Therapeutic Potential of Endothelial Progenitor Cells in Cardiovascular Diseases

Victor J. Dzau, Massimiliano Gnecchi, Alok S. Pachori, Fulvio Morello, Luis G. Melo

Abstract—Endothelial dysfunction and cell loss are prominent features in cardiovascular disease. Endothelial progenitor cells (EPCs) originating from the bone marrow play a significant role in neovascularization of ischemic tissues and in re-endothelialization of injured blood vessels. Several studies have shown the therapeutic potential of EPC transplantation in rescue of tissue ischemia and in repair of blood vessels and bioengineering of prosthetic grafts. Recent small-scale trials have provided preliminary evidence of feasibility, safety, and efficacy in patients with myocardial and critical limb ischemia. However, several studies have shown that age and cardiovascular disease risk factors reduce the availability of circulating EPCs (CEPCs) and impair their function to varying degrees. In addition, the relative scarcity of CEPCs limits the ability to expand these cells in sufficient numbers for some therapeutic applications. Priority must be given to the development of strategies to enhance the number and improve the function of CEPCs. Furthermore, alternative sources of EPC such as chord blood need to be explored. Strategies for improvement of cell adhesion, survival, and prevention of cell senescence are also essential to ensure therapeutic viability. Genetic engineering of EPCs may be a useful approach to developing these cells into efficient therapeutic tools. In the clinical arena there is pressing need to standardize the protocols for isolation, culture, and therapeutic application of EPC. Large-scale multi-center randomized trials are required to evaluate the long-term safety and efficacy of EPC therapy. Despite these hurdles, the outlook for EPC-based therapy for cardiovascular disease is promising. (Hypertension. 2005;46:7-18.)

Key Words: coronary artery disease ■ endothelial progenitor cells ■ genetic engineering ■ myocardial infarction ■ neovascularization ■ vascular repair

It is now well-established that endothelial dysfunction underlies all of the major cardiovascular diseases. In normal conditions, the vascular endothelium produces and secretes substances that modulate vascular tone and protect the vessel wall from inflammatory cell infiltration, thrombus formation, and vascular smooth muscle cell proliferation. Pathologic conditions such as hyperlipidemia, hyperglycemia, and hypertension impair the ability of the vascular endothelium to produce vasodilatory and anti-adhesion moieties and increase the production of vasoconstrictor, pro-adhesion, and pro-thrombotic molecules, leading to elevated vascular tone, enhanced cell adhesion, proliferation of media smooth muscle cells, and propensity toward thrombosis. Endothelial cell loss and turnover are accelerated in the presence of hemodynamic and biochemical alterations and are a prominent feature of vascular injury resulting from percutaneous coronary intervention. The loss of endothelial function and integrity sets in motion the cascade of events that lead to atherosclerosis and restenosis after percutaneous revascularization. Given the role of endothelial cell loss in the pathogenesis of vascular diseases, the attention has recently turned to the development of strategies to enhance rapid endothelial recovery. Several studies have suggested that circulating endothelial progenitor cells (CEPCs) originating in the bone marrow play a significant role in endogenous neovascularization of ischemic tissues and in the re-endothelialization of injured vessels. In addition, EPC mobilization and proliferation was reported to contribute to the salutary effects of 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors and estrogen. EPC transplantation has been shown to induce new vessel formation in ischemic myocardium and hind limb and to accelerate re-endothelialization of injured vessels and prosthetic vascular grafts in humans and in various animal models, demonstrating their therapeutic potential as a cell-based strategy for rescue and repair of ischemic tissues and injured blood vessels. Furthermore, EPCs are amenable to genetic manipulation, underscoring their usefulness as vectors for local delivery of therapeutic genes. However, despite the excitement regarding the possible clinical use of EPC, recent studies have shown that age and other risk factors for cardiovascular disease reduce the availability of EPC and...
Impair their function to varying degrees, thus limiting their therapeutic usefulness in these patient populations. Furthermore, the relative scarcity of CEPCs and their finite proliferative potential limits the ability to expand these cells in sufficient numbers for some therapeutic applications. In this article, we discuss the biology of EPCs, their therapeutic potential in the treatment of cardiovascular diseases, and the limitations facing their use in the clinic. We end with a discussion of the outstanding issues and a perspective on future developments in the field.

