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Recommendations of the National Heart, Lung, and Blood Institute Nanotechnology Working Group

Denis B. Buxton, PhD; Stephen C. Lee, PhD; Samuel A. Wickline, MD; Mauro Ferrari, PhD;
for the Working Group Members

Abstract—Recent rapid advances in nanotechnology and nanoscience offer a wealth of new opportunities for diagnosis and therapy of cardiovascular, pulmonary, and hematologic diseases and sleep disorders. To review the challenges and opportunities offered by these nascent fields, the National Heart, Lung, and Blood Institute convened a Working Group on Nanotechnology. Working Group participants discussed the various aspects of nanotechnology and its applications to heart, lung, blood, and sleep (HLBS) diseases. This report summarizes their discussions according to scientific opportunities, perceived needs and barriers, specific disease examples, and recommendations on facilitating research in the field. An overarching recommendation of the Working Group was to focus on translational applications of nanotechnology to solve clinical problems. The Working Group recommended the creation of multidisciplinary research centers capable of developing applications of nanotechnology and nanoscience to HLBS research and medicine. Centers would also disseminate technology, materials, and resources and train new investigators. Individual investigators outside these centers should be encouraged to conduct research on the application of nanotechnology to biological and clinical problems. Pilot programs and developmental research are needed to attract new investigators and to stimulate creative, high-impact research. Finally, encouragement of small businesses to develop nanotechnology-based approaches to clinical problems was considered important. (*Circulation*. 2003;108:2737-2742.)

Key Words: nanotechnology ■ cardiovascular diseases ■ lung ■ blood diseases ■ sleep

The burgeoning new field of nanotechnology, opened up by rapid advances in science and technology, creates myriad new opportunities for advancing medical science and disease treatment. In the near future, nanotechnology will play an increasingly significant role in the everyday practice of cardiologists, pulmonologists, and hematologists. Nanotechnology and nanoscience focus on materials at the atomic, molecular, and supramolecular level, aiming to control and manipulate these new materials by precisely configuring atoms and molecules, producing novel molecular assemblies and designing systems of self-assembly to create supramolecular devices on the scale of an individual cell and smaller.

In his prescient address to the American Physical Society in 1959, Richard Feynman foresaw that “at the atomic level, we have new kinds of forces and new kinds of possibilities, new kinds of effects. The problems of manufacture and reproduction of materials will be quite different.” Because the behavior of materials, structures, and devices at the nanoscale (in the range of 1 to 100 nm) differs from the macroscopic world, nanostructures display unique mechanical, electrical, chemical, and optical properties. Understanding and controlling such properties is challenging, but harnessing them will provide exciting new opportunities for research, diagnosis,

and therapy of heart, lung, blood, and sleep (HLBS) disorders. The nanoscale is prevalent in natural systems, because many important functional components of living cells fit within this size classification, but few nanoscale drugs or diagnostic, therapeutic, or repair devices have been developed. Nanoscale properties allow high densities of function in small packages to minimize invasiveness and facilitate “smarter” therapeutic interventions with increased specificity of delivery and action, decreased side effects, and the capability to respond to external stimuli and report to external receivers.

The National Heart, Lung, and Blood Institute (NHLBI) wishes to foster the application of nanotechnology to HLBS research and disorders. A Request for Information (RFI) was developed, with advice from scientists and physicians with interests in nanotechnology, to canvas the broader scientific community on approaches to developing and applying nanotechnology to HLBS disorders. A Working Group consisting of scientists, engineers, and physicians with expertise across nanotechnology, nanoscience, and HLBS medicine met on February 28, 2003, using the RFI responses as the starting point for their discussions.

The Working Group was charged with assessing the field of nanotechnology and suggesting appropriate directions for

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research. Nanotechnology is broadly construed as research and technology development at the atomic and molecular levels intended to create, understand, and use nanoscale (ie, typically <100 nm) structures, devices, and systems having novel properties and functions associated with their size and structure. However, the Working Group cautioned against overly rigid or restrictive definition of nanotechnology, emphasizing the continuum of scale from the nanoscale to the microscale. Nanocomponents will also be important in microscale devices, which are therefore included under the nanotechnology rubric. The "top down" approach, encompassing miniaturization of current technology, and the "bottom up" approach, taking advantage of self-assembly or directed assembly of molecules into nanostructures, offer complementary routes for addressing biomedical problems. It was also believed that nanoscience, in addition to nanotechnology, will make important contributions to HLBS research and medicine.

