38

FACTORS GOVERNING PULMONARY RESPONSE TO INHALED PARTICULATE MATTER

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38.1 INTRODUCTION

The biological response of the human respiratory system to inhaled aerosol particles depends on many physical and chemical characteristics of the particles, such as particle size, shape, and surface chemistry. Many aerosol measurement instruments and methods described in this book were developed to characterize such physicochemical properties of airborne particulate matter to understand and evaluate its impact on the human health. This chapter describes how the physiochemical properties of particles determine the site of particle deposition in the respiratory tract, the length of time particles remain in the lung, and their reactivity with lung cells. These factors govern the initiation and progression of pathogenic processes leading to disease. The role of unique physiochemical properties of nanoparticles and their impact on pulmonary response is also discussed.

Pneumoconiosis is a Greek term for "dust lung." It denotes types of pulmonary diseases caused by inhalation of dusts. Such lung diseases may be classified as obstructive lung disease, restrictive lung disease, or carcinogenic disease. Obstructive lung diseases are characterized by an increase in airway resistance and a decline in airflow through the large conducting airways. This results in a decline in dynamic lung volumes, that is, the rate of air movement into and out of the lungs, such as FEV_1 (forced expiratory volume during the first second of exhalation). Obstructive lung diseases include asthma, bronchitis, and emphysema. Chronic obstructive pulmonary disease (COPD) includes chronic bronchitis and emphysema. Restrictive lung diseases are characterized by a loss of lung volume and result in a decline in static lung volumes, such as vital capacity. An example of dust-induced restrictive lung disease is pulmonary fibrosis. Pulmonary carcinogenic diseases include bronchogenic cancer of the conducting airways, parenchymal cancer in the alveolar region of the lung, and mesothelioma, which is a malignancy of cells lining the lung.

Inhalation of airborne dusts in the workplace has long been associated with this wide spectrum of occupational lung diseases (Department of Health and Human Services [DHHS, National Institute for Occupational Safety and Health (NIOSH)] 1986). The type and severity of pulmonary response depends on the following: (1) airborne concentration of particles and duration of exposure, (2) the fraction of airborne dust that possesses an aerodynamic diameter small enough to enter the respiratory tract, (3) the fraction

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of inhaled particles that deposits in the respiratory tract and the site of deposition within the tract, (4) the retention time of deposited particles in respiratory tract, and (5) the bioactivity of the deposited particles. These factors governing the pulmonary response to inhaled particles are discussed in this chapter.

Over the past two decades, there has been great interest in the emerging area of nanotechnology. Nanotechonology is the manipulation of matter at the near-atomic scale to produce new structures, materials, and devices. Engineered nanoparticles are defined as having at least one dimension <100 nm. Because of their small size, nanoparticles have a high particle surface area/mass and exhibit physicochemical properties that differ dramatically from fine-sized particles of the same composition. These unique properties are being exploited for a number of applications, including integrated sensors, semiconductors, energy storage and transmission devices, structural materials, drug delivery systems, bone grafting, medical imaging, sunscreens, cosmetics, paints, and coatings. Therefore, nanotechnology has the potential to transform many industries ranging from medicine to manufacturing. In light of the endless number of potential applications, nanotechnology is predicted to grow into a US$1 trillion global industry, employing 2 million workers worldwide, within the next decade (Roco 2004). Since nanoparticles exhibit unique physicochemical properties, it would be reasonable to expect that these nanoparticles would interact with biological systems in ways which may be dramatically different from fine-sized particles of the same composition. Factors to be considered when comparing the pulmonary response resulting from exposure to a nanoparticle versus a fine-sized particle of the same composition are: (1) deposition fraction, (2) interstitialization, (3) surface area dependent bioactivity, and (4) translocation to systemic sites. Therefore, the potential for nanoparticles to represent a unique health concern upon inhalation is discussed in this chapter as well.

38.2 ANATOMIC REGIONS OF THE LUNG

The respiratory tract is divided into three anatomical regions. (1) The extrathoracic or nasopharyngeal region includes the nasal cavity, nanopharynx, oral pharynx, pharynx, and larynx. The nasopharyngeal region of the respiratory tract serves to humidify inhaled air. Particle-induced disease of the extrathoracic region would include rhinitis. (2) The tracheobronchial region includes the trachea, main bronchi, lobar bronchi, segmental bronchi, bronchioles, and terminal bronchioles. This region is called the conducting zone. In a human, the conducting zone includes 16 generations of airways (Table 38-1).

