

GENERATION AND CHARACTERIZATION OF ENGINEERED NANOPARTICLES FOR ENVIRONMENTAL AND BIOLOGICAL EXPOSURE RESEARCH

Meng-Dawn Cheng¹, Douglas Lowndes², and David B. Geohegan²

Oak Ridge National Laboratory, Environmental Sciences Division¹, Center for Nanophase Material Sciences²,
Condensed Matter Sciences Division, PO Box 2008, MS 6038, Oak Ridge, Tennessee 37831, USA

Abstract

Nanoparticles (D_p in one or more dimensions ≤ 100 nm) have been part of the human environment at moment we started using fire, because combustion of fossil fuel releases polyaromatic hydrocarbons that serve as the nuclei for nanoparticle formation. The recent explosion of nano-hyphenated materials (e.g., engineered nanoparticles) has resulted in excitement for environmentalists, as if a new environmental pollutant is emerging. Nevertheless, their concern is legitimate and has a strong basis because knowledge regarding the biological and environmental impacts of engineered nanoparticles is lacking. Engineered nanoparticles are different from diesel engine particles (DEPs), for example, because engineered nanoparticles have tunable size, morphology, and chemistry while DEPs do not. The toxicity of engineered nanoparticles and nanophase materials may depend on the properties of the materials that are yet to be fully determined. Furthermore, techniques and standards for assessing risk and toxicity from exposure to engineered nanoparticles have not been agreed upon among researchers in federal agencies, industry, and academia. Important techniques needed for assessing exposure and impacts on biological systems (human species, animals, aquatic species, microbial community, and plants) remain to be developed and verified. The lack of metrology for nanotoxicological evaluation contributes much of the confusion in the current exposure/risk assessment framework, causes uncertainty in the prediction of toxicity of interested nanophase materials, and adds to the challenge of environmental and toxicological research. The lack of assessment technology is a critical issue for investors, either federal agencies who fund the research or industries that expect to profit from nanotechnology. We present a review of the existing technologies suitable for generation, characterization, and exposure of nanoparticles for biological and environmental research, and advances in techniques we have developed and are currently using in nanoparticle exposure research.

Keywords: *Engineered Nanoparticles, Exposure, Biological Responses*

Introduction

Nanoparticles are discrete entities dispersed in space and time that in at least one dimension (D) have size less than 100 nm (e.g., 0D nanodots, 1D nanowires, and 2D nanotubes). Generally speaking, nanoparticles are not new species in the outdoor and indoor environments. Nanoparticles have been produced in the atmosphere since the beginning of troposphere formation and play an important part in atmospheric aerosol and cloud cycles (Seinfeld and Pandis, 1998). Man's ability to process and manipulate materials has improved to the point where creation (directly or indirectly) of particles on the nanometer scale has become part of daily activity. For example, engines emit nanoparticles in a high temperature combustion environment. Atmospheric chemical reactions of man-made polluting species such as nitrogen oxides, sulfur dioxide, and reactive organic gases produce nanoparticles. Nanoparticles are chemically complex and known to cause a wide range of health concerns. Adverse health risks due to exposure to complex atmospheric particles (e.g., Donaldson et al., 1998; Ferin et al., 1992), occupational dusts (e.g., quartz) (Knaapen et al., 2002; Schins et al., 2002), engine exhausts (Cheng, and others), and coal fly ash particles (Gilmour et al., 2004) are well documented. Epidemiological studies over the past half of a century on the health effects of airborne particles in the environment and workplaces have yielded a large volume of data associating cardiovascular, respiratory, developmental impairments, and lung cancer. Recent findings by Somers et al. (2004) is the first to report heritable mutations in mice when two groups of mice were exposed, one to HEPA (High-Efficiency Particulate Air) treated air, and the other to the same air without HEPA treatment.

The emerging ability to manufacture new materials at the nanometer scale, with distinctive properties not found in their counterparts of larger sizes (i.e., micrometer scale or bulk), has led recently to many technological advances and heavy investment in research and development. Companies including DuPont, BASF, L'Oreal, HP, Mitsubishi, Toyota, Proctor and Gamble,

and others have invested funds in R&D. The estimated investment in fiscal year 2004 alone is US \$8.6 billion (ZdNETnews.com, 8/15/2004). Engineered nanoparticles are a special class of nanophase materials that have wide applications to nanotechnology. Their properties are complex, and size-dependent, so that engineered nanoparticles have tunable size, morphology, and chemistry that are created for a specific purpose and/or functionality. Ideally they are highly uniform, unlike the nanoparticles mentioned in the previous paragraph.

