REVIEW

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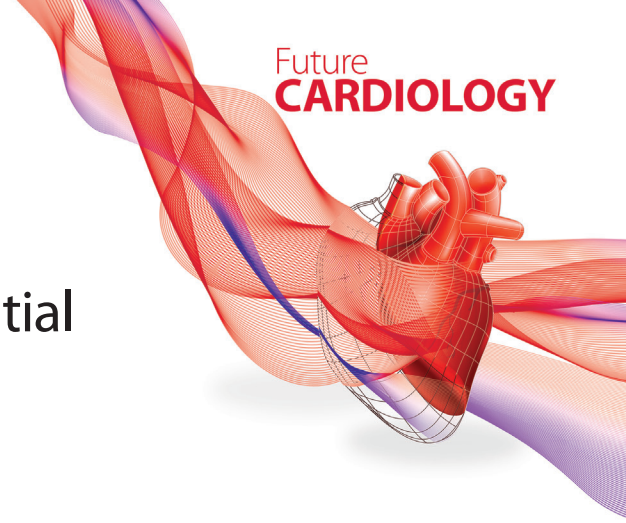

Vascular injury and repair: a potential target for cell therapies

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ABSTRACT Whether due to atherosclerotic disease or mechanical intervention, vascular injury is a frequently encountered pathology in cardiovascular medicine. The past decade has seen growing interest in the role of circulating endothelial progenitor cells in vessel recovery postinjury. Despite this, the definition, origin and potential role of endothelial progenitor cells in vascular regeneration remains highly controversial. While animal work has shown early promise, evidence of a therapeutic role for endothelial progenitor cells in humans remains elusive. To date, clinical trials involving direct cell administration, growth factor therapy and endothelial cell capture stents have largely been disappointing, although this may in part reflect limitations in study design. This article will outline the pathophysiological mechanisms of vascular injury with an emphasis on endothelial progenitor cell biology and the potential therapeutic role of this exciting new field.

KEYWORDS
• atherosclerosis • cell therapy • endothelial progenitor cells • percutaneous coronary intervention • vascular injury

Vascular injury is the central mechanism in the initiation, progression and clinical consequences of atherosclerosis. Damage to blood vessels may arise either directly as a result of the disease process itself or following mechanical disruption induced by interventional procedures, such as surgery or angioplasty.

This results in a spectrum of healing responses that can lead to plaque growth, restenosis and, ultimately, vessel occlusion. Modification of vascular injury and repair processes is a key area in the development of novel therapies for atherosclerosis and the optimization of existing surgical and endovascular interventions. This article will discuss the different causes of vascular injury in atherosclerosis and mechanical intervention, as well as the processes of repair, with a particular focus on the role and therapeutic potential of endothelial progenitor cells.

Atherosclerosis

Vascular injury in atherosclerosis is characterized by lipid and inflammatory cell infiltration into the vessel wall. Plaque progression and clinical outcome are determined by a complex interplay between both systemic (e.g., blood pressure, serum cholesterol and smoking) and local (e.g., cellular and rheological) factors. While atherosclerosis is predominantly a disease of the intima, there are also important contributions from the vascular media and adventitia.

• Intima & endothelium

The endothelium has many roles in the maintenance of vascular homeostasis. Alterations in endothelial function occur early in the atherosclerotic process, often preceding clinically detectable disease,

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and have repeatedly been shown to predict the burden of vascular disease [1–3].

Disruption of cell–cell connections and increased vascular leak are observed in the normal aging process associated with arterial stiffening [4]. This process is accelerated in the presence of vascular injury and leads to further alterations in vascular structure and function. For example, the permeability of the endothelium to circulating cells and biologically active macromolecules increases early in the pathophysiology of atherosclerosis, with cholesterol ingress playing a major role. In apoE-deficient mice, increased endothelial permeability correlates with disturbed gap junction structure and cholesterol infiltration on electron microscopy [2].

As with cholesterol, inflammatory cell infiltration of the vessel wall is believed to play a key role in atherosclerosis. Circulating monocytes migrate into the plaque, where transformation into tissue macrophages is associated with uptake of oxidized LDL particles and, ultimately, transformation into 'foam cells'. Foam cells have a powerful chemotactic effect on vascular smooth muscle cells. While subsets of monocytes have been described [4,5], the importance of these distinct populations in arterial disease has only recently been explored.

Monocytes can be subdivided into distinct populations described as 'classical' (~90%; $CD14^{++}CD16^{-}$) and 'nonclassical' ($CD14^{Low}CD16^{++}$). While the relative contributions of these two cell populations to plaque biology remain to be defined, classical monocytes are thought to promote local inflammation through phagocytosis and cytokine production. By contrast, nonclassical monocytes may have an anti-inflammatory role through collagen deposition and plaque stabilization [6].

It is generally believed that classical and nonclassical monocytes transform into M1 and M2 macrophages, respectively, although the local inflammatory milieu may play a role in determining cell fate. In keeping with the function of classical and nonclassical monocytes, M1 and M2 macrophages are thought to be pro- and anti-inflammatory, respectively, with M1 macrophages promoting erosion of the fibrous cap and plaque instability [7].

As well as distinct populations of macrophages and monocytes, the equilibrium between subpopulations of T lymphocytes may also have an important bearing on plaque progression. Th1 lymphocytes are atherogenic and secrete a proinflammatory cytokine profile (IFN-γ, TNF-α, MIP-1 and IL-12), promoting macrophage accumulation and activation, vascular smooth muscle cell apoptosis, degradation of the collagen matrix and plaque instability. By contrast, the opposing Th2 population secretes an anti-inflammatory cytokine profile (IL-4, IL-6, IL-10 and IL-13), attenuating the Th1 response and promoting plaque stability [8].

