Implications of Early Structural-Functional Changes in the Endothelium for Vascular Disease

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Abstract—By location, between the blood and tissues and the multiple functions, the endothelial cells (ECs) play a major role in securing body homeostasis. The ECs sense all variations occurring in the plasma and interstitial fluid, and respond (function of intensity), initially by modulation of their constitutive functions, then by dysfunction, expressed by temporarily altered functions and a phenotypic shift, and ultimately by injury/death. In dyslipidemia/hyperglycemia, the initial response of EC is the modulation of 2 constitutive functions: permeability and biosynthesis. Increased transcytosis of plasma β-lipoproteins leads to their accumulation within the hyperplasic basal lamina, interaction with matrix proteins, and conversion to modified and reassembled lipoproteins (MRL). This generates a multipart inflammatory process and EC dysfunction characterized by expression of new cell adhesion molecules and MCP-1 that trigger T-lymphocytes and monocyte recruitment, diapedesis, and homing within the subendothelium where activated macrophages become foam cells. The latter, together with the subendothelial accrual of MRL, growth factors, cytokines, and chemokines, and accretion of smooth muscle cells of various sources lead to atheroma formation; in advanced disease, the EC overlaying atheroma take up lipids, become EC-derived foam cells, and the cytotoxic ambient ultimately conducts to EC apoptosis. Understanding the mechanisms of EC dysfunction is a prerequisite for EC-targeted therapy to reduce the incidence of cardiovascular diseases. (Arterioscler Thromb Vasc Biol. 2007;27:266-274.)

Key Words: atherosclerosis ■ cardiovascular disease ■ dysfunction ■ endothelial cell ■ hyperlipemia ■ oxidative stress

A thin squamous type of epithelium almost undetected by light microscopy and considered initially a nonessential cellophane-like sheet, the endothelial cells (ECs), have earned the respect of biologists and pathologists in a relatively short 50 years time.

Biologists discovered that the ECs are endowed with few copies of all cellular organelles including clathrin-coated pits and vesicles, but as characteristic feature they are physiologically equipped with an unusually high number of plasmalemmal vesicles, now termed caveolae, transendothelial channels made up of one or more vesicles fused simultaneously to both EC fronts, specific storage granules, the Weibel-Palade bodies, and differentiated microdomains on the plasmalemma. As social cells, they establish homotypic and heterotypic intercellular junctions that connect ECs one to another or to neighboring subjacent cells: pericytes in capillaries and postcapillary venules and smooth muscle cells (SMCs) in arterioles and large vessels.

Caveolae, a specialized caveolin-rich microdomain of the EC plasmalemma, are endowed with numerous receptors implicated in the body homeostasis (ie, receptors for low-density lipoprotein [LDL], high-density lipoprotein, albumin, transferrin, IL-1, p53) that execute numerous functions ascribed to EC such as transcytosis, endocytosis and message center (intracellular signal transduction). There is evidence that in epithelial cells the precursors of caveolae are the sphingolipid–cholesterol-rich specialized microdomains (rafts) of the plasma membrane that float in a glycerophospholipid-rich environment recruiting a specific set of membrane proteins and regulating signal transduction by cell surface molecules. There are reasons to believe that lipid rafts are present in the EC plasmalemma, as well. Recently, it was reported that in cultured endothelium, E-selectin is localized in membrane lipid rafts and that, on ligation, E-selectin clusters and redistributes in a caveolin-1–rich plasma membrane fraction.

The Weibel Palade bodies, considered initially as storage and secretory granules for von Willebrand factor (vWF), are now reported to deposit also P-selectin, IL-8, eotaxin-3, endothelin-1, and angiopoietin-2; the regulated exocytosis of these bioactive components play a role in inflammation, hemostasis, and vascular tone.

