FORUM SERIES
Research Strategies for Safety Evaluation of Nanomaterials, Part III: Nanoscale Technologies for Assessing Risk and Improving Public Health

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Risk assessment in the environmental health sciences focuses on understanding the nature of environmental exposures and the potential harm posed by those exposures which in turn is determined by the perturbation of biological pathways and the individual’s susceptibility to damage. While there are extensive research efforts ongoing in these areas, progress in each is currently slowed by technological limitations including comprehensive assessment of multiple exposures in real time and dynamic assessment of biological response with high temporal and quantitative resolution. This Forum article discusses recent technological innovations capitalizing on the emergent properties of nanoscale materials and their potential adaptation to improving individual exposure assessment, determination of biological response, and environmental remediation. The ultimate goal is to raise the environmental health science community’s awareness of these possibilities and encourage the development of improved strategies for assessing risk and improving public health.

Key Words: nanoscale materials; risk assessment; individual exposure assessment; biological response; public health.

A July 2005 report from the Environmental Working Group illustrates the need for improved approaches to assessing risk in humans (Houlihan et al., 2005). This study examined cord blood from 10 newborns along with 3 adult controls for the presence of 413 known or suspected toxicants at a cost of $10,000 per sample not to mention a significant investment in time and infrastructure. The results indicate that 287 of these compounds are found in cord blood and 329 in adult but raise several questions including the level of exposure to the remaining 75,000 industrial chemicals as well as other environmental exposures such as dietary factors and the biological response to those exposures. Given the high costs per analyte of existing exposure assays answers to those questions will require fundamental technological advancements. The fact that 212 of the compounds found in newborns were chemicals that were either banned or severely restricted in the U.S. reflects the need for improved remediation strategies as well.

Recent integration of computational and engineering approaches and in particular nanotechnology—the engineering of materials and devices on a scale of 1 to 100 nm—with biomedical research promises improved understanding of exposures and biological responses as they relate to human health and disease. With the reduction in scale to the molecular level numerous advantages such as decreased utilization of reagents, the ability to multiplex assays, improved and unanticipated physical and chemical functions, increased portability, and reduced requirements for energy utilization have arisen. Because of these advantages, nanotechnology is applicable at a broad range of biological and ecological scales, from the submicron to the kilometer, and the use of these technologies in the environmental health sciences both possible and attractive.

Environmental health scientists have long assumed a paradigm, outlined in Figure 1, by which environmental exposures lead to disease through a cascade of events. Environmentally induced disease originates with the presence of the toxicant at some source in the environment. While source contamination is of ecological concern, the environmental health sciences field focuses on source contaminations that lead to eventual individual exposures. Exposed individuals absorb the toxicant primarily through dermal, respiratory, or oral routes and the toxicant is subsequently distributed within the body where it may be metabolized, sequestered in particular organs, or excreted. By definition, the toxicant will affect one or more biological processes leading to altered function and phenotypic

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changes, ultimately resulting in the development of overt disease. While this appears to be a straight-forward cascade of events, it is clear that there is considerable interplay between exposures and responses such that "real world" exposures to mixtures result in widely varied biological effects from individual exposures studied in a laboratory setting. Moreover, it is evident that some individuals have either increased susceptibility or resistance to various toxicants through either polymorphisms or other predisposing factors such as diseases. It is also apparent that some compounds likely induce biological effects that are not pathologic adding additional complexity to risk assessment. We feel that nanotechnologies can aid in our understanding of individual exposure and biological response to improve risk assessment and ultimately guide and perhaps act directly as intervention strategies to improve public health.

