

NANOPARTICLES AND ENDOTHELIUM: AN UPDATE ON THE TOXICOLOGICAL INTERACTIONS

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Abstract

Adverse effects of environmental nanoparticles are emerged due to their escape from the macrophage surveillance and access to microcirculation. Endothelium acts as a physiological barrier lining up the vessel walls and controls the transfer of nanoparticles between the blood and interstitial space. Similarly, the blood–brain barrier at the level of brain microcirculation is the major site of blood–nervous system exchange and constitutes the physical barrier. However, the loss of endothelial function and integrity may result in the uncontrolled passage of the nanoparticles. Despite of some adverse properties, nano-sized materials continue to be used inevitably in daily life. However, their mechanism of action in the exposed organism and their potential toxic effects are still being investigated in order to take preventive measures to save the human health. Thus, this review focuses on the possible output of the interaction of endothelium with the nanomaterials and the significance of the barriers during the exposure to nanoparticles.

Rezumat

Efectele adverse cauzate de nanoparticulele din mediul înconjurător se datorează eludării de către acestea a controlului asigurat de macrofage și intrarea lor în microcirculație. Endoteliul care formează pereții vaselor sanguine acționează ca o bariera fiziologică și controlează transferul nanoparticulelor din compartimentul circulator în spațiul interstițial. În mod similar, bariera hemato-encefalică de la nivelul microcirculației cerebrale este *situs*-ul major unde are loc schimbul dintre componenta circulatorie și sistemul nervos. Pierderea funcționalității și integrității endoteliale poate duce la trecerea necotrolată a nanoparticulelor. În ciuda efectelor adverse, nano-materialele continuă să fie folosite în mod uzual. Mecanismele de acțiune ale nanoparticulelor în organismele expuse și potențialul lor toxic sunt încă studiate intens pentru identificarea modalităților de prevenție în domeniul sănătății. Prezentul *review* evaluează efectele posibile ale interacției endoteliului cu nanomaterialele și semnificația acestor bariere endoteliale în timpul expunerii la nanoparticule.

Keywords: Nanoparticle, endothelium, protein corona, shear stress

Introduction

Although nanotechnology has a substantial contribution to science, quality of life and with its promising applications in the industry, to generate substantial financial gains, products which are innocuous in the bulk form can elicit toxicity due to the altered chemical and physical properties [91]. Adverse effects of nanoparticles on human health largely depend on individual features such as genetic factors and existing disease, as well as exposure status, and nanoparticle size, shape, agglomeration state, and electromagnetic properties. Nanoparticles are less efficiently removed than larger particles and can translocate through the circulatory and lymphatic systems to many tissues [10]. The size of the airborne particles and their surface area determine the potential to elicit

inflammatory injury, oxidative damage, and other biological effects. These effects are more significant for fine and ultrafine particles because they can penetrate deeper into the airways of the respiratory tract and are largely retained in the lung parenchyma. Thus the size and dissolubility of particulate matter define its toxicity through the mechanisms of oxidative stress and inflammation [99]. Surface chemistry appears to play an important role in ultrafine particle toxicity. Ultrafine particles have very high size-specific deposition when inhaled as singlet ultrafine particles rather than as aggregated particles. It appears also that ultrafine particles, after deposition in the lung, largely escape alveolar macrophage surveillance and gain access to the pulmonary capillaries [69]. Actually Nemmar *et al.* detected ultrafine technetium – 99m labelled

carbon nanoparticles (particle size < 100 nm) in blood already at the first minute, reached a maximum between ten and twenty minutes, and remained at this level up to 60 minutes after the inhalation by healthy volunteers [65]. Following exposure to particles, the impairment of endothelium-dependent dilation in the systemic microcirculation coincides with enhanced oxidative stress, increase in myeloperoxidase (MPO) activity and endothelial adhesion of polymorphonuclear leukocytes [66].

Epidemiological studies reveal a strong association between air pollution exposure and increased cardiovascular morbidity and mortality due to acute myocardial infarctions and deep vein thrombosis [7, 63]. Additionally, regarding the adverse effects of environmental nanoparticles, such as ultrafine particles, consumers worry about potential risks when using products containing nano-sized materials. Many products contain nano-sized materials relevant for oral exposure that mainly comprise cosmetics such as, lipsticks, toothpaste, and food packaging materials, storage life sensors, food additives, juice clarifiers, as well as, sunscreen, skin creams which may accidentally get into the gastrointestinal tract [30].

Endothelial internalization of nanoparticles

Endothelial cells lining the lumen of blood vessels serve as a physiological barrier that controls nanoparticle transfer from the vasculature into the tissue. Indeed, endothelium is particularly important in controlling the passage of macromolecules and fluid between the blood and interstitial space. The loss of this function is a result of tissue inflammation. Thus, the efficient transfer of many water insoluble substances from the blood into the interstitium relies on endothelial permeability, and often requires specific carrier proteins [58]. Nanoparticles may induce endothelial system inflammation and subsequent dysfunction either by escaping from phagocytosis and interacting directly with the endothelial monolayer or the nanoparticles may be phagocytized by monocytes and provoke oxidative stress responses [117].

