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STATE-OF-THE-ART PAPER

Endothelial Progenitor Cells in Cardiovascular Disorders

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The important role of the vascular endothelium in cardiovascular health is increasingly recognized. However, mature endothelial cells possess limited regenerative capacity. There is therefore much interest in circulating endothelial progenitor cells (EPCs) among the scientific community, especially into their purported role in maintenance of endothelial integrity and function, as well as postnatal neovascularization. It has been suggested that these cells might not only be responsible for the continuous recovery of the endothelium after injury/damage, but also might take part in angiogenesis, giving the hope of new treatment opportunities. Indeed, there is accumulating evidence showing reduced availability and impaired EPC function in the presence of both cardiovascular disease and associated comorbid risk factors. Thus, many studies into the potential for use of EPCs in the clinical setting are being undertaken. The goal of this review article is to provide an overview of data relevant to the clinical role of EPCs and perspectives for treatment of cardiovascular disorders. (J Am Coll Cardiol 2007; 49:741–52) © 2007 by the American College of Cardiology Foundation

The crucial role played by the endothelium in cardiovascular biology is becoming increasingly appreciated (1). Indeed, endothelial injury has been implicated in atherosclerosis, thrombosis, and hypertension, and the balance between endothelial injury and endothelial recovery is of paramount importance for reducing cardiovascular events (2). However, mature endothelial cells possess limited regenerative capacity (3,4). There is therefore growing interest into circulating endothelial progenitor cells (EPCs), especially into their purported role in maintenance of endothelial integrity, function, and postnatal neovascularization (5). Other studies are also providing intriguing and encouraging insight into the potential use of EPCs in the clinical setting. Indeed, there is accumulating evidence for reduced availability and impaired EPC function in the presence of both cardiovascular disease and associated comorbid risk factors.

The goal of this review paper is to provide an overview of data relevant to the clinical role of EPCs and perspectives for treatment of cardiovascular disorders. A search strategy and a detailed discussion of the pathophysiological aspects of EPCs (that is, EPC definition, links to angiogenesis, and so on) is provided as an online-only Appendix.

Physiological factors and EPCs. Because of the rarity of EPCs and the difficulties in identification, limited information is available about the normal range and functional characteristics of different types of EPCs in humans.

The available data suggest that age may affect the availability and function of EPCs (6–8). Aging is associated with a reduced number of circulating EPCs in patients with coronary artery disease (CAD). For example, Vasa et al. (9) reported age-associated depression in circulating CD34/kinase insert domain receptor (KDR)-positive cells in a mixed group of healthy probands and CAD patients. Scheubel et al. (7) have reported an age-dependent loss of circulating EPCs in stable CAD. Moreover, the number of EPCs mobilized after coronary artery bypass grafting was significantly decreased in older patients.

The aging-associated impairment of cardiac angiogenic capacity in older mice, estimated as neovascularization of cardiac allografts, can be restored by implantation of bone-marrow-derived EPCs from young adult animals (8). Progression of atherosclerosis in apolipoprotein $E^{-/-}$ mice with persistent hypercholesterolemia seems delayed by chronic administration of bone marrow-derived progenitor cells from young mice (6). This treatment was much less effective when donors were older animals with atherosclerosis, indicating that progressive age-dependent reduction in EPCs may accelerate the development of atherosclerosis, particularly in the presence of risk factors (e.g., hypercholesterolemia) (6).

Multiple factors seem to be involved in the aging-associated deterioration of EPC quantity and function (Table 1). The chronic exposure to risk factors continuously damages endothelial cells and requires their intensive replacement. Conversely, risk factors possibly affect EPC mobilization, integration in injured vascular sites, and angiogenic capacity. The EPC dysfunction may also be result

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Abbreviations and Acronyms

ADMA = asymmetric dimethylarginine
CABG = coronary artery bypass grafting
CAD = coronary artery disease
EPC = endothelial progenitor cell
G-CSF = granulocyte colony-stimulating factor
KDR = kinase insert domain receptor
LDL = low-density lipoprotein
MNC = mononuclear cell
NO = nitric oxide
PDGF = platelet-derived growth factor
VEGF = vascular endothelial growth factor

of their accelerated senescence and apoptosis, as well as exhaustion of the pool of progenitor cells available in the bone marrow (10–12).

Reduced levels of angiogenic and mobilizing cytokines have been related to age-dependent impairment of EPC mobilization in vivo. Indeed, vascular endothelial growth factor (VEGF) and nitric oxide (NO) production have been reported to decrease with age (7,11,13–16), and these factors play synergistic roles in the mobilization, migration, proliferation, and survival of endothelial cells (11,15). The alteration of constitutive human telomerase reverse transcriptase activity can also affect the regenerative capacity of EPC (17). Thus, the impaired ability of EPCs themselves for mobilization by adequate stimuli may occur.

It is well known that physical training improves endothelial function, exercise tolerance, and collateralization in patients with CAD (18,19), chronic heart failure (20,21), and peripheral artery disease (22,23). Exercise upregulates circulating EPCs in patients with CAD (24), and increases the number of EPCs in bone marrow, peripheral blood, and the spleen (at least in mice) (25). The upregulation of EPCs by exercise may be dependent on endothelial NO and VEGF levels or a decreased rate of EPC apoptosis (24). In a recent clinical study, physical exertion in patients with peripheral arterial occlusive disease resulted in a 5.2-fold increase in EPCs and improvement of their function. However, subischemic exercise training in revascularized patients did not affect EPC number, although in vitro vascular tube formation was enhanced (26). These data imply that a positive impact of regular physical training on cardiovascular performance may be attributable at least partly to the improved behavior of EPCs (26).