Technologies for Environmental Sensing

Among the major challenges in the environmental health sciences is the development of approaches to comprehensively monitor exposures in real time. Currently, personal exposure assessment is most often performed through laboratory based body burden assessments directed at specific analytes such as the Center for Disease Controls biannual National Report on

FIG. 1. The figure illustrates the general paradigm for the evolution of disease as a result of environmental exposures. The process begins with the presence of a toxin in an environmental source and an individual’s exposure to that toxin. Following exposure, the body internalizes the toxin and it is distributed throughout the body where it alters biological signaling processes leading to defects in organ function and ultimately to the development of disease pathology. Nanotechnologies can further our understanding of these processes by providing information on the presence of toxins within the environment, an individual’s personal exposure to those toxins, and the presence of the toxin within the body. "Biological" sensors can provide information on the effects of the toxin on biological processes and feedback on whether those effects are leading to the emergence of a disease state. In such ways nanotechnology is a highly useful tool for assessing the risk from environmental exposures. At the same time, nanotechnologies can be used to transform a toxin to less toxic metabolites and potentially to intervene to halt or reverse the development of disease. Thus in addition to their use as a risk assessment tool, they provide a potentially valuable tool in directly improving public health.
Human Exposure to Environmental Chemicals (CDC, 2005). These assays suffer from many limitations including expense, high demands for both sample volume and infrastructure, and very limited temporal resolution. These limitations can, in some instances, be offset by the use of portable exposure monitors such as those illustrated in Figure 2. These are limited to monitoring one or a few potential toxicants at a time, and therefore provide only a snapshot of exposures. They are, however, among the most powerful tools for exposure assessment currently available on the commercial market. Nanotechnology provides a potential solution due to increases in specificity, decreases in reagent utilization, and improvements in throughput that occur with decreased particle size. It is currently possible to develop micro- and nano-scale arrays—primarily based on affinity reagents—that can detect specific sets of harmful agents in the environment. Fundamental to the development of nanoscale sensors for individual exposure assessment is the advancement of technologies for analyte detection.

In recent years, as nanotechnology has come to prominence, biology has served as the template for a wide range of devices. Among these are sensors inspired by decades of work on the conductance properties of ion channels. Stochastic sensors function on the principle that, in the simplest sense, a sensor modeled on an ion channel has two defined states—occupied and unoccupied (open and closed)—readily distinguished by a readout of the channel conductance and so are capable of translating an analog “random” signal (analyte presence) into a digital “on/off” signal. Initial work on stochastic ion channel based sensors has focused on the staphylococcal α-hemolysin (α-HL) which has many attractive features including its relatively large single channel conductance and a known three-dimensional structure consisting almost entirely of β-sheets. This β-sheet structure is compatible with a large degree of remodeling to introduce analyte binding sites or specificity filters such that there is a change in channel conductance if the analyte is present (Fig. 3). This ability to engineer analyte specificity offers significant advantage over alpha helical channels (Bayley and Cremer, 2001). To date, these stochastic sensors have been tailored to monitor divalent metals, anions, and a broad range of organic molecules.

Other groups have sought ways of improving electrical biosensors. One such approach has been the use of ion channel switches which are engineered to interact with highly specific antibodies or ligand receptors in the bilayer rather than binding analyte directly, facilitating their adaptation to a broad range of analytes (Cornell et al., 1997). It has been estimated that this approach increases the analyte access of a given channel by a factor of 10^3, increasing both sensitivity and speed of detection in dilute samples. These sensors have been applied to detecting a broad range of hormones, such as thyroid stimulating hormone, and pharmacological agents such as gramicidin, digoxin, and amiloride (Cornell et al., 1997; Yin et al., 2003).

Another electrical sensing approach uses microfluidic chips to detect particles moving through a small channel 7–9 μm long and 1 μm wide. When a colloidal particle moves through the channel, there is a change in the conductance across the pore; when the colloid has an affinity ligand bound, the increased volume further alters the conductance, yielding a quantifiable signal (Fig. 4) (Saleh and Sohn, 2003). To date this system has
been applied only to detecting biotin-streptavidin interactions, but in principle, it should work for any chemical with a well-characterized affinity probe.

