Nanoparticles, Lung Injury, and the Role of Oxidant Stress

Amy K. Madl, Laurel E. Plummer, Christopher Carosino, and Kent E. Pinkerton

Center for Health and the Environment, University of California, Davis, California 95616; email: akmadl@ucdavis.edu, kepinkerton@ucdavis.edu

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Abstract

The emergence of engineered nanoscale materials has provided significant advancements in electronic, biomedical, and material science applications. Both engineered nanoparticles and nanoparticles derived from combustion or incidental processes exhibit a range of physical and chemical properties that induce inflammation and oxidative stress in biological systems. Oxidative stress reflects the imbalance between the generation of reactive oxygen species and the biochemical mechanisms to detoxify and repair the damage resulting from reactive intermediates. This review examines current research on incidental and engineered nanoparticles in terms of their health effects on lungs and the mechanisms by which oxidative stress via physicochemical characteristics influences toxicity or biocompatibility. Although oxidative stress has generally been thought of as an adverse biological outcome, this review also briefly discusses some of the potential emerging technologies to use nanoparticle-induced oxidative stress to treat disease in a site-specific fashion.

INTRODUCTION

The emergence of engineered nanoscale materials has shown promise for significant advancements in the fields of imaging, electronics, and therapeutics (1–7). Although nanotechnology may be an emerging field, the study of particles less than 100 nm in diameter (also known as ultrafine particles) has been ongoing for decades. Nanoparticles come from many different sources; they exist naturally in the environment (e.g., in forest fires, viruses, and volcanoes), are produced as by-products of industrial or combustion processes (e.g., in engines, power plants, and incinerators), and are intentionally made for various industrial or consumer product applications (e.g., as pigments and chemical catalysts). The emergence of nanotechnology has added a new type of nanoparticle to this list, namely engineered nanoparticles. Nanotechnology has been defined as a field that "involves a wide range of technologies that measure, manipulate, or incorporate materials and/or features with at least one dimension between approximately 1 and 100 nanometers (nm). Such applications exploit the properties, distinct from bulk/macroscopic systems, of nanoscale components" (8).

Engineered nanomaterials being developed today are produced in a variety of compositions (e.g., metal, elemental semiconductor, compound semiconductor, and metal oxide), shapes (e.g., spiral, wire, belt, spring, pillar, and helix), and structures (e.g., core/shell and single composition). Similarly, nanoparticles derived from combustion or incidental processes exhibit a range of physical and chemical properties. Incidental nanoparticles generated from combustion processes have the potential for aggregation, forming accumulation mode particles with very unique physical and chemical characteristics (9–11). Although transition metals and polycyclic aromatic hydrocarbons (PAHs) are primary components of these ambient particles, both engineered and incidental nanoparticles can be high in carbon and metal content. Research has shown clear relationships between these different physicochemical characteristics of nanoparticles and the induction of inflammation and oxidative stress in biological systems.

Oxidative stress reflects the imbalance between the generation of reactive oxygen species (ROS) and the biochemical mechanisms to detoxify and repair the damage resulting from reactive intermediates. The imbalances in the cellular oxidative state can lead to the generation of peroxides and free radicals, which in turn can damage proteins, lipids, and DNA. Because ROS act as cellular messengers in redox signaling, oxidative stress can lead to interferences or disruptions in normal cell signaling. Due to the belief that oxidative stress plays an important role in several neurodegenerative and cardiovascular diseases and is likely involved in the development of age-related cancer, a significant amount of research has been invested over the past several decades to better understand the pathophysiological effects of oxidative stress and the implications for the natural history of disease processes.

The purpose of this review is to examine the role of oxidative stress in the health effects of nanoparticles on the lung. Current research on well-studied nanoparticles, such as ambient ultrafine particles, as well as of emerging engineered nanomaterials, such as carbon nanotubes (CNTs), is evaluated in terms of their health effects on the lung and the mechanisms by which oxidative stress via physicochemical characteristics influences the toxicity or biocompatibility of nanoparticles. Readers are directed to recent reviews on methods to measure oxidative stress from exposure to nanoparticles and on proposed screening approaches to predict nanoparticle toxicity or biocompatibility (12, 13). Although oxidative stress has generally been thought of as an adverse biological outcome, this review also briefly touches on some of the potential emerging technologies to use nanoparticle-induced oxidative stress to treat disease in a site-specific fashion (e.g., in Alzheimer's disease).

MECHANISMS OF OXIDATIVE STRESS

Nanoparticle-induced oxidative stress is thought to occur through a number of different mechanisms as a result of the intrinsic properties of nanoparticles, as well as through extrinsic nanoparticle-cell interactions (**Figure 1**). Nanoparticles can have oxidant-generating properties. Transition metals, which can be major or trace contaminants of incidental or engineered nanoparticles, can catalyze the production of the hydroxyl radical from hydrogen peroxide via Fenton-like reactions, which in turn can initiate lipid peroxidation. Stable free radical intermediates present on reactive particle surfaces (such as those seen with quartz) and redox-active groups (e.g., quinones) on functionalized nanoparticles can be sources of intrinsic particle oxidant sources (14–18). Cells, such as macrophages and neutrophils, can act as potent generators of ROS in response to their interactions with nanoparticles. Mitochondria are the major source of ROS through normal

