International Journal of Pharmaceutics 356 (2008) 239–247

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Inhaled nanoparticles—A current review

Wei Yang [a], Jay I. Peters [b], Robert O. Williams III [a,*]

[a] College of Pharmacy, University of Texas at Austin, TX, USA
[b] Department of Medicine, Division of Pulmonary Diseases/Critical Care Medicine, University of Texas Health Science Center at San Antonio, TX, USA

ARTICLE INFO

Article history:
Received 4 December 2007
Received in revised form 2 February 2008
Accepted 11 February 2008
Available online 16 February 2008

Keywords:
Nanotechnology
Nanoparticle
Nanotoxicology
Pulmonary delivery
Poorly water-soluble drug
Protein/peptide drug

ABSTRACT

The field of nanotechnology may hold the promise of significant improvements in the health and well being of patients, as well as in manufacturing technologies. The knowledge of this impact of nanomaterials on public health is limited so far. This paper briefly reviews the unique size-controlled properties of nanomaterials, their disposition in the body after inhalation, and the factors influencing the fate of inhaled nanomaterials. The physiology of the lung makes it an ideal target organ for non-invasive local and systemic drug delivery, especially for protein and poorly water-soluble drugs that have low oral bioavailability via oral administration. The potential application of pulmonary drug delivery of nanoparticles to the lungs, specifically in context of published results reported on nanomaterials in environmental epidemiology and toxicology is reviewed in this paper.

Contents

1. Overview of nanomaterials 239
2. Characteristics of nanomaterials 240
3. The lungs as a delivery target for nanomaterials 240
4. Deposition of nanomaterials in the respiratory tract 241
5. Fate of inhaled nanomaterials in the lung 241
6. Systemic translocation of inhaled nanomaterials 242
7. Factors influencing fate of nanomaterials 243
8. Potential application of nanomaterials in drug delivery 244
9. Delivery devices 244
10. Pulmonary delivery of therapeutic nanomaterials 244
11. Conclusion 245
References 246

1. Overview of nanomaterials

In the current era of nanoscience, the use of nanotechnologies in commercial applications is increasing in many scientific disciplines, including electronics, sporting goods, tires, stain-resistant clothing, cosmetics, and medicine for diagnosis, imaging and drug delivery.

'Nanoscience' and 'nanotechnologies' have been defined by the Royal Society and Royal Academy of Engineering (Dowling et al., 2004; Borm et al., 2006b) as follows:

> "*Nanoscience is the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where the properties differ significantly from those at a larger scale*"; likewise, "*Nanotechnologies are the design, characterization, production and application of structures, devices and systems by controlling shape and size at nanometer scale*".

Nanomaterials, the building blocks for nanotechnology, are engineered materials with one or more components with at

* Corresponding author at: College of Pharmacy (Mailstop A1920), University of Texas at Austin, Austin, TX 78712-1074, USA. Tel.: +1 512 471 4681; fax: +1 512 471 7474.
E-mail address: williro@mail.utexas.edu (R.O. Williams III).

0378-5173/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2008.02.011

least one dimension measuring 100 nm or less. They include nanoparticles, nanofibers and nanotubes, composite materials and nano-structured surfaces. Nanoparticles, as a subset of nanomaterials, are currently defined as single particles with a diameter less than 100 nm. Agglomerates of nanoparticles can be larger than 100 nm in diameter but may be de-agglomerated with weak mechanical forces or by dispersing in a solvent. Nanofibers and nanotubes have two dimensions measuring less than 100 nm but the axial dimension can be much larger.

2. Characteristics of nanomaterials

The main differentiating characteristic of nanomaterials is their size, which falls in the transitional zone between individual atoms or molecules and the corresponding bulk materials (Hoet et al., 2004). Size reduction can modify the physical and chemical properties of nanomaterials distinctively from their bulk and molecular counterparts. It is known that for a group of airborne particles with fixed mass (10 mg/m^3) and unit density (1 g/cm^3), as the particle size decreases to less than 100 nm, the number of particles increases exponentially along with the surface area, as shown in Fig. 1. This allows a greater proportion of atoms or molecules to be orientated on the surface rather than within the interior of the material, hence allowing adjacent atoms and substances to interact more readily. The surface-to-volume ratio determines the potential number of reactive groups; the intrinsic properties of materials at the nano-sized level are emphasized compared to their larger bulk counterparts. The enhanced activities could be either beneficial (e.g., antioxidation, carrier capacity for drugs, increased uptake and interaction with biological tissues) or disadvantageous (e.g., toxicity, instability, induction of oxidative stress) depending on the intended use (Oberdorster et al., 2005; Nel et al., 2006).

Independent of particle size, the charges carried by the materials in contact with cell membranes and the chemical reactivity of the materials play a dominant role when the particles react with other substances or tissues (Lee et al., 1986).

Due to the attractive properties of nanomaterials (summarized in Table 1), such as high strength, conductivity, solubility, durability and reactivity, they have been used in a variety of applications, including fillers, opacifiers, catalysts, semiconductors, cosmetics, microelectronics, and drug carriers (Meyer et al., 2001; Oberdorster et al., 2005). However, as production and use of engineered nanomaterials have expanded, the potential impact to the environment and human health must be investigated and confirmed (Dowling, 2004).

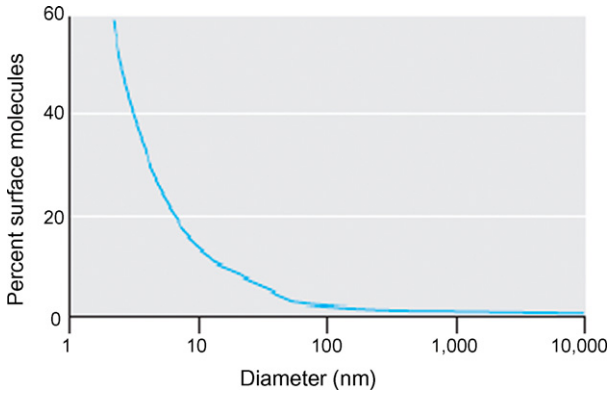

Fig. 1. Relationship between particle size and number of molecules displayed on particle surface. The percentage of molecules displayed on the surface of the particles to the total molecules in the particles increases exponentially while particle diameter decreases in the range of 1–100 nm. At a particle diameter of 30 nm, about 10% of its molecules are displayed on the surface; whereas at 10 and 3 nm particle diameter, 20% and 50% of the total molecules in the particles may display on the surface, respectively. Increase the ratio of atoms or molecules on the surface to the total molecules of a material may enhance the chemical and biological properties of nanomaterials. The enhanced activities could be either beneficial (antioxidation, carrier capacity for drugs, increased uptake and interaction with biological tissues) or disadvantageous (toxicity, instability, induction of oxidative stress) depending on the intended use. Adapted from Oberdorster et al. (2005) with permission.

Table 1
Unique features of nanomaterials (Borm and Kreyling, 2004)

Size: 20–50 nm enters CNS
<70 nm, able to escape defense system in vivo
High surface to mass ratio
High strength, conductivity, solubility, durability and reactivity
Catalytic promotion of reactions
Ability to adsorb and carry other compounds
Ability to escape defense system in vivo
Ability to cross cellular and sub-cellular membranes
Surface coating (e.g., lecithin, albumin)
Enhance uptake by Type I/II pneumocytes
Transcytosis across capillary
Charged particle (higher inhaled deposition)

Particle size and surface area of the nanomaterials are important characteristics from a toxicological viewpoint. Carbon black nanoparticles of similar mass and composition but with different specific surface areas (300 m^2/g versus 37 m^2/g) were studied. It was found that the biological effects, such as, inflammation, genotoxicity, and histology were related to surface area and not particle mass. Similar findings have been reported regarding tumorigenic effects of inhaled particles. It was shown that the tumor incidence correlated better with specific surface area than with particle mass (Driscoll et al., 1997; Oberdorster and Yu, 1999). It is recognized that biologically available surface area is probably the most critical parameter for the effects of the nanomaterials. Additionally, particle surface chemistry, biodegradability, number, shape, and solubility are all found to be significant factors in determining harmful biological effects (Brown et al., 2001; Hoet et al., 2004; Maynard and Kuempel, 2005).

