Abstract: The discovery of endothelial progenitor cells (EPCs) in human peripheral blood advanced the field of cell-based therapies for many pathological conditions. Despite the lack of agreement about the existence and characteristics of EPCs, autologous EPC populations represent a novel treatment option for complications requiring therapeutic revascularization and vascular repair. Patients with diabetic complications represent a population of patients that may benefit from cellular therapy yet their broadly dysfunctional cells may limit the feasibility of this approach. Diabetic EPCs have decreased migratory prowess and reduced proliferative capacity and an altered cytokine/growth factor secretory profile that can accelerate deleterious repair mechanisms rather than support proper vascular repair. Furthermore, the diabetic environment poses additional challenges for the autologous transplantation of cells. The present review is focused on correcting diabetic EPC dysfunction and the challenges involved in the application of cell-based therapies for treatment of diabetic vascular complications. In addition, ex vivo and in vivo functional manipulation(s) of EPCs to overcome these hurdles are discussed. (Circ Res. 2010;106:854-869.)

Key Words: diabetes ■ endothelial progenitor cells ■ bone marrow ■ angiogenesis
ional activation with proinflammatory and proliferative phenotype that ultimately disrupts vascular integrity resulting in increased permeability. In general, diabetic microvascular complications are typically associated with dysregulation of vascular remodeling and vascular growth with decreased responsiveness to ischemic/hypoxic stimuli and impaired or abnormal neovascularization.

Lack of endothelial regeneration and impaired angiogenesis contribute to the progression of diabetic micro and macrovascular complications. Formation of stable vasculature in response to tissue injury is an essential event for the restoration of blood flow and the repair of the affected tissue areas. Currently, clinical management of diabetic complications relies exclusively on pharmacological therapeutics that minimally affect endothelial repair or regeneration in most cases, and, therefore, these treatments have modest influence on end organ dysfunction. Hence, there is a need for therapeutic interventions that actually accelerate repair of dysfunctional endothelium in the end organ and restore blood flow to result in functional tissue generation.

After the identification of “putative” endothelial progenitor cells (EPCs),1 diabetic vascular complications are now thought to be, in part, the result of the reparative dysfunction of these cells. In response to these intriguing findings, a rapid progression of EPCs from the “bench to the bedside” occurred via translational studies even in the absence of a consensus about the true identity of the “EPC.” These seminal discoveries indeed paved the way for cell-based therapeutics for patients with vascular complications; however, a few caveats must be considered for autologous transplantation of cells in diabetic patients. The cells themselves are known to be dysfunctional, and the diabetic environment poses a variety of challenges that need to be considered. Otherwise, cell-based therapies may not be beneficial and may actually result in worsening of existing vascular complications in a patient population most in need of help.

The present review provides a brief introduction to the characteristics of EPCs and their dysfunction in diabetes and focuses mainly on the challenges involved in the application of cell-based therapies for diabetic vascular complications. In addition, a variety of possible ex vivo and in vivo manipulation(s) of EPCs to overcome these hurdles are discussed.

**Putative EPCs**

Based on the observations that Dacron arterial prosthesis in humans were endothelialized,2,3 it was hypothesized that a subpopulation of cells in the circulation support the replenishment of endothelial cells (ECs) within the vasculature and, in part, sustained neovascularization in response to tissue injury. This concept was similar to the phenomenon of hematopoietic recovery and persistent replenishment of blood cells by circulating hematopoietic stem cells (HSCs). Before these observations, adult neovascularization or de novo formation of blood vessels was thought to occur exclusively by angiogenesis, which is formation of blood vessels by existing vessels, rather than by vasculogenesis, formation of blood vessels from stem or progenitor cells (Figure 1).4,5

This long-held paradigm regarding the origins of neovascularization was shifted by the observations of Asahara et al1 that bone marrow (BM)-derived EPCs and this concept has been strongly supported by other studies.1,13 In animal models of ischemia, heterologous, homologous, and autologous EC progenitors incorporated into sites of active angiogenesis, and this led to their use for augmentation of collateral vessel

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**Non-standard Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AGE</td>
<td>advanced glycation end products</td>
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<tr>
<td>BM</td>
<td>bone marrow</td>
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<tr>
<td>BMC</td>
<td>bone marrow–derived progenitor cell</td>
</tr>
<tr>
<td>CFU</td>
<td>colony-forming unit</td>
</tr>
<tr>
<td>CXCR</td>
<td>CXC chemokine receptor</td>
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<tr>
<td>EC</td>
<td>endothelial cell</td>
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<tr>
<td>eEPC</td>
<td>early endothelial progenitor cell</td>
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<tr>
<td>eNOS</td>
<td>endothelial NO synthase</td>
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<tr>
<td>EPC</td>
<td>endothelial progenitor cell</td>
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<tr>
<td>G-CSF</td>
<td>granulocyte colony-stimulating factor</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>granulocyte/macrophage colony-stimulating factor</td>
</tr>
<tr>
<td>HSC</td>
<td>hematopoietic stem cell</td>
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<tr>
<td>IGF</td>
<td>insulin-like growth factor</td>
</tr>
<tr>
<td>INOS</td>
<td>inducible NO synthase</td>
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<tr>
<td>MAPK</td>
<td>mitogen-activated protein kinase</td>
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<tr>
<td>MMP</td>
<td>matrix metalloproteinase</td>
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<tr>
<td>MSC</td>
<td>mesenchymal stem cell</td>
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<tr>
<td>NPDR</td>
<td>nonproliferative diabetic neuropathy</td>
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<tr>
<td>OEC</td>
<td>outgrowth endothelial cells</td>
</tr>
<tr>
<td>PDR</td>
<td>proliferative diabetic neuropathy</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
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<tr>
<td>SDF</td>
<td>stromal cell–derived factor</td>
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<tr>
<td>SMC</td>
<td>smooth muscle cell</td>
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<tr>
<td>STZ</td>
<td>streptozotocin</td>
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<tr>
<td>VEGF</td>
<td>vascular endothelial growth factor</td>
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<tr>
<td>VEGFR</td>
<td>vascular endothelial growth factor receptor</td>
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</table>