One major consequence of atherosclerotic change in the arterial wall is a predisposition to thrombosis. In health, the endothelium secretes various antithrombotic substances, such as nitric oxide, prostacyclin and thrombomodulin, as well as the procoagulant von Willebrand factor and plasminogen activator inhibitor. Imbalances in these pro- and anti-coagulant factors accompany vascular injury and endothelial dysfunction, favoring a prothrombotic phenotype [9,10].

• Media

While structural changes in the media are not prominent in atherosclerosis, functional changes in vascular smooth muscle cells and their migration into the intima are. Early in atherosclerosis, vascular smooth muscle cells migrate into plaque and, depending upon the phenotype that they assume, exert both positive and negative effects on remodeling. Initially, vascular smooth muscle cells were thought to divide themselves between fibroproliferative cells, secreting collagen and other extracellular matrix proteins, and contractile cells, which are neither proliferative nor secretory.

This is now believed to be oversimplistic, and broad phenotypic differences within the fibroproliferative population are likely to result in both pro- and anti-inflammatory influences [11]. The phenotypic profile of the vascular smooth muscle population is determined by autocrine, paracrine and mechanical factors and is comprehensively reviewed elsewhere [12]. As the largest producer of VEGF in the atherosclerotic plaque [13], vascular smooth muscle cells promote vessel ingrowth from the adventitia, providing a conduit for inflammatory cell ingress and a mechanism for plaque hemorrhage, processes that are central to the progression of the atherosclerotic lesion.

• Adventitia

The adventitia was previously thought to be an inert matrix of connective tissue supporting the vasculature with little role in the pathophysiology of atherosclerosis; however, there has been a recent change in this paradigm. In contrast to

the traditional 'inside-out' model based around intimal inflammation, an 'outside-in' model of atherogenesis has been proposed, with adventitial fibroblast activation and differentiation into myofibroblasts occurring as an early event.

These latter cells migrate into the media and generate reactive oxygen species, which, when present in excessive concentrations, are associated with cellular damage. Increased adventitial fibroblast activation and perivascular lymphocyte infiltration occurs in both hypertension and atherosclerosis and precedes alterations in endothelial function [14]. While it is unlikely that the adventitia alone initiates atherogenesis, it is not the inert connective tissue layer that it was once thought to be.

• Plaque rupture

Rupture of atherosclerotic plaques with associated thrombus formation is the main cause of myocardial infarction and sudden death in patients with coronary artery disease. Most plaque rupture events result in nonocclusive thrombus and are asymptomatic; however, thrombosis and intraplaque hemorrhages are important mechanisms by which lesions increase in volume and stenosis progresses.

Rupture occurs in plaques with a large lipid-rich necrotic core and thin fibrous cap, usually at areas of foam cell aggregation. Exposure of the lipid-rich, highly thrombogenic core to the vessel lumen causes thrombosis on the surface of the plaque. Endothelial injury and denudation is the norm under the thrombus, but it is unclear whether this precedes plaque rupture or is a consequence of the thrombotic event. Following plaque rupture and thrombosis, the cascade of cellular events is largely similar to that seen in mechanical intervention **(Figure 1)**, with platelet and neutrophil influx into the vessel wall as an early event.

This is followed by a more chronic inflammatory response involving macrophage, lymphocyte and vascular smooth muscle cell infiltration. Organization and incorporation of the thrombus into the vessel wall with reconstitution of the fibrous cap and endothelial cell layer leads to plaque growth and, potentially, a reduction in lumen calibre.

• Defective vascular repair mechanisms are responsible for disease progression in atherosclerosis

Inflammation promotes plaque instability and progression, but also attenuates the normal mechanisms of repair. Re-endothelialization, crucial to vessel healing and repair, is inhibited by a proinflammatory cytokine profile and promoted by an anti-inflammatory profile [15].

Efforts to modify this inflammatory process showed early promise, with both glucocorticoid and anti-TNF-α therapy [16] inhibiting atherosclerosis in preclinical models. To date, these benefits have failed to translate into the clinical arena, with a lack of cardiovascular efficacy in trials using these drugs with licensed indications for rheumatological or dermatological conditions [17]. This may reflect failure to identify the correct biological target or, more likely, the complex interplay between inflammation and cellular repair processes, such that targeting a single immunological mediator is unlikely to influence the pathogenesis of atherosclerosis.

Mechanical trauma

With the ever-expanding use of endovascular intervention, iatrogenic vascular injury has become an increasingly common problem. The process of vascular injury and repair in this setting shares a number of similarities with, as well as having important differences from, that seen in atherosclerosis. With reported rates of restenosis and stent thrombosis following coronary intervention as high as 11 and 2%, respectively [18], understanding and modifying these adverse responses to vascular injury is crucial if we are to realize the full potential of catheter-based therapies.

• Intima

High-pressure inflation of angioplasty balloons denudes the vessel wall, causing crush injury with cell loss and exposure of the subendothelial matrix **(Figure 1)**. This results in propagation of the coagulation cascade and platelet aggregation, with a thin layer of thrombus forming over the injured segment even in the presence of heparin and dual antiplatelet therapy. At 6–12 weeks following stent implantation, the thrombus begins to resolve and endothelial cells begin to cover the stented section, with full endothelial coverage of the stent taking place by 3 months.