Cardiovascular risk factors and EPCs. An increasing body of evidence suggests that cardiovascular risk factors affect the number and properties of EPCs. An inverse correlation is found between the number (and functional activity) of EPCs and cardiovascular risk factors among apparently healthy people and in patients with CAD (9,10). The number of EPCs correlates with endothelial function and is a better predictor for this than the patient's combined Framingham risk factor score (10).

Lipids. Multiple studies have consistently reported an association between lipid metabolism and the biology of human EPCs. The numbers of EPC colony forming units are significantly reduced in relatively healthy subjects with elevated serum cholesterol levels (10). In CAD, low-density lipoprotein (LDL) cholesterol inversely correlates with the number of circulating EPCs (9). In addition, the functional characteristics of isolated EPCs, such as proliferation, migration, adhesion, and in vitro vasculogenic capacity, are also impaired in patients with hypercholesterolemia (9,27).

Exposure of cultured EPC to oxidized LDL induces a dose-dependent impairment of their functional activity, accelerates the rate of EPC senescence, possibly by telomerase inactivation, and can be associated with up to a 70% reduction in EPC numbers (28,29). In addition, oxidized LDL impairs VEGF-induced EPC differentiation via the deactivation of Akt (30). Plasma levels of high-density lipoprotein cholesterol and triglycerides positively correlate with the number of EPC colony-forming units, but not

Table 1 Cardiovascular Risk Factors and Endothelial Progenitor Cells

Study	Risk Factor	Patients	Effects on EPC Number	Effect on Function
Vasa et al. (9)	LDL	CAD	↓ $CD34^+/KDR^+$ cells, NE CFU	↓ Migration
	Hypertension		NE	↓ Migration
	Smoking		↓ Circulating $CD34^+/KDR^+$ cells, ↓ in culture	NE on migration
Hill et al. (10)	Total cholesterol, LDL	Healthy	↓ CFU	ND
Chen et al. (27)	Total cholesterol	CAD	↓ In culture	↓ Proliferation, migration, adhesion, in vitro vasculogenic capacity
Pellegatta et al. (31)	HDL, triglycerides	Healthy	↓ CFU	ND
Loomans et al. (36)	Diabetes	Type 1 diabetes melitus	↓ In culture	↓ In vitro vasculogenic capacity
Tepper et al. (37)	Diabetes	Type 2 diabetes melitus	↓ In culture	↓ In vitro vasculogenic capacity
Pistrosch et al. (38)	Diabetes	Type 2 diabetes melitus	NE	↓ Adhesion
Kondo et al. (42)	Smoking	Healthy	↓ Circulating $CD45low/CD34^+/CD133^+/KDR^+$ cells	ND
Chen et al. (44)	Homocysteine	Healthy	↓ In culture	↓ Proliferation, migration, adhesion, in vitro vasculogenic capacity
Thum et al. (46)	ADMA	CAD	↓ Circulating $CD34^+/CD133^+$ cells, ↓ CFU	↓ Differentiation, in vitro vasculogenic capacity, NO synthase activity

ADMA = asymmetric dimethylarginine; CAD = coronary artery disease; CFU = colony-forming units of EPCs; EPC = endothelial progenitor cell; HDL = high-density lipoprotein; KDR = kinase insert domain receptor; LDL = low-density lipoprotein; ND = no data; NE = no effect; NO = nitric oxide.

with the number of $CD34^+/CD133^+$-positive progenitor cells (31).

Hypertension. Among various risk factors, hypertension is shown to be the strongest predictor of EPC migratory impairment (9). Angiotensin II diminishes telomerase activity in EPCs and accelerates the onset of EPC senescence through an increase in oxidative stress. Some controversy does exist about the effects of angiotensin II on in vitro EPC proliferation. Although angiotensin II inhibited EPC proliferation in one study, it enhanced VEGF-induced EPC proliferation in another (32,33). Angiotensin II also potentiates VEGF-induced network formation by EPCs, probably by upregulation of KDR (32).

Diabetes mellitus. Diabetes mellitus, another important cardiovascular risk factor, is a disease in which impairment of ischemia-induced neovascularization has been described (34,35). The number of EPCs is reduced in both type 1 and type 2 diabetes (36,37). Furthermore, marked EPC dysfunction may underlie new mechanisms involved in the pathogenesis of vascular complications in diabetic patients. Indeed, EPC proliferation, adhesion, and angiogenic properties are impaired in this setting (36–38).

EPCs can facilitate angiogenesis in a paracrine fashion by secretion of angiogenic factors to mobilize bone-marrow progenitors and to activate mature endothelial cells (39,40). Of note, the media from EPC culture of type 1 diabetic patients not only possesses evidence of reduced angiogenic capacity, but also contains an inhibitor for in vitro tube formation (36). Interestingly, diabetes was not associated with enhanced apoptosis in this study. Tepper et al. (37) showed an impaired ability of mature endothelial cells to incorporate into tubules in type 2 diabetes. In both studies, decreased number and dysfunction of EPCs was inversely related to the levels of hemoglobin A1c, implying that the degree of glycemic dysregulation was associated with EPC pathophysiology.

Further evidence of the negative impact of hyperglycemia on EPCs was provided by Kränkel et al. (41), who showed that cultivation of peripheral blood mononuclear cells (MNCs) from healthy donors under hyperglycemic conditions was associated with significant reduction in EPC numbers, inhibition of NO production, and matrix metalloproteinase-9 activity, as well as an impairment of the migrational and integrative capacities of the cells.