Furthermore, the ECs that constitute a mass of ~1 kg spread throughout the body (estimated total area of ~7000 m² ~6×10¹³ cells) possess an innate heterogeneity, expressed by differences in their structure and function according to the vessel they are lining or tissues in which they reside. The structural heterogeneity consists in the number of caveolae
(that is highest in capillaries) and their generated channels, the presence of fenestrae with diaphragms (in visceral capillaries) or lacking diaphragms (in kidney glomeruli and liver sinusoidal capillaries), and the complexity of interendothelial junctions that are more elaborate in arterial than in venular segments of the vasculature.\textsuperscript{8,9} In addition, variations exist in the frequency of Weibel Palade bodies' (lowest in microvessels) capacity to participate in neovascularization, which is characteristic for capillary and venular EC (expressing vascular endothelial growth factor receptors),\textsuperscript{10} the response to shear stress,\textsuperscript{11} and the distribution of surface enzymes and frequency of membrane receptors. The latter can be schematically classified in receptors for vasoactive molecules, endocytic receptors (including scavenger receptors), and transcytotic receptors. Their distribution vary according to the vascular bed involved, i.e., histamine and serotonin receptors are prevalent in venular endothelium, albumin receptors in capillaries, homing receptors and scavenger receptors in high ECs in postcapillary venules,\textsuperscript{12} and transcytotic receptors for plasma macromolecules in capillary endothelium.\textsuperscript{13} The structural heterogeneity of ECs confer the cells the ability to sense, monitor, command, and modulate their differentiated functions in relation to the tissues in which they reside.

In addition, the biologists revealed that the vascular endothelium is endowed with a complex machinery to actively sort and gate permeant molecules to the right destination: caveolae, coated pits, and vesicles are equipped to function as cargo-carriers that perform endocytosis or transcytosis. Experiments using simultaneously 2 different electron opaque ligands led to the postulate that caveolae, although morphologically identical, may represent 2 functionally distinct entities: endocytic caveolae and transcytotic caveolae.

By endocytosis (nonspecific or specific, receptor-mediated), carrier plasma macromolecules, such as transferrin, ceruloplasmin, LDL, and albumin, are directed to the lysosomal compartment, where after enzyme degradation provide amino acids, cholesterol, phospholipids, or fatty acids to be used for the cell metabolism. Transcytosis, a term and concept coined in 1979 by N. Simionescu\textsuperscript{14} has 2 basic characteristics: (1) it is performed either by a nonspecific process (fluid phase, absorptive) or by a specific, receptor-mediated mechanism; and (2) it is a basic process common to most epithelial cells.\textsuperscript{13} For a given molecule, the ratio between endocytosis and transcytosis may vary according to the pathophysiological state of the ECs; the insights into the fine-tuning and the alteration of the transport machinery in various pathologies remain to be established. The likely pathways used for transport of plasma molecules may be either transcellular (via caveolae and the ensuing channels) or paracellular (through endothelial junctions). Recent molecular biology data revealed that caveolae are endowed with the molecular machinery needed for their fusion/fission from the plasmalemma, and specific docking within the cells. As such, most data infer the cargo-vesicles and their generated channels as the main cellular instruments for transcytosis of macromolecules (albumin, LDL, insulin, transferring, and metalloproteases), whereas the paracellular pathway is generally used for transport of water and ions, except for the postcapillary venules, in which 30% of the junctions are open to a space of 6 nm, allowing transfer of other small molecules. Furthermore, the ECs have a large array of paracrine, endocrine, and autocrine functions, as depicted in Figure 1.

Pathologists discovered that, the heterogeneity of ECs concur to a blood vessel-specific pathology, i.e., atherosclerotic plaques develop in arterial lesion-prone areas, thrombosis in veins, and vascular leakage occurs in venules. Moreover, the ECs adapt continuously and amend their functions to respond to changes occurring in the plasma, interstitial fluid, or their surroundings. The response of ECs to the modified microenvironment is gradual and is dependent on the extent and intensity of the aggressive factors: the initial response of cells to insults is the modulation of constitutive functions; this is followed by EC dysfunction, and only ultimately by injury and apoptosis\textsuperscript{15} (Figure 1).

