Nanomaterial interactions with and trafficking across the lung alveolar epithelial barrier: implications for health effects of air-pollution particles

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Abstract Studies on the health effects of air-pollution particles suggest that injury may result from inhalation of airborne ultrafine particles (<100 nm in diameter). Engineered nanomaterials (<100 nm in at least one dimension) may also be harmful if inhaled. Nanomaterials deposited on the respiratory epithelial tract are thought to cross the air-blood barrier, especially via the expansive alveolar region, into the systemic circulation to reach end organs (e.g., myocardium, liver, pancreas, kidney, and spleen). Since ambient ultrafine particles are difficult to track, studies of defined engineered nanomaterials have been used to obtain valuable information on how nanomaterials interact with and traffic across the air-blood barrier of mammalian lungs.

Since specific mechanistic information on how nanomaterials interact with the lung is difficult to obtain using in vivo or ex vivo lungs due to their complex anatomy, in vitro alveolar epithelial models have been of considerable value in determining nanomaterial-lung interactions. In this review, we provide information on mechanisms underlying lung alveolar epithelial injury caused by various nanomaterials and on nanomaterial trafficking across alveolar epithelium that may lead to end-organ injury.

Keywords Epithelial transport · Nanoparticle trafficking · Pulmonary toxicity · Lung injury · Ultrafine particles · Air pollution

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Introduction

The development of nanotechnology increases concerns about the health effects of these very small particles, whether from exposure via inhalation or other routes. However, great benefits are anticipated from engineered nanomaterials that will help enable significant advances in various biomedical applications, including drug delivery, imaging, biomarkers, and biosensors. The dimensions of manufactured nanomaterials can be controlled from one to several hundred nanometers (nm), with a narrow size distribution. The Food and Drug Administration (and other institutions including the National Nanotechnology Initiative) currently define nanomaterials to be 1–100 nm in at least one dimension (regardless of shape), which is the definition we use throughout this review. Of note, the Environmental Protection Agency groups airborne particles into two categories: “inhalable coarse particles” (larger than 2.5 μm and smaller than 10 μm in diameter) and “fine particles” (2.5 μm in diameter and smaller) (<http://www.epa.gov/air/particlepollution/index.html>). According to this information, ultrafine particles ($\text{PM}_{0.1}$) are included within the $\text{PM}_{2.5}$ definition. Engineered nanomaterials, due to their small size, large surface area to volume ratios, highly reactive surfaces, and other physicochemical properties (e.g., toxic metals and metal oxides) can display characteristics very different from larger materials of the same composition (Oberdorster et al. 2005b; Xia et al. 2006; Yang et al. 2008). Some sources of airborne nanomaterials of interest include: (a) ultrafine particles released into the air by emissions from specific processes (e.g., combustion reactions or automobile engines), (b) nanoparticles emanating from volcanic ash, and (c) manufactured nanoparticles accidentally released into the air at work places.

Health effects of ultrafine particles (and engineered nanomaterials)

Health effects of ultrafine particles and engineered nanomaterials:

The supposition that inhaled engineered nanomaterials (and ultrafine particles) pose a health risk is based on accumulating epidemiologic and toxicological evidence (Bakshi et al. 2008; Beckett et al. 2005; Frampton et al. 2004; Kreyling et al. 2006a; Monteiro-Riviere and Tran 2007; Pietropaoli et al. 2004). There is an association between inhaled ultrafine particles and adverse effects on the cardiovascular system (e.g., myocardial infarction) (Frampton 2001; Gold et al. 2000; Goldberg et al. 2000; Henneberger et al. 2005; Kreyling et al. 2006a; Liao et al. 1999; Nel et al. 2006; Oberdorster et al. 1995, 2002, 2005a, Peters et al. 2000,

2001), especially in the populations most prone to thrombotic events (Nemmar et al. 2002, 2003, 2004) and/or predisposed by genetic susceptibility, age and/or disease (Kreyling et al. 2006a, b). There is evidence that some of these ultrafine particles are found in heart, bone marrow, blood vessels, and other organs after exposure to ambient air-containing nanomaterials (Beckett et al. 2005; Frampton 2001; Frampton et al. 2004; Kreyling et al. 2002, 2006a, b; Nemmar et al. 2002, 2003, 2004; Oberdorster et al. 2002, 2004; Takenaka et al. 2001, 2004; Timonen et al. 2006). However, the quantity of ultrafine particles found in blood and extrapulmonary organs varied widely in different studies, and correlation of data obtained using rodents with humans remains undetermined. A number of reviews and commentaries regarding applications and potential hazards/toxicity of nanomaterials, especially pertaining to the lung, have appeared recently (Borm et al. 2006a, b; Card et al. 2008; Furuyama et al. 2009; Nel et al. 2006; Oberdorster et al. 2005a, b; Sauer 2009; Xia et al. 2009).

Current paradigm for health effects of ultrafine particles/engineered nanomaterials:

The current paradigm in nanotoxicology is that physicochemical properties of engineered nanomaterials play a key role in toxicology/injury to cells and tissues/organs, as hypothesized for inhaled ambient particles in general. Several studies have reported effects of physicochemical properties (e.g., size, shape, composition, and surface charge density) of particles on macrophage phagocytosis (Moghimi and Hunter 2001; Moghimi et al. 2001, 2005; Storm et al. 1995). Most studies used rodents or other animals to demonstrate that inhaled ultrafine particles or engineered nanomaterials enter the systemic circulation and reach end organs at low rates (Berry et al. 1977; Derfus et al. 2004; Hamoir et al. 2003; Heckel et al. 2004; Kam et al. 2004; Kato et al. 2003; Konig et al. 1993; Kreyling et al. 2002; Mehta et al. 2004; Nemmar et al. 2002; Oberdorster 2002; Oberdorster et al. 2002, 2005a, b; Rejman et al. 2004; Silva et al. 2005). A few human studies have been carried out with conflicting findings. When radiolabeled ultrafine carbon particles (~5–10 nm, “Technegas”) as a surrogate for ultrafine particles were inhaled by non-smoking healthy humans, radioactivity in blood reached a maximum between 10 and 20 min (Nemmar et al. 2002). It was also reported that radioactivity is present in blood immediately after inhalation of $^{99\text{m}}\text{Tc}$ -labeled Technegas in humans (Kawakami et al. 1990). However, studies of deposition/clearance (2 h) of an aerosol of $^{99\text{m}}\text{Tc}$ (~60 nm diameter) in humans indicated very little radioactivity accumulated in liver (<1–2% of inhaled radioactivity) (Brown et al. 2002). As seen above, ultrafine particles/nanomaterials appear to be translocated from airspaces into the systemic circulation, although the underlying mecha-

nisms, rates of transfer and subsequent distribution profiles in downstream end organs remain to be elucidated.

Inhaled nanomaterials can be deposited in different parts of the respiratory system (i.e., nasal, tracheobronchial, and alveolar regions) in a manner dependent in large part on particle size (Politis et al. 2008). In general, the aerodynamic radius of a given particle is governed by the physical size, shape, and density of the particle. It is widely accepted that particles with aerodynamic diameters between 1 and 5 μm are most likely to deposit in respiratory airways, but only those in the 2–3 μm range are efficiently deposited in the peripheral (including alveolar) region of the lung. Nanosized particles (10–100 nm) in general show much higher deposition rates in the alveolar region. Particles larger than 10 μm are mostly deposited in the upper respiratory tract (including back of the mouth, larynx, and trachea), whereas particles between 0.1 and 0.5 μm are exhaled from the lungs without much deposition in the lung (Kleinstreuer et al. 2008). Adverse effects of inhaled ultrafine particles include direct injury to lung (cells) (Knol et al. 2009), induction of lung inflammation (which may further affect downstream end organs), or translocation of inhaled particles from lung airspaces into the systemic circulation, eventually reaching other organs (Muhlfeld et al. 2008; Mutlu et al. 2007). The most likely route for entry of inhaled nanomaterials into the systemic circulation is across the alveolar epithelium with its very large surface area and thin barrier thickness (Furuyama et al. 2009; Nemmar et al. 2001; Nemmar et al. 2002; Oberdorster 2001; Yang et al. 2008). Nanoparticles are reported to reach the deep lung, while nanomaterials deposited in upper airways may be cleared via the mucociliary system in the lung towards the pharynx and eventually reach the gastrointestinal tract (and from there the systemic circulation as well) (Hoet et al. 2004). More in depth knowledge about the mechanisms by which engineered nanomaterials injure and/or are translocated into/across the alveolar epithelium is thus of considerable importance for understanding interactions of nanomaterials with the lung (and other organs).

Nanomaterials may exert greater toxicity due to much larger total surface area when compared with micrometer-sized materials of the same chemical compositions at an equal mass. Larger surface area of the nanomaterials may in turn lead to increased probability to react with enzymes or proteins in cells to cause inflammation or injury (Wallace et al. 2007; Oberdorster et al. 2005b). For example, very low solubility and higher toxicity levels were reported for nanosized carbon black and TiO_2 when compared with larger particles of the same composition (Donaldson et al. 2005). In some cases, specific mineral components may be the predominating toxic component in nanomaterials (especially ultrafine air-pollution particles). For example,

fine-sized crystalline silica (e.g., quartz dust) is much more reactive and causes more severe inflammation when inhaled into the lungs than TiO_2 (Oberdorster et al. 1994). Similarly, different degrees of lung inflammation and injury were reported for nanosized particles (of NiO , Co_3O_4 , TiO_2 , and carbon black) instilled into rat lungs (Dick et al. 2003), where the severity of lung injury appears to correlate with the particles' abilities to generate free radicals and cause oxidant injury. It appears that size (and thus total surface area), composition of particles, and surface reactivity are all important determinants of the toxicity of nanomaterials. Also dispersants for various nanomaterials (that tend to aggregate in biological fluids, including lung lining fluid and blood) have begun to receive scrutiny among researchers in the nanomaterial field. This issue is innately related to investigations of how nanomaterials interact with (and subsequently internalize into and traffic across) cells. These issues are important for understanding the underlying mechanisms for nanomaterial interactions in various biological tissues and barriers. Further systematic studies remain to be performed to reach definitive conclusions on toxicology and biomedical applications of nanomaterials.

