Smoking and Endothelial Progenitor Cells: A Revision of Literature

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Abstract: Accumulating evidence indicates that circulating endothelial progenitor cells (EPCs) derived from bone marrow contribute to reendothelialization of injured vessels as well as neo-vascularization of ischemic lesions in either a direct or an indirect way. Moreover, the number and/or the functional activity of EPCs are inversely correlated with risk factors for cardiovascular disease. Among the different risk factors, cigarette smoking is a major cause of reducing the numbers and function of circulating EPCs.

This review is a revision of recent literature on EPC alteration associated with smoking. In particular, we show the recent observation on the effects of active and second hand smoke (SHS) exposure on EPC number and functional activity. This review also considers the effects of nicotine and other smoke compounds on EPC number and activity, in *in vitro* and *in vivo* models.

Keywords: Smoking, endothelial progenitor cells, nitric oxide, nicotine.

INTRODUCTION

In 1997, Asahara *et al*. [1] demonstrated for the first time that peripheral blood of adult humans contains bone marrow-derived progenitor cells with properties similar to those of embryonal angioblasts. These precursor cells have the potential to differentiate into mature endothelial cells and therefore they have been termed endothelial progenitor cells (EPCs). Recent studies in animals and humans suggest the effective contribution of EPCs to reendothelialization and neo-vascularization [2] by both induction and modulation of vasculogenesis and angiogenesis in areas with reduced oxygen supply or by stimulating the reendothelialization of injured blood vessels [3].

The purpose of this review is to describe the relationship between EPCs and smoking compounds.

ENDOTHELIAL PROGENITOR CELLS

EPCs were defined as non-endothelial cells that show clonal expression (the ability of a single cell to multiply), stemness characteristics (proliferative capacity and resistance to stress) and capability to differentiate into endothelial cells [4]. EPCs can be broadly subdivided into 3 inter-related classes. One class of EC precursors is cells that are likely to be hemangioblasts. In humans this includes $CD133^+$, $CD34^+$, and vascular endothelial growth factor receptor-2^+ ($VEGFR\text{-}2^+$) cells from bone marrow and blood. Functional and biochemical analysis of EPCs isolated by culturing peripheral blood mononuclear cells established two sub-populations of cells with endothelial phenotype: early and late EPCs, with different morphology, proliferation rate, survival behaviours, gene expression profiles, secreting activity [5], leading to different function *in vitro*. Early EPCs appear within 4–7 days of culture, show a limited proliferating potential for long term culture and disappear after 2 weeks in *in vitro* conditions. Furthermore, early EPCs have an elongated and spindle shape, express both endothelial and monocytic markers (CD31; von Willebrand Factor, vWF; vascular endothelial-cadherin, VE-cadherin; kinase-derived receptor, KDR; CD31) [6] and release proangiogenic growth factors, such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF), resulting in an enhanced angiogenesis [5, 6].

Late EPCs develop after 2-3 weeks after plating and show cobblestone appearance similar to mature endothelial cells, expressing only endothelial markers [7]. They show long life span and rapidly replicate from several cells to a colony and become a monolayer with almost full confluence.

Despite such differences, they equally contribute to neovasculogenesis *in vivo*, because early EPCs secrete angiogenic cytokines that may activate adjacent endothelial cells and enhance angiogenesis, whereas late EPCs only supply a sufficient number of endothelial cells based on their high proliferation rate [8].

Numerous studies correlated the concentration of $CD34^+$, $CD34^+/VEGFR\text{-}2^+$, or $CD34^+/CD133^+/VEGFR\text{-}2^+$ cells with the risk of adverse cardiovascular outcomes, and in general, an inverse correlation with each of these subsets and the highest risk category exists [9, 10].

Circulating EPCs home to sites of ischemia and vascular injury as a repair mechanism to damaged endothelium and contribute to reendothelialization and neovascularization [4]. EPC-mediated vascular repair has been shown to be associated with normalization of endothelial function and restoration of blood flow at the site of injury. Indeed, numerical and functional impairment of EPCs has been linked to endothelial dysfunction [11], increased atherosclerotic disease risk [12] and higher cardiovascular [10] and cerebrovascular morbidity and mortality.