The endothelial lining of blood vessels in different organs differs with respect to morphology and permeability and is classified as continuous, fenestrated or discontinuous. Furthermore, the mediator release, antigen presentation or stress responses of endothelial cells vary between species, different organs and vessel classes. While pulmonary endothelium exhibits a constitutive expression of adhesion molecules and the pulmonary microcirculation, which is less permeable to protein and water flux, endothelial cells of the blood-brain barrier exhibit a different phenotype with no fenestrations, extensive tight junctions and sparse

pinocytotic vesicular transport [79]. Actually the physiological upper pore size limit of capillary walls of the most non-sinusoidal blood capillaries for the trans-capillary passage of lipid-insoluble endogenous and non-endogenous macromolecules ranges from 5 to 12 nm. Therefore, macromolecules larger than the physiologic pore size upper limits do not accumulate within the respective tissues' interstitial spaces [86]. Finally, there may be relevant differences even between adjacent endothelial cells. Hence, the vascular endothelium transports solutes with a range of molecular radius (M_r) from 0.1 nm to 11.5 nm [82]. Disruption of the endothelial barrier leads to vascular hyper-permeability and protein-rich oedema which is a key hallmark of inflammation. Transport of the macromolecules occurs by means of transcellular and paracellular pathways. In healthy, non-inflamed vessels, endothelial cell-cell contacts significantly restrict the paracellular permeability, whereas transcellular transport from the blood to the perivascular interstitium occurs *via* caveolae. Increased paracellular permeability induced during inflammation is due to the opening of inter-endothelial cell-cell junctions and disruption of endothelial cell-matrix contacts within the vasculature. In addition, caveolae-mediated trans-endothelial transport (transcytosis) of macromolecules through the microvascular endothelial barrier is also an important mechanism that is responsible for inflammation-evoked pulmonary vascular hyper-permeability and protein-rich oedema formation [92]. Src kinase signalling is a critical "switch" regulating caveolae-mediated and inflammation-evoked increase in trans-endothelial transport of the plasma protein [37]. Vascular endothelial cell cadherin is incorporated into endothelial adherent junctions at the cell surface, where it forms homodimeric associations with adjacent cells and contributes to the barrier function of the vasculature [20]. Nanomaterials trigger intracellular signalling cascades *via* specific interaction with vascular endothelial cell-cadherin, resulting in nanomaterial-induced endothelial cell leakiness. Subsequent to exposure to nanoparticles, immediately tens of microns-sized gaps develop between the cells on a continuous monolayer of endothelial cells [87]. As cadherins are positioned at the cell-cell interface, they are most likely important players in mechano-transduction pathways [94].

Nanoparticles entering the lung *via* air pollution have significant adverse effects. Recent toxicological studies have confirmed that nano-sized or ultrafine particles reach deep into the alveolar region of the lungs. Chaotic alveolar flow is characterized by stagnation saddle points associated with transport, mixing, and ultimately the deposition. Susceptibility to the inflammatory effects of ultrafine particles significantly increases in older age and with a

compromised/sensitized respiratory tract. Severity of inflammation is related to the size of surface areas per mass [69, 97]. Repeated exposure may result in significant pulmonary accumulation of ultrafine particles and then could pass from the lungs into bloodstream and extra-pulmonary organs in humans [60]. Moreover, the ultrafine particles (particle size < 100 nm) can carry large amounts of toxic compounds, such as hydrocarbons and metals, on their surfaces. They remain airborne for a long time period [111] and penetrate deeply into the respiratory tract. Endothelial cell-to-cell junctions are vital for the formation and integrity of blood vessels. Adherens junctions and tight junctions are formed by transmembrane adhesive proteins [68]. In contrast Wiebert *et al.* could not detect a quantitatively important translocation of 100-nm particles to the systemic circulation from the healthy lungs [107]. In contrast to fine particles, inhaled ultrafine particles seem to follow different routes in the organism. Cardiovascular effects observed in epidemiological studies triggered the discussion on enhanced translocation of ultrafine particles from the respiratory epithelium towards circulation [44]. Translocated fraction of these particles is about an order of magnitude less than that of 15 nm [43].

On the other hand, the blood-brain barrier (BBB) consists of a largely impermeable network of capillary endothelium cells connected by tight junctions. The BBB at the level of brain micro vessel endothelium is the major site of blood-central nervous system (CNS) exchange and constitutes the physical barrier formed by the endothelial tight junctions [1]. It is estimated that more than 98% of small molecular weight drugs and practically 100% of large molecular weight drugs developed for CNS pathologies do not readily cross the BBB [72]. Extensive transmission electron microscopy imaging shows that all of the nanoparticles are internalized, to different extents, by the BBB and accumulated along the endo-lysosomal pathway [112].