Since the composition and physicochemical properties of inhaled ambient particles are complex, variable and poorly defined, mechanistic information on how inhaled ambient particles exert their effects and/or transit from airspaces into the systemic circulation/end organs is not easily obtained. By contrast, engineered nanomaterials can mimic many of the properties (e.g., size and some toxicities, especially borne by the metal content) of ultrafine air-pollution particles and can be tagged (i.e., labeled with fluorescent or radioactive markers). Thus, studies of engineered nanomaterials (with defined size, surface charge density, composition and shape) are attractive alternatives to study toxicity/trafficking phenomena of ambient ultrafine particles in the lung. We provide below a review of lung studies on toxicity/trafficking of several classes of engineered nanomaterials, insofar as they relate to nanomaterial interactions with lung alveolar epithelium.

Metal/metal oxide nanoparticles:

Metal nanoparticles, including gold (Au), silver (Ag), and platinum (Pt) nanoparticles, are in wide use for various applications (Cohen et al. 2007; Eichmann et al. 2008; Li et al. 2007; Lok et al. 2007; Niemeyer and Mirkin 2004). However, exposure of lungs to metal nanoparticles may pose risks of significant injury (Bakshi et al. 2008; Park et al. 2007; Takenaka et al. 2004). Studies of distribution of inhaled Au nanoparticles in rat lungs indicate that alveolar macrophages, alveolar epithelial type I (ATI) cells and II (ATII) cells all contained Au in small endosomal compart-

ments, suggesting that Au nanoparticles eventually cross the air-blood barrier and reach the systemic circulation (Takenaka et al. 2006). Ag nanoparticles were shown to be rapidly cleared from lung airspaces (Takenaka et al. 2001), in that appearance of Ag in blood following inhalation was rapid and extrapulmonary organs (e.g., liver, kidney, spleen, brain, and heart) contained detectable Ag. Interestingly, agglomerated Ag nanoparticles in macrophages appear undissolved, suggesting smaller Ag nanoparticles may evade engulfment while agglomerated Ag nanoparticles are taken up into macrophages. How these metal nanoparticles gain access into lung epithelial cells, and their fates inside the cells and/or eventual translocation into blood, are poorly understood. It is likely that physicochemical characteristics of these metal nanoparticles, including size (Niemeyer and Mirkin 2004), charge (Goodman et al. 2004; McIntosh et al. 2001), shape (McIntosh et al. 2001; Wang et al. 2008) and composition, and the cell types with which they are interacting, dictate their intracellular/trans-cellular fates.

Many metal oxide nanoparticles (MONP) appear to cause toxicity in various cells and tissues (Brunner et al. 2006; Franklin et al. 2007; Gojova et al. 2007; Hussain et al. 2005; Jeng and Swanson 2006; Karlsson et al. 2004, 2005, 2006, 2008a, b; Sayes et al. 2007a, b; Wagner et al. 2007; Wiseman et al. 2006; Xia et al. 2006). While Fe_2O_3 , CeO_2 , and ZrO_2 do not appear to be very toxic, ZnO and CuO show comparatively much stronger cytotoxicity. For example, ZnO and CuO nanoparticles (71 and 42 nm, respectively) led to both lowered cell viability and DNA damage in A549 cells at concentrations $>40 \mu\text{g/mL}$ (Karlsson et al. 2008a). Similarly, Lin et al. (2008) reported that exposure of A549 cells to ZnO nanoparticles (70 and 420 nm) at concentrations $>10 \mu\text{g/mL}$ induces increases in oxidative stress, lipid peroxidation, cell membrane disruption and DNA damage. Lam et al. (1988) studied pulmonary function of guinea pigs following exposure to freshly generated ultrafine ZnO and found a significant correlation between wet-to-dry ratio of lung weights and cumulative ZnO, concomitantly observing gross damage (e.g., cellular infiltrates in alveoli) in peripheral regions of the lungs. However, little is known about mechanisms of internalization of MONP, followed by interaction with mitochondria and other organelles, in cells of the lung or other end organs.

Silica nanoparticles:

Amorphous silica (SiO_2) nanoparticles (doped with fluorescent dyes) have been widely utilized as labeling agents in biological applications (Anglin et al. 2008; Wang et al. 2006). Although there are some reports (Sayes et al. 2007b; Hefland et al. 2001) showing differential toxicity between

amorphous and crystalline silica particles, further studies are required to definitively address the issue. It is well known that lungs are especially vulnerable to injury following inhalation of crystalline silica nanoparticles, resulting in silicosis. Evans et al. (Evans et al. 2006) reported that intratracheal instillation of amorphous silicon dioxide nanoparticles (Carbosil, $\sim 7 \text{ nm}$) into the rat lung led to significant increases in air-blood barrier permeability, inflammation, and edema formation, with a strong correlation of plasma viscosity vs ATI cell injury (reflected by a rat ATI cell marker found in lung lavage fluid). It is unknown if ATII cells are similarly injured. These reports suggest that cytotoxicity of silica nanoparticles (SNP) may result in increased oxidant stress, lipid peroxidation/cell membrane leakage, and cell death.

Quantum nanocrystals/dots:

Various studies on cytotoxicity of quantum nanocrystals/dots (QD) suggest that physicochemical properties of QD (e.g., size, surface charge density, shape (e.g., rods vs dots or spheres), and surface coating material) play key role(s), while oxidative, photolytic and mechanical stability of QD may contribute to the severity of cytotoxicity (Hardman 2006). Some core metals of QD (e.g., Cd) are well known to be highly toxic to mammalian cells at relatively low concentration and can be released from QD during interactions with cells. Thus, preventive approaches to limit leaching of toxic metal(s) from QD have been devised (e.g., surface coating/doping of QD with non-toxic substances) (Erogbogbo et al. 2008; Su et al. 2009). In addition, shell/capping materials (i.e., used to coat or put an outer layer to the metal core of QD) appear to play an important role in decreasing QD cytotoxicity (Zhang et al. 2006). QD-induced cytotoxicity appears to be related to oxidant stress (Derfus et al. 2004; Lovric et al. 2005a, b; Shiohara et al. 2004). Very recently, it was reported that cytotoxic effects were drastically decreased when the high-bandgap ZnTe shell was used to protect a CdSe core (i.e., CdSe/ZnTe QD), in that QD after ~ 2 weeks of intravenous injection into mice were cleared from blood (into urine) due to its small size, without histological evidence for damage in major organs of mice after 2–4 weeks from injection (Law et al. 2009). Functional groups/materials (e.g., surfactants) on the surface of QD appear to play a role in QD-induced cytotoxicity (Ryman-Rasmussen et al. 2007a, b). Several reports suggest that, in addition to toxic metal ions (e.g., Cd) leaching from the QD core, intact QD may cause injury (Cho et al. 2007; Male et al. 2008; Stern et al. 2008), suggesting that QD-induced cytotoxicity cannot be attributed solely to leaching of toxic ions. These reports suggest that both shell and core compositions of QD may be important determinants of cytotoxicity.

A number of *in vivo* studies have shown that QD may be accumulated in many end organs. 24 h after intratracheal instillation of positively and negatively charged CdTe QD (bearing amino- and carboxyl-groups on their surfaces, respectively) into hyperlipidemic ApoE^{−/−} mice, development of acute pulmonary inflammation/edema and hepatic necrosis were observed (Jacobsen et al. 2009). Biodistribution and toxicology studies of intravenously injected PEG-coated CdSeTe/ZnS QD in IRC (CD-1) mice indicate that accumulation of QD in spleen, liver, and kidneys can be detected for at least 28 days following intravenous injection, and that QD trigger renal toxicity by disrupting mitochondrial function (Lin et al. 2008). Difference in toxicological profiles observed with Lin et al. (2008) and Law et al. (2009) studies for CdSeTe/ZnS QD may be due to the difference in QD surface modification (PEG-coated vs. non-coated) used in these two studies. Also, PEGylation of the QD surface most likely increased the time for blood circulation following intravenous injection of QDs due in part to decreased opsonization, leading to less uptake by the reticuloendothelial system and decreased renal clearance of QDs. Following oral administration of CdSe/ZnS QD in Wistar rats, QD were in the digestive system without evidence for elimination via either urine or feces (Karabanovas et al. 2008). Intravenous injection into Balb/c mice of CdSe/ZnS QD led to pulmonary vascular thrombosis, with distribution of QD mainly in lung, liver, and blood (Geys et al. 2008). Interestingly, it was also noted in this study that negatively charged QD appear to trigger coagulation much more than positively charged QD, and that the thrombotic effect of QD was diminished with pretreatment of animals with injection of heparin intravenously (Geys et al. 2008). These reports suggest that QD-associated (cyto)toxicity may be governed by several factors, including leaching, coating and surface charge. With respect to *in vivo* clearance of QD, Choi et al. (Choi et al. 2007) reported that hydrodynamic diameter of QD<5.5 nm resulted in rapid urinary excretion, whereas QD>15 nm exhibited poor renal clearance and were distributed in liver, lung, spleen, intestine, and kidneys. Pharmacokinetic and dynamic information on quantum nanocrystals in the systemic circulation (either via direct infusion into tail vein or translocated across lung air-blood barrier(s) following inhalation/instillation into the lung) remain to be clarified.

