The field of nanotechnology is growing explosively and impinging on all walks of life. This is reflected in the appearance of nanotechnology in consumer products; in March 2011, the Project on Emerging Nanotechnologies, a partnership between the Woodrow Wilson International Center for Scholars and the Pew Charitable Trusts, reported more than 1300 consumer products containing nanotechnology on the market, a growth of 520% since March 2006. The health care field is also being affected; a search on “nanoparticle” in ClinicalTrials.gov pulls up more than 80 trials, primarily in the cancer field but also including such diverse areas as antibacterial agents, dental composites, wound dressings, imaging agents, and stent coatings.

What does “nanotechnology” refer to? The National Nanotechnology Initiative defines nanotechnology as the understanding and control of matter at dimensions between approximately 1 and 100 nm, where unique phenomena enable novel applications. Encompassing nanoscale science, engineering, and technology, nanotechnology involves imaging, measuring, modeling, and manipulating matter at this length scale. There are 2 approaches to nanotechnology, termed “top-down” and “bottom-up.” Top-down approaches involve the use of tools to make nanoscale features, for example, nanopatterning of surfaces on devices to change their surface properties. Bottom-up approaches involve self-assembly of small components to make nanoscale particles or structures.

National Heart, Lung, and Blood Institute Programs of Excellence in Nanotechnology

Early last decade, the National Heart, Lung, and Blood Institute (NHLBI) recognized the promise of nanotechnology for improving the diagnosis and treatment of heart, lung, and blood diseases, and in 2003 the institute organized a working group to give advice on how to harness this potential. The working group brought together physical scientists such as chemists and material scientists, along with biological scientists and clinicians from the heart, lung, and blood fields. The highest-priority recommendation of the working group was the formation of multidisciplinary centers that would promote collaborations between the biological and physical sciences. This led to a request for applications to support the NHLBI Programs of Excellence in Nanotechnology (PENs), which provided $54 million to fund 4 centers for 5 years, starting in spring 2005. Programs were funded at the Massachusetts General Hospital (Principal Investigator (PI) Ralph Weissleder); Washington University/University of California Santa Barbara/University of California Berkeley (PI Karen Wooley); Emory University/Georgia Institute of Technology (PI Gang Bao); and the Burnham Institute/University of California Santa Barbara (PI Jeffrey Smith). Annual meetings that brought together all the PEN investigators, coordinated through an administrative coordinating center at Washington University, played an important role in sharing technology across the centers.

The first iteration of the PEN program included projects on pulmonary and hematopoietic applications, but cardiovascular disease, and in particular the detection of vulnerable plaque, was the main focus of applications. Approximately 240 publications came out of the PENs over the 5-year funding period, making a significant contribution to the rapid expansion of NHLBI-funded nanotechnology publications that occurred between 2005 and 2010 (Figure 1A). The impact of the PENs was also seen in citations of NHLBI-funded nanotechnology papers, with PEN-supported publications accounting for approximately 56% of citations in 2008 and 2009 (Figure 1B). The PENs also provided interdisciplinary training for approximately 165 postdoctoral fellows and graduate students.

Renewal of the PENs

In 2009, the NHLBI released a Broad Agency Announcement to fund the renewal of the PENs. For the renewal, an increased emphasis was placed on translation of the technologies being developed toward preclinical and clinical application. The contracts were awarded in summer 2010, funding 4 teams spread across 17 institutions for a total of $65 million over the 5-year period.

- Massachusetts General Hospital (Ralph Weissleder, MD) leads a consortium of 6 Boston-area institutions, including Harvard Medical School, the Harvard School of Engineering and Applied Sciences, Massachusetts Institute of Technology, Brigham and Women’s Hospital, and the Broad Institute of Harvard and MIT. The group is developing nanomaterials to diagnose and treat cardiovascular diseases and create a point-of-care system for the rapid detection of pulmonary infections.
- Georgia Institute of Technology (Gang Bao, PhD) is collaborating with Emory University and the University of California, Davis, to develop nanoparticle-based tools to image and deliver therapeutics to atherosclerotic plaque and to enhance stem cell repair of damaged heart tissue.
- Washington University (Michael Welch, PhD) and Texas A&M University (Karen Wooley, PhD) head a collabora-
tion that also includes the University of California, Santa Barbara, the University of California, Berkeley, and the University of Texas–Southwestern Medical Center. Their work will include the nanoparticle-based diagnosis and treatment of acute lung diseases as well as imaging and treating cardiovascular diseases.