Numerous studies have demonstrated that the systemic administration of EPCs improves endothelial dysfunction, reduces neointima formation after endothelial denudation and impairs atherosclerotic lesion progression in mice [13-15]. However, a potential limitation for the use of autologous cells is the decline in the number and function of stem cells. This outcome is seen particularly in patients with CAD, diabetes and severe heart failure [16].

EPCS AND CARDIOVASCULAR RISK FACTORS

Endothelial dysfunction is a common link for all vascular risk factors although some of these are able to induce changes early and with a major intensity.

Numerous clinical studies demonstrated a close association between the degree of endothelial dysfunction and the risk of vascular events [12]. On the other hand, risk factors for athero-

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1381-6128/10 $55.00+.00

sclerosis, such as diabetes [17], ageing [18], smoking [19], hypercholesterolemia [20], obesity [21], and hypertension [22], interfere with endothelial response, promoting endothelial dysfunction and atherosclerosis. A common feature of both endothelial dysfunction and atherosclerosis might be oxidative stress [23]. When vascular risk factors are corrected, the endothelial damage may be reversed or continued [24].

The number and/or the functional activity of circulating EPCs seem to be inversely correlated with risk factors for cardiovascular disease [11, 25], such as age [26], diabetes [27], hypertension [28], smoking [29], family history for CAD [30] , dyslipidemia [31], physical inactivity [32], as well as the overall number of risk factors. In young, healthy subjects, the ability of EPCs to form CFU correlates with flow-mediated brachial artery reactivity [11], and in patients with CAD a negative correlation of EPC with the severity of CAD, was demonstrated [30]. Moreover, individual risk factors seem to differentially affect the number and migratory capacity of EPCs [25]. Risk factors have all been shown to be associated with impaired function of mature ECs. Therefore, the impairment of circulating EPCs may contribute to an insufficient regeneration of the endothelium, leading to endothelial dysfunction.

It was demonstrated that reduced numbers of EPCs predict future cardiovascular events and proposed that low EPCs number and functionality reflect an impaired endogenous repair capacity [9, 30]. Although the close correlation of EPC numbers to cardio-vascular mortality suggests fundamental importance, the exact pathophysiological role of these cells in humans remains unclear.

Among different risk factors, Vasa *et al.* [25] demonstrated that cigarette smoking is the major factor, which contributes to reduced numbers of circulating EPCs, but did not have a significant effect on EPC migration.

However, other studies failed to obtain such evidence and some even reported the opposite. For example, Xiao *et al.* [33] showed that EPC number did not significantly differ between subjects with and without a history of previous CAD.

Such discrepancies may be due to different methods used to characterise and quantify putative EPCs, and may be explained by differences in study design, study population and risk factor levels [34]. Indeed, recently, Jung *et al.* [35] observed that only overweight in adolescents influences EPCs in early life, whereas, all other classical risk factors (smoking, hypertension, cholesterol, etc) failed to predict the number of EPCs.

CIGARETTE SMOKING

In developed countries, cigarette smoking is the leading modifiable risk factor associated with premature death caused by pathological alterations of different body organs. Smokers die on average 8 years earlier than non-smokers, and this increased mortality is mainly attributed to the promoting effect of cigarette smoke exposure on the incidence of ischemic vascular diseases [36]. Although the association between smoking and atherosclerosis is well established, the precise mechanisms involved are not completely understood.

Cigarette smoking has previously been shown to be associated with oxidative stress [37], a potential mediator of endothelial dysfunction [38] and with increased blood thrombogenicity [39] and inflammatory response[40] which are characteristics of endothelial dysfunction, Fig. (**1**).

