NanoHealth Enterprise Initiative

Engineered Nanomaterials Research and Training

Executive Summary

The properties that emerge at the nanoscale—size, surface-area-to-mass ratio, shape, crystal structure, surface chemistry, and surface defects—elicit electrical, optical, magnetic, and biological properties that enable novel applications in medical, industrial, and consumer products. The same unique chemical and physical properties that make engineered nanomaterials (ENM) so potentially useful also make their interactions with biological systems difficult to anticipate and critically important to explore.

The NIH proposes a broad-based program that would employ state-of-the-art technologies in research to examine the fundamental physicochemical interactions of ENM with biological systems at the molecular, cellular, and organ level. To support and promote this investigative work, an informatics framework to identify cross-cutting design principles, as well as training for the next generation of nanotechnology scientists are proposed.

Framework of the NanoHealth Enterprise

The NanoHealth Enterprise would comprise an integrated, interdisciplinary program that draws upon the expertise and interests of the NIH institutes and centers, in partnership with private industry, to address critical research needs for the safe development of nanoscale materials and devices.

- **Materials Science Research**—characterization of the physical and chemical properties of ENM in relevant biological systems;
- **Basic Biology Research**—determination of the relationship of nanoscale size and physicochemical properties to biological response at the cellular, molecular, and systemic levels;
- **Pathobiology Research**—investigation of the relationship of nanoscale size and physicochemical properties to ENM-induced pathophysiologic endpoints and the development of disease;
- **Informatics**—a data-sharing framework to store and structure information on the potential environmental, health and safety risks of ENM's with data-mining, query, and search capabilities; and
- **Training**—education of scientists to work on cross-disciplinary and interdisciplinary nanotechnology research teams, and to develop research programs that integrate materials science, biology, and pathobiology research.

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NanoHealth Enterprise Initiative

Engineered nanomaterials (ENM), with their precise design and engineering attributes and novel physicochemical properties, represent a significant breakthrough in material design and development for medicine, industry, and consumer products. Global demand for ENM and nano-enabled devices is expected to exceed $1 trillion by 2015. This increased production provides increased opportunities for unintentional exposures and unanticipated consequences of intentional use. Despite the proliferation of these materials, little is known about their interaction with biological systems, a condition that has generated significant concern. The same unique chemical and physical properties that make nanomaterials so potentially useful also make their interactions with biological systems difficult to anticipate and critically important to explore.

The NIEHS in partnership with the Trans-NIH Task Force, invites participation in a broad-based initiative that integrates research in material sciences, basic biology, and pathobiology with training. This initiative will employ state-of-the-art technologies to enable research to examine the fundamental physicochemical interactions of ENM with biological systems at the molecular, cellular, and organ level, as well as associated pathophysiologic processes. This research is critical for design of ENM with maximum human and environmental biocompatibility and safety. It will provide the biomedical research community with new knowledge of molecular, cellular, and organ system biology and identify clinically relevant properties of ENM. Moreover, this highly integrated research and training program would support U.S. goals for commercialization and innovation in nanotechnology through the safe development of ENM.

Background on ENM

The dimensions and quantum properties that emerge at the nanoscale enable novel applications. In addition to size, the surface area—to–mass ratio, shape, crystal structure, surface chemistry, and surface defects of ENM elicit electrical, optical, and magnetic properties, as well as biological behavior not observed in corresponding materials at the macroscale.

The nanoscale size of ENM enhances systemic transport, for example transport across the blood-brain barrier, across endothelial membranes into the vasculature, and through nasal sensory neurons to the olfactory bulb and the brain. And evidence continues to document nanoscale particulate translocation through intact stratum corneum, although these data are somewhat controversial. The large surface area–to–mass ratio of single ENM particles supports increased chemical reactivity per unit of material. However, the hydrophobicity of many ENM causes single particles to form aggregates and agglomerates, which may have a size-dependent decrease in propensity for systemic transport, altered chemical reactivity due to decreased surface area–to–mass ratio, and may or may not retain their quantum properties. Aggregates also may form or dissolve *in vivo*, thus complicating analysis of biological response and highlighting the effect of the microenvironment on ENM behavior. The nanoscale size and surface area–to–mass ratio of ENM challenge traditional concepts of dose and biological response. In a traditional dose–response relationship, the dose of a material is measured in units of mass and that mass measurement is related to the magnitude of the biological response. Several studies have explored the relationship of mass measurement of nanomaterials and the surface area of that mass to the biological response. The results suggest that, for certain ENM, surface area is a more accurate correlate of biological response than mass measurement, whereas other data suggest that neither surface area nor mass, but surface reactivity, is the critical parameter. The ENM characteristics and exposure conditions for which surface area or surface reactivity is the more accurate measure of dose have not yet been defined.