Isolation, Characterization, and Genetic Modification of EPCs

The isolation and characterization of endothelial progenitor cells from peripheral blood was first reported by Asahara et al in 1997. Since the original report several other groups, including ours, have confirmed the original finding and have reported various modifications of the methods for the isolation, characterization, and culture of EPCs. The cells are thought to originate from a common hemangioblast precursor in the bone marrow; however, nonhematopoietic mesenchymal precursors in the bone marrow (multipotent adult progenitor cells) have also been reported to transdifferentiate into EPCs in culture. Other studies have reported that myeloid/monocyte lineage cells (CD14+) can differentiate into cells with EPC characteristics. In addition, some tissues harbor stem cells that may differentiate into various lineages including endothelial cells. Thus, CEPCs appear to be a heterogenous group of cells originating from multiple precursors within the bone marrow and present in different stages of endothelial differentiation in peripheral blood. For this reason, the accurate characterization of EPCs is difficult because many of the cell surface markers used in phenotyping are shared by hematopoietic stem cells and by adult endothelial cells. In culture, EPCs emerge as late (2 weeks) outgrowth colonies after plating in endothelium specified medium. Typically, the cells are defined on the basis of expression of cell surface markers such as CD34, Flk-1, and CD-133. The cells have high proliferative potential, albeit for a finite number of cell divisions. As the cells differentiate, they acquire endothelial lineage markers, vascular endothelium-cadherin, PECAM-1 (CD31), von Willebrand factor, endothelial nitric oxide synthase (eNOS), and E-selectin, and incorporate acetylated low-density lipoprotein cholesterol. The loss of hematopoietic stem cell marker CD133 expression coincides with EPC differentiation into cells with functional characteristics of adult endothelial cells.

We have reported a streamlined method for isolation, cultivation, and expansion of EPCs from peripheral blood based on density centrifugation and selective adherence to fibronectin-coated plastic dishes (Figure 1). The unfractio-
ated mononuclear cells (MNCs) are cultivated in medium enriched with endothelial specific growth factors such as vascular endothelial growth factor (VEGF). Within days of plating, colonies of adherent cells proliferate rapidly to form a monolayer with the cobblestone morphology typical of endothelium. After 2 weeks, the cells adopt endothelial-like characteristics such as expression of von Willebrand factor, uptake of acetylated low-density lipoprotein cholesterol, and the ability to assemble into vascular tube-like structures (Figure 2). Using this approach, we are able to expand the circulating cells in culture to yield sufficient number for autologous transplantation onto injured blood vessels and prosthetic grafts in rabbits23.

EPCs are highly amenable to genetic modification with viral vectors, rendering them useful as vehicles for delivery of therapeutic genes.23–25 We use a pseudotyped murine stem cell retroviral vector for ex vivo genetic modification of EPCs.23,24 The pseudotyped murine stem cell retroviral vector transduces EPC with nearly 100% efficiency, without any noticeable effects on cell phenotype or engraftment in vivo.23 Furthermore, because the retroviral genome integrates into the host genome, it can lead to long-lasting transgene expression. We have documented transgene expression in vivo up to 1e month after transplantation of the genetically modified cells. One potential shortcoming of retroviral vectors is their proneness to transcriptional silencing, which may shorten the duration of transgene expression.51 In addition the retroviral DNA randomly integrates into the host genome, posing a potential risk of oncogenesis.52 Other viral vectors such as adenovirus, lentivirus, and herpes virus have also been reported to transduce EPCs, but they have been used far less extensively than retroviral vectors.

**Endothelial Progenitor Cells in Cardiovascular Disease and Aging**

**EPC and Cardiovascular Diseases**

Differences in EPC number and function have been observed in a number of pathologies (Table 2). An inverse correlation was recently reported between the number and migratory activity of CEPCs and risk factors for coronary artery disease.27 In a group of 45 men with various degrees of cardiovascular risk, as defined by the combined Framingham risk factor score, the number of CEPCs correlated with endothelial function.28 Interestingly, these investigators found that the number of CEPC was a better predictor of endothelial function than the presence or absence of traditional risk factors, suggesting that the abundance of CEPC may be a useful marker of vascular function and overall cardiovascular risk. In patients with severe coronary artery disease, the colony-forming capacity and migratory activity of bone marrow-derived CD34+/CD133+ MNCs was markedly reduced and associated with reduced neovascularization after transplantation in ischemic hind limb of nude rats, despite no difference in the total number of hematopoietic progenitor cells between patients and healthy control subjects.55 EPC (CD133+/Flk-1+) was also reported to be inversely related to the severity of congestive heart failure. Reduced number of EPCs was also seen in cardiac transplantation patients with...
TABLE 2. Diseases Characterized by Alterations in EPC Levels and Function

<table>
<thead>
<tr>
<th>Disease Type</th>
<th>Effect on EPC N</th>
<th>Effect on EPC Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>↓</td>
<td>↓</td>
<td>11, 12, 27, 28, 55</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>↓</td>
<td>ND</td>
<td>56, 60</td>
</tr>
<tr>
<td>Unstable angina</td>
<td>↓</td>
<td>ND</td>
<td>59</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>↑</td>
<td>↓</td>
<td>60</td>
</tr>
<tr>
<td>Vascular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>↓</td>
<td>↓</td>
<td>27, 55</td>
</tr>
<tr>
<td>Acute vascular injury and inflammation</td>
<td>↑</td>
<td>ND</td>
<td>14, 15, 17, 18, 32, 80, 81</td>
</tr>
<tr>
<td>Peripheral limb ischemia</td>
<td>↑</td>
<td>ND</td>
<td>10, 25, 30, 34, 43</td>
</tr>
<tr>
<td>Transplant arteriopathy</td>
<td>↓</td>
<td>ND</td>
<td>57</td>
</tr>
<tr>
<td>In-stent restenosis</td>
<td>↓</td>
<td>ND</td>
<td>58</td>
</tr>
<tr>
<td>Hypertension</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>↓</td>
<td>↓</td>
<td>26</td>
</tr>
<tr>
<td>Diabetes</td>
<td>↓</td>
<td>↓</td>
<td>62, 63</td>
</tr>
<tr>
<td>Renal failure</td>
<td></td>
<td></td>
<td>65</td>
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</tbody>
</table>

