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Review

Diesel exhaust particles and endothelial cells dysfunction: An update

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ABSTRACT

Epidemiological studies have shown a consistent positive correlation between exposure to particulate matter (PM) and increased mortality largely due to increased rates of cardiovascular morbidity and mortality. Diesel exhaust particles (DEPs) are major constituents of atmospheric PM and have been shown to cause disruption of the endothelial cell monolayer integrity, thereby affecting organ functions. Endothelial cells are very active metabolic components of biological tissue that performs a number of important physiological functions. Therefore, anything that compromises the integrity and functions of the endothelium will lead to organ dysfunction and disease. This review focuses on scientific evidence that link DEP exposure to endothelial cell dysfunction in various pathophysiological conditions affecting the cardiovascular system. The various mechanisms involved in the DEP-induced endothelial cell dysfunction are also addressed together with the preventive and therapeutic approaches to overcoming these challenges.

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1. Introduction

Exposure to ambient particulate matter, a major component of air pollution, has been consistently shown in various epidemiological studies to be positively correlated with increased morbidity and mortality caused by various diseases, including ischemic heart disease (Haikerwal et al., 2015; Hankey et al., 2012) and chronic obstructive pulmonary disease (Brook et al., 2010). Air pollution could also lead to the onset of myocardial infarction, aggravates symptoms of angina and enhanced exercise-induced myocardial ischemia (Mills et al., 2007). Cumulative evidence has suggested that the largest portion of

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air-pollution-related mortality is due to cardiovascular diseases (Brook et al., 2010). Though air pollution is a very complex mixture of compounds in the gaseous and particulate phases, increasing evidence attributes the cardiovascular effects of air pollution to its particulate matter components (Brook et al., 2010). Therefore, both acute and chronic exposures to air PM aggravate cardiovascular morbidity and mortality. Acute exposure to PM has been linked with the onset of acute myocardial infarction (Mills et al., 2007), discharge of implanted automatic cardioverter defibrillators, hospitalizations for ischemic strokes, and decompensated congestive heart failure (Shah et al., 2013). Repeated exposure could lead to vascular inflammation, oxidative stress and promote atherosclerotic plaque expansion or rupture (Mills et al., 2011; Montiel-Davalos et al., 2010) (Fig. 1). The PM components of air pollution are classified into different size fractions based on their aerodynamic diameter as; PM_{10} (thoracic particles, <10 μm), $PM_{2.5-10}$ (coarse particles, 2.5 to 10 μm), $PM_{2.5}$ (fine particles, <2.5 μm) and UFP (ultrafine particles, <0.1 μm) (reviewed by Araujo and Nel, 2009) (Table 1). Because of their different sizes, these particles have different abilities to cause harmful and deleterious effects with the tendency that the smaller particle size are more favored to cause cardiovascular effects (Araujo and Nel, 2009).

Combustion has been recognized as one important source for the generation of both particulate and gaseous air pollutants (Mills et al., 2011). Several epidemiological studies have identified combustion-derived nanoparticles (CDNP) as an important component contributing to the adverse health effects of PM (Bauer et al., 2011; Mills et al., 2011; Montiel-Davalos et al., 2010; Liu et al., 2009). Several researchers have demonstrated the toxicity of the CDNP such as diesel soot (Berlo et al., 2010), welding fume (Badding et al., 2014), carbon black (Saputra et al., 2014) and nanoparticle coal fly-ash (Nadadur et al., 2009). The sources of exposure to these CDNP include workplace as in the case of welding fume and in carbon black production. CDNP has been shown to be responsible for the induction of the adverse vascular effects of diesel exhaust inhalation in a randomized, double blind crossover study involving 16 healthy volunteers in a 2 h exposure to diesel exhaust (Mills et al., 2011). CDNP could promote both chronic atherogenesis and acute atherothrombosis (Fig. 1). CDNP, like other nanoparticles, readily agglomerate and accumulate which decrease the particle size without affecting their surface areas. The insoluble CDNP can escape from the site of deposition in the lungs and translocate to the target organs via the blood (reviewed by Naseem et al., 2014).

Diesel exhaust particles is an example of CDNP-derived from the combustion of diesel oil from motor vehicle engines and various industries and are ubiquitously present in the urban ambient air and significantly contribute to the fine and ultrafine PM size fractions in urban dwellings (reviewed by Araujo and Nel, 2009). Since the endothelial cell monolayers form the inner layer that line the interior surface of blood vessels, they serve as the first point of contact with the vascular wall for toxic chemicals that could access the circulating blood. They have been widely used to characterize the responses of the microvasculature to various types of physiological stimuli and pathological stresses. Endothelial cells have also been used to elucidate the mechanisms that underlie microvascular dysfunction and various tissue injuries (reviewed by Ierssel et al., 2014). The recognition that abnormal endothelium responses to toxic chemicals in the circulating system is usually accompanied by tissue and organ dysfunction and disease, has led to intense research in developing *in situ* and *in vivo* model systems to study endothelial cell function. In this review, we examine the various *in vitro* and *in vivo* studies that have implicated DEP and its chemical constituents in endothelial cell dysfunction and their mechanisms of action in causing endothelial dysfunction. In addition, we examine the various preventive and rescue interventions that could help in maintaining the endothelial cell integrity against the adverse effect of DEP. This knowledge will contribute to our understanding of the deleterious effects of DEP-induced endothelial cell dysfunction and will help us to better understand the various points of interventions to dosify organ dysfunction and disease elicited by DEP effects on endothelial cells.

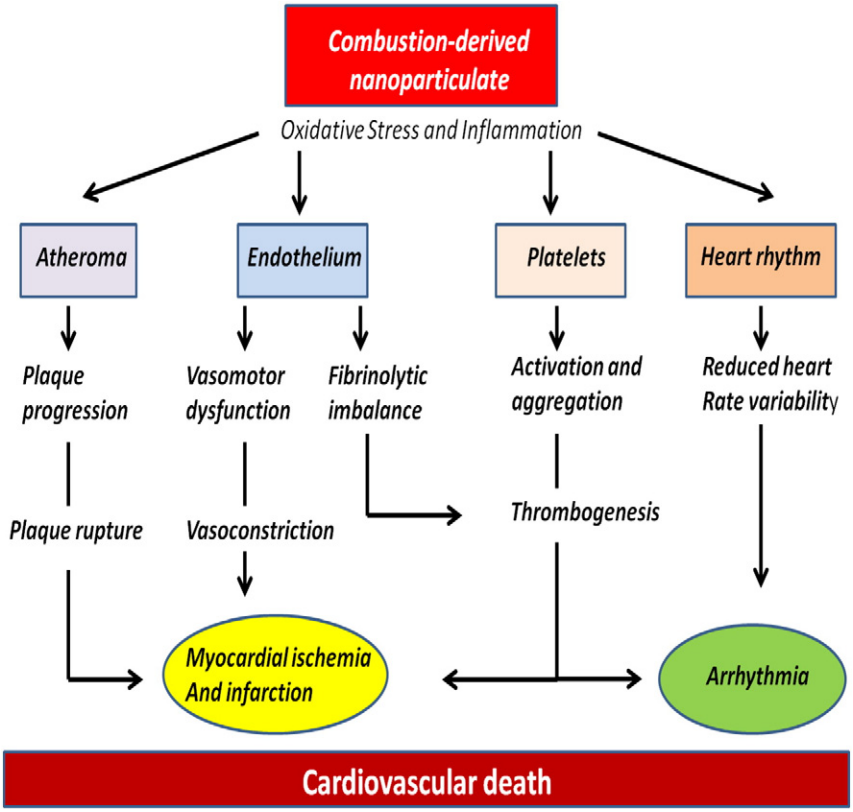

Fig. 1. The mechanism involve in the combustion-derived nanoparticulate matter induction of acute and chronic cardiovascular disease. Adapted from Mills et al., 2007.

Table 1
Particulate matter classification based on size.