Other risk factors. Smoking is a significant predictor of reduced circulating and cultured endothelial progenitors (9). The number of circulating EPCs correlates inversely with the number of cigarettes consumed (42). The EPCs from heavy smokers also die prematurely during the early phase of culture (42). Similarly, smoking cessation is associated with an increase of EPC numbers, and these changes are most marked in those who smoked the least (42). However, if smoking is resumed, EPC numbers rapidly decrease to levels seen before smoking cessation (42). Of note, nicotine effects on the activity and function of EPCs seems to be dose dependent. Lower doses of nicotine have a positive influence on EPC numbers, proliferation, migration, and in vitro vasculogenesis with the peak effect at concentrations of nicotine 10^{-8} mol/l, similar to that found in the blood of smokers (43). However, cytotoxicity was observed at higher nicotine concentrations (43).

Homocysteine, which is another common cardiovascular risk factor, was shown to decrease numbers and impair activity of EPCs from human peripheral blood (44). Asymmetric dimethylarginine (ADMA), an endogenous NO synthase inhibitor, contributes to endothelial dysfunction and inhibition of angiogenesis and is an independent biomarker of future major adverse cardiovascular events or death (45). Of note, circulating ADMA levels inversely correlate with the number of progenitor cells, and ADMA inhibits EPC function, at least in vitro (46).

Table 2 Cardiovascular Disorders and Endothelial Progenitor Cells

Study	Patients	Effects on EPC Number	Effect on Function
Heeschen et al. (47)	Stable CAD	↓ CFU	↓ Migration, in vivo vasculogenic capacity
George et al. (49)	Unstable angina	↑ CFU	NE adhesion
Massa et al. (48)	Myocardial infarction stable CAD	↑ $CD34^+/KDR^+$ cells NE	ND
Shintani et al. (51)	Myocardial infarction	↑ Circulating $CD34^+$ cells, ↑ CFU	ND
Valgimigli et al. (57)	Heart failure	CFU and $CD34^+/CD133^+/KDR^+$ cells, ↑ in NYHA functional class I, ↓ in NYHA functional class III–IV	ND
Foresta et al. (58)	Erectile dysfunction	↓ Circulating EPCs	ND
George et al. (59)	Diffuse in-stent restenosis	↓ CFU	ND
Simper et al. (60)	Transplant arteriopathy	↓ CFU	ND
Taguchi et al. (61)	Cerebrovascular atherosclerosis	$CD34^+/CD133^+$ cells, ↓ in cerebral infarction, no correlation with the degree of atherosclerosis	ND
Ghani et al. (62)	Stroke	↓ CFU	ND

NYHA = New York Heart Association; other abbreviations as in Table 1.

EPCs and cardiovascular diseases. Abnormalities in quantity and function of EPCs have been shown in a number of studies of various cardiovascular disorders (Table 2).

Stable CAD. Despite numbers of circulating $CD34^+$/$CD45^+$ and $CD133^+$/$CD34^+$ progenitor cells and EPCs in patients with severe chronic CAD being similar to those in control subjects, in vitro functional capacity of bone-marrow MNCs is significantly reduced and transplantation of bone-marrow MNCs from patients with CAD into ischemic nude-mice high limb showed a markedly impaired ability to restore tissue perfusion (47,48).

Unstable CAD. In patients with unstable angina, an increase in numbers of EPC colony-forming units, but no change in adhesive properties, has been shown; however, the number of EPCs were reduced by almost 50%, after clinical stabilization (49). Correlations were also noted between systemic C-reactive protein (CRP) levels and circulating EPC numbers, but not with their adhesive capacity, implying that systemic inflammation may play a role in the mobilization of EPCs in patients with unstable angina (49). On the contrary, CRP was found to inhibit EPC proliferation, survival, differentiation, and function, suggesting a possible role in the development of cardiovascular disease (50).

In myocardial infarction, the number of circulating EPCs is markedly increased from the early phase of the disease to peak levels on day 7 (48,51). Subsequently, EPC numbers reduce and become similar to levels seen in control subjects within 60 days (51). Of note, plasma levels of VEGF (a growth factor associated with angiogenesis) are closely related to circulating EPC numbers, and levels also peak on day 7 (51). These data show the important role for VEGF in EPC mobilization in acute coronary syndromes. However, given that most patients with myocardial infarction are treated with EPC mobilizing drugs, such as statins or the angiotensin-converting enzyme inhibitors, the primary driving factor for peripheral EPC elevation in myocardial infarction is uncertain. In a rat model, the number and function of EPCs were depressed after myocardial infarction in those given placebo, whereas treatment with either an angiotensin-converting enzyme inhibitor or a statin was associated with significant stimulation of the amount and activity of the EPCs (52). Moreover, mesenchymal stem cells, which also possess the potential to differentiate to endothelial cells, are decreased on day 7 after acute ST-segment elevation myocardial infarction (53).

The functional role of the bone marrow cells in myocardial infarction may be attributable not only to their angiogenic properties and release of growth factors and cytokines, but also to their ability to restore the population of cardiac progenitor cells by selective homing to specific areas of myocardial injury and conversion to the phenotype of cardiac side-population cells (54). Bone marrow-derived hematopoietic cells may generate cardiomyocytes (albeit at a low frequency) within the infarcted myocardium in some animal models (55), although others fail to show transdifferentiation of hematopoietic stem cells into cardiac myocytes after myocardial infarction (56).