ND indicates not determined.

vasculopathy57 and in patients with in-stent restenosis.58 In contrast, elevated EPC levels were reported in patients with unstable angina59 and in patients with acute myocardial infarction60 in association with elevation in plasma C-reactive protein levels. Interestingly, C-reactive protein reduces EPC proliferation, survival, differentiation, and function,61 suggesting that the ability of C-reactive protein to inhibit EPC differentiation and function may play a role in the development of cardiovascular disease.

Diabetes, a major condition associated with cardiovascular diseases, also adversely affects endothelial function and number.62,63 EPC are markedly reduced in patients with either type I62 or type II63 diabetes. Furthermore, the EPCs from diabetic patients showed reduced capacity to induce angiogenesis in vitro.62,63 These defects in EPC function may underlie some of the vascular complications associated with diabetes, such as endothelial dysfunction, that predisposes to diffuse atherosclerosis and impaired neovascularization after ischemic events. In this regard, Schatteman et al64 showed that transplantation of CD34+ -derived angioblasts from non-diabetic mice markedly accelerates blood flow restoration in ischemic hind limb of diabetic mice in association with enhanced neovascularization. Chronic renal failure, another disease well known to predispose to coronary artery disease and heart failure, is characterized by enhanced coronary atherosclerosis and impaired angiogenesis. In patients with renal failure on hemodialysis, the number and colony-forming capacity of EPCs recovered from venous blood was decreased by >40%.65 In addition, the EPCs from these patients showed reduced migratory activity and impaired ability to assemble into vascular tubes, suggesting that EPC deficiency may play a role in the progression of the disease.

EPC and Aging
Age appears to affect EPC availability and function as well.66,67 It has been reported that in young patients with stable coronary artery disease after coronary artery bypass grafting, the number of CEPCs increases, whereas the opposite occurs in older patients.66 The age-related deficiency in the number of CEPCs was not related to differences in cardiovascular risk factor or cardiac function and it may be caused, at least in part, by reduced levels of angiogenic and mobilizing cytokines. This deficiency may be at the root of impaired neovascularization of ischemic tissues and attenuated re-endothelialization of injured tissues commonly observed in older patients.68,69 Evidence for this hypothesis was provided by an elegant study by Eldeberg et al.67 who showed that neovascularization of cardiac allografts in aged mice occurred only after transplantation of bone marrow-derived EPCs from young animals. Cardiovascular disease further compounds the effects of aging on EPC number and function. The presence of cardiovascular risk factors increases the rate of EPC senescence, even in the absence of overt disease.28 Chronic treatment of apolipoprotein E−/− mice with bone marrow-derived progenitor cells form young mice without atherosclerosis attenuates the progression of atherosclerosis of animals maintained in an atherogenic diet,26 despite underlying hypercholesterolemia, suggesting that progressive depletion of EPCs with aging may precipitate the development of atherosclerosis, particularly in the presence of risk factors such as hypercholesterolemia. Reduction in progenitor cell mobilization with age may be caused by defects in the bone marrow stem cell niche and in the production of angiogenic cytokines and chemokines. VEGF and nitric oxide production have been reported to decrease with age,66,70–74 and it is known that these 2 factors play synergistic roles in the mobilization, migration, proliferation, and survival of endothelial cells.72,73

The alterations in EPC number and properties seen in aging and cardiovascular disease may be caused by a combination of factors. The chronic exposure to risk factors and presence of underlying cardiovascular disease accentuates endothelial injury, which may require continuous replacement of damaged endothelial cells. This may lead to exhaustion of the pool of progenitor cells available in the bone marrow, which may be exacerbated by accelerated senescence and apoptosis of the remaining cells.28,72,75 In addition, the reduced availability of mobilizing, homing, and differentiation/survival signals may limit the ability of EPC to repair injured tissues. Paradoxically, the functional impairment of EPC by cardiovascular disease and aging may limit their therapeutic usefulness in the patients who need it most.