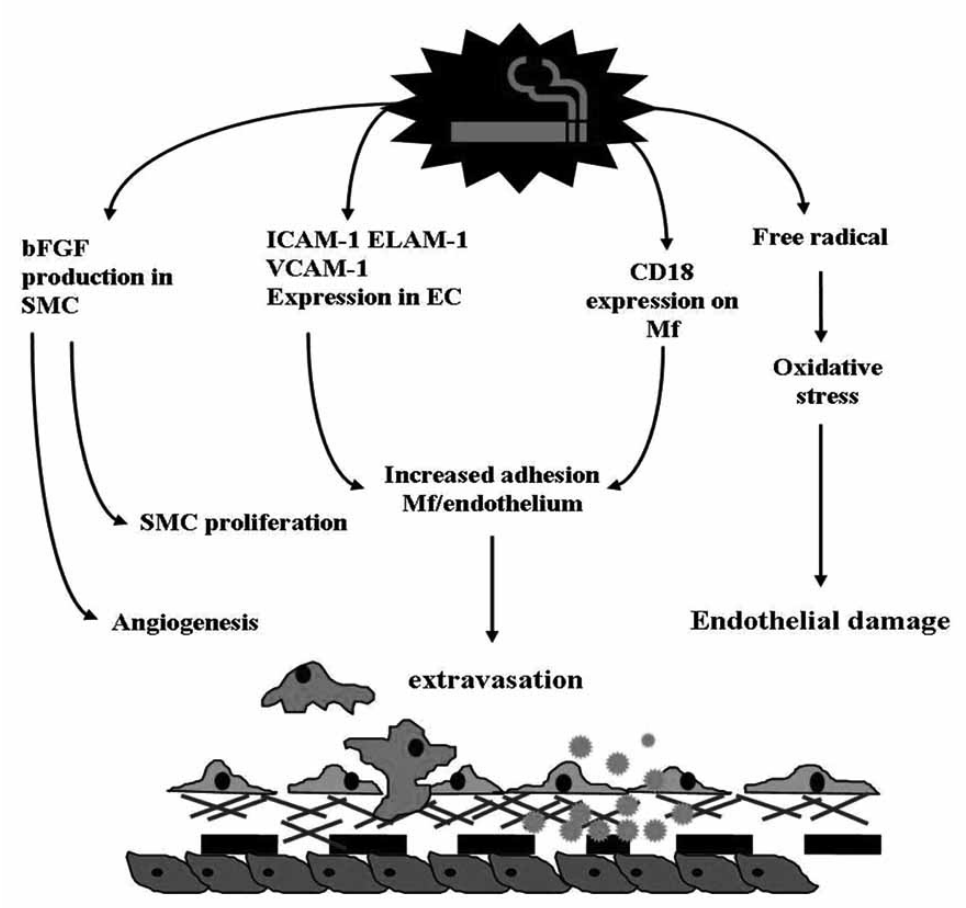

Fig. (1). Cigarette smoking effects. Cigarette smoking is associated with oxidative stress, thrombogenicity and inflammatory response which are characteristics of endothelial dysfunction. SMC= smooth muscle cell; Mf= macrophage; EC= endothelial cell.

Vascular endothelium and cigarette smoking are linked through a negative response that leads to formation of an atherosclerotic plaque as a final result of their interaction. However, there is an important difference between active or passive smokers in terms of endothelial changes. In active smokers, it is hard to assess the initial steps of endothelial damage since usually there are advanced alterations caused by several factors (e.g. platelet activation, lipid changes and inflammation) which are superimposed on the changes associated with endothelial dysfunction strongly related to passive exposure (Table **1**). Active smokers can display endothelial dysfunction when studied immediately after smoking [41, 42] although even these observations are influenced by other damaging factors, particularly platelet function and morphology changes. In contrast, passive smoking exposure documents the initial changes in endothelial function.

Table 1. Changes Caused by Cigarette Compounds on Vascular Endothelium

1. Functional changes	Impaired nitric oxide (NO) release Impaired flow-mediated dilation Vasoconstriction
2. Structural changes	Endothelial activation Platelet function changes Inflammatory responses Coagulation-fibrinolysis cascade activation Atherosclerotic plaque/thrombi formation
3. Combined functional and structural changes	

The toxic effects of passive smoking are capable of causing endothelial dysfunction usually followed by structural alterations even after a brief exposure (hours or minutes) that are similar to those caused by active smoking [43]. Moreover, endothelial changes occur early with initial exposure. However, endothelial disorders are usually transient after the first exposure to passive smoking [41, 44-47].