### Modulation of Endothelial Constitutive Functions Is the Initial Cell Response to Insults Such as Hypercholesterolemia and/or Hyperglycemia

In arterial lesion-prone areas, adjustments of the EC’s 2 constitutive functions, namely the selective permeability and biosynthetic capacity, are the first cell rebuttal to insults like hyperlipemia and/or hyperglycemia. On a large variety of in vivo and in situ experiments, using LDL or \( \beta \)-very-low-density lipoprotein (radiolabeled, fluorescent, or tagged to gold particles), it was demonstrated that in hyperlipemic animals, the plasma lipoproteins (Lp) concentration gradient generates a prominent increase in transcytosis of Lp.\textsuperscript{16–23} The latter, in conjunction with the interaction of lipoproteins with the subendothelial matrix and the reduced efflux from the vessel wall, concur to their accumulation in the subendothelium (Figure 2). This event takes place almost concurrently with changes in the biosynthetic capacity of ECs that is evident both structurally and functionally. The cells switch to a secretory phenotype characterized by many copies of the rough endoplasmic reticulum, Golgi apparatus, and numerous caveolae, which correlates well with the progressive development of a multilayered basal lamina in the meshes of which transcytosed LDL is steadily entrapped (Figure 3). A reticular basal lamina was detected in some normal vessels,\textsuperscript{24} but not to the extent found in hyperlipemic rabbits, hamsters, and human atheroma.\textsuperscript{25–27}

### Transcytosed Lipoproteins Amass Within the Subendothelium as Modified and Reassembled Lipoproteins

Studies on hyperlipemic animals revealed that the first change occurring in aortic lesion-prone areas and heart valves is the progressive accumulation within the subendothelium of transcytosed Lps that differ structurally and chemically from native Lps.\textsuperscript{23,25,28} This event takes place before monocyte diapedesis and foam cell formation. Electron microscopy revealed the heterogeneity of these particles, which appear (in situ and after isolation from animals and human aorta) as vesiculated, aggregated, or fused particles (Figure 2) rich in unesterified cholesterol (as revealed by exposure to filipin).
Initially called “extracellular liposomes,” additional biochemical studies showed that, relative to native Lp, these particles underwent physico-chemical alterations (slight proteolysis, lipolysis, oxidative modifications), and were named modified and reassembled lipoproteins (MRLs). This observation was further confirmed on Watanabe heritable hyperlipemic rabbits (WHHL) and cholesterol-fed rabbits and in intimal thickening of human aorta. Although the exact location and the mechanism(s) of MRL formation are not completely understood, one can safely assume that the process may take place either on LDL interaction with ECs, or during transcytosis or within the subendothelial space by extended interaction with matrix components and cells. Another alternative that I favor is that the LDL modifications and MRL formation may take place gradually in all these locations. As the first structural modification observed in hyperlipemia (as well as in combined hyperlipemia/hyperglycemia), one can hypothesize that MRLs are fundamental to the early stage of atheroma formation. Thus, subsequent to endothelial transport of ApoB Lp, subtle or minimal modifications render the MRL prone to interact and form complexes with intima proteoglycans that in turn induce further alterations of these particles and provide the residence time and Lp retention in the arterial wall. This argument is strengthened by previous reports that in vitro, purified arterial proteoglycans (PG), particularly those from lesion-prone areas bind LDL, but chiefly LDL isolated from patients with coronary artery disease, presumably slightly modified Lp that have increased propensity to bind PG.