Other engineered nanomaterials:

Carbonaceous nanomaterials (e.g., fullerenes and carbon nanotubes) represent a major class of new materials which are widely utilized. Carbon nanotubes (CNT) are produced in great quantity and have already found many applications, including in bionanomedicine. However, it is not clear if shorter CNT (up to a few microns in length) are safe, since most toxicology studies were performed using longer CNT

(up to several hundred microns in length). The effects of aspect ratio (i.e., the ratio between the length and diameter) of CNT on cytotoxicity/trafficking in the lung alveolar epithelium largely remain unknown. Depending on specific procedures to produce them, CNT vary significantly in length and diameter. They are virtually insoluble in water and are hydrophobic/lipophilic (and thus partition easily into lipid bilayers of cell plasma membranes). Single-walled CNT (SWCNT) have diameters between ~0.7 and ~3 nm and multi-walled CNT (MWCNT) have diameters between ~10 and ~200 nm. However, they have a strong tendency to bundle together in “rope shapes” which contain multiple nanotubes of highly aggregated form due in part to van der Waals forces. Therefore, CNT are thought to exhibit fiber-related toxicology similar to asbestos (Donaldson et al. 2006; Helland et al. 2008; Hurt et al. 2006; Jia et al. 2005; Lam et al. 2004, 2006; Muller et al. 2005, 2008a, b; Shvedova et al. 2003, 2005, 2008; Singh et al. 2006; Smart et al. 2006; Soto et al. 2007; Wang et al. 2004; Warheit 2006; Warheit et al. 2008; Wick et al. 2007; Xu et al. 2008). It is attractive to postulate that shorter CNT (i.e., with smaller aspect ratio between length and diameter) may be less harmful to cells.

In addition to fiber-like features of CNT, a number of toxic metals including Co, Fe, Ni, and Mo (all of which have documented toxic effects) are present in CNT, making it difficult to determine if observed toxicological effects are due to these metal contaminants or to the fiber-like features of the CNT (Donaldson et al. 2006; Helland et al. 2007; Hurt et al. 2006; Muller et al. 2005; Shvedova et al. 2003, 2005, 2008). Purification of CNT, free of these metals, is required to address this confounding problem (Donaldson et al. 2006; Moon et al. 2004). On an equal mass basis, SWCNT showed greater toxicity in lungs than carbon black and quartz (Hurt et al. 2006; Jia et al. 2005). Inhaled SWCNT led to formation of granulomas (fibrotic lesions) and alveolar wall thickening with inflammation (Donaldson et al. 2006; Lam et al. 2004, 2006). When pathogenic bacteria were inhaled into mouse lungs in combination with SWCNT, bacterial clearance was significantly slower, indicating that SWCNT, in addition to their primary toxicity, may also diminish general resistance to pathogenic organisms (Shvedova et al. 2008). By contrast, surfactant-dispersed SWCNT led to no major effects when inhaled into mouse lungs, although pristine SWCNT was very toxic (Mutlu et al. 2010). No significant organ distribution of CNT in mice was noted after inhalation into lungs (Singh et al. 2006). Further in depth studies of CNT effects on lungs will be required to better understand health effects of inhaled CNT.

Spherical fullerenes are a family of carbon allotropes (including graphite, diamond, carbon nanotubes and graphene sheet). Spherical fullerenes are called buckyballs (or buckminsterfullerenes, named for R. Buckminster Fuller of

geodesic dome fame (Kroto et al. 1985)). Fullerenes in pristine form can be made in various sizes (mass ranging from C_{60} through C_{84}), are insoluble in water, have unique properties (including oxidant generation, phototoxicity, and entrapment of noble gases and metals) under controlled experimental conditions, and can be derivatized to render them hydrophilic (Bakry et al. 2007; Bosi et al. 2004; Camps and Hirsch 1997; Deguchi et al. 2007; Partha et al. 2007). While many studies have reported that exposure to C_{60} causes toxicity in various cell types in vitro as well as in vivo animal studies (Gharbi et al. 2005; Lewinski et al. 2008; Sayes et al. 2004, 2005, 2007a, b; Scrivens and Tour 1994; Yamago et al. 1995), some in vivo studies found no effects of fullerenes (Sayes et al. 2007b). Cytotoxicity can result from oxidative stress, leading to potential downstream effects including lipid peroxidation, DNA and protein adduction and cell death (Lewinski et al. 2008; Porter et al. 2006; Sayes et al. 2004, 2005, 2007a, b; Scrivens and Tour 1994; Spohn et al. 2009; Tokuyama et al. 1993; Usenko et al. 2008; Yamawaki and Iwai 2006). No significant cytotoxicity was found when murine and guinea pig macrophages were exposed for several hours to pristine C_{60} (up to $226 \mu\text{g}/\text{cm}^2$) (Lewinski et al. 2008). Porter et al. (2006, 2009) studied the effects of C_{60} in human monocyte macrophages and found no significant cytotoxicity. Sayes et al. (Sayes et al. 2007a) analyzed pulmonary toxicity of nano- C_{60} (colloidal suspension of aggregates of pristine C_{60} in water) and $C_{60}(\text{OH})_{24}$ in rats at 1 day and 3 months after exposure and found little lung toxicity, except for some inflammation (measured in bronchoalveolar lavage) on day 1. Interestingly, it was also reported that nano- C_{60} is much more cytotoxic (~3–4 orders of magnitude) to human dermal fibroblasts, lung epithelial cells and normal human astrocytes when compared with identical exposure of these cell types to a highly water-soluble fullerol of $C_{60}(\text{OH})_{24}$ (Isakovic et al. 2006; Lewinski et al. 2008; Sayes et al. 2004). Baker et al. (2008) reported only marginal influences of C_{60} on rat lung tissue. Spohn et al. (2009) reported that tetrahydrofuran (THF)-suspended C_{60} showed adverse effects on A549 cells in vitro or *Daphnia magna* in vivo due to THF solvent, while water-suspended C_{60} had no effect. Gharbi et al. (2005) reported that n C_{60} may be applied as a powerful liver protective agent against tetrachloride intoxication in rats, attributed to the free radical scavenging properties of C_{60} . Further studies are needed in order to understand the mechanistic interactions of fullerene interactions with and trafficking across lung air-blood barriers.

Mechanisms underlying toxicity and injury to cells by ultrafine particles/engineered nanomaterials:

It is generally believed that formation of excess cellular free radicals induced by oxidative stress leads to cellular

toxicity. Accumulation of peroxidative products and antioxidant depletion in the cell due to nanomaterials may be the major cause for loss of cell viability. For example, nanomaterials of C_{60} , MWCNT, Ag, TiO_2 , Fe_2O_3 , Al_2O_3 , ZrO_2 , Si_3N_4 , carbon black, and MnO_2 are reported to cause cytotoxicity in in vitro cell studies (Bottini et al. 2007; Gurr et al. 2005; Hussain et al. 2005; Sayes et al. 2005; Soto et al. 2007). Following intratracheal instillation of SWCNT containing variable levels of residual metals in mice lungs, all animals developed epithelioid granulomas in a dose-dependent manner (Lam et al. 2004), whereas instillation of SWCNT into rat lungs led to a transient inflammatory response and injury with non-dose-dependent multi-focal granulomas. Acute inflammation and granulomatous responses were found near dense SWCNT aggregates in the lung, while early onset of progressive diffuse interstitial fibrosis and alveolar wall thickening appears to be associated with well dispersed SWCNT localized away from SWCNT aggregates (Shvedova et al. 2005). By contrast, when equal mass-based doses of ultrafine carbon black or fine crystalline silica dust were utilized, weaker inflammation and damage without apparent granulomas or cell wall thickening were found. These reports illustrate the difficulties inherent in animal studies, which may include species differences, uneven distribution (including agglomeration) of nanomaterials in lung airspaces following instillation/inhalation, purity of engineered nanomaterials and laboratory-to-laboratory variations in techniques and experimental design/execution.

Trafficking properties of ultrafine particles/engineered nanomaterials

Ultrafine particles and engineered nanomaterials can translocate across the alveolar epithelial barrier and subsequently appear in lymphatics and/or systemic circulation (Ferin et al. 1992; Geiser et al. 2005; Oberdorster et al. 1992). A number of nanomaterials (e.g., TiO_2 and SiO_2) were shown to be internalized into human epithelial cells, without cytotoxic effects, while pro-inflammatory stimulation and impairment of proliferative activity were noted for Co and silica nanoparticles. These toxic nanomaterials may demonstrate increased translocation rates due to their toxic effects (e.g., oxidant stress or opening of tight junctions) on or severe injury (e.g., inflammation and/or destruction of epithelial and endothelial cells) to the air-blood barrier of distal lung airspaces.

It has been reported that inhaled ultrafine particles appear in extrapulmonary organs following intratracheal instillation or inhalation in rodents, suggesting translocation across respiratory epithelial barriers. However, the quantity of ultrafine particles found in blood and extrapulmonary

organs varied widely in different studies, and correlation of rodent data obtained with human data remains undetermined. A number of reviews and opinion articles regarding applications and potential hazards/toxicity of nanomaterials, especially pertaining to lungs, have appeared recently (Borm et al. 2006b; Card et al. 2008; Nel et al. 2006; Oberdorster et al. 2005a, b). Several studies have reported effects of physicochemical properties (e.g., size and charge) of particles on macrophage phagocytosis. However, few attempts have been made to study interactions of nanoparticles with non-phagocytic cells. Most studies used rodents or other animals to demonstrate that inhaled ultrafine particulates or nanospheres enter the systemic circulation and reach end organs at low rates. A few human studies have been carried out with conflicting data.