The main compounds of environmental tobacco, particularly nicotine and carbon monoxide (CO), determine endothelial cell activation by triggering a mechanism of atherosclerosis originally functional but later structural [48].

Nicotine plays a strong role in causing atherosclerosis not only by endothelial changes but also by a several anatomical and metabolic alterations like platelet aggregation, blood coagulation-fibrinolysis cascade activation, lipid metabolism and EPC changes [49-51].

Nicotine has a highly debated effect on cell proliferation and tissue healing. In fact, while cigarette smoke has been shown to decrease wound healing [52-54], recent studies have documented the effect of topical nicotine on improving dermal wounds [51, 55, 56].

As nicotine's effect in wound healing is at least mediated in part through the modulation of angiogenesis [57, 58], ischemic or infarct tissues may benefit from nicotine's ability to stimulate new blood vessel formation [59]. However, while its beneficial effects are still under investigation, important findings regarding nicotine's acceleration of atherosclerosis, tumor angiogenesis, cell proliferation and resistance to apoptosis put its systemic use into question.

A direct activity of CO [60, 61] capable to induce a series of changes which recognize either a functional mechanism or later structural changes should be considered when endothelial functional disorders due to passive smoking exposure are analyzed.

The main mechanisms by which exposure to passive smoking induce endothelial function changes consist of reduced availability in vasodilator metabolites, particularly NO, although hypoxia caused by increased carboxyhaemoglobin levels and sympathetic-adrenergic stimulation also play a role influencing other cardiac parameters related to heart function [41].

Thus, heart rate variability significantly changes following passive smoking exposure [62]. Heart rate variability is measured by analyzing beat to beat variations that can be identified by variations in R–R interval on the ECG. Impaired heart rate variability may trigger malignant arrhythmias as well as sudden cardiac death [43, 62]. Barnoya and Glantz [43] also affirmed that 2 h exposure to passive smoking was associated with a 12% reduction in heart rate variability and this reduction could be associated with an increased risk of ventricular fibrillation or tachycardia. Restoration of baseline heart rate variability could be recorded 2 h after exposure. Malignant ventricular arrhythmias with possible reproducibility of themselves have been shown to characterize acute passive smoking exposure especially for those subjects with a pre-existing myocardial infarction. Moreover, these problems are a close consequence of elevated catecholamine release also in children [19, 41-43].

CO follows usually 2 ways to damage vascular endothelium: it may activate catecholamine release and, then, induce functional disorders of the cardiovascular system; moreover, it can exert a directly toxic influence [61]. Usually, however, the latter influence more often causes anatomical changes than functional disorders. Mechanisms by which CO causes functional disorders are not yet fully known. Probably, functional stimulation is often the result of combined direct action on endothelium and activation of the adrenergic system with accompanying endothelial dysfunction. However, hypoxia can be the factor capable of triggering functional alterations since it particularly activates the adrenergic system and changes endothelial metabolite concentrations [61].

EC activation is a very important factor of functional disorders. As mentioned above, physiologically endothelial function [63] is the result of a balanced response between vasodilator chemicals as NO and vasoconstrictors as endothelin. Decreased release of NO following different stimuli, and consequently the prevailing of vasoconstrictor response, cause endothelial dysfunction, an important marker of early vascular damage that leads to atherosclerosis.

Passive smoking can induce endothelial dysfunction even after acute exposure [44-46]. Celermajer *et al*. [47] compared endothelial function of the arteries in 3 groups of healthy individuals. The first group included active smokers, the second group lifelong non-smokers who were exposed to environmental tobacco smoke, and the third group consisted of subjects exposed irregularly to passive smoke. Effective exposure to passive smoking was considered of at least 1 h per day anywhere (i.e. home, work or both environments). Endothelial function was assessed by vascular ultrasonography measuring brachial arterial vascular reactivity. Passive smokers had significantly impaired arterial endothelial function with reduced NO release. Furthermore, impaired availability of NO can stimulate platelet aggregation [64, 65] that initiates vascular damage leading to atherosclerosis.