The early thrombus presents an inflammatory milieu with a cellular population made-up largely of activated platelets and neutrophils, later giving way to monocytes and vascular smooth muscle cells [19,20]. The briskness of

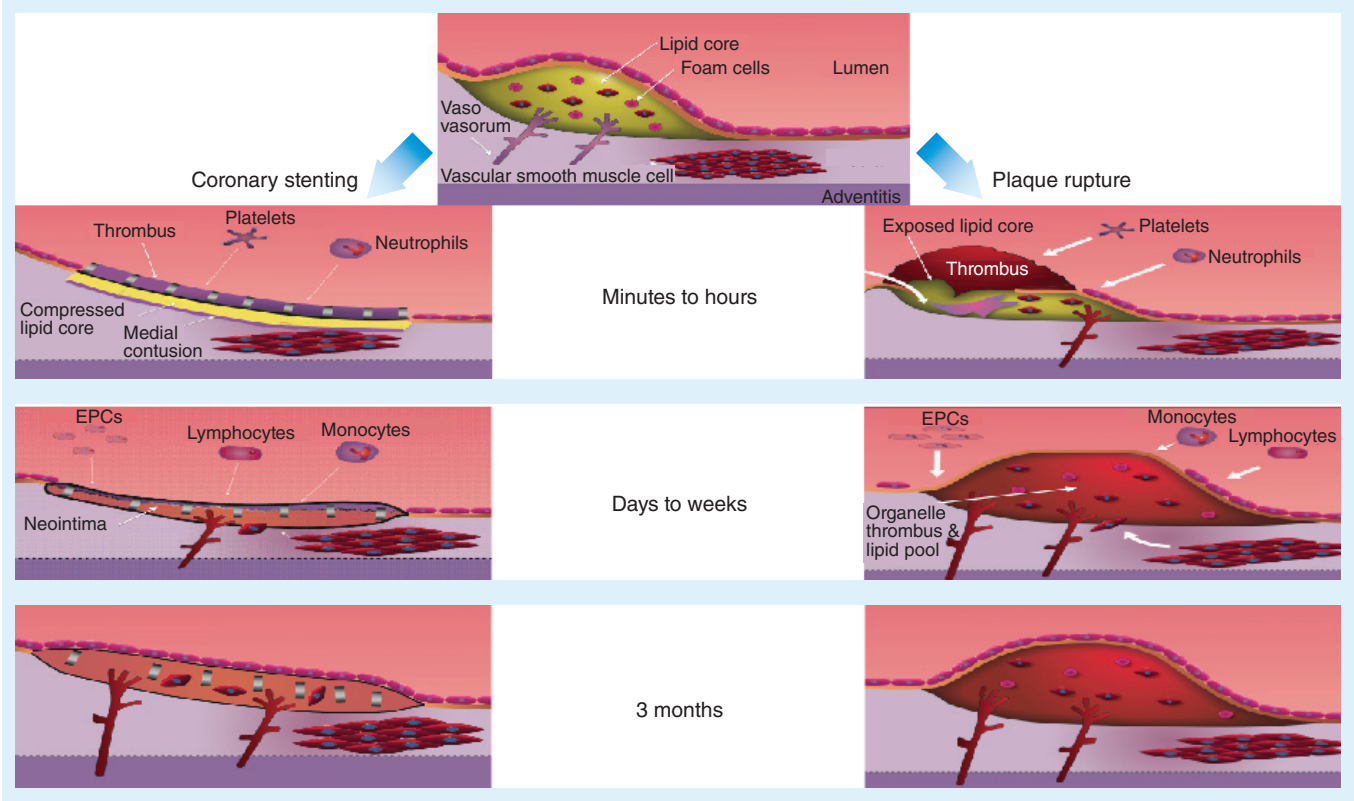

Figure 1. Sequential changes in an atherosclerotic artery following plaque rupture and coronary stenting. At baseline, the atherosclerotic plaque is characterized by a lipid-rich, thrombogenic core containing vascular smooth muscle cells and foam cells, which is separated from the blood by a fibrous cap and endothelial cells. At minutes to hours, in the ruptured plaque, thrombus forms due to exposure of the lipid core. The endothelium is denuded and there is an acute inflammatory response with an influx of platelets and neutrophils into the vessel wall. The process is broadly similar in coronary stenting, with thrombus formation adjacent to exposed stent struts and a similar influx of platelets and neutrophils. However, in contrast to plaque rupture, there is compression of the lipid pool and an increase in lumen diameter. Medial contusion is often seen in stented arteries. At days to weeks, the profile of inflammatory cells becomes more chronic, with lymphocyte and monocyte influx into the vessel wall as well as smooth muscle cell migration into the intima, with subsequent deposition of extracellular matrix constituting the neointima. Following both stent implantation and plaque rupture, overlying thrombus becomes organized into the vessel wall. Endothelial progenitor cells reconstitute the endothelial layer, restoring vasomotor and thrombomodulatory function, as well as suppressing the inflammatory response. At 3 months, the endothelial monolayer has been reconstituted. In the stented artery, lumen size is increased. By contrast, following plaque rupture, negative remodeling may result in a reduction in lumen size and subsequent restriction of blood flow.
EPC: Endothelial progenitor cell.

this inflammatory response most likely plays role in determining vascular smooth muscle cell and monocyte behavior and, as such, the course of vessel healing and the risk of restenosis. Moreover, the magnitude of this response is proportional to the degree of vessel trauma.