Endothelial Progenitor Cells, Endogenous Repair, and Rescue in Cardiovascular Disease
Multiple lines of evidence suggest that EPC are recruited to sites of injury where they participate in the repair of damaged tissues.7,9,10,21,30,33,41,76,77 For example, the bone marrow-derived mononuclear cells (BM-MNCs), containing also the EPC population, home to ischemic myocardium,12,21,60 brain,78 and hind limb,10,25,30,34,43 where they participate in neovascularization. Marked increase in mobilization and
homing of CD34+ cells was seen in patients with myocardial infarction2,60 or hind limb43 ischemia. Furthermore, in patients with inoperable coronary artery disease, mobilization and homing of EPCs to the ischemic myocardium increased up to 9 weeks after VEGF gene transfer, in parallel with increase in circulating levels of VEGF,11 and in patients with critical limb ischemia VEGF gene transfer led to marked increase in CEPCs.43

Regeneration of endothelium is a fundamental process in vascular repair.1,7,8 Mature endothelial cells have limited ability to regenerate damaged endothelium because these cells are terminally differentiated.79 Accessory mechanisms such as EPCs may play a significant role in vascular repair and healing8,32,76,80 and strategies aimed at rapid endothelial recovery should reduce cardiovascular events associated with endothelial cell loss, including thrombosis, restenosis, and hypertension.8 EPCs were reported to repopulate implanted vascular grafts and damaged blood vessels as part of endogenous repair mechanism.13,14,15,17,18,32,80,81

Therapeutic Potential of the Endothelial Progenitor Cells

Studies in animal models of tissue ischemia and vascular injury have confirmed the therapeutic efficacy of EPCs in rescue of ischemia,10,11,19–21,25,30,31,34,35,38,84 repair of blood vessels, and in bioengineering of prosthetic grafts,13,15,17,22–24,32 Several small-scale trials have provided preliminary evidence of feasibility and safety of EPC transplantation in patients in myocardial and limb ischemia (Table 3).

Cell Therapy for Myocardial and Peripheral Ischemic Disease Using EPCs

Cell-based neovascularization has been achieved either by directly injecting purified EPC into the ischemic region19–21,30 or by mobilizing the cells using cytokines or chemokines such as VEGF, granulocyte colony-stimulating factor (G-CSF), or stromal derived factor (SDF).10,11,43,44,83–85 Various studies have confirmed the efficacy of EPC transplantation in inducing neovascularization of ischemic myocardium and hind limb (Table 3),19–21,25,30,34,43 Kocher et al21 showed that intravenous delivery of human CD34+ cells into athymic nude rats with myocardial infarction leads to marked angiogenesis in the peri-infarct region, resulting in decreased myocyte apoptosis, reduced interstitial fibrosis, and recovery of left ventricular function. Transplantation of CD31+ cells from peripheral blood improved left ventricular perfusion and function in a porcine amaroid valve model.86 We showed recently that transplantation of whole80 or CD34+-selected human peripheral blood mononuclear cells (PB-MNCs) into nude rats immediately after myocardial infarction led to significant neovascularization and improved function in the infarcted myocardium.86 Others showed that transcendocardial delivery of unfractionated autologous BM-MNCs induced collateral formation and rescued ventricular function in hibernating porcine myocardium.87–90 Orlic et al reported that implantation of bone marrow-derived Lin−/c−kit+ cells, a subpopulation of BM-MNCs, into the infarct border enhanced new vessel formation.91 In rats with limb ischemia, local intramuscular delivery of autologous BM-MNCs restored blood flow and exercise in association with enhanced neovascularization of the ischemic muscle.92 Interestingly, in one study93 the transplanted peripheral blood MNCs did not incorporate into the new capillaries, but contributed to new vessel formation by secreting pro-angiogenic cytokines. This observation suggests that a paracrine effect is probably an important mechanism contributing to the increased neovascularization observed after EPC transplantation.

Mobilization of EPC with cytokines or conventional pharmacological agents used in treatment of cardiovascular disease such as statins has been reported to enhance angiogenesis of ischemic tissues. Orlic et al94 showed that mobilization of bone marrow cells by G-CSF and SCF led to decreased postinfarction mortality and functional recovery in mice with myocardial infarction in association with significant regeneration and angiogenesis of the infarcted myocardium. In athymic nude mice with hind limb ischemia, local injection of SDF-1 stimulated homing of systemically delivered human PB-MNCs to the ischemic muscle and induced vasculogenesis.44 Other groups reported that statin therapy increases the number of CEPC in animal models and in patients with stable coronary artery disease,27,47,48 suggesting that the therapeutic effect of these drugs may be mediated, at least in part, via mobilization of EPCs.