Knowledge of the effects of nanomaterials on biological systems is limited due to the relative novelty of this technology, and little has been done to assess the risks of nanomaterials to the biological systems. The current paradigm in environmental epidemiology is that adverse health effects of fine and ultrafine particulates, such as those found in air pollution and some workplaces, are driven by the ultrafine particle fraction, indicating that exposure to materials in the nano-size range could cause significant public health problems, such as pulmonary and cardiovascular diseases (Donaldson et al., 2005; Powell and Kanarek, 2006a,b).

3. The lungs as a delivery target for nanomaterials

The lungs, skin and intestinal tract are in direct contact with the environment. These organs are likely to be a first port of entry for nanomaterials into the body. Epidemiological studies showed a positive correlation between increases in atmospheric particulate concentrations and the short-term increases in morbidity and mortality (Borm and Kreyling, 2004; Powell and Kanarek, 2006a). Inhalation is the most significant exposure route for airborne nanoparticles (Hoet et al., 2004; Oberdorster et al., 2005). The lung consists of two functional parts, the airways (trachea, bronchi, and bronchioles) and the alveoli (gas exchange areas). The conducting zone consist of the first 16 generations of airways comprised of the trachea (generation 0), which bifurcates into the two main stem bronchi, and subdivides into progressively smaller-diameter bronchi and bronchioles. The respiratory zone consists of all structures that participate in gas exchange and begins with the respiratory bronchioles (Weibel, 1963). The human lungs con-

tain about 2300 km of airways and 500 million alveoli (Stone et al., 1992). The surface area of the human lungs is estimated to be approximately 75–140 m^2 in adults (Gehr et al., 1978; Smith and Bernstein, 1996; Groneberg et al., 2003). The pseudostratified epithelia that constitute the barrier to absorption into the bloodstream are markedly different in airways and alveoli of the lungs. The airways are composed of a gradually thinning columnar epithelium, with the bronchial epithelium of 3–5 mm and bronchiolar epithelium of 0.5–1 mm in thickness (Weibel, 1963; Patton, 1996). In the tracheobronchial region the epithelium is protected by a mucus layer (Courrier et al., 2002). Any particle deposited in this area is transported away from the lung by mucociliary clearance (Gehr et al., 1996), or diffuse through the thick mucus to reach the epithelium cells. In contrast, the alveoli have a thin, single cell layer. The distance from the air in the alveolar lumen to the capillary blood flow is less than 400 nm. The large surface area of the alveoli and the intimate air–blood contact in this region make the alveoli less well protected against inhaled substances, such as nanoparticles, as compared to the airways (Courrier et al., 2002).

4. Deposition of nanomaterials in the respiratory tract

There are three principal mechanisms that lead to pulmonary deposition: inertial impaction, gravitational sedimentation and Brownian diffusion, as summarized in Table 2. The inertial impaction occurs during the passage through the oropharynx and large conducting airways if the particles possess a mass median aerodynamic diameter (MMAD) more than 5 μm. When the MMAD of particles ranges from 1 to 5 μm, they are subject to sedimentation by gravitational force that occurs in smaller airways and respiratory bronchioles. Sedimentation is influenced by breath-holding. Particles with a MMAD of less than or equal to approximately 0.5 μm, they are deposited significantly by diffusion, based on the Brownian motion (Martonen and Katz, 1993; Ariyananda et al., 1996; Courrier et al., 2002).

The site, extent and efficacy of particle deposition after inhalation is influenced primarily by three factors (aerosol properties and physiology) during breathing: (a) particle/droplet size (diameter), density, surface properties, or shape (i.e. fibers) (Vincent et al., 1985); (b) anatomy of the upper and lower airways and the alveolar structure; (c) ventilatory parameters with impact on the particle deposition are breath pattern (including breath-holding and presence of expiratory flow limitation), flow rates and tidal volume, determining the airflow velocity and the residence time in the respiratory tract (Newman et al., 1982; Martonen and Katz, 1993; Byron and Patton, 1994).

Next to morphological characteristics and ventilation parameters, the particle size and geometry is most important (Martonen and Katz, 1993). The particle size is commonly referred to the aerodynamic diameter, which is a variable depending on the shape, density and size of the object. If aerosols contain different particles, the size distribution is usually characterized by MMAD, which is particularly important to determine whether the particles will be efficiently deposited deep into alveolar region (Byron and Phillips, 1990; Groneberg et al., 2003). A successful deposition into deep lung requires the particles be small enough to avoid deposition by inertial impaction on upper airways and can pass through the lower airways, meanwhile be large enough to avoid exhalation (Tsapis et al., 2002; Gill et al., 2007). The optimal particle size for achieving delivery deep into alveolar region has been established to be an aerodynamic diameter between 1 and 3 μm (Byron, 1986). In general, aerosol particles measuring less than 1 μm can be exhaled up to 80% after inspiration without being deposited, because of their low inertia (Heyder et al., 1986; Heyder and Rudolf, 1984). The deposition of particles in the lung; however, is bi-model and ultrafine particles (less than 100 nm) appear to settle effectively to the alveolar region with a fractional deposition of around 50%, as calculated from mathematical modeling of monodisperse particles after slow inhalation with a breath hold (see Fig. 2) (Byron, 1986; Courrier et al., 2002; Patton, 2005). This has been confirmed by controlled clinical studies evaluating deposition and effects of laboratory-generated ultrafine particles. High-deposition efficiencies in the total respiratory tract of healthy subjects were found, and deposition was even greater in subjects with asthma or chronic obstructive pulmonary disease (Jaques and Kim, 2000; Chalupa et al., 2004).

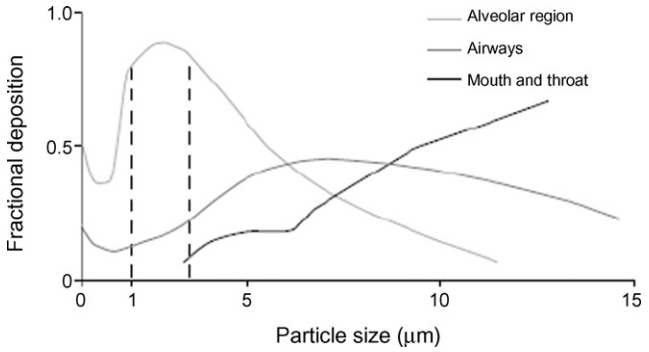

Fig. 2. The effect of particle size on the deposition of aerosol particles in the human respiratory tract following a slow inhalation and a 5 s breath hold. Larger particles deposit in the airways or mouth and throat, whereas smaller particles deposit in the alveolar region. Particles <1 μm can be exhaled, thereby reducing deep lung deposition. Reproduced from Patton (2005) with permission.

Nanoparticle deposition in the respiratory tract is determined predominantly by diffusional displacement due to the thermal motion of air molecules interacting with particles in the inhaled and exhaled air streams (Schurch et al., 1990; Geiser et al., 2003). Depending on the particle size, shape and ventilation parameters, deposition occurs in all regions of the lung: the airways and the alveoli. With decreasing particle diameter below about 500 nm, the deposition increases in all regions of the lung because of the increasing diffusional mobility (Byron, 1986). Nanofibers with a small diameter will penetrate deeper into the lungs, while very long fibers (more than 20 μm) are predominantly located in the upper airways (Oberdorster, 2002; Oberdorster et al., 2002).

5. Fate of inhaled nanomaterials in the lung

The fate of inhaled nanomaterials depends on regional distribution in the lung, because disposition within the lung is a complex function of the kinetics of absorption and non-absorptive clearance mechanisms (Sakagami, 2006). Once nanomaterials are deposited onto the lining of the respiratory tract, they first con-

Table 2
Mechanism of aerosol deposition (Courrier et al., 2002; Byron, 1986)

Site	Size (μm)	Mechanism	Comment
Large airways	5–9 (slow inhalation), 3–6 (fast inhalation)	Impaction	Most deposition in segmental airways
Smaller airways	1–5	Gravitational sedimentation	Improved with slow and deep breathe
Respiratory bronchioles	1–3	Gravitational sedimentation	Improved with slow and deep breathe
Alveoli	≤0.5	Brownian diffusion	Most exhaled

tact the mucous layer within the airways or the surfactant-lining fluid layer within the alveolar region. Airway mucus (about 5 μm in depth) is a complex aqueous secretion of airways, comprising electrolytes, proteins, glycoproteins (e.g., mucins) and debris of cells (Widdicombe and Widdicombe, 1995). The components vary much depending on environmental and disease states. The surfactant-lining layer (10–20 nm in thickness) that covers the alveolar surface is composed of 90% in weight of phospholipids and 10% in weight of specific proteins (Goerke, 1998; Johansson et al., 1994). Both airway and alveolar surface liquids are coated with at least a monolayer of highly surface active lung surfactant, which are primarily water-insoluble long-chain phospholipids. They form liquid crystals but not micelles in aqueous media (Patton, 1996) to maintain the functions of the lungs: facilitation of gas exchange and prevention of alveoli collapse by reducing the lung air interface surface tension (Veldhuizen et al., 1998; Schief et al., 2003).