The specific aspects of design and synthesis of ENM that create unique physicochemical properties also will influence biological activity. Several studies suggested that ENM with highly derivatized surfaces permit cell growth and differentiation, while other materials perturb homeostasis and activate protective response pathways. For example, certain nanostructures, or fullerenes (unsubstituted C60), but not others (hydroxylated C60(OH)24), cause in vitro cell death. Also, the neuroprotective activity of one type of fullerene (tris-malonyl-C60) is attributed to its ability to scavenge free radicals and inhibit all three isoforms of nitric oxide synthase, albeit with different potencies. Other studies have shown that ENM cause oxidative stress, inflammation, mitochondrial perturbations, and membrane disruption. Also, carbon nanomaterials cause double strand DNA breaks in cells-free systems and, thus, introduce the possibility of point mutations, mitotic recombination, and chromosomal loss and translocation *in vivo*. Examples of these responses exist for all major classes of nanomaterials: fullerenes, metal and metal oxide nanoparticles, carbon-based nanoparticles and nanotubes, and macromolecules such as dendrimers.

Pathobiological changes have been observed in ENM–exposed rodent models, including development and exacerbation of vascular, pulmonary, and neurological disease. Several laboratories have demonstrated that carbon nanotubes accelerate atherosclerotic disease in hyperlipidemic ApoE knockout mice, and that carbon nanoparticles are thrombogenic. ENM show potential for use as neuroprotective agents in neuronal tissue engineering and as drug delivery systems that cross the blood–brain barrier, however, preliminary studies have shown that fullerenes induce potentially harmful lipid peroxidation in the brains of large mouth minnows. In murine models, ENM exposure has induced pathobiology

consistent with atherosclerosis and cardiac inflammation, fibrosis, granulomatosis, and emphysema.

Scope of the Initiative

This initiative outlines an integrated, interdisciplinary program that draws on the expertise and interests of the NIH institutes and centers, and addresses critical research needs for the safe development of ENM and nanoscale devices. The initiative has the following components:

- Materials Science Research — characterization of the physical and chemical properties of ENM in relevant biological systems;
- Basic Biology Research—determination of the relationship of nanoscale size and physicochemical properties to biological response at the cellular, molecular and systemic levels;
- Pathobiology Research—investigation of the relationship of nanoscale size and physicochemical properties to ENM-induced pathophysiologic endpoints and the development of disease;
- Informatics – a data-sharing framework to store and structure information on the potential environmental, health and safety risks of ENMs with data-mining, query, and search capabilities; and
- Training Program—education of scientists to work on cross-disciplinary and interdisciplinary nanotechnology research teams, and to develop research programs that integrate materials science, biology, and pathobiology research.

A partnership of NIH institutes, federal agencies, and private organizations is needed to fully embrace this initiative to pursue the very best science, avoid duplication of effort, leverage investment, and minimize the time from research to application. The structure of this initiative will be flexible in order to accommodate new projects, new partners, and research needs that emerge during the course of the research and training activities. The NIH recognizes the importance of nanomaterials safety research to the public, and will seek to incorporate a stakeholder advisory group into the research framework.

Materials Science Research

The rapid pace of nanotechnology development has created the need for targeted research to address questions that benefit the entire field and improve the quality of data and data reporting.

Dose Metrics. The large surface area–to–mass ratio of ENM, as well as highly reactive surface chemistry, suggest that traditional mass measurements may be insufficient or inappropriate to understand the relationship of dose to biological response. While mass remains an important measurement, evidence has been published for and against the inclusion of surface area, surface reactivity, particle number, and particle size distribution. More recent studies suggest that the surface electron energy status and the number of surface defects per particle are more accurate predictors of the magnitude of biological response. Still others suggest that crystal structure and shape will be critical components of a dose metric. The number of possible parameters and the lack of substantive research make delineation of the dose metric a complex question that can be addressed through targeted studies with clearly defined goals.