The function of the conducting zone is to transport inhaled air to the gas exchange region of the lung, that is, the respiratory zone. The volume of the conducting zone is relatively small, accounting for 150 ml of air in an adult man. This lung volume is referred to as the anatomical dead space, since no gas exchange occurs in the conducting zone airways. Particle-induced diseases of the conducting airways include obstructive lung disease and bronchogenic cancer. (3) The pulmonary region of the lung is called the respiratory zone. It contains airway generations 17–23 and includes the respiratory bronchioles, alveolar ducts, and alveoli (Table 38-2).

TABLE 38-1 Branching of Human Airways in the Conducting Zone

Generation	Airway	Number of Airways
0	Trachea	1
1	Main bronchi	2
2	Lobar bronchi	4
3	Segmental bronchi	
4–15	Bronchioles	
16	Terminal bronchioles	

TABLE 38-2 Branching of Human Airways in the Respiratory Zone

Generation	Airway	Number of Airways
17–19	Respiratory bronchioles	
20–22	Alveolar ducts	
23	Alveoli	900 million

Note that airway branching starts with one trachea and ends in 900 million alveoli. As a result of this sequential branching, the respiratory zone contains as much as 97% of the lung volume (>5 L) in an adult male. The surface area of airways in the respiratory zone is 85 m^2. This large surface area serves to maximize gas exchange in the respiratory zone between the air spaces and the pulmonary capillaries located in the alveolar septa in close proximity to the airspaces (i.e., a diffusion distance of 0.2–0.6 μm). Particle-induced diseases of the pulmonary region of the lung include parenchyneal cancer, interstitial fibrosis, and emphysema. Interstitial fibrosis is manifested by enhanced collagen deposition in the alveolar walls or septa due to particle-induced damage and activation of fibrogenic mediators. Interstitial fibrosis results in an increase in alveolar septal thickness. The net result is an increase in the diffusion distance between alveolar air spaces and the blood in the pulmonary capillaries and a decrease in the rate of gas exchange. Emphysema is damage and loss of alveolar septa, which may be due to particle-induced hypersecretion of digestive enzymes from alveolar phagocytic cells. Since pulmonary capillaries are located in the alveolar walls, loss of alveolar septa would decrease the surface area available for diffusion of gas and decrease gas exchange.

38.3 PARTICLE DEPOSITION

Deposition of inhaled particles in various regions of the respiratory tract is determined by the aerodynamic diameter of the particle (see Chapter 2). Equivalent aerodynamic diameter for a given particle is the diameter of a spherical particle of unit density (1 g/cm^3) that exhibits the same terminal settling velocity in air. For fibers, Stokes diameter (for definition see Chapter 2) increases with increasing aspect ratio, that is, length to diameter ratio. Therefore, long fibers exhibit a greater aerodynamic diameter than short fibers of the same width (Oberdorster 1996).

Particles are classified by the US Environmental Protection Agency according to aerodynamic diameter (Table 38-3) as ultrafine, fine, coarse, and supercoarse fraction.

