Title	**Arterial endothelial cells: still the craftsmen of regenerated endothelium**
Author(s)	**Hagensen, MK; Vanhoutte, PM; Bentzon, JF**
Citation	**Cardiovascular Research, 2012, v. 95 n. 3, p. 281-289**
Issued Date	**2012**
URL	**http://hdl.handle.net/10722/164492**
Rights	**This is a pre-copy-editing, author-produced PDF of an article accepted for publication in Cardiovascular Research following peer review. The definitive publisher-authenticated version Cardiovascular Research, 2012, v. 95 n. 3, p. 281-289 is available online at: http://cardiovascres.oxfordjournals.org/content/95/3/281**

Arterial endothelial cells: still the craftsmen of regenerated endothelium.

Hagensen. Endothelial progenitor cells in arterial regeneration

Mette K. Hagensen[1], MSc, PhD, Paul M. Vanhoutte[2], MD, PhD, Jacob F. Bentzon[1], MD, PhD.

[1] Atherosclerosis Research Unit, Institute of Clinical Medicine and Department of Cardiology, Aarhus University Hospital, Skejby, Denmark.

[2] Department of Pharmacology and Pharmacy, Li Ka Shing Faculty of Medicine, Hong Kong, China and Department of BIN Fusion Technology, Chonbuk National University, Jeonju, Korea.

Corresponding author:

Mette Kallestrup Hagensen, MSc, PhD.

Atherosclerosis Research Unit, Institute of Clinical Medicine and Department of Cardiology,

Aarhus University Hospital, Skejby

Brendstrupgaardsvej

8200 Aarhus N, Denmark

Phone: +45 78459030 Fax: +45 89496011

Email: mette.hagensen@ki.au.dk

Word count: 7495 Abstract word count: 145 Figures: 3

ABSTRACT

For more than a decade, a prevailing hypothesis in research related to arterial disease has been that circulating endothelial progenitor cells (EPC) provide protection by their innate ability to replace dysfunctional or damaged endothelium. This paradigm has led to extensive investigation of EPCs in the hope of finding therapeutic targets to control their homing and differentiation. However from the very beginning, the nomenclature and the phenotype of EPCs have been subject to controversy and there are currently no specific markers that can unambiguously identify these cells**.** Moreover, many of the initial observations that EPCs differentiate to endothelial cells in the course of arterial disease have been criticized for methodological problems. The present review discusses the contrasting experimental evidence as to the role of EPCs in contributing to relining of the endothelium and highlights some of the methodological pitfalls and terminological ambiguities that confuse the field.

Keywords: Endothelial progenitor cells, endothelial cell, atherosclerosis, allograft vasculopathy, mechanical injury

1. INTRODUCTION

The healthy endothelium forms a continuous lining of the vascular system that controls the passage of nutrients and oxygen from the blood into tissues, serves removal of cellular and metabolic waste products, and regulates arterial reactivity through synthesis and release of vasoactive molecules.[1] The basal rate of endothelial cell (EC) proliferation in healthy blood vessels is low but certain regions display a higher rate.[2] Moreover, EC proliferation increases with age and the presence of cardiovascular risk factors.[3]

Common for cardiovascular diseases is the loss of normal endothelial function. Prolonged or exaggerated endothelial activation leads to dysfunction and loss of integrity with apoptosis and necrosis.[1, 4] At the atherosclerosis-prone regions of the apolipoprotein E deficient (apoE$^{-/-}$) mouse, for example, endothelial turnover rate and proliferation is increased early before the development of atherosclerotic plaques.[5]

The means whereby the uninterrupted endothelial lining is maintained and regenerated have been debated for a long time (**Figure 1**). In the mid-1900's, several experiments showed that regeneration of damaged endothelium involves EC mitosis.[6, 7] However, other investigators also reported that the endothelium may be derived from other types of cells, including circulating cells in the blood and undifferentiated cells from the subendothelial space.[8] A number of careful studies in the 1970s further supported the dominant role of local EC mitosis and migration in endothelial regeneration after vascular damage,[3, 9, 10] although they provided little information concerning the actual mechanisms that control the movement and proliferation of ECs at injury sites.[11] Additionally, it was shown that small areas of endothelial injury can be repaired solely through EC migration while larger areas of injury are repaired by both proliferation and

migration.[9, 12] These findings are in line with indirect evidence obtained in wound healing assays which supports the role of EC proliferation and migration in endothelial repair.[13] Moreover, pathological processes that cause damage to the endothelium can also cause detachment of ECs resulting in a very low number of circulating mature ECs in the bloodstream which might participate in endothelial repair.[14] None of these studies, however, were able to determine whether local proliferation is the only mechanism underlying EC renewal and regeneration.

In a landmark study, Asahara and colleagues[13] isolated putative endothelial progenitor cells (EPC) from the peripheral blood. During short-term culturing, some of these $CD34^+$ mononuclear blood cells acquired endothelial-like characteristics, and they homed to sites of angiogenesis when injected intravenously into animals with hind limb ischemia. The foundation for research in EPC-mediated repair of the endothelium was laid (**Figure 1**). During the last decade, numerous studies have undertaken to define the role of EPCs in vascular disease as well as their potential use as a biomarker of cardiovascular disorders. However, attempts to identify and describe EPCs and their biological properties have yielded conflicting results. As discussed in the present review, much of the controversy may be fostered by inconsistent terminology and common methodological pitfalls.

2. ORIGIN AND CHARACTERIZATION OF EPCS

EPCs can reside in the bone marrow (BM), the peripheral blood, the vascular adventitia and/or the endothelium itself.[14-16] Their identification and characterization has been based on a variety of techniques that fall roughly into two categories: flow cytometric assays for cell surface markers and cell culture assays. Asahara and colleagues[13] defined EPCs as

cells positive for the hematopoietic stem cell (HSC) marker CD34 and the vascular endothelial growth factor receptor 2 (VEGFR2 or KDR in humans). Since CD34 is not exclusively expressed in HSCs but also in mature ECs, the immature HSC marker AC133 has been used to better discriminate between EPCs and mature ECs.[17] Both populations ($CD34^+$ or $AC133^+$) may differentiate into cells that express EC markers *in vitro*[13, 18] and enhance neovascularization when injected in animal models after ischemia.[13, 19] However, although $CD34^+VEGFR\text{-}2^+AC133^+$ cells are widely accepted to represent "true EPCs" in humans,[20] they have never been proven to differentiate into ECs *in vivo*. Moreover, recent studies show that mobilized adult peripheral blood $CD34^+VEGFR\text{-}2^+AC133^+$ cells in fact represent an enriched population of $CD45^+$ hematopoietic precursors, which do not differentiate to ECs *in vitro*.[21]

Based on cell culture assays, two distinct EPC phenotypes derived from human peripheral blood mononuclear cells have been described.[22] When seeded in culture dishes, colonies of cells with an elongated and spindle shape were observed similar to that of the EPCs first reported by Asahara and colleagues[13]. These cells die within four weeks and were called "early" EPCs in contrast to a cell population displaying an endothelial-like cobblestone shape after two to three weeks, surviving up to twelve weeks and named endothelial colony forming cells (ECFCs) or "late" EPCs.[23] The rare circulating ECFCs were recently found to be $CD146^+CD34^+CD31^+CD45^-AC133^+$ cells by flow cytometry, similar to mature circulating and resident ECs.[24] The origin of ECFCs is not yet clear, but similar cells can be identified in primary cultures of umbilical vein and aortic ECs, suggesting that they may dislodge into the blood from the vessel wall.[25]