EPC Cell Therapy for Pulmonary Hypertension

EPC-based cell therapy might be beneficial as a treatment of pulmonary hypertension. Chord blood-derived human EPCs overexpressing adrenomedullin markedly reduced pulmonary vascular resistance and improved survival rates after intravenous administration into nude rats with monocrotaline-induced pulmonary hypertension compared with control animals treated with either saline or EPCs alone.95 Similarly, intraparenchymal injection of autologous EPCs in dogs with pulmonary hypertension brought about significant improvements in mean pulmonary artery pressure, cardiac output, and pulmonary vascular resistance96

Endothelial Cell Therapy for Vascular Repair and Bioengineering of Grafts

An emerging area in which endothelial progenitor cell transplantation and genetic manipulation may have therapeutic potential is in repair of damaged vessels and in the bioengineering of prostheses and artificial organs.13–17,22–24,81,97–100 Autologous EPC transplantation may be used to promote rapid re-endothelialization and restoration of homeostasis in blood vessels injured during revascularization procedures22–24,97–100 or for seeding of prosthetic grafts, stents, or engineered blood vessels to create a bioactive endothelial layer.22,23,97,98 We showed recently that transplantation of autologous PB-EPCs leads to rapid re-endothelialization of balloon-denuded rabbit carotid arteries, resulting in significant reduction of neointimal hyperplasia.23 Using a similar approach, Gulati et al99 reported that transplantation of cultured autologous MNCs at the time of injury markedly reduced neointima hyperplasia in association with rapid re-endothelialization of the damaged vessel. Oth-
<table>
<thead>
<tr>
<th>Target</th>
<th>Donor</th>
<th>Recipient</th>
<th>Type and Source of Cells</th>
<th>Method of Delivery</th>
<th>Therapeutic Effects</th>
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<tr>
<td>Myocardial ischemia</td>
<td>Swine</td>
<td>Autologous</td>
<td>CD31+, peripheral blood</td>
<td>Transendocardial with NOGA mapping</td>
<td>† Rentrop score, † EF, † capillary density</td>
<td>86</td>
</tr>
<tr>
<td>Myocardial ischemia</td>
<td>Swine</td>
<td>Autologous</td>
<td>MNC, bone marrow</td>
<td>Transendocardial</td>
<td>† capillary density, † collateral flow, † myocardial contractility</td>
<td>90</td>
</tr>
<tr>
<td>Hibernating myocardium</td>
<td>Swine</td>
<td>Autologous</td>
<td>MNC, peripheral blood</td>
<td>Transendocardial</td>
<td>† EF, † capillary density, † capillary density, † flow</td>
<td>87</td>
</tr>
<tr>
<td>Myocardial ischemia</td>
<td>Rat</td>
<td>Autologous</td>
<td>MNC, bone marrow</td>
<td>Intramyocardial</td>
<td>† capillary density</td>
<td>88</td>
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<tr>
<td>Myocardial ischemia</td>
<td>Human</td>
<td>Nude rat</td>
<td>CD34+, peripheral blood</td>
<td>Intramyocardial</td>
<td>† EF, † capillary density, † fibrosis</td>
<td>90</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>Human</td>
<td>Nude rat</td>
<td>CD34+, bone marrow</td>
<td>Tail vein injection</td>
<td>† EF, † capillary density, † fibrosis</td>
<td>21</td>
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<tr>
<td>Myocardial infarction</td>
<td>GFP mouse</td>
<td>Syngenric mouse</td>
<td>Lin− c-kit+, bone marrow</td>
<td>Peri-infarct region</td>
<td>† LVDP, † capillary density, † infarct,</td>
<td>91</td>
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<td>Mouse</td>
<td>Autologous</td>
<td>bone marrow mobilization</td>
<td>Homing</td>
<td>† EF, † capillary density, † remodelling</td>
<td>94</td>
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<td>Hind limb ischemia</td>
<td>Rat</td>
<td>Autologous</td>
<td>MNC, bone marrow</td>
<td>Intramuscular injection in gastrocnemius</td>
<td>† capillary density, † blood flow,</td>
<td>92</td>
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<tr>
<td>Hind limb ischemia</td>
<td>Rabbit</td>
<td>Autologous</td>
<td>MNC, bone marrow</td>
<td>Intramuscular injection in thigh</td>
<td>† capillary density, † blood flow</td>
<td></td>
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<tr>
<td>Hind limb ischemia</td>
<td>Human</td>
<td>Athymic mice</td>
<td>MNC, peripheral blood</td>
<td>Intracardiac injection</td>
<td>† capillary density, † blood flow</td>
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<td>Human</td>
<td>Athymic mice</td>
<td>MNC, peripheral blood overexpressing VEGF</td>
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<td>Nude rat</td>
<td>MNC, peripheral blood</td>
<td>Intramuscular injection in thigh</td>
<td>† capillary density, † blood flow</td>
<td>93</td>
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<td>Myocardial infarction</td>
<td>Human</td>
<td>Autologous</td>
<td>CD133+, bone marrow</td>
<td>Infarct border</td>
<td>† EF, † collateral flow (SPECT)</td>
<td>107</td>
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<td>Myocardial infarction</td>
<td>Human</td>
<td>Autologous</td>
<td>MNC, bone marrow</td>
<td>Intracoronary balloon catheter</td>
<td>† infarct size, † wall motion</td>
<td>108</td>
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<tr>
<td>Myocardial infarction</td>
<td>Human</td>
<td>Autologous</td>
<td>MNC, bone marrow, peripheral blood</td>
<td>Intracoronary balloon catheter</td>
<td>† contractility, † myocardial perfusion, † remodeling, † EF</td>
<td>109–112</td>
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<td>Myocardial infarction (BOOST trial)</td>
<td>Human</td>
<td>Autologous</td>
<td>CD34+/CD117+/AC133+</td>
<td>Intracoronary during PCA</td>
<td>† EF, † LV wall thickness, † ESV</td>
<td>113</td>
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<td>Myocardial infarction (Unstable angina)</td>
<td>Human</td>
<td>Autologous</td>
<td>MNC, bone marrow</td>
<td>Transendocardial with NOGA mapping</td>
<td>† angina episodes, † wall thickening, † wall motion, † EF</td>
<td>114, 115</td>
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<td>Human</td>
<td>Autologous</td>
<td>CD34+</td>
<td>1: intracoronary, 2: G-CSF</td>
<td>† EF, † exercise time, † myocardial perfusion</td>
<td>120</td>
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<tr>
<td>Hind limb ischemia</td>
<td>Human</td>
<td>Autologous</td>
<td>MNC, bone marrow</td>
<td>Intramuscular injection in gastrocnemius</td>
<td>† ankle-brachial index, † pain-free walking, † transcutaneous PO2</td>
<td>116</td>
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AVDO$_2$ indicates arteriovenous oxygen difference; EF, ejection fraction; G-CSF, granulocyte colony-stimulating factor; MNC, mononuclear cells; PO$_2$, partial pressure of oxygen.
ers have shown the ability of transplanted EPCs to restore endothelial function in damaged vessels.\textsuperscript{99,100} We have also demonstrated the suitability of EPC to seed prosthetic grafts. Seeding of EPCs led to rapid endothelialization of expanded polytetrafluoroethylene (ePTFE) grafts after carotid interpositional grafting.\textsuperscript{23} Using a similar strategy, Kaushal et al\textsuperscript{22} showed that implantation of EPCs into decellularized porcine iliac vessels implanted as coronary interposition grafts reconstituted a functional endothelial layer that conferred improved vasodilatory function and prolonged patency of the grafts. EPCs may also be useful for genetic engineering of stents. Shirota et al\textsuperscript{97} reported that EPCs are capable of efficiently seeding photo-cured gelatin-coated metallic and microporous thin segmented polyurethane stents, suggesting that seeding of stents before implantation may provide a strategy for prevention of in-stent restenosis and thrombosis.