The American Conference of Governmental Industrial Hygienists (ACGIH) follows a different convention; particle size fractions are classified as inhalable, thoracic, and respirable (ACGIH 2001). Inhalable particles are those which can enter any part of the human respiratory tract upon inhalation, that is, particles with a 50% sampling mass cutpoint (aerodynamic diameter) of 100 μm. Thoracic particles can penetrate past the larynx into the thorax and are <10 μm in aerodynamic diameter. Respirable particles can penetrate past the conducting airways to the pulmonary region of the lung and are <3.5 μm in aerodynamic diameter. In 1993, the international definition of a respirable particle in humans was set at an aerodynamic diameter of <4 μm (CEN 1993). The particle nomenclature developed by ACGIH reflects the deposition pattern of inhaled particles in the respiratory tract of humans. The relationship of deposition fraction (the fraction of inhaled particles that deposits in the nanopharyngeal, tracheobronchial, or pulmonary region of the respiratory tract) to particle aerodynamic diameter has been reported (Task Group on Lung Dynamics 1966). (See Chapter 25 for more detail.) Particle deposition is governed by mechanisms of impaction, sedimentation, interception, and diffusion. Impaction occurs when the momentum of a particle in an airstream prevents a rapid change in direction at bends in the nasal cavity or at airway bifurcations. Sedimentation occurs as particles fall out of the airstream and land on airway surfaces as a result of gravitational forces on a particle. Interception occurs when a particle travels close to and touches an airway wall and sticks to the fluid lining the airway. This process is of greater significance for fibers than for spherical particles, while impaction and sedimentation are increasingly important as the aerodynamic diameter of a particle increases above 0.5 μm. Small particles (<0.5 μm) have little mass and thus little momentum in an airstream. Thus, sedimentation and impaction become unimportant. Rather, thermodynamic properties, such as Brownian motion, govern the deposition of small particles. This is called diffusional deposition. For particles with an aerodynamic diameter of >10 μm, thoracic and pulmonary deposition is essentially zero, with all airborne particles of this size being filtered from inhaled air in the nasal region of the respiratory tract. The highest deposition fraction for fine particles in the tracheobronchial region of an adult human is ≈7% for 4-μm diameter particles. The highest deposition fraction for fine particles in the pulmonary region of an adult human is ≈32% for 2-μm diameter particles. Both tracheobronchial and pulmonary deposition due to aerodynamic properties decrease at a particle diameter of 0.2–0.3 μm. Below this diameter, that is, as particles become ultrafine on nano-sized (<0.1 μm), thermodynamic properties of the particle dominate, and deposition in the tracheobronchial and pulmonary regions increases rapidly to ≈20% and 70%, respectively, for 20-nm particles. Below 10 nm, nasopharyngeal deposition rapidly increases. Deposition curves have also been developed for rats (Kreyling 2003) and other laboratory animals, such as the hamster, mouse, guinea pig, and rabbit (Raabe et al. 1988). Since airways are smaller in lab animals than humans, the deposition versus aerodynamic diameter curves are shifted to the left, that is, to smaller particle diameters.

TABLE 38-3 Particle Classification by EPA

Particle Type	Aerodynamic Diameter
Ultrafine	<0.1 μm
Fine	0.1–2.5 μm
Coarse	2.5–10 μm
Supercoarse	>10 μm

From these deposition curves and the airborne concentration of the particle in questions, one can calculate lung burden as follows:

$$\underset{\text{dose}}{\text{Deposited}} = \underset{(\text{mg/m}^3)}{\text{mass concentration}} \times \underset{(\text{m}^3/\text{min})}{\text{minute ventilation}} \times \underset{(\text{min})}{\text{exposure time}} \times \text{deposition fraction} \quad \text{(Eq. 38-1)}$$

A resting value for minute ventilation for an adult male is 7500 mL/min. During light work minute ventilation can increase to 20,000 mL/min, thus, resulting in an increased lung burden (Galer et al. 1992). Minute ventilation can increase tenfold during vigorous exercise (Foos et al. 2008). Lung burden for laboratory animals commonly used in inhalation studies can be calculated using average minute ventilation values for the given species (Cosfill and Widdicombe 1961) and species-specific deposition curves (Raabe et al. 1988). Recently, a working panel of the Association of Inhalation Toxicologists has developed a formula to calculate the minute ventilation of laboratory

TABLE 38-4 Surface Area of the Alveolar Region of the Lung

Species	Alveolar Epithelial Surface Area (m^2)
Human	102.20
Rat	0.40
Mouse	0.05
Guinea pig	0.68
Hamster	0.21

animals (Alexander et al. 2008):

$$\text{Minute ventilation} = 0.608 \times (\text{body weight})^{0.852} \quad \text{(Eq. 38-2)}$$
$$(1/\text{min}) \qquad (\text{kg})$$

For risk assessment, it is useful to normalize pulmonary lung burden of a test particle in studies using animal models to that in humans. This normalization is accomplished by expressing lung burden as deposited mass of particles/surface area of the alveolar epithelium (mg/m^2) as reported by Stone et al. (1992) and shown in Table 38-4.