Usually, functional disorders of the cardiovascular system following passive smoking exposure have been described as an effect of either isolated catecholamine stimulation or endothelial dysfunction alone. There is evidence that functional disorders are usually the consequence of a complex synergy of these 2 mechanisms capable of exerting their action separately but, at the same time, potentiating their effects by the relationship that links them. In re-

sponse to epinephrine, the endothelium [43] releases endothelin that causes vasoconstriction; then, vasoconstriction induces endothelial dysfunction that, otherwise, could be caused directly by some components of passive smoking. Functional disorders from passive smoking exposure are the result of at least 3 different factors which interact: adrenergic system stimulation, endothelial dysfunction, and endothelial dysfunction induced by catecholamine release. Passive smoking may act on 2 main levels: catecholamine release and endothelial dysfunction.

Functional changes caused by passive smoking tend to improve slightly after a long-term smoking exposure cessation – usually at least 1 year – especially those manifestations related to endothelial dysfunction. Endothelium-dependent vasodilatation has been shown to be better in those passive smokers who had ended their exposure when they were compared with current exposed non-smokers [66] although this parameter was not fully restored in the first group.

It is worth noting that functional disorders characterized by endothelial dysfunction, when they are repeated and untreated, lead to the appearance of anatomically irreversible alterations which lead to atherosclerotic plaque formation.

EPCS AND SMOKING

EPC Levels in Smokers

Endothelial reactivity predicts future cardiac events, and the restoration of endothelial function by smoking cessation might imply a reduction of future cardiovascular events in chronic smokers. Hill *et al*. [11] demonstrated that endothelial reactivity and circulating EPCs are correlated, so that the measurement of circulating EPCs may predict future cardiac events.

Multivariate analysis of the individual risk factors revealed smoking as the major independent predictor for the reduction of EPCs levels [25], probably due to increased apoptosis of premature progenitor cells.

Kondo *et al.* [29] were the first to assess the number of circulating $CD45^{low}CD34^{+}$ $CD133^{+}$ (progenitor cells [PCs]) and circulating $CD45^{low}CD34^{+}CD133^{+}VEGFR2^{+}$ (EPCs) in chronic smokers and non-smoking subjects. EPCs from heavy smokers have been shown to be incapable of forming colonies and died prematurely in culture media (within 4-5 days). The number of circulating PCs/EPCs was significantly reduced in 15 chronic smokers (10 light smokers, < 20 cigarettes/day, and 5 heavy smokers, ≥ 20 cigarettes/day) compared with 14 non-smokers. In addition, they examined whether smoking cessation influenced the levels of circulating PCs/EPCs. Short-term smoking cessation (4 weeks) led to a rapid restoration of EPC counts. Moreover, this recovery was greater in light smokers compared to heavy smokers. Furthermore, they observed that the level of the increase in EPCs after smoking cessation was slightly higher among nicotine patch users than non-users. However, this difference was not significant. They proposed that smoking may affect the bone marrow environment, and PC/EPC mobilizations from the bone marrow could be decreased by smoking through the inhibition of NO release by eNOS, which plays an important role in EPCs mobilization from bone marrow [67, 68].

Recently, Heeschen *et al*. [69] demonstrated that nicotine stimulates angiogenesis at sites of ischemia. Therefore, the effect of nicotine may account for the small increase in EPC levels in nicotine patch users. However, these studies were performed in a non-smoking setting.