Several lines of evidence support the hypothesis that MRL may be the key event and first change occurring in atherogenesis: (1) although in normal artery wall there is constant...
Endothelial Cell Dysfunction Is the Outcome of a Lipid Disorder and an Inflammatory Reaction that Ultimately Result in Atheroma Formation

One can postulate that the dual assault on ECs, namely, the alteration of plasma lipid homeostasis and the subendothelial accrual of MRL, generate as a defense reaction a multipart inflammatory process. The latter is expressed by the synthesis of new or more EC surface adhesion molecules, novel secreted molecules such as monocyte chemoattractant protein-1 (MCP-1), and further modifications of native endothelial cell attributes having, as consequence, the EC dysfunction. The latter is portrayed structurally, as a localized prominent EC shift to a secretory phenotype, and functionally by increased secretion of basal lamina and matrix components, synthesis of novel molecules, impaired nitric oxide (NO) bioavailability, imbalanced synthesis of procoagulation/anticoagulation factors in favor of the former, increased secretion of vWF and plasminogen activator inhibitor-1, and decreased synthesis of prostacyclin.

Expression of New Cell Adhesion Molecules

Compelling evidence indicates that in dyslipidemia the ECs express new cell adhesion molecules, such as intercellular adhesion molecule-1, E-selectin, and P-selectin (which function in the selective recruitment of monocytes), and vascular cell adhesion molecule (VCAM)-1 (that trigger monocyte adherence to ECs by interaction with VLA-4 and CCR2 monocyte surface receptors). The ECs also synthesize MCP-1 and IL-8, which are chemoattractant for leukocytes. In addition, pro-inflammatory cytokines and components of MRL (oxidized phospholipids and short-chain aldehydes) induce activation of VCAM-1 gene, mediated by the nuclear factor-kB, another link between dyslipidemia and inflammation. The local release of cytokines and chemokines in lesion-prone areas and the activation of cells that express receptors for these factors are critical in the complex process of atheroma formation: tumor necrosis factor-α has a major role in the induction of EC adhesion molecules, IL-10 acts on EC and SMC activation, and the pro-inflammatory cytokines stimulate the secretion of EC’s Weibel Palade body–stored P-selectin, VCAM-1, and intercellular adhesion molecule-1.

Monocytes recruitment and adhesion is part of the multifaceted inflammatory process in which the EC adhesion molecules in concert with MCP-1 and eotaxin function in the selective recruitment of the circulating inflammatory cells to the developing plaque. Chemokines, the family of secreted proteins that induce chemotaxis through the activation of G-protein-coupled receptors, recruit monocytes or lymphocytes to the site of plaque formation, providing the presence of the leukocyte complementary receptor. MCP-1 contributes critically to monocyte chemotaxis acting through their CCR2 receptors. A trio of CXC chemokines function in lymphocyte recruitment and eotaxin is chemoattractant for mast cells (via CCR3 receptors); both are overexpressed in human atherosclerosis along with other chemokines: MCP-1, T-cell–derived CC chemokines (MIP-1α, MIP-1β, RANTES, I-309), the transmembrane chemokines CXCL16 and fractalkine, secreted by ECs, SMCs, and monocytes/macrophages. Fractalkine is a special chemokine that as a transmembrane protein function as an efficient EC adhesion molecules for monocytes and T cells (by an integrin-independent mechanism) and on cleavage by specific metalloproteases becomes soluble fractalkine, operating as chemotactic for these cells. Recently, it was reported that the EC membrane-bound fractalkine has a role in platelet activation and adhesion; moreover, in early atherosclerosis, activated platelets, using a P-selectin mechanism, secrete and deliver RANTES and PF4 chemokines to the EC surface, enhancing monocyte recruitment by activated endothelium. The large collection of chemokines advocates for the complexity of the process involved in the efficient and specific recruitment of inflammatory cells to the site of plaque formation.

Monocytes diapedesis takes place through the EC junctions, specifically in focal areas of MRL deposition in hyperplasic basal lamina (Figure 4). This is followed by...
homing within the subendothelium, where they undergo phenotypic modulations and become “activated” macrophages, expressing scavenger receptors (SR-A and CD-36). Unlike LDL receptors, the latter function in the nonregulated uptake of MRL, accumulation of intracellular lipids, and ultimately formation of macrophage-derived foam cells (Figure 5).