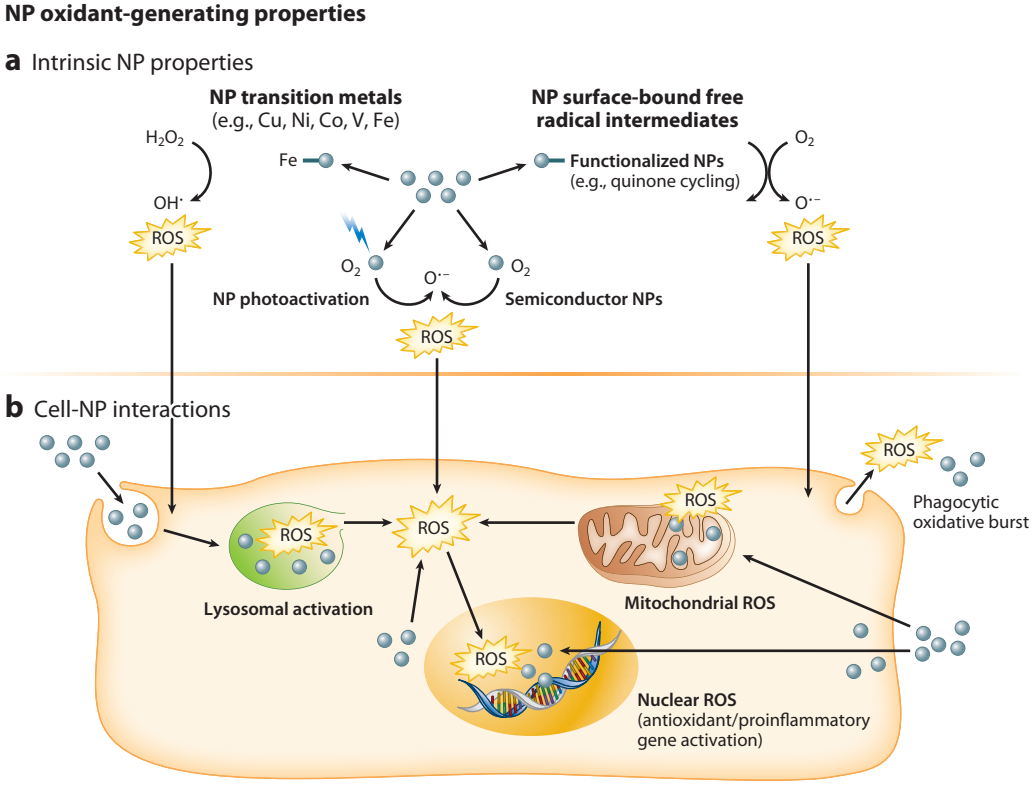

Figure 1

(*a*) Nanoparticles (NPs) exhibit intrinsic oxidant-generating properties. Nanoparticles may contain transition metals due to particle engineering or as by-products capable of generating ROS through Fenton-like chemical reactions. Free radical intermediates present on reactive nanoparticle surfaces and redox-active groups (e.g., quinones) on nanoparticles are capable of redox cycling, producing superoxide or hydroxyl radicals. Nanoparticles with semiconductor properties can generate superoxide via electrons jumping from the conduction band to oxygen, and photocatalytic-capable nanoparticles facilitate the creation of electron-hole pairs, generating ROS such as superoxide or hydroxyl radicals. (*b*) Nanoparticles also generate and contribute to oxidative stress through direct and indirect cellular interactions. Nanoparticle interaction and damage to internal cellular structures such as lysosomes, mitochondria, and the nucleus can lead to cellular damage and oxidative stress. Through direct gene interaction with nanoparticles or nuclear oxidative stress, activation of signaling pathways for antioxidant or prooxidant responses may be upregulated. Additionally, nanoparticles may indirectly interact with cells to alter ROS production and emission through modified cellular phagocytic activity and oxidative burst. Adapted from References 27 and 31.

mechanisms of cellular respiration; however, if imbalances occur between oxidant generation and the expression of antioxidant enzymes and proteins, oxidant stress can ensue. Recent studies also suggested that ROS-producing mitochondria can prompt inflammasome activation of phagocytic cells, thus providing the cell-signaling link between mitochondria and inflammation (16, 19). Ultimately, whether ROS originate from nanoparticles themselves and/or through the cellular response to nanoparticles, oxidative stress and the accumulation of oxidative products can lead to cell damage and death when the equilibrium between pro- and antioxidants is disrupted.

Many cellular and enzymatic players generate endogenous sources of ROS. A primary source of ROS is the generation of a wide variety of oxidants through phagocytic respiratory bursts of macrophages and neutrophils. Much understanding of cell-based synthesis of ROS was developed through the investigation of antimicrobial systems used by leukocytes in which bacterial killing is facilitated by the generation of a variety of highly effective bactericidal agents, including the superoxide anion, hydrogen peroxide, and halo-oxygen species such as HOCl. The respiratory burst is a metabolic event in which cells manufacture large quantities of highly reactive oxidants in response to a stimulus and is a cyanide-insensitive, rapid utilization of oxygen and glucose. Leukocytes, such as neutrophils and macrophages, produce ROS during phagocytosis or via stimulation with a wide variety of agents through the activation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (20). The capacity for this rapid response is regulated by protein phosphorylation of a key subunit, $p47^{phox}$, a 45-kDa oxidase component that is extensively phosphorylated when the oxidase is activated. This subunit moves to the phagosomal membrane, where, as it organizes with the remaining components of the active enzyme, it generates electrons to reduce molecular oxygen (21). The organized oxidase complex acts through a catalytic subunit ($gp91^{phox}$, also known as NOX2) to bind NADPH, extract electrons, and transport them across the phagosomal membrane to react with oxygen through an iron-heme complex, thus secreting superoxide into the lumen (22). Differentiated macrophages (such as alveolar macrophages) also generate ROS by using a NOX2-based metabolism but lack the myeloperoxidase (MPO) present in neutrophils that is necessary for HOCl generation. Macrophages also generate nitrogen-based radicals through nitric oxide synthase and its interaction with ROS. It is now becoming clear that nonphagocytic cells use similar ROS-generating enzyme complexes for both innate defense and intracellular signaling (23).