To understand the direct effect of smoking on EPC number and functional activities, Michaud *et al.* [70] studied the effect of smoking on EPCs in healthy subjects without symptoms associated with atherosclerosis and who did not present any other conventional cardiovascular risk factors. ROS formation was significantly increased in EPCs isolated from smokers. Although this was not associated with an increased cellular death in culture, high levels of oxidative stress in smokers could potentially influence the mobilization and/or the survival of EPCs *in vivo*. They also demonstrated that lower serum antioxidant levels in smokers correlate with reduced availability of NO and EPC functional activity. The study showed that cigarette smoking does not only influence the absolute number of EPCs, but also significantly modulates their functional activities. In fact, cellular proliferation and migration in response to VEGF were significantly impaired in EPCs isolated from smokers. Moreover, EPCs isolated from smokers had a significant reduction in the expression of the VEGF receptor KDR, which mediate VEGF-induced proliferation, migration, and angiogenesis in ECs [71]. Thus, the authors demonstrated that potential mechanisms responsible for the negative effect of smoking on EPCs include increased oxidative stress, decreased NO availability and impaired EPC differentiation towards an endothelial phenotype.

More recently, Nakamura *et al*. [72] have shown that smoking abolishes ischemic preconditioning stimulus-induced augmentation of endothelium-dependent vasodilation through a reduction of the number of circulating progenitor cells, and an impairment of their function in smokers compared with nonsmokers.

Oxidative stress is associated with reduced NO production and endothelial dysfunction following cigarette smoke exposure [73]. The involvement of oxidative stress on EPC dysfunction was confirmed by Kim *et al.* [74]. They demonstrated that reduced circulating EPC numbers and endothelial function in smokers were significantly ameliorated by consuming green tea. Green tea is a free radical scavenger and has abundant catechin, which has a powerful antioxidant action [75]. Moreover, it is known that catechins activate eNOS through the Akt-dependent pathway and improve endothelial vasorelaxation reactions [76]. The authors supposed that beneficial effects of green tea on EPCs number occur through eNOS activation and antioxidant activity of green tea.

Turgeon *et al*. [77] tested the hypothesis that antioxidant therapies, such as Probucol and antioxidant vitamins, stimulate EPC function and improve ischemia-induced neovascularization following cigarette smoke exposure. The mechanisms involve beneficial effects on oxidative stress levels in ischemic tissues together with an improvement of EPC functional activities.

The results are summarized in Table **2**.

Effects of Passive Smoke on EPCs

The effects of SHS on the cardiovascular system are remarkably similar in magnitude to those caused by chronic active smoking [43]. Although the dose of smoke delivered during exposure to SHS is 10 to 100 times lower than that delivered during active smoking, the relative risk of CAD is 1.31 in passive smokers compared with 1.78 in active light smokers [78]. Importantly, the concentration of numerous toxins has been shown to be dramatically (up to 100-fold) elevated in sidestream smoke when compared with mainstream smoke, underscoring the potential adverse impact of SHS [79]. Acute and chronic exposure of non-smokers to SHS decreases endothelial function near to the levels observed in chronic active smokers [80].

Heiss *et al.* [81] tested the hypothesis that the number and function of EPCs might also be adversely affected by acute brief SHS exposure in non-smokers. The authors tested the vascular effects of a brief (30 min) controlled exposure to SHS on EPCs in healthy non-smokers. Results showed that a brief exposure to real-world levels of SHS leads to a mobilization of dysfunctional EPCs in response to acute vascular injury that persists for more than 24 h. Moreover, they observed that, in contrast to previous studies showing persistently decreased levels of EPCs in chronic smokers [11, 25, 70], SHS exposure led to increased numbers of EPCs in circulating blood in the first 24 h. Although there were more circulating EPCs after SHS exposure, these cells showed severe functional impairment. The authors showed that *in vitro* treatment with plasma isolated after SHS exposure decreased NO production and chemo-

Table 2. Effects of Active and Second Hand Smoke on EPC Number and Functional Activity

	Changes in Number/Function of EPCs	Investigators
Active smoke	↓ EPC number	[25]
	↓ EPC number, ↓ CFU-EPCs	[29]
	↓ EPC number, ↓ EPC proliferation, migration and differentiation	[70, 72]
Second hand smoke	↑ EPC mobilization, ↓ EPC functional activity	[81]

taxis but increased proliferation of EPCs isolated from non-exposed subjects. In contrast to the results described above, in a mouse lung cancer model, second hand smoking was associated with significant increases in EPC number and an accelerated tumor angiogenesis and growth [82].