This study showed that the putative EC progenitor cells or angioblasts could be isolated from human peripheral blood by magnetic bead selection on the basis of cell surface antigen expression and in vitro these cells differentiated into ECs. Specifically, CD34+VEGFR-2+ mononuclear cells were shown to express EC-associated markers such as CD31, vascular endothelial growth factor (VEGF) receptor (VEGFR)-2, Tie-2, and E-selectin when cultured on fibronectin. The EPC population of Asahara et al also expressed endothelial NO synthase (eNOS). However, both CD34 and VEGFR-2 antigens are present on ECs, and so, to exclude differentiated circulating ECs from a study population, now CD133 has been included as a marker limited to primitive cells but not expressed on ECs. To date, many antigenic markers including CD34, CD133, CD45, VEGFR-2, CD133, CXC chemokine receptor (CXCR)4, CD14, and CD31 have been used to identify EPC populations. Cell populations characterized by these surface markers have been shown to become angiogenic or to support angiogenesis in vitro or in vivo assays.6–11 An elegant study by Loomans et al12 in mice suggested eNOS expression is a reliable marker to identify bone marrow (BM)-derived EPCs and this concept has been strongly supported by other studies.1,13
growth to ischemic tissues. These pivotal studies were corroborated by many investigators and by the discovery that these EPCs can be found in BM niches and mobilized to areas of tissue injury/repair (Figure 2). Later, the protocol of Asahara et al was slightly modified by eliminating the cell-sorting step and the so-called colony-forming unit (CFU)-Hill or CFU-EC was developed as a commercial kit to quantify EPCs and enumeration of CFU-EC correlated closely with cardiovascular risk factors.

Recent studies examining the relationship between EPC number/function and presence of vascular disease support that CD34+ cells or cells that express both CD34 and VEGFR-2 are reliable markers for the “putative” EPC phenotype and that cells with these surface markers indeed predict defective vasoregenerative capacity in a given clinical condition. CD34+VEGFR-2 cell counts predicted the occurrence of cardiovascular events in a 10 month follow-up study involving 120 patients. Later, protocol of Ashara et al was slightly modified by eliminating the cell-sorting step and the so-called colony-forming unit (CFU)-Hill or CFU-EC was developed as a commercial kit to quantify EPCs and enumeration of CFU-EC correlated closely with cardiovascular risk factors.

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endothelial differentiation from BM-derived cells from normal and 3 month streptozotocin (STZ)-diabetic rats. These studies raise questions about the value of culture-expanded EPCs as valid diagnostic or prognostic tools.

Despite the lack of consensus on the identity of EPCs based on surface expression of antigenic markers and the clinical relevance of in vitro expanded progenitor populations, most investigators agree that the following criteria must be satisfied by a cell in order for it to be considered an EPC. The cell must mobilize from niches in response to ischemic stimuli, homing to areas of ischemia and participation in neovessel formation. Although there is an ongoing debate about the exact identity of EPCs, evidence has been accumulating from preclinical and clinical studies that multiple populations of progenitor cells whether freshly isolated or in vitro expanded may have therapeutic efficacy (Table).40–43

Paracrine Function of EPCs
The degree of vascular engraftment by administered putative EPCs/BM-derived progenitor cells (BMCs) varies among studies but ranges from none to a high percentage.1,13,25,26 Regardless of the number of cells that physically integrate into the vessel wall and the antigenic/phenotypic characteristics they express, circulating BMCs participate in vascular repair and promote vascular growth by releasing proangiogenic factors. An elegant study by Majka et al30 reported that human CD34+/H11001 cells, myeloblasts, erythroblasts, and megakaryoblasts release numerous growth factors, cytokines, and chemokines that regulate the process of hematopoiesis by autocrine and paracrine mechanisms; however, this study did not evaluate vascular repair. Harraz et al13 showed that CD34+/H11002 or CD34+/H11001 cells can incorporate into the endothelium of blood vessels in mouse ischemic limbs; however, this required the presence/coinjection of CD34+/H11001 cells, clearly indicating paracrine modulation of CD14+ populations by CD34+ populations. Direct evidence for the paracrine interaction between subsets of EPCs was also provided by Krenning et al,45 who showed that CD34+ cells modulate proliferation and endothelial differentiation of CD14+ cells by releasing hepatocyte growth factor, interleukin-8, and monocyte chemoattractant protein-1. In yet another study, culture-expanded EPCs derived from human monocytes/macrophages exhibited low potential for proliferation and endothelial differentiation but were capable of...
secrating proangiogenic factors VEGF, hepatocyte growth factor, granulocyte colony-stimulating factor (G-CSF), and granulocyte/macrophage (GM)-CSF.\(^6\) Conditioned medium of culture-expanded EPCs produced a strong migratory response in ECs in vitro that was abolished by neutralizing antibodies to stromal cell–derived factor (SDF)-1 and VEGF.\(^7\) Recently, CD34\(^+\) cells were shown to secrete interleukins, growth factors and chemokines that are capable of accelerating vascular network formation in vivo and enhancing the healing of ischemic ulcers in diabetic mice.\(^48\)

For a detailed account of paracrine mechanisms in adult stem cell signaling and therapy, refer to the recent reviews by Gnecci et al\(^{49}\) and Burchfield and Dimmeler.\(^{50}\)

Studies by Katusic and colleagues provided a mechanistic basis for the paracrine modulation of vascular function and accelerated angiogenesis by EPCs. In their studies, culture-expanded human EPCs released interleukin-8 and demonstrated a mitogenic effect on vascular ECs.\(^{51}\) Paracrine factors released by ex vivo expanded rabbit EPCs conferred cerebrovascular protection by increasing prostaglandin I\(_2\) production via cyclooxygenase-2/prostaglandin I\(_2\) synthase and by reducing thromboxane A\(_2\) production but did not change eNOS or inducible (i)NOS levels.\(^{52}\) Culture-expanded human EPCs were shown to express cyclooxygenase-1 and secrete prostaglandin I\(_2\) that increase angiogenesis via activation of peroxisome proliferator-activated receptor-\(\delta\).\(^{53}\)