Exogenous mobilization of EPC from the bone marrow may provide a less cumbersome and potentially more effective strategy to enhance re-endothelialization of damaged vessels. Bhattacharya et al\textsuperscript{101} and Shi et al\textsuperscript{102} reported that mobilization of bone marrow by exogenous G-CSF enhanced endothelialization and patency of small caliber prosthetic grafts. We showed that treatment with G-CSF before balloon injury of rat carotid arteries led to accelerated re-endothelialization and concomitant reduction in neointima of the injured vessels, in association with an increase in the number of CEPCs.\textsuperscript{13} Others have reported that statin therapy\textsuperscript{15,17} and estrogen\textsuperscript{18} increases the number of EPCs and reduces neointima hyperplasia in animal models of arterial injury, presumably by stimulating eNOS activity.\textsuperscript{103} Interestingly, statins also reduce senescence and stimulate proliferation of PB-EPCs by regulating the activity of telomerase and cell cycle genes.\textsuperscript{89,104} Thus the therapeutic potential of endothelial progenitor cells could potentially be enhanced by noninvasive pharmacological manipulation and used to accelerate re-endothelialization of injured vessels and inhibit neointimal hyperplasia after revascularization procedures.

Potential of Genetic Engineering of Endothelial Progenitor Cells

The ability to genetically modify EPCs with expression vectors provides the opportunity to use the cells as vehicles for local delivery of therapeutic genes and allows the design of strategies to enhance the biological properties of EPCs. For example, the EPCs could be genetically modified to overexpress antithrombotic, vasodilatory, or antiproliferative genes to improve the function of the implanted cells in injured vessels or prostheses and to prevent thrombosis and restenosis.\textsuperscript{53,105} or to increase the capability of the cells to synthesize angiogenic, vasodilatory, or cytoprotective factors for maintenance and survival of the grafts.\textsuperscript{24} We reported recently that the therapeutic effect of EPCs could be enhanced by overexpressing eNOS.\textsuperscript{24} Our results showed that the denuded carotid vessels treated with the eNOS-expressing EPCs had enhanced inhibition of neointima and reduced incidence of thrombosis compared with vessels treated with control vector (Figure 3). We postulated from these observations that the transplantation of autologous EPCs expressing vasculoprotective genes may be a useful strategy to prevent postintervention complications such as thrombosis and restenosis after revascularization procedures.