The results are summarized in Table **2**.

EFFECTS OF CIGARETTE SMOKE COMPOUNDS ON EPCS

Risk factors for CAD such as smoking are known to increase oxidative stress, a well-established stimulus for apoptotic cell death [83]. Moreover, numerous reports indicated that nicotine or smoking causes endothelial injury and thus might impair neovascularization [84]. However, recent studies have shown that nicotine increases the EC number, reduces apoptosis, and increases capillary network formation *in vitro*, as well as enhances neovascularization in different murine models [51, 85].

Tobacco smoke is a complex mixture of more than 4,000 chemical constituents, and the effect of nicotine delivered *via* the use of tobacco may be quite different. Moreover, the effect of the other constituents on EPCs is still under evaluation. Although the association between cigarette smoking and atherosclerotic vascular diseases is well established, the precise mechanisms involved, possibly also including indirect effects, are not yet completely understood.

Nicotine

Nicotine is an important constituent of cigarette smoke. Nicotine has been reported to increase proliferation and tube formation of ECs in an *in vitro* assay [69]. Investigations of the effect of nicotine on ECs indicated that nicotine may be associated with cell loss and desquamation [86], suggesting cellular toxicity in response to nicotine and a correlation among constituents of cigarette smoke with cellular injury. Given the well-established role of EPCs participating in neovascularization, Wang *et al.* [51] demonstrated a concentration-dependent effect of nicotine on EPC number and functional activities. In particular, they showed that nicotine increased EPC number and promoted EPC proliferative, migratory, adhesive, and *in vitro* vasculogenesis capacity at physiologically relevant concentrations, maximal at concentrations of nicotine (10^{-8} mol/L) similar to those in the blood of smokers (typical nicotine levels are 60-100 nmol/L). However, they observed cytotoxic effects at higher nicotine concentrations (> 10^{-6} mol/L); so there are different actions at different concentrations. The mechanisms by which nicotine increases EPC numbers and activity remains to be determined. The authors suggest that nicotine could increase the number of circulating EPCs through decreasing apoptosis of premature progenitor cells. In accordance with Wang's work, Heeschen *et al.* [85] have shown a proangiogenic effect of nicotine. They used a hind-limb ischemic murine model in their study. They observed that systemic exposure to nicotine augmented the number of EPCs in the bone marrow and spleen and that this increase is associated with a marked increase in angiogenesis in ischemic tissue. Furthermore, systemic administration was more effective than local administration of nicotine. Intramuscular administration of nicotine induced a 46% increase in neovascularization as compared with controls; local injection of nicotine did not lead to detectable cotinine levels in the peripheral blood, whereas systemic treatment with nicotine *via* the drinking water increased cotinine levels. Moreover, they showed that the angiogenic pathway was mediated by endothelial nicotinic acetylcholine receptors (nAChR).

Sugimoto *et al.* [87] investigated whether nicotine administration, i.e. using the dose of nicotine replacement therapy (approximately $2X10^{-6}$ M), improves blood flow recovery in ischemic hindlimbs *via* a favorable effect on EPC bioactivities following EPC transplantation therapy. The authors demonstrated that nicotine promoted blood flow in murine ischemic hind-limb following *ex vivo*-expanded EPC transplantation. In accordance with Wang *et al.* nicotine dose-dependently increased EPC number as well as proliferative, migratory, adhesive, and *in vitro* vasculogenesis capacity. Moreover, they showed that the antiapoptotic effect of nicotine on cultured EPCs was mediated by nAChR.

Polycyclic Aromatic Hydrocarbons

The observation that nicotine can mobilize EPCs seems to conflict with reports that human smokers have fewer circulating EPCs [25, 29]. Low levels of circulating EPCs in smokers could be caused by a number of mechanisms. Cigarette smoke contains more than 4000 known constituents, including large amounts of free radicals and prooxidants. Kondo [29] observed that attaching EPCs from heavy smokers died during the early phase of culture. This suggests that components of cigarette smoke, yet undetermined, may exert acute toxicity towards EPCs.