The inflammatory cells, through the factors they secrete within the plaque, send molecular messages: macrophage-derived foam cells secrete cytokines, growth factors, tissue factor, IFN-γ, MMPs, and produce reactive oxygen species (ROS); lymphocytes secrete CD-40L. These messages govern the plaque formation that includes clonal accumulation of SMCs within the intima. Recent evidence indicates that besides migration of the existing SMCs from the vessel’s media to the intima, circulating bone marrow cells and the vascular progenitor cells present in the adventitia of all arteries, are other sources of intimal SMCs. In coronary arteries, SMCs form a fibrous cap that, if afflicted by MMPs and IFN-γ, may lead to the plaque rupture.

Impairment of NO Bioavailability: A Cause and a Result of EC Dysfunction

Endothelial-derived NO is a critical molecule for the vessel’s normal function as well as for the progression/reversal of atherosclerosis. NO is constitutively generated from L-arginine by the enzymatic action of endothelial NO synthase (eNOS), is released in response to shear stress or activation of different receptors and diffuses (in physiological conditions) rapidly and freely to SMCs. The multifaceted biological functions of NO include its vasodilator property, and the inhibitory effect on SMC growth, nuclear transcription of cell adhesion molecules, platelets aggregation, and leukocyte adhesion to ECs. The eNOS is considered a...
protective enzyme, not only for its role in NO synthesis but also because its inhibition is associated with the production of ROS (eNOS is a NADPH consuming enzyme). Inflammatory cytokines (tumor necrosis factor-α and IL-6) can directly activate endothelial NADPH oxidases and this may lead to eNOS uncoupling.54

Insults to ECs reduce or abolish the NO functions. In hypercholesterolemia, the decline in endothelial NO bioavailability is attributed to: (1) the decreased expression of eNOS; (2) the lack of substrate or cofactors for eNOS and a deficient activation of eNOS caused by altered cellular signaling;55,56 (3) a diminished capacity of activated ECs to synthesize and/or release NO; or (4) ROS inactivation of synthesized NO.57,58 In the latter case, the superoxide anions may be generated by the eNOS itself and by the NADH/NAD(P)H oxidase system.59-60 Quenching of NO generates unbalance levels of NO/endothelin-1, manifested by impaired EC vasodilation property and antithrombotic activity.

Oxidative Stress-Induced EC Dysfunction

ROS include free radicals such as the superoxide anion (O$_2^-$), hydroxyl radical (HO), nitric oxide (NO), and lipid radicals or strong oxidants like hydrogen peroxide (H$_2$O$_2$), peroxynitrite (ONOO$^-$), and the hypochlorous acid (HOCl). Cellular production of one ROS leads to the production of several others by a radical chain reaction.58

Evidence exists that ROS induce endothelial dysfunction by affecting eNOS expression or by inactivation of NO through the formation of lipid peroxidation products and peroxynitrite radicals that disturb directly the EC membrane.61-63

In ECs, the enzymatic systems that contribute to the increase production of ROS are xanthine oxidase, NADH/NAD(P)H oxidase, and eNOS. Within the atherosclerotic plaque, the inflammatory cells and SMCs are a source of superoxide possibly via angiotensin II-activated NAD(P)H oxidase.64,65 Among others, the endothelial dysfunction may result from an imbalance between the oxidant stress and a depletion of the antioxidant reserve. Antioxidant enzymes, especially the 3 isoforms of superoxide dismutase, modulate basal levels of superoxide and protect against EC dysfunction and the ensuing vasomotor response. One of the isoforms, the extracellular superoxide dismutase, is synthesized by vascular SMCs, released and localized in the extracellular space between endothelium and SMCs having the role to protect NO that diffuses from ECs to SMCs.66 There is convincing evidence on the major role of superoxide on NO-mediated vasomotor tone; in addition, it was demonstrated recently, that O$_2^-$ and other ROS can modulate an NO-independent relaxation of the vessel wall.66 Overall, the oxidative stress is implicated in most cardiovascular diseases and ROS have a major role in vascular endothelial cell signal transduction.66