While a positive correlation between various periprocedural inflammatory markers and subsequent restenosis has been demonstrated, this has not been a universal finding (Table 1). Cell therapy designed to facilitate the early restoration of a functional endothelium has the potential to improve vasomotor and thrombomodulatory function, critical to the prevention of stent thrombosis, as well as attenuating the local inflammatory response-a driver for restenosis.

Following the initial acute inflammatory phase of vascular injury, a more chronic cellular profile supervenes, with monocytes, lymphocytes and vascular smooth muscle cells as the main protagonists. It is during this period that neointima formation occurs. Made up largely of vascular smooth muscle, proteoglycan and collagen matrix, the neointima reaches a maximum volume over 3–6 months following mechanical injury and is at the heart of the restenotic process.

Central to the deposition of extracellular matrix and neointimal formation is vascular smooth muscle cell entry into the cell cycle, migration into the intima and adoption of a synthetic phenotype. These synthetic vascular smooth muscle cells are rich in endoplasmic reticulum and are highly proliferative and migratory, in contrast with the population within the healthy vessel wall, which is rich in contractile elements and has low proliferative and migratory capabilities.

• Media

Medial injury is frequent following stenting, with one autopsy study finding medial fracture to be present in half of the arteries that were studied [42]. In both porcine [43] and human [42,44] studies, the extent of vascular injury during percutaneous coronary intervention correlates directly with neointima formation and restenosis. The mechanism behind this is unclear, but more extensive injury increases inflammatory cell infiltrate [42], likely increasing vascular smooth muscle cell activation and extracellular matrix deposition.

In summary, balloon angioplasty causes endothelial denudation and an acute inflammatory reaction in the vessel wall, which in turn determines the likelihood of restenosis within the vessel. Acceleration of re-endothelialization and modification of the inflammatory response may hold the key to preventing restenosis and improving outcomes post-percutaneous coronary intervention.

• Vascular grafts

Mechanical injury and its consequences are not limited to percutaneous intervention. While arterial conduits have excellent longevity, with 90% patency at 10 years, approximately 50% of saphenous vein are occluded by the same time point [45].

Exposure of the venous conduit to the higher-pressure arterial circulation causes an increase in radial tissue and surface shear stress (the tangential frictional force of the blood flowing

Table 1. Inflammatory cells and mediators involved in restenosis.

Peripheral blood marker	Correlation to restenosis	Ref.
Neutrophils	More avid infiltration following stenting than balloon angioplasty alone and correlates with degree of neointima formation as well as late-lumen loss	[19,20]
Eosinophils	Seen in restenotic coronary arteries adjacent to stent struts and lipid pools, but a prominent role in restenosis is not established	[20]
Platelets	Mean platelet volume (larger volume denotes increased platelet activation) preangioplasty predicts restenosis	[21]
IL-1	Production by peripheral blood monocytes predicts restenosis rates and inhibition of IL-1 action in porcine stenting reduces neointima formation	[22,23]
IL-6	Proinflammatory, prominent in the early post-stenting period. Positive correlation between IL-6 level and restenosis in some studies, but not others	[24–27]
High-sensitivity C-reactive protein	Periprocedure levels correlate positively with restenosis	[26,28–30]
TNF-α	Concentration correlates positively with rate of restenosis	[26,31–32]
PDGF	Promotes vascular smooth muscle cell chemotaxis and proliferation and PDGFR is overexpressed in restenotic lesions	[33–36]
TGF-β	Potent monocyte and neutrophil chemotractant that promotes matrix deposition. Overexpressed in restenotic lesions	[37]
Plasminogen activator inhibitor	Patients with restenotic disease have high levels of plasminogen activator inhibitor immediately postangioplasty and out to 3 months	[38]
MIP-1	Promotes monocyte and vascular smooth muscle cell chemotaxis and proliferation, is increased postballoon arterial injury in animals and is positively correlated with restenosis rates	[39,40]
Platelet-activating factor	Promotes neutrophil adhesion to endothelium and fibrin and experimental inhibition reduces neointimal hyperplasia, but no evidence of correlation between its levels and restenosis	[41]

over the endothelium). This leads to endothelial damage followed by arterialization of the vessels, with vascular smooth muscle cell migration into the vessel wall alongside an acute and chronic inflammatory cell influx. The end result is extracellular matrix deposition and neointima formation, sharing many similarities with the changes seen following balloon injury [46].

An exaggerated response to injury, early thrombosis (due to operative endothelial injury) and accelerated atherosclerosis account for the three major pathologies leading to graft failure. A recent meta-analysis suggested that atraumatic handling of grafts at the time of surgery improves long-term patency rates [47], emphasizing the importance of the initial injury and inflammatory response for determining outcomes in vascular intervention. Pharmacological strategies to modify vein graft longevity have been largely disappointing, with aspirin and statins being the only agents proven to inhibit graft failure [48,49]. Targeting graft failure with novel cell-based therapies has the potential to improve graft patency and clinical outcomes.

Endothelial progenitor cells

Following vascular injury, reconstitution of a functional endothelium is a critical step in the recovery of the vessel, allowing the return of vasomotor and thrombomodulatory potential, as well as modifying the inflammatory process within the vascular wall. The mechanisms by which this happens, the specific cells involved and their roles in the reparative process remain controversial.