Nanoparticles as promising drug delivery agents, can transport across the BBB and increase and facilitate the uptake of appropriate drugs in the brain [101]. Currently, three predominant synthetic carriers are being studied to transport therapeutics across the BBB: liposomes, metallic nanoparticles, and polymersomes [51].

Engineering nanocarriers with higher uptake by targeted cells and with low cellular interaction in the systemic circulation, is a challenge and is much more complicated *via* the BBB. In a two-stage nanocarrier-system, at first step conjugation of angiopep to the surface of the designing lipid nanoparticle for targeting the low-density lipoprotein receptor-related protein-1 is expressed

on the BBB. In the second step, the positively charged lipid nanoparticles are masked with a negatively charged polyethylene glycolated cleavable lipopeptide. This lipopeptide contains a recognition sequence for matrix metalloproteinases (MMPs), a class of enzymes often expressed in the inflammatory BBB conditions and tumour microenvironment [9]. Angiopep is applied as a ligand specifically binding to low-density lipoprotein receptor-related protein (LRP) which is overexpressed on BBB. Angiopep-conjugated nanoparticles exhibit higher cellular uptake and LRP gene expression in brain cells. Therefore, to allow transport of large-molecule drugs across the BBB, the drugs are encapsulated in nanoparticles [38]. Also, a transcytosis-targeting peptide permits polyethylene-glycol modified liposomal nanoparticle to penetrate through monolayers of brain-derived endothelial cells [4]. Another recent approach used receptor mediated transcytosis for targeting transferrin receptors with 80-nm gold nanoparticles designed with an acid-cleavable linkage between transferrin and nanoparticles core. Using an experimental mouse model it was demonstrated that these targeted nanoparticles, bind to transferrin receptors on the blood side of BBB and, upon acidification during the transcytosis, release the nanoparticle into the brain [16].

Nanoparticle interaction with plasma proteins

Despite titanium dioxide, silicon dioxide and zinc oxide nanoparticles having similar surface charges in buffer, bound different plasma proteins. When these nanoparticles enter a biological system such as human plasma they selectively absorb biomolecules developing the biomolecular corona on their surface [55]. Protein absorption to nanoparticles may determine their interaction with cells and tissues [21]. The nanoparticle's composition and surface chemistry dictate the extent and specificity of protein binding [2]. The non-specific interaction between nanoparticles and plasma proteins occurs immediately after nanoparticles enter the blood, resulting in the formation of the protein corona that thereafter "functionally" replaces the original nanoparticle, meaning that all the induced effects of the nanoparticles are the ones induced by the bio-corona and not by the nanoparticle by itself. In order to minimize the uncontrolled effect developed by proteins accumulating on the nanoparticle's surfaces there are several experimental tactics. One of them is the successful protective application of the preformed albumin corona that will inhibit the plasma proteins adsorption and will decrease the complement activation, and ultimately prolong the blood circulation time and reduce the toxicity of the nanoparticles [75]. Many nanometer-sized particles proposed for clinical use contain heavy metals. The

charge of the coating has a profound effect on the adsorption of serum proteins as well as the hydrodynamic diameter (HD); purely anionic or cationic charge is associated with an increase in HD of over 15 nm after incubation with serum [14]. Nanoparticle HD is a critical parameter in designing potential diagnostic and therapeutic agents. The mammalian vasculature has an average pore size of ≈ 5 nm. Below this value, relatively fast equilibrium is reached between agents injected intravenously and the extracellular space, but above this value, transport across the endothelium is remarkably slow [14]. The charge of the coating has a profound effect on the adsorption of serum proteins as well as the HD; purely anionic or cationic charge was associated with an increase in HD of over 15 nm after incubation with serum [14]. Although neutral colloidal semiconductor nanocrystals quantum dots do not bind serum protein, it is not possible to synthesize them with an HD less than ≈ 13.2 nm [98]. These ligands provide a possibility of preparing quantum dots and gold nanoparticles that facilitate their effective use in bioassays and live cell imaging [59]. Zwitterionic-coatings render nanoparticles more biocompatible. Such coatings greatly reduce the rate and/or extent of non-specific adsorption of proteins and lipids to the nanoparticle surface, thereby inhibiting production of the "biomolecular corona". Additionally, *in vivo* studies have demonstrated that zwitterionic coating increases the circulatory lifetimes of larger-sized nanoparticles, while those with hydrodynamic diameters of ≤ 5 nm exhibit small-molecule-like pharmacokinetics, remaining sufficiently small to pass through the fenestrae and slit pores during glomerular filtration within the kidneys, and enabling efficient excretion *via* the urine [77].

