Overview of Current Toxicological Knowledge of Engineered Nanoparticles

Vincent Castranova, PhD

Objective: Nanotechnology is the manipulation of matter on a near-atomic scale to produce nanoparticles with unique properties, allowing new commercial applications. Since nanoparticles exhibit unique physicochemical properties, they are likely to exhibit biological activity significantly different from fine-sized particles of the same chemical composition. Therefore, evaluation of the biological effects of nanoparticles is critical. **Methods:** The article lists the major objectives of nanotoxicology and briefly reviews the literature concerning biological responses to pulmonary exposure. **Results:** Interactions of nanoparticles with biological systems depend on particle size, shape, oxidant generation, surface functionalization, and rate of dissolution. Pulmonary, cardiovascular, and central nervous system responses to pulmonary exposure to nanotitanium dioxide and carbon nanotubes are described. **Conclusions:** Significant biological responses occur in animal models after pulmonary exposure to certain nanoparticles. Control of exposure appears prudent to protect worker health. **Clinical Significance:** Nanotechnology is synthesizing a wide range of nanoparticles, which exhibit unique physicochemical properties. These unique properties make unique biological activity likely. If certain nanoparticles induce adverse effects in vitro or in animal models, then occupational health surveillance and exposure control may be prudent steps in the protection of worker health.

During the Clinton administration, Congress enacted the National Nanotechnology Initiative to foster research in a new field, nanotechnology, and to stimulate the commercial development of new products resulting from such research. Nanotechnology is the manipulation of matter on a near-atomic scale to produce new structures, materials, and devises. Nanotechnology is projected to grow into a trillion dollar industry employing millions of workers worldwide within the next decade.[1] Indeed, a wide variety of novel applications and products are being developed for commercial use in cosmetics, electronics, sensors, structural materials, sporting goods, sunscreens, antimicrobial products, paints, coatings, energy storage devices, conductive fabric, bone grafting, medical imaging, and targeted drug delivery.[2]

At the core of nanotechnology is the synthesis of engineered nanoparticles, which are defined as particles having one dimension less than 100 nm. Engineered nanoparticles are created with tightly controlled size, shape, surface features, and chemistry. Since a large fraction of the particle's atoms are on its surface, nanoparticles exhibit unique physicochemicals, which are distinctly different from those of fine-sized particles of the same chemical composition. Because of the small size and low density of nanoparticles, aerosolization is likely during energetic processes, such as voxtexing, weighing, sonication, mixing, and blending. Therefore, worker exposure via inhalation is anticipated during production, use, and disposal of nanoparticles.[3]

The unique physiochemical properties of nanoparticles are driving nanotechnology and the development of unique products and applications. Nevertheless, these unique physiochemical properties are likely to result in unique bioactivity. *Nanotoxicology* is the systemic evaluation of the interaction of nanoparticles with biological systems, the quantification of resulting responses, and the elucidation of mechanisms determining the interactions and responses to nanoparticles on the molecular, cellular, tissue, organ, and whole body levels. The objectives of nanotoxicology are to

1. Determine the relationships between physicochemical properties of nanoparticles and their bioactivity,
2. Identify responses at the primary site of exposure as well as in distal organs, and
3. Determine the dose and time dependence of these biological responses.

The following is a brief review of selected areas of knowledge development in nanotoxicology.

RELATIONSHIP BETWEEN NANOPARTICLE CHARACTERISTICS AND BIOACTIVITY

A major challenge for toxicological assessment in nanotechnology is the large and rapidly growing number of possible nanoparticles to be tested for biological activity. It is not feasible to conduct a full assessment of bioactivity for every possible nanoparticle. Therefore, it is critical to develop a matrix of relationships between specific physicochemical properties and resultant bioactivity. An understanding of such relationships would allow the prediction of possible health effects in the absence of complete toxicity data. This knowledge can be applied to develop prevention strategies to protect worker health.