Paracrine function of EPCs in diabetes has not been extensively studied, but the literature suggests that diabetic EPCs have impaired paracrine function in vitro and in vivo (Figure 3). Diabetic EPCs may not be able to release adequate levels of factors in the diabetic environment or alternatively, the diabetic environment may degrade these factors before they are able to promote neovascularization. Awad et al\(^{54}\) reported an interesting observation that obesity and diabetes together differentially affect primitive CD34\(^+\) and monocytic CD14\(^+\) cell function and furthermore, convert the proangiogenic phenotype to antiangiogenic in CD34\(^+\) cells, whereas CD14\(^+\) cells are affected to a lesser extent. These findings can be explained on the basis of paracrine release of antiangiogenic factors by cells of obese/diabetic origin. We observed that the release of SCF and hepatocyte growth factor by diabetic CD34\(^+\) cells in to the conditioned medium was lower whereas the secretion of transforming growth factor-\(\beta\)1 was higher compared to the nondiabetic cells, and, moreover, conditioned medium of diabetic cells did not support the proliferation and migration of nondiabetic cells in vitro.\(^{55}\)

### Table. Antigenic Characteristics of Different Progenitor Populations That Have Been Evaluated for Therapeutic Angiogenesis

<table>
<thead>
<tr>
<th>Antigenic Markers</th>
<th>Donor</th>
<th>Test System(s)</th>
<th>Functional End Point(s)</th>
<th>Proposed Mechanism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34(^+)/CD45(^-)/Flk-1(^*)</td>
<td>Mouse or Rabbit HLI</td>
<td>None</td>
<td>Direct Differentiation(^1)</td>
<td></td>
</tr>
<tr>
<td>CD34(^+)</td>
<td>Human Diabetic</td>
<td>Diabetic mouse HLI</td>
<td>Limb perfusion,</td>
<td>Vascular incorporation(^26)</td>
</tr>
<tr>
<td>CD34(^-) CD117(^-) GATA (^-)</td>
<td>Rat MI</td>
<td>Capillary density, myocardial function and remodeling</td>
<td>Direct differentiation and paracrine effects(^161)</td>
<td></td>
</tr>
<tr>
<td>CD34(^++)/cKit(^+)/Sca1(^+)</td>
<td>Mouse MI</td>
<td>None</td>
<td>Direct differentiation(^162)</td>
<td></td>
</tr>
<tr>
<td>CD34(^-) or CD34(^+)</td>
<td>Mouse HLI</td>
<td>Limb perfusion</td>
<td>Direct differentiation and vascular incorporation(^13)</td>
<td></td>
</tr>
<tr>
<td>CD34(^-) or CD34(^+)</td>
<td>Diabetic mouse wounds</td>
<td>Vascularity</td>
<td>Paracrine effects(^23)</td>
<td></td>
</tr>
<tr>
<td>CD34(^-) or CD34(^+)</td>
<td>Mouse MI</td>
<td>Infarct size, myocardial perfusion</td>
<td>Direct differentiation(^163)</td>
<td></td>
</tr>
<tr>
<td>CD34(^+)</td>
<td>Obese-diabetic mouse</td>
<td>Mouse HLI</td>
<td>Limb perfusion and autoamputation(^54)</td>
<td></td>
</tr>
<tr>
<td>CD133(^+)</td>
<td>Diabetic ischemic wounds in mice</td>
<td>Wound closure</td>
<td>Paracrine effects(^48)</td>
<td></td>
</tr>
<tr>
<td>CD34(^+) or CD14(^+)</td>
<td>Diabetic mouse HLI</td>
<td>Limb perfusion</td>
<td>Paracrine effects(^10)</td>
<td></td>
</tr>
<tr>
<td>Culture-expanded cells</td>
<td>Mouse MI</td>
<td>Myocardial function</td>
<td>Direct differentiation and vascular incorporation(^164)</td>
<td></td>
</tr>
<tr>
<td>Rabbit carotid balloon injury</td>
<td>Mouse HLI</td>
<td>Endothelial function; neointima formation</td>
<td>Direct differentiation(^165)</td>
<td></td>
</tr>
</tbody>
</table>

Refer to the recent review by Sekiguchi et al\(^{42}\) for preclinical studies in models of MI and controlled clinical trials.
Figure 3. Diabetic dysfunction in the BM mobilization of stem/progenitor cells and paracrine regulation of ischemic vascular repair. In normal conditions, factors released by ischemic/injured tissue causes mobilization of BM cells, which, when transmigrated into areas of ischemia, release proangiogenic factors and physiological levels of NO and ROS that modulate repair mechanisms by activating vascular endothelium in the surrounding areas, by recruiting more BM cells and by modulating ischemic environment. In diabetic conditions, signals arising from the vascular injury are weaker, resulting in reduced mobilization of BM cells into circulation. Those few progenitor cells reaching the areas of ischemia are either not able to release proangiogenic factors or release antiangiogenic or proinflammatory factors and nonphysiological levels of NO and ROS that delay or inhibit vascular repair.

and CD34+/CD31+ cell populations compared to normal subjects. These results clearly indicate a generalized reduction in putative endothelial progenitors with different levels of maturity/differentiation. In contrast, Lee et al62 demonstrated an increase in c-Kit+ and CD34+ cells in advanced PDR; however, the results were based on a small sample size and a limited characterization of comorbidities and drug treatment that may have had an effect on progenitor numbers.

Kusuyama et al63 showed that in newly diagnosed patients with type 1 diabetes, the number of EPCs was significantly related to hemoglobin A1c and blood sugar levels before treatment and, therefore, concluded that diabetes reduces the number of circulating EPCs according to its severity. The decline in the number of circulating progenitor cells is linearly correlated with the severity of diabetes56,59 and degree of glucose control has been shown to be negatively correlated with circulating progenitor numbers.57,63 Therefore, it is clear that improvement of glycemic control significantly increases the number of circulating EPCs which are vascular protective.