For biomedical applications, prevention of non-specific uptake by reticulo-endothelial system in order to increase the half-life of the nanoparticles in circulation is important. The reason is that nanoparticles can deliver their loaded drugs to the intended tissues and are not cleared by the system. Hence grafting poly-(ethylene glycol) (PEG) onto particle surfaces, reduces additional biomolecule binding [103] subsequently camouflaged with membrane lipids derived from erythrocytes [36]. In this manner nanoparticles are transformed into biomimetic carriers, and the bio-corona is now a "self" structure that can surpass reticulo-endothelial system [27].

When the nanoparticles are experimentally coated with antibodies targeting vascular cell adhesion molecule-1 (VCAM-1) and E-selectin in equal proportions, a more uniform vascular distribution is achieved. When the nanoparticles have a diameter ranging from 0.1 to 2.0 μm and are decorated with antibodies directed toward three endothelial

adhesion molecules, namely intravascular cell adhesion molecule-1 (ICAM-1), VCAM-1, and E-selectin, their surface density depends on the local wall shear stress [35].

Shear stress and endothelial activation

Endothelial cells are exposed to a variety of hemodynamic forces that are created by blood flow and by the pulse wave which is defined as shear stress. Shear stress acts at the interface between flowing blood and the vessel wall. Endothelial cells recognize the shear stress as a mechanical stimulus. This signal is transmitted into the cells, thereby triggers a variety of cellular responses that involve alterations in cell morphology, cell function, and gene expression [5]. A variety of cell-membrane molecules including ion channels, G proteins, growth factor receptors, tyrosine kinase receptors, adhesive proteins, caveolae, the cytoskeleton, the glycocalyx, and the primary cilia are involved in shear stress-induced signal transduction pathways [6]. Shear stress and endothelial activation are major determinants of particle uptake. Even moderate shear stresses typically encountered in the venous system strongly reduce particle uptake compared with static conditions. In contrast a pronounced particle uptake is observed in inflamed endothelial cells [39]. Shear stress regulates the uptake of nanoparticles by endothelial cells and induces the compartmentalization of nanoparticles in human endothelial cells. The activation of cells with tumour necrosis factor-alpha (TNF-alpha) stimulates the inflammation in endothelium. The shear stress-induced nanoparticles uptake is independent of the adhesion molecules which are expressed by activated endothelium, at least for nanoparticles in the size not exceeding 50 nm [85]. Vascular endothelial cells are directly and continuously exposed to fluid shear stress generated by blood flow. The transmission of hemodynamic forces throughout the endothelium and the mechanotransduction mechanisms lead to biophysical, biochemical, and gene regulatory responses of endothelial cells [19]. Shear stress determines the shape and orientation of endothelial cells. Morphological changes in shear stress are accompanied by coordinated shape changes resulting in varying degrees of alignment with flow direction. Eventually, actin filaments become rearranged into bundles of stress fibres and aligned in the direction of the shear stress [105, 110]. The nuclear pore allows for the passage of biomolecules smaller than 9 nm by passive diffusion [14], whereas active transport is required for molecules of up to approximately 39 nm [73]. At first nanoparticles are trapped in endocytic vesicles and then subsequently transported by molecular motors along microtubule tracks to the

perinuclear area [85]. Surface protonation of nanoparticles in the endolysosomal compartment followed by their reversible and pH-dependent aggregation is responsible for their escape into the cytoplasm [61]. This phenomenon could also be attributed to the possible interactions of nanoparticles with the cytoplasmic proteins, thereby forming a "protein corona". However, in experiments with fixed and permeabilized cells, in which cell-membrane-associated uptake mechanisms are eliminated and putative physical subcellular barriers are preserved [109]. Some of the nanoparticles are found internalized into the cells under both static and flow conditions. In addition, fixation and permeabilization also ensure that the barriers to particle localization are mainly a function of size. Permeabilization did not affect the ability of particles to enter through the outer cell membrane, whereas the size of the particles determined their passage through the nuclear membrane [109]. Negatively charged nanoparticles do not infiltrate into the cytoplasm of both live cells and fixed/permeabilized cells under static conditions. However, under the influence of shear stress, the nanoparticles are bound to the cell membrane in live cells and confined to the cytoplasm in fixed/permeabilized cells. This indicates that the fixation/permeabilization of the cell membrane and a mechanical (shear) force are needed to infiltrate the cytoplasm. Inability of the nanoparticles to penetrate the live plasma membrane despite the applied shear stress could be attributed to their large size. Smaller nanoparticles penetrate deeper into cells and induce toxicity at far quicker rates than their larger counterparts [78]. On the other hand, cytotoxicity of a nanoparticle may depend on either the higher negative charge on the surface or to their preferential accumulation in the cytoplasm [85].