In vivo, both types of EPCs apparently show similar vasculogenic capacity[22] but since ECFCs have higher proliferation potency and may incorporate into perfused vessels they are considered by some to represent true EPCs.[26] In a model of hind limb ischemia, ECFCs was shown to enhance revascularization in synergy with early EPCs.[27]

Recent observations suggest that the formation of "EC" colonies after culture of peripheral blood cells, used to demonstrate early EPCs, should be interpreted with caution. Prokopi and colleagues demonstrated that conventional methods for isolating mononuclear cells after 7 days of culture lead to contamination with platelet microparticles (MPs).[28] These are taken up in mononuclear cells, which thereby acquire artefactual "EC" characteristics such as the presence of CD31 and von Willebrand Factor (vWF), binding of lectins and even angiogenic properties.[28]

Overall, the use of different marker combinations and culture assays to isolate or measure progenitor cells indicate that the term EPC does not represent a single, well-defined cell type. Rather, the term is used to describe a number of different and heterogeneous cell populations that circulate in the blood or can be derived through short- or long-term culturing of blood cells.

3. EPCS AND CARDIOVASCULAR DISEASE

Consistent with their putative function in endothelial turnover and repair, several reports indicate that the concentration of EPCs, defined in various ways, is associated with cardiovascular disease. Thus, risk factors for atherosclerosis correlate inversely with the number of EPCs in the blood *in vivo* and with the results of *in vitro* assays of EPC function.[29-35] Furthermore, a low number of EPCs, defined as $CD34^{+}VEGFR2^{+}$ cells, is

associated with the presence of coronary artery disease (CAD), and predicts the occurrence of cardiovascular events and death from cardiovascular causes in patients.[30, 31] Using flow cytometry and short-term culture assays to measure EPC numbers, similar correlations have been obtained between reduced EPC numbers and the extent of atherosclerosis in other vascular beds than the coronaries,[34] allograft vasculopathy in transplanted hearts,[33] and diffuse in-stent restenosis after percutaneous coronary intervention (PCI).[35] By contrast, Güven and colleagues reported that the number of EPCs was actually increased among patients with significant CAD when EPCs were defined by their ability to give rise to endothelial-cell like colonies during long-term culture (late EPCs).[36]

Stem cells usually exist in a quiescent state but start to differentiate and to be mobilized into the systemic circulation upon specific stimulation. For example, putative EPCs can be mobilized in patients with vascular diseases by granulocyte-colony stimulating factor, statins or by exercise.[37] Whether the associations between EPC number and cardiovascular disease reflect alterations in consumption rate or regulation of the release of putative EPCs is unknown.

4. *IN VIVO* EVIDENCE FOR ENDOTHELIAL CELL ORIGIN

Before classifying cells as EPCs, it is essential to validate their homing and differentiation potential *in vivo*. The ability to produce cells with endothelial characteristics *in vitro* is indicative, but the capacity to incorporate into an endothelial lining and conduct EC functions is a far more stringent criterion which cannot be tested outside the living organism. If EPCs exist and undertake physiologically relevant repair

of the endothelium, it should be possible to detect their contribution by labelling circulating cells with a genetic tracking marker and analyse their fate using specific markers for phenotype and high-resolution microscopy of the blood vessel wall (**Figure 2**).[38, 39] This approach also has the advantage that it bypasses the errors involved with isolation of EPCs, if they exist, and the confusion regarding appropriate phenotypic markers of these cells.

5. EPCS IN MODELS OF ARTERIAL DISEASE

Most arterial diseases involve injury to or death of ECs.[4] During the development of atherosclerosis, ECs are continuously injured and replaced (i.e. turnover)[1] while under other circumstances, EC injury may be more pronounced and cover a larger area to be repaired (i.e. regeneration). The process of EC turnover may differ from the one involved in endothelial regeneration.

Circulating EPCs may contribute to the generation of microvascular ECs at sites of neovascularization,[13, 40] and to relining of the endothelium after various kinds of arterial injuries, including mechanical removal,[41-43] and allograft vasculopathy,[41, 44, 45] as well as to vein graft atherosclerosis.[46] Although atherosclerosis is by far the most important arterial disease worldwide, only few studies have been conducted on the role of putative EPCs in the EC turnover associated with this disease.

5.1. EPCs in atherosclerosis

Only indirect evidence exists supporting the prevailing understanding that circulating EPCs provide protection against atherosclerosis by their ability to replace dysfunctional

ECs.[5, 47] The few studies in which atherosclerotic mice received BM- or blood-derived cells assumed to contain EPCs intravenously yielded discrepant results. Thus, Silvestre *et al.*[48] and George *et al.*[49] reported that transplantation of BM-derived cells accelerated atherosclerosis while Zoll and colleagues[50] found that injection of EPCs had no effect on the development of the disease. In yet another study, the intravenous injection of BM-derived progenitors from young non-atherosclerotic into mature atherosclerotic apoE$^{-/-}$ mice exerted a vascular protective effect.[51] Some of the injected cells adhered to the surface of atherosclerosis-prone areas of the aorta, an observation which lead to the suggestion that they may reduce atherosclerosis by replacing senescent ECs in plaques.[51] Others, however, observed that the injection of fluorescently-labeled BM- and spleen-derived EPCs in apoE$^{-/-}$ mice, resulted in the predominant accumulation of these cells within the lipid core of the plaques and not within the endothelium.[49]

In a transgenic TIE2-lacZ mouse model of vein graft atherosclerosis expressing β-galactosidase positive (β-gal^{+}) in ECs, the endothelium was partly repaired by BM-derived EPCs.[46] In vein grafts from TIE2-LacZ mice transplanted into the carotid artery of wild-type mice, the number of β-gal^{+} cells was reduced markedly while such cells were evenly distributed on the surface of wild-type vein segments when transplanted into TIE2-LacZ mice.[46] Moreover, β-gal^{+} cells were seen on wild-type veins transplanted into mice carrying BM cells from TIE2-LacZ mice.[46] These data are in line with the observation that, in areas prone to lesion development in apoE$^{-/-}$ mice transplanted with BM from TIE2-lacZ mice, 3% to 4% of cells displaying progenitor markers were β-gal^{+}.[5] Only one study so far has analyzed the origin of the plaque endothelium during the actual development of atherosclerosis in hyperlipidemic apoE$^{-/-}$ mice. Not a single circulatory

EPC-derived EC was present at the plaque surface.[38] This finding was further corroborated by studies of endothelial regeneration after mechanical injury to advanced plaques in old apoE$^{-/-}$ mice, where the regenerated endothelium was derived exclusively from the local blood vessel wall, presumably through proliferation of flanking ECs (**Figure 2**).[38]

Plaque neovascularization may contribute to plaque growth and increase plaque vulnerability,[52, 53] but due to the lack of animal models featuring plaque neovessels, not much are known about the contribution of EPCs to this process.