The group identified areas of opportunity and challenges to further development associated with the application of nanoscience and nanotechnology to improved diagnosis, treatment, and prevention of HLBS disorders. It also developed prioritized recommendations to expedite the application of nanotechnology to biological questions and improved patient care.

Opportunities

Nanotechnology is relatively new, but although the full scope of contributions these technological advances will make to HLBS medicine is unexplored, recent advances suggest nanotechnology will have a profound impact on disease prevention, diagnosis, and treatment. The Working Group identified 4 bionanotechnology areas (listed alphabetically) as the most promising areas of discovery and application.

Biosensors and Diagnostics

Currently, diagnostics are largely confined to *in vitro* use, with diagnostic tests performed individually in centralized clinical laboratories. Nanotechnology has enormous potential both for multiplexing *in vitro* diagnostic tests and for allowing miniaturization of sensors for use *in vivo*. The former applications are likely to predominate in the short term, but *in vivo* devices that report health problems in real time could be powerful tools for disease management.

Nanotubes or nanowires, which are starting to find use as components of very small computer circuits, show promise for diagnostic testing. They can be used to measure pH or decorated with specific capture molecules to detect minute quantities of biological and chemical species.¹ Nanocantilevers can measure the content of specific DNA moieties² or can be used for simultaneous rapid monitoring of multiple serum protein markers.³ Bioengineered nanopores allow sequence-specific detection of individual DNA strands with single-base resolution,⁴ and similar pores may be useful as components of quantitative sensors for cell signaling molecules.

Quantum dots are highly fluorescent semiconductor nanocrystals that are excited by a broad range of wavelengths but have narrow, tunable emission spectra. Linked to antibodies

or DNA probes, they can detect specific protein or DNA targets. Their narrow emission spectra allow numerous probes to be used simultaneously for multiplexed high-throughput screening.⁵

In vivo nanosensors show promise for real-time monitoring of biological signals, such as the release of proteins or antibodies in response to cardiac or inflammatory events. Eventually, nanosensors may even be used in conjunction with signaling and therapeutic delivery devices for comprehensive *in vivo* screening and treatment. For example, inhalable nanoparticles could sense local ventilatory status, releasing drugs at appropriate local dosage in response to physiological and pathophysiological stimuli.

Drug Delivery and Therapeutics

The current generation of drugs is largely based on small molecules with a mass of 1000 Da or less that circulate systemically. Common deleterious consequences of systemic biodistribution include toxicity to nontarget tissues, difficulty in maintaining drug concentrations within therapeutic windows, and metabolism and excretion of drugs, all of which can reduce efficacy. Drug solubility and cell permeability issues are also common with small molecules and biologics. Nanotechnology-based delivery systems could mitigate these problems by combining tissue- or organ-specific targeting with therapeutic action. Multifunctional nanodelivery systems could also combine targeting, diagnostic, and therapeutic actions.

Research has already shown that drugs can be encapsulated in nanospheres⁶ or erodible self-assembled structures⁷ or conjugated to well-defined multivalent macromolecules such as dendrimers (highly branched polymers).⁸ These mechanisms can improve bioavailability and enable continued release, thereby controlling the initial dose, improving effectiveness, and widening the therapeutic window. Potential targets include proliferating smooth muscle cells, neoplastic cells, inflammatory mediators, proteins expressed in viral infections, or even distinct subcellular localizations such as mitochondria⁹ and other cytoplasmic organelles.¹⁰ Specific targeting of drugs should mitigate systemic toxicity, and encapsulating or conjugating drugs to nanoscale carriers can protect them from systemic metabolism or excretion. In addition, nanoparticulate or macromolecular targeting systems can be used to give triggered release in response to internal triggers such as pH¹¹ or to externally administered signals such as ultrasound,¹² near-infrared light,¹³ magnetic fields, or radiofrequency pulses.¹⁴ For example, magnetic microparticles or nanoparticles that bind to specific cells could be heated with an alternating magnetic field to kill neighboring cells thermally.¹⁵

Imaging

Established imaging modalities such as CT, MRI, and ultrasound focus primarily on anatomy and physiology. The emerging field of molecular imaging uses novel reagents and methods to image specific molecular pathways noninvasively *in vivo*, particularly pathways involved in disease processes. As these technologies mature, noninvasive diagnostics with high-affinity homing molecules should become faster,

cheaper, and more accurate, allowing physicians to detect early disease and improve patient outcomes.