Heart failure. The numbers of EPCs are elevated in patients with acute heart failure, which significantly correlates with levels of the cytokine tumor necrosis factor alpha (57). Differences in the quantity of EPCs can be related to the stage of heart failure, with relatively higher numbers in the early stage of heart failure (New York Heart Association functional class I and II), with levels progressively decreasing with New York Heart Association functional class III and IV heart failure (57). Higher levels of brain natriuretic peptide are associated with depression of circulating EPCs with no effect of medical therapy or etiology of heart failure (57).

Other disease states. Reduced numbers of EPCs are found in patients with erectile dysfunction (58), those with in-stent restenosis (59), and in cardiac transplantation patients with vasculopathy (60). The number of EPCs does not seem to be associated with the degree of cerebrovascular atherosclerosis per se (61). However, EPC levels are significantly decreased in patients after stroke (62) and in those with atherosclerotic patients (including ones without clinical stroke) in whom areas of cerebral infarction as determined by positron emission tomography were found (61). In the latter study, EPC numbers also correlated with regional blood flow in areas of chronic hypoperfusion of the brain (61).

Effects of drug therapies on EPCs. Drug therapies may influence EPC physiology, as summarized in Table 3. These changes need to be placed in context to explain the possible therapeutic benefit(s) of these drugs, and to justify the effects on clinical outcomes, good or bad.

3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase inhibitors (statins). Many primary and secondary prevention trials have suggested that statins possess favorable (pleiotropic) effects, which include the improvement of endothelial function and an anti-thrombotic effect, independent of their impact on cholesterol reduction (63,64).

Along with direct effects on endothelial cells, stimulation of EPC activity may be an additional mechanism of the beneficial influence of statins on endothelial performance. Indeed, different statins have been shown to enhance the proliferative capacity of EPCs in vitro (12,65,66). Moreover, the effect of statins seems to be comparable with VEGF (66), which is known to augment the number of EPCs (67,68).

Statins stimulate EPC proliferation through the cell cycle regulatory genes (12). Additionally, statins induce EPC differentiation via the PI 3-kinase/Akt pathway (65,66), as well as enhance adhesiveness by increased integrin expression (69), and improve migratory activity by upregulation of the telomere repeat-binding factor TRF2 in EPCs (70,71). As previously mentioned, the pleiotropic effects of statins on EPC activity are independent of their impact on reduction in LDL cholesterol, as shown by comparison of simvastatin and

Table 3 Drug Therapies and Endothelial Progenitor Cells

Study	Patients	Therapy	Effects on EPC Number	Effect on EPC Function
Llevadot et al. (65)	ND	Simvastatin	↑ Proliferation	↑ Migration
Dimmeler et al. (66)	Healthy	Simvastatin, mevastatin, atorvastatin	↑ Proliferation	ND
Vasa et al. (70)	CAD	Atorvastatin	↑ EPC number	↑ Migration
Assmus et al. (12)	Healthy	Atorvastatin	↑ Proliferation	↓ Senescence
		Mevastatin	NE	↓ Senescence
Walter et al. (69)	ND	Simvastatin	ND	↑ Adhesion
Spyridopoulos et al. (71)	Healthy	Atorvastatin, mevastatin	ND	↑ Migration
Landmesser et al. (72)	Heart failure	Atorvastatin, ezetimibe	↑ In culture NE	ND
Bahlmann et al. (73)	Diabetes melitus	Olmesartan, irbesartan	↑ In culture	ND
Imanishi et al. (32)	Healthy	Valsartan	ND	↓ Senescence
Min et al. (74)	CAD	Ramipril	↑ In culture	↑ Proliferation, migration, adhesion, in vitro vasculogenic capacity
Bahlmann et al. (83)	Renal anemia, healthy	Erythropoietin	↑ Circulating $CD34^+/CD45^+$ cells, ↑ in culture	↑ In vitro vasculogenic capacity
Heeschen et al. (81)	CAD	Erythropoietin levels	Correlate with EPC number	Correlate with migration capacity
Pistrosch et al. (38)	Diabetes	Rosiglitazone	↑ In culture	↑ Migration
Foresta et al. (77)	Healthy	Vardenafil	↑ Circulating EPCs	ND
Zhu et al. (78)	ND	Puerarin	↑ In culture	↑ Migration, adhesion, in vitro vasculogenic capacity
Chen et al. (79)	ND	*Ginkgo biloba*	↑ In culture	↑ Migration, adhesion, in vitro vasculogenic capacity
Butzal et al. (80)	ND	Rapamycin	↓ In culture	↓ Differentiation, adhesion, ↑ apoptosis

Abbreviations as in Table 1.

ezetimibe (72). Finally, atorvastatin or mevastatin dose-dependently inhibit the onset of EPC senescence in culture (12). Thus, one may potentially consider the use statins to augment the functional potential of EPCs for transplantation therapy.

The renin-angiotensin-aldosterone system. The renin-angiotensin-aldosterone system is an important pathophysiological mechanism related to many cardiovascular disorders, and may also be involved in EPC (dys)function (32,33). Indeed, treatment with the angiotensin II receptor antagonists, olmesartan or irbesartan, significantly increases the number of EPCs (73). Also, valsartan was reported to reduce angiotensin II accelerated senescence of EPCs via upregulation of telomerase activity (32). The administration of ramipril, an angiotensin-converting enzyme inhibitor, increases the number and improves the functional capacity of EPCs in patients with CAD, independent of any impact on blood pressure (74).

Estrogens. No direct studies of effect of estrogen therapy on EPCs in humans are available, but increased blood estrogen levels in women do correlate with numbers of circulating EPCs (75). In an animal carotid injury model, estradiol treatment showed stimulatory effects on EPC mobilization, proliferation, mitogenic activity, and migration activity, as well as inhibited EPC apoptosis (76).