At this point in the development of a knowledge base in nanotoxicology, the following physicochemical properties are believed to be important determinants of biological response:

1. Particle size
2. Particle shape
3. Oxidant generation
4. Surface functionalization
5. Rate of dissolution

A growing body of data indicates that particle size is an important factor in driving the biological response to particles. The National Institute for Occupational Safety and Health (NIOSH) laboratory has evaluated the pulmonary response to intratracheal instillation of well-dispersed fine versus nano titanium dioxide (TiO_2) particles.[4] On an equal-mass exposure basis, nano-TiO_2 was as much as 41-fold more potent than fine TiO_2 in causing lung inflammation, lung damage, inflammatory cytokine/chemokine production, and oxidant generation by alveolar macrophages. If lung burden were normalized to total particle surface area deposited, the potency of nano and fine TiO_2 was not significantly different. Particle size also affected the fate of the particles after pulmonary exposure.[4] Fine-sized TiO_2 was avidly phagocytized by alveolar macrophages, while nano-TiO_2 exhibited a significantly greater ability to evade phagocytosis and enter the alveolar walls. The importance of particle size to bioactivity also impacts the pulmonary response to agglomerated versus more dispersed nanoparticles. The NIOSH laboratory reported that intratracheal instillation of a well-dispersed suspension of

From the National Institute for Occupational Safety and Health, Morgantown, WV.
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Address correspondence to: Vincent Castranova, PhD, NIOSH, 1095 Willowdale Road, Morgantown, WV 26505; E-mail: vic1@cdc.gov.

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carbon black nanoparticles resulted in an 8-fold greater response on an equal mass burden basis than a poorly dispersed suspension of agglomerated carbon black nanoparticles.[5] Furthermore, the agglomeration status of single-walled carbon nanotubes has been shown to affect both deposition site and pulmonary response.[6] On aspiration, micrometer-sized agglomerates deposit at the proximal alveolar region of mouse lungs and induce granulomatous lesions. In contrast, aspirated well-dispersed single-walled carbon nanotube structures deposit in the distal alveoli, rapidly enter the alveolar walls, and induce interstitial fibrosis. These data indicate that bioactivity of a nanomaterial is dependent not only on the primary size of the nanoparticle but also on the degree at which the nanoparticles are agglomerated, that is, the physical size of the nanoparticle structures, as they interact with biological systems.

Data from NIOSH studies indicate that nanoparticle shape is also a critical determinant of bioactivity. Porter et al[7] reported that TiO_2 nanoparticles in the form of long belts were significantly more toxic in vitro and more inflammatory in mice at 1 day postexposure than an equal mass of TiO_2 nanospheres of the same chemical composition and diameter. Similarly, Shvedova et al[8] reported that high aspect ratio single-walled carbon nanotubes were 23-fold more inflammatory 1 day after aspiration in mice than an equal mass of spherical carbon nanoparticles (carbon black). The high aspect ratio of long, thin carbon nanotubes has raised concern that carbon nanotubes may induce pulmonary responses similar to asbestos.[9]

Nel et al[10] has proposed that oxidant stress may be a critical parameter determining bioactivity. Indeed, a strong correlation ($R^2 = 0.95$) has been demonstrated between the ability of eight different spherical particles to stimulate oxidant production by alveolar macrophages in vitro and their potency to cause pulmonary inflammation 1 day after intratracheal instillation in a rat model.[11] Nevertheless, carbon nanotubes appear to be an exception to the oxidant stress paradigm. Raw single-walled carbon nanotubes, containing 30% iron by weight, generate a substantial hydroxyl radical signal measured by electron spin resonance spectroscopy in an acellular system in the presence of hydrogen peroxide. In contrast, purified single-walled carbon nanotubes (0.2% iron) do not generate hydroxyl radicals. In agreement with the oxidant stress paradigm, raw single-walled carbon nanotubes were highly toxic and caused oxidant stress to cells in culture, while purified single-walled carbon nanotubes were significantly less cytotoxic.[12] Nevertheless, the oxidant stress paradigm does not predict the pulmonary response to single-walled carbon nanotubes in a mouse model. Indeed, Shvedova et al[8,13] found that the level of pulmonary inflammation 1 day after aspiration of raw single-walled carbon nanotubes by mice was not significantly different than the inflammation reported after pulmonary exposure to an equal mass (10 μg per mouse) of purified single-walled carbon nanotubes. Therefore, while oxidant generation appears to be an important factor to determine the pulmonary response to some types of nanoparticles (nanometals and nanospheres), it appears to be of minor importance to the pulmonary response to carbon nanotubes, where particle shape or aspect ratio appears to drive bioactivity.