- Mount Sinai Medical School (Zahi Fayad, PhD) and Massachusetts Institute of Technology (Robert Langer, PhD) are collaborating with New York University, Columbia University, and Brigham and Women’s Hospital. The group is focused on developing therapies for early- and late-stage cardiac disease, treatment for atherosclerotic plaque to prevent heart attack, and delivery of regeneration factors to repair heart tissue damaged by heart attack.

- Additional information on the PEN program and links to the 4 PENs can be found at the Administrative Coordinating Center website, http://www.nhlbi-pen.net.

Other NHLBI Support for Nanotechnology
In addition to the PENs, NHLBI funds nanotechnology through a number of other mechanisms. Research support for small businesses is provided through SBIR and STTR Bioengineering Nanotechnology Initiative Funding Opportunity Announcements (FOAs).3,4 The NIH also has specific Nanoscience and Nanotechnology in Biology and Medicine FOAs for R01 and R21 nanotechnology projects5,6 in which NHLBI participates. The Bioengineering Research Partnership program7 funds larger translational multidisciplinary research projects, encouraging the participation of industrial partners, and has funded several nanotechnology applications. Finally, investigator-initiated applications contribute to a robust nanotechnology portfolio at the NHLBI. For the 2009 fiscal year, the NHLBI funded $23.7 million in nanotechnology research through the regular appropriation and committed an additional $5.7 million in American Recovery and Reinvestment Act funds, for a total of $29.4 million.

Research Supported by the NHLBI
Developmental nanotechnology research supported by the NHLBI falls into 5 main areas: in vivo imaging, therapeutic delivery, regenerative medicine, in vitro diagnostics, and enabling technologies. In addition, NHLBI researchers use nanotechnology tools to address fundamental questions in cardiovascular biology. Examples of NHLBI-supported research in each of these areas are described below.

In Vivo Imaging
A number of NHLBI-supported groups have used nanoparticle-based molecular imaging tools to explore plaque composition, with the goal of detection of vulnerable plaque at high risk for rupture. Briley-Saebo and colleagues8 used antibodies targeted to oxidation-specific epitopes to direct lipid-coated ultrasmall iron particles (<20 nm) to atherosclerotic lesions. Specific uptake of the targeted particles into atherosclerotic plaques was shown by MRI and confirmed by immunohistochemistry (Figure 2). Uptake of targeted particles was greatly reduced by competitive inhibition with free antibody, whereas nontargeted particles showed no uptake.

Another strategy to identify inflamed plaque is to assess macrophage burden. In a study supported by the PEN program and other NHLBI grants, Morshige et al9 used macrophage-targeted superparamagnetic iron oxide nanoparticles in cholesterol-fed New Zealand White rabbits 6 months after balloon injury. Using MRI, they demonstrated uptake of nanoparticles into abdominal aortas and showed that the MRI signal, which correlated with macrophage content by histology, was decreased by treatment with rosvustatin.

Therapeutic Delivery
Peripheral arterial disease exerts a large burden on the US population, both economically and in terms of morbidity and mortality. Furthermore, in many patients, revascularization is not an option, and so these clinical and economic burdens are likely to grow rapidly. Protein- and gene-based growth factor therapies have shown promise in animal studies but have been disappointing in clinical trials, probably reflecting at least in part a failure to maintain a high enough growth factor concentration for biological activity.10 In a study supported by the Emory/Georgia Tech PEN program, Golub et al11 encapsu-
lated vascular endothelial growth factor in poly(lactic-co-glycolic acid) nanoparticles to provide sustained release. Mice with experimental hind-limb ischemia receiving vascular endothelial growth factor–nanoparticle treatment showed significantly greater increases in total vessel volume and vessel connectivity compared with free vascular endothelial growth factor or saline treatments11 (Figure 3).

Regenerative Medicine
Stem cell therapies for repair after myocardial infarction have been shown to be beneficial in both preclinical and clinical settings. However, the hostile microenvironment in infarct and peri-infarct myocardium affects survival of transplanted cells, which may in turn limit the efficacy of cell therapy. Padin-Iruegas and colleagues,12 supported by investigator-initiated NHLBI funding, used insulin-like growth factor-I tethered to self-assembling peptide nanofibers to potentiate the efficacy of exogenous cardiac progenitor cells after myocardial infarction. Combination therapy with insulin-like growth factor-I nanofibers and cardiac progenitor cells was more effective than either therapy singly in treating acute myocardial infarcts in rats.