Particle	Aerodynamic diameter (μm)	Sources	Mode of generation	Atmospheric half-life
Thoracic particles (PM_{10})	<10	–	–	–
Coarse particles ($PM_{2.5–10}$)	2.5–10	Disturbed soil suspension (mining, farming, unpaved roads), plant and animal fragments, construction	Mechanical disruption (crushing, grinding, abrasion of surfaces), evaporation of sprays, suspension of dusts	Minutes to hours
Fine particles ($PM_{2.5}$)	<2.5	Power plants, oil refineries, wildfires, residential fuel combustion, tailpipe and brake emissions	Gas-to-particle conversion by condensation, coagulation (accumulation mode)	Days to weeks
Ultrafine particles (UFP)	<0.1	Fuel combustion (diesel, gasoline) and tailpipe emissions from mobile sources (motor vehicles, aircraft, ships)	Fresh emissions, secondary photochemical reactions (nucleation mode)	Minutes to hours

Source: *Adapted from* Araujo and Nel, 2009.

2. DEP chemical constituents, compositions and active components

Diesel exhaust (DE) is a highly complex mixture containing thousand of compounds- fine particles, vapors and toxic organic materials, which are produced when diesel fuel is, burned (NTP and Department of Health and Human Services, 2011). The Diesel engine exhaust has been classified by the International Agency for Research on Cancer (IARC) as a human carcinogen (Group 1: carcinogenic to humans) based on human, animal, and experimental data (Benbrahim-Tallaa et al., 2012; IARC, 2012) and a potential occupational carcinogen (Vermeulen et al., 2014) and an estimate 25% of all hazardous particulate air pollution from fuel combustion comes from diesel engines. More than 40 chemicals in DE are considered toxic air contaminants (TACs) by the state of California (CARB, 2011). The toxic air contaminants in diesel exhaust include carbon monoxide, sulfur dioxide, arsenic, acetaldehyde, benzene, formaldehyde, inorganic lead, manganese compounds, mercury compounds, methanol, phenol and cyanide compounds. The diesel fumes released into the atmosphere contain 100 times more sooty particles than fumes from gasoline engines for the same load and engine conditions (Lucking et al., 2011). Exposure to high concentrations of diesel exhaust may cause respiratory diseases, fatigue, impair sense of smell, irritation of the eye, nose and throat, headache, drowsiness, nausea and heartburn. DE exposure causes lung cancer in humans as evidence in case–control studies involving 12,315 workers in non-metal mining facilities in the US (Attfield et al., 2012; Silverman et al., 2012). DE also enhanced thrombus formation and increased platelet activation (Lucking et al., 2011) (Fig. 1). We have shown that exposure of Apo E deficient mice to DE at ≈250 μg/m^3 for 2 weeks led to enhanced systemic lipid peroxidation in the bronchoalveolar lavage fluid that was accompanied by the development of dysfunctional prooxidant and proinflammatory HDL (Yin et al., 2013a). Whole body exposure of Apo E null mice to whole diesel exhaust (WDE) at a concentration of about 1 mg/m^3 for 6 h/day, 5 days/week for 2, 5 or 8 weeks induced CD 31 expression, decreased endothelial nitric oxide (NO) expression in the aortic wall, increased ischemic and non-ischemic hind-limbs, increased expression of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor (HIF) −1α and induced angiogenesis and vasculogenesis (Xua et al., 2009).

2.1. Chemical constituents of DEP

The composition of DEP varies with age and source. Though some of the older DEP preparations seem less potent than the newer ones, these differences seem not to correlate with the content of carcinogenic polycyclic aromatic hydrocarbons (PAHs) in the DEP. Generally, DEPs are made up of soots, PAHs, redox active semi-Quinones and transition metals. They contain carbon with large surface areas which readily absorb chemicals like PAHs, aldehydes, quinones and heavy metals like copper, nickel, iron and chromium. The organic compounds such as quinone and PAH constitute about 30% of the weight of DEP and have been shown to be potentially carcinogenic in humans (NTP and Department of Health and Human Services, 2011). Indeed, diesel exhaust particles have been classified as a human carcinogen (Group 1) (IARC, 2012). Schuetzle (1983) did the sampling, analysis of the contents of different PAHs in the diesel exhaust particulate of a motor vehicle and reported that the highest PAHs in DEP are the nonpolar PAH; hydrocarbons and alkylbenzene (920.00 ppm/1000) followed by moderately polar PAH; phthalates, HC contaminants (340.00 ppm/1000) and PAH ketones (147.00 ppm/1000) (Table 2). Recently, Popovicheva et al. (2014) analyzed the chemical composition of diesel particles from a 4 cylinder diesel engine Opel Astra X20DTL (1998) model and reported that the particles are dominated by alkanes aliphatic C–H groups with relatively low carbonyl CO groups in ketones, aldehydes, esters, carboxylic acids, lactones, aromatic $–NO_2$ and $–NO_3$ groups in nitro compounds and SO_4^{2-} ions in inorganic salts. These studies show that hydrocarbons, which include the aliphatic hydrocarbon and the nonpolar polycyclic aromatic hydrocarbons, are the major chemical constituents of DEP. Singh et al. (2004) carried out the physical and chemical analyses of automobile-derived DEP (A-DEP) and the National Institute of Standards Technology standard reference material (SRM 2975) generated from forklift engine, and reported that A-DEPs with 10 times more extractable organic materials and less than one-sixth the amount of elemental carbon compared with SRM 2975, exhibits a different phenotype in CD-1 mice in relation to SRM 2975. This emphasized the need for chemical, physical and source characterization of particle samples derived from a variety of generation and collection conditions for toxicity testing to be meaningful.

2.2. Active components of DEP

Even though few *in vitro* studies comparing the effects induced by organic extracts of the DEP and the residual particulate left after extraction, have reported that the organic extracts of DEP may be more potent for activating proinflammatory responses (Totlandsdal et al., 2012, 2013, 2014; Øvrevik et al., 2015), both the organic and particulate components of DEP generally play an important role in DEP-induced effects (reviewed by Øvrevik et al., 2015). In addition, exposure to DEPs or its organic chemicals have been shown in several studies to produce significant levels of cytotoxic and proinflammatory effects in vascular cells such as endothelial cells and macrophages (Krishnan et al., 2012; Lawal et al., 2015; Yin et al., 2013b). However, it is still unclear which of the compound or group of compound in the organic fraction of DEP is responsible for this proinflammatory response. Recently we have shown that the methanolic–organic fractions of DEP induced cytotoxicity, oxidative stress, and pro-inflammatory responses in Human Microvascular Endothelial Cells (HMEC) (Lawal et al., 2015) and bovine aortic endothelial cells (BAEC) (Yin et al., 2013b). An earlier study has reported that the polar organic extracts of PM induced cytotoxicity and IL-6 responses in BEAS-2B cells, but no response was observed with the nonpolar organic PM-extracts (Fuentes-Mattei et al., 2010). Some studies have shown that the polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs are important active components of organic extracts of DEP (Totlandsdal et al., 2012, 2014) and may be responsible for most of its effects on endothelial cells. PAHs and nitro-PAHs constitute major environmental contaminants in urban ambient air and have both been shown to be mutagenic and carcinogenic (Topinka et al.,

Table 2
Concentration of various PAH compounds and PAH derivatives in the nonpolar and moderately polar fractions of a diesel particulate extract from vehicle exhaust emissions.