Similar observations have been made in experimental studies that reported decreased numbers of BMCs in circulation of diabetic rodents in response to ischemic injury compared to nondiabetic animals and this was associated with reduced eNOS expression in these cells.64,65 The reduction in circulating progenitor cells may signal diabetic dysfunction of BM or an inability to respond to signals from injured vasculature/ischemic tissue (Figure 3). Selective depletion of functional hematopoietic progenitor cells in the BM was also observed in the patients with postinfarction heart failure, suggesting increased demand and turnover. In contrast, Nguyen et al66 found an increased number of myofibroblast progenitors in these patients, suggesting that the reduction may be limited to cardio- and vasculoprotective progenitors. Thus, reduced numbers of functional EPCs in circulation have been identified as an important pathology in diabetic patients.

Diabetic BM dysfunction has been poorly studied. Diabetic neuropathy may be a determinant of this diabetic BM defect. We recently reported that decreased numbers of BMCs in the circulation are accompanied by increased numbers of “trapped” cells in the BM of type-2 diabetic rats. In physiological conditions, the release of BMCs into the circulation follows a definitive circadian pattern, which is regulated by sympathetic neuronal activity. Diabetic peripheral neuropathy affects the BM and is manifested as a reduced number of nerve endings within the BM. This leads to an altered circadian rhythmicity of BMC release and reduced numbers of cells in the circulation.67

Changes in signaling of matrix metalloproteinase (MMP)-9, eNOS (see below), and c-kit ligand–receptor may also mediate this BM dysfunction and involvement of additional factors cannot be ruled out. The diabetic defect in the release of BM cells is reversed by glucose normalization early in the disease course, suggesting that the mobilization mechanisms are sensitive to chronic hyperglycemic conditions and early on remain reversible.58,64,68

In contrast, mobilizing cells from BM is not always an ideal option as cells obtained after G-CSF stimulation were found to have reduced in vivo vasoreparative function in patients with ischemic heart disease despite their higher clonal expansion capacity.69 Moreover, mobilization of BMCs for vasoreparation was shown to aggravate underlying vascular dysfunction in some clinical conditions.70 Mobilized cells from diabetic BM have not yet been characterized but need to be investigated systematically to identify the risk/benefit ratio.

Diabetes and EPC Dysfunction

Diabetic EPCs exhibit reduced proliferative potential and migratory function.25,26,60,71 Vascularization was depressed when diabetic EPCs were injected into normal mice.25,72 Moreover, diabetic EPCs show a reduced ability to integrate into EC tubes in vitro.68 A large number of studies point to the defective NO signaling as a mediator of diabetic EPC dysfunction.

NO and Oxidative Stress

NO is generated from the guanidino group of l-arginine and is an NADPH-dependent reaction catalyzed by a family of NO synthase (NOS) enzymes: eNOS, neuronal NOS, and iNOS. Deficiencies in l-arginine supply have been strongly implicated in vascular diseases, including diabetes. If the
supply of L-arginine or the essential cofactor tetrahydrobiop- 
terin is inadequate, NO supplies becomes “uncoupled” and uses 
molecular oxygen as a substrate to form superoxide instead of 
NO. An imbalance between L-arginine availability and NO activity can also occur when cellular transport of L-arginine is 
recycled, catabolism of L-arginine by arginase is increased, 
or competitive inhibition for the active site of NOS by asymmetrical dimethylarginine occurs. In diabetic vascula- 
ture, eNOS was decreased, whereas NADPH oxidase (a major source of superoxide in the vascular endothelium), 
iNOS, and ONOO\textsuperscript{−} were all increased. The shift in redox 
state in diabetes with increased generation of reactive oxygen 
species (ROS) causes interaction of NO with superoxide, 
resulting in loss of bioavailable NO and formation of 
ONOO\textsuperscript{−}. The reduced NO and elevated ONOO\textsuperscript{−} can lead to 
microvascular dysfunction in diabetes.\textsuperscript{77–79} In diabetes, uncoupling of eNOS has been found to be a source of ROS 
generation and, when blocked, mouse models show reduced 
signs of endothelial dysfunction.\textsuperscript{80} Diabetic vascular dysfunction 
in rats was shown to be prevented by administration of 
1400W, a specific iNOS inhibitor, suggesting a major role for 
the iNOS in diabetic complications.\textsuperscript{81,82}

NO-mediated signaling pathways are essential for mobiliza-
tion of BMCs\textsuperscript{83,84}; NO activates MMP-9, releasing soluble 
Kit ligand, which shifts resident BM cells from a quiescent to 
a proliferative niche and stimulates rapid mobilization into 
the circulation.\textsuperscript{84,85} NO regulates migration of stem/progeni-
tor cells into ischemic sites and promotes their survival.\textsuperscript{56,57} It has been shown that diabetic BMCs have decreased eNOS 
activity, and more importantly, that exogenous NO can 
correct the migratory defect in these cells.\textsuperscript{71}

In addition to its effects on NO signaling, hyperglycemia has 
been shown to decrease circulating BMCs by increasing 
their senescence via activation of p38 mitogen-activated 
protein kinase (MAPK)\textsuperscript{88} or Akt/p53/p21\textsuperscript{89} pathways or by downregulation of SIRT1, the mammalian homolog of 
Sir2 (silent information regulator-2).\textsuperscript{90} A recent study showed that hyperglycemia shifts differentiation of BMCs into proin-
flammatory phenotype with decreased EPCs and increased 
macrophages.\textsuperscript{91}

**Increased ROS in EPC Dysfunction**

In physiological conditions, ROS have been shown to be 
involved in cellular signaling mechanisms that result from the 
reversible oxidation of redox-sensitive target proteins. Protein 
tyrosine phosphatases are extremely sensitive to oxidative 
modification which leads to increased phosphorylation and 
activation of many receptor tyrosine kinases.\textsuperscript{92} Overproduction 
of ROS in diabetes caused by increased activation of 
NADPH oxidase has been shown to be involved in the 
initiation and progression of diabetic vascular complications 
by decreasing the bioavailability of NO.\textsuperscript{93,94}