A slightly different approach utilizes protein engineering approaches to create environmental sensors (Hellinga and Marvin, 1998). This strategy uses members of the bacterial periplasmic binding protein family (such as maltose binding protein) which have a central hinge region that collapses around a bound ligand in what has been described as a “venus fly trap.” Incorporating a novel protein engineering algorithm to create site-directed mutants, it has been possible to engineer specific binding sites for a series of targets ranging from L-Lactate and serotonin to toxins such as trinitrotoluene, soman, and the potentially toxic gasoline additive methyl tertiary butyl ether (MTBE) (Allert et al., 2004; Looger et al., 2003, 2001). Detection of analyte binding to the mutant binding proteins is accomplished through optical techniques. One commonly used output is fluorescence resonance energy transfer (FRET), which involves the transfer of energy from one fluorophore to a second located in close physical proximity—the fluorescence of the second probe being inversely proportional to the distance between the two probes. When the
two lobes, each with a fluorophore tag, move relative to each other upon analyte binding the degree of energy transfer is altered and can be easily observed. An alternative approach is to use a single fluorophore tag placed in a location which undergoes a change in microenvironment on analyte binding which in turn results in an alteration of probe intensity. This tactic, like that ion channel based sensors, is highly amenable to the creation of sensor arrays.

An inherently array-based approach uses cell based biosensors using optical imaging. The premise behind these...
biosensors is that bacterial strains can be engineered to respond to distinct toxin exposures with well-defined alterations in cellular processes associated with reporter constructs for optical readout. The major distinction of this approach relative to other sensor techniques is that the readout relates to the functional effects of the toxicant rather than its exact identity. Cell arrays have been created in which tens of thousands of individual cells are placed at the tips of an imaging fiber bundle to detect responses to toxic metals and genotoxins (Biran et al., 2003; Kuang et al., 2004). These studies have also been extended to the development of artificial “olfactory” systems based on cross-reactive patterns of arrays of vapor sensitive fluorescent microspheres (Albert and Walt, 2003; Walt, 2002). This approach allows for exponential growth in the complexity of the system and allows for sensing of undefined vapors by analogy to benchmark patterns.

Dendrimer molecules have also been successfully applied to exposure monitoring. Thin layers combined with mass-sensitive surface acoustic wave (SAW) devices have been applied to detect and accurately quantitate volatile chemicals in vapor streams, which is a critical technical and environmental challenge (Wells and Crooks, 1996). The ability of dendrimer films to recognize various classes of volatile compounds as a function of polymer chemical structure permits development of a pattern recognition database, and enables quick selection of the appropriate sensor for a given situation. Specific functionalization of dendrimers enables the selective detection of live bacteria (Chang et al., 2001) and has also been used in intracellular applications (Baker et al., 2001; Bielinska et al., 2002).

Clearly a significant amount of research effort is being devoted to the use of nano- and micro-scale technologies to create sensor technologies that may be amenable to ultimate use in exposure assessment. In order to make this extension to field deployable devices that can be used of individual risk assessment, a number of different research groups are developing approaches to create “lab-on-a-chip” sensor devices with the goal of developing largely automated platforms for the detection of a broad array of analytes. For the most part, these technologies are all in the early stages of development, finding innovative approaches to introduction of samples from the environment, fractionation and delivery to the sensor itself.

Sampling technologies for airborne exposure monitoring are fairly straightforward approaches to both passive and active sniffing of ambient air and are effective for detecting gases with potential adverse health effects or particulate matter and aerosols. Sampling for water-born exposures can also be straightforward where it can be introduced into plumbing and process streams. For surface exposures, however, the technological demands for sampling are considerably more significant. At the current time it is necessary for swabs to be taken manually, a process that inherently decreases the temporal resolution of the exposure assessment and limits the extension to individual exposure.