Miscellaneous drugs. Enhancement of EPC activity has been shown with treatment with vardenafil (a phosphodiesterase inhibitor) (77), puerarin (78), and *Ginkgo biloba* extract (79). In contrast, rapamycin inhibits proliferation and differentiation of human EPCs in vitro (80). The administration of rosiglitazone, a peroxisome proliferator-activated receptor gamma agonist, in patients with type 2 diabetes not only increases the number and migratory activity of cultured EPCs (38), but also can attenuate the detrimental effects of C-reactive protein on endothelial progenitors (50).

Finally a correlation between erythropoietin levels and EPC numbers, as well as functional activity, has been reported (81,82). The administration of erythropoietin also increases the number of functionally active EPCs in patients with renal anemia, as well as in healthy subjects (83).

EPC transplantation—clinical experience. It is increasingly recognized that EPCs are recruited to sites of injury and participate in the repair of damaged tissues and neovascularization in ischemic myocardium (48,51,84), hind limb (85,86), and brain (87). Many pre-clinical studies have shown therapeutic efficacy of EPCs in ischemic disorders and vascular injury in animal models (86,88,89).

Several small-scale phase 1 trials of bone marrow MNC transplantation in treatment of myocardial infarction, peripheral limb ischemia, severe stable CAD, and heart failure providing preliminary evidence of feasibility and safety of EPC transplantation have been performed (Table 4). For example, Hamano et al. (90) performed autologous bone-marrow MNC implantation during coronary artery bypass grafting (CABG), and long-term improvement of myocardial perfusion was reported in 3 of 5 patients, with no change seen in 2 patients.

Stamm et al. (91) injected autologous $CD133^+$ bone marrow cells into the infarct border during CABG in 6

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Table 4 Clinical Studies of Endothelial Progenitor Cell Therapy

Study	Disorder	n	Control Group	Used Cells	Number of Cells	Method of Delivery	Results
Stamm et al. (91)	CABG after 10 days to 3 months after MI	6	No	BM 133^+	1.5×10^6	Injection in infarct border during CABG	↑ EF, myocardial perfusion
Strauer et al. (92)	Acute MI	10	Yes	BM MNC	$9–28 \times 10^6$	Intracoronary infusion	↓ Infarct area, ESV; ↑ myocardial perfusion, SV
Assmus et al. (TOPCARE-AMI trial) (94)	Acute MI	40	Yes	BM MNC	Included 7.35×10^6 $CD34^+/CD45^+$	Intracoronary infusion	↑ EF, local contractility, myocardial viability, coronary flow reserve; ↓ ESV
Wollert et al. (BOOST trial) (96)	Acute MI	30	Yes	BM MNC	ND	Intracoronary infusion	↑ EF after 6 months but not after 18 months, with accelerated LV function recovery
Fernandez-Aviles et al. (97)	Acute MI	20	Yes	BM MNC	$50–125 \times 10^6$	Intracoronary infusion	↑ EF, local contractility, myocardial wall thickness and thickening; ↓ ESV
Hamano et al. (90)	CABG	5	No	BM MNC	$5–10 \times 10^6$ per point	Injection in myocardium CABG	↑ Myocardial perfusion in 3 of 5 patients
Tse et al. (99)	Refractory stable CAD	8	No	BM MNC	ND	NOGA* mapping-guided intramyocardial injection	↑ Target wall thickening and motion, myocardial perfusion
Perin et al. (100)	Severe ischemic heart failure	21	Yes	BM MNC	25×10^6	NOGA* mapping-guided intramyocardial injection	↑ Myocardial perfusion, EF, physical tolerance ↓ Heart failure and anginal symptoms, ESV
Tateishi-Yuyama et al. (TACT study) (101)	Severe leg ischemia	25 ischemic legs	Yes	BM, PB MNC	ND	Injection into the gastrocnemius muscle	↑ ABI, physical tolerance, number of collateral vessels, tissue perfusion; ↓ rest pain
Janssens et al. (98)	Acute MI	67	Yes	BM MNC	172×10^4	Intracoronary infusion	↓ Infarct size, no change of EF

*Biosense-Webster, Johnson & Johnson Inc., Diamond Bar, California.
ABI = ankle-brachial index; BM = bone marrow; CABG = coronary artery bypass grafting; EF = ejection fraction; ESV = end-systolic volume; LV = left ventricular; MI = myocardial infarction; MNC = mononuclear cells; PB = peripheral blood; SV = stroke volume; other abbreviations as in Table 1.

patients at 10 days to 3 months after myocardial infarction. Improvement of global left ventricular function, diastolic left ventricular dimensions (in 4 patients), and perfusion of the infarcted area (in 5 patients) with no adverse effect 3 to 9 months after surgery was shown. The intracoronary delivery of unfractionated bone marrow MNCs in patients with percutaneous coronary intervention after myocardial infarction has been shown to result in the improvement of local and global left ventricular contractility and geometry at 3 months of follow-up when compared with patients treated with standard therapy for myocardial infarction alone (92).

In the final 1-year follow-up results of the TOPCARE-AMI (Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction) trial (93,94), ex vivo expanded bone marrow MNCs or culture-enriched EPCs derived from peripheral blood MNCs were infused intracoronarily to a randomized group of 20 patients with acute myocardial infarction. After 4 months, significant enhancement of left ventricular ejection fraction and wall motion in the infarct zone, as well as improvement in cardiac geometry, coronary blood flow reserve, and myocardial viability in the injured area and reduction of end-systolic dimensions were observed. The final 1-year results for this trial confirmed a sustained improvement in left ventricular function, as well as reductions in end-systolic volumes and areas of dysfunctional myocardium in treated groups (93). The beneficial effects of MNC transplantation on post-infarct contractility restoration and prevention of remodeling seem to be highly correlated with the migratory capacity of these cells (95).