As much as we have learned about the biological and environmental effects of pollution nanoparticles from various sources, very little is known about the biological and environmental consequences of exposure to engineered nanomaterials. There is currently only handful of papers in the open literature documenting observations on the biological effects of engineered nanoparticles. Semiconductor quantum dots (QDs~ 4-5 nm) have been used for cellular studies, biological labeling, and medical imaging. However, recently, Derfus et al. (2004) found that exposure of their uncoated QDs (made from CdSe) to oxygen in the air or UV radiation freed Cd thereby leading to cytotoxicity (potentially via the cadmium hepatotoxicity mechanism). Only two pulmonary toxicology studies, both on single-walled carbon nanotubes, were reported. Each employed intratracheal instillation technique to inject nanotubes to rats (Warheit et al., 2004; Lam et al., 2004). Both studies demonstrate that long exposure to carbon nanotubes produced greater adverse inflammatory responses compared with larger carbon-based particles of identical composition at equivalent mass concentrations. Oberdörster (2004) first showed that uncoated fullerenes (C_{60}) in water, forming colloidal fullerenes ($_nC_{60}$), induced oxidative stress in the brain of juvenile largemouth bass. Glutathione depletion was also found in the gill. The evidence was clear suggesting the potential damage to aquatic species. More interestingly, the author noticed that water became clearer after fullerenes were added than without, implying that fullerenes might have suppressed biological activity in the aquarium used in experiments. The impact of fullerenes on the

microbial community however needs further study.

Major differences between engineered nanoparticles and those discussed earlier in this paper (e.g., engine exhaust nanoparticles) are the tunable morphology, size, and chemistry that only engineered nanoparticles possess (Colvin, 2003). However, the differences do not make biological exposure studies of engineered nanoparticles any easier than for exhaust nanoparticles. Trace quantities of impurities in engineered materials could easily bias research results, for example, requiring new calibration and assay standards. The extremely small size of nanoparticles demands precise and calibrated aerosol measurement instrumentation whose response to these very fine particles is well established. High chemical reactivity (e.g., the ability to produce radicals readily) or in some cases unusual physical properties (e.g. magnetism) makes their properties challenging to predict. Specifically, it is unclear how biological systems (e.g., human cells, a network of cells, microbial, and animals) will respond when they encounter engineered nanoparticles or environmentally modified nanoparticles.

At this point, there are no agreed techniques and/or procedures on how exposure of nanoparticles should be employed and performed, what biological endpoints should be measured, and what nanoparticle materials should be used to benchmark studies across different agencies and academia. In a sense, metrology for nanotoxicological research remains to be defined. To advance our knowledge and quantify the risk of nanoparticle exposure via various routes, e.g., inhalation, dermal penetration, and ingestion, it is imperative that critical skills and technologies be developed, aiming at understanding of impacts associated with engineered nanoparticles. Techniques for precision particle generation, modification, characterization, and biological exposure are highly needed. This paper discusses advances made in the research group at ORNL, and techniques that exhibit the potential for engineered nanoparticle exposure investigation. The effort to incorporate various techniques into the discussion is by no means exhaustive, so that

other important technologies may have been unintentionally left out.

Generation of Nanoparticles

Biological risk and/or hazard of engineered nanoparticles (e.g., to tissue cells, physiology of whole animals) is primarily determined by the route of exposure (i.e., inhalation, ingestion, and dermal). Colvin (2003) indicates that most engineered nanoparticles are generated by wet chemistry and the particle surface is also modified in the liquid phase. Exposures to these wet nanoparticles via the ingestion and dermal routes are more likely than the inhalation route. This could lead to a less complicated nanoparticle exposure experiment, since the engineered nanoparticles can be introduced to a biological model simply by instillation techniques. The extent of how the wet-processed engineered nanoparticles become airborne has never been investigated. Aerosolization of wet-processed engineered nanoparticles (e.g., colloids) will need to be developed for inhalation research on exposure of the nanoparticles.