Nanomaterials in general may be taken up into cells (and perhaps eventually transcytosed) via phagocytosis and/or “pinocytic” processes, including clathrin-mediated endocytosis, caveolin-mediated endocytosis and/or other processes (e.g., micropinocytosis and macropinocytosis). It is reasonable to speculate that smaller nanoparticles may be internalized into cells via non-specific fluid-phase endocytosis (e.g., micropinocytosis taking place via clathrin- and caveolin-independent processes). Specialized endocytosis, requiring receptors congregated in caveolae or clathrin-coated pits, may also allow extracellular fluid (and thus nanoparticles contained therein) to be internalized along with the ligand-receptor complex (es). In addition, there are several non-endocytic routes by which nanomaterials may enter and traffic across alveolar epithelial barrier, including tight junctions and “holes” in the plasma membranes. We and others have shown that hydrophilic solutes (whose Stokes-Einstein radii range <6 nm) may passively translocate by restricted diffusion via paracellular pathways (Kim and Crandall 1983; Matsukawa et al. 1997). Several reports on passive diffusion of hydrophilic solutes in many epithelial barriers indicate that paracellular pathways can be modeled to have equivalent water-filled pores whose radii range from 5 to 6 nm (Adson et al. 1994; Matsukawa et al. 1997; Wright and Pietras 1974). Thus, it is reasonable to predict that nanospheres >10 nm in diameter are excluded from tight junctions under normal conditions. However, if specific nanomaterials affect the epithelial barrier so as to widen the gap between epithelial cells, nanomaterials may diffuse via such altered tight junctions. Defects (or “holes”) in lipid bilayers have been reported to be created by interactions of certain classes (e.g., cationic dendrimers) of nanomaterials with lipid components of cell membranes or lipid bilayer models (Hong et al. 2004, 2006a, b; Mecke et al. 2005). Diffusion of “hydrophobic” nanomaterials through lipid bilayers of cell plasma membranes is another potential trafficking pathway.

Airspaces of the lung are endowed with various detoxifying enzymes and other defenses (e.g., ciliary escalator system in the conducting airways) against exogenously delivered substances. It is expected that most airborne materials are cleared via some or all of these systems, while those reaching the alveolar region may be presented first to resident professional phagocytes (e.g., macrophages and polymorphonuclear cells) (Geiser et al. 2008; Schlesinger et al. 1982; Yang et al. 2008). It should be pointed out that we do not know much about the fates of much smaller particles (of 1–10 nm size), although these very small particles are not thought to be readily recognized or phagocytized by macrophages (Oberdorster 2000; Politis et al. 2008) which have been implicated in generation of chemokines following ingestion of ultrafine particles or (some) engineered nanomaterials (Donaldson et al. 2005, 2006; Hoet et al. 2004; Oberdorster 2000). Bigger sized particles (or agglomerated nanomaterials/ultrafine particles) >100 nm were transported across the air-blood barrier into the interstitium and vasculature at a much lower rate than particles of smaller size (i.e., 100 nm or less) (Geiser and Kreyling 2010; Kreyling et al. 2009; Yacobi et al. 2008). The amount of ultrafine particles (including engineered nanomaterials utilized as surrogates for ultrafine particles) transported into systemic circulation from lung airspaces may be related to total surface area, in that ultrafine particles are translocated amount at a rate much greater than that for particles with diameter >100 nm of similar mass.

Ferin and Oberdorster (Ferin et al. 1992) showed histologic evidence for the existence of two distinct translocation pathways for inhaled TiO_2 particles in rat lungs. They reported that ultrafine TiO_2 particles found in peripheral lung consist of those that were translocated via airways into the interstitium (and to the systemic circulation?) and others that may have back-tracked from interstitial lymphatics to airway lumen. In this regard, Semmler-Behnke et al. (2007) reported that translocation of iridium nanoparticles to systemic circulation is measurable but the translocated fraction is much less than the fraction back-tracked to luminal spaces of the epithelium.

Nanomaterial interactions with alveolar epithelium utilizing *in vitro* models

Although studies of *in vivo* and/or *in situ* lungs yielded important observations pertaining to health effects of ambient ultrafine particles and engineered nanomaterials, specific mechanistic information cannot be easily extracted from such studies. As an alternative, simpler *in vitro* models of primary cultured rat alveolar epithelial cell monolayers (RAECM) grown on permeable supports (Cheek et al. 1989a, b) have played a pivotal role in the

mechanistic investigation of vectorial transport of ions and solutes across the mammalian alveolar epithelial barrier. RAECM exhibit spontaneous potential difference >10 mV (apical-side negative), resistance (R_t) $>2,000 \Omega \text{ cm}^2$, and short-circuit current (I_{sc}) $\sim 5 \mu\text{A}/\text{cm}^2$ (Cheek et al. 1989b). Utilizing RAECM, we reported new mechanistic information for alveolar epithelial transcytosis and metabolism of various proteins and dextrans (Kim and Crandall 1996; Kim and Malik 2003; Kim et al. 2003, 2004; Wiedera et al. 2003a, b).

Utilizing the in vitro RAECM model, we have recently studied changes in electrical R_t of RAECM following apical exposure to several engineered nanomaterials and ambient ultrafine particle suspensions (UAPS) as functions of concentration and exposure time (Yacobi et al. 2007). Exposure to UAPS containing mixtures of carbon (organic and elemental), nitrate, sulfate and trace elements and metals led to significantly decreased R_t which did not recover to control after 24 h of exposure at apical concentration of ~ 0.04 mg/mL. Decreases in R_t caused by UAPS exposure were dose- and time-dependent, with an apparent EC_{50} of ~ 0.009 mg/mL. Apical exposure of RAECM to UAPS did not affect mannitol or inulin flux across RAECM. R_t of RAECM decreased significantly when RAECM were exposed apically to SWCNT (~ 0.09 mg/mL), with subsequent recovery toward control at 24 h. When up to ~ 0.18 mg/mL of chitosan coated (positively charged) and alginate coated (negatively charged) QD (manufactured in our laboratories) were added to apical fluid of RAECM, positively charged QD decreased R_t significantly with recovery toward control at 24 h of exposure while negatively charged QD had a lesser effect. By contrast, polystyrene nanoparticle (PNP; positively and negatively charged; 20–120 nm in diameters) did not elicit significant changes in R_t of RAECM during 24 h of apical exposure for up to ~ 0.6 mg/mL. LDH release from RAECM exposed apically to UAPS or SWCNT did not change. These findings indicate that apical exposure of RAECM to some nanomaterials leads to disruption of alveolar epithelial barrier properties, especially the transcellular transport pathways (since paracellular pathways were seen not to be disturbed based on inulin or mannitol permeability assessment). Interactions of nanomaterials and their fate(s) in alveolar epithelial cells appear to be highly dependent on specific physicochemical properties (e.g., composition, size, shape and/or surface charge density) of nanomaterials (including ambient ultrafine particles). In line with this deduction, our very recent studies using metal oxide nanoparticles indicated that ZnO nanoparticles irreversibly derange barrier functions of RAECM, due in part to free leached Zn^{2+} , whereas Au and Fe_2O_3 nanoparticles did not affect R_t of RAECM when studied at comparable doses (unpublished observations).

We have also used the RAECM model to investigate trafficking of PNP (20 or 100 (or 120) nm; negatively charged (i.e., carboxylate, sulfate, or aldehyde-sulfate modified) or positively charged (i.e., amidine-modified)) across the alveolar epithelium (Yacobi et al. 2008). PNP flux increased as surface charge density of PNP became less negative (or more positive), in that positively charged PNP were translocated across RAECM ~ 20 –40 times faster than highly negatively charged PNP of comparable size. When PNP trafficking rates were studied as a function of size, larger PNP (100 or 120 nm in diameters) were transported three to four times slower than smaller PNP (20 nm in diameter) of similar charge characteristics. Unexpectedly, PNP trafficking rates tended to reach a plateau when apical PNP concentration approached >0.2 mg/mL. Decreasing experimental temperature from 37°C to 4°C significantly decreased PNP transport by $>80\%$. Following apical exposure of RAECM to PNP for 24 h, confocal microscopy revealed localization of PNP in cell cytoplasm, whereas no PNP were found at cell-cell junctions and nuclei. These findings indicate that PNP translocation across RAECM is predominantly determined by surface charge density of the nanoparticle, with size playing an important role. Significant decreases in PNP trafficking with lowered temperature suggest that PNP may translocate primarily transcellularly utilizing cellular energy-requiring process(es).

Based on these findings on PNP trafficking properties, we explored mechanisms of nanoparticle interactions with and trafficking across RAECM and an artificial lipid bilayer on filter model (ALBF) (Yacobi et al. 2010). RAECM were exposed to PNP (i.e., 20 and 100 (or 120) nm; carboxylate (negatively charged) or amidine (positively charged)-modified) and changes in apical-to-basolateral flux under various experimental conditions were investigated. Similarly, *cis-to-trans* PNP fluxes across ALBF were determined. We found that translocation rates of positively charged PNP were 20–40 times those of negatively charged PNP across both RAECM and ALBF. Interestingly, ALBF were found to mimic properties of RAECM well despite PNP trafficking rates across RAECM two to three times greater than those across ALBF. PNP translocation across RAECM was not affected when tight junctions were disrupted by 2 mM EGTA (which lowered R_t to zero) or when various inhibitors of phagocytosis and endocytosis (e.g., clathrin- and caveolin-dependent processes) were utilized, suggesting that PNP do not cross paracellular pathways or utilize transcellular endocytic mechanisms for transepithelial translocation. In support of this notion, no discernible colocalization of PNP with early endosome antigen-1, caveolin-1, clathrin heavy chain, cholera toxin B or wheat germ agglutinin was noted by confocal microscopy. PNP do not appear to disrupt cell membranes, as measured by leakage of 5-carboxyfluorescein diacetate from alveolar epithelial cells. Sodium ion and

mannitol fluxes across ALBF were not affected in the presence or absence of PNP. These findings indicate that PNP are translocated primarily transcellularly across RAECM, but not via known major endocytic pathways. Translocation of PNP across RAECM thus appears to occur via a diffusional mechanism through the lipid bilayer of plasma membranes of alveolar epithelial cells without causing “permanent” disruption (e.g., creating holes).