Shear stress stimulates nitric oxide (NO) production from endothelial cells by using a variety of protein regulators including caveolin and Ca^{2+} /calmodulin and various protein kinases, protein kinase A and B (Akt), in a time-dependent manner [8]. Shear stress induces an increase in the intracellular concentrations of Ca^{2+} and tetrahydrobiopterin, an essential cofactor of endothelial nitric oxide synthase (eNOS), and activation of protein kinases leads to eNOS activation. In addition to increase in eNOS levels, shear stress also stimulates production of tetrahydrobiopterin by markedly enhancing guanosine triphosphate (GTP) cyclohydrolase-1 activity *via* casein kinase 2-dependent phosphorylation [18, 106]. Production of other potential vasodilators, C-type natriuretic peptide and expression of adrenomedullin mRNA in human endothelial cells increases under shear stress in a time-dependent and shear stress intensity-dependent manner [15]. In contrast, production of the vasoconstrictor, endothelial cell

levels of precursor preproendothelin mRNA and endothelial angiotensin-converting enzyme (ACE) expression decreases in response to shear stress [80, 88].

By using dynamic cell cultures, *in vivo* situation is simulated more accurately and thereby can be applied as a novel system to investigate the performance of *in vivo* nanoparticle systems, more reliably [81]. When compared with the experiments in ordinary static conditions, under flow conditions sedimentation of gold nanoparticle aggregates onto the endothelial cells are decreased and lessen the cytotoxicity [29]. In this respect it is reasonable that physiologically relevant and realizable microfluidic configurations stress-induced intracellular transport across the lipid membrane can be achieved by the gating of reconstituted mechanosensitive channels, which can be useful for designing drug delivery in medical therapy and understanding complicated mechanotransduction [71].

Exposure to diesel exhaust ultrafine particles

The average exposure to outdoor ultrafine particles (UFPs) with the diameter less than 100 nm in Asian cities is about four-times more than that in European cities but impacts on human health are largely unknown [46]. In the urban aerosol, UFPs concentrations are tens of thousand times more than in nonurban aerosol. They are deposited deep in the lung tissues, translocate to endothelium and skip the innate clearance mechanisms. However, after retention for a long time, these can infiltrate into the interstitium and permeate into the cells. In particular traffic-linked UFPs are toxic to the respiratory, cardiovascular, and nervous systems [47]. Especially diesel exhaust particles which include particles of size smaller than ten micrometres are considered as "probably carcinogenic" (International Agency for Research on Cancer group 2A) and a number of studies showed their genotoxic effects [22]. Diesel exhaust particles directly affect the endothelial cell-cell junctions. After exposure, increasing concentrations of diesel exhaust particles cause increasing redistribution of vascular endothelial-cadherin away from the cell-cell junctions toward intracellular locations [11]. Diesel exhaust particles also down-regulate tight junction protein, *Zonula Occludens-1* (ZO-1), to a greater extent. Increase in endothelium permeability is implicated in inflammatory cell transmigration into sub-endothelial layers with relevance to the initiation of atherosclerosis [50].

Epidemiological studies have shown that there is a strong correlation between atherosclerosis and chronic exposure to UFPs. A higher level of redox active organic compounds and metals containing UFPs induce the expression of pro-inflammatory genes such as interleukin-8 (IL-8), monocyte

chemoattractant protein-1 (MCP-1) and VCAM-1 in addition to the induction of superoxide radical production and up-regulation of stress response genes in endothelial cells [50]. In particular exposure to diesel exhaust UFPs have been recently identified as an important cardiovascular risk factor. Exposure to diesel exhaust decreases the acetylcholine/sodium nitroprusside vasodilation ratio and is impaired NO-mediated endothelial vasomotor function by promoting reactive oxygen species generation in endothelial cells [104]. Thus acute diesel exhaust appears to uncouple NOS, increasing reactive oxygen species generation and causing endothelial dysfunction, potentially because of depletion of tetrahydrobiopterin limiting its bioavailability [13]. Exposure to diesel exhaust particles of human brain microvascular endothelial cells display significantly higher oxidative stress and this may indicate a potential role of diesel exhaust particles in neurotoxicity [95]. Nevertheless, proinflammatory cytokines released from particle-laden monocyte-derived macrophages appear to exacerbate endothelial activation after diesel exhaust particles exposure [89].

Endothelin-1 (ET-1)-mediated coronary artery constriction is augmented in arteries from the diesel exhaust-exposed rats. Diesel exhaust exposure diminishes the endothelin type B receptor activation of endothelial NOS and augments the endothelin type B-dependent vasoconstriction [12]. Motorcycle exhaust particles (MEP) exposure induce both mRNA and protein expression of VCAM-1 and ICAM-1 *via* nuclear factor- κ B (NF- κ B) in human endothelial cells. MEP exposure also causes hydrogen peroxide and superoxide formation. While the carbon black nanoparticles could induce VCAM-1 and ICAM-1 protein expression, polycyclic aromatic hydrocarbons only increase the expression of ICAM-1 but not that of VCAM-1 in endothelial cells [49]. Diesel exhaust particulate extracts inhibit nuclear respiratory factor-1 (NRF-1) transcription and endothelial cell viability. Considering NRF-1 is a key nuclear transcription factor regulating genes involved in mitochondrial activity and biogenesis, diesel exhaust particulate extracts may adversely affect mitochondrial function leading to endothelial dysfunction [56]. When comparing the hazards of particles from combustion of biodiesel blends and conventional diesel in old and improved engines, particles emitted from the improved engines are smaller in size and more potent with respect to reactive oxygen species production and DNA damage, but similarly potent concerning cytokine mRNA expression. Furthermore, particles emitted from combustion of biodiesel blends are larger in size, and less or equally potent than particles emitted from combustion of conventional diesel