Self-assembling nanoparticles attached to peptide ligands or antibodies show great promise for detecting targets such as fibrin,¹⁶ smooth muscle cells,¹⁷ or apoptotic cells.¹⁸ Further developments may include multifunctional targeting, in which specificity is enhanced by the use of unique constellations of markers.¹⁹ Polyvalent or multivalent probes may also improve signal-to-background ratio by increasing the efficiency and specificity of target binding.

Other targeted probes include prodrug-like activatable “smart” probes, administered in an inactive, quenched form but activated chemically in the target environment to an unquenched form, for example, by metalloproteinase cleavage. This approach has been used with caged fluorescent probes and near-infrared imaging²⁰ and with paramagnetic and superparamagnetic MRI probes.^{21,22}

Quantum dots (see “Biosensors and Diagnostics”) also possess potential for cellular imaging.²³ Their bright fluorescence, which permits detection of a small number of dots per daughter cell even after cell division, makes them particularly promising for cell tracking, eg, tracking lymphocyte movements through the blood and lymphatic systems.

Targeted multivalent dendrimers and macromolecular conjugates can carry chromophoric “antennae” capable of performing both imaging and therapeutic delivery functions,²⁴ using, for example, multiphoton excitation with infrared light capable of deep penetration in human tissue. Dendrimer-based contrast agents have already shown promise for imaging the lymphatics²⁵ and kidneys²⁶ by MRI.

Tissue Engineering and Biomaterials

Progress with synthetic materials for repair of blood vessels, heart valves, and lung structures has been disappointing, leading to a growing focus on the alternative approach of tissue engineering. Microstructured and nanostructured materials may play a critical role in tissue engineering, but this area is still in its infancy. Tissue-engineered implants could result in lower rates of implant rejection and better regulation of adhesive properties, improving adherence of cells and decreasing biofouling of implanted devices. Nanoscale synthetic biomaterials show great promise as scaffolding for regeneration of damaged cellular membranes, tissues, and bones. Studies using ultrafine fibers, morphologically similar to the extracellular matrix of natural tissue, have shown that cells attaching to the fibers maintain their shape, and growth is directed by the fiber structure.²⁷ Self-assembling biomaterials that provide sustained release of cholesterol can promote fibroblast spreading,²⁸ which suggests a general approach for tissue engineering by local release of growth factors and other biomolecules. Patterned substrates prepared by microcontact printing can direct *in vitro* generation of differentiated cardiac myofibers²⁹ or, in conjunction with self-assembling monolayers, create micropatterned endothelial cells.³⁰ Three-dimensional microfluidic systems have also been used to generate micropatterns of cells on planar substrates.³¹

An additional approach is the use of microtechnologies and nanotechnologies to direct tissue remodeling *in vivo*. Microspheres that provide sustained perivascular release of elastase

can create a chemotactant gradient across the arterial wall to direct smooth muscle cell migration away from the lumen, reducing pathological neointima formation in a balloon injury model.³² Directed migration could also attract cells to injury sites for repair purposes.

Needs and Barriers

The Working Group identified a number of needs that must be met and barriers that must be overcome for nanotechnology to benefit HLBS research and medicine.

Interdisciplinary Interactions

Investigators with nanotechnology skills rarely focus on HLBS research, and investigators focused on HLBS disorders are generally unskilled in the development and use of nanomaterials and nanotechnologies. A crucial need exists to foster partnerships between the 2 communities, to establish shared goals and resources. The creation of multidisciplinary programs will be essential to realization of the potential of nanotechnology. The first step may be to establish a shared scientific vocabulary and language to facilitate exchange of ideas. In addition, training programs incorporated into the multidisciplinary programs should train young scientists to bridge the gap between the cultures. For large programs to be established, financial stability must exist, which requires long-term funding. It is critical that programs be clinically driven and oriented to translating biological nanotechnology/science into diagnosis and therapy of HLBS diseases.

The multidisciplinary nature of the field may benefit from changes in the way academic programs are organized, researchers are evaluated, and grants are reviewed and awarded. National Institutes of Health peer review is effective in assessing scientific merit but less successful when applied to technology- or design-based applications. Tailored reviews recognizing the importance of design- and technology-driven applications are critically important. Recognition of independent contributions to collaborative projects within academic and research institutions was also a concern, because multiple principal investigators on a grant may not be recognized. This is particularly important for young investigators, for whom the absence of appropriate recognition for collaborative work can profoundly inhibit tenure and promotion prospects.

