional trials involving Endothelial Progenitor Cells listed on ClinicalTrials.org.