concerning reactive oxygen species production, DNA damage and mRNA of chemokine (C-C motif) ligand-2 (CCL2) and IL-8, ICAM-1 and VCAM-1 expression in human endothelial cells [34]. Combustion exhausts emitted from motor vehicles and industries represent a major source of nanoparticles in the atmosphere. However, flame-generated organic carbon nanoparticles simulate fresh combustion emissions near roadways. Flame-generated organic carbon nanoparticles also induce a time-dependent increase of proinflammatory lysophospholipid production in endothelial cells leading to apoptotic cell death [74]. In order to display the toxicity to vascular endothelium, UFPs should translocate from the respiratory epithelium towards circulation by different transfer routes and mechanisms, resulting in distribution throughout the body, including the brain, with potential neurotoxic consequences [93]. In one study by Krishnan *et al.*, 15 healthy subjects inhaled filtered air (FA) or exposed to diesel exhaust in two-hour sessions on different days with a minimum 2-week washout period. Short-term diesel exhaust exposure results in hemoconcentration and thrombocytosis, which are important determinants of acute cardiovascular events. Multiplex assay showed a non-significant increase in IL-1 β and IL-6 immediately post exposure followed by MPO and endothelial activation molecules [45]. In a double-blind, crossover, controlled exposure study, 27 adult volunteers were exposed in randomized order to FA and diesel exhaust (100 or 200 $\mu\text{g}/\text{m}^3$ of fine particulate matter). Short-term exposure to diesel exhaust is associated with acute endothelial response and vasoconstriction of a conductance artery [76].

Nanoparticle toxicity and endothelium

Endothelial cells lining the lumen of blood vessels serve as a physiological barrier controlling nanoparticle movement from the vasculature into the tissue. Synthetic amorphous silica is gaining popularity as the material of choice in the fabrication of nanoparticles for use in imaging diagnostics, medical therapeutics, and tissue engineering because of its biocompatible nature. However, recent evidence suggests that silica nanoparticles show a concentration- and size-dependent toxic effect. Silica nanoparticles display surface interactions, rather than mass effect on vasodilator function of aortic vessels [3]. Furthermore, silica nanoparticles significantly reduce endothelium-dependent vasodilation. The degree of attenuation is related to nanoparticle surface area, rather than size [28]. The accumulation of the silica nanoparticles in the lung mostly occurs in capillaries rather than in pulmonary cells, consequently pro-inflammatory mediators, TNF-alpha, IL-6, IL-8 and macrophage inflammatory protein-1alpha (MIP-

lpha), are released by endothelial cells after inhalation of silica particles [62, 114]. Following the silica nanoparticles penetration from the plasma membrane, they rapidly stimulate the release of cytoprotective NO and, to a greater extent, production of cytotoxic ONOO(-). The low [NO]/[ONOO(-)] ratio indicates increased nitroxidative/oxidative stress and correlates closely with endothelial inflammation and necrosis [17]. Silica nanoparticles-induced reactive oxygen species generation that causes oxidative damage followed by the production of malondialdehyde (MDA) as well as the inhibition of superoxide dismutase (SOD) and glutathione peroxidase (GPx). Both necrosis and apoptosis are increased significantly after 24 h exposure. The mitochondrial membrane potential (MMP) obviously decreases in a dose-dependent manner. The toxicity mechanisms of silica nanoparticles on endothelial cells are through DNA damage response [23]. Actually silica nanoparticles could induce endothelial dysfunction through oxidative stress *via* c-Jun N-terminal kinase (JNK), p53 and NF-κB pathways. These suggest that exposure to silica nanoparticles may be a significant risk for the development of cardiovascular diseases [54]. 50 nm and 500 nm amorphous silica nanoparticles cause a decrease in endothelium-dependent relaxation of isolated small mesenteric arteries and systemic inflammation and coagulation events by altering vascular reactivity [64]. The disruption of F-actin cytoskeleton occurred as an initial event in silica nanoparticles-treated endothelial cells. The depolarized mitochondria, autophagic vacuole accumulation, microtubule-associated protein 1 light chain 3 (LC3)-I/LC3-II conversion, and the down-regulation of cellular adhesion molecule expression were all involved in the disruption of endothelial cell homeostasis. The vascular endothelial growth factor receptor2/ phosphoinositide3kinase/Akt/mammalian target of rapamycin (VEGFR2/PI3K/Akt/mTOR) and VEGFR2/mitogen-activated protein kinases/extracellular signal regulated kinase1/2/mTOR (VEGFR2/MAPK/Erk1/2/mTOR) signalling pathway was involved in the cardiovascular toxicity triggered by silica nanoparticles [24].