New Technology, Materials, and Infrastructure

In emerging fields, scientific discovery is frequently limited by access to new technological developments and reagents. The interdisciplinary nature of therapeutic nanotechnology may make the requisite investment too large to be feasible for individual laboratories. New molecular entities for intracellular and extracellular targeting and new pharmacologically active molecules are also required, and laboratories outside pharmaceutical companies rarely have resources needed to generate extensive compound libraries. Centralized facilities could greatly stimulate nanotechnology application to HLBS research and medicine by providing broad access to resources in a cost-effective way, allowing novel, high-risk projects to be undertaken without inordinate initial costs. Animal models are also costly yet essential for development of applications that offer clinical benefits. Provision of preclinical toxicology screening facilities could be an efficient solution. Expensive

equipment needs may also constitute a significant barrier that collaborations between academia and industrial partners may help to overcome.

Safety and Manufacturing

US Food and Drug Administration approval is essential for clinical applications of nanotechnology, but substantial regulatory problems may be encountered in the approval of nanotechnology-based products. Pharmaceuticals, biologicals, and devices are all regulated differently by the Food and Drug Administration, and it is not yet clear how emerging nanotherapeutics will be evaluated. These uncertainties may constitute a barrier for initial approval, but once regulations are established and the requirements for approval are defined, the process should be straightforward. The Working Group also discussed the need for a central resource to acquire basic safety data such as biodistribution, pharmacokinetics, efficacy, and toxicity for nanoparticles and other macromolecules.

Manufacturing feasibility could also hinder translation of new nanotechnologies into usable treatments. Concerns related to suitability for “scale up” and mass production must be considered early in the research and development phase of new nanotechnology devices and therapeutics, but commercial production issues should not dominate decisions regarding research into early clinical applications of nanotechnology. Although the Working Group was not complacent regarding manufacturing issues, it anticipated that commercial opportunity would drive manufacturing innovation. For example, mass production of nanocrystal-based diagnostic tools initially appeared improbable, but the exciting potential benefits stimulated commercially viable manufacture.

Industry Interest

Technology transfer to industrial partners is often critical to the introduction of new research into the clinical setting. Pharmaceutical companies traditionally focus on small-molecule therapeutics applicable to all potential patients. The genomic era and resultant implications for individualized therapies will increase demand and facilitate development of novel, individualized therapeutic technologies. Nanotechnology-based devices that provide site-specific drug delivery and efficacy monitoring may contribute to the development of “personalized” medicine, which takes advantage of improved understanding of the molecular basis of individual variation. Establishment of collaborations between academic researchers and industry and demonstration that the development of nanoscale and personalized therapeutics will be profitable are essential to realizing this potentially vast benefit to the public.

Disease-Specific Examples

The Working Group also identified specific NHLBI disease target areas that present opportunities for immediate application of nanotechnology. Because clinical applications of nanotechnology are likely to be relatively expensive, the Working Group focused on serious disease states for which current care is clearly inadequate. This list is not inclusive but is intended to highlight selected disease areas for which new methodologies offer great promise.

Unstable or Vulnerable Plaque

The diagnosis and treatment of unstable plaque is an area in which nanotechnology could have an immediate impact. Research is under way using probes targeted to plaque components for noninvasive detection of patients at risk.¹⁶ In an extension of this approach, targeted nanoparticles, multi-functional macromolecules, or nanotechnology-based devices could deliver therapy to a specific site, localized drug release being achieved either passively (by proximity alone) or actively (through supply of energy as ultrasound, near-infrared, or magnetic field). Targeted nanoparticles or devices could also stabilize vulnerable plaque by removing material, for example, oxidized LDL. Devices able to attach to unstable plaques and warn patients and emergency medical services of plaque rupture would facilitate timely medical intervention.

Tissue Repair, Engineering, and Remodeling

Nanotechnology may facilitate repair and replacement of blood vessels, myocardium and myocardial valves, and lung tissue. It also may be used to stimulate regenerative processes such as angiogenesis. Cellular function is integrally related to morphology, so the ability to control cell shape in tissue engineering is essential to ensure proper cellular function in final products. Precisely constructed nanoscaffoldings and microscaffoldings are needed to guide tissue repair and replacement in vessels and organs. Nanofiber meshes may enable vascular grafts with superior mechanical properties to avoid patency problems common in synthetic grafts, particularly small-diameter grafts. Cytokines, growth factors, and angiogenic factors can be encapsulated in biodegradable microparticles or nanoparticles and embedded in tissue scaffolds and substrates to enhance tissue regeneration. Scaffoldings capable of mimicking cellular matrices should be able to stimulate the growth of new heart and lung tissue and direct revascularization.