There are several molecules in cigarette smoke that are toxic to ECs (e.g. cadmium, ROS) [88] and may also impair EPCs.

Grevenynghe *et al.* [89] investigated the effects of polycyclic aromatic hydrocarbons (PAHs), on EPCs. Indeed these environmental contaminants, found at high levels in tobacco smoke [90], trigger deleterious cardiovascular effects [91, 92], through, at least partly, targeting vascular cells, including mature endothelial cells and smooth muscle cells [93, 94]. The results showed that *in vitro* exposure to PAHs such as benzo(α)pyrene (BP), which constitute major components of cigarette extract [95], markedly impairs the development of EPC cultures from peripheral blood mononuclear cells. In addition, the researchers evaluated the effects of various PAHs, and only 2 of 5 constituents tested were found to impair survival of EPCs. Moreover, they showed that the receptor for PAHs (AhR), is involved in PAH toxicity towards EPCs.

LDL Subfraction

Tang *et al.* [96] hypothesized that the effects of smoking on EPCs are, at least in part, lipid-mediated. They demonstrated that L5, a highly electronegative LDL subfraction present in the plasma of smoking subjects, can inhibit the differentiation of EPCs derived from circulating monocytes. In healthy non-smokers with normal lipid and glucose profiles, L5 is either absent or scanty. Moreover, L5 inhibited EPC telomerase activity, resulting in accelerated senescence. They proposed that circulating L5 of cigarette smokers

Table 3. Effects of Smoking Compounds

Compounds	Changes in Number/Function of EPCs	Investigators
Nicotine	$\leq 10^{-8}$ mol/L: ↑ EPC number, ↑ EPC proliferation, migration, adhesiveness; $>10^{-6}$ mol/L: ↓ EPC survival	[51, 87]
	↑ EPC number	[85]
Polycyclic aromatic hydrocarbons	↓ EPC survival	[89]
LDL subfraction	↓ EPC proliferation and differentiation	[96]

LDL = low density lipoprotein

activates the lectin-like oxidized LDL receptor 1 (LOX-1), which restricts Akt phosphorylation and other steps necessary to promote normal EPC maturation. L5 isolated from smoking subjects also induces EPC apoptosis in a concentration-dependent manner, whereas sustained exposure to low-dose smoker L5 inhibits EPC proliferation and differentiation without inducing apoptosis.

These findings provide a new insight into the mechanism of smoking-related EPC damage. The results on EPCs and smoking compounds are summarized in Table **3**.

CONCLUSIONS

Observations reported in this review raise some interesting questions:

Firstly, there is a strongly negative relationship between smoking, either active or passive, and the endothelium. Smoking promotes several alterations consisting of functional and structural disorders like impaired NO release, inflammatory response, platelet activation and atherosclerotic plaque and thrombus formation.

Secondly, cigarette smoking induces impairment in the repair of endothelial damage, as EPCs, irrespective of their number, are dysfunctional and possibly incapable of restoring endothelial function.

Finally, the effects of tobacco on EPCs are still incompletely known, since there are more than 4,000 smoke constituents, potentially affecting EPC biology, either directly or indirectly.

ABBREVIATIONS

BP	=	Benzo(α)pyrene
CAD	=	Coronary artery disease
CFU	=	Colony-forming units
ECs	=	Endothelial cells
eNOS	=	Endothelial nitric oxide synthase
EPCs	=	Endothelial progenitor cells
FMD	=	Flow-mediated dilation
LDL	=	Low density lipoprotein
LOX-1	=	Lectin-like oxidized low density lipoprotein receptor 1
nAchR	=	Nicotinic acetylcholine receptor
NO	=	Nitric oxide
PAD	=	Peripheral arterial disease
PAHs	=	Polycyclic aromatic hydrocarbons
ROS	=	Reactive oxygen species
SHS	=	Second hand smoke
VEGFR-2	=	Vascular endothelial growth factor receptor-2

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Received: May 11, 2010 **Accepted: June 2, 2010**