It was found that regardless of the nature of the nanomaterials surfaces, they will be submersed into the lining fluids after their deposition (Geiser et al., 2003). Study of interactions between different nanoparticles and lung surfactant film indicated that the smaller the size of nanoparticle, the more can be incorporated into the surfactant film. However, the surface pressure of the surfactant film does not change significantly with the incorporation of nanoparticles, i.e. the size-dependent incorporation of nanoparticles does not destabilize the lung surfactant film (Stuart et al., 2006). D-Alpha-tocopheryl polyethylene glycol 1000 succinate (TPGS)-coated nanoparticles also do not destabilize the model surfactant film, suggesting potential application of nanoparticles to lungs (Mu and Seow, 2006).

Once deposited within the lung lining fluid, there are separate biokinetics for lung absorption and non-absorptive clearances. The kinetics of dissolution of inhaled particulates determines whether the inhaled nanomaterials will dissolve in the epithelial lining fluid for lung absorption or whether such nanomaterials will undergo non-absorptive clearances (Borm et al., 2006a). Inhaled nanomaterials that are either lipid soluble, or soluble in intracellular or extracellular fluids undergo chemical dissolution in situ. Low molecular weight hydrophobic molecules are thought to be rapidly absorbed (within seconds) by passive diffusion through the lung epithelial membrane (Patton and Byron, 2007). The kinetics of diffusion in the alveoli is much faster than that in the small airways, mainly because lung absorption mostly occurs from the air-side surface of the alveoli to the pulmonary capillaries. The alveoli has a thin monolayer (0.1–0.4 μm) composed of extremely broad and thin Type I cells and small compact Type II cells, and a large surface area (more than 100 m^2). Only a small portion of inhaled nanoparticles is absorbed from the tracheobronchial airways which have a much thicker layer of column-shaped epithelial cells (10–60 μm) and lower surface area (1–2 m^2) (Byron and Patton, 1994). This is supported by Fick's law. Low molecular weight hydrophilic molecules can be absorbed by active transport via specific transporters, or by passing through the tight junctions (Patton, 1996). The kinetics of active absorption should depend upon the lung-regional expression and functionality of receptors or transporters. It was recently reported that the absorption of large molecule immunoglobulins of the IgG class (150 kDa) might occur in the upper airways by receptor-mediated transcytosis of IgG (Spiekermann et al., 2002; Bitonti et al., 2004). Solutes and soluble components may be eventually cleared into blood and lymphatic circulation.

Inhaled nanomaterials that are insoluble in mucus and lining fluid, are not able to be rapidly absorbed, and may undergo physical translocation. This is different depending on lung region in which the nanoparticles have been deposited (Oberdorster et al., 2005). Immersion of the inhaled, slowly dissolving or insoluble nanomaterials in the fluid lining the lungs may enable them to be closely associated with epithelial cells and cells of the host-defense system for particle–cell interaction (Geiser et al., 2003). Subsequently, several post-defense mechanisms, including the mucociliary escalator transport, phagocytosis by macrophages and endocytosis, are involved in the removal of deposited nanoparticles and to maintain the lung mucosal surfaces (Gumbleton, 2001; Arredouani et al., 2004).

The mucociliary escalator dominates clearance of nanoparticles from the upper airways. Nanoparticles that consist of slowly dissolving or insoluble materials in the airway mucus will be partly moved by action of the ciliated epithelial cells pushing the mucus along with the nanoparticles that deposited on the airway walls to the larynx, where they are swallowed to the gastro-intestinal tract or excreted through the mouth (Heyder et al., 1986). The deposited nanoparticles may also be removed by coughing within 1–2 days (Patton, 1996). However, Schurch et al. (1990) showed that mucus clearance can be overcome by nanoparticles, possibly due to rapid displacement of particles to the airway epithelium via surface energetics.

Clearance of the slowly dissolving and insoluble nanoparticles from the alveoli is predominantly by macrophage phagocytosis and endocytosis (Sibille and Reynolds, 1990). The air-side surface of each of the 500 million alveoli in the human lungs is routinely monitored by 12–14 alveolar macrophages in the lung lining fluid (Stone et al., 1992). The uptake of deposited particles by alveolar macrophages depends on the particle size and composition of coating material. Particles of 1–3 μm in diameter are far better taken up than those of 6 μm by macrophages, which have cell diameters about 15–22 μm (Chono et al., 2006). Particles of less than 0.26 μm can escape from phagocytosis by macrophages (Lauweryns and Baert, 1977). Due to the small size, the chance of nanoparticles undergoing phagocytosis in the alveoli is much lower than micron-sized particles. The remaining nanoparticles will interact with the non-phagocytic cells of the epithelium, and the endocytic events are regulated by clathrin-coated pits and caveolae, as well as scavenger receptors (e.g., scavenger receptor SR-A). It has been suggested that caveolae and coated pits preferentially transport small and large particles, respectively, but this needs to be further verified in vivo (Rejman et al., 2004). Caveolae are indentations of the plasma membrane lined with caveolin-1, and are abundantly expressed on lung capillaries and Type I alveolar cells. Macromolecules or particles of several nanometers in radii may be transported within caveolae from lung to blood (Gumbleton, 2001; Rejman et al., 2004).

Transport via pores, as suggested for lung–blood substance exchange, is another possible route of disposition of inhaled nanomaterials. Inspiratory expansion and expiratory contraction of lung alveoli may lead to the opening and closing of the caveolae. These openings measure between 40 and 100 nm in size and are thought to be involved in the transport of macromolecules, such as proteins, across the alveolar-capillary barrier (Patton, 1996). Additionally, a reactive nanomaterial surface will be able to initiate chemical interactions between nanoparticles and membranes by inducing lipid peroxidation at the interface, causing changes in membrane permeability and dynamics (Nel et al., 2006). Thus, depending on size and surface reactivity, nanoparticles may be transported across cellular and sub-cellular membranes by different mechanisms. As a result, most nanoparticles will be no longer retained as free particles on the epithelium as inhaled and deposited.

6. Systemic translocation of inhaled nanomaterials

Recently, it was reported that inhaled nanomaterials may also influence organs other than the lungs. Inhaled ultrafine technetium

(99 mTc) labelled carbon particles, which are very similar to the ultrafine fraction of actual pollutant particles, diffused into the systemic circulation of hamsters within 5 min. Nemmar et al. (2001) concluded that phagocytosis by macrophages and/or endocytosis by epithelial and endothelial cells may be responsible for particle-translocation to the blood, along with other mechanisms. There are recent reports that inhaled nanoparticulates have been found in the brain, probably traveling from the nasal nerves (Donaldson et al., 2004). This suggests that nanoparticles may travel to sites away from the site of deposition in the lungs.

However, no definite conclusion about the systemic translocation of inhaled nanoparticles can be drawn to date, based on the conflicting results of human and animal studies. It was reported that rapid translocation to the liver (more than 50%) of 13C-labelled nanoparticles with a diameter of 26 nm occurred within 24 h following inhalation in a rat model (Oberdorster et al., 2002). In another rat study, only less than 1% iridium nanoparticles (15–20 nm in diameter) were found in secondary organs of rats, but the nanoparticles were distributed widely throughout the body to such organs as liver, spleen, kidneys, brain and heart (Kreyling et al., 2002). Kato et al. (2003) have provided morphological data showing that inhaled polystyrene particles are transported into the pulmonary capillary space, presumably by transcytosis; nevertheless, other research groups did not find any detectable particulates in the body other than the lungs (Brown et al., 2002; Mills et al., 2006).

The variable results from extrapulmonary translocation of experimental nanoparticles may due to differences in the chemical composition, particle size, surface characteristics, labelling materials and experimental models reported in the different studies. Taken together evidence from the in vivo studies for alveolar translocation of inhaled nanomaterials, supports that this pathway exists in humans; however, the extent of extrapulmonary translocation is determined by characteristics of the nanomaterials. Systemic translocation of the inhaled nanomaterials could better explain the epidemiological findings of adverse cardiovascular effects found in communities with air pollution (Pekkanen et al., 2002).