Duan *et al.* showed that nano-silicon dioxide is internalized into endothelial cells in a dose-dependent manner. Thereafter, the level of NO, the activities of NOS and eNOS significantly decreased in a dose-dependent way, while the activity of inducible NOS (iNOS) markedly increased. The expression of C-reactive protein, as well as the production of proinflammatory cytokines (TNF-alpha, IL-1β, and IL-6) significantly elevated. Moreover, it has been indicated that nano-silicon dioxide has an inhibitory effect on the PI3K/protein kinase B (Akt)/ mTOR signalling pathway. Duan's findings demonstrated that nano-silicon dioxide could disturb the NO/NOS system, induce

inflammatory response, activate autophagy, and eventually lead to endothelial dysfunction *via* the PI3K/Akt/mTOR pathway. This indicates that exposure to nano-silicon dioxide is a potential risk factor for cardiovascular diseases [25].

Carbon nanomaterials have multiple applications in various areas. However, exposure to carbon nanoparticles may be a risk for the development of vascular diseases due to injury and dysfunction of the vascular endothelium [32].

The carbon black (CB) particles exposure is associated with increased surface expression of ICAM-1 and VCAM-1 in endothelial cells. The CB particles exposure increases reactive oxygen species production and damages to the cell membranes, whereas these particles do not alter the mitochondrial enzyme activity or the NO production. Overall, exposure to nano-sized CB particles activates endothelial cells and generates oxidative stress, which is associated with vasomotor dysfunction [102]. Thus single-walled carbon nanotube exposure induces oxidative stress, thereby altering ICAM-1 and VCAM-1 expression. Carbon nanotube induced nuclear NF-κB/p65 translocation can be inhibited by N-acetylcysteine. This indicates that elevated ICAM-1 and VCAM-1 expression is mediated by oxidative stress in aortic endothelial cells, and may play important inflammatory roles in carbon nanotube-induced vascular endothelium damage [116]. Exposure to multi-wall carbon nanotubes increases intracellular reactive oxygen species and MDA levels, as well as decreasing SOD activity and GPx levels [30].

Atherosclerosis is a chronic inflammatory disease that remains as the leading cause of death in the number of countries. Amongst the risk factors for endothelial cell inflammation and the development of atherosclerosis have been identified inhalation of ultrafine particles. Recently, engineered nanoparticles such as titanium nanoparticles have attracted much attention due to their wide range of applications. Titanium nanoparticles exposure increase cellular oxidative stress and DNA binding of NF-κB. Further, phosphorylation of Akt, ERK, JNK and p38 is increased in endothelial cells exposed to titanium nanoparticles. These nanoparticles also significantly increase induction of mRNA and protein levels of VCAM-1 and mRNA levels of MCP-1. Titanium nanoparticles can induce endothelial inflammatory responses *via* redox-sensitive cellular signalling pathways [33].

Exposed to fine titanium particles, endothelium-dependent arteriolar dilation is significantly decreased, and this dysfunction is augmented in endothelial cells exposed to nano-titanium particles. However endogenous endothelial NO production is similarly decreased after inhalation of either fine or nano-titanium in a dose-dependent manner. Microvascular

oxidative stress is significantly increased among both exposure groups. The loss of bioavailable NO after particles exposure is at least partially caused by elevations in local oxidative stress, MPO activity, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity [67]. In the recent studies, it has been indicated that, while iron, copper, chromium, vanadium and cobalt undergo redox-cycling reactions and possess the ability to produce reactive radicals such as superoxide anion radicals and NO, metals like mercury, cadmium and nickel, the primary route for their toxicity is depletion of antioxidant capacity [40, 100]. Likewise, exposure of aortic endothelial cells to yttrium oxide (Y_2O_3) or zinc oxide (ZnO) nanoparticles significantly up-regulates mRNA levels of the inflammatory markers IL-8, ICAM-1, and MCP-1 [31]. Indeed, transmission electron microscopy revealed that the iron (III) oxide (Fe_2O_3), Y_2O_3 , cerium oxide (CeO_2) and ZnO nanoparticles are internalized by human endothelial cells and are often found within intracellular vesicles [41]. Silver nanoparticles (AgNPs) are an important class of nanomaterials as they are widely used for industrial and medical purposes [42]. However, exposure to AgNPs, could inhibit proliferation, damage the cell membrane and seriously induce apoptosis of human endothelial cells whose dysfunction could be ascribed to the activation of NF- κ B pathways and oxidative stress [90]. AgNPs induce NO-dependent proliferation through activation of eNOS by phosphorylation of Serine 1177 [83]. Simultaneously, the inflammatory cytokines, adhesion molecules, and chemokines of human endothelial cells are up-regulated [90]. AgNPs, with a smaller size and higher reactivity, may induce much higher toxicity to endothelial cells compared with particulate matter, thus may be a potential hazardous factor for early atherosclerosis [90]. The studies with cerebral microvasculature identified the involvement of pro-inflammatory mediators that can increase BBB permeability. The results show a size-dependent increase in BBB permeability correlates with the severity of immunotoxicity. Thus, larger Ag-NPs has significantly less effect on rat brain micro-vessel endothelial cells, whereas the smaller particles induce significant effects on all the end points at lower concentrations and/or shorter times leading to neurotoxicity [96]. Similarly, nano-sized cupric oxide (copper (II) oxide; CuO) causes a dose- and time-dependent increase in plasminogen activator inhibitor-1 (PAI-1) expression and p38 phosphorylation due to the generation of reactive oxygen species in the exposed pulmonary microvascular endothelial cells [113]. Bone marrow endothelial progenitor cells (EPCs) are susceptible to acute exposure to inhaled nickel nanoparticles. Tube formation and chemotaxis, but not proliferation, of bone marrow EPCs was