The balance between the pathological and protective mechanisms of ROS is most apparent in the normal biochemical machinery of the mitochondria. Mitochondria are major producers of ROS and may also be major targets for oxidative damage. This delicate balance between ROS generation in meeting cellular energy demands versus perturbations that lead to dysfunction, cell death, and disease has been a major area of research to better understand the fundamental attributes of mitochondria and the role of mitochondrial dysfunction in generating disease (24, 25).

Cellular pathogenesis can be initiated and enhanced by mitochondrial oxidative stress. Mitochondrial ROS are generated in the respiratory chain during ATP synthesis due to leakage of electrons from mitochondrial complexes I, II, and III (26). Although cellular and mitochondrial antioxidants maintain basal levels of ROS, perturbations can lead to mitochondrial oxidative stress, which in turn can result in interruption of the energy supply, calcium imbalances, the release of lethal proteins, and a culmination of changes that lead to apoptosis or necrosis. Although severe mitochondrial oxidative stress can produce pathological dysfunction that leads to apoptosis or necrosis, minor ROS generation is thought to result in protective preconditioning against a subsequent severe oxidative stress attack (26).

A hierarchical oxidative stress model has been proposed as a possible explanation of the differential effects of minor versus severe oxidative stress. In this hierarchical oxidative stress model, minor oxidative stress triggers antioxidant protection, whereas higher oxidant stress initiates specific signaling and gene expression pathways that can cause cellular and organelle injury and

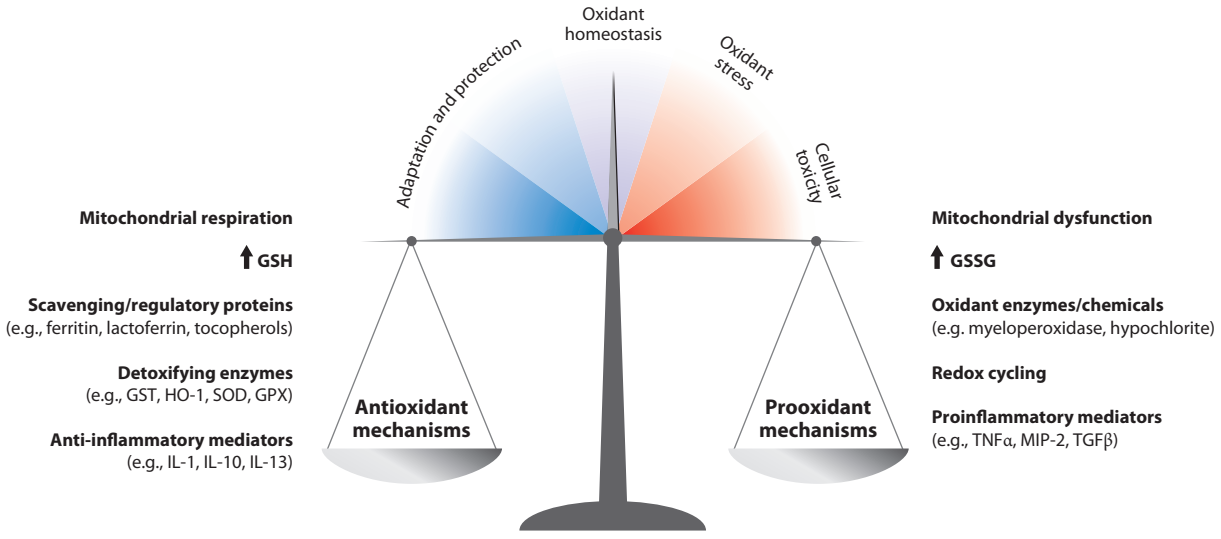

Figure 2

Oxidative stress reflects the imbalance between the generation of reactive oxygen species (ROS) and the biochemical mechanisms to detoxify and repair damage resulting from reactive intermediates. Antioxidant defense, inflammation, and toxicity follow a continuum from adaptation and compensation of oxidant stresses to an equilibrium whereby the protective antioxidant mechanisms break down or are overwhelmed. Abbreviations: GPX, glutathione peroxidase; GSH, glutathione; GSSG, glutathione disulfide; GST, glutathione-*S*-transferase; HO-1, heme oxygenase 1; IL, interleukin; MIP-2, macrophage inflammatory protein-2; SOD, superoxide dismutase; TGFβ, transforming growth factor β; TNFα, tumor necrosis factor α.

cell death (27–29). Oxidative stress is an imbalance or disequilibrium of the redox state of a cell; cellular glutathione (GSH) and glutathione disulfide (GSSG) are the primary regulators of the redox balance. The extent and rate by which GSH/GSSG levels are changed can determine whether stress responses are protective or injurious in nature (30–32). **Figure 2** illustrates the careful balance between antioxidant and prooxidant mechanisms and the implications for maintaining biochemical homeostasis or for leading to oxidant disruption that translates into cellular toxicity. In the hierarchical oxidative stress model, antioxidant defense, inflammation, and toxicity are defined as the three tiers along the continuum of adaptation and compensation of oxidant stresses to an imbalance whereby the protective antioxidant mechanisms break down or are overwhelmed. In the first tier, protective antioxidant and detoxifying enzymes are induced. Heme oxygenase, glutathione-*S*-transferase (GST), NADPH quinine oxidoreductase, catalase, superoxide dismutase (SOD), and glutathione peroxidase are examples of enzymes that influence and regulate cellular oxidant capacity (31, 33–36). In the second tier of the oxidant stress paradigm, proinflammatory processes are initiated when ROS production surpasses the protective antioxidant mechanisms. These proinflammatory effects are mediated by redox-sensitive mitogen-activated protein kinase and nuclear factor-κB cascades, which initiate the expression of cytokines, chemokines, and adhesion molecules involved with the inflammatory process in the lungs (28, 30–33). In the third tier of the oxidant stress model, cytotoxicity involving perturbation of mitochondrial permeability and electron transfer in organelle respiration results in cellular apoptosis or necrosis (31, 32, 37).