In fact, a wide variety of nanomaterials, engineered nanoparticles and nanostructures, are also synthesized by the dry (i.e., aerosol) route in the gas phase. These techniques include plasma (Bica, 1999), evaporation-condensation (Singh et al., 2002; Veranth et al., 2003), laser ablation (Bekyarova et al, 2002a; 2002b; 2003; Geohegan et al., 2001), spray pyrolysis (Miller et al., 2001; Kim et al., 2003), and electrospray (Lenggoro et al., 2002; Cheng, 2004), for example. The engineered nanoparticles produced by aerosol techniques can be delivered directly to biological receptors in an in vitro or in vivo test without going through the liquid phase.

Special techniques have been developed to monitor and understand nanomaterials growth in the gas/plasma phase. For instance, time-resolved optical imaging and spectroscopy studies have been developed at ORNL (see Figure 1) to provide a unique facility in the Condensed Matter Sciences Division (and soon the Center for Nanophase Material Sciences)

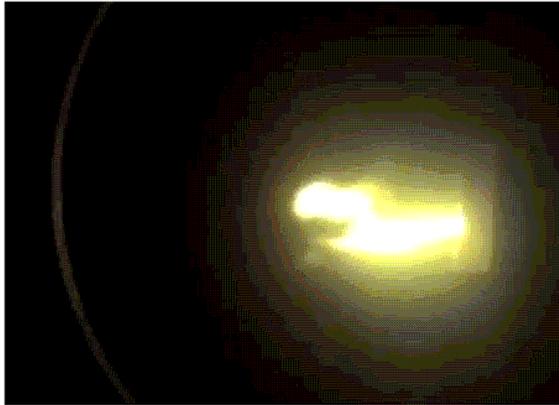


Figure 1. Laser vaporization production of nanomaterials at high rates using a 600W industrial Nd: YAG laser in a specially-constructed system located at ORNL.

(<http://www.cnms.ornl.gov>) for the characterization of laser-generated plasma plumes for nanoparticle and nanotube synthesis, including photoluminescent silicon-oxide nanoparticles, single-wall carbon nanotubes (SWNTs), and single-wall carbon nanohorns (SWNHs). Through these *in situ* diagnostics, plume properties can be controlled to provide reproducible conditions for synthesis of multigrams quantities of nanomaterials.

It is well known to the atmospheric aerosol community that the physical and chemical properties of nanoparticles can be altered, significantly, once the nanoparticles encounter the liquid phase. For example, soluble species like sulfate account for 60-80% of the PM_{2.5} mass in the Southeast of the US (Tanner et al., 2001; Cheng and Tanner, 2002). Wetting of engineered aerosol nanoparticles modifies the size and chemistry of the engineered nanoparticles. Coating on engineered nanoparticles may delay or prevent change of the physical and chemical properties to occur; it is unknown how stable the coating will be under environmental conditions. Investigation of the true biological responses to engineered nanoparticles would require preservation of the nanoparticle properties (size, morphology, and chemical composition, especially the surface chemistry) without potential modification by the liquid phase.

It is likely the situation will be more complicated once engineered nanoparticles are released into the environment than in workplaces, because of the multitude of modifying factors; e.g., water vapor, nitrogen oxides, sulfur oxides, reactive organic gases, radicals, UV, and ambient airborne particles. The surface of engineered metal nanoparticles can be oxidized in the air by oxygen (Derfus et al., 2003). It is unknown at this point regarding the stability of the properties of various engineered nanoparticles and of the surface coatings currently used on nanoparticles such as ZnO and TiO₂ in cosmetics and sunscreen lotions.

Nanoparticles prepared by various colloidal methods in the liquid phase cannot be used directly for an inhalation exposure study, and first must be dispersed into the air. Techniques do exist to aerosolize colloidal particles. For example, a tandem differential mobility analyzer