impaired in the nickel nanoparticle exposure [53]. Thus, inhalation of nickel nanoparticles results in functionally impaired EPCs and reduced number in the bone marrow, which may lead to enhanced progression of atherosclerosis [52]. Nanoparticle-triggered endothelial dysfunction is hypothesized to be a dominant mechanism in the development of the diseases. One of the most widely used metals, nano-sized different oxidation stages particles of iron are found to induce cytoplasmic vacuolation, mitochondrial swelling and cell death in human aortic endothelial cells (HAEC). The up-regulation of ICAM-1 and IL-8 expression following the adhesion of monocytes to the HAECs provoke oxidative stress and mediate severe endothelial toxicity. Therefore, intravascular iron oxide nanoparticles may induce endothelial system inflammation and dysfunction by directly interacting with the endothelial monolayer. At first, phagocytized nanoparticles by monocytes may be dissolved and impact the endothelial cells as free iron ions later provoke oxidative stress. All of which are considered to promote atherosclerosis [112].

The other ubiquitously used metal aluminium nanoparticles lead to the increased mRNA and protein expression of VCAM-1, ICAM-1, and endothelial-leukocyte adhesion molecule-1 (ELAM-1) in human endothelial cells. Furthermore, human endothelial cells treated with alumina particles are more adherent for activated monocytes and may initiate proinflammatory response [70]. Mercury is the most dangerous one among the all heavy metals [84] and it can associate with sulphides and natural organic matters to form chemical species that include organic-coated mercury sulphide nanoparticles as reaction intermediates of heterogeneous mineral precipitation. Methylmercury production in bacteria cultures exposed to mercury nanoparticles is six times greater than in cultures treated with microscale particles. In contrast to the larger particles, the methylation rate of mercury derived from nanoparticles is highly significant in the aqueous phase [115]. This indicates that mercury-induced vascular endothelial dysfunction involves lipid signalling enzyme activities [57]. Additionally, mercury also activates vascular endothelial cell phospholipase D and even very low levels of chronic mercury exposure promote endothelial dysfunction as a result of the decreased NO bioavailability [108].

Conclusions

Although the organism intends to prevent the adverse outcomes of nanoparticles, detrimental effects on the immune, cardiovascular systems, liver, kidney and brain are unavoidable depending on the following conditions. As a matter of fact,

nanoparticles can easily cross the endothelium, BBB and/or produce damage to the barrier integrity by altering endothelial cell membrane permeability depending on their size, surface charge and structure [26]. Following exposure to nanoparticles, the impairment of endothelium-dependent dilation in the systemic microcirculation coincides with enhanced oxidative stress. Thus, the loss of controlling the passage of macromolecules between the endothelium and interstitial space is a result of inflammation. Inhibiting production of the biomolecular corona provides the remarkably fast transport of nanoparticles across the endothelium. On the other hand, dynamically changing mechanical forces that are generated by the blood flow are major determinants of endothelial activation and nanoparticle uptake *in vivo*. UFPs are deposited deep in the lung tissues, translocate to endothelium and skip the innate clearance mechanisms. Many nanoparticles show a concentration- and size-dependent toxic effect. They also display surface interactions, rather than mass effect on endothelial cells. Nanoparticle-induced oxidative damage is followed by the production of reactive and potentially mutagenic lipid peroxides, as well as the consumption of antioxidant capacity. Thus, reducing the exposure to nanoparticles or supporting the antioxidant status of the organism and the investigation of the physico-chemical properties of the nanoparticles *versus* their mechanism of action on the host may help to take preventive measures against the destructive effects of these materials.

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