Compound	Fraction concentration, ppm/1000
Nonpolar fractions	
1. PAH	
Phenanthrenes and anthracenes	1.1
Methyl (phenanthrenes and anthracenes)	2.6
Dimethyl (phenanthrenes and anthracenes)	5.8
Pyrene	3.1
Fluoranthene	2.5
Methyl (pyrenes and fluoranthenes)	1.4
Chrysene	0.18
Cyclopenta (c, d) pyrene	0.03
Benzo (g, h, i) fluoranthene	0.24
Benzo(a) anthracene	0.95
Benzo(a) pyrene	0.07
Subtotal	18.0
Other PAHs, heterocyclic	62.0
Hydrocarbons and alkylbenzenes	920.0
Total	1000.0
Moderately polar fractions	
1. PAH ketones	
Fluorenones	43.2
Methylfluorenones	4.8
Dimethylfluorenones	1.8
Anthrones and phenanthrones	17.9
Methyl (anthrones and phenanthrones)	17.7
Dimethyl (anthrones and phenanthrones)	14.4
Fluoranthones and pyrones	13.4
Benzanthrones	2.1
Xanthones	3.6
Methylxanthones	1.8
Thioxanthones	17.0
Methylthioxanthones	9.3
Subtotal	147.0
2. PAH Carboxaldehydes	
Fluorine carboxaldehydes	17.6
Methyl fluorine carboxaldehydes	3.9
(Phenanthrene and anthracene) carboxaldehydes	28.2
Methyl (anthracene and phenanthrene carboxaldehydes)	17.7
Dimethyl (anthracene and phenanthene carboxaldehydes)	4.8
(BaA, Chrysene and triphenylene carboxaldehydes)	4.4
Naphthalene dicarboxaldehydes	3.5
Dimethyl naphthalene carboxaldehydes	3.5
Trimethyl naphthalene carboxaldehydes	10.5
(Pyrene and fluoranthene) carboxaldehydes	17.3
Xanthenes carboxaldehydes	6.6
Dibenzofuran carboxaldehydes	4.2
Subtotal	122.2
3. PAH acid anhydrides	
Naphthalene dicarboxylic acid anhydrides	31.2
Methyl naphthalene dicarboxylic acid anhydrides	11.3
Dimethylnapthalene dicarboxylic acid anhydrides	5.0
(Anthracene and phenanthrene) dicarboxylic acid anhydrides	6.6
Subtotal	54.1
4. Hydroxy-PAH	
Hydroxyfluorene	14.8
Methylhydroxyfluorene	4.1
Dimethylhydroxyfluorene	16.7
Hydroxy (anthracenes and phenanthrenes)	6.3
Hydroxymethyl (anthracenes and phenanthrenes)	9.5
Hydroxydimethyl (anthracenes and phenanthrenes)	14.4
Hydroxyfluoreneone	22.4
Hydroxyxanthone	14.1
Hydroxyxanthene	10.8
Subtotal	113.1
5. PAH quinines	
Fluorene quinines	8.1
Methylfluorene quinines	6.2
Dimethylfluorene quinines	5.3
(Anthracene and phenanthrene) quinines	20.4
Methyl (anthracenes and phenanthrene) quinines	22.4
(Fluoranthene and pyrene) quinines	2.1
Naptho (1,8) pyrene-1,3-dione	6.8
Subtotal	71.3
6. Nitro-PAH	
Nitrofluorenes	0.34
Nitro (anthracenes and phenanthrenes)	0.71
Nitrofluoranthrenes	0.05
Nitropyrenes	1.5
Methylnitro (pyrenes and fluoranthenes)	0.25
Subtotal	2.9
Other oxygenated PAHs	83.4
PAH carry-over (from non-polar fraction)	66.0
Phthalates, HC contaminants	340.0
Total	1000.0

Sources: Adapted from Schuetzle, 1983.

2012). Considerable evidence had shown that the human endothelium is a major target tissue for PAHs released majorly into the environment through tobacco smoke and have been implicated in the etiology of atherosclerosis (reviewed by Messner and Bernhard, 2014; reviewed by Ross et al., 2014; Watanabe et al., 2013). Benzo (a) pyrene, a PAH and the most extensively studied member of this group has been shown in previous studies to induce expression of the pro-inflammatory gene MCP-1 and to induce DNA damage in HUVEC. 1-nitropyrene (1-NP), the most abundant nitro-PAHs in diesel exhaust, has been shown to be involved in the mutagenicity of diesel exhaust particulate matter (Steiner et al., 2014). It has also been shown to induce DNA damage, increased levels of reactive oxygen species and decreased cell viability in HUVEC (Anderson et al., 2009). The importance of PAHs in the prooxidative and proinflammatory effects of PM was also emphasized in a comparative study investigating the potential of two PMs, with different PAH contents; the DEP SRM 2975 and the wood smoke particles (WSP), to activate the endothelial cells and to induce monocyte adhesion in the cells. In this study, Forchhammer et al. (2012) co-cultured HUVECs with THP-1 cells and then exposed the co-culture cells to DEP (SRM 2975) and WSP at concentrations of 1–100 μg/ml for 3–24 h. They reported that DEP SRM 2975, with lower PAHs content in comparison with

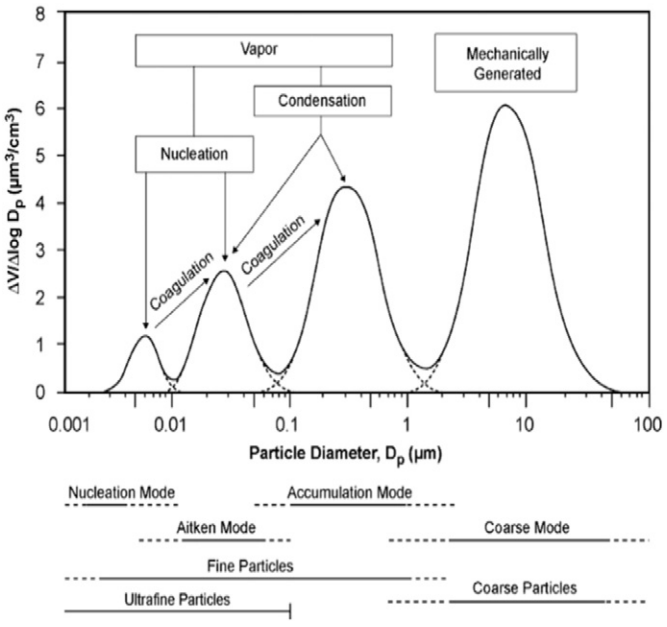

Fig. 2. Particle size distribution that may be observed in diesel exhaust from traffic. Four modes are involved in generating the sizes; nucleation, aitken, accumulation and coarse mode. The growth mechanisms and major formation of the four modes of ambient particles are also shown. V, volume; D_p, particle diameter. Adapted from Araujo and Nel, 2009.

WSP, does not induce THP-1 monocytes adhesion onto HUVEC nor does it increase expression of TNF and IL8 mRNA in THP-1 cells when compared with wood particles. In addition, DEP SRM 2975 does not elevate ROS production nor does it induce oxidative DNA damage in sharp contrast to WSP.

2.3. Diesel exhaust particle number and size distribution

Diesel exhaust particle (DEP) size distribution from internal combustion engines has been receiving increased attention since 1990s because of the known adverse health effects of the fine and ultrafine particulates. Both engine design and after treatment has been used as a control strategy for diesel emission by evaluating their effectiveness in the control of the finest fractions of diesel particulates and particle number emissions. Particle size and number are important parameters in evaluating the potential of DEP to cause adverse health effects. As mentioned earlier, ambient particulate matter is divided into different categories based on their aerodynamic diameter, which is the diameter of a 1 g/cm^3 density sphere of the same settling velocity in air as the measured particle. Diesel particulates nearly all have a size of significantly less than 1 μm and therefore represent a mixture of fine, ultrafine, and nanoparticles.

Using the PM sampling technique in which diesel exhaust sample was diluted at temperature above 52 °C, diesel particulate matter was found to consist of solid components such as elemental carbon and ash, and liquids such as water, condensed hydrocarbons and sulfuric acid. The size distribution of DEP shows that particulate formation begins with a nucleation process through homogeneous and heterogeneous nucleation and this occurs both in the engine cylinder (as in the case of carbon and ash) and in the dilution tunnel (for hydrocarbons, sulfuric acid, water). This nucleation process is followed by agglomeration of the nuclei particles (Fig. 2). DEP has a bimodal size distribution character which corresponds to the particle nucleation and agglomeration mechanisms. The particle size distribution includes the small nuclei mode particles and the larger accumulation mode particles. Most of the mass of diesel particles is contained in the accumulation mode while most of the particle number is in the nuclei mode. This implies that, diesel particulate matter is made up of numerous small particles holding very little mass, mixed with relatively few larger particles which contain most of the total mass. Only a small fraction of diesel particulates resides in the coarse mode. The nuclei mode particles are made up of mainly volatile condensates (such as hydrocarbons, sulfuric acid) and little solid material (Pirjola et al., 2015). The diameter of nuclei mode particles falls less than 40–50 nm (0.04–0.05 μm). These size ranges, thus placed the nuclei mode particles entirely in the nanoparticle range and since the diesel particulate in the nuclei mode account for more than 90% of the total particle count in DEP, although accounting for 0.1–10% of the total PM mass, it shows that most of the DEP components can easily penetrate deep into the cells and may become biologically available in the organs via the blood system.