5.2. EPCs in allograft vasculopathy

In 2002 Quaini and colleagues[54] reported that recipient-derived cardiomyocytes, smooth muscle cells and ECs were common in sex-mismatched (female-to-male) heart transplants. Multiple studies have been conducted to study this phenomenon in simplified rodent models where vessel allografts are transplanted into the arterial system of recipients without immunosuppression to protect the donor cells. In these systems, the endothelium of the allograft efficiently regenerates with recipient-derived cells. The hypothesis that the majority of these recipient-derived cells originates from circulating EPCs that home and differentiate into mature ECs in the allografts has attracted particular interest.[55, 56] Using a mouse model in which the expression of β-gal was regulated by the endothelial-specific TIE2 promoter, Hu *et al.*[45] observed that ECs of neointimal lesions in allografts of aortic segments are derived from β-gal^{+} circulating EPCs with approximately 30% of ECs being of BM origin.[45] By contrast, Hillebrands and colleagues[44] reported that the contribution of BM-derived EPCs was only minimal (1 to

3%) when investigating the origin of ECs by performing orthotopic aortic allografting in BM-chimeric recipient rats; they did, however, not determine the potential contribution from non-BM-derived EPCs. Taken in conjunction, these studies point to a contribution of non-BM- and BM-derived EPCs in transplant atherosclerosis rather than ECs from the nearby blood vessel wall, as inferred mainly from the absence of observable inward migration fronts of ECs at selected early time points after allografting.[44, 45] By contrast, the majority of the reported data favors the view that ECs in allografts are derived from migrating ECs from the flanking vasculature.[9, 10] When an allografted blood vessel or heart is interpositioned in a recipient, it is not obvious to ascertain whether cells originate, from the flanking vasculature versus circulating EPCs. However, the use of an approach that distinguishes between different cell sources (**Figure 3**) demonstrated that migrating vascular ECs rather than circulating EPCs are the source of recipient-derived ECs in a murine model of hyperlipidemia-accelerated allograft vasculopathy (AV).[39]

5.3. EPCs in endothelial regeneration

Re-endothelialization after mechanical vascular injury, including PCI, is essential for inhibition of neointima formation and restoration of vascular homeostasis.[57, 58] Several studies in experimental models have concluded that circulating EPCs may be involved in this process.[42, 43, 59-61] Walter *et al.*[42] and Werner *et al.*[43] demonstrated that in mice and rats statins facilitated re-endothelialization of injured arterial segments with a resultant reduction in neointimal thickening, and that this was associated with an increased contribution of EPC-derived ECs mobilized from the BM. Mobilization of EPCs has also been implicated in the promoting effect of several other interventions on endothelial

regeneration, including estrogen therapy,[59] exercise,[61] and systemic heme oxygenase 1-overexpression.[60] By contrast, diabetes was found to impair EPC function. Thus, Ii *et al.* reported that transplantation of diabetic BM into non-diabetic mice resulted in delayed re-endothelialization after wire-induced carotid denudation presumably due to impaired incorporation of BM-derived ECs.[61]

Other studies, however, have questioned that differentiation of ECs from circulating EPCs is the underlying mechanism explaining these findings. Tsuzuki and colleagues[62] concluded to the absence of contribution of BM-derived cells to re-endothelialization in a wire-induced endothelial denudation model of the common carotid artery of mice transplanted with green fluorescence protein positive (GFP^+) BM. Furthermore, Itoh and colleagues[63] observed that re-endothelialization after injury of a confined area of the pial artery occurred by stepwise re-growth of endothelium from the edges of the injured area, a pattern that does not permit to accept homing of circulating EPCs as a major mechanism for endothelial regeneration. Several studies reporting important contributions of EPCs have observed similar patterns of re-growth of endothelium from the edges as demonstrated by Evans blue injection.[59, 61, 64]

Using a mechanical arterial injury in TIE2-GFP^+ transgenic mice, to establish whether circulating non-BM-derived EPCs or the nearby healthy endothelium contributed to EC regeneration, revealed that endothelial regeneration does not involve homing and differentiation of circulating EPCs into ECs.[65] Instead, the endothelium regenerated by GFP^+ cells as a function of time, evolving from the anastomosis sites towards the center of the transplant. Thus, endothelial regeneration is mediated by migration and possible proliferation of ECs from the adjacent healthy vessel wall.

5.4. EPCs and neovascularization

Circulating cells have been reported to incorporate into and facilitate neovascularization of ischemic tissues.[40, 66] In ischemic hindlimbs of mice transplanted with BM cells expressing lacZ under the regulation of an EC lineage-specific promoter (Flk-1 or Tie-2), Asahara et al. found $lacZ^+$ cells to be incorporated into capillaries between skeletal myocytes.[40] Moreover, after ligation of the left coronary artery, EPCs were found to be incorporated into foci of neovascularization at the border of the infarct.[40] Circulating EPCs have also been reported to contribute to the endothelial lining of microvessels in wound healing,[67] tumor growth,[40] and corneal neovascularization.[68]

The observation that BM-derived EPCs may facilitate and incorporate into neovessels in ischemic muscle offers hope that one could harness this mechanism to treat chronic refractory myocardial ischemia or improve functional recovery after myocardial infarction.[69, 70]

Using a variety of cell sources and delivery strategies, the effect of transplanting autologous BM-derived cells to the suffering myocardium has been tested in clinical studies. Some of these, but not all,[69] have shown significant improvements in specified endpoints such as angina frequency or exercise tolerance.[71] Whether the positive effects, when observed, involve stable integration of the transplanted cells and differentiation to ECs has been debated. Putative EPCs have been shown to be able to facilitate neovascularization through secretion of paracrine factors[66, 72, 73]; e.g. injecting EPC-conditioned medium alone into a rat model of chronic hindlimb ischemia increased capillary density, blood flow and muscle performance significantly.[73] Whether mediated

by paracrine signaling or not, experiments in both myocardial infarction and limb ischemia models have shown that persistence of the transplanted EPCs and their differentiation to eNOS expressing cells are necessary for functional improvement.[74, 75]

6. METHODOLOGICAL CHALLENGES

The seminal descriptions of circulating EPCs have inspired a large number of studies investigating various aspects of this cell population, including their phenotypic characterization, mobilization and homing, and their value as a blood biomarker of vascular health.[15, 17, 18, 21, 22, 29-31, 41] A number of those, however, have fundamentally questioned the theory that these "EPCs" act as a source of arterial endothelium *in vivo* and have pointed out a number of potential methodological pitfalls that may have led to incorrect conclusions.[14, 16, 20, 21, 28, 38, 39, 65, 76-79] The key methodological issues in the EPC field are probably not different from those initiating other controversies in the progenitor cell field being rooted in the failure to reach clear single-cell resolution, and in the use of unspecific cell markers or detection systems.[38, 80]

Detection of EPC contribution to the endothelium has been based on models in which a population of cells, e.g. BM or circulating cells, assumed to be enriched for EPCs, is tagged with a transgene encoding a reporter molecule, such as eGFP or β-gal. Incorporation of tagged cells in the endothelial lining and their expression of characteristic endothelial proteins is the criterion used to detect EPC-derived arterial ECs. In many vascular diseases, homing of BM-derived cells is an inherent part of the pathophysiological process. After vascular injury, hematopoietic cells may transiently

cover the denuded area before being replaced by regenerated endothelium[16], and such cells may easily be mistaken for BM-derived ECs early after injury. Furthermore, in atherosclerosis, there is an ongoing passage of BM-derived inflammatory cells through the endothelial layer. Moreover, some of these cells may reside in the subendothelial space in close proximity to ECs.[38, 81] Choi and colleagues[82] have shown that dendritic cells are abundant in the normal mouse aorta and localized beneath the endothelium in the subintimal space. Moreover, dendritic cells are abundant in the intima of atherosclerosis-predisposed regions of the wild-type C57BL/6 mouse aorta.[83] If clear single-cell resolution is not reached, a dendritic or other BM-derived cell lying close to an EC, may appear as a single BM-derived EC. Furthermore, the use of widefield rather than confocal microscopy can contribute to the above problem since it is not possible to visualize thin optical sections of cells in tissue sections when using the former. A cell with a tracking marker overlying a cell with a phenotypic marker can therefore be misinterpreted as "co-localization".