EPCs have also been used as vectors for delivery of pro-angiogenic factors. The genetically modified EPC appear to contribute to the new vessel growth by proliferating and differentiating at the site of implantation, and by secreting...
growth factors that stimulate growth of pre-existing vessels. Overexpression of mobilizing cytokines and chemokines such as G-CSF and SDF-1 may be used to further potentiate the angiogenic effect of locally implanted EPCs by potentiating the recruitment and homing of other progenitor cells to the sites of ischemic injury. Genetic engineering may also be useful in the design of strategies to improve cell adhesion and survival and prevention of senescence. For example, overexpression of human telomerase reverse-transcriptase was reported to enhance the proliferative and migratory capacity of EPCs in response to VEGF stimulation, leading to improved neovascularization of ischemic limb.29 The combination of genetic modification and endogenous mobilization of EPCs may have synergistic effects,106 but the development of a strategy that will target mobilization of EPCs exclusively presents a difficult challenge.

**Clinical Application of Endothelial Progenitor Cells**

Several small-scale feasibility and safety clinical trials have been performed recently to evaluate the use of bone marrow cell transplantation in treatment of myocardial infarction and peripheral limb ischemia (Table 3).107–113 All the results published so far indicate that such therapeutic approach is feasible and safe. Moreover, in the case of myocardial ischemia, an improvement in ventricular function in association with enhanced coronary perfusion has been reported after cell therapy (Table 2). Stammt et al.107 injected autologous AC133+/CD133+ bone marrow cells into the infarct border during coronary artery bypass grafting in 6 patients that had experienced earlier myocardial infarction. The authors reported improved perfusion of the infarcted area and significant enhancement of global left ventricular function 3 to 9 months after surgery and no incidence of adverse events in 5 of the 6 patients. Similarly, intracoronary delivery of unfractiated autologous mononuclear bone marrow cells in patients undergoing PCA 5 to 9 days after myocardial infarction led to a reduction in infarct size and improvement in ventricular function and chamber geometry for up to 3 months after transplantation compared with patients treated with standard therapy for myocardial infarction alone.108 Intracoronary infusion of ex vivo-expanded BM-MNCs or PB-MNCs to a randomized group of 20 patients with reperfused acute myocardial infarction 4 days after infarction (Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction [TOPCARE-AMI] trial) led equally to significant improvements in global left ventricle ejection fraction and wall motion in the infarct zone and reduced end systolic dimensions at 4 months.109 The improved echocardiographic parameters were accompanied by increases in coronary flow reserve in the infarct artery and in myocardial viability in the infarct zone.109 Subsequently, the authors reported increase in global ejection fraction and decreases in end-systolic volume and infarct size at the 4-month follow-up period in the cell-treated patients, suggesting the infusion of progenitor cells attenuates postinfarct remodeling.110 Interestingly, the beneficial effects of progenitor cell infusion in post infarction functional recovery and prevention of remodeling was highly correlated with the migratory capacity of the progenitor cells,110 suggesting that the ability of the cells to migrate to the infarct site is a major determinant of infarct remodeling and regeneration. The recently published final 1-year follow-up results for this phase I trial showed sustained improvement in left ventricular function, reduction in end-systolic volumes, and smaller infarct sizes in the 2 groups of patients treated with cell therapy.111 Wollert et al reported recently the results of the BOOST randomized controlled trial evaluating the therapeutic effect of autologous bone marrow cell transfer in patients with myocardial infarction.112 In this trial, 60 patients with myocardial infarction were randomized into 2 groups of 30 patients after PCA and treated with medical therapy for myocardial infarction alone or with medical therapy and intracoronary infusion of autologous CD34+ bone marrow cells. The authors reported improvement in global left ventricular ejection fraction in the patients treated with the bone marrow cells 6 months after cell transfer.112 Furthermore, cell administration did not lead to adverse cardiac events or in-stent restenosis, thus indicating that the method of cell delivery is safe. However, the total number of cells administered and the mechanisms responsible for the functional improvement after cell delivery were not identified in this study. Comparable results were also reported by Fernandez-Aviles et al113 using intracoronary delivery of CD34+/CD117+/CD133+ cells in patients with reperfused myocardial infarction. However, it is not clear whether the cells used by Wollert et al112 are the same as those used in the later study.113 Two other groups have reported that transcatheter delivery of autologous BM-MNCs using NOGA mapping led to significant improvements in left ventricular perfusion and performance and reduced incidence of ischemic episodes in patients with end-stage ischemic heart disease114 or stable angina.115

Recently, autologous BM-MNCs were injected into the gastrocnemius muscle of patients with unilateral or bilateral leg ischemia caused by severe peripheral artery disease.116 Four weeks after cell transplantation, ankle−brachial indexes were significantly improved in the legs of patients treated with BM-MNCs but not in patients treated with saline. Rest pain and pain-free walking were also reported to be significantly improved during the 24-week duration of the study. The authors suggest that BM-MNC transplantation may be a safe and effective strategy for treatment of peripheral ischemic disease.