Inhaled nanoparticles may end up in systemic circulation and the lymphatic system once they reach the pulmonary interstitial sites following transcytosis across alveolar epithelial cells (Oberdorster et al., 2005). There is uncertainty regarding the real contribution of the lung's lymphatic pathway to systemic appearance following inhalation of nanoparticles. In the respiratory system, a vast network of lymphatic vessels drains both the airways and the alveolar regions and terminates in the hilar and mediastinal lymph nodes (Corry et al., 1984). Lymphatic drainage is responsible for alveolar clearance of deposited drugs and particulates up to a certain particle diameter, i.e. 500 nm (Leak and Ferrans Lee, 1991; McIntire et al., 1998). In vivo pharmacokinetic studies of radiolabelled solid lipid nanoparticles (mean diameter of 200 nm) revealed significant lymphatic uptake and a high rate of distribution in periaortic, axillar and inguinal lymph nodes after inhalation in rats (Videira et al., 2002). For the deposited nanoparticles that are insoluble in the lining fluid of the lungs, they are taken up less efficiently by the macrophages (Chono et al., 2006). The phagocytosed nanoparticles may be destroyed once within the lysosomes of phagocytic cells. Therefore, it is evident that for the nanoparticles consisting of protein drug, macrophage engulfment usually means eventual digestion of the protein (Lombry et al., 2004). The nanoparticles sequestered by the macrophages may also be transported to regional lymph nodes and may subsequently migrate to systemic circulation. Particle-loaded macrophages were seen in pulmonary lymphatic vessels and in hilar lymph nodes of animals following instillation of particulates into the airways (Corry et al., 1984). It has been shown that transfer of nanoparticles to the lymph nodes of the lung generally increases with increasing molecular weight greater than 10–20 kDa, whereas molecules less than 10 kDa are unlikely to be involved in this pathway (Muranishi et al., 1996). The extent of elimination of inhaled nanoparticles from the different pathways is highly dependant on the nanomaterial characteristics (e.g., particle size, coating, surface charges), the amount of inhaled nanoparticles, and potential degradation by lysosomal enzymes before transport to the lymphatic circulation.

7. Factors influencing fate of nanomaterials

Clearance of inhaled nanoparticles from the lungs depends mainly on particle size and, by implication, on particle surface characteristics. It was reported following 3 months exposure of rats to ultrafine (~20 nm) and fine (~200 nm) titanium dioxide (TiO_2) particles by inhalation, the ultrafine particles were cleared significantly more slowly, and showed more translocation to interstitial sites and to regional lymph nodes as compared to the fine TiO_2 particles (Oberdorster et al., 1994). Particles between about 20 and 50 nm in diameter may enter into the central nervous system and cells. In addition, alveolar macrophages on the surface of the lungs appear not be able to recognize particles of less than 70 nm as being "foreign", thus allowing them to gain access to the pulmonary interstitium, and then capillary blood flow (Moghimi and Hunter, 2001).

Particle shape may also interfere with the clearance mechanisms. Nanofibers measuring more than 20 μm in one axis are too long to be phagocytosed (fibers longer than the diameter of the alveolar macrophage) and will be cleared very slowly, staying in the lungs for months and possibly years. They induce a rather general non-specific pulmonary inflammatory response, including release of chemokines, cytokines, reactive oxygen species, and other mediators, which can result in sustained inflammation and eventually fibrotic changes (Borm and Kreyling, 2004; Hoet et al., 2004).

Surface coating of nanoparticles was found to effect particle uptake. Albumin, lecithin, polysorbate 80 or peptide attachments can enhance nanoparticle uptake into cells, whereas polyethylene glycol interferes with nanoparticle uptake into the liver (Somasundaran et al., 2004). Kato et al. administered intact or lecithin-coated insoluble polystyrene latex beads (240 nm in diameter) intratracheally to rats using an air jet nebulizer. Scanning electron micrographs of the rat lungs showed that both lecithin-coated and -uncoated beads were incorporated into alveolar macrophages. Some of the ingested beads in the alveolar macrophages were sequestered within lysosomes. Types I and II alveolar epithelial cells and monocytes in the capillary lumen selectively incorporated only lecithin-coated beads. These findings suggest that alveolar epithelial cells can incorporate exogenous particles, which are then transferred from the alveoli to intravascular spaces by transcytosis. The interaction between cells and the lecithin-coated particles may involve cellular ligands to recognize the lecithin by virtue of its molecular charge or hydrophilicity. Also, as observed with lecithin, albumin coating of inhaled nanoparticles appeared to facilitate nanoparticle endocytosis (Kato et al., 2003).

Moreover, surface electrostatic charge is an important factor influencing the deposition of inhaled nanoparticles. Charged nanoparticles have higher deposition efficiencies as compared to neutrally charged nanoparticles. Moderately lipophilic compounds with a positive charge at physiological conditions, such as pentamidine and verapamil, are preferentially bound to lung tissue (Byron, 1993). Polycationic macromolecules show a strong interaction with cell membranes in vitro. Three polycationic paint components exhibited considerable cytotoxicity (LD50 generally below 100 mg/mL for an incubation period of 20–24 h) in primary cultures of rat and human Type II pneumocytes, alveolar macrophages and human erythrocytes. The authors argued that the

multiple positive charges play an important role in the toxic mechanism (Hoet et al., 1999, 2001). It was found that nanoparticles with polar surfaces showed different translocation rates across respiratory epithelium and into circulation in a hamster model (Nemmar et al., 2001).

8. Potential application of nanomaterials in drug delivery

Learning from environmental toxicology studies, nano-sized air pollutants, especially the spherical solid materials, easily enter the lungs and reach the alveoli, and subsequently are cleared from the lungs by different clearance mechanisms. However, due to their small size, nano-sized particles are not likely to be detected around the lung epithelial barriers. They will translocate into systemic circulation and target other organs. Since the definition for the cut-off size of airborne nanoparticles is the same as that of engineered nanoparticles (100 nm), they should share the same biokinetics upon inhalation into the lungs. Furthermore, the high surface-to-volume ratio of natural airborne and engineered nanomaterials renders them more reactive, even though they are inert as larger particles. Therefore, any possible effects of the nanomaterials may be amplified once entering the body via inhalation. On the other hand, the extrapulmonary toxicity induced by inhaled nano-sized air pollutants may also provide evidence for systemic delivery of nano-sized pharmaceutical agents by inhalation, for the medicines not suitable for oral or parenteral administration to improve bioavailability and patient compliance.

Due to rapid advances in nanotechnology and biotechnology, nanoparticles have been considered as an effective form for delivery, and have been studied extensively to deliver the new generation of protein-, gene-based macromolecular therapeutic agents into the body, since many of the components of living cells are constructed at the nano-level, such as ribosomes, membrane transporters, receptors and cell signaling systems (Labhasetwar, 2005). Nanoparticles fall in the same size range of the biological entities; therefore they can readily interact with molecules on both the cell surface and within the cell (Rao et al., 2004; Moore, 2002). Furthermore, drugs that are deposited within the lungs in nanoparticulate form have a greater chance to escape from the clearance mechanisms by the lung defense systems, compared to microparticulate form (Chono et al., 2006; Schurch et al., 1990). Thus, drug-bearing nanoparticles have the potential to deliver drugs efficiently to the epithelium, while avoiding unwanted mucociliary clearance. In the pharmaceutical area, most nanoparticles described in the literature for drug delivery are between 50 and 500 nm in diameter (Yokoyama, 2005).

Nanoparticles are useful to deliver water-insoluble drugs. Despite high potency, the effectiveness of water-insoluble drugs can be severely limited because the solubility is too low to reach therapeutic systemic concentrations. However, when their size is reduced to nano-level, the increased particle surface-to-volume ratio helps to enhance solubility and dissolution rate in an aqueous environment. Nanoparticulate forms of drug could have an enormous benefit by significantly improving systemic bioavailability (defined as the rate and extent of therapeutically active drugs reaching the systemic circulation) and allowing a more rapid onset of therapeutic action (Shargel and Yu, 1999).