BMCs express NADPH oxidase isoforms and it has been 
suggested that low levels of ROS in the BM play an essential 
role in preserving primitive HSCs in the hypoxic environ-
ment; slightly elevated levels promote mobilization of HSCs in 
the early stages of postischemic neovascularization, but 
excessive ROS production causes senescence and impairs 
self-renewal of HSCs.\textsuperscript{95–98} Expression of antioxidant en-
zymes catalase, glutathione peroxidase, and manganese sup-
eroxide dismutase (MnSOD) is higher in circulating EPCs, 
whereas basal levels of ROS are lower compared to that in 
ECs, suggesting that EPCs are resistant to oxidative stress.\textsuperscript{99,100} In contrast, Ingram et al\textsuperscript{101} reported that clono-
genic proliferative EPCs derived from adult peripheral blood 
are more sensitive to oxidative stress and exhibit decreased 
clonogenic capacity and angiogenic function in the presence 
of oxidants. Our studies show that human CD34\textsuperscript{+} cells of 
diabetic origin show higher levels of NADPH oxidase-
dependent ROS production and decreased NO bioavailability. 
We further observed that decreasing NADPH oxidase activa-
tion results in reduced ROS production, increased bioavail-
able NO levels, and improved EPC migration in response to 
SDF-1 or VEGF.\textsuperscript{102}

**Advanced Glycation End Products**

Advanced glycation end products (AGEs) are modified pro-
teins or lipids that are nonenzymatically glycated and oxi-
dized after chronic exposure to sugars associated with diabe-
tes. AGEs affect cellular function by altering intracellular 
mechanisms via activating the receptor for AGEs, RAGE, as 
well as by modifying the extracellular environment by the 
formation of cross-links between key molecules in the base-
ment of membrane/extracellular matrix. Several studies sug-
gested that AGEs directly impair the vasoreparative function 
of EPCs. Incorporation of CD34\textsuperscript{+} cells into endothelial 
sprouting was impaired in the presence of pathological AGE 
concentrations because of enhanced apoptosis of cells via 
activation of p38 and p44/42 MAPKs or extracellular signal-
regulated kinase 1/2 MAPK pathways with the activation of 
nuclear factor κB.\textsuperscript{103,104}

Other studies implicated oxidative stress and downregula-
tion of Akt and cyclooxygenase-2 in the AGE-mediated 
apoptosis, impaired migration, and reduced tube formation in 
EPCs that were reversed by anti-RAGE antibody.\textsuperscript{105,106} In 
diabetes, the modification of extracellular matrix proteins by 
AGEs may impair or prevent interaction of EPCs with the 
vascular wall, thereby adversely affecting the initial trigger-
ing events in the process of re-endothelialization and angio-
genesis.\textsuperscript{107} This may be the case when autologous cells are 
“corrected” for their diabetic dysfunction ex vivo before their 
reinfusion for therapy. This is one of the challenges that need 
to be addressed when autologous cell transplantation is 
considered for therapeutic reendothelialization in diabetes. 
For a detailed account of the biological effects of AGES and 
strategies to alleviate their effects in diabetes refer to the 
recent review by Negre-Salvayre et al.\textsuperscript{108}

**Diabetic Microvascular Complications**

**Retinopathy**

Retinopathy is the most common of all diabetic complica-
tions, with almost all diabetic patients developing background 
retinopathy or nonproliferative diabetic retinopathy (NPDR), 
the non–vision-threatening form of diabetic retinopathy that 
is characterized by “microaneurysms,” hemorrhages, and 
exudates. The later stages of NPDR are accompanied by 
vasodegeneration characterized by retinal ischemia attribut-
able to areas of nonperfusion. These ischemic areas appear as acellular capillaries in the histological examination, a hallmark feature of diabetic retinopathy.

As discussed in the preceding sections, diabetes is associated with both widespread dysfunction and reduced numbers of EPCs. Thus, it is not surprising that several studies have detected reduced EPC number in patients with NPDR. Brunner et al conducted a case–control study that compared 90 patients with type 1 diabetes with and without retinopathy and concluded that in type 1 with retinopathy, EPCs underwent stage-related regulation. In NPDR, a reduction of EPC numbers was observed, whereas in PDR, a dramatic increase of mature EPCs was observed. This is in keeping with the hypothesis that the vasodegenerative phase of NPDR is associated with reduced reparative function of these cells. In contrast, Lee et al demonstrated an increase in c-Kit+CD34+ cells in advanced PDR; however, the study was small in sample size and there was limited characterization of comorbidities and drug treatments that could influence progenitor numbers. Kusuyama et al examined EPCs from peripheral blood obtained from 11 patients with type 1 diabetes with PDR, 12 age- and gender-matched type 1 diabetics without retinopathy, and 11 age- and sex-matched nondiabetic controls. They found that the number of colony-forming units per 1×10^6 monocytes was increased in patients with PDR when compared with patients without retinopathy. Nondiabetic controls showed an intermediate number of EPC counts that were nonetheless significantly increased with respect to patients without retinopathy.

Taken together, these results can be explained by the hypothesis that in contrast to EPCs in healthy individuals that rescue and maintain the existing retinal capillary bed, in diabetic patients, the reduced number and reduced clonogenic potential of EPCs might predispose these patients to development of the vasodegenerative phase of diabetic retinopathy. Once the damage is widespread, inflammation increases and chemokines are produced by the ischemic retina; the BM may respond/compensate by increasing the production of EPCs, which, in the pathological setting of a growth factor-rich vitreous and retina, may result in aberrant neovascularization characteristic of PDR.

No studies to date have evaluated the potential of EPCs in the treatment of patients with diabetic retinopathy. However, animal studies have provided a better understanding of possible vascular repair by EPCs in retinopathy. Participation of BM cells in the retinal repair has been documented in mouse models; homing to areas of retinal injury and differentiation of BMCs into ECs, microglia, and astrocytes have been observed. High numbers of EPCs contributing to both repair as well as pathological neovascularization in the eye have been observed in rodent models. This may be attributable to the particularly quiescent nature of the resident retinal vasculature (typical retinal EC turnover occurs every 4 years), thus facilitating the contribution of circulating cells to the newly forming vessel. In human–mouse chimeric models of ocular vascular injury, we observed that healthy human CD34+ cells effectively repair injured mouse/rat retina by directly participating in re-endothelialization of acellular capillaries (Figure 4).