Injurious effects of nanomaterials on the alveolar epithelial barrier could be due to loss of cell plasma membrane integrity, disruption of cell-cell borders (including tight junctions) and/or mitochondrial dysfunction due to increased intracellular reactive oxygen species. It is possible that disruption of lung alveolar epithelial barrier integrity following exposure to UAPS is caused by the complex and variable composition of air-pollution particles. Ultrafine particles may act as a more potent catalyst for specific reactions with lung epithelium, leading to increased lung toxicity and inflammation (Oberdorster 2001).

As discussed above, nanomaterials may be taken up into cells (and perhaps eventually transcytosed) through endocytotic pathways (Conner and Schmid 2003; Doherty and McMahon 2009). There are also non-endocytic mechanisms for nanomaterial trafficking across cell monolayers (tight junctions), through defects in the cell plasma membrane, or by passive or facilitated diffusion across the plasma membrane. Nanomaterials smaller than 10 nm may diffuse between epithelial cells into interstitium (and eventually reaching the systemic circulation) from airspaces, while particles larger than 10 nm are expected to be excluded from tight junctions under normal conditions (Yacobi et al. 2010). However, injury to the epithelial barrier following exposure to nanomaterials could lead to increased diffusion of luminal substances (including nanomaterials) via dysfunctional tight junctions. Geiser et al. (Geiser et al. 2005) has shown that ultrafine particles cross cell membranes through non-endocytic pathways, while others reported endocytic mechanisms (e.g., caveolin-mediated) for nanomaterial transport utilizing various *in vitro* models (Kemp et al. 2008; Orr et al. 2009; Xia et al. 2008). The limited data available on nanomaterial interactions with cells in the deep lung suggest that multiple mechanisms (e.g., endocytosis, direct penetration across cell membrane or diffusion) may be involved in nanomaterial translocation. Due to the complexity and variability of nanomaterials, coupled with heterogeneity of cells involved, further detailed and systematic studies remain to be performed.

Concluding remarks

Although there is a relative paucity of specific information on nanomaterial interactions with lung, at least some

inhaled nanomaterials can be translocated across the lung air-blood barrier. Some nanomaterials (e.g., carbon nanotubes and quantum dots) are reported to be toxic to lung (cells), leading to generation of inflammatory responses and/or generation of excess reactive oxygen species. Inhaled toxic nanomaterials may directly trigger pulmonary inflammation leading to systemic end-organ inflammation or may physically reach the end organ and trigger local inflammation. Limited experimental findings from *in vitro* studies indicate that nanomaterials translocate across alveolar epithelium through non-endocytic or endocytic pathways (e.g., caveolin-mediated endocytosis) with trafficking mechanisms that are highly dependent on nanomaterial physicochemical characteristics. Although the rates of nanomaterial trafficking may be low, the absolute amount translocated could be very significant (e.g., millions to billions of specific nanoparticles) which could have major deleterious effects on end-organ function. Alveolar epithelial cytotoxicity of nanomaterials appears to be strongly dependent on nanomaterial physicochemical properties.

Future directions

Even though our knowledge regarding nanomaterial interaction with and trafficking across the lung is limited, human beings are exposed to nanomaterials every day, incidentally or accidentally, and are prone to subsequent health effects. The science of nanotechnology is rapidly developing, leading to significant advances in biomedical applications such as drug delivery, molecular imaging, biomarkers and biosensors. Literature data available currently do not support conclusive correlations between data obtained *in vitro* and *in vivo* on nanoparticle interactions (including toxicology) with and trafficking across lung alveolar epithelium. For design of future studies, doses (and dosage rates) utilized in both *in vitro* and *in vivo* in a given animal species have to be considered carefully, since short- vs. long-term studies cannot be easily resolved. The limited lifespans of *in vitro* models (up to about 1 week), complex anatomy of the lung (and multiple organs and biological spaces (e.g., interstitial, lymphatic, and blood)), and inherent difficulties in mechanistic studies of toxicity and injury utilizing animal models (e.g., species differences, uneven distribution (including agglomeration) of nanomaterials in lung airspaces following instillation/inhalation, purity of engineered nanomaterials and laboratory-to-laboratory variations in experimental protocols and experimental design/execution) make direct comparisons between data obtained in the two settings difficult. *In vitro* and *in vivo* experiments utilizing nanomaterials with

defined physiochemical properties (e.g., composition, size, surface charge density and shape) are required to establish policies in safety guidelines for human exposures.

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References

- Adson A, Raub TJ, Burton PS et al (1994) Quantitative approaches to delineate paracellular diffusion in cultured epithelial cell monolayers. *J Pharm Sci* 83:1529–1536
- Anglin EJ, Cheng L, Freeman WR et al (2008) Porous silicon in drug delivery devices and materials. *Adv Drug Deliv Rev* 60:1266–1277
- Baker GL, Gupta A, Clark ML et al (2008) Inhalation toxicity and lung toxicokinetics of C₆₀ fullerene nanoparticles and microparticles. *Toxicol Sci* 101:122–131
- Bakry R, Vallant RM, Najam-ul-Haq M et al (2007) Medicinal applications of fullerenes. *Int J Nanomedicine* 2:639–649
- Bakshi MS, Zhao L, Smith R et al (2008) Metal nanoparticle pollutants interfere with pulmonary surfactant function in vitro. *Biophys J* 94:855–868
- Beckett WS, Chalupa DF, Pauly-Brown A et al (2005) Comparing inhaled ultrafine versus fine zinc oxide particles in healthy adults: a human inhalation study. *Am J Respir Crit Care Med* 171:1129–1135
- Berry JP, Arnoux B, Stanislas G et al (1977) A microanalytic study of particles transport across the alveoli: role of blood platelets. *Biomedicine* 27:354–357
- Borm P, Klaessig FC, Landry TD et al (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 PJ, Robbins D, Haubold S et al (2006b) The potential risks of nanomaterials: a review carried out for ECETOC. *Part Fibre Toxicol* 3:11
- Bosi S, Feruglio L, Da Ros T et al (2004) Hemolytic effects of water-soluble fullerene derivatives. *J Med Chem* 47:6711–6715
- Bottini M, D'Annibale F, Magrini A et al (2007) Quantum dot-doped silica nanoparticles as probes for targeting of T-lymphocytes. *Int J Nanomedicine* 2:227–233
- Brown JS, Zeman KL, Bennett WD (2002) Ultrafine particle deposition and clearance in the healthy and obstructed lung. *Am J Respir Crit Care Med* 166:1240–1247
- Brunner TJ, Wick P, Manser P et al (2006) In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility. *Environ Sci Technol* 40:4374–4381
- Camps X, Hirsch A (1997) Efficient cyclopropanation of C₆₀ from malonates. *JCS Perkin Trans* 1:1595–1596
- Card JW, Zeldin DC, Bonner JC et al (2008) Pulmonary applications and toxicity of engineered nanoparticles. *Am J Physiol Lung Cell Mol Physiol* 295:L400–L411
- Cheek JM, Evans MJ, Crandall ED (1989a) Type I cell-like morphology in tight alveolar epithelial monolayers. *Exp Cell Res* 184:375–387
- Cheek JM, Kim KJ, Crandall ED (1989b) Tight monolayers of rat alveolar epithelial cells: bioelectric properties and active sodium transport. *Am J Physiol* 256:C688–C693
- Cho SJ, Maysinger D, Jain M et al (2007) Long-term exposure to CdTe quantum dots causes functional impairments in live cells. *Langmuir* 23:1974–1980
- Choi HS, Liu W, Misra P et al (2007) Renal clearance of quantum dots. *Nat Biotechnol* 25:1165–1170
- Cohen MS, Stern JM, Vanni AJ et al (2007) In vitro analysis of a nanocrystalline silver-coated surgical mesh. *Surg Infect (Larchmt)* 8:397–403
- Conner SD, Schmid SL (2003) Regulated portals of entry into the cell. *Nature* 422:37–44
- Deguchi S, Yamazaki T, Mukai SA et al (2007) Stabilization of C₆₀ nanoparticles by protein adsorption and its implications for toxicity studies. *Chem Res Toxicol* 20:854–858
- Derfus AM, Chan WCW, Bhatia SN (2004) Intracellular delivery of quantum dots for live cell labeling and organelle tracking. *Adv Materials* 16:961–969
- Dick CA, Brown DM, Donaldson K et al (2003) The role of free radicals in the toxic and inflammatory effects of four different ultrafine particle types. *Inhal Toxicol* 15:39–52
- Doherty GJ, McMahon HT (2009) Mechanisms of endocytosis. *Annu Rev Biochem* 78:857–902
- Donaldson K, Tran L, Jimenez LA et al (2005) Combustion-derived nanoparticles: a review of their toxicology following inhalation exposure. *Part Fibre Toxicol* 2:10
- Donaldson K, Aitken R, Tran L et al (2006) Carbon nanotubes: a review of their properties in relation to pulmonary toxicology and workplace safety. *Toxicol Sci* 92:5–22
- Eichmann SL, Anekal SG, Bevan MA (2008) Electrostatically confined nanoparticle interactions and dynamics. *Langmuir* 24:714–721
- Erogbogbo F, Yong KT, Roy I et al (2008) Biocompatible luminescent silicon quantum dots for imaging of cancer cells. *ACS Nano* 2:873–878
- Evans SA, Al-Mosawi A, Adams RA et al (2006) Inflammation, edema, and peripheral blood changes in lung-compromised rats after instillation with combustion-derived and manufactured nanoparticles. *Exp Lung Res* 32:363–378
- Ferin J, Oberdorster G, Penney DP (1992) Pulmonary retention of ultrafine and fine particles in rats. *Am J Respir Cell Mol Biol* 6:535–542
- Frampton MW (2001) Systemic and cardiovascular effects of airway injury and inflammation: ultrafine particle exposure in humans. *Environ Health Perspect* 109(Suppl 4):529–532
- Frampton MW, Utell MJ, Zareba W et al. (2004) Effects of exposure to ultrafine carbon particles in healthy subjects and subjects with asthma. *Res Rep Health Eff Inst* (126):1–47; discussion 49–63
- Franklin NM, Rogers NJ, Apte SC et al (2007) Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and ZnCl₂ to a freshwater microalga (*Pseudokirchneriella subcapitata*): the importance of particle solubility. *Environ Sci Technol* 41:8484–8490
- Furuyama A, Kanno S, Kobayashi T et al (2009) Extrapulmonary translocation of intratracheally instilled fine and ultrafine particles via direct and alveolar macrophage-associated routes. *Arch Toxicol* 83:429–437
- Geiser M, Kreyling WG (2010) Deposition and biokinetics of inhaled nanoparticles. *Part Fibre Toxicol* 7:2
- Geiser M, Rothen-Rutishauser B, Kapp N et al (2005) Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. *Environ Health Perspect* 113:1555–1560
- Geiser M, Casaulta M, Kupferschmid B et al (2008) The role of macrophages in the clearance of inhaled ultrafine titanium dioxide particles. *Am J Respir Cell Mol Biol* 38:371–376
- Geys J, Nemmar A, Verbeke E et al (2008) Acute toxicity and prothrombotic effects of quantum dots: impact of surface charge. *Environ Health Perspect* 116:1607–1613
- Gharbi N, Pressac M, Hadchouel M et al (2005) C₆₀ fullerene is a powerful antioxidant in vivo with no acute or subacute toxicity. *Nano Lett* 5:2578–2585