Although it had been well understood that the generation of ROS plays an important role in the pathogenesis of highly surface-reactive particles like quartz and asbestos (38–41), it was not until research on nanosized and fine-sized low-toxicity, low-solubility particles (e.g., titanium dioxide, carbon black, and polystyrene beads) that particle size was also appreciated to have

an important role in ROS-mediated cytotoxicity (42–48). For example, exposure to polystyrene nanoparticles, which are thought to be relatively inert because they do not spontaneously produce ROS, results in lysosomal leakage, ROS production, and mitochondrial damage that lead to apoptosis in murine macrophages (29, 31). This work clearly showed that nanoparticles lacking surface chemical reactivity can induce ROS through biological interactions that directly or indirectly target the mitochondria. Since the early studies of low-toxicity, low-solubility particles, researchers have sought to better understand the role and mechanisms by which oxidative stress is generated, biochemical processes are disrupted, and cellular damage is induced from incidental and engineered nanosized particles. The subsequent sections of this article address pulmonary health effects resulting from exposure to incidental ambient nanoparticles and engineered CNTs and what is currently understood in regard to the involvement of oxidative stress in the pathology associated with these two types of nanoparticles.

INCIDENTAL NANOPARTICLES

Health Effects and Lung Injury

Particulate air pollutants are complex mixtures derived from a variety of anthropogenic and natural sources. Ambient particulate matter (PM) can cause effects on the respiratory system that are similar to the effects of particulates found in occupational settings. However, extrapulmonary changes (e.g., cardiovascular effects) have also been documented from peak exposures to ambient PM. Whereas research on the effects of ambient PM over the past few decades has focused on the PM in coarse- and fine-sized fractions, considerable efforts have recently been expended to understand the role that ultrafine particles may play in either contributing to or exacerbating cardiopulmonary disease in normal and susceptible populations. However, particularly in the era of nanotechnology, researchers now refer to ambient ultrafine particles as nanosized particles produced by incidental means to make a distinction from engineered nanoparticles.

Dissimilarly sized fractions of ambient PM have very different physicochemical characteristics, partly because of their emission sources. Coarse particles are generally composed of natural materials (e.g., minerals, silicates, pollen) derived from weathering and disturbance of earth soils, whereas fine particles usually originate from anthropogenic sources (e.g., combustion processes, industrial emissions) and are composed of a mixture of elemental and organic carbon, sulfate, nitrate, minerals, and metals. Ultrafine PM is also generated through combustion processes but can quickly aggregate to form larger-sized particles (9–11).

Episodes of intense particulate air pollution have caused increased morbidity and mortality for at least a half-century. The Donora death fog of 1948 and the London fog of 1952 were notable air pollution events that led to more than a few thousand excess deaths. More recently, the work from the Harvard Six Cities Studies in 1993 showed that peak particulate air pollution events led to increased deaths from lung cancer and cardiopulmonary disease (49). Over the past decade, more than 100 studies from more than 35 different cities have investigated the acute effects of ambient PM; these studies have shown increased hospital admissions and deaths from cardiopulmonary disease (e.g., asthma, chronic obstructive pulmonary disease, arrhythmia, heart attack) (50–52). The effects appear to best correlate with $PM_{2.5}$ (PM 2.5 μm or less in diameter), with an increased mortality of 0.5 to 1.5% for every incremental concentration increase of 5 $\mu g/m^3$ (51). These studies have served as a basis for the National Ambient Air Quality Standard for PM (10).

There is considerably less information about the chronic effects of ambient particulate pollution. Although lung tissue remodeling occurs from exposure to ambient PM, these changes tend to be less prevalent and severe compared with those observed in occupational settings. Autopsy

studies have provided some means for assessing the potential long-term effects of exposure to ambient PM. Findings from these studies have suggested that long-term exposures to ambient PM can lead to remodeling of the lungs, with the most significant changes occurring in the respiratory bronchioles and bronchoalveolar duct junctions or centriacinar regions of the lungs. Pinkerton et al. (53), for example, evaluated lungs of deceased young males in California's Central Valley who died of nonrespiratory causes. The individuals had been exposed to ambient conditions consisting primarily of mineral and carbonaceous dusts. Dust deposition was observed principally in tissue sections at the terminal bronchioles and at first-generation respiratory bronchioles, with less deposition observed in the upper airways. There were significant wall thickening via inflammatory cell accumulation (dust-laden macrophages), increased collagen deposition, and smooth muscle cell hypertrophy, resulting in terminal and first-generation respiratory bronchiole structural remodeling. Changes in lung structure and particle retention appeared to be inversely proportional to the distance from the center of the lung acinus; particle retention decreased from the first generation to the second generation to the third generation of respiratory bronchioles. The effects seen in the respiratory bronchioles were also observed in the lungs of both smokers and nonsmokers, with more severe changes occurring in smokers. Pinkerton et al. suggested that there might be synergistic effects between ambient PM and cigarette smoke, but despite any interactions, respiratory bronchiole remodeling as a result of exposure to ambient PM could be detected irrespective of the smoking status (53).