Figure 4. Human CD34+ of nondiabetic, but not diabetic, origin integrate into degenerate vasculature in mouse eyes damaged by ischemia/reperfusion injury (Caballero et al). Two days after intravitreal or systemic administration, integration of diabetic cells into existing vasculature was not observed (A), whereas cells of nondiabetic origin show extensive integration into small and medium sized vessels (yellow in the composite images) (B). Insets show separate red and green channels used to make the composite images. C, CD34+ cells home to an area of injury and traverse toward the ischemic region of the capillary (arrows). Copyright 2007 American Diabetes Association. From Diabetes 2007;56:960–967. Reprinted with permission from The American Diabetes Association.

Nephropathy
Whereas the evidence for EPCs taking part in the pathogenesis of diabetic retinopathy is quite robust, the evidence for involvement in diabetic nephropathy is less strong, although a role for endothelial dysfunction has been documented in the development of diabetic nephropathy. In addition to the 3 different types of endothelium (vascular, peritubular, and glomerular), the kidney contains glomerular mesangial and tubular epithelial cells. Studies are yet to be carried out to identify stem/progenitor cell phenotypes that regenerate/differentiate into one or more of these cell types. The participation of BMCs in kidney repair has been shown in different experimental studies; however, these findings were challenged by a study using a chimeric mouse model. In addition, Lee et al observed homing of very few of the human mesenchymal stem cells (MSCs) in the glomeruli of STZ-diabetic NOD/SCID mice following intracardiac administration of the MSCs. More systematic studies need to be carried out to support the use of cell-based therapies in diabetic nephropathy.

Neuropathy
Peripheral neuropathy is a common complication of diabetes, which may lead to foot ulcers that can result in amputation. Diabetes-induced impairment of the microcirculation precedes diabetic neuropathy. Therefore, re-endothelialization or vascular repair may in fact be essential for the restoration of nerve function. A few studies have tested the therapeutic potential of stem/progenitor cells in diabetic neuropathy. Using intramuscular injection of mononuclear cells derived from peripheral blood or BM, Hasegawa et al showed significant amelioration of diabetic neuropathy in rats with 4 weeks of STZ-diabetes. This study further showed this beneficial effect was not accompanied by angiogenesis but
was blocked by treatment with VEGF-neutralizing antibody. In rats with 6 weeks of STZ-diabetes, Naruse et al. observed amelioration of neuropathy associated with improved angiogenesis following intramuscular administration of human umbilical cord blood-derived 7-day culture-expanded EPCs. Recent study in rats with 12-weeks of STZ-diabetes treated with rat BMCs reported restoration of nerve function that was accompanied by revascularization but colocalization of injected cells with ECs was not observed. Jeong et al. showed that intramuscular injection of murine BM-derived culture-expanded EPCs restored physiological responses and neural vascularity as well as transdifferentiation into ECs was observed in mice with 12 weeks of STZ-diabetes. This study also showed evidence for the durability of engrafted EPCs for >12 months. Together, these studies suggest that the cell therapy as an innovative option for the treatment of diabetic neuropathy; however, no studies involving use of autologous diabetic cells have been reported.

**Diabetic Macrovacular Complications**

Diabetes with or without clinical obesity is a high risk factor for the progression of atherosclerosis, which, in turn, manifests as coronary artery disease or peripheral vascular disease and recent studies observed close association between EPCs and incidence of atherosclerosis. It is not clear whether EPCs promote or alleviate the progression and severity of atherosclerosis. Controversial results from experimental studies could be attributable to the lack of uniformity in the age and gender of the animals used because these 2 factors seem to influence the impact of BMCs on the development of atherosclerosis. Early case reports suggested that BMCs may worsen restenosis; however, later studies showed no evidence for this outcome. 

George et al. evaluated the effects of intravenous injection of BMCs or spleen-derived culture-expanded EPCs in apolipoprotein E knockout mice. Treatment with BMCs increased aortic sinus lesion size with increased plaque area; whereas treatment with EPCs resulted in an increased lipid core of the plaque with thinner fibrous cap and increased number of infiltrating CD3 cells. Both treatments resulted in decreased interleukin-10 levels, and, in addition, interleukin-6 and monocyte chemoattractant protein-1 were seen in the group that received BMCs. In a cholesterol-fed rabbit model of neointimal formation and inflammation, balloon-denuded and radiated iliac arteries were evaluated following treatment with peripheral blood EPCs or BMCs. No signs of decrease in neointimal thickening were observed after treatment with either population of cells twice at one and 2 weeks after denudation and radiation of arteries, whereas the study by Ma et al. in a similar rabbit model with denuded common carotid artery, reported significant decrease in stenosis by 2 weeks after treatment with EPCs and beneficial effect remained similar up to 15 weeks. The question that is yet to be clarified is which model is best as a representative model of human atherosclerosis, keeping in mind that not all studies in humans result in clear outcomes. A recent study by Subramaniyam et al. in patients with peripheral artery disease showed beneficial effects of mobilization of BMCs with GM-CSF. Numbers of leukocytes and CD34+ cells were significantly increased by 2 weeks of GM-CSF treatment, with an increased number of CFUs, suggesting that the mobilized cells are functional. Improved endothelial function and increased exercise capacity were observed 12 weeks after GM-CSF treatment. In contrast, the study by Horie et al. using autologous implantation of mobilized peripheral blood mononuclear cells was inconclusive, and this was attributed to low numbers of CD34+ cells and decreased survival attributable to reasons other than peripheral limb ischemia.

Significant progress has been accomplished in applying cell-based therapies in patients with coronary artery disease or myocardial ischemia and different ongoing clinical trials evaluated and confirmed the safety of cell-based therapies.