- Gojova A, Guo B, Kota RS et al (2007) Induction of inflammation in vascular endothelial cells by metal oxide nanoparticles: effect of particle composition. *Environ Health Perspect* 115:403–409
- Gold DR, Litonjua A, Schwartz J et al (2000) Ambient pollution and heart rate variability. *Circulation* 101:1267–1273
- Goldberg MS, Bailar JC III, Burnett RT et al (2000) Identifying subgroups of the general population that may be susceptible to short-term increases in particulate air pollution: a time-series study in Montreal, Quebec. *Res Rep Health Eff Inst* 97:7–113, discussion 115–120
- Goodman CM, McCusker CD, Yilmaz T et al (2004) Toxicity of gold nanoparticles functionalized with cationic and anionic side chains. *Bioconjug Chem* 15:897–900
- Gurr JR, Wang AS, Chen CH et al (2005) Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. *Toxicology* 213:66–73
- Hamoir J, Nemmar A, Halloy D et al (2003) Effect of polystyrene particles on lung microvascular permeability in isolated perfused rabbit lungs: role of size and surface properties. *Toxicol Appl Pharmacol* 190:278–285
- Hardman R (2006) A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. *Environ Health Perspect* 114:165–172
- Heckel K, Kieffmann R, Dorger M et al (2004) Colloidal gold particles as a new in vivo marker of early acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 287:L867–L878
- Hefland RB, Schwarzel PE, Johansen BV et al (2001) Silica-induced cytokine release from A549 cells: importance of surface area versus size. *Hum Exp Toxicol* 20:46–55
- Helland A, Wick P, Koehler A et al (2007) Reviewing the environmental and human health knowledge base of carbon nanotubes. *Environ Health Perspect* 115:1125–1131
- Helland A, Wick P, Koehler A et al (2008) Reviewing the environmental and human health knowledge base of carbon nanotubes. *Ciê Saúde Colet* 13:441–452
- Henneberger A, Zareba W, Ibaldo-Mulli A et al (2005) Repolarization changes induced by air pollution in ischemic heart disease patients. *Environ Health Perspect* 113:440–446
- Hoet PH, Nemmar A, Nemery B (2004) Health impact of nanomaterials? *Nat Biotechnol* 22:19
- Hong S, Bielinska AU, Mecke A et al (2004) Interaction of poly(amidoamine) dendrimers with supported lipid bilayers and cells: hole formation and the relation to transport. *Bioconjug Chem* 15:774–782
- Hong S, Hessler JA, Banaszak Holl MM et al (2006a) Physical interactions of nanoparticles with biological membranes: the observation of nanoscale hole formation. *Chem Health Saf* 13:16–20
- Hong S, Leroueil PR, Jnaus EK et al (2006b) Interaction of polycationic polymers with supported lipid bilayers and cells: nanoscale hole formation and enhanced membrane permeability. *Bioconjugate Chem* 17:728–734
- Hurt RH, Monthieux M, Kane A (2006) Toxicology of carbon nanomaterials: status, trends, and perspectives on the special issue. *Carbon* 44:1028–1033
- Hussain SM, Hess KL, Gearhart JM et al (2005) In vitro toxicity of nanoparticles in BRL 3A rat liver cells. *Toxicol In Vitro* 19:975–983
- Isakovic A, Markovic Z, Todorovic-Markovic B et al (2006) Distinct cytotoxic mechanisms of pristine versus hydroxylated fullerene. *Toxicol Sci* 91:173–183
- Jacobsen NR, Moller P, Jensen KA et al (2009) Lung inflammation and genotoxicity following pulmonary exposure to nanoparticles in ApoE^{-/-} mice. *Part Fibre Toxicol* 6:2
- Jeng HA, Swanson J (2006) Toxicity of metal oxide nanoparticles in mammalian cells. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 41:2699–2711
- Jia G, Wang H, Yan L et al (2005) Cytotoxicity of carbon nanomaterials: single-wall nanotube, multi-wall nanotube, and fullerene. *Environ Sci Technol* 39:1378–1383
- Kam NWS, Jessop TC, Wender PA et al (2004) Nanotube molecular transporters: internalization of carbon nano-tube-protein conjugates into mammalian cells. *J Am Chem Soc* 126:6850–6851
- Karabanovas V, Zakarevicius E, Sukackaite A et al (2008) Examination of the stability of hydrophobic (CdSe)ZnS quantum dots in the digestive tract of rats. *Photochem Photobiol Sci* 7:725–729
- Karlsson HL, Nygren J, Moller L (2004) Genotoxicity of airborne particulate matter: the role of cell-particle interaction and of substances with adduct-forming and oxidizing capacity. *Mutat Res* 565:1–10
- Karlsson HL, Nilsson L, Moller L (2005) Subway particles are more genotoxic than street particles and induce oxidative stress in cultured human lung cells. *Chem Res Toxicol* 18:19–23
- Karlsson HL, Ljungman AG, Lindbom J et al (2006) Comparison of genotoxic and inflammatory effects of particles generated by wood combustion, a road simulator and collected from street and subway. *Toxicol Lett* 165:203–211
- Karlsson HL, Cronholm P, Gustafsson J et al (2008a) Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. *Chem Res Toxicol* 21:1726–1732
- Karlsson HL, Holgersson A, Moller L (2008b) Mechanisms related to the genotoxicity of particles in the subway and from other sources. *Chem Res Toxicol* 21:726–731
- Kato T, Yashiro T, Murata Y et al (2003) Evidence that exogenous substances can be phagocytized by alveolar epithelial cells and transported into blood capillaries. *Cell Tissue Res* 311:47–51
- Kawakami K, Iwamura A, Goto E et al (1990) Kinetics and clinical application of ^{99m}Tc-Technegas. *Kaku Igaku (Japan)* 27:725–733
- Kemp SJ, Thorley AJ, Gorelik J et al (2008) Immobilization of human alveolar epithelial cells to investigate nanoparticle uptake. *Am J Respir Cell Mol Biol* 39:591–597
- Kim KJ, Crandall ED (1983) Heteropore populations of bullfrog alveolar epithelium. *J Appl Physiol* 54:140–146
- Kim KJ, Crandall ED (1996) Models for investigation of peptide and protein transport across cultured mammalian respiratory epithelial barriers. *Pharm Biotechnol* 8:325–346
- Kim KJ, Malik AB (2003) Protein transport across the lung epithelial barriers. *Am J Physiol Lung Cell Mol Physiol* 284:L247–L259
- Kim KJ, Matsukawa Y, Yamahara H et al (2003) Absorption of intact albumin across rat alveolar epithelial cell monolayers. *Am J Physiol Lung Cell Mol Physiol* 284:L458–L465
- Kim KJ, Fandy TE, Lee VH et al (2004) Net absorption of IgG via FcRn-mediated transcytosis across rat alveolar epithelial cell monolayers. *Am J Physiol Lung Cell Mol Physiol* 287:L616–L622
- Kleinstreuer C, Zhang Z, Donohue JF (2008) Targeted drug-aerosol delivery in the human respiratory system. *Annu Rev Biomed Eng* 10:195–220
- Knol AB, de Hartog JJ, Boogaard H et al (2009) Expert elicitation on ultrafine particles: likelihood of health effects and causal pathways. *Part Fibre Toxicol* 6:19
- Konig MF, Lucocq JM, Weibel ER (1993) Demonstration of pulmonary vascular perfusion by electron and light microscopy. *J Appl Physiol* 75:1877–1883
- Kreyling WG, Semmler M, Erbe F et al (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
- Kreyling WG, Semmler-Behnke M, Moller W (2006a) Health implications of nanoparticles. *J Nanopart Res* 8:543–562
- Kreyling WG, Semmler-Behnke M, Moller W (2006b) Ultrafine particle-lung interactions: does size matter? *J Aerosol Med* 19:74–83

