Feature

Ophthalmology on the Nanofrontier

By Annie Stuart, Contributing Writer

The Lilliputian world of nanotechnology may one day stand shoulder to shoulder with biotechnology as an industrial and medical colossus.

***EyeNet* spoke with eight hard-at-work nanoexperts.**

With a turn of irony, infinitesimal particles invisible to the human eye may represent the very future of eye medicine. “We’re beginning to embrace a paradigm-shifting scientific approach to engineering and discovery,” said Marco A. Zarbin, MD, PhD, professor and chairman of the Institute of Ophthalmology and Visual Science at the University of Medicine and Dentistry of New Jersey. Working on a molecular scale, Dr. Zarbin and his colleagues are plotting a Lilliputian Revolution.

A nanometer is one billionth of a meter. Broadly defined, nanotechnology designs and engineers structures less than 100 nanometers in size, the scale at which organic molecules and compounds operate inside living cells. “We have the power to design biologically living structures that solve mechanical problems,” Dr. Zarbin said.

Such living systems would be quite different from those typified by relatively static machines, which only respond when someone presses a button or a lever. According to Carlo D. Montemagno, PhD, dean of the college of engineering at the University of Cincinnati, we’ve already learned a great deal about the nature of a living system: “It’s a robust one that allows it to be adaptive to changing conditions, to accept localized failure and keep on functioning, to use the interaction of molecules to pass information.”

For physicians and bioresearchers, the prospects are exciting: Nanomedicine—an offshoot of nanotechnology—could help solve multifaceted problems with minimal iatrogenic consequences. “Think about what doctors and surgeons do every day,” said Dr. Zarbin. “We make holes in the eye. We do things that are damaging to the tissue we’re trying to preserve. What we’re currently doing is like using an excavator to clear the china off a dining room table.”

Top

Vast Potential of the Ultrasmall

It’s safe to say nanomedicine will bring transformative change, said Dr. Montemagno. That’s because it offers control over how molecules interact with one another—giving them the ability to respond to their environment with sentientlike behavior. Here are four key properties of nanoparticles:

Large surface area. Nanomaterials are surface-rich objects in relation to volume, said Robert Ritch, MD, professor of clinical ophthalmology at New York Medical College; surgeon director and chief, glaucoma services at The New York Eye & Ear Infirmary. “If you have a collection of molecules the size of a golf ball, most of the molecules are inside the golf ball and only a tiny proportion are on its surface,” he said. “But if you have a construct that is 100 nanometers across with molecules in it, then most of those molecules are at the surface, where they can interact and engage in chemical reactions.”

Altered functionality. With a shrinking of scale come surprising changes in functionality. Molecules at the nanoscale behave differently, said Dr. Zarbin. Carbon becomes 100 times stronger than steel, for example, and gold can melt at room temperature.

Molecular self-assembly. “What distinguishes nanotechnology is the precision assembly of matter,” said Dr. Montemagno. “It’s basically putting molecules where you want them to be, to do what you want them to do, when you want them to do it.” Ubiquitous in nature, molecular self-assembly offers a qualitative advance in molecular engineering; in some cases, self-assembled materials even biodegrade once their mission is complete.[1]

Cost-effectiveness. Although research is currently costly, nanotechnologies have the potential to become quite cost-effective. In the semiconductor industry, for example, lithography on the nanoscale allows for mass production at a low cost. In medicine, targeted therapies delivered in minute quantities to precise targets may have few or no adverse effects and dramatically lower the cost of long-term drug therapy, said Dr. Zarbin.

Top

Glory, Hope and Fear

Like any new science in the spotlight, nanotechnology has generated its share of popular myths, including oversimplification, unfounded fears and the promise of immediate wealth.

Just a miniature version of the real world, right? Nanotechnology is the art of designing and building machines in which the specifications are determined down to the molecule.[2] That scale is not merely smaller than the familiar world, but conceptually different. "Being small is a prerequisite," said Dr. Montemagno, "But it doesn't constitute true nanotechnology." It is not a simple matter of taking the macroscopic world and reducing it, agreed Dr. Zarbin. "That would be like thinking computers simply give you a new way to type. The idea of nanotechnology is that you have a microscopic world and everything is actually different in that world."