The findings from Pinkerton and colleagues (53) have also been observed in other PM-exposed populations. Subjects living in high-PM areas in Canada showed increased particle deposition in the respiratory bronchioles and at airway bifurcations; such particle deposition correlated with airway remodeling (54, 55). These studies have shown correlations of high ambient PM exposure with particle deposition and airway remodeling in the lungs, with indications that fine particles ($PM_{2.5}$) and aggregations of ultrafine particles [$PM_{0.1}$ (PM less than or equal to 0.1 μm in diameter)] are likely the particle size fractions contributing to these effects because of their prevalence in lung tissue digests (53, 56). Further studies comparing individuals exposed to nonoccupational, high ambient PM have indicated that ultrafine particles retained in the bronchiole airway walls are associated with fibrogenic small airway remodeling and may produce chronic airflow obstruction (57). Although these results may suggest possible effects from long-term exposures to ambient particulate air pollution, other confounders (e.g., genetics, lifestyle, smoking, occupational exposures, other ambient pollutants) may contribute to these effects and may not be fully accounted for in individuals' case histories (53, 55–59).

As a means to understand the potential chronic effects of ambient PM, researchers have utilized tracheal explants as a model system for controlling the administered PM dose to a particular region of the lungs. More specifically, particles (collected ambient PM, mineral dusts, and diesel exhaust PM) administered into the airway lead to the expression of mediators promoting fibrosis and smooth muscle hyperplasia. The expression occurs without exogenous inflammatory cells and suggests that PM may directly cause epithelial cell injury, airway remodeling, and possible obstruction even in the absence of inflammation (60, 61). Furthermore, studies of concentrated ambient particles (CAPs) have shown both pulmonary and extrapulmonary effects, including upregulation of proinflammatory genes and markers of oxidative stress in the lungs, as well as systemic effects that suggest an increased risk of atherosclerosis (59, 62, 63).

Role of Oxidative Stress

Oxidative stress is one of the primary mechanisms by which ambient particulate air pollution exerts adverse health effects. Of the varying sizes of ambient PM, nanosized particles are thought to be

potentially the most hazardous due to their small size; large surface area; and high relative content of redox-cycling organic chemicals with the ability to penetrate, deposit in, and be retained in the deep lung (28, 31). Many of the components of nanosized ambient PM, such as metals and organic carbon compounds, can generate ROS through Fenton and Haber-Weiss chemistry, as well as through redox cycling of organic chemicals (e.g., quinones), which form superoxide radicals (30, 31). The ability of ultrafine particles ($PM_{0.1}$) to generate more free radicals than do coarse particles [PM_{10} (PM greater than or equal to 10 μm in diameter)] or fine particles ($PM_{2.5}$), as measured by induction of heme oxygenase and depletion of intracellular GSH, may be due to ultrafine particles having a large surface area for adsorption of ROS-generating components (64). Inherent ROS generation is commonly associated with transition metals, such as iron, copper, nickel, cobalt, and vanadium. These metals are associated with anthropomorphic sources, with iron having an order-of-magnitude-greater concentration in most samples from polluted regions (65). Transition metals catalyze the production of the hydroxyl radical from hydrogen peroxide via Fenton-like reactions that are enhanced in the presence of physiological concentrations of ascorbate (**Figure 1**) (66). The use of metal chelators or antioxidants to ameliorate the oxidative stress induced by PM has shown that metals play an important role in the prooxidant and proinflammatory effects of ambient PM (31, 67).

In addition to transition metals, environmental PM contains PAHs and quinones that undergo redox cycling to generate ROS. During the burning of hydrocarbons, radicals formed early in combustion interact, forming PAHs, including carcinogens, from less complex structures. PAHs aggregate into nanoparticles, which can extend into the branched-chain structures observed as black smoke or soot (68). Quinones are derived from PAH components and likely include compounds such as 1,4-naphthoquinone; 5,12-naphthacenequinone; bez[*a*]anthracene-7,12-dione; and anthracene-9,10-dione. These quinones undergo cyclic reduction reactions with oxygen, followed by oxidative coupling with either NADPH or iron to form semiquinones, leading to the formation of superoxide radicals (69). Quinones not only are by-products of fuel combustion but also are generated by the enzymatic conversion of PAH in the lungs (31, 70, 71).

The toxic potential of PM in ambient air or from combustion processes correlates with the chemical composition and the capacity to induce oxidative stress (31, 72, 73). Studies of CAPs show that nanosized particles, which contain a significant amount of PAHs and quinones, have a greater potential to induce oxidative stress in macrophages and epithelial cells than does coarse PM, which is composed mostly of crustal elements (31, 72). Similarly, quinones and PAHs in diesel exhaust particles contribute to oxidant injury of the lung (31, 74–76). Oxidative stress generated from organic chemicals on the surfaces of combustion particles may be responsible not only for proinflammatory effects, but also for adjuvant effects in the respiratory tract, which can lead to nonspecific and allergic inflammatory processes (31, 73). Interestingly, pretreatment of SOD or nitric oxide synthase inhibitors significantly diminished inflammatory cell infiltration and mucus and nitric oxide production and increased airway hyperreactivity (effects involved in the pathogenesis of asthma), emphasizing the role of oxidant stress in inflammatory processes and pathological outcomes (31, 77–79). Similarly, thiol antioxidants (e.g., *N*-acetylcysteine) suppressed adjuvant effects of diesel exhaust particles on ovalbumin-induced allergic responses (31, 80). The importance of the role of antioxidant and enzyme detoxification pathways is further emphasized by the observation of increased nasal allergic and allergen-specific IgE responses in individuals who exhibit the GST M1-null genotype following exposure to diesel exhaust particles (31, 81). In conclusion, although recent research has provided a better understanding of the potential drivers of oxidative stress from ambient PM, the complexity of particle-cell interactions and the associated intrinsic and extrinsic sources of ROS still leaves much to be evaluated in terms of defining the role and molecular pathways by which oxidative stress leads to PM-induced disease processes.