Many promising techniques exist for fluid handling in sensor platforms. One particularly promising approach is the use of electrowetting based nanodroplet control. In this approach, an electric current is applied in a localized but nonuniform manner to a droplet, causing the droplet to move towards, or away from, the electrodes. Using an array of electrodes, the droplets can be moved very rapidly with extremely fine control and droplets can be mixed in a precise manner (Paik et al., 2003a; Pollack et al., 2002). An alternative approach to control nanoliter sized droplets is through dielectropheretic manipulation, in which the droplet is suspended on an electrical field that provides a comparable degree of control over the droplet. This approach eliminates the potential difficulty of adherence of samples to the chamber in other nanofluidic approaches but sacrifices speed in the process (Velev et al., 2003). Regardless of the approach used to control the samples in an automated lab-on-a-chip, it remains imperative to detect the presence of analyte and approaches based on electrical readout, affinity, fluorescence, or cell function as discussed above remain the most promising approaches, alone or in combination.

An ultimate goal is to enable comprehensive exposure studies with real-time, remote assessment of exposure; achieving such a goal will require not only advances in sensor and sample handling technologies but also informatics and remote sensing infrastructure support. These comprehensive exposure studies should extend beyond environmental monitoring to encompass monitoring at an individual level, detecting exposures and tissue distributions of toxins and environmental agents.

Technologies for Detecting Biological Responses to Exposure

The central dogma of risk assessment is that risk is a function of both exposure and hazard. We have discussed above the potential of nano- and micro-scale technologies in improving understanding of exposure and will turn our attention to the potential for addressing the question of “hazard” or biological response to exposure. At the heart of the environmental health sciences is evidence-based assessment of environmental threats to human health and the reduction of environmental exposures to toxins and toxicants based on an understanding of the mechanism or mode of action. The field has long assumed that a given exposure leads to a cascade of responses (Fig. 1) from internalization, distribution to target organs, initial alterations of gene expression and protein function, alterations in metabolite levels and ultimately to the development of altered phenotype and overt disease. At each stage in this cascade, researchers actively seek markers for monitoring the process, as well as identifying potential markers which correlate to individual susceptibilities and the means to detect them. This paradigm is not novel; researchers have been using a variety of approaches to understand the pathways leading from exposure to disease for many years. However, the emergent properties of many nanoparticles make them attractive candidates for advancing these studies. A number of strategies are being used that capitalize on the use of nanofluidics, lab-on-chip and...
unique properties of nanoscale materials including unique electrical and magnetic properties. However, it is our view that the most promising possibilities are the enhanced optical properties that emerge in well ordered semiconducting nanocrystals, quantum dots, dendrimers, and dendrimer nanocomposites which can provide information with temporal spatial and quantitative resolution that cannot be matched using other technologies including other applications of nanotechnology.

The striking optical properties of quantum dots relate in large part to quantum confinement, which is the function of composition, size and shape. Nanocrystals, in many ways, behave as if they are an atom rather than a molecule, sharing electrons throughout the crystal. Because their structural size is smaller than their Bohr radius, the emission spectra is dependent on the size and shape (Buhro and Colvin, 2003) of the particle; therefore, the use of size selected quantum dots allows for the fine tuning of the emission properties and the use of multiple quantum dots—each having distinct, well-defined emission spectra. Coupled with the remarkable brightness of the probes, their narrow emission bands, long fluorescence lifetime, and resistance to photobleaching, quantum dots are becoming extremely useful tools for fluorescence spectroscopy. Moreover, in addition to their size tunable emission properties, the emission spectra is also directly controlled by the composition of the nanocrystal core [CdSe (tunable emission between 350-500 nm) vs. CdS (500-700 nm) vs. InP (650-900 nm), etc.], further increasing the multiplexability.