Routes of Exposure and Magnitude of Uptake. The behavior of nanomaterials in air and water differs from that of macroscale materials. For example, nanoparticles in air behave as a gas and may remain suspended indefinitely. In contrast, several laboratories have reported that nanoscale particles, unlike macroscale particles, penetrate intact stratum corneum and reach the epidermis and dermis, locations in which ENM may induce a cellular response or enter the blood stream or lymph. These differences in particle behavior at the nanoscale level will directly impact the internalized dose that is available to the body. Additional confounding factors, such as the effects of shape and electrical charge on uptake, have not yet been evaluated. Systematic analysis of size, shape, and electrical charge on ENM uptake for each of the exposure routes would inform these questions.

Minimal Information Reporting Standards. The complexity of dose metrics, coupled with the complexity and non-uniformity of design and execution of nanotechnology experiments, demonstrate the need to define more clearly how biological data on ENM are obtained. Minimal information reporting standards have been developed for microarray data, and are under development for proteomic data. Minimal information reporting standards ensure that data can be easily interpreted, compared, and verified, and should facilitate the entry of such data into multi-user databases and enable better data analysis. While not simple to achieve, minimal information standards should improve reporting in new fields with large amounts of rapidly accumulating data, and provide a basis for comparison of data sets and resolution of data conflicts.

Basic Biology Research

Although it is known that the human body is capable of a limited set of responses to exogenous and endogenous forms of stress, and that these responses can be evaluated through well-established biological assays, unique ENM properties suggest the possibility of novel biochemical, molecular, and cellular behavior in response to exposure. The Basic Biology Research component of this initiative will pursue critical questions that identify the molecular and cellular processes through which essential homeostatic mechanisms such as oxidative stress, inflammation, apoptosis, mitochondrial damage, and DNA repair are altered, and seek to link the novel size and size-dependent physicochemical properties of ENM with molecular and cellular responses.

Molecular and Cellular Studies. Cellular homeostasis is maintained by tightly controlled and integrated molecular networks. ENM–induced cell phenotypes provide indirect evidence for ENM interaction with fundamental cellular processes such as cell cycling, vesicular trafficking, transcription, respiration, and energy metabolism, and with cell-specific processes such as apoptosis, inflammation, and immunity. ENM could impair a functional pathway or,

through biocompatible design and engineering, facilitate repair of a dysfunctional pathway. Interaction of ENM with proteins, lipids, and nucleic acids in these critical pathways has not been investigated and represents a significant, unmet research need.

Membrane Dynamics. The barrier function of cell membranes is crucial for maintenance of cell structure and metabolism. The plasma membrane defines the intracellular space, and intracellular membranes create enclosed, functionally specialized compartments. These hydrophobic, lipid bilayers block transit of polar entities, however, cells have evolved multiple transport mechanisms to move ions and molecules into and out of cells and compartments, as well as transmembrane signaling pathways to communicate information across membranes. Disruption of membranes and membrane-supported events has profound consequences for cells and organ systems.

Many ENM are hydrophobic and able to partition easily into cell membranes, potentially disrupting the organization of the lipid bilayer, and its structural relationship to the membrane proteins, and consequently, the function of the membrane proteins. ENM may enter cells passively through endocytic transport or, if they fall within transport criteria, traverse channels and pores. Several laboratories report receptor-mediated uptake of ENM, carbon nanotube intercalation into plasma membranes, and cell-specific differences in ENM toxicity based on membrane behavior. For example, fullerenes cause fibroblast toxicity through lipid peroxidation of the plasma membrane and macrophage toxicity through lipid peroxidation of intracellular membranes and the plasma membrane. Differences in membrane composition and dynamics translate into differences in cell and organ system ENM uptake, intracellular sequestration, and systemic transport. While these pathways can be exploited for improved ENM drug delivery, they may
also adversely affect cellular structure and function.