The route of administration is as important as the drug itself for therapeutic success. Nano-based approaches to drug delivery are focused on crossing a particular physical barrier, such as the gastro-intestinal epithelium for absorption of macromolecules, blood–brain barrier; or on finding alternative and acceptable routes for the delivery of drugs expensive and vulnerable to the gastro-intestinal environment. Pulmonary delivery of drugs at the nano-level is a non-invasive promising means to provide not only local lung effects but possibly high systemic bioavailability.

9. Delivery devices

Aerosols are an effective method to deliver therapeutic agents to the lungs. Nebulizers, metered dose inhalers (MDIs), or dry powder inhalers (DPIs) are commonly used to generate aerosols (Newman, 1991; Thompson, 1998). Despite the above mentioned advantages of nanoparticles, the use of a drug-bearing nanoparticle itself for delivery to lungs is severely limited because their low inertia causes them to be exhaled after inspiration. Moreover, their small size leads to particle aggregation due to their high surface energy, making handling of nanoparticles very difficult (Hinds, 1998). Therefore, the drug-bearing nanoparticles require carriers with MMADs suitable for efficient pulmonary delivery.

In contrast to the conventional micron-sized particulate drug formulations for nebulizers, the drug-bearing nanoparticles in an aqueous colloidal dispersion is more easily incorporated into the "respirable percentage" of aerosolized droplets (McCallion et al., 1996). Therefore, more nanoparticles can be enveloped into the aerosol droplets and delivered to deep lung. For instance, assuming the particles are spherical, if the volume fraction of particles in the carrier solvent is 0.01, only about 1/100 of 3 μm carrier droplets will contain a 3 μm particle; whereas each carrier droplet would contain about ten 300 nm particles. Thus, nanoparticle colloidal dispersions, relative to microparticle dispersions, have the potential to increase the rate of drug absorption by promoting more uniform drug distribution throughout the alveoli (Ostrander et al., 1999; Jacobs and Muller, 2002).

For pulmonary delivery of drug formulations in solid form, micron-sized powder particles containing the drug-bearing nanoparticles were designed for deep lung delivery by using MDIs and DPIs. Sham et al. (2004) developed a platform for aerosol delivery of nanoparticles by preparing carbohydrate (e.g., lactose, mannitol) carrier particles containing nanoparticle clusters using spray-drying technique. Carrier particles can be made with an appropriate MMAD to optimize alveolar deposition. Dispersion of the lactose carrier containing either gelatin or polybutylcyanoacrylate nanoparticles by a DPI showed a fine particle fraction (FPF) of about 40% and MMAD of 3 μm. Upon reaching the deep lung and contacting with the aqueous lining fluid of the lung epithelium, the carrier particles dissolved and released the nanoparticles. A novel type of effervescent carrier particle containing nanoparticles, with a MMAD suitable for deep lung delivery, was reported by Ely et al. (2007). Incorporation of effervescent technology into carrier particles adds an active release mechanism for the nanoparticles after pulmonary administration using DPI. Nanoparticles were observed to be distributed throughout the gas bubble that caused by the effervescent reaction when exposed to humidity. Another idea reported for pulmonary delivery of nanoparticles is forming trojan particles, which can be formed by incorporation of nanoparticles into a thin-walled micron-sized large porous particles (LPPs) (Tsapis et al., 2002). LPPs, characterized by geometric sizes larger than 5 μm and mass densities around 0.1 g/cm^3 or less, offer advantage of higher aerosolization efficiency over conventional inhaled therapeutic aerosol particles (Edwards et al., 1998). In addition, LPPs with geometric diameters of 10–20 μm can penetrate deep into the lungs and avoid macrophage engulfment by virtue of their large size (Edwards et al., 1998; Edwards and Dunbar, 2002). The trojan particles reportedly have several attractive features: they are comprised solely of nanoparticles; they are readily redispersed as nanoparticles in solution, yet the trojan particles are readily dispersed as aerosols. By using these micron-sized carriers to deliver nanoparticles to deep lung, benefits of aerosolization properties of

micron particles and the drug release and delivery advantages of nanoparticles can be combined.

10. Pulmonary delivery of therapeutic nanomaterials

Drug-loaded nanoparticles have the potential to be used for pulmonary delivery of therapeutics for treating lung diseases locally and exerting systemic actions. Delivery of therapeutic agents to the site of action for lung diseases may allow for efficient treatment of chronic lung infections, lung cancers, tuberculosis and other respiratory pathologies (Gelperina et al., 2005).

In vivo studies have observed an accumulation of nanoparticles in tumor sites after intravascular administration (Brigger et al., 2002), due to the leaky blood vessel structure of tumors. Such properties make nanoparticles a very attractive delivery vehicle for lung cancer treatment. Polysorbate 80-coated nanoparticles which were loaded with doxorubicin (DOX) have been developed to treat lung cancer. The nanoparticles were then incorporated into inhalable carrier particles by a spray freeze-drying technique (Azarmi et al., 2006). DOX-loaded nanoparticles had a particle size of 173 ± 43 nm after re-dissolving the carrier particles. Cytotoxicity was assessed by incubated cultured monolayer of two lung cancer cell lines (H460 and A549) with DOX-nanoparticle (powder form) at the concentration of 0.625 μg/ml for 24 h. The DOX-loaded nanoparticles showed enhanced cytotoxicity in a concentration-dependent manner, compared to free DOX. This indicates that DOX-loaded nanoparticles are more effective than free drug. The enhanced activity of DOX-loaded nanoparticles resulted from the nanoparticles being more readily internalized by an endocytosis mechanism compared to a passive diffusion mechanism of DOX into cells. This study supports the approach of lung cancer treatment using nanoparticles as a drug delivery vector. The carrier particles containing DOX-loaded nanoparticles may be delivered by a dry powder inhaler. Development of inhalable nanoparticles loaded with bioactive molecules is a new delivery platform which can allow targeting of lung-specific diseases in the future.

Solid lipid nanoparticles (SLN) are lipophilic particulates consisting of spherical solid lipid matrixes, and can be used as an efficient and non-biotoxic drug carrier for drug delivery (Mehnert and Mader, 2001). Recently, SLN have been proposed as carriers of either diagnosis or therapeutic reagents upon encapsulation of cytotoxic drugs. Videira et al. (2002) prepared lipid nanoparticles using gliceryl behenate by a melt homogenization technique and radiolabelled with 99 mTc using the lipophilic chelator D,L-hexamethylpropyleneamine oxime (HMPAO). Thus obtained SLNs have a mean diameter of 180–220 nm. Biodistribution studies were carried out following ultrasonic nebulization of an aqueous dispersion of the 99 mTc-HMPAO-SLN and administration of the aerosols to rats by inhalation using a mask. Dynamic images were obtained up to 4 h post-inhalation, and showed a significant uptake of the radiolabelled SLN into the lymphatic system after inhalation, and a high rate of distribution in periaortic, axillar and inguinal lymph nodes. The results revealed an important role of the lymphatic pathway in the uptake of inhaled nanoparticulates. This study suggested the possibility of pulmonary delivery of radiolebelled SLN as a lymphoscintigraphic agent and direct delivery of cytotoxic drugs to target lung cancer, which may metastasize through lymphatic drainage.

In vivo pulmonary delivery of 5-fluorouracil (5-FU) in lipid-coated nanoparticles (LNPs) system to a hamster model was recently reported (Hitzman et al., 2006). The 5-FU lipid-coated nanoparticles consisted of a core composed of 20% (w/w) 5-FU, 20% (w/w) FITC–dextran, and 60% (w/w) poly-(glutamic acid) with a shell composed of 33% (w/w) cetyl alcohol and 67% (w/w) tripalmitin. The cores measure 600 nm in diameter and the shells measure 200 nm in thickness. The LNPs were suspended at 5 mg/mL in 0.01% Pluronic F68 aqueous solution and atomized into droplets using an ultrasonic driver. The produced droplets were dried and then directed into a nose-only rodent aerosol exposure chamber for inhalation by hamsters at a dose of 30 mg LNPs/kg body weight (1.5 mg 5-FU/kg body weight). The pharmacokinetics of the 5-FU lipid-coated nanoparticle and total 5-FU in the lung, trachea, larynx, esophagus, and serum was studied. It was found that effective local targeting as well as sustained efficacious concentrations of 5-FU in the expected tumor sites were achieved. The results suggest using 5-FU containing lipid-coated nanoparticles for treating squamous cell carcinoma of the lung.