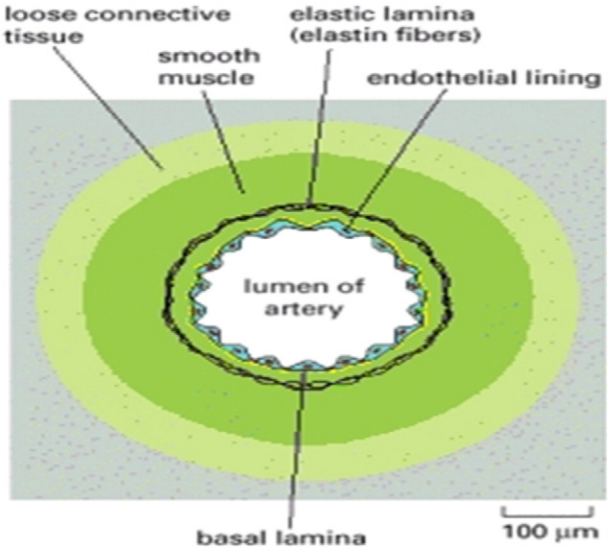

Fig. 3. Endothelial cell location in the artery wall. Adapted from Albert et al., 2002.

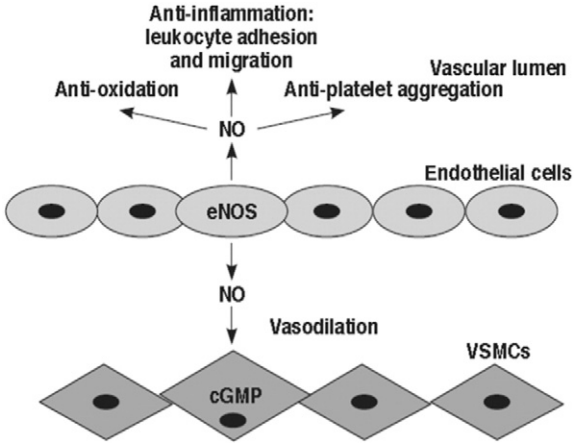

Fig. 4. NO production and physiological effect. NO synthesized by eNOS in the endothelial cells diffuses into the vascular smooth muscle cells (VSMCs) and activates second messenger, cyclic guanosine monophosphate (cGMP), leading to relaxation and consequent vasodilation of the VSMC. Vascular homeostasis is also regulated by the anti-oxidation, anti-inflammatory and anti-platelet aggregation effect of NO. Adapted from Mudau et al., 2012.

3. Endothelial cells: functions, dysfunctions and relevance to cardiovascular injury

The endothelium is the thin layer of cells that lines the interior surface of blood vessels and forms the physical and biological barrier between circulating blood in the lumen and the rest of the vessel wall (Fig. 3). Thus, the endothelial cells (EC), serve as a first point of contact with the vascular wall for the toxic chemicals that could access the circulating blood. It lines the whole circulating system of the heart to the smallest capillary, regulates vascular tone and participates in vascular homeostasis (reviewed by Goldenberg and Kuebler, 2015). The endothelial function reflects the balance between the repair and injury to the endothelium and can be accessed by the reduction of NO bioavailability and production, plasma level of asymmetric dimethylarginine (ADMA), nitrotyrosine upregulation, upregulation of endothelin-1, quantifying the detachment of mature endothelial cells and derived microparticles and as well as the number and functional state of circulating endothelial progenitor cells (reviewed by Mudau et al., 2012). Endothelial dysfunction, also referred to as endothelial activation, has been implicated in atherogenesis and plaque progression (Eckers and Haendeler, 2015). Vascular tone, cellular adhesion, thrombo resistance, smooth muscle proliferation, and vessel wall inflammation are regulated by a wide range of factors produced by the endothelium in response to physical and chemical signals. Endothelial expression of proinflammatory, proliferative, procoagulant factors and vasoconstrictors are indices of atherosclerosis (Vitiello et al., 2014) and these become pronounced when endothelial is switched from a quiescent state to one that involves the host defense response. The switch in signaling from the Nitric Oxide (NO)-mediated quiescent state of cellular processes in the endothelium toward cellular processes activation by the redox signalling is a very important process in endothelial activation [reviewed by Mudau et al., 2012]. The nitric oxide (NO) produced by the endothelium plays an important role in endothelium vascular relaxation, in normal vascular physiology and helps in the inhibition of inflammation, cell proliferation and thrombosis thereby maintaining the integrity of the

vascular wall. The activation of endothelium NO synthase (eNOS) generates NO from L-arginine and this diffuses into the vascular smooth muscle cells to synthesized cyclic GMP (cGMP) by activating guanylate cyclase enzyme (reviewed by Mudau et al., 2012) (Fig. 4). However, in the presence of increased levels of reactive species, such as peroxynitrite ($ONOO^-$) and decrease arginine, eNOS fails to dimerize, leading to the uncoupling of the enzyme and the production of superoxide anions (O_2^-). Excessive O_2^- production can thus react with NO producing more peroxynitrite. Like NO, reactive oxygen species can migrate rapidly throughout the endothelial cell and react with cysteine groups in proteins to cause structural and functional alterations (reviewed by Mudau et al., 2012). These alterations can mediate different processes in the cells, such as phosphorylation of transcription factors, induction of transcription genes and nuclear chromatin remodeling and protease activation. Though eNOS under a normal cellular process helps to maintain the endothelium in the quiescent state, but can switch to generate ROS (such as superoxide and hydrogen peroxide) to initiate endothelium activation.

In the presence of cardiovascular risk factors, the endothelium can become dysfunctional and activated from the quiescent state to host defense response state. At this state, the endothelium activates the molecular process to produce chemokines, cytokines and adhesion molecules which interact with leukocytes and platelets to initiate inflammation in targeted tissues to clear microorganisms or toxic molecules. Although endothelial activation and redox signalling are part of host defense against toxic insults, they may however, contribute to atherogenesis and clinical events under certain conditions. Risk conditions such as hypercholesterolemia, hypertension, smoking, diabetes, aging and other inflammatory indices which may produce long-term dysregulation of NO and ROS production may be proatherogenic (Bhatt et al., 2011; Herrera et al., 2010). Oxidative stress and inflammation appear to be the common underlying mechanisms for the development of endothelial dysfunction in all the cardiovascular risk factors. NADPH oxidase, xanthine oxidase, cyclooxygenase (COX) and mitochondria have been identified as important sources of ROS upregulation in cardiovascular risk factor (Chhaba, 2009; Osto and Cosentino, 2010). It appears there is a casual relationship between oxidative stress and inflammation. Under physiological conditions, vascular inflammation is regulated via endothelium released of NO (Osto and Cosentino, 2010), but under oxidative stress, vascular inflammatory signalling pathway is amplified with the increase release of ROS leading to overexpression of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1). These inflammatory cytokines consequently lead to EC expression of adhesion molecules such as VCAM-1, ICAM-1, MCP-1 and interleukin-6 (IL-6), which are markers of endothelial activation (reviewed by Mudau et al., 2012). In addition, oxidative stress may contribute to vascular disease by induction of sustained endothelial activation. However, no matter their source, the interaction between ROS and NO may result in a cascade of events that may further endothelial activation and inflammation. This prolonged or repeated exposure to cardiovascular risk factors can overwhelm the protective machinery of antioxidant and anti-inflammatory systems within the endothelial cells, resulting in endothelial dysfunction and its consequent detachment into circulation.