Periodicity of the Biological Response to ENM. ENM present an opportunity to characterize a precisely engineered series of structurally and chemically related materials. Materials cientists report nanoperiodic relationships for dendrimers and quantum dots and hypothesize that nanoperiodicity is a feature of ENM that can be exploited for applications in industry and medicine. Size, shape, and chemical reactivity are considered critical components of nanoperiodicity, as are features that influence periodic patterns in physical properties, chemical stoichiometry, and steric effects, among others. This aim will investigate the hypothesis that physicochemical periodicity of ENM will result in biological periodicity, a concept that may be thought of as a stepped progression in a biological parameter as a function of a stepped change in a physical parameter. Early evidence for biological periodicity is suggested by the ENM dose–oxidative stress response, a correlative relationship that implies scalable and potentially controllable relationships between ENM and a biological activity.

Pathobiology Research

Research has documented that ENM can alter basic homeostatic pathways, such as inflammation, and oxidative stress pathways that may affect the development and exacerbation of disease. Vascular and pulmonary pathophysiology is linked, in part, to the efficiency of particle clearance, a process that in ENM is influenced by particle size and composition. Several laboratories have reported that single metal nanoparticles and small agglomerates evade macrophage engulfment, whereas quantum dots were observed in endocytic vesicles weeks to months after exposure. In rodent studies, inhaled nanoscale metal oxides caused significant increases in air-blood barrier permeability, macrophage accumulation, disruption of alveolar septa, and type II pneumocyte hyperplasia up to one year after a single aerosol exposure. ENM exposure has induced pathobiology consistent with atherosclerosis and cardiac inflammation. Additional pathways activated by ENM and associated with disease include cell cycle, apoptosis, immunity, and complement cascades. These data are derived from many classes of ENM, but no systematic study has yet identified the physicochemical properties of ENM that link these pathways to disease. Studies supported under this component of the initiative will extend the understanding of ENM disruption of homeostasis to ENM-induced pathophysiology.

Informatics

In order for the large amounts of data that will be collected in conjunction with this initiative to foster sound risk assessment and risk management decision making, the data must be standardized, stored, structured, and shared in an organized manner. To this end, a data-sharing framework for ENM's will be created. This framework will aggregate and store the data obtained from characterizing the physical, chemical, structural, mechanical, and biological properties of nanomaterials.

The informatics infrastructure will provide a structured repository for the data collected by the three research communities. It will also serve as a forum for communication between materials science, basic biology, and pathobiology researchers and facilitate modeling and evaluation of materials in new environments. Such a resource is expected to accelerate the transition from developmental research to health-related translational research; provide a forum for biologists and toxicologists to provide feedback to the developers of nanomaterials; and promote data standards, metrology standards, and validation standards for the development of nanoscale materials. The informatics component will thereby enable improved data integration; facilitate and incentivize data sharing; allow for efficient search and querying of data on engineered nanomaterials; provide the foundation for computerized decision support and modeling systems; and enable data-mining to discover new insights into structure-function relationships.

Training Program

In order to design and implement the kinds of cross-disciplinary and interdisciplinary research that will be necessary to fully understand how the novel attributes of ENM contribute to their biologic effects, a cadre of new investigators who are able to work in interdisciplinary ENM

research teams must be developed. These individuals will need to have training in several disciplines including exposure assessment, molecular and cellular biology, physical chemistry, materials science, engineering, and disease-based research, that, to date, have had little interaction. To develop meaningful collaborations, these researchers will need to have strong expertise in their chosen fields as well as knowledge in these other fields. Training opportunities will need to be developed at the predoctoral level where young investigators can be cross-trained in several disciplines, as well as at the postdoctoral level where such investigators can complement their previous training in one discipline with training in another. In addition, career development programs to allow new and more established faculty to expand their skills and, hence, their capacity to work in interdisciplinary teams would be appropriate. Many of these training opportunities could be developed within the context of established NIH training grants and fellowship programs. However, given the breadth of expertise envisioned, joint programs with other federal agencies or with private organizations or professional societies might be necessary.

Summary

Findings obtained through this initiative will establish standards for material sciences, determine biological thresholds, and address disease-based concerns for the emerging field of nanotechnology. This will be accomplished through scientific discovery followed by publication of standard setting reports in high quality, peer-reviewed journals, thereby creating a level of discourse that will facilitate debate of the controversial issues in this field. Data will be integrated into a data-sharing framework that will enable improved collaboration amongst materials scientists and biologists, promote rapid and targeted ENM development, and facilitate risk assessment. Fundamental research will inform the development of industrial, medical, and consumer products, and provide critical data for regulatory agencies.