It must be pointed out that all the trials mentioned had several weaknesses. Namely, they consisted of a limited number of patients, were single-center, not blinded, and almost all of them were uncontrolled. Moreover, in most of these studies the cell population was not preselected; rather, the whole bone marrow MNC fraction was injected without further purification. Thus the results to date, although encouraging, should be considered preliminary. There is pressing need for large multicenter, controlled trials to test the efficacy of preselected pure EPC transplantation in the treatment of cardiovascular diseases.
Potential Problems With Therapeutic Use of EPCs

Although the preclinical and clinical studies reviewed here generally lend support to the therapeutic potential of autologous EPCs in the treatment of tissue ischemia and repair of injured blood vessels, the clinical application of EPCs is limited by several factors. First, the scarcity of EPCs makes it difficult to expand sufficient number of cells for therapeutic application without incurring the risk of cell senescence and change in phenotype. Furthermore, EPCs from patients with cardiovascular diseases display varying degrees of functional impairment. Aging and diabetes markedly reduce the availability and impair the function of EPCs. Because older and diabetic patients are the most vulnerable populations for cardiovascular diseases, this severely restricts the ability to treat with autologous EPCs the patients who theoretically need them most. The purity and developmental stage of the cells used for transplantation are important factors. Yoon et al reported recently that injection of total bone marrow cells into the heart of infarcted rats could potentially lead to severe intramyocardial calcifications. In contrast, animals receiving the same number of clonally expanded bone marrow cells did not show myocardial calcification. Thus, this finding brings attention to the potential risks of transplanting unselected bone marrow cells and cautions against their premature use in the clinical setting.

Exogenous mobilization of bone marrow with hematopoietic growth factors and other endothelial cell growth factors may recruit progenitor cells to sites of occult neoplasia, leading to vascularization of dormant tumors. In addition, mobilization could potentially accelerate progression of atherosclerotic plaque by recruiting inflammatory and vascular smooth muscle cell progenitor cells into the plaque, contributing to neointima hyperplasia and transplant arteriopathy. Increased rate of in-stent restenosis led recently to the cancellation of the MAGIC clinical trial using G-CSF for endogenous mobilization of progenitor cells in patients with myocardial infarction. Finally, there has been one study that has shown evidence that EPC may themselves contribute to allograft vasculopathy by promoting neovascularization of the plaque. However, another study failed to show evidence that EPCs contribute significantly to transplant arteriosclerosis.

Outstanding Issues and Perspective for Future Direction

Despite the encouraging results regarding the therapeutic potential of EPCs, several issues currently stand in the way of their wide clinical application. Strategies need to be developed to enhance the number of EPCs to allow the harvesting of adequate number for therapeutic application. The limited ability to expand PB-MNC–derived EPCs in culture to yield sufficient number for clinical application indicates that alternative sources of cells (ie, chord blood) or strategies to increase their number endogenously need to be explored. We believe that further characterization of the biology of EPCs, the nature of the mobilizing, migratory and homing signals, and the mechanisms of differentiation and incorporation into the target tissues need to be identified and further characterized. Strategies to improve retention and survival of the transplanted cells need to be developed as well. The issues of the timing of cell administration, the appropriate clinical condition, the optimal cell number, and, most importantly, the safety of cell transplantation must be defined. There is urgent need to standardize the protocols for isolation, cultivation, and therapeutic application for cell-based therapy. Finally, large-scale randomized, controlled, multi-centric trials will be essential to evaluate the long-term safety and efficacy of EPC therapy for treatment of tissue ischemia and vessel repair amid concerns of potential side effects such as neovascularization of occult neoplasias and the development of age- and diabetes-related vasculopathies. Despite these hurdles, the outlook for EPC-based therapies for tissue ischemia and blood vessel repair appears promising. Genetic engineering of EPC may provide an important strategy to enhance EPC mobilization, survival, engraftment, and function, thereby rendering these cells efficient therapeutic modalities for cardiovascular diseases.

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12. Kong D, Melo LG, Gnechchi M, Zhang L, Mostoslavski G, Liew CC, Pratt RE, Dzau VJ. Cytokine-induced mobilization of circulating endo-
This image contains a page from a scholarly article discussing the role of endothelial progenitor cells in vascular biology. The page includes numerous references to studies investigating the potential of these cells in repairing injured arteries, reducing neointimal formation, and enhancing reendothelialization as therapeutic strategies. The text touches on various aspects of endothelial progenitor cell biology, including their mobilization, differentiation, and homing response to injury. The article also highlights the use of genetically engineered cells to enhance regenerative properties and the potential for therapeutic neovascularization in ischemic conditions. Overall, the page provides a comprehensive overview of the multifaceted role of endothelial progenitor cells in cardiovascular health and disease.


Therapeutic Potential of Endothelial Progenitor Cells in Cardiovascular Diseases
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