ENGINEERED NANOMATERIALS

Health Effects and Lung Injury

Nanoscale (<100-nm-diameter) particles resulting from manufacturing processes (e.g., ultrafine titanium dioxide, carbon black) or combustion processes (e.g., vehicle exhaust, air pollution) have been studied extensively for decades. However, the potential biocompatibility and toxicity of engineered nanomaterials have only recently received attention from the scientific community. Carbon-based engineered nanoparticles, such as single-walled CNTs (SWCNTs), multiwalled CNTs (MWCNTs), and fullerenes, have received notable attention due to their superior electronic, optical, mechanical, chemical, and even biological properties. Questions have been raised as to whether the unique properties of these materials may exert biological effects distinct from those of their parent material (82, 83). CNTs are hollow graphite tubes that can be visualized as a single sheet of graphite rolled to form a cylinder. CNTs are composed of either a single layer (i.e., SWCNTs) or multiple layers of individual SWCNTs stacked within one another (i.e., MWCNTs) and are manufactured by either electrical arc discharge, laser ablation, or chemical vapor deposition processes (84, 85).

The type of carbon nanoparticle (i.e., SWCNT, MWCNT, or fullerene), the method of processing (i.e., refined or unrefined), the presence of residual transition metal catalysts, and the functionality of different reactive groups are a few parameters that researchers have tested in cultured cells to better understand which physicochemical characteristics influence toxicity (86–89). SWCNTs, for example, appear to have greater toxic effects on cultured human fibroblasts than do MWCNTs, active carbon, carbon black, and graphite carbon. In addition, acid treatment (a method of CNT refinement and removal of residual metal catalysts) of SWCNTs produced more toxicity than did its unrefined counterpart (89). These findings are supported by other studies that show that acid treatment and subsequent functionalization of SWCNTs or fullerenes influence the extent of toxicity on human lung tumor cell lines and primary immune cells (86–88). The addition of carbonyl, carboxyl, or hydroxyl groups on the surfaces of CNTs induces cell death in lung tumor cells (87). Functionalization of SWCNTs with water-soluble functional groups appears to influence cellular specific uptake and tolerance by primary immune cells, whereas nonfunctionalized CNTs induce oxidative stress and apoptosis in a variety of cell systems (90–95).

Whereas clear toxicological differences between carbon nanoparticles functionalized with different chemical moieties have been observed with in vitro cell systems, these same responses are not always seen when the same material is administered in vivo. The different toxicity responses of in vitro versus in vivo studies have been specifically observed in the context of functionalized fullerenes administered to different human cell lines (dermal fibroblasts, lung epithelial cells, astrocytes) compared with pulmonary responses of rats administered the same material by intratracheal instillation (88, 96). As for in vivo studies conducted on CNTs, most investigators report inflammation, progressive fibrosis, and granulomas in rodents exposed to CNTs via intratracheal installation or pharyngeal aspiration. More specifically, as a result of these exposures, acute dose-dependent changes in alveolar wall thickness, immune cell recruitment, and indicators of cellular damage and oxidative stress (as measured by levels of inflammatory cells, cytokines, and protein in bronchoalveolar lavage) were observed (97–101). CNTs also produce pulmonary function deficits, impairment of bacterial clearance, aortic plaques, and atherosclerotic lesions (99, 102–104).

In an attempt to understand how different physical and chemical parameters contribute to toxicological effects, researchers have evaluated the impact of the method of CNT production, as well as the influence of milling CNTs or altering the content and type of metal catalyst on toxicity in animals (98, 105, 106). Results suggest that all the various formulations of CNTs

produce pulmonary lesions (97). A relatively recent published study shows that these effects can be exacerbated by feeding animals a vitamin E–deficient diet, thereby reducing the antioxidant capacity (GSH, ascorbate, α-tocopheral) of the lungs while enhancing acute inflammation and fibrotic responses (101).

A few inhalation studies of SWCNTs and MWCNTs using a variety of aerosol delivery systems have recently attempted to address whether the pulmonary effects of CNTs can be attributed to the method of administration (e.g., pharyngeal aspiration or intratracheal instillation) or to the toxicity of the particle itself (107–115). In these recent studies, differences were apparent in the pattern and extent of pathology across the different types of nanomaterials (SWCNTs versus MWCNTs), as well as in approaches for delivery to the respiratory tract (aspiration versus inhalation). MWCNTs delivered by intratracheal instillation or pharyngeal aspiration produce inflammation and fibrosis biochemically and histologically at delivered doses of up to 5 mg per rat (98, 116), whereas inhalation of aerosolized MWCNTs produces mixed results. For example, no pulmonary lesions were observed following exposure to 100 mg/m^3 MWCNTs for 6 h (109) or to 5 mg/m^3 MWCNTs for 14 days (108). In fact, in one study, inflammatory responses in the spleen were more sensitive to MWCNT exposures than those observed in the lungs (108). In mice exposed by inhalation to 0.3, 1, or 5 mg/m^3 MWCNTs for 7 or 14 days (followed for 7 and 14 days postexposure), alveolar macrophages in bronchoalveolar lavage and in lung tissue sections contained black particles, but without elevations of white blood cell counts in bronchoalveolar lavage or oxidant stress markers or pathology in the lungs. Changes in immunosuppression markers (e.g., T cell antibody and proliferative response) and cytokine gene expression of interleukin (IL)-10 and NAD(P)H oxidoreductase, however, were observed in the spleen (108).