Early theories proposed that re-endothelialization occurred from mature endothelium in areas bordering endothelial loss. However, by as early as the 1960s, animal studies of vascular grafting [50,51] challenged the traditional paradigm of reconstitution by ingrowth by demonstrating islands of endothelial cells that were remote from the site of anastomoses. While this early work suggested a circulating endothelial progenitor cell in the peripheral blood, it is only following Asahara's work in 1997 [52] that this hypothesis has really developed.

Over a decade of research has explored the role of progenitor cells in vascular repair and the paradigm has been refined in many respects. One of the most important shifts has been the distinction drawn between the early and late outgrowth endothelial cells. Early-outgrowth cells, also known as endothelial cell colony-forming units (EC-CFUs), arise from the mononuclear portion of peripheral blood following 5–7 days of culture on fibronectin. They are largely made up of monocytes and lymphocytes [53] and highly express the pan-leukocyte cell marker CD45; however, following culture in angiogenic conditions, they express mature endothelial surface markers, as well as demonstrating the ability to uptake acetylated LDL. Hill and colleagues reported an inverse relationship between EC-CFU concentrations and Framingham risk score [53].

These observations, alongside the fact that EC-CFU concentrations are raised following acute vascular injury [53–55], suggested a role for these early-outgrowth cells in endothelial regeneration. However, it is now clear that these cells have low proliferative potential and are incapable of forming mature endothelial cells. Early-outgrowth cells, therefore, likely consist of proangiogenic monocytes and lymphocytes supporting vascular repair indirectly through phagocytic and secretory actions, with a separate population directly responsible for endothelial reconstitution following injury, termed 'late-outgrowth endothelial progenitor cells'. Late-outgrowth endothelial progenitor cells, also known as endothelial cell-forming cells (ECFCs), are capable of forming mature endothelium and are more likely to be responsible for endothelial reconstitution following injury. ECFCs differ from early-outgrowth cells in that they have higher proliferative potential and express markers of mature endothelium, such as CD31 and KDR (the extracellular domain of VEGFR), rather than hematopoietic markers, and form cobblestone-like sheets similar to mature endothelium in culture.

• Definition

The phenotypic definition of the progenitor cell (from which late-outgrowth cells arise) remains controversial. Attempts to define this population depend upon selecting surface markers of cellular naivety and an endothelial phenotype prior to culture under angiogenic conditions, with the appearance of late-outgrowth cells considered to be evidence that the progenitor cell is contained within the selected cell populations.

Endothelial progenitor cells were traditionally defined by Asahara as expressing CD34, a marker of hematopoietic stem cells, and KDR,

Table 2. Cell surface markers proposed for the definition of endothelial progenitor cells.

Surface marker	Description	Expression	Proposed role of positive cell population in endothelialization
CD133	Transmembrane glycoprotein, function uncertain	Immature hematopoietic cells	Supportive role in vascular regeneration, but unlikely to give rise to mature endothelium
CD14	Cell surface marker for bacterial lipopolysaccharide	Monocytes	Supportive role in vascular regeneration, but unlikely to give rise to mature endothelium
C-Kit (CD117)	Cell surface receptor for stem cell factor	Hematopoietic and multipotent stem cells	Not expressed on mature endothelial cells, but progenitor cells may arise from this population
CD146	Transmembrane glycoprotein, function uncertain	T lymphocytes, mesenchymal stem cells, endothelial cells and smooth muscle	May contain endothelial progenitor cells, giving rise to mature endothelial cells
CD45	Transmembrane receptor regulating various aspects of the cell cycle and differentiation	Hematopoietic cells	Marker of cells with a supportive role in vascular regeneration, but unlikely to give rise to mature endothelium
Tie-2	Cell surface marker for angiopoietin	Multipotent stem cells, leukocytes, monocytes and endothelial cells	Tie-2-positive monocytes likely support angiogenesis; contribution of the Tie-2-positive population to the formation of mature endothelium unclear
CD34	Adhesion molecule important for cellular migration, other functions uncertain	Multipotent stem cells	Likely to be expressed on progenitor cells, giving rise to mature endothelial cells, but heterogeneous population
CD31	Adhesion molecule thought to be important for transendothelial migration of inflammatory cells	Platelets, leukocytes and mature endothelial cells	Marker of mature endothelial cells
Kinase domain receptor	Surface receptor for VEGF	Mature endothelium	Expressed on mature endothelium and late-outgrowth endothelial cell populations; endothelial progenitor cells may reside within this population

found on mature endothelial cells [52]. However, this combination of markers still identifies a heterogeneous population containing cells that give rise to mature endothelium: those with a supportive role in vascular regeneration and those unrelated to the process. Additional markers have been used in an attempt to further define this population **(Table 2)**.

CD133 was proposed as an additional marker of cellular naivety with cells that were copositive for CD34, KDR and CD133 considered to be endothelial progenitor cells [56,57]. However, recent work has shown that endothelial cells cannot be raised from a $CD133^+$ population [1,58] and that CD133 depletion increases the efficiency with which endothelial progenitor cell populations are raised [1]. CD133 likely represents a population of primitive hematopoietic progenitor cells that do not contribute directly to endothelial repair. Practically speaking, CD34/KDR/CD133 triple-positive cells are very rare in the circulation [56–58] and thus not attractive candidates for cellular therapy.

Expression of the pan-leukocyte marker CD45 denotes a hematopoietic population that is highly expressed in early-outgrowth populations, but incapable of raising late-outgrowth endothelial cells [59]. CD34 is a pan-stem cell marker that is not specific for endothelial progenitors, but late-outgrowth cells likely arise

form the $CD34^+$ portion of the mononuclear fraction and CD34 enrichment enhances ECFC yield in culture [1].