Lung Inflammatory Diseases

The diagnosis, treatment, and prevention of inflammatory lung diseases such as asthma and emphysema may all be improved by nanotechnology. Inhaled nanoscale biosensors could examine lung microenvironments, enabling detection of proinflammatory signals early in disease. Similarly, nanoparticles capable of sensing alveolar function could release drugs only when needed, restricting drug delivery to affected areas in heterogeneous disease conditions. Lipid-based nanoparticles are being considered for site-specific delivery of antifungal therapy to the pulmonary epithelium, which would mitigate nephrotoxicity. A similar approach shows promise for asthma drugs, enhancing local delivery and retention, controlling release, and limiting systemic effects such as glucocorticoid leakage into the bloodstream.

Thrombosis and Hemorrhagic Disorders

Patients would benefit greatly from nanotechnological devices that could monitor the body for the onset of thrombotic or hemorrhagic events. Multifunctional devices could detect events, transmit real-time biologic data externally, and deliver anticoagulants or clotting factors to buy critical time. Another likely role for nanoprobe is in cell tracking; quantum dots and superparamagnetic particles could be valuable

for tracking hematopoietic and other stem cells to determine efficiency of cell delivery and survival.

Sleep Apnea and Related Cardiovascular Consequences

Because sleep apnea is a cause of irregular heartbeat, hypertension, heart attack, and stroke, it is important that patients be diagnosed and treated before these highly deleterious sequelae occur. For patients suspected of experiencing sleep apnea, in vivo sensors could constantly monitor O₂ blood concentrations and cardiac function to detect problems during sleep. In addition, heart-specific antibodies tagged with nanoparticles may allow doctors to visualize heart movement while a patient experiences sleep apnea to determine both short- and long-term effects of apnea on cardiac function.

Recommendations

The four highest-priority recommendations made by the Working Group for stimulating nanotechnology applications to HLBS disorders are discussed below in order of their ranking.

Multidisciplinary Centers

The formation of programs bringing together scientists from complementary disciplines to create nanotechnology research centers was the highest-priority recommendation of the Working Group. Program members would not necessarily be located in the same geographical location, and program composition should be biologically and clinically driven. Centers should be dynamic, adapting to the changing research milieu as the field develops. Inclusion of industrial partners is highly encouraged. The overriding goal of these multidisciplinary teams would be to create nanotechnology applications for HLBS medicine. Each center would have an obligation to train young scientists and physicians to understand the technological, scientific, and clinical aspects of applying nanotechnology to HLBS disorders. The centers would be expected to make materials, reagents, and devices available to other researchers and disseminate technological advances.

Investigator-Initiated Research

Individual investigators should be encouraged to apply nanotechnology and nanoscience to HLBS disorders, with a particular emphasis on clinically oriented research. This effort will broaden the scope of nanotechnology research, allowing researchers who are not members of larger teams to pursue individual, cutting-edge ideas.

Exploratory and Developmental Research

Due to the cutting-edge nature of research in nanotechnology, short-term or small-scale exploratory research should be fostered. The focus should be on innovative high-risk, high-impact projects, and preliminary data should not necessarily be required for initial support. An important goal of this initiative would be to attract investigators from outside the HLBS fields and to bring their expertise to bear on HLBS-related problems. Projects could involve the establishment of a new methodology or application of a new technology.

Small Business Opportunities

The Working Group also noted the potential benefits of stimulating research and development by small businesses

through the Small Business Innovation Research/Small Business Technology Transfer program. Such an effort could harness the considerable talents and capabilities of the small business community to use nanotechnology to solve problems in HLBS research and disease.

Appendix

Working Group Members

Chair: Mauro Ferrari, PhD, Biomedical Engineering Center, Ohio State University. Members: Jean Frechet, PhD, Department of Chemistry, University of California, Berkeley; Jeffrey Fredberg, PhD, Department of Environmental Health, Harvard School of Public Health; Bruce Furie, MD, Beth Israel Deaconess Medical Center; Pascal Goldschmidt, MD, Department of Medicine, Duke Clinical Research Institute; Stephen Lee, PhD, Biomedical Engineering Center, Ohio State University; Viola Vogel, PhD, Department of Bioengineering, University of Washington; Jennifer West, PhD, Department of Bioengineering, Rice University; Samuel Wickline, MD, Cardiovascular Division, Washington University School of Medicine; Karen Wooley, PhD, Department of Chemistry, Washington University.

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