- Kreyling WG, Semmler-Behnke M, Seitz J et al (2009) Size dependence of the translocation of inhaled iridium and carbon nanoparticle aggregates from the lung of rats to the blood and secondary target organs. *Inhal Toxicol* 21(Suppl 1):55–60
- Kroto HW, Heath JR, O'Brien S et al (1985) C₆₀-Buckminsterfullerene. *Nature* 318:162–163
- Lam HF, Chen LC, Ainsworth D et al (1988) Pulmonary function of guinea pigs exposed to freshly generated ultrafine zinc oxide with and without spike concentrations. *Am Ind Hyg Assoc J* 49:333–341
- Lam CW, James JT, McCluskey R et al (2004) Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. *Toxicol Sci* 77:126–134
- Lam CW, James JT, McCluskey R et al (2006) A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks. *Crit Rev Toxicol* 36:189–217
- Law WC, Yong KT, Roy I et al (2009) Aqueous-phase synthesis of highly luminescent CdTe/ZnTe core/shell quantum dots optimized for targeted bioimaging. *Small* 5:1302–1310
- Lewinski N, Colvin V, Drezek R (2008) Cytotoxicity of nanoparticles. *Small* 4:26–49
- Li D, He Q, Cui Y et al (2007) Immobilization of glucose oxidase onto gold nanoparticles with enhanced thermostability. *Biochem Biophys Res Commun* 355:488–493
- Liao D, Creason J, Shy C et al (1999) Daily variation of particulate air pollution and poor cardiac autonomic control in the elderly. *Environ Health Perspect* 107:521–525
- Lin P, Chen JW, Chang LW et al (2008) Computational and ultrastructural toxicology of a nanoparticle, Quantum Dot 705, in mice. *Environ Sci Technol* 42:6264–6270
- Lok CN, Ho CM, Chen R et al (2007) Silver nanoparticles: partial oxidation and antibacterial activities. *J Biol Inorg Chem* 12:527–534
- Lovric J, Bazzi HS, Cuie Y et al (2005a) Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots. *J Mol Med* 83:377–385
- Lovric J, Cho SJ, Winnik FM et al (2005b) Unmodified cadmium telluride quantum dots induce reactive oxygen species formation leading to multiple organelle damage and cell death. *Chem Biol* 12:1227–1234
- Male KB, Lachance B, Hrapovic S et al (2008) Assessment of cytotoxicity of quantum dots and gold nanoparticles using cell-based impedance spectroscopy. *Anal Chem* 80:5487–5493
- Matsukawa Y, Lee VH, Crandall ED et al (1997) Size-dependent dextran transport across rat alveolar epithelial cell monolayers. *J Pharm Sci* 86:305–309
- McIntosh CM, Esposito EA 3rd, Boal AK et al (2001) Inhibition of DNA transcription using cationic mixed monolayer protected gold clusters. *J Am Chem Soc* 123:7626–7629
- Mecke A, Majoros IJ, Patri AK et al (2005) Lipid bilayer disruption by polycationic polymers: the roles of size and chemical functional group. *Langmuir* 21:10348–10354
- Mehta D, Bhattacharya J, Matthay MA et al (2004) Integrated control of lung fluid balance. *Am J Physiol Lung Cell Mol Physiol* 287:L1081–L1090
- Moghimi SM, Hunter AC (2001) Capture of stealth nanoparticles by the body's defences. *Crit Rev Ther Drug Carrier Syst* 18:527–550
- Moghimi SM, Hunter AC, Murray JC (2001) Long-circulating and target-specific nanoparticles: theory to practice. *Pharmacol Rev* 53:283–318
- Moghimi SM, Hunter AC, Murray JC (2005) Nanomedicine: current status and future prospects. *FASEB J* 19:311–330
- Monteiro-Riviere NA, Tran CL (2007) Nanotoxicology: characterization, dosing, and health effects. Informa Healthcare, New York
- Moon MH, Kang D, Jung J et al (2004) Separation of carbon nanotubes by frit inlet asymmetrical flow field-flow fractionation. *J Sep Sci* 27:710–717
- Muhlfeld C, Rothen-Rutishauser B, Blank F et al (2008) Interactions of nanoparticles with pulmonary structures and cellular responses. *Am J Physiol Lung Cell Mol Physiol* 294:L817–L829
- Muller J, Huaux F, Moreau N et al (2005) Respiratory toxicity of multi-wall carbon nanotubes. *Toxicol Appl Pharmacol* 207:221–231
- Muller J, Decordier I, Hoet PH et al (2008a) Clastogenic and aneugenic effects of multi-wall carbon nanotubes in epithelial cells. *Carcinogenesis* 29:427–433
- Muller J, Huaux F, Fonseca A et al (2008b) Structural defects play a major role in the acute lung toxicity of multiwall carbon nanotubes: toxicological aspects. *Chem Res Toxicol* 21:1698–1705
- Mutlu GM, Green D, Bellmeyer A et al (2007) Ambient particulate matter accelerates coagulation via an IL-6-dependent pathway. *J Clin Invest* 117:2952–2961
- Mutlu GM, Budinger GR, Green AA et al (2010) Biocompatible nanoscale dispersion of single-walled carbon nanotubes minimizes *in vivo* pulmonary toxicity. *Nano Lett* 10(5):1664–1667
- Nel A, Xia T, Madler L et al (2006) Toxic potential of materials at the nanolevel. *Science* 311:622–627
- Nemmar A, Vanbilloen H, Hoylaerts MF et al (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
- Nemmar A, Hoet PH, Vanquickenborne B et al (2002) Passage of inhaled particles into the blood circulation in humans. *Circulation* 105:411–414
- Nemmar A, Hoylaerts MF, Hoet PH et al (2003) Size effect of intratracheally instilled particles on pulmonary inflammation and vascular thrombosis. *Toxicol Appl Pharmacol* 186:38–45
- Nemmar A, Hoylaerts MF, Hoet PH et al (2004) Possible mechanisms of the cardiovascular effects of inhaled particles: systemic translocation and prothrombotic effects. *Toxicol Lett* 149:243–253
- Niemeyer CM, Mirkin CA (2004) Nanobiotechnology. Wiley-VCH, Weinheim
- Oberdorster G (2000) Acute pulmonary effects of ultrafine particles in rats and mice. Health Effects Institute, Cambridge
- Oberdorster G (2001) Pulmonary effects of inhaled ultrafine particles. *Int Arch Occup Environ Health* 74:1–8
- Oberdorster G (2002) Toxicokinetics and effects of fibrous and nonfibrous particles. *Inhal Toxicol* 14:29–56
- Oberdorster G, Ferin J, Gelein R et al (1992) Role of the alveolar macrophage in lung injury: studies with ultrafine particles. *Environ Health Perspect* 97:193–199
- Oberdorster G, Ferin J, Lehnert BE (1994) Correlation between particle size, *in vivo* particle persistence, and lung injury. *Environ Health Perspect* 102(Suppl 5):173–179
- Oberdorster G, Gelein RM, Ferin J et al (1995) Association of particulate air pollution and acute mortality: involvement of ultrafine particles? *Inhal Toxicol* 7:111–124
- Oberdorster G, Sharp Z, Atudorei V et al (2002) Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. *J Toxicol Environ Health A* 65:1531–1543
- Oberdorster G, Sharp Z, Atudorei V et al (2004) Translocation of inhaled ultrafine particles to the brain. *Inhal Toxicol* 16:437–445
- Oberdorster G, Maynard A, Donaldson K et al (2005a) Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part Fibre Toxicol 2:8
- Oberdorster G, Oberdorster E, Oberdorster J (2005b) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect* 113:823–839
- Orr G, Panther DJ, Cassens KJ et al (2009) Syndecan-1 mediates the coupling of positively charged submicrometer amorphous silica