Monocytes expressing Tie-2 (a cell-surface receptor for angiopoietin) support angiogenesis in preclinical models and their concentrations are increased in chronic ischemic conditions, but their role in the formation of mature endothelium is unclear. KDR is highly expressed on mature endothelial cells, and late-outgrowth endothelial cell populations can be raised from the KDR^+ population. CD146 is widely expressed on immature cells, but is also seen on mature endothelial cells, and it has been suggested that endothelial progenitor cells may express CD146, with the $CD146^-$ fraction of mononuclear cells being incapable of forming endothelial outgrowth cells [1].

• Origin

Alongside the controversy regarding the phenotype of endothelial progenitor cells, there is uncertainty regarding their anatomical origin. The bone marrow was initially thought to be the source of endothelial progenitor cells, with studies of non-sex-linked bone marrow recipients showing donor cells incorporated into recipient vessels [60]. However, recent evidence suggests that while the bone marrow may supply cells that support angiogenesis (and hence may appear in vessel walls), it does not contain a population from which mature endothelial cells arise [1,61–62].

This led to suggestions of a population of cells resident within the vasculature from which endothelial progenitor cells arise, and a distinct 'vasculogenic zone' has been proposed to exist between the smooth muscle of the media and the adventitia. Consistent with this, progenitor cells have been demonstrated in the walls of both embryonic [63] and mature blood vessels [64,65].

It is our belief that endothelial progenitor cells arise from the $CD34^+CD45^-$ portion of peripheral blood mononuclear cells and that these cells are derived from the vasculature and not bone marrow. This is based on observations that the late-outgrowth endothelial progenitor cell yield from mononuclear culture is dramatically increased by CD45 depletion and abolished by positive selection [59] and that neither G-CSF-mobilized peripheral blood nor bone marrow aspirate are capable of raising late-outgrowth endothelial cell colonies [1].

• Pericytes & vascular smooth muscle cells

In order to maintain health, the endothelium requires the support of pericytes and vascular smooth muscle cells. These cells surround the endothelial layer and provide mechanical support in terms of extracellular matrix deposition and radial contractile strength, prevent vessel leakage and secrete various paracrine mediators, such as VEGF. Vascular smooth muscle cells support larger vessels, while pericytes support arterioles, capillaries and venules.

Although isolated endothelial cells can form tube-like structures *in vitro*, these structures require the support of the perivasculature in order to maintain health and regress without it [66]. Therefore, while reconstitution of the endothelial layer of an injured artery (i.e., one in which the perivascular support structure is already in place) may be possible using endothelial cells alone, tissue engineering with the aim of new vessel formation will need to take into account the important role of pericytes and vascular smooth muscle cells.

Modifying the repair process

As our understanding of the processes of vascular injury and repair has increased, a number of therapeutic strategies to modify this process have been proposed. These include the modification of progenitor cell populations with pharmacological agents and growth factors, direct stem cell administration and stent-based therapies.

Alongside attempts to directly harness endothelial progenitor cells, various cardioprotective therapies in routine clinical use have been shown to affect endothelial progenitor cell biology. The definition of progenitor cells in these studies is variable, with some looking at early-outgrowth cells, some defining endothelial progenitors as those exhibiting LDL uptake and lectin binding and others defining them by cell surface markers ($CD34^+KDR^+$ and $CD34^+CD133^+KDR^+$).

• Pharmacological agents

Angiotensin has an inhibitory effect on endothelial progenitor cell function [67]. Angiotensin-converting enzyme inhibitors and angiotensin receptor antagonists reduce restenosis and target vessel revascularization in clinical studies [68,69], as well as increasing endothelial progenitor cell numbers in various animal studies [70,71]. Similarly, mineralocorticoid receptor antagonism has been shown to increase progenitor cell

numbers in experimental [72] and clinical [73] studies.

Statins, alongside antiplatelet agents, are the most widely used drugs in vascular disease. Among their pleotropic effects, statin use is associated with the mobilization of endothelial progenitor cells and a reduction in senescence [74–80]. Other agents have been shown to influence progenitor cell function, but their role is less well established. Calcium channel blockers have been shown to increase endothelial progenitor cell numbers in step with improvements in noninvasive measurements of endothelial function, both dependent [81] and independent [82] of effects on blood pressure. β-blockers increase endothelial progenitor cell numbers and function in animal models of hypertension [83]. PPAR-γ agonists increase endothelial progenitor cell numbers and migratory activity [84].

• Growth factors

Exogenous G-CSF increases the circulating concentrations of putative endothelial progenitor cell populations $CD34^+CD133^+$ [85], $CD133^+KDR^+$ [85], $c\text{-}Kit^+KDR^+$ and $CD34^+CD133^+KDR^+$ [86], as well as increasing the numbers of early-outgrowth colonies [85,86]. These observations would perhaps suggest a role for G-CSF in promoting vascular repair, and indeed, there is evidence in mice of accelerated re-endothelialization with this treatment [87,88].

However, there has been concern regarding the increased neointima formation and restenosis with G-CSF (an effect seen by Yoshioka *et al.* in mice treated with bare metal but not drug-eluting stents [88]). This effect was seen in the MAGIC trial, which randomized patients undergoing bare metal stenting to intracoronary CD34 cell infusion following mobilization with G-CSF alone or standard care [89]. The trial was stopped early due to an unexpectedly high rate of restenosis in the active treatment limbs.