The choice of markers to establish EC type is equally important. A number of studies have used CD31 as an EC marker, but since this protein is also expressed by monocytes[84] the detection of BM-derived $CD31^+$ cells is not surprising. More specific markers such as vascular endothelial (VE)-cadherin, endothelial nitric oxide synthase (eNOS) and vWF should be used as markers for ECs. Even though vWF is also found in platelets and in the subendothelial extracellular space, it is specific for ECs when identified within nucleated cells in the arterial wall, typically with a characteristic granular cytoplasmic staining pattern.[85]

Reporter transgenes driven by the endothelial-specific angiopoietin receptor TIE2 promoter have often been used to track BM derived ECs.[5, 40, 42, 46, 59, 86, 87] The use of a reporter molecule that is only expressed in cells of the endothelial lineage obviates the need for single-cell resolution, but puts great dependence on the endothelial specificity of the promoter. However, a subpopulation of monocytes that circulates in the peripheral blood and is recruited to sites of angiogenesis and in regenerating tissues also expresses TIE2.[88, 89] These cells localize in close proximity to ECs and can therefore easily be mistaken for BM-derived ECs.

7. EPC OR ONLY PUTATIVE EPC

As said above, in most arterial diseases inflammation takes place, in which mobilization, circulation, homing, and local differentiation of BM-derived leukocytes play important roles in disease progression. Since many EPC populations contain monocytes, it therefore can be expected that when injecting putative EPC populations into animals some of these cells will home to sites of vascular damage. Dil-Ac-LDL uptake and lectin binding together with endothelial marker expression is the phenotypic profile that is commonly used for EPC identification. However, monocytes express a similar phenotypic profile when cultured under specific conditions *in vitro*. When Rohde and colleagues,[77] cultured monocytes for four to six days under angiogenic conditions, the cells lost CD14/CD45 and displayed a commonly accepted EPC phenotype [including LDL uptake, lectin binding, CD31/CD105/CD144 expression and formation of cord-like structures] that made them indistinguishable from putative EPCs. These characteristics of monocytes/macrophages have also been reported by others.[78, 90] Noticeably, monocytes already express most tested endothelial genes and proteins at even higher levels than the

so-called EPCs, and colony-forming unit ECs (CFU-EC) formation is strictly dependent on monocyte presence.[77] The putative EPC population of colony-forming mononuclear cells in human peripheral blood, similar to those described by Asahara and colleagues in mice[13, 40], later appeared to be composed mainly of inflammatory and immune mononuclear cells rather than true EPCs[20, 26, 76, 77, 91, 92] and such cells home to sites of arterial injury and atherosclerosis.

8. PARACRINE EFFECT

An alternative explanation for the association between EPCs and endothelial regeneration may be that some of the cells contained in the "EPC" cell populations exert pro-angiogenic paracrine effects without actually differentiating into ECs.[20, 81, 89, 91] They may adhere and migrate through the endothelium into the blood vessel wall and there release growth factors and chemokines that stimulate endothelial regeneration by resident ECs. Putative "EPCs" obtained in short-term culture assays (early EPCs) express monocyte/macrophage markers and secrete multiple potent angiogenic growth factors[76] that enhance EC migration[93] and protect against oxidative stress-induced apoptosis.[94] Consistent with such paracrine function, serial injections of conditioned media harvested from peripheral blood-derived EPCs into an ischemic hind limb ameliorated local perfusion by promoting neovascularisation and vascular maturation.[95] TIE2-expressing monocytes have been reported to exert similar functions. Indeed, during tumor angiogenesis, $TIE2^+$ monocytes are located adjacent to new blood vessels without integrating into the endothelium.[89] Induced apoptosis of these cells through activation of

a suicide gene blocked tumor angiogenesis indicating that they stimulate angiogenesis though paracrine signaling.[89]

The term EPC was initially used in the literally correct way for immature precursor cells capable of differentiating into mature ECs *in vivo*.[13, 40-44, 46] Now the term has gradually been redefined to include circulating angiogenic cells without an endothelial fate. Thus, an alternative explanation for the conflicting results reported in the literature could be due to paracrine signaling to local ECs by blood -derived pro-angiogenic cells.[79] However, to avoid confusion, the literally correct definition of a progenitor cell as an immature precursor cell capable of differentiating into a mature cell type *in vivo* should be retained.

9. CONCLUSION

After more than ten years of enthusiastic research in EPC biology, researchers worldwide still do not agree on how this cell population should be defined. Despite the fact that a number of studies show an inverse correlation between the number and functional activity of EPCs in the blood and the development of cardiovascular disease, this correlation does not imply causation.

The present review has identified limitations and pitfalls in the EPC literature and also pointed out methodological challenges. Although there have been numerous attempts to define the exact EPC phenotype it has become obvious instead that one deals with a heterogeneous population of cells with different phenotypes and *in vitro* potential. Instead of being cells that differentiate into ECs, these putative EPCs rather represent a population of monocytes sharing many characteristics of ECs *in vitro* and *in vivo* affect existing cells through paracrine mechanisms. These putative EPCs do not contribute

directly to regeneration of the endothelium in arterial diseases. Thus, as of today, the paradigm from the 1970s, which states that ECs in arterial disease are repaired and regenerated by proliferation and migration of ECs from the local and adjacent vasculature, remains valid. The time probably has come to abandon the term *EPC* and instead to focus more on investigating the paracrine potential of this heterogeneous population of cells and the mechanisms responsible for EC proliferation and migration from the existing endothelium.

FUNDING

This work was supported by The Danish Council for Independent Research, Medical Sciences.

CONFLICT OF INTEREST

None.

REFERENCES

(1) Vanhoutte PM. Regeneration of the endothelium in vascular injury. *Cardiovasc Drugs Ther* 2010;**24**:299-303.

(2) Schwartz SM, Benditt EP. Clustering of replicating cells in aortic endothelium. *Proc Natl Acad Sci U S A* 1976;**73**:651-653.

(3) Schwartz SM, Gajdusek CM, Reidy MA, Selden SC, III, Haudenschild CC. Maintenance of integrity in aortic endothelium. *Fed Proc* 1980;**39**:2618-2625.

(4) Falk E. Pathogenesis of atherosclerosis. *J Am Coll Cardiol* 2006;**47**:C7-C12.

(5) Foteinos G, Hu Y, Xiao Q, Metzler B, Xu Q. Rapid endothelial turnover in atherosclerosis-prone areas coincides with stem cell repair in apolipoprotein E-deficient mice. *Circulation* 2008;**117**:1856-1863.

(6) Poole JC, Sanders AG, Florey HW. The regeneration of aortic endothelium. *J Pathol Bacteriol* 1958;**75**:133-143.