**Wound Healing and Ulcers**

Effective wound healing is an orchestrated response involving angiogenesis, enhanced cellularity, reepithelialization, and glandularization, which is indicative of cutaneous regeneration. Specificity of cellular phenotypes governing these different events is not yet known, but the phenomenon of wound healing is severely impaired in diabetes. Earlier studies in cell-based therapies for diabetic wound healing were focused on using fibroblasts; however, recent preclinical studies explored the benefits of BMCs. Healthy, nondiabetic CD34+ cells were shown to accelerate vascularization and wound healing in diabetic mice; murine diabetic BMCs inhibited angiogenesis but produced wound healing in diabetic mice suggesting that these 2 events involve distinct mechanisms perhaps involving distinct cellular phenotypes. Murine BM-derived MSCs or their conditioned medium were shown to enhance wound healing with cutaneous regeneration that was not observed with CD34+ cells. BM stromal cells also have been shown to improve wound closure following topical or systemic administration in diabetic rats. Human fetal-derived CD133+ cells produced effective wound healing in diabetic mice. Recently, murine lin- cells were found to be effective in diabetic wound healing and were shown to remain in wound areas for up to 28 days compared to lin+ cells that were not found after 7 days. These studies suggest that healthy progenitors may serve to enhance wound healing in diabetic patients.

**Diabetic Erectile Dysfunction**

Erectile response is a neurovascular response involves both central and peripheral components. The peripheral component of this response is known to be impaired in diabetes and has been considered as a risk factor and a predictor of future cardiovascular events, suggesting that the diabetic impairment of erectile response precedes coronary artery disease. Experimental studies were reported showing the potential of cell-based therapy in correcting erectile dysfunction; however, to date, there are no studies reported in diabetic erectile dysfunction. Immortalized neural crest stem cells, K10 cells, transplanted in rat penile corpus cavernosum were shown to differentiate into cells with both EC- and smooth muscle cell (SMC)-specific markers. Human MSCs transduced with
retroviral vector encoding v-myc were shown to differentiate into endothelial and SMCs. However, these 2 studies have not evaluated functional/erectile responses. In a rat model of age-associated erectile dysfunction, Bivalacqua et al studied erectile responses in rat at 7 and 21 days after intracorporal injection of ex vivo-expanded rat MSCs that were or were not transduced with adenoviral vector encoding eNOS. Functional responses were shown to be improved in 21 days, but not in 7 days. The injected cells expressed EC- and SMC-specific markers at both the 7 and 21 day time points.

**Autologous Cell Therapy in Diabetic Vascular Complications**

Despite strong lines of evidence supporting the concept of therapeutic angiogenesis by putative EPCs, few studies have used diabetic models, particularly, testing autologous transplantation. As described above, diabetic cells have impaired reparative function with complex underlying mechanisms. Cells for treatment of diabetic vasculopathy should be equipped with cellular and molecular armamentarium to withstand the in vivo diabetic microenvironment. Their migration to the site of repair and the ability to transmigrate, differentiate, and engraft into a blood vessel should be minimally affected by the diabetic environment. Several approaches have been proposed, but few have been tried in diabetic cells and evaluated in diabetic models. Hypoxic preconditioning of EPCs was shown to enhance their angiogenic potential. However, it is questionable whether diabetic EPCs would be as responsive as normal cells to hypoxia.

**Neovascularization: When Is There Too Little or Too Much?**

Perhaps one of the most perplexing aspects of diabetes vascular dysfunction is how the retina can respond to the same insults with pathological neovascularization and the other vascular beds consistently demonstrate reduced angiogenesis. This “paradox” remains at the center of key questions in the field. So how can this be reconciled? One explanation is that the diabetic retina and vitreous represents a unique environment in which high concentrations of complementary growth factors (VEGF, insulin-like growth factor [IGF]-1, fibroblast growth factor, SDF-1, erythropoietin) act in a synergistic manner in contiguous environments (retina and vitreous) that provide scaffolding (remnant “hyaloid”) for new vessel formation. This unique structural arrangement and an accompanying plethora of inflammatory cells likely set up an ideal environment for neovessel formation. Once the damage is widespread, inflammation increases and chemokines are produced by the ischemic retina; the BM responds by increasing the production of EPCs, which in the pathological setting of the growth factor rich vitreous and retina may result in pathological neovascularization. Thus, the cooperation of circulating EPCs with resident retinal vasculature and circulating inflammatory monocytes results in “too much” neovascularization, leading to PDR. In contrast, in nonocular vascular beds, the level of neovascularization is markedly reduced, in part, because of progressive basement membrane modification by AGEs, which contributes to impairment of EPC reparative function after diabetes-related endothelial injury.

**Ex Vivo Manipulation of Diabetic Cells**

**Genetic Modification of Cells**

Accumulating evidence indicates that biologically modified EPCs to release proangiogenic factors may be a potent therapeutic tool compared to unmodified cells. In this regard, Iwaguro et al transduced murine EPCs ex vivo with adenoviral vector encoding VEGF gene and demonstrated that VEGF-expressing cells had enhanced angiogenic potential in in vitro and in vivo assays. Murasawa et al have used a similar approach to delay human EPC senescence by transducing progenitor cells with hTERT (human telomerase reverse transcriptase) and observed increased capillary density and blood flow in the mouse hindlimb ischemia. EPCs that were transduced to overexpress eNOS were shown to inhibit neointimal hyperplasia in a rabbit model of carotid artery balloon angioplasty. Choi et al have targeted glycogen synthase kinase-3β in human early EPCs and late outgrowth EPCs and reported that this genetic modification enhanced their vasoregenerative potential in mouse hindlimb ischemia. Human MSCs transduced with the prosurvival gene Akt-1 showed enhanced viability and greater functional repair in a mouse infarct model. We recently identified that expression of IGFBP-3 (IGF-1 binding protein-3) in stem/progenitor cells or in the area of vascular injury dramatically enhanced the homing and vascular repair.