However, a recent meta-analysis of postinfarct patients treated with G-CSF has shown a neutral effect on restenosis regardless of whether bare metal or drug-eluting stents were used [90]. With both negative [91,92] and positive [93,94] findings reported, the role for G-CSF in coronary artery disease is uncertain, but early safety concerns have not been borne out in later trials.

• Cell therapies

Treatment with endothelial progenitor cells has been demonstrated to reduce neointimal hyperplasia, accelerate re-endothelialization and improve vasomotor function in experimental models of arterial injury [95–97]. These studies largely used unselected monocyte populations with or without modification by culture in an endothelial growth medium for several days. As such, the populations used were heterogeneous, with the inherent problems of this approach.

To date, cell therapy trials have focused on myocardial regeneration following acute myocardial infarction. Although increased left ventricular ejection fraction is often the primary outcome in these trials, many have also investigated the effects of cell therapy on myocardial perfusion, and some have looked more directly at revascularization with stem cell therapy **(Table 3)**. While myocardial regeneration and angiogenesis are distinct from vascular repair, these studies hint at the regenerative potential of progenitor cells. Encouraging though these early trials have been, they have suffered from small numbers and often lacked appropriate controls.

Interestingly, a recent meta-analysis suggested that the effect of cell therapy on left ventricular function was small and the effect size appears larger due to the inclusion of studies with flawed experimental designs [106]. It is hoped that upcoming Phase III clinical trials, such as RENEW [107], will address some of these shortcomings.

Another problem of these studies is that they utilize a nonspecific population of mononuclear cells derived either from direct bone marrow extraction or from peripheral blood following G-CSF mobilization, with $CD34^+$ selection being the only refinement, if any. The result is that it is difficult to differentiate between the direct beneficial effects of re-endothelialization by progenitor cells and the paracrine action of the angiogenic monocytes and lymphocytes present in peripheral blood.

Endothelial progenitor cells may be contributing to this process, but may be excluded by CD34 selection and are likely to be underrepresented in both bone marrow- and G-CSF-mobilized blood. As such, the therapeutic potential of a selected endothelial progenitor cell-based therapy may have been underestimated.

• Tissue engineering & vascular conduits

Endothelial cell -based products may also have potential therapeutic roles in the tissue

Table 3. Trials of cellular therapies in cardiovascular disease.

Author (year)	Cell therapy product	Delivery	Control	Population (n)	Outcome	Ref.
Lasala *et al.* (2010)	BM-derived cells mononuclear cell populations plus 'progenitor population' ($CD34^+CD133^+CD144^+$)	Intramuscular	No	Chronic lower limb ischemia (11)	↑ Limb perfusion (digital subtraction angiography); ↑ quality of life score; ↑ ankle–brachial pressure index	[98]
Lasala *et al.* (2011)	Marrow-derived MSC population plus MNC fraction from BM aspirate	Intracoronary	No	Stable CCS III/IV angina (10)	↑ perfusion (single-photon emission tomography); ↓ frequency of angina	[99]
Murphy *et al.* (2011)	BM aspirate. Analysis of population by CD133, CD34, KDR positivity conducted but not reported	Intramuscular	No	Critical limb ischemia (29)	↑ amputation-free survival; ↓xperfusion (PET)	[100]
Erbs *et al.* (2005)	G-CSF-mobilized 'CPCs' with high CD34, CD133, KDR and CXCR4 positivity	Intracoronary	Yes	Coronary artery disease (26)	↑ coronary flow reserve; ↑ ejection fraction; → in-stent restenosis	[101]
Boyle *et al.* (2006)	G-CSF-mobilized $CD34^+$ cells	Intracoronary	No	Stable coronary artery disease (5)	↑ collateral flow on repeat angiography; ↓ angina frequency	[102]
Tuma *et al.* (2011)	BM-derived MNCs and $CD34^+$ fraction	Coronary sinus infusion	No	Refractory angina (14)	↓ angina frequency; ↑ myocardial perfusion (single-photon emission tomography); ↑ walk time	[103]
Losordo *et al.* (2011)	G-CSF-mobilized PB $CD34^+$ cells	Intramyocardial	Yes	Refractory angina (167)	↓ angina frequency; ↑ walk time; → perfusion	[104]
Losordo *et al.* (2012)	G-CSF-mobilized PB $CD34^+$ cells	Intramuscular	Yes	Critical limb ischemia (28)	↓ amputation; ↓ rest pain; ↑ walk time	[105]

↑: Increase; ↓: Decrease; →: Unchanged; BM: Bone marrow; CCS: Canadian Cardiovascular Society; CPC: Circulating progenitor cell; MNC: Mononuclear cell; MSC: Mesenchymal stem cell; PB: Peripheral blood.

engineering of vascular conduits for the treatment of ischemic vascular conditions and vascular trauma. At present, large-caliber vessels can generally be replaced or bypassed with conduits made from synthetic polymers. The absence of an intact functional endothelial layer at implantation increases the risk of conduit failure due to thrombotic occlusion, infection or rejection. In smaller vessels (<6 mm), synthetic conduits are prone to thrombosis, and as such, autologous vessels (either venous or arterial) are preferred.

Tissue engineering using decellularized scaffolds either seeded with cells of the vessel wall or designed to capture these *in situ* offers a potential solution to these problems. This approach has been shown to be both feasible and effective in the preclinical setting [108] and in an early clinical model. In a series of ten hemodialysis patients with limited vascular access options [109], grafts constructed from autologous fibroblasts and extracellular matrix were implanted as arteriovenous shunts for dialysis access. These grafts demonstrated patency rates equivalent to those seen in fistulae constructed solely from native vessels.