- particles with actin filaments across the alveolar epithelial cell membrane. *Toxicol Appl Pharmacol* 236:210–220
- Park S, Lee YK, Jung M et al (2007) Cellular toxicity of various inhalable metal nanoparticles on human alveolar epithelial cells. *Inhal Toxicol* 19(Suppl 1):S59–S65
- Partha R, Lackey M, Hirsch A et al (2007) Self assembly of amphiphilic C₆₀ fullerene derivatives into nanoscale supramolecular structures. *J Nanobiotechnology* 5:6
- Peters A, Liu E, Verrier RL et al (2000) Air pollution and incidence of cardiac arrhythmia. *Epidemiology* 11:11–17
- Peters A, Frohlich M, Doring A et al (2001) Particulate air pollution is associated with an acute phase response in men; results from the MONICA-Augsburg Study. *Eur Heart J* 22:1198–1204
- Pietropaoli AP, Frampton MW, Hyde RW et al (2004) Pulmonary function, diffusing capacity, and inflammation in healthy and asthmatic subjects exposed to ultrafine particles. *Inhal Toxicol* 16 (Suppl 1):59–72
- Politis M, Pilinis C, Lekkas TD (2008) Ultrafine particles (UFP) and health effects. Dangerous. Like no other PM? Review and analysis. *Global NEST J* 10:439–452
- Porter AE, Muller K, Skepper J et al (2006) Uptake of C₆₀ by human monocyte macrophages, its localization and implications for toxicity: studied by high resolution electron microscopy and electron tomography. *Acta Biomater* 2:409–419
- Porter AE, Gass M, Bendall JS et al (2009) Uptake of nontoxic acid-treated single-walled carbon nanotubes into the cytoplasm of human macrophage cells. *ACS Nano* 3:1485–1492
- Rejman J, Oberle V, Zuhorn IS et al (2004) Size-dependent internalization of particles via the pathways of clathrin- and caveolae-mediated endocytosis. *Biochem J* 377:159–169
- Ryman-Rasmussen JP, Riviere JE, Monteiro-Riviere NA (2007a) Surface coatings determine cytotoxicity and irritation potential of quantum dot nanoparticles in epidermal keratinocytes. *J Invest Dermatol* 127:143–153
- Ryman-Rasmussen JP, Riviere JE, Monteiro-Riviere NA (2007b) Variables influencing interactions of untargeted quantum dot nanoparticles with skin cells and identification of biochemical modulators. *Nano Lett* 7:1344–1348
- Sauer UG (2009) Animal and non-animal experiments in nanotechnology—the results of a critical literature survey. *ALTEX* 26:109–128
- Sayes CM, Fortner JD, Guo W et al (2004) The differential cytotoxicity of water-soluble fullerenes. *Nano Lett* 4:1881–1887
- Sayes CM, Gobin AM, Ausman KD et al (2005) Nano-C₆₀ cytotoxicity is due to lipid peroxidation. *Biomaterials* 26:7587–7595
- Sayes CM, Marchione AA, Reed KL et al (2007a) Comparative pulmonary toxicity assessments of C₆₀ water suspensions in rats: few differences in fullerene toxicity in vivo in contrast to in vitro profiles. *Nano Lett* 7:2399–2406
- Sayes CM, Reed KL, Warheit DB (2007b) Assessing toxicity of fine and nanoparticles: comparing in vitro measurements to in vivo pulmonary toxicity profiles. *Toxicol Sci* 97:163–180
- Schlesinger PA, Stillman MT, Peterson L (1982) Polyarthritis with birefringent lipid within synovial fluid macrophages: case report and ultrastructural study. *Arthritis Rheum* 25:1365–1368
- Scrivens W, Tour JM (1994) Synthesis of ¹⁴C-labeled C₆₀, its suspension in water, and its uptake by human keratinocytes. *J Am Chem Soc* 116:4517–4518
- Semmler-Behnke M, Takenaka S, Fertsch S et al (2007) Efficient elimination of inhaled nanoparticles from the alveolar region: evidence for interstitial uptake and subsequent reentrainment onto airway epithelium. *Environ Health Perspect* 115:728–733
- Shiohara A, Hoshino A, Hanaki K et al (2004) On the cyto-toxicity caused by quantum dots. *Microbiol Immunol* 48:669–675
- Shvedova AA, Castranova V, Kisin ER et al (2003) Exposure to carbon nanotube material: assessment of nanotube cytotoxicity using human keratinocyte cells. *J Toxicol Environ Health A* 66:1909–1926
- Shvedova AA, Kisin ER, Mercer R et al (2005) Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice. *Am J Physiol Lung Cell Mol Physiol* 289: L698–L708
- Shvedova AA, Fabisiak JP, Kisin ER et al (2008) Sequential exposure to carbon nanotubes and bacteria enhances pulmonary inflammation and infectivity. *Am J Respir Cell Mol Biol* 38:579–590
- Silva VM, Corson N, Elder A et al (2005) The rat ear vein model for investigating in vivo thrombogenicity of ultrafine particles (UFP). *Toxicol Sci* 85:983–989
- Singh R, Pantarotto D, Lacerda L et al (2006) Tissue biodistribution and blood clearance rates of intravenously administered carbon nanotube radiotracers. *Proc Natl Acad Sci USA* 103:3357–3362
- Smart SK, Cassady AI, Lu GQ et al (2006) The biocompatibility of carbon nanotubes. *Carbon* 44:1034–1047
- Soto K, Garza KM, Murr LE (2007) Cytotoxic effects of aggregated nanomaterials. *Acta Biomater* 3:351–358
- Spohn P, Hirsch C, Hasler F et al (2009) C₆₀ fullerene: a powerful antioxidant or a damaging agent? The importance of an in-depth material characterization prior to toxicity assays. *Environ Pollut* 157:1134–1139
- Stern ST, Zolnik BS, McLeland CB et al (2008) Induction of autophagy in porcine kidney cells by quantum dots: a common cellular response to nanomaterials? *Toxicol Sci* 106:140–152
- Storm G, Belliot SO, Daemen T et al (1995) Surface modification of nanoparticles to oppose uptake by the mononuclear phagocyte system. *Adv Drug Deliv Rev* 17:31–48
- Su Y, He Y, Lu H et al (2009) The cytotoxicity of cadmium based, aqueous phase—synthesized, quantum dots and its modulation by surface coating. *Biomaterials* 30:19–25
- Takenaka S, Karg E, Roth C et al (2001) Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. *Environ Health Perspect* 109(Suppl 4):547–551
- Takenaka S, Karg E, Kreyling WG et al (2004) Fate and toxic effects of inhaled ultrafine cadmium oxide particles in the rat lung. *Inhal Toxicol* 16(Suppl 1):83–92
- Takenaka S, Karg E, Kreyling WG et al (2006) Distribution pattern of inhaled ultrafine gold particles in the rat lung. *Inhal Toxicol* 18:733–740
- Timonen KL, Vanninen E, de Hartog J et al (2006) Effects of ultrafine and fine particulate and gaseous air pollution on cardiac autonomic control in subjects with coronary artery disease: the ULTRA study. *J Expo Sci Environ Epidemiol* 16:332–341
- Tokuyama H, Yamago S, Nakamura E et al (1993) Photoinduced biochemical activity of fullerene carboxylic acid. *J Am Chem Soc* 115:7918–7919
- Usenko CY, Harper SL, Tanguay RL (2008) Fullerene C₆₀ exposure elicits an oxidative stress response in embryonic zebrafish. *Toxicol Appl Pharmacol* 229:44–55
- Wagner AJ, Bleckmann CA, Murdock RC et al (2007) Cellular interaction of different forms of aluminum nanoparticles in rat alveolar macrophages. *J Phys Chem B* 111:7353–7359
- Wallace WE, Keane MJ, Murray DK, et al (2007) Phospholipid lung surfactant and nanoparticle surface toxicity: lessons from diesel soots and silicate dusts. *J Nanopart Res* 9:23–38
- Wang H, Wang J, Deng X et al (2004) Biodistribution of carbon single-wall carbon nanotubes in mice. *J Nanosci Nanotechnol* 4:1019–1024
- Wang L, Wang K, Santra S et al (2006) Watching silica nanoparticles glow in the biological world. *Anal Chem* 78:646–654

- Wang Y, Camargo PH, Skrabalak SE et al (2008) A facile, water-based synthesis of highly branched nanostructures of silver. *Langmuir* 24:12042–12046
- Warheit DB (2006) What is currently known about the health risks related to carbon nanotube exposures? *Carbon* 44:1064–1069
- Warheit DB, Sayes CM, Reed KL et al (2008) Health effects related to nanoparticle exposures: environmental, health and safety considerations for assessing hazards and risks. *Pharmacol Ther* 120:35–42
- Wick P, Manser P, Limbach LK et al (2007) The degree and kind of agglomeration affect carbon nanotube cytotoxicity. *Toxicol Lett* 168:121–131
- Widera A, Beloussow K, Kim KJ et al (2003a) Phenotype-dependent synthesis of transferrin receptor in rat alveolar epithelial cell monolayers. *Cell Tissue Res* 312:313–318
- Widera A, Kim KJ, Crandall ED et al (2003b) Transcytosis of GCSF-transferrin across rat alveolar epithelial cell monolayers. *Pharm Res* 20:1231–1238
- Wiseman DA, Wells SM, Wilham J et al (2006) Endothelial response to stress from exogenous Zn^{2+} resembles that of NO-mediated nitrosative stress, and is protected by MT-1 overexpression. *Am J Physiol* 291:C555–C568
- Wright EM, Pietras RJ (1974) Routes of nonelectrolyte permeation across epithelial membranes. *J Membr Biol* 17:293–312
- Xia T, Kovochich M, Brant J et al (2006) Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. *Nano Lett* 6:1794–1807
- Xia T, Kovochich M, Liong M et al (2008) Cationic polystyrene nanosphere toxicity depends on cell-specific endocytic and mitochondrial injury pathways. *ACS Nano* 2:85–96
- Xia T, Li N, Nel AE (2009) Potential health impact of nanoparticles. *Annu Rev Public Health* 30:137–150
- Xu Z, Hu PA, Wang S et al (2008) Biological functionalization and fluorescent imaging of carbon nanotubes. *Appl Surf Sci* 254:1915–1918
- Yacobi NR, Phuleria HC, Demaio L et al (2007) Nanoparticle effects on rat alveolar epithelial cell monolayer barrier properties. *Toxicol In Vitro* 21:1373–1381
- Yacobi NR, Demaio L, Xie J et al (2008) Polystyrene nanoparticle trafficking across alveolar epithelium. *Nanomedicine* 4:139–145
- Yacobi NR, Malmstadt N, Fazlollahi F et al (2010) Mechanisms of alveolar epithelial translocation of a defined population of nanoparticles. *Am J Respir Cell Mol Biol* 42:604–614
- Yamago S, Tokuyama H, Nakamura E et al (1995) In vivo biological behavior of a water-miscible fullerene: ^{14}C labeling, absorption, distribution, excretion and acute toxicity. *Chem Biol* 2:385–389
- Yamawaki H, Iwai N (2006) Cytotoxicity of water-soluble fullerene in vascular endothelial cells. *Am J Physiol Cell Physiol* 290:C1495–C1502
- Yang W, Peters JI, Williams RO 3rd (2008) Inhaled nanoparticles—a current review. *Int J Pharm* 356:239–247
- Zhang T, Stilwell JL, Gerion D et al (2006) Cellular effect of high doses of silica-coated quantum dot profiled with high throughput gene expression analysis and high content cellomics measurements. *Nano Lett* 6:800–808