The early obstacle in using quantum dots related to their inorganic core, which in addition to being toxic, had low water solubility (Chan and Nie, 1998). It was also a challenge to functionally couple the particle for biological application. These problems have largely been addressed by the addition of an outer surface layer which minimally impacts on the fluorescent properties but dramatically increases both water solubility and the ability to conjugate the quantum dot to any number of affinity probes, proteins, or nucleic acids while conferring some degree of protection from cytotoxicity (Bruchez et al., 1998; Chan and Nie, 1998). Dendrimers have also successfully been used for stabilization of quantum dots (Balogh, 2002). More recently the problems of fluorescence quenching by the surface coating have been addressed and the ability to conjugate quantum dots has improved markedly leading to the development of in vivo imaging modalities (Akerman et al., 2002; Ballou et al., 2004; Dubertret et al., 2002). These innovations have made it possible to use quantum dots to increase the power of a number of biochemical, molecular biological and physiological experiments, including tracking the movements of proteins within the cell, characterizing the expression of cell determinant markers on the cell surface, and other studies illustrating the utility in understanding the biological response to exposures, potentially in whole organism studies.

Qdot approaches to date have largely focused on targeting the identification of macromolecules. Another family of fluorescent nanoparticles is aiding biomedical researchers in a different way: understanding intracellular signaling processes through monitoring small molecules. A process has been developed to encapsulate organic fluorophores in a polymer shell, enhancing their spectral properties while decreasing the toxicity of the dye towards the cell; a product called Probes Encapsulated by Biologically Localized Embedding (PEB- BLES). This group has used either fluorophores that are sensitive to intracellular ions (such as Mg$^{2+}$, Ca$^{2+}$, Zn$^{2+}$, K$^+$, H$^+$, Cl$^-$), metabolites, or other signaling molecules (glucose, NO, dissolved oxygen) allowing for real-time intracellular monitoring of multiple analytes with very high quantitative and spatial resolution in isolated cells providing another level of biological organization to studies of environmentally induced disease (Brasuel et al., 2003; Clark et al., 1999a,b; Koo et al., 2004; Park et al., 2003; Sumner et al., 2002; Xu et al., 2002).

The power of these optical techniques as well as others, such as metal oxide nanoparticles, comes from the very high degree of spatial resolution and the ability to image at a single particle or molecule level since individual particles fluoresce brightly enough to be observed in common confocal microscopy formats. In addition, these techniques are amenable to multiplex analyses for simultaneous real time quantification of multiple signals. A further level of power in assessing the biological response to exposures relates to the development of in vitro assays allowing massively parallel detection of biomarkers including both array based transcript and protein chips and commercial products for automated control of assay plates with thousands of wells and high-precision tools such as picoliter delivery systems. These platforms allow for the rapid generation of massive amounts of data on the biological effects of toxins in vitro or in isolated cell systems. The challenge becomes one of informatics tools for the interpretation of this information and in the ability to extend these studies to whole organism systems.

**Nanoscale Interventions for Improving Public Health**

Beyond their clear utility for improving risk assessment, nanoscale materials are also seeing application in direct interventions to improve public health both through therapeutic strategies and environmental remediation. Recent years have seen the emergence of nano-engineered drug delivery strategies. One prominent example is the development of Abraxane, a nano-formulation of the breast-cancer therapeutic Taxol which received FDA approval in January of 2005. This protein nano-bead conjugated pharmaceutical has greatly increased water solubility allowing for elimination of the toxicity associated with the solvent vehicle and improved therapeutic index. The benefit of Abraxane relies on the nano-scale formulation rather than on the emergent properties of the nanomaterials as a therapeutic modality. There is indication, however, that nanomaterials can also be used as direct therapeutic interventions. One such example is the
development of gold-coated mica nanoshells that are selectively delivered to the tumor and heat dramatically when the patient is exposed to infrared illumination effectively cooking the tumor (Hirsch et al., 2003). These nano-therapeutics and others have garnered significant media and public interest; however, it is preferable to prevent the development of disease rather than to treat it and the prevention of exposure is a promising means to prevent diseases.