In contrast to the negative pathological changes in the above-discussed studies, other researchers have reported thickening of alveolar walls following exposure to 32 mg/m^3 MWCNTs for 15 days (107), lung injury and fibrosis under conditions of preexisting allergic inflammation following exposure to 100 mg/m^3 MWCNTs for 6 h (109), inflammation and granuloma formation following exposure to 0.5 or 2.5 mg/m^3 MWCNTs for 90 days or 0.4 mg/m^3 MWCNTs for 13 weeks (113, 114), and subpleural fibrosis and mononuclear cell aggregates following a single 6-h exposure to 30 mg/m^3 MWCNTs (115). To evaluate the effects of inhaled CNTs, researchers have used a variety of systems, such as a nebulizer, jet mill, and powder generator, to aerosolize these carbon-based nanomaterials with median mass aerodynamic diameters (less than 2 μm) within the respirable size range.

In comparison, studies of SWCNTs have reported pulmonary inflammation, interstitial fibrosis, and granulomas following exposure by instillation, pharyngeal aspiration, and inhalation (97–101, 110, 117). Two studies published by researchers at the National Institute for Occupational Safety and Health suggested that administration of dispersed SWCNTs, by either aspiration or inhalation, increases collagen and alveolar wall thickness compared with less dispersed forms of SWCNTs delivered by aspiration (110, 117). SWCNTs may elicit these responses by ROS generation mediated through the presence of residual iron catalysts that are decorated on the surfaces of the SWCNTs as well as through the release of inflammatory mediators. Although MWCNTs are clearly recognized by alveolar macrophages (108, 109), the evasion of SWCNTs from macrophage phagocytosis may contribute to their facilitated translocation to the interstitium, where collagen production is stimulated. Whether macrophage clearance and transepithelial migration, or differences in physicochemistry, can explain the dissimilar pathological patterns in the respiratory tract between MWCNTs and SWCNTs will require further investigation.

Because of the elongated and fibrous nature of CNTs, some researchers have suggested analogies between these engineered nanoparticles and asbestos. In fact, two studies have compared

peritoneal responses of MWCNTs to asbestos following intraperitoneal injection. In one study, inflammatory and granuloma responses in the peritoneal cavity were assessed following an intraperitoneal injection (50 μg/mouse) of MWCNTs, amosite asbestos (long fibers >10–20 μm long or short fibers <5 μm long), or nanoparticle carbon black (118). The granulomatous inflammation was greater for long MWCNTs and amosite formulations than for formulations containing mostly short MWCNTs or amosite fibers. Although the responses observed with long MWCNTs and amosite fibers were similar, the study did not address whether the inflammatory or granulomatous changes would lead to mesotheliomas (118). Although highly criticized for the dosing regimen and animal model selection, Takagi et al. (119) reported mesotheliomas in p53 heterozygous mice following single 3-mg intraperitoneal injections of MWCNTs or crocidolite fibers but reported that mice injected with fullerenes did not develop mesotheliomas (119). In contrast, a 2-year bioassay showed no mesotheliomas as a result of single intraperitoneal injections of MWCNTs (with and without structural defects) in mice, whereas significant incidence rates of mesothelioma tumors were observed with intraperitoneal injections of crocidolite (120). Despite the findings in these three studies, further research is needed to evaluate whether any pathological changes in the appropriate animal model can lead to tumors following acute exposure to CNTs via relevant routes of administration (e.g., inhalation) (118, 121, 122).

Role of Oxidative Stress

In a manner similar to that of incidental ambient nanoparticles derived from anthropogenic sources, engineered nanoparticles can produce ROS and oxidative stress through intrinsic physicochemical properties of the nanoparticles, as well as through interactions of nanoparticles with immune and epithelial cells in the lungs. There are various proposed mechanisms by which engineered nanoparticles produce ROS (28, 31). First, electron-hole pairs through UV activation may participate in electron donor or capture interactions that generate superoxide or hydroxyl radicals. Second, semiconductor properties may lead to electrons jumping from the conduction band to oxygen to generate superoxide. Third, dissolution of the nanoparticle to release metal ions may catalyze ROS generation, and lastly, transition metals on the nanoparticle surface may generate superoxide radicals via Fenton chemistry (28, 31). Also, in a manner similar to that of ambient nanosized particles, engineered nanoparticles may produce oxidant injury via nanoparticle-cell interactions and ROS derived from inflammatory processes and mitochondrial dysfunction.

Given some of the physicochemical similarities (e.g., the presence of transition metals, high aspect ratio, potential biopersistence) of engineered nanoparticles (e.g., CNTs, titanium oxide nanobelts, silver nanowires) to other high-aspect-ratio particles such as asbestos, researchers have sought to understand how the role of oxidative stress integrates with some of the health effects seen in the lung following exposure to CNTs. Studies have generally focused on oxidative responses from the presence of residual transition metal catalysts associated with the production of SWCNTs and MWCNTs and/or from the recruitment of phagocytic cells as a result of phagolysosomal activation. As-produced (iron-rich) SWCNTs are more effective than purified SWCNTs (in which iron is removed through acid treatment) in stimulating hydroxyl radicals in zymosan-stimulated RAW 264.7 macrophages, in increasing intracellular ROS, and in decreasing mitochondrial membrane potential in rat macrophages and human lung cells (16, 123, 124). Although several studies have shown that the presence of transition metals (e.g., iron) is important in determining the redox-dependent responses of macrophages, CNTs have other characteristics that make them unique from other toxicants such as asbestos. Specifically, CNTs may have the ability to quench and scavenge free radicals; this ability may be related to the presence of structure defects and may be associated with genotoxic and inflammatory potential (16, 105, 125).

With the massive oxidizing potential of phagolysosomes, oxidative modification of phagocytized CNTs may occur. MPO, a potent oxidant enzyme source in neutrophils, biodegraded SWCNTs in an acellular system (16, 126). Treatment of biodegradation products of SWCNTs with MPO failed to induce pulmonary inflammatory responses in mice after pharyngeal aspiration (16, 126). Additionally, the clearance of SWCNTs from MPO-deficient mice was markedly reduced compared with clearance in wild-type animals (15, 16). Other carbon-based nanoparticles undergo similar MPO-catalyzed modifications and degradation (16, 127). Hypochlorite and MPO reactive intermediates may be pathways in which the oxidative biodegradation process occurs (15, 16, 127, 128). The biodegradation of SWCNTs has tremendous implications for biopersistence and may result in long-term health effects from prolonged exposures.