38.4 CLEARANCE

Adverse pulmonary response to inhaled particles is a function of the length of time in which particles interact with lung tissue. Therefore, retention of inhaled particles is an important concept. Retention is defined as follows:

$$\text{Retention} = \text{deposition} - \text{clearance} \quad \text{(Eq. 38-3)}$$

The rate at which particles are cleared varies with deposition site along the respiratory tract. Particles entrapped in the anterior one-third of the nasal cavity are cleared by blowing the nose. Particles deposited on the reminder of the nasopharyngeal region are moved by mucociliary clearance rearward to the throat, where they are swallowed or coughed out of the respiratory tract. Mucus flow in the nasopharyngeal region is rapid (1 cm/min), and the half time ($T_{\frac{1}{2}}$) for particle clearance in the nanopharyngeal region is 6–7 h [International Commission on Radiation Protection (ICRP) 1994]. Particles deposited in the tracheobronchial region are moved toward the throat by mucociliary clearance. The rate of mucus-mediated clearance is faster in the large airways of the conducting zone than the small bronchioles in the conducting zone. Morrow et al. (1967) suggested three values of $T_{\frac{1}{2}}$ of 0.5, 2.5, and 5 h that best define clearance in the large, intermediate, and small conducting airways, respectively. Chronic cigarette smoking has been reported to decrease the rate of mucociliary clearance in the tracheobronchial region (Vastag et al. 1986). The most important mechanism of clearance of particles deposited in the pulmonary region is through phagocytosis by alveolar macrophages and subsequent migration of particle-laden cells to the mucociliary escalator. The $T_{\frac{1}{2}}$ for clearance of particles from the pulmonary region of the lung is 60–80 days in the rat (Snipes 1989). Alveolar clearance is much slower in humans, with a $T_{\frac{1}{2}}$ of $\approx$1 year (Bailey et al. 1985).

Phagocytosis of particles by alveolar macrophages is a rapid process. Ingestion times for silica or bacteria by rat alveolar macrophages have been calculated to range from 10–25 min (Camner et al. 2002). The rate of phagocytosis is increased by the addition of 1 mM $CaC1_2$ or $MgCl_2$ and opsonization of particles by serum, with the active component being complement C3 (Stossel 1973). Pre-exposure of macrophages to concentrated ambient particles has been shown to decrease the subsequent uptake of bacteria (Zhou and Kobzik 2007). A similar depression of bacterial uptake was reported in macrophages pre-exposed to single-walled carbon nanotubes (Shvedova et al. 2008). Ultrafine carbon black and ultrafine titanium dioxide appear to inhibit uptake of latex beads by alveolar macrophages to a greater extent than an equal mass dose of fine carbon black or titanium dioxide (Renwick et al. 2001). This inhibition of phagocytosis was not due to a cytotoxic effect of the pretreated particles.

The relative slow clearance of particles from the pulmonary region of the lung involves the following steps:

1. *Recruitment of alveolar macrophages to the particle deposition site.* In the case of bacteria, a chemotactic peptide, such as *n*-formyl-methionyl-leucyl-phenyalanine (FMLP), is released by the microbes, which attracts phagocytes to the deposition site (Boukili et al. 1989; Opalek et al. 2007). In the case of mineral dusts, particles can stimulate alveolar epithelial cells to secrete chemotactic chemokines, such as RANTES (regulated on activation, normal T expressed and secreted), MCP-1 (monocyte chemoattractant protein-1), and GMCSF (granulocyte-macrophage colony-stimulating factors) (O'Brien et al. 1998).
2. *Particle recognition and uptake.* Scavenger receptors, in particular, MARCO (macrophage receptor with collagenous structure) are critical for opsonin–independent recognition and uptake of mineral particles, such as silica, titanium dioxide, iron oxide, etc. (Palecanda et al. 1999; Palecanda and Kobzik 2001; Thakur et al. 2009).
3. *Migration of particle-laden alveolar macrophages from the deep lung to the mucociliary escalator in the conducting zone airways.* While the first two steps of particle clearance in the pulmonary region of the lung are relatively rapid, this cell migration is relatively slow. When particle burdens are high, alveolar macrophages become loaded with insoluble particles and macrophage mobility is depressed. As a result, the migration rate of particle-laden alveolar macrophages to the mucociliary escalator is decreased.