One hypothesis for the promotion of cardiovascular disease by PM is the activation of lung autonomic nervous system (ANS), after pulmonary exposure, resulting in ANS imbalance, leading to pathological alterations in vasoconstriction, endothelial dysfunction, hypertension, platelet aggregation, increased heart rate variability and increased arrhythmia potential (reviewed by Chin, 2015). In a healthy state, appropriate blood vessel tone is maintained between the release of vasodilatory factor from the endothelium and vasoconstricting factor from the ANS nerve terminal (Harris and Mathews, 2004). The vascular smooth muscle cells, which lie between the ANS nerve terminal and the endothelial cells lining the blood vessel lumen, maintain the appropriate vessel tone due to the balance between the ANS activity and endothelial cell function. However, imbalance between the vasoconstricting activity of ANS and the vasodilatory activity of endothelium has been reported as an early indicator in the development of cardiovascular disease (Harris and Mathews, 2004). Oxidative stress has been identified as one factor that provides a link between ANS regulation and endothelial function (Harris and Mathews, 2004). The systemic oxidative stress induced by PM exposure, due to the activation of the lung autonomic nervous system, can promote oxidative stress in the vasculature and other organs such as the heart and the liver (Brook et al., 2010), as has been shown in pregnant mice exposed to DEPs (Weldy et al., 2014) and this induced oxidative stress can lead to endothelial dysfunction with consequent cardiovascular effect.

4. DEP and endothelial cell injury: problems, bioavailability and mechanisms

4.1. Problems

Air pollution constitutes the 13th leading cause of death in the world, and death due to atherosclerotic cardiovascular disease is the leading cause of mortality in the Western world (WHO, 2009). According to the World Health Organization (WHO), cardiovascular diseases/ischemic heart disease were the leading cause of death worldwide in 2004 and cardiovascular-related mortalities are likely to escalate to 23.4 million by the year 2030. Cardiovascular diseases are responsible for about 195 deaths per day in South Africa between 1995 and 2004, and it is expected to increase by 41% in the working age group in South Africa population by the year 2030 (in a review by Mudau et al., 2012). Recently, the WHO's State Report on noncommunicable diseases, from 2014, identified cardiovascular diseases as the number one cause of death worldwide (Eckers and Haendeler, 2015). Several studies have shown that urban dwellers expose to high ambient PM levels have a higher incidence of cardiopulmonary death (Brook et al., 2010) and both acute and chronic exposure to PM may promote cardiovascular ischemic events. Acute exposure may lead to acute myocardial infarction, ischemic stroke and congestive heart failure leading to hospitalizations. 10 $\mu g/m^3$ increases in ambient $PM_{2.5}$ was found to cause a 4.5% daily increase in acute ischemic coronary events in 12,865 subjects in an Intermountain Heart Collaborative study (reviewed by Araujo and Nel, 2009). In a randomized, crossover study were 20 men with prior myocardial infarction were exposed to diesel exhaust for an hour, diesel exhaust was found to promote the myocardial ischemia and inhibits the endogenous fibrinolytic capacity in the subjects (Mills et al., 2007). Chronic exposure to PM may also lead to atherosclerosis as evidenced in a study where an association between long-term residential exposure to high traffic levels of $PM_{2.5}$ and coronary atherosclerosis, assessed by coronary artery calcification scores, was established. In a long-term follow-up study by the American Cancer Society, 10 $\mu g/m^3$ increases in long-term exposure to $PM_{2.5}$ caused a 12% increase in cardiovascular deaths mostly due to ischemic heart disease, with arrhythmias and heart failure constituting smaller percentages (reviewed by Araujo and Nel, 2009). There are well established facts in both animal and human studies that show that exposure to diesel exhaust particles is linked with accelerated progression of atherosclerotic plaques (Araujo and Nel, 2009; Møller et al., 2011).

Different *in vitro* models, using cell culture and experimental animal models have been used to characterize the toxic effects of different DEPs and their detailed mechanisms of action. These studies have examined among others the effects of DEPs on DNA damage, cell proliferation, production of cytokines and chemokines, cytotoxicity, oxidative stress and differentiation and/or capacity of immune cells to defend against infections (Forchhammer et al., 2012; Frikke-Schmidt et al., 2011). Since the chemical and physical properties of DEP collected depend on factors such as engine technology, fuel type, load, temperature and filtration devices, different studies have examined a range of different DEP samples of varying composition on endothelial cells (EC) (Tables 3 & 4).

Table 3
In vitro studies evaluating the effect of DEP chemical constituents on endothelial cells.

Study	Particles (Mode/dose of administration)	Cell line	Assessment parameter/pathway	Effects
Bauer et al. (2011)	Silica nanoparticles (SiO_2NP; 1000–30,000 NP/cell), 24–48 h	HUVEC	Cell viability, cytotoxicity, apoptosis/necrosis	Decreased cell viability, increased LDH released, increased necrosis
Forchhammer et al. (2012)	DEP (SRM 2975) & Wood Smoke Particles; 1–100 μg/ml; 3–24 h	HUVEC co-cultured with THP-1 monocyte cell line	ROS production, monocyte adhesion, inflammation, oxidative damage	SRM 2975 does not cause THP-1 monocyte adhesion, increased VCAM-1 expression, does not cause increased TNF and IL-8 Mrna expression in THP-1, no effects on ROS production, induced DNA oxidative damage and oxidized guanines.
Frikke-Schmidt et al. (2011)	DEP (whole particle suspension); carbon black; 1–100 μg/ml; 3–24 h	HUVEC	Levels of oxidative stress, expression of cell adhesion genes	Time-dose dependent increased oxidative stress, increased DNA damage, increased VCAM-1 & ICAM-1 genes.
Lawal et al. (2015)	DEP methanolic extract (SRM 2975 & Auto-DEP); 5–50 μg/ml; 1–4 h	HMEC	Cell viability & cytotoxicity, oxidative stress levels, inflammatory & UPR gene response, adhesion molecule expression, HO-1 response	DEP decreased cell survival, increased cytotoxicity, increased ROS levels (oxidative stress), increased proinflammatory & UPR gene expressions, increased adhesion molecules (ICAM-1 & VCAM-1) genes expressions, increased HO-1 expression.
Li et al. (2009)	Diesel UFP suspension (collected from a 1998 Kenworth truck); 12–5–50 μg/ml; 1–6 h	HAEC	Superoxide ion level, protein carbonyl level, HO-1 expression, JNK activation	UFP increased Superoxide anion and protein carbonyl levels, increased HO-1 expression, activates JNK protein.
Tobwala et al. (2013)	Whole DEP (SRM 1650b) suspension; 10–50 μg/ml; 3–24 h	Human brain microvascular endothelial cells (HBMVEC)	ROS production, GSH levels, Glutathione peroxides, Glutathione reductase, lipid peroxidation	DEP SRM 1650b caused a dose-dependent increased in ROS, decreased GSH levels, decreased glutathione reductase, glutathione peroxides and increased lipid peroxidation.
Tseng et al. (2015a)	DEP	HUVEC	Oxidative stress, ATP level, apoptosis, cell viability, P13/Akt activity, HUVEC tube cell permeability	DEPs significantly deplete ATP of HUVEC tube cells, cause depolarization of their actin cytoskeleton, inhibit P13/Akt activity, induce endothelial apoptosis, decreased cell survival.
Tseng et al. (2015b)	AUTO-DEP (1–100 μg/ml; 1–24 h)	HUVEC	ROS, TNFα, IL-6, HO-1, IκB-α, p65 expressions; capillary VE-Cadherin redistribution, GSH/GSSG levels	DEP increased ROS, TNFα, IL-6, IκB-α, p65, HO-1 expression levels. Caused capillary VE-Cadherin redistribution, decreased GSH/GSSG ration.
Tsou et al. (2010)	Zinc oxide (ZnO) particles (50 & 100 nm; 0.1–75 μg/ml), 24 h	HUVEC	Cytotoxicity, cell proliferation, glutathione levels	Dose-dependent increased in oxidized glutathione levels, dose-dependent increased in ICAM-1 expression, significant increased in NF-kβ.
Weldy et al. (2011)	Whole DEP suspension	Mouse lymph node endothelial cell line (SVEC4-10) co-cultured with macrophage RAW264.7 cell line	ENOS, INOS, MCP-1, GCLC and GSH levels	DEP induced ENOS, INOS, MCP-1 and GCLC mRNA in SVEC4-10. In co-culture, DEP increased Inos and MCP-1 Mrna, increased GSH levels but decreased Enos.
Yin et al. (2013b)	DEP methanolic extract (Auto-DEP); 5–40 μg/ml; 1–24 h	BAEC; RAW264.7	Cell viability (MTT), oxidative stress (ROS levels, DCFH-DA), Paraoxonase activity, HDL functionality	DEP decreased cell viability and generate ROS in a concentration- and time-dependent manner. Inhibit paraoxonase activity and caused HDL dysfunctionality.