The randomized, controlled BOOST (Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration Trial) (96) evaluated the therapeutic effect of bone marrow cell transfer in 60 patients with myocardial infarction undergoing percutaneous coronary intervention. This controlled trial showed an increase in global left ventricular ejection fraction and systolic wall motion in the border zone at 6 months after autologous bone marrow MNC transfer (96). However, the difference in left ventricular contractility between groups was not significant after 18 months of follow-up, despite an acceleration of left ventricular function recovery by cell therapy.

Fernandez-Aviles et al. (97) published similar results of intracoronary infusion of bone marrow MNCs with estimated numbers of $CD34^+$, $CD117^+$, and $CD133^+$ subpopulations to patients with myocardial infarction after successful thrombolysis and stenting. Significant improvement of the end-systolic volume and the ejection fraction were found, as well as an increase in the end-diastolic and end-systolic thickness of the infarcted wall, as measured by magnetic resonance imagining at 6 months. All patients remained free of major cardiac symptoms or events at follow-up.

In a randomized, double-blind, placebo-controlled study, intracoronary autologous bone marrow MNCs were infused after percutaneous coronary reperfusion in 67 patients with

acute myocardial infarction (98). Although stem cell transplantation was associated with a reduction in infarct size, no significant improvement of left ventricular function was observed during the 4-month follow-up period. In this study, cells were delivered within 24 h after myocardial infarction, which is significantly earlier when compared with others, providing some evidence of the importance of timing of stem cell transplantation.

Selective delivery of EPCs to ischemic areas of the heart may have advantages. For example, a nonfluoroscopic left ventricular electromechanical mapping system-guided delivery of bone marrow MNCs to ischemic myocardium is an effective and safe treatment modality in selected patients. Improvement in symptoms, myocardial perfusion, and function at the ischemic region on magnetic resonance imaging during the 3-month follow-up period after MNC implantation was shown in 8 patients with stable angina refractory to maximum medical therapy (99). Another prospective, nonrandomized, open-label study of transendocardial injections of autologous bone marrow MNCs in patients with end-stage ischemic heart disease confirmed the safety and effectiveness of the treatment; at the 2- and 4-month follow-up period, 21 patients in the treatment group experienced less heart failure and fewer anginal symptoms, as well as an enhancement in myocardial perfusion and pump function and the improvement of cardiac geometry (100).

Leg ischemia caused by severe peripheral artery disease was the target of the TACT (Therapeutic Angiogenesis Using Cell Transplantation) study (101). Four weeks after autologous bone-marrow MNC injection into the gastrocnemius muscle, ankle-brachial indexes were significantly improved in the legs of patients treated with cells but not in patients treated with placebo (saline). There was a striking increase in the number of visible collateral vessels and recovery of blood perfusion in the treated legs during the 24-week duration of the study. Rest pain and pain-free walking also significantly improved during the follow-up period. Of note, legs injected with peripheral blood MNCs showed much smaller increases in the ankle-brachial index.

Importantly, the therapy based on transplantation of cells with endothelial potential seems to be safe because no deterioration in cardiac performance or adverse cardiac events, including proarrhythmia or an increased rate of in-stent restenosis, have been reported in the abovementioned studies (90–101).

Although these initial studies have shown optimism for the potential of EPCs as a therapeutic area, some important questions have arisen with respect to the design of clinical trials of EPC therapy:

1. *Which cell population should be transplanted, and should bone marrow or peripheral blood be the preferred source of cells*?
   There is not currently enough convincing evidence regarding which of the progenitor cell populations is the most potent for stimulating neovascularization and regeneration of ischemic tissue. Indeed, the $CD34^+$ stem-cell fraction takes part in postnatal revascularization (89,102). On the other hand, $CD34^{-302}$ cells enhance $CD34^+$ cell-mediated angiogenesis (100,102). Although the underlying mechanisms of this process are not clearly understood, they may be related to secretion of angiogenic cytokines and chemokines (39,84), or transdifferentiation of bone marrow mesenchymal stem cells and stromal cells into endothelial lineage, cardiomyocytes, and smooth muscle cells (103–105). This presumes that several different fractions of bone marrow MNCs may contribute to the regeneration of necrotic myocardium and vessels and increase regenerative potential. Conversely, peripheral blood progenitors being mobilized from bone marrow may possess higher functional activity.
   The TOPCARE-AMI trial showed that bone marrow MNCs and culture-enriched circulating EPCs had a similar positive effect on post-infarct myocardial recovery and perfusion enhancement. Controversially, in the TACT study (101), peripheral blood MNCs showed much smaller effectiveness in improvement of ischemic leg perfusion; however, because 500 ml of bone marrow were used for preparation of transplanted mononuclear cells and the number of $CD34^+$ cells, including EPC subfraction, is much lower in fresh peripheral blood MNCs than in bone marrow MNCs, it may be difficult to compare their relative clinical effectiveness. Thus, both purified EPC fractions and bone marrow MNCs, containing also the EPC population, may be effective for treatment of ischemic disorders.
2. *Which delivery method is the most efficient*?
   EPCs are a relatively rare cell population, and when given intravenously, only a very small fraction of infused cells reach the target region. It seems logical to choose a delivery route that provides maximal cell concentration in the damaged tissue. Injection of progenitor cells in the infarct border during CABG or into the gastrocnemius muscle in peripheral vascular disease, or intracoronary infusion and transendocardial intramyocardial injection, have been successfully applied methods (91–97,99–101). However, it remains premature to draw a conclusion about optimal delivery route given the small number of involved patients and the lack of comparative studies.
3. *What time point is optimal for the cell transplantation in acute ischemic states*?
   The peak of inflammatory response in myocardial infarction is observed in the first days with excessive production of cytokines, growth factors, and extracellular matrix proteins mediating myocardial repair. These molecules seem to be involved in the natural processes of mobilization, differentiation, and homing of bone marrow precursors. For example, VEGF is at its peak concentration at 7 days after myocardial infarction, and the decline of adhesion molecules (intercellular adhesion molecules, vascular cell adhesion molecules) follows shortly after-