Although in its infancy in the clinical arena, tissue engineering, either in the form of preformed multilayered vessels coated with an endothelial cell product or resorbable scaffolds that promote *in situ* chemotaxis and seeding of endogenous endothelial cells, has the potential to revolutionize conduit choice and vascular grafting in man.

• Endothelial progenitor cell capture stents

As the migration of endothelial progenitor cells to the site of vascular injury is accepted as central to the process of vascular healing, antibody-coated 'capture' stents have been developed in an attempt to accelerate re-endothelialization and attenuate neointimal hyperplasia.

The first-generation Genous R-stent™ (Orbus Neich, Amersfoort, The Netherlands) is a bare metal stent coated with anti-CD34 antibodies that enhances the binding of cells

expressing CD34. Results of clinical trials using this device have been varied. There was early promise with a small-scale trial comparing the Genous R-stent with a bare metal control, finding reduced rates of restenosis [110]. The eHEALING registry of 4996 patients reported low rates of target lesion revascularization (4.4%) and late-stent thrombosis (0.3%) at the 1-year follow-up [111]. However, the TRIAS trial of 622 patients, which randomized patients in a 1.1 ratio to the Genous R-stent or a drug-eluting stent, was terminated early with rates of target vessel failure of 17.4 and 7.0% in the these groups, respectively [112].

Whilst CD34-coated stents accelerate re-endothelialization, the cell population recruited to the site of injury is heterogeneous and is likely to include vascular smooth muscle and hematopoeitic and proinflammatory cells, in addition to endothelial progenitor cells [113]. This may, in part, explain the excess of restenosis observed in clinical trials.

Attempts have been made to address this by using both different progenitor cell capture coatings and combining antibody coatings with antiproliferative agents. The use of more endothelial-specific coatings, such as vascular endothelial cadherin [113] and VEGF [114], have shown favorable results compared with CD34-coated stents in porcine models.

In addition to alternative antibodies, polymer microarray technology has identified novel biosynthetic polymers that promote endothelial cell attachment and may promote re-endothelialization while minimizing platelet adherence [115]. The REMEDEE study randomized 180 patients to either the COMBO™ stent (Orbus Neich; CD34 antibody and sirolimus coating) or an everolimus drug-eluting stent and showed no difference in restenosis at 12 months[116], with the REMEDEE registry planning to follow 1000 patients treated with the COMBO stent over 5 years.

Stent-based manipulation of endothelial progenitor cell biology is an appealing prospect, but the ideal substrate for endothelial progenitor cell capture remains to be defined, and the best results may be achieved through combination with an antiproliferative drug.

Conclusion

A greater understanding of the processes of vascular injury and repair has led to improved outcomes in cardiovascular disease over the last few decades, and manipulation of the cell populations involved will be central to continued success. While results to date have been mixed, they give cause for optimism as our understanding of the origin, phenotype and function of the endothelial progenitor cell improves. Endothelial progenitor cell biology is an exciting new field with the potential to maximize benefits from vascular intervention and optimize outcomes in chronic ischemic conditions.

Future perspective

When the manipulation of endothelial progenitor cell biology truly moves into the sphere of routine clinical practice, it is likely to be on a number of fronts. Pharmacological manipulation of progenitor cell populations to promote vessel repair, whether through the use of monoclonal antibodies or otherwise, holds promise. Trials of endothelial progenitor cell capture stents are already underway and likely to bear fruit, although optimal results will require combination with antiproliferative agents and the targeting of a more specific cell surface marker than CD34. Direct administration of progenitor cells remains expensive, with limited efficacy having been demonstrated. Expansion into the routine clinical arena will likely require the development of semiautomated culture systems and further definition of the optimum population to administer.

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EXECUTIVE SUMMARY

Atherosclerosis

- Atherosclerosis is a dynamic condition with inflammation at its core and involves all layers of the vessel wall.

Mechanical trauma

- The cellular profile and timeline in mechanical injury are distinct from those seen in atherosclerosis.
- The acute inflammatory response is critical to determining the fate of the vessel wall following injury and is a potential therapeutic target.

Vascular grafts

- Changes seen in vascular conduits following grafting are similar to those seen in stent-injured arteries and the degree of initial trauma and inflammation again appear to be important in determining the course of changes in the vessel wall.

Endothelial progenitor cells

- Endothelial progenitor cells are critical to both the reconstitution of the endothelial monolayer and attenuation of the postinjury inflammatory response.
- Endothelial colony-forming units may have some supportive phagocytic/ paracrine role in angiogenesis, but by themselves do not reconstitute damaged endothelium.
- The optimum definition of an endothelial progenitor cell in terms of cell surface markers remains controversial.
- Endothelial progenitor cells are not bone marrow derived and likely reside in a niche within the vasculature.

Modifying the repair process

- Pharmacotherapies with proven cardiovascular efficacies cause favorable changes in endothelial progenitor cell profiles.
- Anti-inflammatory agents show some promise in reducing restenosis following mechanical injury to blood vessels, but there is insufficient evidence to recommend their use at present.
- There is no role for G-CSF postangioplasty, but initial concerns regarding its safety appear to be unfounded.
- Endothelial progenitor cell capture stents have yet to prove themselves, but the combination of an antiproliferative agent alongside a specific cell surface target offers promise.
- Cell therapy for ischemic disease remains expensive, with limited demonstrable efficacy to date. Refining the manufacturing process and the populations used are likely to address both of these issues.

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