Recent work has shown that CNTs are generally durable materials but may undergo biological modification (16, 129). Testing the durability of four types of CNTs in simulated biological fluid demonstrated that durable CNTs with short or tightly bundled aggregates with no long, isolated fibers were less inflammogenic than the longer CNT counterparts (16, 129). Other studies have shown that biodegradation leads to physical and/or chemical modifications. SWCNTs with carboxylated surfaces in artificial phagolysosomal fluid can reduce SWCNT length and result in the accumulation of carbonaceous debris. These biodegradation processes have implications for the hazardous potential of these materials.

CNTs may induce ROS through NADPH oxidase–dependent pathways. In NADPH oxidase–deficient mice, SWCNT exposure resulted in increased accumulation of neutrophils, increased production of proinflammatory cytokines, decreased numbers of anti-inflammatory and profibrotic cytokines, and less collagen deposition compared with wild-type control mice (16, 110). Similarly, deficiency in antioxidant molecules resulted in an increased sensitivity to the inflammatory effects of SWCNTs. Lower levels of antioxidants in vitamin E–deficient mice were associated with greater acute inflammation and enhanced profibrotic responses (increases in transforming growth factor β and collagen deposition) following exposure to SWCNTs (16, 101). In summary, the research thus far suggests that multiple factors (e.g., CNT chemistry, biodegradation, and antioxidant capacity/susceptibility) may influence oxidative stress following exposure to CNTs. Further research is needed to understand which variables affect or are associated with any progression of pulmonary disease.

NANOTHERAPEUTICS AGAINST OXIDATIVE INJURY

One noteworthy aspect of toxicity testing of nanoparticles is that cellular toxicity may actually be a desired outcome and intended use of the nanoparticles, particularly if the target is tumor cells. Although research in the area of biocompatible CNT design is still in its infancy, investigators are functionalizing these nanoparticles to have sufficient biocompatibility, functionality, distribution, retention, and specificity in hopes that these nanomaterials can be utilized as carriers of biological and therapeutic molecules (84, 85). The ways in which researchers are manipulating the CNT chemistry for pharmaceutical applications include entrapment of active components within the CNT matrix or bundle, functional attachment of the compound on the exterior walls of the CNT, and the use of CNT channels as nanocatheters (84, 85). These approaches, as well as the dispersive agents used to solubilize the CNTs, can have dramatic effects on the clearance and retention of these nanomaterials (130, 131).

Researchers are investigating ways in which nanomaterials can be used to target and mitigate or augment endogenous oxidative stress as potential preventative or treatment modalities for disease. One example is the potential use of nanomaterials to transport metal-chelating agents to pathological sites of significant and abnormal metal accumulation within Alzheimer's disease

patients (132). Transition metal accumulations in Alzheimer's disease brains are thought to have an important role in local oxidative reactions and pathological lesions. Metal-chelating agents with the assistance of nanocarriers that can cross the blood-brain barrier (regardless of their size and hydrophilicity), selectively bind, remove, and "redox silence" transition metals provide promising potential for therapeutic intervention (132). Engineered nanomaterials may also act as targeted enhancers of oxidative stress for tumor or cancer therapy. Chemical modification of fullerenes can target mitochondria, inducing significant mitochondrial ROS formation and leading to enhanced apoptosis and necrosis (26). Alternatively, minor mitochondrial ROS formation is thought to precondition by preventing the propagation of mitochondrial ROS during oxidative insults, thus providing potential opportunities for the development of nanoantioxidants (26). Thus, ROS-targeted strategies for either enhancement or prevention of oxidative stress may offer useful approaches for therapeutic interventions in the treatment of cancer, mitochondrial disease, and aging diseases such as Alzheimer's disease.

CONCLUSION

In summary, emerging research on engineered nanoparticles (e.g., CNTs) as well as on nanoparticles in ambient air particle pollution has demonstrated that oxidative stress can be an important mechanism for toxicity and potential health effects in the lung. There are a number of pathways and intrinsic/extrinsic nanoparticle properties by which an imbalance in the prooxidant and antioxidant equilibrium can result in an oxidative stress state. The presence of transition metal catalysts, organic compounds that can undergo redox cycling, and other surface chemistries (e.g., functionalization) can influence the intrinsic potential of nanoparticles to produce oxidative species. Additionally, the biodegradability and biopersistence of nanoparticles are likely to influence nanoparticle-cell interactions, which in turn may have direct effects on the cellular or organelle oxidative status (e.g., mitochondrial cell signaling) or have indirect effects through inflammatory processes (e.g., oxidative burst and phagolysosome activation) and enzymatic detoxification pathways. Genetic or epigenetic phenotypes that influence prooxidant or antioxidant capacity of an individual (e.g., polymorphisms in GST) may have implications for the susceptibility of oxidative stress and potential health effects following exposure to engineered or incidental nanoparticles. Past experience with particles or fibers like asbestos and silica would suggest that characteristics such as surface reactivity, morphology, and biopersistence that influence ROS generation, persistent inflammation, impaired macrophage clearance, and fibrotic lesions are likely important parameters for driving the hazards of CNTs. Additional research in the area of engineered nanomaterials will likely continue to focus on the role of transition metals in ROS generation, particle length and morphology in influencing particle fate and transport, and surface chemistry (e.g., functional groups, dangling bonds) in directing cellular responses.

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