ward (92). Transplantation of active progenitors in this period may exacerbate undesirable effects of inflammation on regenerative processes in the myocardium. Indeed, no improvement of left ventricular function was observed when bone marrow MNCs were delivered 1 day after myocardial infarction (98).

In one animal study, fetal rat cardiomyocytes were implanted into cryoinjured adult rat hearts immediately, 2 weeks later, and 4 weeks later (106). Negative results of immediate cell transplantation were reported, whereas the best results have been obtained when progenitors were implanted after 2 weeks, suggesting that early cells were not successful because of the excessive inflammatory process in the first days after infarction, whereas a 4-week delay was probably less effective because of scar expansion. In clinical studies in which cell transplantation was performed at 4 to 10 days and at up to 3 months, some positive results were shown and this approach would seem to be reasonable, but larger controlled multicenter trials are required.

How do EPCs improve neovascularization? How many endothelial progenitors really incorporate in vascular structures? In different ischemic models, the rate of incorporation of bone marrow-derived cells ranges from 0% to 57% and achieves 80% in vascular grafts (88,107,108). Although the basal incorporation rate of progenitor cells is low (109), ischemic tissues (myocardial [110], hind limb [88], cerebral [87,111]) and models of vascular injury (112,113) usually show involvement of EPCs in the vascular wall.

Most studies report homing of bone marrow progenitors in neocapillaries, but they are also found among stromal cells (89), fibroblasts, pericytes, and primarily leukocytes (107) at the sites of neovascularization. Some even suggest that in the adult organism, bone marrow-derived progenitors may primarily function as supporting cells (107). Of note, different factors potentially may affect the rate of incorporation of EPCs into the vascular wall, and the type or source of cells seems to be important. For example, implantation of ex vivo purified bone marrow MNCs, their subfractions, or culture-expanded EPCs is associated with a higher incorporation rate of cells than endogenously mobilized bone marrow cells (88,110,114). In one study, bone marrow MNCs but not peripheral blood MNCs were incorporated into neocapillaries (115). Outgrowth culture-expanded EPCs seem to show similar rates of incorporation into intima in the rabbit carotid injury models, as compared with earlier culture-modified EPCs (expanded on day 7 to 12) (113,116). The severity of ischemia may also significantly affect the incorporation of EPCs in different models of injury/ischemia (117), as does the pattern of local cytokine/chemokine levels. For example, stromal cell-derived factor 1 significantly increases EPC incorporation in the ischemic hind limb neovasculature (118). Similarly, there is increased recruitment of bone marrow MNCs to the sites of VEGF-induced neovascularization, but not into the newly formed vessel (119). Finally, treatment with HMG-CoA reductase inhibitors (and probably other drugs) may affect not only the mobilization, but also the homing of bone marrow progenitors. For example, simvastatin-enhanced corneal vasculogenesis was associated with an increase in incorporation of bone marrow-derived cells from 7.3% to 25.7% (65). On the whole, the rate of incorporation of EPCs into the endothelial monolayer is relatively low, indicating the important role of angiogenic factors produced by these cells to this process (120).

Quo vadis? The initial results from the studies summarized above have shown the feasibility and safety of bone marrow cell transplantation in treatment of myocardial infarction and peripheral limb ischemia, and no significant complication associated with this treatment has yet been reported. Furthermore, none of the patients after cell therapy had malignant arrhythmias, which seems to be a major limitation of injecting skeletal myoblast-derived cells directly into the myocardium (121). The concerns over the safety of granulocyte colony-stimulating factor to induce EPC mobilization and angiogenesis indicate the importance of appropriate control in further studies.

Although available clinical studies of EPC transplantation show beneficial results in terms of improvement in ischemic tissue perfusion and myocardial contractility, preventing remodeling after myocardial infarction, these studies are limited: a small number of patients were studied, randomization was not blinded, and few centers were involved. Furthermore, the exact profile of the cells used for treatment needs to be accurately determined. Ideally, a specific cell population or combination should be tested. Thus, despite encouraging preliminary results, there remains significant work to be done in terms of larger-scale multicenter controlled trials. The feasibility and safety of cell treatment should also be proved by long-term follow-up results.

Furthermore, there are some important obstacles in the large-scale clinical use of EPCs. First, EPCs are relatively rare cells, and expansion of sufficient numbers of a definite subpopulation from peripheral blood is hardly possible. Second, in vitro enumeration of progenitor cells for a quantity sufficient for therapeutic implantation is associated with a change in phenotype and differentiation and the risk of cell senescence, and may require artificial cell preactivation or stimulation (89,102). The reduced availability and functional properties of EPCs in older patients is limiting, especially in those with cardiovascular risk factors and comorbidities in which further suppression of EPCs is seen. It is in this very group of patients that advances in this technology would be most helpful. Finally, there remains a (theoretical) risk of undesirable recruitment of implanted cells by tumors or the retina, leading to vascular proliferation.