Table 4
Animal studies evaluating the effects of DEP on the vasculature.

Study	PM fraction	Mode/dose of administration	Animal model/Sex	Diet	Tissue	Assessment parameter/pathway	Effect
Davel et al. (2012)	$PM_{2.5}$	Inhalation; 600 μg/m^3; 2 weeks	Wistar/Male	Chow	Pulmonary artery	Vascular reactivity, oxidative stress, proinflammatory cytokines	Increased vascular oxidative stress, enhanced protein expression of Cu/Zn- and Mn-superoxide dismutase, decreased expression of eNOS synthase, impairs endothelium-dependent vasodilation of pulmonary arteries, high TNF-α levels
Miller et al. (2013)	DEP (SRM 2975)	Oropharyngeal aspiration (instillation); 1 mg/ml (35 μl, twice daily)	Apo E deficient mice	Chow	Lung, liver, thoracic aorta, brachiocephalic artery	Atherosclerotic plaque size, plasma lipid peroxidation, proinflammatory gene expressions, antioxidant responses	DEP increased plaque size formation, no effects on pulmonary and systemic inflammation, increased serum cholesterol, increased expression of antioxidant genes.
Robertson et al. (2012)	Whole DEP (SRM 2975) suspension	Intratracheal instillation; 0.5 mg; 6–24 h	Wistar rats/male	Chow	Bronchoalveolar lavage fluid (BALF), blood	Endothelium-dependent vasodilation, pulmonary and systemic inflammation	DEP induced both systemic and pulmonary inflammation, but did not impair endothelial function.

Bauer et al. (2011) reported that exposure of HUVEC to silica nanoparticles (SiO_2NP) of a dose range from 1000 to 30,000 NP/cell for 24 & 48 h induced necrosis, decreased cell viability and increased cytotoxicity (LDH released) in a dose and size-dependent manner.

Their data show that SiO_2NP-induced activation and dysfunction of EC is reflected by release of procoagulant factor (Von Willebrand factor) and necrotic cell death, which shows that exposure of EC to NP is a significant risk factor for the development of ischemic heart disease. Tsou et al. (2010) examined the mechanism involve in zinc oxide (ZnO) particles induction of inflammatory responses in HUVEC exposed to ZnO (50 & 00 nm size) concentration between 0.1–75 μg/ml for 24 h. ZnO at a concentration of ≤45 μg/ml caused dose-dependent increases in oxidized glutathione levels and ICAM-1. They reported that ZnO particles induce ICAM-1 expression via NF-ĸB signalling and it acts synergistically with TNF-α to induce ICAM-1 expression. Hence, it was concluded that ZnO modulates the inflammatory response of EC via NF-ĸB signalling pathway. This study highlights the importance of NF-ĸB signalling pathway as an important target in the treatment of cardiovascular disease caused by particulate matter and its chemical composition (see Fig. 5).

One key regulator of vascular tone and ultimately vascular homeostasis is nitric oxide (NO) generated from L-arginine by endothelial nitric oxide synthase (eNOS) (Fig. 4). Clinically, endothelial dysfunction is assessed as impaired endothelial-dependent vasomotion. Hence, a reduction in endothelium-derived NO vasodilators bioavailability accompanies by relative or absolute abundance of vasoconstrictors is a hallmark of endothelial dysfunction (Eckers and Haendeler, 2015). Therefore the induction of NO and upregulation of eNOS expression could be considered beneficial to the cardiovascular system. DEP has been shown to cause an upregulation in the expressions of eNOS and inducible nitric oxide synthase (iNOS) in lymph node endothelial cell line SVEC4-10 (Weldy et al., 2011). However, NO has been reported to possess some angiogenic effect which could play a very important role in tumor invasion (Hongbao and Ma, 2015). The role of NO and eNOS induction in EC was assessed by exposing primary cultured HUVEC to different PAHs at a range of 0.1–100 μM for 24 h and determine the level of intracellular Ca^{2+}, NO production and eNOS activation and expression (Li et al., 2004). They reported that low molecular weight PAHs induced the activation of eNOS mRNA and eNOS protein expression in a Ca^{2+}-dependent manner. The high molecular weight (HMW) PAHs do not cause these effects and therefore the atherogenic effects of PAHs may be attributed to the high molecular weight components. It should however be noted that despite the increase in eNOS expression and activity caused by low molecular weight PAHs, co-exposure with the HMW PAHs might enhance the carcinogenic effects of the latter, since NO could play important role in tumor invasion (Hongbao and Ma, 2015).

The effects of engine loads and speed on DEP chemical constituents and vascular activities were examined by Sumanasekera et al. (2007). DEP extracts (DEPEs) were prepared from a truck at different speeds (S DEPEs) and loads (L DEPEs) and their effects on intracellular signaling in HUVEC cells were examined. Their data show that while DEPEs from increasing loads (L-DEPEs) stimulate phosphorylation of MAPK, AKT and eNOS with increase NO production, DEPEs from increasing speed only stimulate the phosphorylation of AKT and eNOS. Their data show that depending on the engine loads and speeds, the vascular effects elicited by DEP may affect signalling pathways to different extent.

4.2. Bioavailability

Since elevated air pollutants in ambient air target the human vascular endothelium causing an increased incidence of cardiovascular morbidity and mortality (Bauer et al., 2011; Brook et al., 2010; Xua et al., 2009), the bioavailability of DEP in the endothelium could play a very important role in the etiology of air pollutants-related cardiovascular events. Since DEPs have particulates sizes in the fine, ultrafine and nanoparticle range, it can be postulated that the inhaled ultrafine particulate

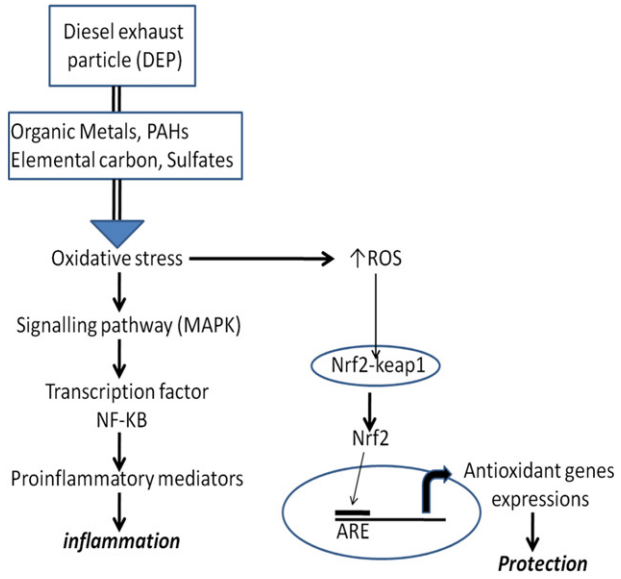

Fig. 5. Mechanism of DEP-induced damage in endothelial cells and cellular protection. Modified from Donaldson et al., 2005.

matter (PM) and nanoparticles in DEP, present in air pollution increases cardiovascular mortality by passing into the systemic circulation and possibly affecting EC function. DEPs have a mean diameter of 0.2 μm or less and therefore are easily respirable with the ability to deposit in the airways and alveoli. However, whether DEP or its chemical constituents enters the systemic circulation and become available at the vasculature and at the site of its action in the endothelium is debatable and require further investigation. Few studies have provided data supporting the availability of DEP in the endothelium and have eluded the toxic and cardiovascular effects of these particles to their presence at the endothelium. In a study carried-out by Chao et al. (2011), HUVEC were exposed to DEP for 24 h and its effects were examined on endothelial tube VE-Cadherin. Their data show that DEP triggers increasing redistribution of VE-Cadherin away from the cell–cell junctions toward intracellular locations. It is possible that DEP translocates into the alveolar capillaries *in vivo* and so may have a direct effect on endothelial cell–cell junctions. These findings suggest that it is likely that DEP interacts with endothelial cells, at least with the advential side of the monolayer, even if the particles don't make it into the lumen of the vessel.