This phenomenon is called "particle overload," and the resulting depression of particle clearance from the pulmonary region of the lung has been associated with persistent alveolar inflammation, interstitial fibrosis, and lung cancer in rat models of pulmonary response to poorly soluble particles of low cytotoxicity [International Life Sciences Institute (ILSI) 2000]. Morrow (1988) has proposed that particle-induced depression of clearance is the result of volumetric overload of alveolar macrophages. This theory predicts that mobility would begin to be retarded when 6% of the alveolar macrophage volume was filled with phagocytosed particles, and complete cessation of macrophage-dependent clearance would occur when 60% of macrophage volume was occupied by engulfed particles.

Clearance from the pulmonary region of the lung is also influenced by particle dissolution and translocation. Particle dissolution has been recognized as an important factor in the clearance of fibers from the lung (ILSI 2005). Depending on the composition of fibers, these particles may dissolve in lung fluids. Leaching of minerals from the fiber causes weak points and breakage along the fiber length. Therefore, fibers initially too long to be engulfed by alveolar macrophages would break into short fibers, which can then be cleared by macrophages. Evidence indicates that the pathogenicity of fibers is related to fiber biopersistence, which is inversely related to phagocytic clearance by alveolar macrophages. Fibers longer than the macrophage diameter are poorly cleared due to frustrated phagocytosis, that is, repeated failed attempts to engulf long fibers by macrophages leading to persistent macrophage release of reactive products. Fiber dissolution in lung fluids results in breakage of long fibers into short fibers that can be phagocytosed. Therefore, durable asbestos fibers are human carcinogens, while low durability glass wool, slag wool, rock wool, and para-aramid fibrils have been classified as noncarcinogens [International Agency for Research on Cancer (IARC) 1997, 2002]. *In vitro* assay systems for fiber durability (Mattson 1995) and *in vivo* measurement of biopersistence (European Commission 1999) have been described.

Particles may also clear from the lung by entering the tracheo-bronchial lymphatic system. Ultrafine particles have been shown to migrate to lymph nodes at a more rapid rate than fine particles of the same composition (Oberdorster et al. 1994; Sager et al. 2008). However, migration to the lymphatics represents a small fraction of pulmonary clearance.

38.5 PARTICLE CHARACTERISTICS INFLUENCING BIOACTIVITY

As discussed previously, particle diameter affects the deposition site and lung burden. This in turn determines the type of adverse pulmonary response that may occur. For fibers, particle length and durability impact pathogenicity. Several other physiochemical properties of inhaled particles have been shown to govern particle-cell/tissue interaction and bioactivity. These factors include: (1) generation of reactive oxidants by the particle, (2) particle surface charge, (3) the crystalline structure of the particle, (4) the particle aspect ratio, (5) soluble metals adsorbed onto the particle, (6) adsorbed organic compounds, and (7) adsorbed microbial products.

The ability of particles to generate free radicals has been implicated in the induction of tissue injury and lung disease (Brigham 1986; Kehrer 1993). Vallyathan and Shi (1997) have reviewed data supporting the hypothesis that generation of reactive oxygen species (ROS) by particles plays an important role in the initiation and progression of particle-induced lung disease. Weitzman and Graceffa (1984) used electron spin resonance (ESR) to show that amosite, crocidolite, and chrysotile asbestos can generate hydroxyl radicals ($^{\bullet}OH$) in the presence of hydrogen peroxide (H_2O_2). This radical generation was inhibited by the presence of the iron chelator, deferoxamine, implicating radical generation by a Fenton-like action:

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \qquad \text{(Eq. 38-4)}$$

Quartz also generates $^{\bullet}OH$ via a Fenton-like reaction (Vallyathan et al. 1988; Fubini 1987). Freshly fractured quartz exhibits siloxyl radicals ($Si^{\bullet}$ and $SiO^{\bullet}$) on the cleavage planes, which enhance the ability to generate $^{\bullet}OH$ (Vallyathan et al. 1988). This enhanced $^{\bullet}OH$ generation has been associated with enhanced pathological activity of freshly fractured silica compared to aged silica in a rat inhalation model (Vallyathan et al. 1995). Coal mine dust has been reported to generate $^{\bullet}OH$ in the presence of H_2O_2, and the degree of $^{\bullet}OH$ generation has been associated with the prevalence of coal workers' pneumoconiosis (CWP) in different coal mining regions (Dalal et al. 1995). In addition, oxygen free radicals generated by diesel exhaust particles have been associated with the toxicity of diesel emissions (Sagai et al. 1993). Generation of reactive oxygen species due to metal contaminants as also been related to the pulmonary toxicity of residual oil fly-ash (Lewis et al. 2003) and welding fume (Taylor et al. 2003). In summary, the relationship between the ability of a particle to generate reactive oxidants and activation of cell injury and death, the activation of signaling pathways and genes controlling production of inflammatory cytokines and growth factors, DNA damage, cell proliferation, fibrosis, and cancer is supported by a large body of evidence (Castranova and Vallyathan 2004; Chen and Castranova 2004). Indeed, the induction of oxidant stress has recently been proposed as the leading paradigm for the evaluation of the potential toxicity of a new class of particles, that is, engineered nanoparticles (Nel et al. 2006).

Surface charge of a particle can affect particle-cell interaction and, thus, bioactivity. At a pH = 7.0, quartz exhibits a negative surface charge, with the ratio for −SiOH to SiO^- groups on the crystal surface being 30 : 1 (Nolan et al. 1981). Pretreatment of crystalline silica with metal cations decreases the zeta potential to zero and prevents silica-induced hemolysis. In addition, pretreatment of crystalline silica with aluminum salts prevents the inflammatory response of rats after pulmonary exposure (Brown et al. 1989). The bioactivity of negatively charged particles is now understood to be due to particle-cell interaction mediated by scavenger receptors on alveolar macrophages, which stimulates cellular production of ROS and apoptosis (Hamilton et al. 2008).

The crystalline structure of a particle can affect particle-cell interaction and, thus, bioactivity. Silica occurs in nature in several crystalline forms, that is, quartz, tridymite, cristobalite, coesite, and stishovite. Silica can also exist in amorphous forms, such as glass from molten silica, aerogels formed from silica condensation from the vapor phase, or silica gels obtained by precipitation from liquid solutions. Mandel and Mandel (1996) have related the biological activity as measured by *in vitro* hemolysis (Stalder and Stober 1965) and *in vivo* inflammation and fibrosis (Wiessner et al. 1988) of crystalline silica polymorphs (quartz ≈ tridymite > cristobalite > coesite > stishovite) to the 0–0 distance and the vertical offset (distance from the highest to lowest oxygen atom array) of the crystal lattice. In contrast to crystalline silica, amorphous silica is generally viewed as a low toxicity particle (Warheit et al. 1995).

Aspect ratio, that is, the length to diameter ratio, is critical to the biological activity of fibrous particles. Indeed, the Stanton hypothesis predicts that fiber pathogenicity is the direct function of fiber length (Stanton et al. 1981). Blake et al. (1998) exposed alveolar macrophages to size-selected fractions of fiberglass and showed that cytotoxicity dramatically increased once fiber length exceeded the diameter of the macrophages, thus leading to frustrated phagocytosis. A meta-analysis of epidemiology studies concerning asbestos-induced lung cancer and mesothelioma reported that cancer and mesothelioma incidence were strongly related to inhalation of fibers greater than 10 μm in length (Berman and Crump 2008). For this reason, dissolution and breakage of long fibers into shorter structures, which can be effectively phagocytosed and cleared, is an important factor in reducing the pathogenicity of a fiber, as discussed previously.

In contrast to fibers where high solubility results in increased clearance and reduced toxicity, this is not the case with zinc oxide nanoparticles, where cytotoxicity is due to soluble Zn rather than the particulate form (Xia et al. 2008). In addition, soluble metals on particles, such as residual oil fly-ash (ROFA) or welding fume, have been associated with pulmonary toxicity (Dreher et al. 1997; Taylor et al. 2003; Antonini et al. 2004a). Pulmonary exposure to either welding fume or ROFA has been shown to increase the susceptibility of the lung to infections (Hatch et al. 1985; Antonini et al. 2004b). Soluble metals have been shown to play an important role in the ability of ROFA to increase bacterial infectivity and decrease bacterial clearance (Antonini et al. 2004b; Roberts et al. 2004). This decrease in bacterial clearance was related to a soluble metal-dependent decrease in nitric oxide production and bacterial killing by alveolar macrophages (Antonini et al. 2002). In the case of welding fume, soluble chromium has been reported to play an important role (Antonini and Roberts 2007).