**Ex Vivo Treatment With Small Molecules**

Ex vivo treatment of BMCs with a novel molecule, AVE9488, that increases eNOS expression by stimulating eNOS promoter activity, enhanced the angiogenic function of EPCs in mouse hindlimb ischemia. Similar effects were produced by ex vivo treatment with IGF-1, which indeed reversed age-associated decrease in human and murine EPCs. However, one should be very cautious when adopting this approach in diabetic cells, because without correcting the diabetic oxidative environment, an increase in NO production by iNOS may result in higher production of reactive nitrogen species that further increase oxidative stress. Sphingosine-1-phosphate and its synthetic equivalent, FTY720, were shown to stimulate EPC function in restoring blood flow in mouse ischemic limbs via activating CXCR4-coupled signaling pathway. Active p38 MAPK downregulates the number of BMCs in response to high glucose or tumor necrosis factor-α and ex vivo treatment with the p38 MAPK inhibitor SB203580 has been shown to increase the proliferation of EPCs that can be used for transplantation.

Sorrentino et al showed that treatment of human diabetic EPCs with the peroxisome proliferator-activated receptor-γ agonist rosiglitazone improved their angiogenic function in a mouse model of carotid injury by reducing NADPH oxidase–dependent ROS production and enhancing bioavailable NO levels. Agents or molecular maneuvers that can reduce oxidative stress as well as increase eNOS expression/activation would be of immense value in correcting diabetic EPC dysfunction.
In Vivo Manipulation of Diabetic Cells

In vivo manipulation of EPCs may involve modifying the diabetic milieu or the EPC niches to release functioning cells for homing to the areas of repair; however, possible options are very few. Treatment with growth hormone has been shown to reverse age-associated impairment of EPC function with increased eNOS expression, and this treatment resulted in elevated IGF-1 levels in mice and humans.\(^1\) Although mobilization of BM cells using G-CSF for therapeutic angiogenesis requires more thorough safety evaluation,\(^69,70\) studies using AMD3100 appear to be encouraging.\(^153\) AMD3100 is a small molecule antagonist of chemokine receptor CXCR4 and has been shown to mobilize BM cells in mice and humans.\(^154-156\) In vivo treatment with AMD3100 or local administration of cells mobilized by AMD3100 accelerates blood flow to ischemic limbs in 12 week STZ-diabetic mice.\(^157\) However, these studies need to be carried out in mice with different durations of diabetes, because it has been hypothesized that BM microenvironment may initially provide protection to resident EPCs from adverse diabetic milieu of the circulation and becomes dysfunctional only with longer durations of diabetes (Grant, MB, unpublished). Another CXCR4 antagonist, SDF-1BP2G, has better angiogenic function compared to AMD3100 but has not yet been studied in diabetic models.\(^157\)

Manipulation of Diabetic Host Environment

A successful outcome of the cell-based therapy also depends on the host environment. An optimal “fertile” environment is essential for the adhesion and transmigration of cells that require interaction of cell surface molecules with extracellular matrix proteins. The oxidative diabetic milieu modifies the host environment, and the manipulation of which is required to enhance the reparative function of cells. Treatment with antioxidants or anti-AGE agents is an option. Treatment with insulin, glitazones, or statins may directly or indirectly make the host environment favorable for the therapeutic behavior of the cells.

Future of Cell-Based Therapies

Attempting translational studies before understanding the full identity and characteristics of EPC phenotype is likely fraught with difficulties. Which cell type is appropriate for a particular complication is still a difficult question to be answered. Although the study by Tendera et al\(^158\) found no difference in the efficacy of unselected BMCs and CD34+CXCR4+ cells, preclinical studies and ongoing clinical trials support the use of CD34+ cells compared to the total mononuclear leukocytes or unselected BMCs, although a higher dose of CD34+ cells was shown to be less efficacious than lower doses.\(^42,159,160\) As described above, autologous cell therapy in diabetics requires the ex vivo modification of EPCs for a better angiogenic outcome regardless of the cell type chosen. At the same time, PDR needs treatments inhibiting new retinal blood vessel formation, including blocking EPC recruitment and engraftment therein. Conversely, other sites and disease conditions may actually need an increase of EPC activity toward tissue repair, eg, in myocardial ischemia or wound healing. The previous sections have emphasized the role of NO in maintaining normal function of EPC/BMCs and ways to maintain balanced NO and ROS levels. All BMC populations may have a key role in the repair process, and it is likely that combinations of progenitor cells will be needed, as will the simultaneous optimization of the diabetic environment into which these cells will be placed. Such a complex approach will likely be needed to optimally treat diabetic patients with vascular complications.

Acknowledgments

We thank Dr Lynn C. Shaw for the preparation of schematics for this manuscript.

Sources of Funding

This work was supported by NIH grants EY007739 and EY012601 (to M.B.G.).

Disclosures

None.

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The Promise of Cell-Based Therapies for Diabetic Complications: Challenges and Solutions
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Circ Res. 2010;106:854-869
doi: 10.1161/CIRCRESAHA.109.213140

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/106/5/854

An erratum has been published regarding this article. Please see the attached page for:
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In the article that appears on page 854 of the March 19, 2010, issue, the legend for Figure 2 should not contain a permission to reprint material from The American Diabetes Association. The legend for Figure 4 should contain a permission to reprint material from The American Diabetes Association. The legend for Figure 4 should read as follows:

Human CD34⁺ cells of nondiabetic, but not diabetic, origin integrate into degenerate vasculature in mouse eyes damaged by ischemia/reperfusion injury (Caballero et al²⁵). Two days after intravitreal or systemic administration, integration of diabetic cells into existing vasculature was not observed (A), whereas cells of nondiabetic origin show extensive integration into small and medium sized vessels (yellow in the composite images) (B). Insets show separate red and green channels used to make the composite images. C, CD34⁺ cells home to an area of injury and traverse toward the ischemic region of the capillary (arrows). Copyright 2007 American Diabetes Association. From Diabetes 2007;56:960–967. Reprinted with permission from The American Diabetes Association.

Reference 49 is incorrect and should appear as follows:


The authors and publisher regret these errors. These errors have been noted in the online version of the article, which is available at http://circres.ahajournals.org/cgi/content/full/106/5/854.

Reference


DOI: 10.1161/RES.0b013e3181e1963e