4.3. Mechanisms

Different mechanisms have been adduced for the adverse health effects of DEP. The induction of oxidative stress and inflammatory response are considered to be important events in the adverse health effects of exposure to PM (Lawal et al., 2015; Montiel-Davalos et al., 2010) and $PM_{2.5}$ and PM_{10} particulate sizes have been reported to induce oxidative stress and apoptosis in HUVEC cells (Montiel-Davalos et al., 2010). Thus, the cardiovascular and pulmonary diseases associated with the adverse health effects of PM_{10} (Liu et al., 2009; Miller et al., 2009) have been attributed to its ability to generate oxidative stress. The free radicals generated by PM_{10} are reported to be responsible for the inflammatory effects observed *in vitro* and *in vivo* studies (Montiel-Davalos et al., 2010). Some markers of oxidative stress, such as ROS (Montiel-Davalos et al., 2010; Sanchez-Perez et al., 2009), TNF-α release (Overocker and Pfau, 2012), and DNA adduct formation (Chirino et al., 2010; Frikke-Schmidt et al., 2011), have been used to explain the mechanism of oxidative stress induced by PM_{10}. Thus, the oxidative stress-induced by DEP has been attributed in part to both its PM_{10} and ambient UFP (Montiel-Davalos et al., 2010) components.

DEPs have been widely used as a standard model pollutant and its proinflammatory effects have been extensively studied. DEP induction of oxidative stress and its consequent cellular effects in target cells are explained according to the hierarchical oxidative stress hypothesis. The increase ROS production overwhelms the antioxidant defense leading to GSH depletion and GSSG accumulation with a drop in the GSH/GSSG ratio (Chirino et al., 2010; Tseng et al., 2015b). This depletion results in activating redox-sensitive signalling pathways such as the different MAP kinases and the NF-KB cascade with the consequent activation of cytokines and adhesion molecule expression (Fig. 5). Several studies have shown that DEP indeed induce NF-KB, JNK and P38MAPK activities which are connected to cytokine and chemokine production (Lee et al., 2012; Li et al., 2009; Montiel-Davalos et al., 2010).

Increased ROS production, leading to oxidative stress (OS) has adverse implication on endothelial and vascular functions and has significantly contributed to vascular disease. Various studies have shown that increased ROS production and OS in the vascular wall are involved in the development of endothelial dysfunction and cardiovascular diseases by various cardiovascular risk factors such as diabetes, hypertension and hypercholesterolemia (Chhaba, 2009; Osto and Cosentino, 2010). Some of the markers of OS such as increase lipid peroxidation, oxidative damage to DNA and other macromolecules and induction of antioxidant enzymes have been shown in various studies to be elevated in endothelial cells exposed to diesel exhaust particles (Tobwala et al., 2013; Tseng et al., 2015b; Weldy et al., 2011). In one study, exposure of human brain microvascular endothelial cells (HBMEC) to 10–50 μg/ml DEP SRM 1650b increased lipid peroxidation, decreased Glutathione (GSH) levels, decreased glutathione peroxidase and glutathione reductase activities and also caused a dose-dependent increased in ROS levels (Tobwala et al., 2013). DEP has also increased total GSH and glutamate cysteine ligase catalytic subunit (GCLC) mRNA in mouse lymph node endothelial cell line (SVEC4-10) co-cultured with macrophage cell line RAW264.7 (Weldy et al., 2011) and increased HO-1 expression and protein carbonyl production in human aortic endothelial cells (HAEC) (Li et al., 2009).

Previous *in vitro* and *in vivo* studies (Tables 3 & 4) have shown that ambient PM and/or DEP can induce OS via increased ROS production in vascular cells, such as EC (Forchhammer et al., 2012; Li et al., 2010;

Table 5
Studies showing the effects of antioxidants in modulating DEP-induced effects in EC.

Diesel type	Test system	Concentrations	Antioxidant source	Endpoints	Citation
DEP SRM 2975 & DEP EU4	HUVEC	1–100 μg/ml; 3–24 h	Vitamin c, desferrioxamine	Oxidative stress levels, cell adhesion molecules expressions. Vit c attenuates ROS production; desferrioxamine protects DNA from oxidative damage and attenuates the expression of cell adhesion molecules.	Frikke-Schmidt et al. (2011)
DEP SRM 2975 & AUTO-DEP	HMEC	5–50 μg/ml; 1–4 h	Modulation of HO-1 expression & activity	Cell viability, ROS levels, proinflammatory (IL-8) & UPR gene responses (MCP-1), adhesion molecules (ICAM-1 & VCAM-1) responses	Lawal et al. (2015)
Diesel UFP (1998 Kenworth truck)	HAEC	12.5–50 μg/ml; 1–6 h	N-acetylcysteine (NAC)	Superoxide anion level, protein carbonyl level, HO-1 expression	Li et al. (2009)
DEP (AUTO-DEP)	HUVEC	1–100 μg/ml; 1–24 h	N-acetylcysteine (NAC)	ROS levels, HO-1, TNF-α, IL-6, IKB-α, p65 expression levels, GSH/GSSG ratio	Tseng et al. (2015b)
DEP (AUTO-DEP)	BAEC & RAW 264.7	5–40 μg/ml; 1–24 h	HDL	Cell viability, ROS level, paraoxonase activity, HDL functionality	Yin et al. (2013b)

Montiel-Davalos et al., 2010). UFP component of the DEP has also been shown to induce vascular oxidative stress in HAEC (Li et al., 2009). Recently, we have shown in our lab that exposure of HMEC to 25 μg/ml of DEP SRM 2975 and Auto-DEP for 4 h induced significant oxidative stress, which was accompanied by increased cytotoxicity and decreased cell viability (Lawal et al., 2015). Moreover, other studies have shown that DEP can elicit ROS production in various EC such as human umbilical vein endothelial cells (Tseng et al., 2015a, 2015b), human brain microvascular endothelial cells (Tobwala et al., 2013), human aortic endothelial cells (Li et al., 2009), and bovine aortic endothelial cells (Yin et al., 2013b). The ROS production by DEP has been linked to the activation of the endothelial NADPH oxidase (Mo et al., 2009) and/or leaks in electron of the mitochondrial electron transport complexes and the activation of the JNK pathway (Li et al., 2009). Animal studies have also shown increased oxidative stress and expression of vascular antioxidant genes after DEP PM exposure accompanied by increased atherosclerotic plaque size and plasma lipid peroxidation, which are markers of increased atherosclerosis and cardiovascular disease (Davel et al., 2012; Miller et al., 2013).

The integrity of the adherens junctions is important in maintaining the cell–cell permeability barrier and cell functions. ROS has been reported to stimulate the permeability of the cell–cell barrier adherens junctions by inducing the release of vascular permeability factor/VEGFA (vascular endothelial growth factor A) (Tseng et al., 2015a). Disruption of adherens junction leading to vascular permeability has deleterious effects on vasculature such as acute myocardial infarction and atherosclerosis. DEP has been reported to increase vascular permeability in HUVEC tube cells by inducing oxidative stress, followed by ATP depletion, depolarization of their actin cytoskeleton and endothelial apoptosis (Tseng et al., 2015a). The disruption of the adherens junctions of the EC may cause the DEP to slip between the cells, via a redistribution of the VE-cadherin network, and thereby accessing the circulatory system to cause adverse effects.