A critical step in the expression of bioactivity of a nanoparticle is the biophysicochemical interaction of the nanoparticle surface with biological systems.[14] Since the surface activity of nanoparticles is considered critical to bioactivity, it has been proposed that functionalization of the surface of nanoparticles would alter bioactivity and that this may be a practical approach in the development of "safe" nanoparticles. This concept received support from the work of Sayes et al,[15] in which hydroxylization [C_{60} $(OH)_{24}$] significantly decreased the cytotoxicity of fullerenes (C_{60}) in fibroblast, lung epithelial cell, and astrocyte in vitro models. Unfortunately, such functionization of fullerenes did not alter their inflammatory potential in rat lungs 1 day to 3 months after intratracheal instillation, that is, both C_{60} and C_{60} $(OH)_{24}$ exhibited similar levels of transient inflammation.[16]

The pulmonary and systemic response to pulmonary exposure to nanoparticles is believed to be related to the rate at which the particle dissolves. For example, fiber pathogenicity is related to the durability of the fiber, which impacts the biopersistence of such particles in the lung.[17] In contrast, many of the effects of residual oil fly ash have been associated with its soluble metal component.[18] Sager et al[19] have demonstrated that zinc oxide nanoparticles exhibit a high rate of dissolution, which accounts for the rapid clearance of zinc from the lung and translocation to systemic organs. Doping zinc oxide nanoparticles with iron results in a substantial decrease in the rate of dissolution.[20] Iron-doped zinc oxide nanoparticles are far less toxic to cells in culture and cause significantly less lung damage and inflammation in rat lungs at 1 to 30 days after intratracheal instillation.

In summary, there is a growing nanotoxicology database relating bioactivity to a specific physicochemical property of a nanoparticle. As such information is expanded, it will allow one to predict the relative pathogenicity of a given nanoparticle with given properties. This will allow control banding approaches for developing prevention strategies for worker protection.

RESPIRATORY AND SYSTEMIC RESPONSES TO PULMONARY EXPOSURE TO SELECTED NANOPARTICLES

Significant airborne levels of nanoparticles have been associated with various processes (vortexing, weighing, sonication, mixing, blending, and reactor cleanout) in nanotechnology workplaces.[21–24] The following section will briefly review the pulmonary, cardiovascular, and central nervous system responses resulting from pulmonary exposure to TiO_2 nanoparticles or multi-walled carbon nanotubes (MWCNT).

Sager et al[4] have reported that intratracheal instillation of rats to a well-dispersed suspension of nano-TiO_2 caused a dose- and time-dependent pulmonary inflammation and damage. Substantial pulmonary responses were observed after exposure to 0.26 mg of TiO_2 per rat, with responses increasing in a near-linear manner through 1.04 mg per lung. Responses were maximal at 1 to 7 days postexposure and only partially returned toward control (a decrease from the peak response of 25% to 50%) at 42 days postexposure. Substantial pulmonary fibrosis was not noted over this time period.

Inhalation exposure of rats to nano-TiO_2 has also been reported to cause systemic microvascular dysfunction at 1 day postexposure.[25] Intravital microscopic analysis of the ability of arterioles in the shoulder muscle to respond to dilators indicates that significant inhibition of normal dilatory response after inhalation of nano-TiO_2 at lung burdens from 7 to 40 μg. Complete inhibition of dilatory function of systemic arterioles was observed at a lung burden of 400-μg nano-TiO_2, at which dose, no gross changes in bronchoalveolar lavage markers of pulmonary inflammation or damage were noted. Le Blanc et al[26] have reported similar, sensitive inhibition of the ability of coronary arterioles to respond to dilators in rats 1 day after inhalation of 10-μg of nano-TiO_2. These results suggest that pulmonary exposure to nano-TiO_2 may result in elevated peripheral resistance and decreased oxygen delivery to the heart, which may have adverse impact under exercise conditions.