In Vitro Diagnostics
Nanotechnology is being used in a wide range of biosensor platforms, both in research and commercial application. The Weissleder laboratory, with funding from the PEN program and other NIH sources, has developed hand-held magnetic resonance biosensor devices that can perform measurements on unprocessed biological samples. The devices detect changes in MR signal resulting from bringing magnetic nanoparticles closer together, for example, by clustering on molecular targets through affinity ligands or solubilizing clustered nanoparticles, for example, by enzymatic cleavage of cross-linking peptides.13 The methodology is very flexible and is applicable to proteins, enzymes, nucleic acids, small molecules, cells, and organisms. Swirski et al14 used the MR biosensor device to differentiate monocyte subset dynamics, taking advantage of the fact that the 2 major monocyte subsets express similar levels of macrophage colony stimulating factor receptor but differ markedly in phagocytic capacity. They were able to quantify small changes in rare leukocyte subset populations rapidly and with small sample volumes (1 μL) incompatible with flow cytometry.

Enabling Technologies
Developing tools that can measure and characterize nanoparticles is important for moving nanotechnology toward clinical translation. Supported by the PEN program, researchers at the Burnham Institute and University of California, Santa Barbara, developed a versatile high-throughput label-free nanoparticle
The microfluidic device can detect, quantify, and size 500,000 individual nanoparticles per second, can be used on biological fluids such as plasma, and could be readily adapted to a multichannel design on an inexpensive disposable chip to allow sizing of a wide range of particle sizes.

Nanoparticle toxicity is an important consideration in designing imaging agents and therapeutics, as well as in understanding how environmental exposures affect the body. Shaw and colleagues developed a multidimensional screening method to understand biological effects of nanomaterials, including toxicity. Four cell-based assays were carried out to examine apoptosis, mitochondrial potential, reducing potential, and ATP content. The approach provides robust structure-activity relationships and can be used to inform nanomaterial design and guide in vivo studies.

Nanotechnology Tools for Fundamental Discovery

Nanotechnology tools such as atomic force microscopy (AFM) and optical trapping provide NHLBI researchers with key insights into cardiovascular biology. For example, the Granzier laboratory has used AFM to probe how protein kinase C-α modulates cardiac stiffness and conclude that phosphorylation of a specific serine residue in the sarcomeric protein titin plays a key role. Measurements of the mechanical properties of vascular smooth muscle cells using AFM show increased stiffness in old versus new cells, supporting a novel role for intrinsic changes in vascular smooth muscle cells in age-related vascular stiffness. Optical trapping assays provide unique opportunities to quantify the molecular regulation of muscle biology; Debold et al. used this technology to demonstrate that human actin mutations associated with hypertrophic and dilated cardiomyopathies have differential effects on thin-filament regulatory properties that may explain their divergent physiological effects.

Future Directions

Continued growth of the application of nanotechnology to cardiovascular disease is anticipated, with a number of areas ripe for expansion. The use of nanotechnology to assess vascular inflammation has focused primarily on finding vulnerable plaque to predict coronary events. However, in the future, broader application of the technologies is likely, for example, to assess valvular disease, to predict expansion of aortic aneurysms, and to...
assess transplant rejection. Further exploration of synergies between nanofiber support matrices and progenitor cells for cardiac regeneration is likely. Electrospun nanofibrous grafts show promise for use in coronary artery bypass surgery and arteriovenous access grafting, and strategies such as biofunctionalization may improve endothelialization.

In terms of NHLBI support for nanotechnology research, the current PEN program has 4 more years to run. Because the PEN program has been successful in helping the NHLBI to develop a robust portfolio of basic research to apply nanotechnology for heart, lung, and blood diseases, translational efforts to move the technologies toward clinical application are likely to represent a focus for future funding. However, support for basic research is expected to continue, supported through in part through the bioengineering and nanotechnology-oriented FOAs.

Summary

The application of nanotechnology to the diagnosis and treatment of cardiovascular disease is expected to increase rapidly in coming years. Nanoparticle-based molecular imaging agents targeted to specific disease targets such as cell surface markers, enzymes, or cells such as macrophages will expand our capacity to diagnose disease and to monitor therapy. Nanotechnology offers a number of opportunities for improving drug delivery, including sustained delivery from degradable nanomaterials, improved pharmacokinetics and bioavailability, and targeted delivery to improve effective concentration at the disease site and reduced systemic effects. Sustained delivery of cytokines and growth factors will also play an important role in enhancing regenerative medicine, along with the provision of biomimetic scaffolds that can help to guide growth and proliferation of stem and progenitor cells. The availability of nanotechnology-based multiplexed point of care diagnostics will contribute to personalized medicine, allowing optimized care for the individual patient. The NHLBI’s flagship Programs of Excellence in Nanotechnology is expected to accelerate the translation of these promising technologies toward clinical application. In addition, the NHLBI’s robust portfolio of funding through other FOAs and investigator-initiated applications, including small business grants, will continue to make important contributions to basic discovery and translation.

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