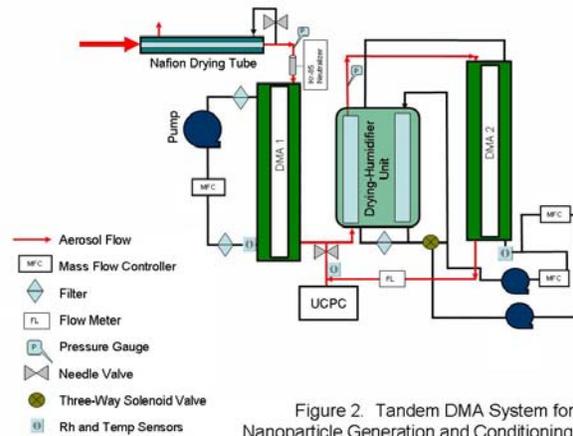


Figure 2. Tandem DMA System for Nanoparticle Generation and Conditioning

technique (TDMA) has been employed to select nanoparticles of a desired size for toxicological tests at ORNL. Figure 2 shows the schematic of a TDMA for spraying engineering nanoparticles for biological exposure research. If one starts with nanoparticles of a broad size distribution, the problem is that the number concentration of “monodisperse” nanoparticles selected with TDMA can be small (~ a few hundred particles per cm³ or less) and is likely to produce insufficient dose for animal tests (i.e., *in vivo*). *In vitro* cellular exposure to be discussed later in this paper (see the Section Exposure) does not

have such a sensitivity limitation and can be employed in the TDMA experiment. It is likely that for precision engineered nanoparticles via a wet process, one can produce large quantity of material. Thus, it is not too difficult to disperse a large quantity (in number) of nanoparticles with minimal loss inside the delivery system that consists of two DMAs.

One can disperse engineered nanoparticles using an electrospray technique operated in a cone-jet mode in a similar experimental configuration to that of Lenggoro et al. (2002) shown in Figure 3. The colloidal solutions can be oxides (silica), metals (gold), and/or polymers (polystyrene

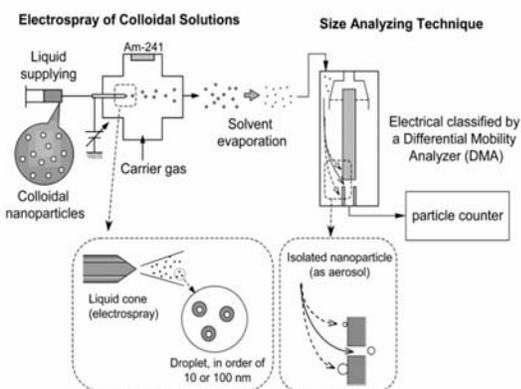


Figure 3. Electrospray of colloidal solutions and sizing of the nanoparticles using DMA. Lenggoro et al., *Langmuir*, 2002, 18, 4584-4591.

latex). Our experience indicates that the challenge in operating the setup is the precision control of solution conductivity using acids or bases, for example. A limitation of obtaining precision nanoparticles by this technique is the quality of the colloidal nanoparticles.

Electrospray can yield a highly monodispersed aerosol. This technique can be used to generate nanometer size particles from metal salt solutions of simple and complex mixture. Cheng (2004) demonstrated the use of a single-jet electrospray device to investigate cellular (human epithelial cells, ATCC A549 cell line) responses to their air exposure to uncoated nanoparticles produced from solutions containing transition metal salts. The mobility diameters of nanoparticles produced were about 11 nm for all the material tested; the nanoparticles were virtually monodisperse (the geometric standard deviation ≤ 1.2). The size distribution measured by a scanning mobility

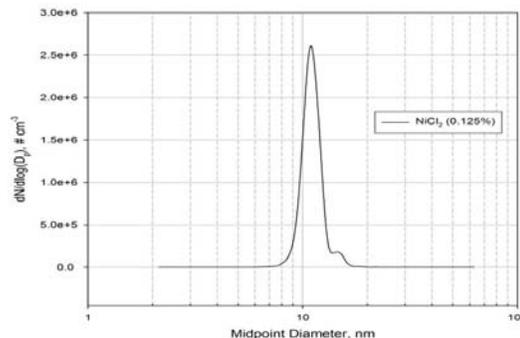


Figure 4. Particle size distribution of nanoparticles produced from 0.125% NiCl_2 solution by an electrospray device. The distribution was measured using a TSI SMPS system equipped with a nano-DMA column.

particle sizer equipped with a nano-DMA is shown in Figure 4. We were able to maintain the number concentration on the order of 10^6 cm^{-3} during any experiment achieving a virtually constant loading of nanoparticles for the inhalation exposure study. The effect of co-species on cellular elicitation of interleukin-8 was clearly shown in Cheng (2004).