Pulmonary exposure to diesel exhaust particles has also been associated with an increased susceptibility to viral or bacterial infection (Hahon et al. 1985; Yang et al. 2001). In this case, adsorbed organic chemicals have been associated with a decrease in alveolar macrophage production of inflammatory cytokines, reactive oxygen species, and nitric oxide in response to bacteria, a bacterial product (LPS), or a fungal product (β-glucan), which results in decreased microbial killing by alveolar macrophages (Castranova et al. 2001; Yang et al. 2001). In addition, the adsorbed organic compounds on diesel exhaust particles are known mutagens and have been proposed to be responsible for the carcinogenicity of diesel particles (Gu et al. 2005).

Another example, where adsorbed components affect the bioactivity of a particle, is organic ducts, such as, cotton dust, silage, or grain dust, where the pulmonary inflammatory potential has been related to bacterial endotoxin contamination of the dusts (Castranova et al. 1996).

38.6 FACTORS INFLUENCING THE BIOACTIVITY OF NANOPARTICLES

Nanoparticles exhibit unique physicochemical properties. Therefore, whether nanoparticles differ substantially from fine-sized particles of the same composition in bioactivity is a critical question. Factors affecting the bioactivity of nanoparticles include: (1) pulmonary deposition, (2) interstitialization, (3) translocation to systemic sites, and (4) surface area dependent bioactivity.

Since nanoparticles are very small, they have essentially no mass and, thus, no momentum in an airstream. Therefore, nanoparticles do not deposit in the respiratory tract by impaction or sedimentation, as is characteristic of fine-sized particles. Rather, nanoparticles exhibit substantial Brownian motion, and respiratory tract deposition is governed by diffusional properties of the nanoparticles. Since nanoparticles are small, they can be inhaled into the deep lung. Once in the alveoli, diffusion within the alveolar airspace results in random collision with the alveolar surfaces and deposition. Alveolar deposition fraction increases dramatically as particle size decreases below 100 nm, approaching 70% in humans for a 20-nm particle. As particle size

decreases below 10 nm, alveolar deposition decreases as nasal deposition predominates. Daigle et al. (2003) have monitored deposition of 8.7- and 26-nm carbon nanoparticles in human volunteers upon inhalation. At rest, 66% and 80% of the inhaled 8.7- and 26-nm particles, respectively, deposited in the respiratory tract. Upon exercise, deposition increased to 83% and 94%, respectively. Since nanoparticles have a significantly higher deposition fraction in the pulmonary region of the lung than fine particles, all other factors being equal, one would predict that nanoparticles would cause a greater pulmonary response than fine particles of the same composition.

Once deposited in the respiratory region of the lung, fine particles are phagocytized and cleared by alveolar macrophages. It appears that the process of particle recognition and engulfment by alveolar macrophages is more efficient for fine particles than for nanoparticles of the same composition (Kreyling and Scheuch 2000). Therefore, nanoparticles are said to "escape" phagocytosis. Thus, a greater fraction of deposited nanoparticles are retained in the lung than fine particles (Ferin et al. 1992). As a result of greater pulmonary deposition and lower phagocytic clearance, nanoparticles exhibit a greater retention in the deep lung than fine-sized particles. In addition, nanoparticles exhibit a greater potential to cross the alveolar epithelial cells and enter the alveolar septa. This enhanced interstitialization has been reported in a rat model for nano versus fine titanium dioxide (Oberdorster et al. 1994: Sager et al. 2008). Mercer et al. (2008a) have studied the deposition, fate, and pulmonary reaction to labeled single-walled carbon nanotubes (SWCNT) in mice following pulmonary exposure by pharyngeal aspiration. Morphometric analysis demonstrated that only a small fraction of SWCNT deposited in the respiratory region was engulfed by alveolar macrophages (10–15%). In contrast, a large fraction of SWCNT (85–90%) rapidly (within 24 h postexposure) entered the alveolar interstitial space (Fig. 38-1). Once within the alveolar septum, SWCNT induced significant, rapid (7 days postexposure) and persistent (through 56 days postexposure) interstitial fibrosis.