Besides the success in delivering macromolecules via inhalation to systemic circulation, the potential of pulmonary delivery of small molecule weight entities that encounter formidable biopharmaceutical challenges with conventional routes (e.g., oral) of administration was also explored. Itraconazole (ITZ), a poorly water-soluble compound, has displayed low and erratic absorption following oral administration. It has been used for treating invasive fungal infections, which quite often gained entry to the body from lung, and may disseminate to the circulation in immune-suppressed patients. Two formulations of amorphous nanoparticulate ITZ using polymers and surfactants as excipients were prepared by spray freezing into liquid (SFL) technology. One is designed for pulmonary delivery (ITZ-pulmonary). Eight milliliters of the aqueous colloidal dispersion of the ITZ-pulmonary nanoparticles (containing 200 mg of ITZ equivalent) was aerosolized by a micropump nebulizer. The aerosols were administered to mice via inhalation exposure for 20 min at a dose of 30 mg/kg body weight of ITZ. The other (ITZ-oral) is for oral delivery (Vaughn et al., 2006). The pharmacokinetic profiles of the two formulations were compared with the commercial product Sporanox® oral solution (itraconazole/Janssen) after repeated dosing. ITZ-pulmonary achieved significantly greater (more than 10-fold) lung tissue concentrations compared to the Sporanox® oral solution and ITZ-oral. There were no statistical differences between the two oral formulations. ITZ-pulmonary achieved significantly greater lung levels per unit serum concentration compared to the orally dosed ITZ compositions. High and sustained lung tissue concentrations were achieved via inhalation of an amorphous nanoparticulate ITZ-pulmonary formulation while maintaining serum levels above the minimum lethal concentration of *Aspergillus fumigatus.* This study and other related research (Vaughn et al., 2007; Alvarez et al., 2007) showed a paradigm of treating disseminated lung infection by pulmonary delivery of nano-sized therapeutics to achieve both high lung local and sufficient systemic drug concentrations to effectively improve survival.

Budesonide, another poorly water-soluble drug has been prepared as a nanosuspension by high-pressure homogenization and delivered by nebulization (Jacobs and Muller, 2002). This budesonide nanosuspension has a mean particle size about 500–600 nm, 99% of the nebulized aerosols were below 3 μm; and the nanosuspension also displayed long-term stability without aggregation or particle growth occurring over the examined period of 1 year. The manufacturing technology is feasible to scale up. These features of the budesonide nanosuspension imply the potential of successful in vivo pulmonary application. However, it is regretted that no in vivo experiments were reported to study the pharmacokinetics and effects of the budesonide nanosuspension.

11. Conclusion

While nanotechnology provides great promises in healthcare, the potential risk imposed by natural and engineered nanomate-

rials to public health has also been of concern. This is due to their enhanced activity at the nano-scale. The potential of the lung as a natural entry for systemic delivery of aerosols of macromolecules that are otherwise vulnerable to enzyme degradation in the gastrointestinal tract, and of water-insoluble drugs, has been recognized in the pharmaceutical field. The integration of nanotechnology and pulmonary delivery of drug aerosols represents a new and exciting frontier for pharmaceutical dosage form design to increase bioavailability and patient compliance, as supported by the results of studies using nanoparticles as either diagnostic or therapeutic agents for lung and systemic diseases.

References

Alvarez, C.A., Wiederhold, N.P., Mcconville, J.T., Peters, J.I., Najvar, L.K., Graybill, J.R., Coalson, J.J., Talbert, R.L., Burgess, D.S., Bocanegra, R., Johnston, K.P., Williams III, R.O., 2007. Aerosolized nanostructured itraconazole as prophylaxis against invasive pulmonary aspergillosis. J. Infect. 55, 68–74.

Ariyananda, P.L., Agnew, J.E., Clarke, S.W., 1996. Aerosol delivery systems for bronchial asthma. Postgrad. Med. J. 72, 151–156.

Arredouani, M., Yang, Z., Ning, Y., Qin, G., Soininen, R., Tryggvason, K., Kobzik, L., 2004. The scavenger receptor MARCO is required for lung defense against pneumococcal pneumonia and inhaled particles. J. Exp. Med. 200, 267–272.

Azarmi, S., Tao, X., Chen, H., Wang, Z., Finlay, W.H., Lobenberg, R., Roa, W.H., 2006. Formulation and cytotoxicity of doxorubicin nanoparticles carried by dry powder aerosol particles. Int. J. Pharm. 319, 155–161.

Bitonti, A.J., Dumont, J.A., Low, S.C., Peters, R.T., Kropp, K.E., Palombella, V.J., Stattel, J.M., Lu, Y., Tan, C.A., Song, J.J., Garcia, A.M., Simister, N.E., Spiekermann, G.M., Lencer, W.I., Blumberg, R.S., 2004. Pulmonary delivery of an erythropoietin Fc fusion protein in non-human primates through an immunoglobulin transport pathway. Proc. Natl. Acad. Sci. U.S.A. 101, 9763–9768.

Borm, P., Klaessig, F.C., Landry, T.D., Moudgil, B., Pauluhn, J., Thomas, K., Trottier, R., Wood, S., 2006a. Research strategies for safety evaluation of nanomaterials. Part V. Role of dissolution in biological fate and effects of nanoscale particles. Toxicol. Sci. 90, 23–32.

Borm, P.J., Kreyling, W., 2004. Toxicological hazards of inhaled nanoparticles—potential implications for drug delivery. J. Nanosci. Nanotechnol. 4, 521–531.

Borm, P.J., Robbins, D., Haubold, S., Kuhlbusch, T., Fissan, H., Donaldson, K., Schins, R., Stone, V., Kreyling, W., Lademann, J., Krutmann, J., Warheit, D., Oberdorster, E., 2006b. The potential risks of nanomaterials: a review carried out for ECETOC. Part. Fibre Toxicol. 3, 11.

Brigger, I., Dubernet, C., Couvreur, P., 2002. Nanoparticles in cancer therapy and diagnosis. Adv. Drug Deliv. Rev. 54, 631–651.

Brown, D.M., Wilson, M.R., Macnee, W., Stone, V., Donaldson, K., 2001. Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. Toxicol. Appl. Pharmacol. 175, 191–199.

Brown, J.S., Zeman, K.L., Bennett, W.D., 2002. Ultrafine particle deposition and clearance in the healthy and obstructed lung. Am. J. Respir. Crit. Care Med. 166, 1240–1247.

Byron, P., Phillips, E., 1990. Absorption, clearance and dissolution in the lung. In: Byron, P.R. (Ed.), Respiratory Drug Delivery. CRC, Boca Raton, FL, pp. 107–141.

Byron, P.R., 1986. Prediction of drug residence times in regions of the human respiratory tract following aerosol inhalation. J. Pharm. Sci. 75, 433–438.

Byron, P.R., 1993. Physicochemical effects on lung disposition of pharmaceutical aerosols. Aerosol Sci. Technol. 18, 223–229.

Byron, P.R., Patton, J.S., 1994. Drug delivery via the respiratory tract. J. Aerosol Med. 7, 49–75.

Chalupa, D.C., Morrow, P.E., Oberdorster, G., Utell, M.J., Frampton, M.W., 2004. Ultrafine particle deposition in subjects with asthma. Environ. Health Perspect. 112, 879–882.

Chono, S., Tanino, T., Seki, T., Morimoto, K., 2006. Influence of particle size on drug delivery to rat alveolar macrophages following pulmonary administration of ciprofloxacin incorporated into liposomes. J. Drug Target. 14, 557–566.

Corry, D., Kulkarni, P., Lipscomb, M.F., 1984. The migration of bronchoalveolar macrophages into hilar lymph nodes. Am. J. Pathol. 115, 321–328.

Courrier, H.M., Butz, N., Vandamme, T.F., 2002. Pulmonary drug delivery systems: recent developments and prospects. Crit. Rev. Ther. Drug Carrier Syst. 19, 425–498.

Donaldson, K., Stone, V., Tran, C.L., Kreyling, W., Borm, P.J., 2004. Nanotoxicology. Occup. Environ. Med. 61, 727–728.

Donaldson, K., Tran, L., Jimenez, L.A., Duffin, R., Newby, D.E., Mills, N., Macnee, W., Stone, V., 2005. Combustion-derived nanoparticles: a review of their toxicology following inhalation exposure. Part. Fibre Toxicol. 2, 10.