Veranth et al. (2003) reported a vaporization-condensation technique in generating nanoparticles for pulmonary toxicology research. The size distribution of nanoparticles generated from an organic solution by this method was quite broad with the geometric standard deviation on the order of 1.4. No prior application of such a technique to engineered nanoparticles has been reported, but it is unlikely that the vaporization-condensation technique can be applied to the dispersion of engineered nanoparticles unless the technique is used in producing the nanoparticles.

Characterization

Knowing the agent that causes the damage to DNA or induces excessive expression of cytokines to a biological system, for instance, is the most conclusive way to derive a cause-effect relationship in biological exposure research, if it is possible. For nanoparticles generated as a by-product; e.g., engine exhaust, the connection has never been exact. The issue is the incomplete, and extremely difficult, characterization of a complex mixture of nanoparticles produced by diesel engine exhausts. Moreover, the diesel engine that generated waste nanoparticles by-

product is imprecise and not highly reproducible in producing nanoparticles. Without a complete characterization, one can only make a partially informed estimate of what might have led to the observed biological endpoints.

The study of exposure to engineered nanoparticles need not have to follow the same path as engine exhaust nanoparticle studies have been over past decades, because the nanoparticles are generated and/or synthesized by design for a specific purpose. The physical and chemical attributes of engineered nanoparticles are generally well characterized because nanoscientists need to know what has been prepared. Such wealth of information is good for biological exposure research; however, the properties characterized for materials research may or may not be sufficient for a biological exposure study, because the focus of individual investigators may be different from each others. Additional samples may have to be taken, and/or new measurement technology developed for characterization of environmental nanoparticles because of potential modification of the particle properties discussed earlier.

A characterization technique should be able to measure the properties of engineered nanoparticles and stability in physiological environments. To what extent which nanoparticles remain encapsulated and stable in the body needs to be determined and evaluated over the span of days and weeks in appropriate tissue growth media and serum. Environmental factors like ambient particles, temperature, humidity, solar radiation, and reactive gases species such as ozone, nitrogen oxide, reactive organic species, and other singlet oxygen species could modify engineered nanoparticles. Can a technique be reliably used for nanoparticle characterization without serious analytical interferences originated from the physiological matrix? Finally, when conducting a biological exposure experiment on engineered nanoparticles, it is preferable to monitor the dose given to a biological system throughout the experiment if the dose is unsteady (fluctuating over time). Will a characterization technique be capable of continuous monitoring?

Here we show the techniques currently known and useful for environmental and biological nanoparticle exposure research in Table 1. During the preparation of this manuscript, a paper on chemical characterization of environmental nanoparticles was published by Bureson et al. (2004). Interested readers are referred to this paper for more details.

We divide the techniques in Table 1 according to the physical and chemical attributes of engineered nanoparticles that they are able to characterize. Many of the analytical techniques listed in Table 1 are commonly used in material science, chemistry, physics, and geochemistry. Specialized configurations can be developed using a single or combination of the techniques listed in the table to improve the sensitivity or increase measurement versatility. For instance, STEM probe has been recently developed to single atom resolution at approximately 0.6 Ångstrom in the length scale (Pennycook, personal communication, 2004). The Electron Energy Loss Spectroscopy (EELS) is used with microscope to provide surface chemistry of single particles.

Important physical attributes of engineered nanoparticles to a biological exposure study are the length scale (i.e., the length of a dimension, surface area, and volume) and morphology (e.g., the nanostructures). For techniques that measure a sphere-equivalent size; e.g., an electrical mobility analyzer or a differential mobility analyzer, the length scale of a nanoparticle is collapsed to one single parameter – the mobility diameter. This piece of information, although is important, may not provide sufficient information regarding the surface morphology and chemical property of the nanoparticles. Electron and scanning probe microscopes can provide a detailed characterization of engineered nanoparticles (i.e., size and morphology), but only for a smaller number of nanoparticles. Combined use of transmission electron microscope, atomic force microscope, and electron energy loss spectroscopy enabled characterization of mixing characteristics of SiO₂ and TiO₂ nanopowder (Wei et al., 2002). Thus, it is imperative to use several techniques

to characterize nanoparticles in a biological response study.