Once within the alveolar interstitium, nanoparticles are in close contact with pulmonary capillaries in the alveolar septa. Thus, it is feasible that nanoparticles can cross the pulmonary capillary endothelial cells, enter the blood, and translocate from the lung to systemic organs where systemic effects may result. Oberdorster et al. (2002) have reported that radiolabeled carbon nanoparticles were found in the liver of rats 24 h after inhalation. Furthermore, radiolabeled carbon nanoparticles were reported in the blood of human volunteers following inhalation exposure (Nemmur et al. 2002). Although translocation of nanoparticles from the lung to systemic sites is possible, the rate of translocation appears to be slow and dependent on the type of nanoparticle evaluated (Kreyling et al. 2002). Indeed, translocation of SWCNT from the lung to systemic sites has not been demonstrated (Mercer et al. 2008b). Even if the translocation of inhaled nanoparticles to the blood is low, pulmonary exposure to titanium dioxide nanoparticles has been demonstrated to cause systemic microvascular dysfunction 24 h after inhalation (Nurkiewicz et al. 2008). This suggests that other mechanisms of signaling between the lung and systemic organs, beside particle translocation, may also be involved (Nurkiewicz et al. 2009).

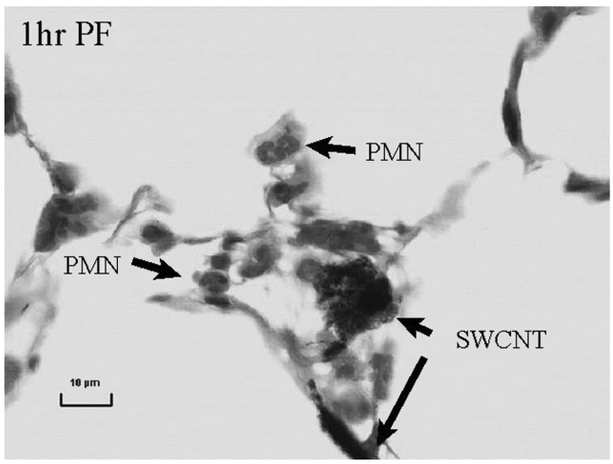

Figure 38-1 Rapid migration of SWCNT into alveolar septa. Mice were exposed to SWCNT (10 μg/mouse) by pharyngeal aspiration and nanoparticle distribution determined by histological evaluation 1 h postexposure. Polymorphonuclear leukocytes (markers of acute inflammation) are noted in the alveolar spaces. Black structures marked by arrows are SWCNT that have migrated into the alveolar walls.

Not only is deposition of nanoparticles in the deep lung greater than fine-sized particles, but nanoparticles may exhibit unique bioactivity due to their small size. On an equivalent mass dose basis, nanoparticles have been reported to be more effective than fine particles of the same composition in stimulating the production of inflammatory cytokines from lung epithelial cells in culture (Monteiller et al. 2007). Likewise, on an equal mass basis, nanoparticles have been shown to be more inflammatory than fine particles after pulmonary exposure in rats (Donaldson et al. 2002). This enhanced pulmonary activity of nanoparticles appears to be related to their high surface area per mass. Indeed, when dose was normalized to equivalent surface area of particles delivered to the lung, the inflammatory potency of nano- and fine-sized particles of the same composition was similar (Oberdorster 2001; Sager et al. 2008). Likewise, inhaled titanium dioxide nanoparticles were shown to be six-fold more potent in altering dilation of systemic microvessels than an equal mass of fine titanium dioxide particles. Again, this difference in cardiovascular potency was resolved when exposures were normalized to equivalent particle surface area delivered to the lung (Nurkiewicz et al. 2008).

38.7 CONCLUSION

In summary, the pulmonary response to an inhaled particle is influenced by its deposition fraction and the site of deposition. Pulmonary response would be minimized if the particle were rapidly cleared and be more pronounced if the particle had a high residence time in the lung. Once in the lung, the unique surface properties of a particle govern particle-cell interactions and affect the bioactivity and degree of pathogenicity of the particle. Nanoparticles are likely to exhibit greater adverse health effects than fine particles of the same composition due to their higher pulmonary deposition, their ability to enter the alveolar septa and perhaps the systemic circulation, and the role of particle surface area in affecting cellular toxicity.

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