Dowling, A., Clift, R., Grobert, N., Hutton, D., Oliver, R., O'neill, O., Pethica, J., Pidgeon, N., Porritt, J., Ryan, J., Seaton, A., Tendler, S., Welland, M., Whatmore, R., 2004. Nanoscience and Nanotechnologies: Opportunities and Uncertainties. The Royal Society and the Royal Academy of Engineering, London, UK.

Dowling, A.P., 2004. Development of nanotechnologies. Mater. Today 7, 30–35.

Driscoll, K.E., Deyo, L.C., Carter, J.M., Howard, B.W., Hassenbein, D.G., Bertram, T.A., 1997. Effects of particle exposure and particle-elicited inflammatory cells on mutation in rat alveolar epithelial cells. Carcinogenesis 18, 423–430.

Edwards, D.A., Ben-Jebria, A., Langer, R., 1998. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J. Appl. Physiol. 85, 379–385.

Edwards, D.A., Dunbar, C., 2002. Bioengineering of therapeutic aerosols. Ann. Rev. Biomed. Eng. 4, 93–107.

Ely, L., Roa, W., Finlay, W.H., Lobenberg, R., 2007. Effervescent dry powder for respiratory drug delivery. Eur. J. Pharm. Biopharm. 65, 346–353.

Gehr, P., Bachenfren, M., Weibel, E., 1978. The normal human lung: ultrastructure and morphometric estimations of diffusion capacity. Respir. Physiol. 32, 121–140.

Gehr, P., Green, F.H., Geiser, M., Im Hof, V., Lee, M.M., Schurch, S., 1996. Airway surfactant, a primary defense barrier: mechanical and immunological aspects. J. Aerosol Med. 9, 163–181.

Geiser, M., Schurch, S., Gehr, P., 2003. Influence of surface chemistry and topography of particles on their immersion into the lung's surface-lining layer. J. Appl. Physiol. 94, 1793–1801.

Gelperina, S., Kisich, K., Iseman, M.D., Heifets, L., 2005. The potential advantages of nanoparticle drug delivery systems in chemotherapy of tuberculosis. Am. J. Respir. Crit. Care Med. 172, 1487–1490.

Gill, S., Lobenberg, R., Ku, T., Azarmi, S., Roa, W., Prenner, E.J., 2007. Nanoparticles: characteristics, mechanisms of action, and toxicity in pulmonary drug delivery—a review. J. Biomed. Nanotechnol. 3, 107–119.

Goerke, J., 1998. Pulmonary surfactant: functions and molecular composition. Biochim. Biophys. Acta 1408, 79–89.

Groneberg, D.A., Witt, C., Wagner, U., Chung, K.F., Fischer, A., 2003. Fundamentals of pulmonary drug delivery. Respir. Med. 97, 382–387.

Gumbleton, M., 2001. Caveolae as potential macromolecule trafficking compartments within alveolar epithelium. Adv. Drug Deliv. Rev. 49, 281–300.

Heyder, J., Gebhart, J., Rudolf, G., Schiller, C.F., Stahlhofen, W., 1986. Deposition of particles in the human respiratory-tract in the size range 0.005-15-Mu-M. J. Aerosol Sci. 17, 811–825.

Heyder, J., Rudolf, G., 1984. Mathematical-models of particle deposition in the human respiratory-tract. J. Aerosol Sci. 15, 697–707.

Hinds, W.C., 1998. Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles. Wiley, New York.

Hitzman, C.J., Wattenberg, L.W., Wiedmann, T.S., 2006. Pharmacokinetics of 5-fluorouracil in the hamster following inhalation delivery of lipid-coated nanoparticles. J. Pharm. Sci. 95, 1196–1211.

Hoet, P.H., Bruske-Hohlfeld, I., Salata, O.V., 2004. Nanoparticles—known and unknown health risks. J. Nanobiotechnol. 2, 12.

Hoet, P.H., Gilissen, L., Nemery, B., 2001. Polyanions protect against the in vitro pulmonary toxicity of polycationic paint components associated with the Ardystil syndrome. Toxicol. Appl. Pharmacol. 175, 184–190.

Hoet, P.H., Gilissen, L.P., Leyva, M., Nemery, B., 1999. In vitro cytotoxicity of textile paint components linked to the "Ardystil syndrome". Toxicol. Sci. 52, 209–216.

Jacobs, C., Muller, R.H., 2002. Production and characterization of a budesonide nanosuspension for pulmonary administration. Pharm. Res. 19, 189–194.

Jaques, P.A., Kim, C.S., 2000. Measurement of total lung deposition of inhaled ultrafine particles in healthy men and women. Inhal. Toxicol. 12, 715–731.

Johansson, J., Curstedt, T., Robertson, B., 1994. The proteins of the surfactant system. Eur. Respir. J. 7, 372–391.

Kato, T., Yashiro, T., Murata, Y., Herbert, D.C., Oshikawa, K., Bando, M., Ohno, S., Sugiyama, Y., 2003. Evidence that exogenous substances can be phagocytized by alveolar epithelial cells and transported into blood capillaries. Cell Tissue Res. 311, 47–51.

Kreyling, W.G., Semmler, M., Erbe, F., Mayer, P., Takenaka, S., Schulz, H., Oberdorster, G., Ziesenis, A., 2002. Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. J. Toxicol. Environ. Health A 65, 1513–1530.

Labhasetwar, V., 2005. Nanotechnology for drug and gene therapy: the importance of understanding molecular mechanisms of delivery. Curr. Opin. Biotechnol. 16, 674–680.

Lauweryns, J.M., Baert, J.H., 1977. Alveolar clearance and the role of the pulmonary lymphatics. Am. Rev. Respir. Dis. 115, 625–683.

Leak, L.V., Ferrans Lee, V.J., 1991. Lymphatics and lymphoil tissue. In: Crystal, R.G., West, J.B., Cherniack, N.S., Weibel, E.R. (Eds.), The Lung: Scientific Foundations. Raven Press, New York, pp. 779–786.

Lee, K.P., Kelly, D.P., Schneider, P.W., Trochimowicz, H.J., 1986. Inhalation toxicity study on rats exposed to titanium tetrachloride atmospheric hydrolysis products for two years. Toxicol. Appl. Pharmacol. 83, 30–45.

Lombry, C., Edwards, D.A., Preat, V., Vanbever, R., 2004. Alveolar macrophages are a primary barrier to pulmonary absorption of macromolecules. Am. J. Physiol. Lung Cell Mol. Physiol. 286, L1002–L1008.

Martonen, T.B., Katz, I.M., 1993. Deposition patterns of aerosolized drugs within human lungs—effects of ventilatory parameters. Pharm. Res. 10, 871–878.

Maynard, A., Kuempel, E., 2005. Airborne nanostructured particles and occupational health. J. Nanoparticle Res. 7, 587–614.

McCallion, O.N.M., Taylor, K.M.G., Thomas, M., Taylor, A.J., 1996. Nebulisation of monodisperse latex sphere suspensions in air-jet and ultrasonic nebulizers. Int. J. Pharm. 133, 203–214.

McIntire, G.L., Bacon, E.R., Toner, J.L., Cornacoff, J.B., Losco, P.E., Illig, K.J., Nikula, K.J., Muggenburg, B.A., Ketai, L., 1998. Pulmonary delivery of nanoparticles of insol-