(7) Spaet TH, Lejnieks I. Mitotic activity of rabbit blood vessels. *Proc Soc Exp Biol Med* 1967;**125**:1197-1201.

(8) Ts'ao C. Myointimal cells as a possible source of replacement for endothelial cells in the rabbit. *Circ Res* 1968;**23**:671-682.

(9) Schwartz SM, Haudenschild CC, Eddy EM. Endothelial regneration. I. Quantitative analysis of initial stages of endothelial regeneration in rat aortic intima. *Lab Invest* 1978;**38**:568-580.

(10) Clopath P, Muller K, Staubli W, Burk RR. In vivo and in vitro studies on endothelial regeneration. *Haemostasis* 1979;**8**:149-157.

(11) Schwartz SM, Gajdusek CM, Selden SC, III. Vascular wall growth control: the role of the endothelium. *Arteriosclerosis* 1981;**1**:107-126.

(12) Reidy MA, Schwartz SM. Endothelial regeneration. III. Time course of intimal changes after small defined injury to rat aortic endothelium. *Lab Invest* 1981;**44**:301-308.

(13) Asahara T, Murohara T, Sullivan A, Silver M, van der ZR, Li T *et al*. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997;**275**:964-967.

(14) Rabelink TJ, de Boer HC, de Koning EJ, van Zonneveld AJ. Endothelial progenitor cells: more than an inflammatory response? *Arterioscler Thromb Vasc Biol* 2004;**24**:834-838.

(15) Urbich C, Dimmeler S. Endothelial progenitor cells functional characterization. *Trends Cardiovasc Med* 2004;**14**:318-322.

(16) Yoder MC. Is endothelium the origin of endothelial progenitor cells? *Arterioscler Thromb Vasc Biol* 2010;**30**:1094-1103.

(17) Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M *et al*. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. *Blood* 2000;**95**:952-958.

(18) Gehling UM, Ergun S, Schumacher U, Wagener C, Pantel K, Otte M *et al*. In vitro differentiation of endothelial cells from AC133-positive progenitor cells. *Blood* 2000;**95**:3106-3112.

(19) Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J *et al*. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med* 2001;**7**:430-436.

(20) Timmermans F, Plum J, Yoder MC, Ingram DA, Vandekerckhove B, Case J. Endothelial progenitor cells: identity defined? *J Cell Mol Med* 2009;**13**:87-102.

(21) Case J, Mead LE, Bessler WK, Prater D, White HA, Saadatzadeh MR *et al*. Human CD34+AC133+VEGFR-2+ cells are not endothelial progenitor cells but distinct, primitive hematopoietic progenitors. *Exp Hematol* 2007;**35**:1109-1118.

(22) Hur J, Yoon CH, Kim HS, Choi JH, Kang HJ, Hwang KK *et al*. Characterization of two types of endothelial progenitor cells and their different contributions to neovasculogenesis. *Arterioscler Thromb Vasc Biol* 2004;**24**:288-293.

(23) Ingram DA, Mead LE, Tanaka H, Meade V, Fenoglio A, Mortell K *et al*. Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. *Blood* 2004;**104**:2752-2760.

(24) Mund JA, Estes ML, Yoder MC, Ingram DA, Jr., Case J. Flow Cytometric Identification and Functional Characterization of Immature and Mature Circulating Endothelial Cells. *Arterioscler Thromb Vasc Biol* 2012;**32**:1045-1053.

(25) Ingram DA, Mead LE, Moore DB, Woodard W, Fenoglio A, Yoder MC. Vessel wall-derived endothelial cells rapidly proliferate because they contain a complete hierarchy of endothelial progenitor cells. *Blood* 2005;**105**:2783-2786.

(26) Yoder MC, Mead LE, Prater D, Krier TR, Mroueh KN, Li F *et al*. Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. *Blood* 2007;**109**:1801-1809.

(27) Yoon CH, Hur J, Park KW, Kim JH, Lee CS, Oh IY *et al*. Synergistic neovascularization by mixed transplantation of early endothelial progenitor cells

and late outgrowth endothelial cells: the role of angiogenic cytokines and matrix metalloproteinases. *Circulation* 2005;**112**:1618-1627.

(28) Prokopi M, Pula G, Mayr U, Devue C, Gallagher J, Xiao Q *et al*. Proteomic analysis reveals presence of platelet microparticles in endothelial progenitor cell cultures. *Blood* 2009;**114**:723-732.

(29) Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA *et al*. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 2003;**348**:593-600.

(30) Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A *et al*. Circulating endothelial progenitor cells and cardiovascular outcomes. *N Engl J Med* 2005;**353**:999-1007.

(31) Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H *et al*. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 2001;**89**:E1-E7.

(32) Chironi G, Walch L, Pernollet MG, Gariepy J, Levenson J, Rendu F *et al*. Decreased number of circulating CD34+KDR+ cells in asymptomatic subjects with preclinical atherosclerosis. *Atherosclerosis* 2007;**191**:115-120.

(33) Simper D, Wang S, Deb A, Holmes D, McGregor C, Frantz R *et al*. Endothelial progenitor cells are decreased in blood of cardiac allograft patients with vasculopathy and endothelial cells of noncardiac origin are enriched in transplant atherosclerosis. *Circulation* 2003;**108**:143-149.

(34) Fadini GP, Sartore S, Albiero M, Baesso I, Murphy E, Menegolo M *et al*. Number and function of endothelial progenitor cells as a marker of severity for diabetic vasculopathy. *Arterioscler Thromb Vasc Biol* 2006;**26**:2140-2146.

(35) George J, Herz I, Goldstein E, Abashidze S, Deutch V, Finkelstein A *et al*. Number and adhesive properties of circulating endothelial progenitor cells in patients with in-stent restenosis. *Arterioscler Thromb Vasc Biol* 2003;**23**:e57-e60.

(36) Guven H, Shepherd RM, Bach RG, Capoccia BJ, Link DC. The number of endothelial progenitor cell colonies in the blood is increased in patients with angiographically significant coronary artery disease. *J Am Coll Cardiol* 2006;**48**:1579-1587.

(37) Vasa M, Fichtlscherer S, Adler K, Aicher A, Martin H, Zeiher AM *et al*. Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. *Circulation* 2001;**103**:2885-2890.

(38) Hagensen MK, Shim J, Thim T, Falk E, Bentzon JF. Circulating endothelial progenitor cells do not contribute to plaque endothelium in murine atherosclerosis. *Circulation* 2010;**121**:898-905.

(39) Hagensen MK, Shim J, Falk E, Bentzon JF. Flanking recipient vasculature, not circulating progenitor cells, contributes to endothelium and smooth muscle in murine allograft vasculopathy. *Arterioscler Thromb Vasc Biol* 2011;**31**:808-813.

(40) Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M *et al*. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res* 1999;**85**:221-228.

(41) Sata M, Saiura A, Kunisato A, Tojo A, Okada S, Tokuhisa T *et al*. Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis. *Nat Med* 2002;**8**:403-409.

(42) Walter DH, Rittig K, Bahlmann FH, Kirchmair R, Silver M, Murayama T *et al*. Statin therapy accelerates reendothelialization: a novel effect involving mobilization and incorporation of bone marrow-derived endothelial progenitor cells. *Circulation* 2002;**105**:3017-3024.