Furthermore, there are instances that real-time monitoring of nanoparticles may be needed; e.g., work safety applications. Many of the techniques listed in Table 1 are for research purposes, not suitable for the monitoring applications. Those suitable are also discussed in the table.

Exposure

Exposure study provides quantitative data for constructing a dose-response curve and is a required step in risk assessment to evaluate the environmental and biological impacts of engineered nanoparticles. If adverse impact is anticipated, successful minimization of exposure (e.g., using engineering controls and/or administrative means) will result in effective reduction of health risk and meaningful protection of workers. However, engineering control of engineered nanoparticles for worker protection is an area that needs significant work of its own. Understanding of nanoparticle effects on human species and other biological species in the environment (e.g., animals, microbial, and plants) would require better knowledge of exposure mechanisms and improved exposure characterization techniques.

Engineered nanoparticles are produced by laser ablation typically in a closed chamber at low pressure ($\sim 10^{-3}$ to 10^{-5} atm), a dry gas or plasma phase process rather than a wet process as in the liquid. The chamber is also continuously vacuum-pumped. Exposure via inhalation and dermal-contacting routes do exist when the particle trapping or control system malfunction, workers open the chamber or the pump exhausts. There are currently used engineering processes for minimizing the exposure potential to workers such as (1) properly trapping and/or burning the pump exhaust; (2) purging the chamber several times before opening; (3) using a “snorkel” at the chamber flange to direct air flow away from the workers; and (4) wearing a respirator. It is possible that all these controls may not be necessary. However, the performance and effectiveness of these common measures remain

to be evaluated. New control technologies for engineered nanoparticles are also remained to be explored and tested.

Exposure of airborne particles (dusts, quartz, etc.) has been traditionally performed by instillation of aqueous suspension of the particles directly to target cells in a Petri dish (in vitro) or to animals (in vivo). The instillation approach may be appropriate if the nanoparticles were prepared by wet processes, since the approach is presumably not altering nanoparticle attributes. This is remained to verified, though. In the case of nanotube exposure, the tumor results obtained from the instillation studies can be difficult to comprehend (e.g., Lam et al., 2004). It is likely nanotubes do not suspend in the air easily, and special treatment is required to enable a successful instillation to rats causing unexpected physiological responses. Comparing traditional instillation and direct air-cell deposition approaches in an exposure study using DEPs, responses measured by immunological biomarkers are inconsistent (Seagrave, 2002). This could be attributed to the lost of dissolvable organic fractions in the aqueous solution.

For well-characterized nanoparticles engineered in a dry process, e.g., laser ablation, passing the nanoparticles through the liquid phase (so traditional instillation approach can be taken) can only introduce unnecessary artifacts to the physical and chemical properties of the nanoparticles. This artifact will become an even more pronounced when studying environmental modifications of engineered nanoparticles in the near future, because the surface of the modified nanoparticles may become oxygenated (via atmospheric chemical reaction, for example).

To minimize the possibility of unintended alteration of the properties of airborne nanoparticles for inhalation exposure research, a novel in-vitro exposure system has been developed by ORNL and was demonstrated by Cheng (2004) (see Figure 5). Nanoparticles prepared by various salt solutions of transition metals were tested, also tested were colloidal suspension consisting of polystyrene latex spheres and various metal salts. It is found that the technique provides a responsive and

repeatable means for investigating cellular responses to nanoparticle exposure. The ORNL system is being used currently to investigate the toxicological effects of engineered aluminum nanospheres on cells, and to other studies involving engine exhaust particles (Cheng et al., 2003). Recognizing the potential of undesirable modifications on particles when passing them through an additional liquid phase, similar developments of the air-cell exposure technique were made in other laboratories around the

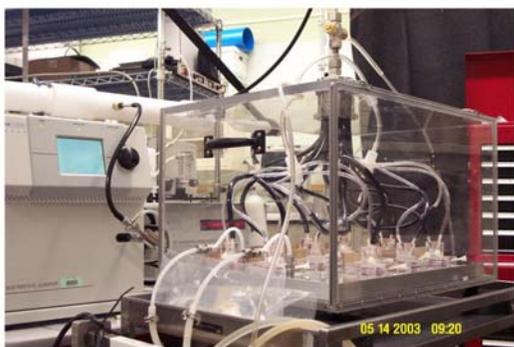


Figure 5. A direct air-cell exposure device developed by ORNL. Adherence cells are cultured on a Polycarbonate transwell membrane. An array of 12 chambers are employed for this prototype device.

world for toxicological study of diesel engine exhaust nanoparticles (Tippe et al., 2002; Morin et al., 1999), but no reports were found on using these techniques for studies involving engineered nanoparticles.