**EC Injury and Apoptosis**

There is now substantial evidence, both in human and animal models, that endothelial injury is a late event in atherosclerosis, and there are reasons to believe that the process is gradual. We have found that with the advancement of experimental hypercholesterolemia (in rabbits and hamsters), presumably as a consequence of extensive or extended offense of aggressive factors, some ECs covering atheroma display warning signs of structural damage manifested by the appearance of luminal pseudopods and intracellular lipid droplets.23,28,67,68 The latter steadily increase in size and number, leading finally to focal formation of EC-derived foam cells (Figure 6) that appear to maintain, also, some attributes of ECs (Weibel-Palade bodies, intercellular junctions, caveolae). The EC-derived foam cells were also obtained in vitro by incubation of aortic ECs with hypercholesterolemic serum. In these conditions, the EC appear heavily loaded with lipid droplets, change the pattern distribution of actin and vinculin, and fail to express intercellular adhesion molecule-1 and VCAM-1 on their surface.69,70

Hyperglycemia alone accelerates the development and progression of atherosclerotic lesions and the rapid formation of EC-derived foam cells.68,71 It remains to be determined the role of EC scavenger receptors in the process and the existence of EC-derived foam cells in human atherosclerotic advanced lesions, a tough task because of the difficulty to preserve these structures after explantation of atheroma or in arteries studied postmortem.

Several reports, as well as our data, indicate that EC death is a late event, occurring only in advanced atherosclerosis.72-74 Apoptosis of ECs is assumed to be caused by the local inflammatory mediators or the cytolytic attack of activated killer T cells,41 cytokines, and oxidized LDL that increased EC synthesis of MMPs (which degrade components of EC basal lamina75) or the oxidative stress.76 Little is known
about the mechanism(s) of EC death. The intracellular signaling that regulate the onset and execution of apoptosis have been elucidated only in part.\textsuperscript{77,78}

**EC: A Therapeutic Target and Therapeutic Tool, More Important than Previously Thought**

All these data, which disclose the ECs as the first affected cells and the last to surrender in various cardiovascular diseases (CVD), recommend EC as a prominent therapeutic target. Endothelial dysfunction may be assessed indirectly by plasma biomarkers that, although generally nonspecific, can give an indication of cellular malfunction. Currently, in hypercholesterolemic or diabetic patients, the indicators of EC dysfunction are the plasma NO metabolites (nitrites, nitrates), the increase concentration of vasoconstrictors (ie, endothelin-1), vWF, and others. To date, there are several categories of drugs used in CVD that diminish indirectly the endothelial dysfunction by upstream effect.

Statins, independent of their action on lipid metabolism, have been shown experimentally to increase the expression of eNOS possible acting at the level of gene expression\textsuperscript{79} and improve endothelial dependent vasomotion after acute coronary syndrome.\textsuperscript{80} In hyperlipemic hamsters, simvastatin increases the plasma antioxidant potential, reduces transcytosis of LDL, and restores the endothelium-dependent relaxation,\textsuperscript{81} an effect likely caused by an increase in endothelial NO synthesis. In general, statins prevent downregulation of eNOS expression induced by atherogenic levels of LDL,\textsuperscript{82} thus having a role in the restoration of EC function.

Some angiotensin-converting enzyme inhibitors (like ramiprilate) increase the expression of eNOS\textsuperscript{83} and reduce the breakdown of bradykinin that, in turn, stimulates bradykinin B\textsubscript{1} receptors to release NO.\textsuperscript{84} Furthermore, angiotensin-converting enzyme inhibitors indirectly increase the NO bioavailability by reducing O\textsubscript{2}\textsuperscript{-} production.\textsuperscript{80} The ECs benefit indirectly from the inhibition of the renin-angiotensin system, which consists of the reduced (angiotensin II-mediated) radical formation and the increase in NO bioavailability.\textsuperscript{85} L-arginine supplementation amends NO-induced EC dysfunction in human diabetes and in hyperlipemic-diabetic hamsters.\textsuperscript{86,87}

A direct approach foreseen in the treatment of CVD is the specific targeting of endothelial altered mechanisms and molecules, because the ECs are major actors involved in all stages of these diseases and have the advantage of being easily accessible for vascular-delivered drugs.