(43) Werner N, Priller J, Laufs U, Endres M, Bohm M, Dirnagl U *et al*. Bone marrow-derived progenitor cells modulate vascular reendothelialization and neointimal formation: effect of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibition. *Arterioscler Thromb Vasc Biol* 2002;**22**:1567-1572.

(44) Hillebrands JL, Klatter FA, van Dijk WD, Rozing J. Bone marrow does not contribute substantially to endothelial-cell replacement in transplant arteriosclerosis. *Nat Med* 2002;**8**:194-195.

(45) Hu Y, Davison F, Zhang Z, Xu Q. Endothelial replacement and angiogenesis in arteriosclerotic lesions of allografts are contributed by circulating progenitor cells. *Circulation* 2003;**108**:3122-3127.

(46) Xu Q, Zhang Z, Davison F, Hu Y. Circulating progenitor cells regenerate endothelium of vein graft atherosclerosis, which is diminished in ApoE-deficient mice. *Circ Res* 2003;**93**:e76-e86.

(47) Werner N, Nickenig G. Influence of cardiovascular risk factors on endothelial progenitor cells: limitations for therapy? *Arterioscler Thromb Vasc Biol* 2006;**26**:257-266.

(48) Silvestre JS, Gojova A, Brun V, Potteaux S, Esposito B, Duriez M *et al*. Transplantation of bone marrow-derived mononuclear cells in ischemic apolipoprotein E-knockout mice accelerates atherosclerosis without altering plaque composition. *Circulation* 2003;**108**:2839-2842.

(49) George J, Afek A, Abashidze A, Shmilovich H, Deutsch V, Kopolovich J *et al*. Transfer of endothelial progenitor and bone marrow cells influences atherosclerotic plaque size and composition in apolipoprotein E knockout mice. *Arterioscler Thromb Vasc Biol* 2005;**25**:2636-2641.

(50) Zoll J, Fontaine V, Gourdy P, Barateau V, Vilar J, Leroyer A *et al*. Role of human smooth muscle cell progenitors in atherosclerotic plaque development and composition. *Cardiovasc Res* 2008;**77**:471-480.

(51) Rauscher FM, Goldschmidt-Clermont PJ, Davis BH, Wang T, Gregg D, Ramaswami P *et al*. Aging, progenitor cell exhaustion, and atherosclerosis. *Circulation* 2003;**108**:457-463.

(52) Langheinrich AC, Kampschulte M, Buch T, Bohle RM. Vasa vasorum and atherosclerosis - Quid novi? *Thromb Haemost* 2007;**97**:873-879.

(53) Moreno PR, Purushothaman KR, Fuster V, Echeverri D, Truszczynska H, Sharma SK *et al*. Plaque neovascularization is increased in ruptured atherosclerotic lesions of human aorta: implications for plaque vulnerability. *Circulation* 2004;**110**:2032-2038.

(54) Quaini F, Urbanek K, Beltrami AP, Finato N, Beltrami CA, Nadal-Ginard B *et al*. Chimerism of the transplanted heart. *N Engl J Med* 2002 January 3;**346**:5-15.

(55) Mitchell RN, Libby P. Vascular remodeling in transplant vasculopathy. *Circ Res* 2007;**100**:967-978.

(56) Xu Q. Stem cells and transplant arteriosclerosis. Circ Res 2008;**102**:1011-1024.

(57) Asahara T, Bauters C, Pastore C, Kearney M, Rossow S, Bunting S *et al*. Local delivery of vascular endothelial growth factor accelerates reendothelialization and attenuates intimal hyperplasia in balloon-injured rat carotid artery. *Circulation* 1995;**91**:2793-2801.

(58) Libby P, Schwartz D, Brogi E, Tanaka H, Clinton SK. A cascade model for restenosis. A special case of atherosclerosis progression. *Circulation* 1992;**86**:III47-III52.

(59) Iwakura A, Luedemann C, Shastry S, Hanley A, Kearney M, Aikawa R *et al*. Estrogen-mediated, endothelial nitric oxide synthase-dependent mobilization of bone marrow-derived endothelial progenitor cells contributes to reendothelialization after arterial injury. *Circulation* 2003;**108**:3115-3121.

(60) Lin HH, Chen YH, Yet SF, Chau LY. After vascular injury, heme oxygenase-1/carbon monoxide enhances re-endothelialization via promoting mobilization of circulating endothelial progenitor cells. *J Thromb Haemost* 2009;**7**:1401-1408.

(61) Ii M, Takenaka H, Asai J, Ibusuki K, Mizukami Y, Maruyama K *et al*. Endothelial progenitor thrombospondin-1 mediates diabetes-induced delay in reendothelialization following arterial injury. *Circ Res* 2006;**98**:697-704.

(62) Tsuzuki M. Bone marrow-derived cells are not involved in reendothelialized endothelium as endothelial cells after simple endothelial denudation in mice. *Basic Res Cardiol* 2009;**104**:601-611.

(63) Itoh Y, Toriumi H, Yamada S, Hoshino H, Suzuki N. Resident endothelial cells surrounding damaged arterial endothelium reendothelialize the lesion. *Arterioscler Thromb Vasc Biol* 2010;**30**:1725-1732.

(64) Sorrentino SA, Bahlmann FH, Besler C, Muller M, Schulz S, Kirchhoff N *et al*. Oxidant stress impairs in vivo reendothelialization capacity of endothelial progenitor cells from patients with type 2 diabetes mellitus: restoration by the peroxisome proliferator-activated receptor-gamma agonist rosiglitazone. *Circulation* 2007;**116**:163-173.

(65) Hagensen MK, Raarup MK, Mortensen MB, Thim T, Nyengaard JR, Falk E *et al*. Circulating Endothelial Progenitor Cells Do Not Contribute to Regeneration of Endothelium after Murine Arterial Injury. *Cardiovasc Res* 2011;**93**:223-231.

(66) Ii M, Nishimura H, Iwakura A, Wecker A, Eaton E, Asahara T *et al*. Endothelial progenitor cells are rapidly recruited to myocardium and mediate protective effect of ischemic preconditioning via "imported" nitric oxide synthase activity. *Circulation* 2005;**111**:1114-1120.

(67) Bauer SM, Goldstein LJ, Bauer RJ, Chen H, Putt M, Velazquez OC. The bone marrow-derived endothelial progenitor cell response is impaired in delayed wound healing from ischemia. *J Vasc Surg* 2006;**43**:134-141.

(68) Murayama T, Tepper OM, Silver M, Ma H, Losordo DW, Isner JM *et al*. Determination of bone marrow-derived endothelial progenitor cell significance in angiogenic growth factor-induced neovascularization in vivo. *Exp Hematol* 2002;**30**:967-972.

(69) Perin EC, Willerson JT, Pepine CJ, Henry TD, Ellis SG, Zhao DX *et al*. Effect of Transendocardial Delivery of Autologous Bone Marrow Mononuclear Cells on Functional Capacity, Left Ventricular Function, and Perfusion in Chronic Heart Failure: The FOCUS-CCTRN Trial. *JAMA* 2012 March 24.

(70) Lipinski MJ, Biondi-Zoccai GG, Abbate A, Khianey R, Sheiban I, Bartunek J *et al*. Impact of intracoronary cell therapy on left ventricular function in the setting of acute myocardial infarction: a collaborative systematic review and meta-analysis of controlled clinical trials. *J Am Coll Cardiol* 2007;**50**:1761-1767.