A better understanding of the EPC biology and identification of the precise mechanisms involved in mobilizing, migration, and homing of endothelial pro-

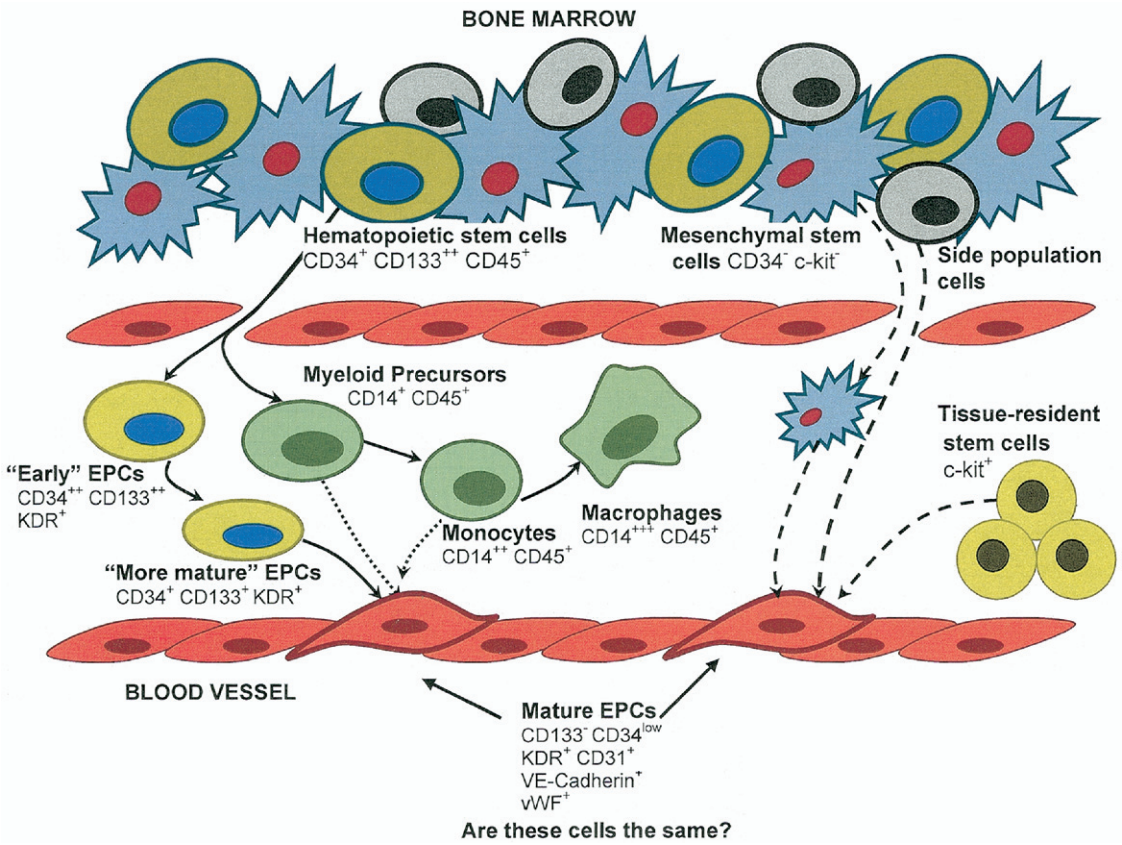

Figure 1 **Potential Origin and Differentiation of Endothelial Progenitor Cells**

EPC = endothelial progenitor cell; KDR = kinase insert domain receptor; VE = vascular endothelial; vWF = von Willebrand factor.

genitors may help to identify optimal regimens for cell preparation and delivery. Combinations of gene-cell therapy should also be considered. Indeed, the results of the first pre-clinical studies, in which efficacy of EPC therapy was enhanced by paracrine factors or factors antagonizing cellular aging and preventing apoptosis, are now available and can be considered promising. For example, ex vivo transfection of EPCs with VEGF before transplantation was shown to improve their capacity to augment neovascularization in a hind limb ischemia model (86). The capacity to augment blood flow and capillary density is also significantly increased after ex vivo transduction of EPCs with human active subunit of the telomerase reverse transcriptase (17). Biological properties of EPCs may also be enhanced by their genetic modification with expression vectors to overexpress antiproliferative, antithrombotic, or vasodilatory genes. This may improve the function of the implanted cells in damaged vessels or prostheses, as well as prevent thrombosis and restenosis, or even increase synthesis of proangiogenic factors to improve perfusion of ischemic regions. The latter may produce other factors to increase the proliferative, migratory, and adhesive capacity of EPCs in response to appropriate stimulus. Endogenous mobilization of EPCs is another possible approach for enhancing postnatal angiogenesis, and its combination with genetic modification of EPCs may have synergistic effects.

Conclusions. The development of the optimal strategy of effective and safe progenitor cell therapy presents a difficult challenge. More studies are needed to discover accurate mechanisms of EPC mobilization, migration, (trans)differentiation, and homing to the target areas. As multiple physiological and pathological factors are involved in EPC regulation, optimal regimens of activation, stimulation, treatment, and probably genetic modification, as well as of EPC protection, must be developed. Improvement and standardization of methods of cultivation is also required. Furthermore, alternative sources of endothelial progenitors such as cord blood and menstrual blood are under investigation. Despite a large number of unanswered questions, the first clinical studies show some promising results. A better understanding of the biology of EPCs will help us to increase the therapeutic potential of EPCs, as well as to apply these cells as therapies in clinical medicine (Fig. 1).

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APPENDIX

For the search strategy, pathophysiological considerations of endothelial progenitor cells, and angiogenic cytokines and chemokines, please see the online version of this article.