uble, iodinated CT X-ray contrast agents to lung draining lymph nodes in dogs. J. Pharm. Sci. 87, 1466–1470.
Mehnert, W., Mader, K., 2001. Solid lipid nanoparticles: production, characterization and applications. Adv. Drug Deliv. Rev. 47, 165–196.
Meyer, M., Persson, O., Power, Y., 2001. Mapping excellence in nanotechnologies, Preparatory study (Nanotechnology expert group and Eurotech data). Report to the European Commission. Brussel, European Commission.
Mills, N.L., Amin, N., Robinson, S.D., Anand, A., Davies, J., Patel, D., De La Fuente, J.M., Cassee, F.R., Boon, N.A., Macnee, W., Millar, A.M., Donaldson, K., Newby, D.E., 2006. Do inhaled carbon nanoparticles translocate directly into the circulation in humans? Am. J. Respir. Crit. Care Med. 173, 426–431.
Moghimi, S.M., Hunter, A.C., 2001. Capture of stealth nanoparticles by the body's defences. Crit. Rev. Ther. Drug Carrier Syst. 18, 527–550.
Moore, M.N., 2002. Biocomplexity: the post-genome challenge in ecotoxicology. Aquat. Toxicol. 59, 1–15.
Mu, L., Seow, P.H., 2006. Application of TPGS in polymeric nanoparticulate drug delivery system. Colloids Surf. B: Biointerf. 47, 90–97.
Muranishi, S., Fujita, T., Murakami, M., Yamamoto, A., 1996. Lymphatic transfer of macromolecules after intrapulmonary administration in the presence or absence of various absorption enhancers in rats. Pharmazie 51, 331–336.
Nel, A., Xia, T., Madler, L., Li, N., 2006. Toxic potential of materials at the nanolevel. Science 311, 622–627.
Nemmar, A., Vanbilloen, H., Hoylaerts, M.F., Hoet, P.H., Verbruggen, A., Nemery, B., 2001. Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. Am. J. Respir. Crit. Care Med. 164, 1665–1668.
Newman, S.P., 1991. Aerosol generators and delivery systems. Respir. Care 36, 939–951.
Newman, S.P., Pavia, D., Garland, N., Clarke, S.W., 1982. Effects of various inhalation modes on the deposition of radioactive pressurized aerosols. Eur. J. Respir. Dis. Suppl. 119, 57–65.
Oberdorster, G., 2002. Toxicokinetics and effects of fibrous and nonfibrous particles. Inhal. Toxicol. 14, 29–56.
Oberdorster, G., Ferin, J., Lehnert, B.E., 1994. Correlation between particle size, in vivo particle persistence, and lung injury. Environ. Health Perspect. 102, 173–179.
Oberdorster, G., Oberdorster, E., Oberdorster, J., 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ. Health Perspect. 113, 823–839.
Oberdorster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Lunts, A., Kreyling, W., Cox, C., 2002. Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. J. Toxicol. Environ. Health A 65, 1531–1543.
Oberdorster, G., Yu, C.P., 1999. Lung dosimetry–considerations for noninhalation studies. Exp. Lung Res. 25, 1–6.
Ostrander, K.D., Bosch, H.W., Bondanza, D.M., 1999. An in-vitro assessment of a NanoCrystal beclomethasone dipropionate colloidal dispersion via ultrasonic nebulization. Eur. J. Pharm. Biopharm. 48, 207–215.
Patton, J.S., 1996. Mechanisms of macromolecule absorption by the lungs. Adv. Drug Deliv. Rev. 19, 3–36.
Patton, J.S., 2005. Unlocking the opportunity of tight glycaemic control. Innovative delivery of insulin via the lung. Diabetes Obes. Metab. 7, S5–S8.
Patton, J.S., Byron, P.R., 2007. Inhaling medicines: delivering drugs to the body through the lungs. Nat. Rev. Drug Discov. 6, 67–74.
Pekkanen, J., Peters, A., Hoek, G., Tiittanen, P., Brunekreef, B., De Hartog, J., Heinrich, J., Ibald-Mulli, A., Kreyling, W.G., Lanki, T., Timonen, K.L., Vanninen, E., 2002. Particulate air pollution and risk of ST-segment depression during repeated submaximal exercise tests among subjects with coronary heart disease: the Exposure and Risk Assessment for Fine and Ultrafine Particles in Ambient Air (ULTRA) study. Circulation 106, 933–938.
Powell, M.C., Kanarek, M.S., 2006a. Nanomaterial health effects. Part 1. Background and current knowledge. WMJ 105, 16–20.
Powell, M.C., Kanarek, M.S., 2006b. Nanomaterial health effects. Part 2. Uncertainties and recommendations for the future. WMJ 105, 18–23.
Rao, G.C., Kumar, M.S., Mathivanan, N., Rao, M.E., 2004. Nanosuspensions as the most promising approach in nanoparticulate drug delivery systems. Pharmazie 59, 5–9.
Rejman, J., Oberle, V., Zuhorn, I.S., Hoekstra, D., 2004. Size-dependent internalization of particles via the pathways of clathrin- and caveolae-mediated endocytosis. Biochem. J. 377, 159–169.
Sakagami, M., 2006. In vivo, in vitro and ex vivo models to assess pulmonary absorption and disposition of inhaled therapeutics for systemic delivery. Adv. Drug Deliv. Rev. 58, 1030–1060.
Schief, W.R., Antia, M., Discher, B.M., Hall, S.B., Vogel, V., 2003. Liquid-crystalline collapse of pulmonary surfactant monolayers. Biophys. J. 84, 3792–3806.
Schurch, S., Gehr, P., Im Hof, V., Geiser, M., Green, F., 1990. Surfactant displaces particles toward the epithelium in airways and alveoli. Respir. Physiol. 80, 17–32.
Sham, J.O.H., Zhang, Y., Finlay, W.H., Roa, W.H., Lobenberg, R., 2004. Formulation and characterization of spray-dried powders containing nanoparticles for aerosol delivery to the lung. Int. J. Pharm. 269, 457–467.
Shargel, L., Yu, A., 1999. Applied Biopharmaceutics & Pharmacokinetics, fourth ed. McGraw Hill, New York.
Sibille, Y., Reynolds, H.Y., 1990. Macrophages and polymorphonuclear neutrophils in lung defense and injury. Am. Rev. Respir. Dis. 141, 471–501.
Smith, S., Bernstein, J., 1996. Inhalation aerosols: physical and biological basis for therapy. In: Hickey, A. (Ed.), Lung Biology Health Diseases. Marcel Dekker, New York, pp. 233–269.
Somasundaran, P., Chakraborty, S., Qiang, Q., Deo, P., Wang, J., Zhang, R., 2004. Surfactants, polymers and their nanoparticles for personal care applications. J. Cosmet. Sci. 55, S1–S17.
Spiekermann, G.M., Finn, P.W., Ward, E.S., Dumont, J., Dickinson, B.L., Blumberg, R.S., Lencer, W.I., 2002. Receptor-mediated immunoglobulin G transport across mucosal barriers in adult life: functional expression of FcRn in the mammalian lung. J. Exp. Med. 196, 303–310.
Stone, K.C., Mercer, R.R., Gehr, P., Stockstill, B., Crapo, J.D., 1992. Allometric relationships of cell numbers and size in the mammalian lung. Am. J. Respir. Cell Mol. Biol. 6, 235–243.
Stuart, D., Lobenberg, R., Ku, T., Azarmi, S., Ely, L., Roa, W., Prenner, E.J., 2006. Biophysical investigation of nanoparticle interactions with lung surfactant model systems. J. Biomed. Nanotechnol. 2, 245–252.
Thompson, P.J., 1998. Drug delivery to the small airways. Am. J. Respir. Crit. Care Med. 157, S199–S202.
Tsapis, N., Bennett, D., Jackson, B., Weitz, D.A., Edwards, D.A., 2002. Trojan particles: large porous carriers of nanoparticles for drug delivery. Proc. Natl. Acad. Sci. U.S.A. 99, 12001–12005.
Vaughn, J.M., Mcconville, J.T., Burgess, D., Peters, J.I., Johnston, K.P., Talbert, R.L., Williams III, R.O., 2006. Single dose and multiple dose studies of itraconazole nanoparticles. Eur. J. Pharm. Biopharm. 63, 95–102.
Vaughn, J.M., Wiederhold, N.P., Mcconville, J.T., Coalson, J.J., Talbert, R.L., Burgess, D.S., Johnston, K.P., Williams III, R.O., Peters, J.I., 2007. Murine airway histology and intracellular uptake of inhaled amorphous itraconazole. Int. J. Pharm. 338, 219–224.
Veldhuizen, R., Nag, K., Orgeig, S., Possmayer, F., 1998. The role of lipids in pulmonary surfactant. Biochim. Biophys. Acta 1408, 90–108.
Videira, M.A., Botelho, M.F., Santos, A.C., Gouveia, L.F., De Lima, J.J., Almeida, A.J., 2002. Lymphatic uptake of pulmonary delivered radiolabelled solid lipid nanoparticles. J. Drug Target. 10, 607–613.
Vincent, J.H., Johnston, A.M., Jones, A.D., Bolton, R.E., Addison, J., 1985. Kinetics of deposition and clearance of inhaled mineral dusts during chronic exposure. Br. J. Ind. Med. 42, 707–715.
Weibel, E., 1963. Morphometry of the Human Lung. Academic, New York.
Widdicombe, J.H., Widdicombe, J.G., 1995. Regulation of human airway surface liquid. Respir. Physiol. 99, 3–12.
Yokoyama, M., 2005. Drug targeting with nano-sized carrier systems. J. Artif. Organs 8, 77–84.