Sriram et al[27] reported that aspiration of TiO_2 nanobelts (30μg per mouse) in mice caused pulmonary inflammation 1 day postexposure. Associated with this pulmonary exposure was a significant elevation of messenger ribonucleic acid (mRNA) levels for markers of inflammation and blood–brain barrier injury in selected regions of the brain.

Aspiration of MWCNT (10 to 40 μg per mouse) in mice has been reported to cause a rapid but transient pulmonary inflammatory and damage response.[28] Response peaked 1 to 7 days postexposure and returned toward control levels at 28 and 56 days postexposure. In

contrast to the transient inflammatory reaction, a persistent (through 56 days) fibrotic response of early onset (7-day postexposure) was noted. Results indicate that acute pulmonary responses to short-term inhalation of MWCNT are similar to those reported after a bolus exposure via aspiration at the same lung burden of MWCNT.[29]

Data from the NIOSH laboratory indicate that inhalation exposure of rats to MWCNT at a lung burden of 17 μg per rat resulted in significant pulmonary inflammation and damage 1 day postexposure. Associated with this pulmonary exposure to MWCNT was complete inhibition of the ability of coronary arterials to respond to dilatory signals.

Aspiration of 80 μg of MWCNT in mice significantly elevated mRNA for inflammatory mediators (interleukin [IL]-1β, IL-6, tumor necrosis factor [TNF]-α, and colony-stimulating factor [Csf]-3) in the olfactory bulb and other selected brain regions at 1 day postexposure.[30] Induction of mRNA for E-selection (a marker of blood–brain barrier injury) was also noted.

Possible mechanisms by which pulmonary nanoparticle exposure results in systemic effects include the following:

1. Translocation of the nanoparticle from the lung to the systemic organ
2. Systemic inflammation
3. Neurogenic signals

Evidence suggests that nanoparticles can translocate to systemic organs. Nevertheless, the rate of translocation is low.[31] Indeed, nano-TiO_2 or MWCNT were below the level of detection in cardiovascular and brain tissue in the NIOSH studies described previously. In contrast, there is evidence that pulmonary exposure to nano-TiO_2 results in potentiation of peripheral blood polymorphonuclear leukocytes, adherence of polymorphonuclear leukocytes to the microvessel walls, and generation of oxidants at the vessel wall.[32] These events have been linked to particle-induced systemic and coronary microvascular dysfunction.[32,33] Lastly, particle-induced systemic and coronary microvascular dysfunction has been linked to neurogenic signals from airway sensory neurons to the cardiovascular tissue.[34]

CONCLUSION

The nanotoxicology literature indicates that the unique physicochemical properties of nanoparticles dictate the interaction with biological systems at the molecular, cellular, organ, and whole body level. Results indicate that nanoparticle size, shape, oxidant-generation capacity, surface functionalization, and rate of dissolution are critical determinants of bioactivity. Structure, function, and mechanistic studies are ongoing with the goal of constructing a matrix of relationships between physicochemical properties and biological response. Such correlations will allow preliminary assessment of relative health hazard for nanoparticles in the absence of a complete toxicological evaluation.

Studies evaluating responses to pulmonary exposure to selected nanoparticles, such as TiO_2 and MWCNT, indicate that reactions are noted both in the organ of exposure, that is, the lung, and in distal organs, such as the cardiovascular and central nervous system. Data indicate that systemic reactions can often be measured at low exposure doses where lung effects are minimal. Therefore, markers of cardiovascular and central nervous response may prove useful biomarkers for worker surveillance. Indeed, volunteers exposed to diesel exhaust exhibit electroencephalography changes, that is, an increase in fast wave activity in the frontal cortex,[35] and microvascular changes, that is, impaired forearm vascular response to dilators,[36] within hours after exposure.

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