(71) Losordo DW, Henry TD, Davidson C, Sup LJ, Costa MA, Bass T *et al*. Intramyocardial, autologous CD34+ cell therapy for refractory angina. *Circ Res* 2011;**109**:428-436.

(72) Miyamoto Y, Suyama T, Yashita T, Akimaru H, Kurata H. Bone marrow subpopulations contain distinct types of endothelial progenitor cells and angiogenic cytokine-producing cells. *J Mol Cell Cardiol* 2007;**43**:627-635.

(73) Di SS, Yang Z, Wyler von BM, Voelzmann J, Diehm N, Baumgartner I *et al*. Novel cell-free strategy for therapeutic angiogenesis: in vitro generated conditioned medium can replace progenitor cell transplantation. *PLoS One* 2009;**4**:e5643.

(74) Ziebart T, Yoon CH, Trepels T, Wietelmann A, Braun T, Kiessling F *et al*. Sustained persistence of transplanted proangiogenic cells contributes to neovascularization and cardiac function after ischemia. *Circ Res* 2008;**103**:1327-1334.

(75) Yoon CH, Koyanagi M, Iekushi K, Seeger F, Urbich C, Zeiher AM *et al*. Mechanism of improved cardiac function after bone marrow mononuclear cell therapy: role of cardiovascular lineage commitment. *Circulation* 2010;**121**:2001-2011.

(76) Rehman J, Li J, Orschell CM, March KL. Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation* 2003;**107**:1164-1169.

(77) Rohde E, Malischnik C, Thaler D, Maierhofer T, Linkesch W, Lanzer G *et al*. Blood monocytes mimic endothelial progenitor cells. *Stem Cells* 2006;**24**:357-367.

(78) Schmeisser A, Garlichs CD, Zhang H, Eskafi S, Graffy C, Ludwig J *et al*. Monocytes coexpress endothelial and macrophagocytic lineage markers and form cord-like structures in Matrigel under angiogenic conditions. *Cardiovasc Res* 2001;**49**:671-680.

(79) Horrevoets AJ. Angiogenic monocytes: another colorful blow to endothelial progenitors. *Am J Pathol* 2009 May;**174**:1594-1596.

(80) Bentzon JF, Falk E. Circulating smooth muscle progenitor cells in atherosclerosis and plaque rupture: current perspective and methods of analysis. *Vascul Pharmacol* 2010;**52**:11-20.

(81) Purhonen S, Palm J, Rossi D, Kaskenpaa N, Rajantie I, Yla-Herttuala S *et al*. Bone marrow-derived circulating endothelial precursors do not contribute to vascular endothelium and are not needed for tumor growth. *Proc Natl Acad Sci U S A* 2008;**105**:6620-6625.

(82) Choi JH, Do Y, Cheong C, Koh H, Boscardin SB, Oh YS *et al*. Identification of antigen-presenting dendritic cells in mouse aorta and cardiac valves. *J Exp Med* 2009;**206**:497-505.

(83) Jongstra-Bilen J, Haidari M, Zhu SN, Chen M, Guha D, Cybulsky MI. Low-grade chronic inflammation in regions of the normal mouse arterial intima predisposed to atherosclerosis. *J Exp Med* 2006;**203**:2073-2083.

(84) Pusztaszeri MP, Seelentag W, Bosman FT. Immunohistochemical expression of endothelial markers CD31, CD34, von Willebrand factor, and Fli-1 in normal human tissues. *J Histochem Cytochem* 2006;**54**:385-395.

(85) Yamamoto K, de W, V, Fearns C, Loskutoff DJ. Tissue distribution and regulation of murine von Willebrand factor gene expression in vivo. *Blood* 1998;**92**:2791-2801.

(86) Aicher A, Rentsch M, Sasaki K, Ellwart JW, Fandrich F, Siebert R *et al*. Nonbone marrow-derived circulating progenitor cells contribute to postnatal neovascularization following tissue ischemia. *Circ Res* 2007;**100**:581-589.

(87) Ruifrok WP, de Boer RA, Iwakura A, Silver M, Kusano K, Tio RA *et al*. Estradiol-induced, endothelial progenitor cell-mediated neovascularization in male mice with hind-limb ischemia. *Vasc Med* 2009;**14**:29-36.

(88) De PM, Venneri MA, Roca C, Naldini L. Targeting exogenous genes to tumor angiogenesis by transplantation of genetically modified hematopoietic stem cells. *Nat Med* 2003;**9**:789-795.

(89) De PM, Venneri MA, Galli R, Sergi SL, Politi LS, Sampaolesi M *et al*. Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. *Cancer Cell* 2005;**8**:211-226.

(90) Fernandez PB, Lucibello FC, Gehling UM, Lindemann K, Weidner N, Zuzarte ML *et al*. Endothelial-like cells derived from human CD14 positive monocytes. *Differentiation* 2000;**65**:287-300.

(91) Sirker AA, Astroulakis ZM, Hill JM. Vascular progenitor cells and translational research: the role of endothelial and smooth muscle progenitor cells in endogenous arterial remodelling in the adult. *Clin Sci (Lond)* 2009;**116**:283-299.

(92) Hirschi KK, Ingram DA, Yoder MC. Assessing identity, phenotype, and fate of endothelial progenitor cells. *Arterioscler Thromb Vasc Biol* 2008;**28**:1584-1595.

(93) Urbich C, Aicher A, Heeschen C, Dernbach E, Hofmann WK, Zeiher AM *et al*. Soluble factors released by endothelial progenitor cells promote migration of endothelial cells and cardiac resident progenitor cells. *J Mol Cell Cardiol* 2005;**39**:733-742.

(94) Yang Z, von Ballmoos MW, Faessler D, Voelzmann J, Ortmann J, Diehm N. Paracrine factors secreted by endothelial progenitor cells prevent oxidative stress-induced apoptosis of mature endothelial cells. *Atherosclerosis* 2010;**211**:103-109.

(95) Di SS, Yang Z, Wyler von BM, Voelzmann J, Diehm N, Baumgartner I *et al*. Novel cell-free strategy for therapeutic angiogenesis: in vitro generated conditioned medium can replace progenitor cell transplantation. *PLoS One* 2009;**4**:e5643.

(96) Thim T, Hagensen MK, Bentzon JF, Falk E. From vulnerable plaque to atherothrombosis. *J Intern Med* 2008;**263**:506-516.

FIGURE LENGENDS

Figure 1. Until the first reports of EPCs in the late 1990s, it was generally assumed that endothelial repair in arterial disease was exclusively due to proliferation and migration of local ECs. By contrast the EPC theory states that EPCs in the circulating blood contribute to endothelial repair by differentiating into ECs. This theory is illustrated here in the context of endothelial turnover in an atherosclerotic plaque. Revised from Thim et al.[96]

Figure 2. Plaque ECs are derived from the local arterial wall. Revised from Hagensen et al.[38] a. Experiment to investigate whether or not BM-derived EPCs contribute to plaque ECs during atherogenesis, An aortic root plaque from an $apoE^{-/-}$ mouse transplanted with BM from an $eGFP^{+}apoE^{-/-}$ mouse. No $eGFP^{+}vWF^{+}$ double-positive cells are present. b. Experiment to investigate whether or not any types of bloodborne EPCs contribute to plaque ECs during atherogenesis. A common carotid artery (CCA) segment from an $apoE^{-/-}$ mouse was orthotopic transplanted into $eGFP^{+}apoE^{-/-}$ mice (isotransplantation except for the eGFP transgene). None of the vWF^{+} cells are $eGFP^{+}$. Green indicates eGFP; red, vWF; blue, nuclei; gray, DIC; L, lumen; BM, bone marrow; AA, aortic arch; CCA, common carotid artery; TCCA, transplanted common carotid artery. Scale bars=50μm.