Environmental remediation represents a primary prevention mechanism for improving public health; if toxins are removed from contaminated soil and groundwater they pose a significantly lower public health risk. Nanostructured materials provide not only high reaction rates for catalytic detoxification, but also potential immobilization and sequestration of toxic compounds. Among the primary determinants of effectiveness is the surface area available for the reaction. Surface area increases with the square of radius while volume increases with the cube; therefore, the smaller the particle the greater the ratio of surface area to volume. For nanoscale materials, a teaspoon volume has the approximate surface area of a football field. Other characteristics of the particle surface are also key determinants of the effectiveness of the remediation tools; these include curvature, strain, and reactivity. It is of central importance to study the reaction mechanisms of contaminant transformations, not only to understand how the nanomaterial remediation strategy works, but also to identify potential by-products or side-reactions so that potentially problematic releases can be avoided before deployment. These tools can be applied both for in the field remediation or, ideally, can be integrated into the process stream to prevent the introduction of toxins into the environment.

Among the highly promising chelation strategies are self-assembled mesoporous ceramics (SAMMS) with pore diameters on nanometer scales and surface areas greater than 1000 m²/g initially reported in 1992 by scientists from Mobil Oil research. A group at the Pacific Northwest National Labs has taken a leading role in developing SAMMS with functionalized surfaces that enable specific chelation of numerous environmental toxins such as metals and anions for use in contaminated site cleanup or in drinking water purification (Kemner et al., 1999; Lin et al., 2001). Other nanoparticles such as dendrimers, nanotubes, or polymer beads have also been successfully used for toxin chelation (Masciangioli and Zhang, 2003). One issue fundamental to all chelation strategies becomes the long-term storage of the sequestered toxin, for which there is no readily obvious solution.

Conversely, catalytic nanoparticles such as nanorefined iron or bimetallic iron-nickel or iron-palladium have proven quite useful for conversion of organic toxins. For example, Fe/Ni particles have been used in dechlorinating trichloroethylene (TCE) (Meyer et al., 2004) while iron nanoparticles have proven useful in a wide range of applications from catalysis of organics to oxidation/reduction of toxic metals into non-toxic forms (Masciangioli and Zhang, 2003). Each of these tools has demonstrated its potential effectiveness as a remediation tool; however, significant effort is needed to ensure the rational design and synthesis of functional materials by design—the planned-out integration of chemical function and physical structure to optimize performance of specific remediation tasks.

Closing Thoughts

There is a need for improvements in environmental risk assessment and nano- and micro-technologies offer exciting possibilities as outlined above. Sensor technologies capitalizing on the high specificity, selectivity, and multiplexability provided by nanoscale sensors will expand the breadth of coverage of exposure assessments while tools based on the unique properties, particularly the optical properties, of nanomaterials will significantly expand our ability to identify the biological responses to human exposures. Moving beyond their likely impacts on risk assessment, nanomaterials have begun to demonstrate their utility in direct interventions to improve public health, both through therapeutic interventions and through environmental remediation to eliminate exposures. Caution must, however, be exercised as on one hand we can exploit the novel properties while on the other there will be unanticipated biological interactions that may have significant deleterious effects on human and ecological health. We will need to carefully examine the environmental fate and transport of nanoeengineered materials in an attempt to determine human exposures and associated risks. We will need to develop new metrics for the assessment of pharmakotoxico kinetic and dynamic properties of the developed particles since these nanomaterials are unlikely to behave in the same manner as small molecules and pharmaceuticals.

It is our hope that by presenting some of the potential applications of these technologies this commentary will serve as a useful resource for the environmental health and toxicology communities and will stimulate efforts by the environmental health sciences community to adapt these technologies to the improvement of human health from environmental threats. Through the development of improved environmental and biological sensors and environmental remediation devices it is hoped that the promise of nanotechnology will become a reality of improved public health.

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