In addition to the OS, DEP has also been shown to induce proinflammatory responses. The proinflammatory effect of the DEP has been reported in several studies (Davel et al., 2012; Robertson et al., 2012; Totlandsdal et al., 2012) and we have recently shown in our lab that DEP induces an inflammatory response in HMEC (Lawal et al., 2015). Increased expressions of inflammatory cytokines such as ICAM-1, VCAM-1 and MCP-1 have also been reported in HUVEC (Frikke-Schmidt et al., 2011; Forchhammer et al., 2012; Tsou et al., 2010), HAEC and SVEC4-10 (Weldy et al., 2011). DEP exposure has also been shown to be highly inflammatory in mice and rats (Davel et al., 2012; Miller et al., 2013; Robertson et al., 2012).

Though the involvement of oxidative stress in the DEP induction of endothelial dysfunction has been established by different studies as outlined above, however, the role of the proinflammatory effect of DEP in endothelial dysfunction is still contentious. For instance, Robertson et al. (2012) reported that despite inducing pulmonary and systemic inflammation in male Wistar rats, DEP exposure does not impair endothelial function. This data were correlated with a later study by Miller et al. (2013) where DEP SRM 2975 produces no effect on pulmonary and systemic inflammation in apo $E^{-/-}$ mice despite an increased expression of antioxidant genes. Thus, the ability of DEP to incite oxidative stress may be a pre-requisite for its induction of inflammatory effects (Fig. 5) and consequent carcinogenic effects since it has been well established that there is a linked between inflammation and cancer (Attfield et al., 2012; Danielsen et al., 2009, 2011).

5. Therapeutic and preventive approaches to DEP-induced endothelial cell dysfunction

Identifying the various endothelial assessment tools (biomarkers) is paramount to find effective anti-endothelial dysfunction therapies against DEP-induced endothelial activation. These biomarkers, mentioned above, will allow the use of therapeutic target for inhibiting the adverse effects of DEP on endothelial cells.

5.1. The use of antioxidants and anti-inflammatory agents

Oxidative stress occurs when the level of pro-oxidants exceed that of the antioxidant defense in the cells. Oxidative stress has been implicated in DEP-induced endothelial dysfunction and therefore attenuating the level of ROS induced by DEP may serve as a therapeutic approach in treatment of adverse effects of DEP in EC. One way of attenuating ROS production is to boost the level of antioxidants present in the cells to exceed the level of the damaging free radical generated by toxic compounds. Indeed, several studies have shown that modulating antioxidant levels in the EC have profound effects on the ability of DEP to induce endothelial damage (Table 5).

Antioxidants are substances that prevent the damaging effects of free radicals using different mechanisms such as scavenging of free radicals, prevention of lipid peroxidation, prevention of protein modification, prevention of DNA damage, inhibition of free radical generating enzymes, activation of internal antioxidant enzymes, and metal ion chelating (reviewed by Pradedova et al., 2011). The antioxidant enzymes in the cells are supplemented by small molecule non-enzymatic antioxidants, some of which are exclusively present in the diet and are vitamins. These small molecule antioxidants include ascorbic acid (vitamin C), glutathione (GSH) and tocopherols (vitamin E). GSH is a non-enzymatic endogenous thiol antioxidant that provides protection against oxidative stress in the body. Therefore, an exogenous source of thiol groups could boost the antioxidant levels of the thiol containing antioxidants in the cells. For example, N-acetylcysteine (NAC) has been reported to attenuate the adverse effects of DEP-induced cytotoxic and oxidative stress in HAEC (Li et al., 2009) and HUVEC (Tseng et al., 2015a, 2015b). It has also been reported that N-acetylcysteine amide (NACA), an amide form of NAC, also protects against oxidative stress in the lung of mice exposed to DEP (Banerjee et al., 2009). Vitamin C, a water soluble, non-enzymatic antioxidant, has also been shown to protect against oxidative stress in EC. It has been reported that vitamin C and desferrioxamine, an iron chelator, protects HUVEC against the oxidative stress-induced damage of DEP and carbon black by attenuating ROS production, DNA oxidative damage and decreasing the expression of ICAM-1 and VCAM-1 (Frikke-Schmidt et al., 2011). We have also shown that by modulating the expression of endogenous antioxidant enzymes, the adverse effects of DEP on EC can be attenuated. For instance, we have shown that the overexpression of heme oxygenase-1 (HO-1) enzyme can attenuate the cytotoxicity and the prooxidative damage of DEP in HMEC (Lawal et al., 2015). Though elevated ROS are always accompanied by upregulation of antioxidant protective gene expression such as HO-1; for example, in HAEC (Araujo, 2012), human microvascular endothelial cells (HMEC) (Lawal et al., 2015), and human umbilical vein endothelial cells (Tseng et al., 2015b); other non-enzymatic molecules in the circulating blood such as albumin, bilirubin, or plasma high-density lipoprotein (HDL) can also participate in antioxidant protection (Araujo, 2012). Yin et al. (2013b) reported that HDL can inhibit DEP oxidative and prooxidative cellular effects in bovine aortic endothelial cells (BAEC) but a dysfunctional HDL fails to protect or could even enhance DEP toxic effects.

One mechanism involved in the induction of antioxidant genes and thus prevent the deleterious effects of ROS is the activation of the Nrf2-keap1 pathway. The ROS induction can trigger-off the release of Nrf2 from its keap1 repressor by the oxidation of the disulfide link between Nrf2 and keap1. The release Nrf2 migrate into the nuclear where it binds to the antioxidant response element (ARE) present at the promoter ends of antioxidant genes such as HO-1, NAD (P) H Quinone Oxidoreductase (NQO1), Glutathione S-transferase (GST) and Glutamylcysteine Ligase Catalytic (GCLC) subunit, to trigger their transcription. Ambient PM can trigger Nrf2-regulated gene response due to its prooxidative and electrophilic chemical contents as have been

shown in the induction of HO-1 in endothelial cells (Lawal et al., 2015), GST and other phase II enzymes in macrophages and epithelial cells by aromatic and polar DEP fractions. However, the use of exogenous antioxidants from plants and other pharmaceutical products, as well as anti-inflammatory agents, can help to boost the antioxidant response of the cells in coping with the excess ROS and inflammation.

5.2. The use of different pharmacological drugs

DEP has been shown to induce the oxidation of LDL (Yin et al., 2013b), which is a vital step in endothelial activation leading to atherosclerosis and cardiovascular disease. The oxidative and inflammatory effects of DEP also cause the reduction in NO level through the formation of peroxynitrite ($ONOO^-$) and decrease arginine resulting in the lowering of vasodilation (discussed above). Drugs with anti-endothelial dysfunction properties, such as statins (e.g. simvastatin, atorvastatin and pravastin), that lower plasma cholesterol levels and improve endothelial-dependent vasodilation (reviewed by Mudau et al., 2012) could be an effective therapy against DEP-induced endothelial dysfunction. Statin also increases eNOS mRNA stability by stimulating PKB/AKt activity and this may probably be responsible for its vasodilation effect.

Angiotensin converting enzyme (ACE) is responsible for the synthesis of angiotensin II and endothelin converting enzyme synthesized endothelin-I. These endothelium-derived factors are both potent vasoconstrictors and thus are biomarkers of endothelial dysfunction mostly generated in the oxidative stress condition. Therefore, the use of angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor (ATI) blockers can be important therapeutic agents against DEP-induced endothelial dysfunction.

6. Future perspective and conclusion

Though much work has been done on the adverse health effect of DEP on the cardiovascular system through disruption of endothelial functions, there is still yet a lot to be done on the protective means of attenuating these effects especially in the use of natural plant sources of antioxidant phytochemicals. Though gene modulation and the use of pharmaceutical products could be of immense importance in dealing with this challenge, however, the cost and availability of these means of treatment may be out of reach for a large population of patients in need of such care. Therefore, it will be of great importance if future studies are directed toward investigating the potential of the use of phytochemicals as it will be cost effective and readily available for the patients especially in countries where the use of medicinal plants is high. In conclusion, though DEP induced EC dysfunction as a step in its atherogenic effects, the use of exogenous compounds that contain antioxidants or that can trigger the migration of the transcription factor Nrf2 from the cytoplasm to the nucleus, could serve a therapeutic resource for the prevention/or attenuation of these deleterious effects of DEP in endothelial cells and so research should be directed toward such area.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found, in online version.

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