By now, MCP-1 and CCR-2 have become therapeutic targets because of their role in the recruitment of inflammatory cells in early stage of atheroma formation; attempts are made to develop specific antagonists to these molecules.\textsuperscript{45} We have reported that in cultured human ECs, aspirin and PPAR-\textalpha activators decrease the high-glucose--induced expression of MCP-1 by a reduction of AP-1 and NF-\kappaB activation through a mechanism dependent on inhibition of ROS.\textsuperscript{88}

Chemokine receptor antagonists, inhibitors of signaling, transcription factor decay, and polymer-coated stents for focal delivery of chemokine antagonists are undergoing studies.\textsuperscript{86} Various classes of drugs are currently designed to act on specific enzymes involved in the intracellular signaling cascade in inflammation.\textsuperscript{89} In addition, specific drugs that preclude the synthesis of different enzyme sources of ROS, such as vascular-specific NAD(P)H oxidase inhibitors, are efficient in prevention of EC dysfunction.\textsuperscript{90} Superoxide dismutase entrapped in liposomes restores the EC-dependent relaxation, increases significantly the NO bioavailability, and is effective in scavenging superoxide anions in experimental diabetes.\textsuperscript{91} Targeting and restoring altered molecular mechanisms of the dysfunctional endothelium in CVD is an unessential therapeutic trial.

A novel therapeutic goal is the stabilization of vulnerable plaque by lowering LDL, increasing high-density lipoprotein, reduction of ROS, and therapeutic actions on inflammatory processes and matrix metabolism, all indirectly acting on EC functions.\textsuperscript{92}

Rapid restoration of injured or denuded arterial ECs could be of great assistance in preventing thrombus formation in plaque rupture and restenosis after balloon catheterization or stent implantation. A promising novel therapeutic option for replacement of damaged EC, ie, re-endothelialization, as well as for neovascularization of ischemic tissues is the use of endothelial progenitor cells (EPCs). The latter may derive from bone marrow (the cells expressing the antigens CD133 and vascular endothelial growth factor receptor 2) or from other sources such as tissue resident, or vessel wall stem cells. Successful exploitation of EPCs is a complex, multi-step process that includes mobilization, homing to specific sites, adhesion, further differentiation, and functional integration.\textsuperscript{93} Moreover, recent attempts are made to use EPCs for endothelialization of stents; rapid mobilization and recruitment of EPCs are currently tested by vascular endothelial growth factor-eluting stents, seeding stents with ECs or EPCs, or coating the stent surface with antibodies against CD34 to attract EPCs that eventually differentiate into functional ECs.\textsuperscript{94} Although thus far, there are contradictory reports on the origin, subtypes, identity, and surface markers, the EPCs hold the promise to become a helpful tool for regenerative medicine.

**Conclusion**

The wealth of data on the attributes and the vital role of the vascular endothelium in health and diseases generated a complex discipline, focused on these apparently frail but physically sturdy and functionally complex cells. Theoretically, one can consider that this discipline includes: **endotheliology**, the investigation of EC normal functions, which warrants the understanding of the molecular mechanisms used by the cells to preserve the body homeostasis; **endotheliopathy**, the search for the alterations of EC innate molecules and mechanisms in vascular diseases, which promises the comprehension of cellular malfunction; and **endotheliotherapy**, the quest for novel drugs targeted specifically to dysfunctional ECs, a promising venue for the reversal/slow-down or treatment of cardiovascular diseases.

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None.

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