Figure 3. Endothelium in allograft vasculopathy (AV) lesions in a murine model is derived from ECs migrating into the lesion from the adjacent arterial wall. Revised from Hagensen et al.[39] To locate the source of the recipient-derived cells, carotid artery segments (1.TCCA) were first isografted from apoE$^{-/-}$ B6 mice into eGFP^{+}apoE$^{-/-}$ B6 mice (a) or from eGFP^{+}apoE$^{-/-}$ B6 mice into apoE$^{-/-}$ B6 mice (b). Four weeks later, the isograft was transected, and a BALB/c carotid allograft was inserted end-to-end (2.TCCA). a. No co-localization of eGFP^{+} and vWF^{+} to individual cells was identified. The recipient-derived eGFP^{+} cells were most likely macrophages. b. Almost all vWF^{+} cells were also eGFP^{+}. Green indicates eGFP; red, vWF; blue, nuclei; gray, DIC; L, lumen; CCA, common carotid artery; TCCA, transplanted common carotid artery. Scale bars=25μm.

FIGURES

Figure 1.

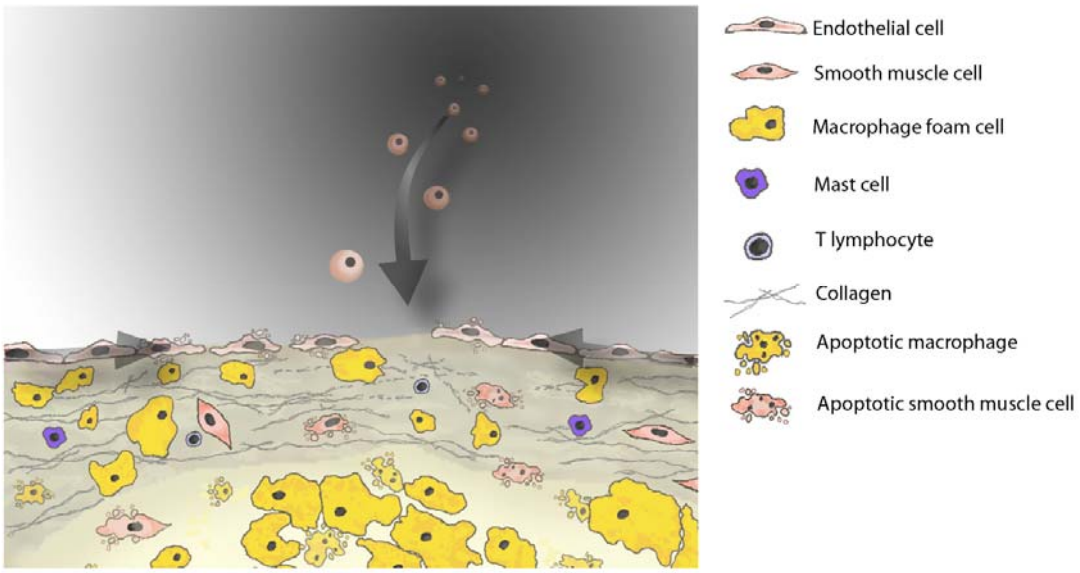

Figure 2.

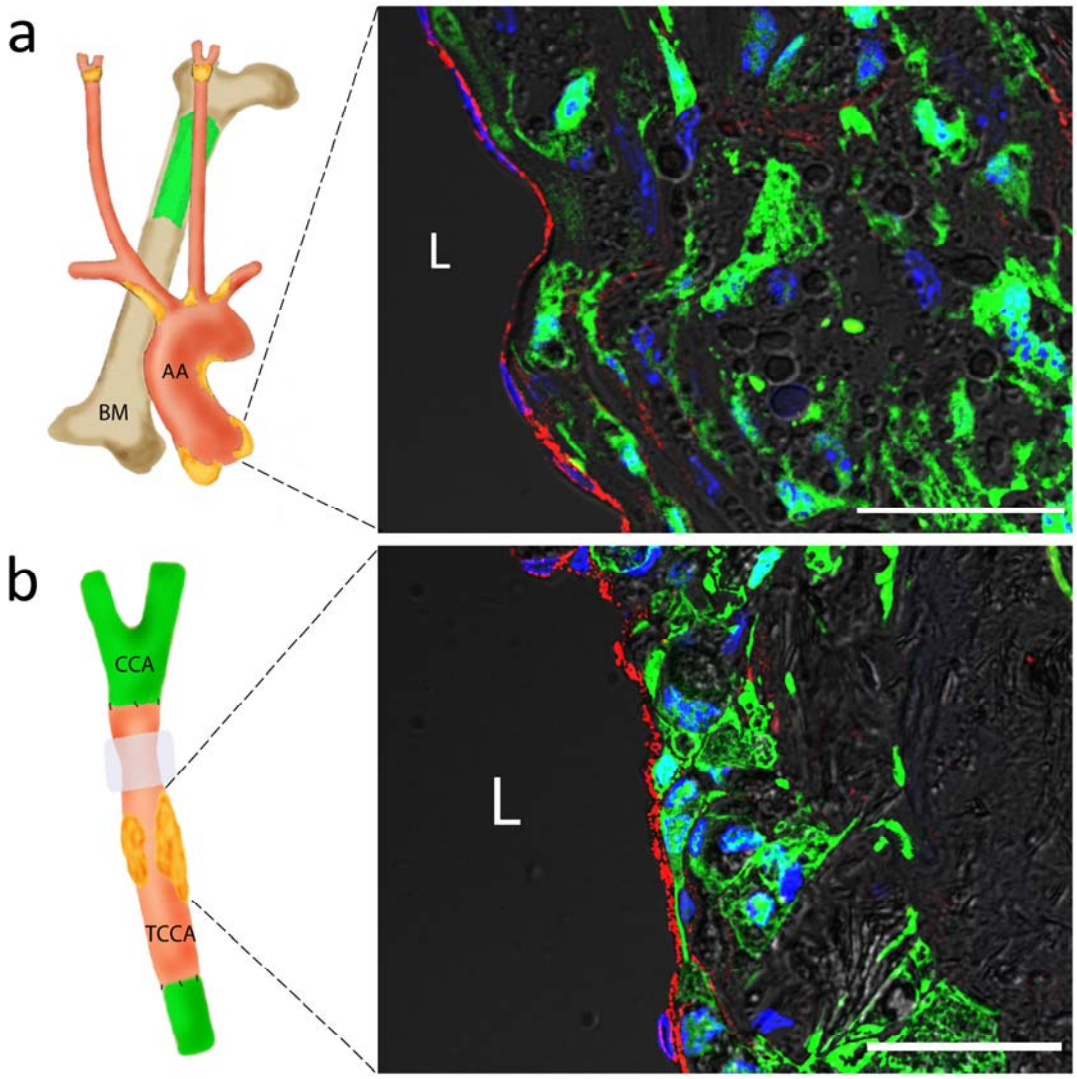

Figure 3.