Conclusion

Investigating cellular exposure to engineered nanoparticles could enable researchers to advance their knowledge on the fundamental biochemical interactions between nanoparticles and a particular cell type or cell community. This is an important first step toward better quantitative characterization and understanding of engineered nanoparticle exposure.

Ultimately, a controlled physiological study using live species will be required to improve our understanding of the true impacts of nanoparticle exposure. Chambers for inhalation exposure research using small animals (i.e., mice, rats, and rodents) have been used for decades, and can be readily adapted in combination with the nanoparticle generation and characterization techniques described previously in this paper.

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Table 1. Material Characterization Techniques for Nanoparticle Exposure

	METHOD OF CHARACTERIZATION	DATA OF INTEREST
Physical Properties	<ol style="list-style-type: none"> 1. Differential and Electrical Mobility 2. Electron Microscopy (TEM, STEM, or SPM like AFM) 3. Light Scattering (Small Angle or Multi-Angle) 4. Photon Correlation Spectroscopy (PCS) 5. Small Angle Neutron Scattering (SANS) 6. BET (Brunauer Emmett Teller) Analysis 7. Aerosol Surface Area Measurement Technique 	<ol style="list-style-type: none"> 1. Single particle measurement of size, can be adapted to measure electrical charge on particles. Near real-time measurement in the gas phase. 1 nm-1μm. 2. Single particle measurement of size, shape, morphology. Automated computer control can facilitate the analysis of a large number of particles (on the order of one particle per second). 3. Single particle measure of size, phase function if multi-angle measurement is made. Near real-time measurement in the gas or liquid phase. 4. Ensemble size distribution of particles, typically done in the liquid phase. Colloidal analysis. 5. 1nm-1μm, special focusing lens, neutron source required. Off-line, non-real-time analysis. 6. Surface area determination. Sample size limitation. 7. Determine aerosol surface area based on charge diffusion to aerosol particles

	METHOD OF characterization	DATA OF INTEREST
Chemical Properties	<ol style="list-style-type: none"> 1. ESCA/XPS (Electron Spectroscopy for Chemical Analysis/X-Ray Photoelectron Spectroscopic System) 2. EDS (Energy Dispersive Spectroscopy), PIXE (Proton-Induced X-Ray Spectroscopy) 3. EELS (Electron Energy Loss Spectroscopy) 4. Raman Spectroscopy, and Surface-Enhanced Raman Spectroscopy 5. XRD (X-Ray Diffraction Spectroscopy) 6. NMR (Nuclear Magnetic Resonance) 7. TGA (Thermal Gravimetric Analysis) 8. LIBS (Laser-Induced Breakdown Microprobe) 9. ATOFMS (Aerosol Time-of-Flight Mass Spec.) 	<ol style="list-style-type: none"> 1. Elemental and chemical bonding info. 2. Elemental/associated with EM, can be activated by proton, electrons, or X-ray. The spatial resolution is $\sim 1 \mu\text{m}$, insufficient for nanoparticle characterization 3. Generally coupled with microscope. Molecular and nanostructure 4. Molecular information, single μm particles, nanostructures, DNA/RNA probes with SERS. 5. Use in powder diffraction, typically require 1% wt or larger sample, crystalline phase identification, structural id. 6. Biological surfaces, microemulsions, liquid crystals, membranes, gas- solid interfaces, and diffusion in heterogeneous systems 7. Defect and impurity analysis, measure weight loss during heating, sensitivity $\sim 0.1 \mu\text{g}$, can be coupled with size-exclusive chromatography 8. Microprobe analysis, elemental composition, bulk, ensemble, or single particle analysis. Elemental composition can be measured in near real-time or off-line using LIBS. Core composition of aerosols, not surface composition. 9. Near real-time analysis of single particles. Surface composition of molecules and metals.