Safety concerns are insurmountable? The strange new world of nanotechnology has roused plenty of naysayers, who are concerned about the "gray goo" problem, said Dr. Ritch. "They envision self-replicating nanorobots that are capable of functioning autonomously in the natural environment, changing us from biomass into nanomass," said Dr. Ritch. Although toxicity is always an issue to be addressed, said Dr. Zarbin, there's no more reason for alarm than with other technologies. "And, in fact, nanotechnology affords incredible opportunities for specificity," he said, making it possible to generate activation signals that don't occur naturally. In addition, many new technologies, such as gene arrays, are available to rapidly screen for toxicity. Safety should not become a paralyzing concern, concluded Dr. Montemagno. "We need to use true scientific inquiry, looking at this with an open mind and not with preconceived ideas of finding dragons or finding nothing," he said.

Profits are right around the corner? Another popular misconception concerns the imminence of the nanorevolution. Acknowledging its great potential, Dr. Montemagno asserted that many people overestimate how quickly the major impacts of this technology will be realized. "There is a huge amount of pressure to make products tomorrow," he said. "But you can't lose your sight of the prize." You can't ignore the basic science and unglamorous tasks required to establish the solid foundation necessary to move forward, he said.

Top

Nano-Ophthalmology

With the potential to imbue inanimate materials with the properties of living systems, ophthalmic medicine of the future could find new tools for problems once considered intractable. Here is a glimpse at nine possibilities:

Sensors. The rich surface-to-volume area of nanomaterials will allow them to collect and report invaluable information, leading to better diagnoses, treatment decisions and feedback control. This might include determining the presence and identification of microbes or concentration of chemicals. Or, they might signal when a cellular event takes place.[3] Biosensor technology is undergoing significant development, said James F. Leary, PhD, SVM, professor of nanomedicine, basic medical sciences and biomedical engineering at Purdue University School of Veterinary Medicine. "Reactive oxygen species biosensors have already been created that can control therapeutic gene delivery," said Dr. Leary, adding that one application is the treatment of retinopathies caused by oxidative stress conditions.[4]

Delivery. "Eye drops have been the standard form of drug delivery for glaucoma for the last 10 years," said Dr. Ritch, noting that they pose inherent problems of consistency and adherence. And Dr. Zarbin added that intravitreal injections of drugs for retinal diseases are wasteful, potentially dangerous and inconvenient for patients and doctors. With the promise of sustained, targeted control, nanodrug delivery will be the low-hanging fruit of nanomedicine, said Dr. Zarbin. We have a paradigm of the future already, said Dr. Leary, with the advent of monoclonal antibody therapies used in the treatment of certain cancers, such as the use of rituximab (Rituxan) for non-Hodgkin's lymphomas. "Nanomedicine will use these directed antibodies—or alternatives to antibodies, such as peptides or aptamers—as the first step," said Dr. Leary. "Then, other more specialized molecules in the nanomedical 'package' can perform more specialized functions such as cell repair."

Specificity. Nanomedicine allows for tiny "smart bombs"—therapies that are cell- and tissue-specific. "Since the drugs are targeted," said Dr. Leary, "there is the very real possibility that we can use thousands of times less drug to get a therapeutic response of equal or greater efficacy, with fewer side effects." This becomes possible with the design of nanosystems consisting of multilayered, specialized parts. The outside layer might contain molecules targeted to a specific cell, and once the system is bound to the cell, other molecules pull it across the membrane. Yet other drug or gene molecules are released once safely ensconced inside the cell.[5] Chemotherapy and radiation will not become obsolete, said Dr. Leary, but will be much more efficient and less damaging in the future. "Now, we probably kill 1,000 good cells for every bad cell we get rid of," he said.

Control. The combination of biosensors and nanoparticles could also allow for controlled release of therapies. A biosensor might be configured to be normally switched off, said Dr. Leary, then turn on only in the presence of diseased molecules binding to the biomolecular switch. "A number of biomolecular in the presence of diseased molecules binding to the biomolecular switch." A number of biomolecular switches controlling downstream therapeutic gene expression have been produced. The difficulties remaining are to build fully integrated nanomedical systems that work in a multistep targeting and delivery process with low error rates."

Regeneration. "No self-respecting engineer would build a machine without feedback control," said Dr. Leary. "We're trying to put it into the delivery of medicine." The combination of molecular biosensors and feedback control will allow the repair or regeneration of diseased cells, rather than just killing them.

"We'll have, on the nanoscale, the equivalent of an insulin pump," said Dr. Leary. If repair isn't possible, the goal would be to induce programmed cell death. The benefit of that, said Dr. Leary, is that apoptosis does not lead to an immune response, which can itself be quite dangerous.[6] The implications of repair capability are astounding. "We've previously accepted many of the ravages of aging as normal. But many symptoms may be the result of unrepaired damage, said Dr. Leary, who optimistically added, "Regenerative nanomedicine techniques will lengthen the lifespan by as much as 50 percent or more—allowing us to stay more youthful and healthy along the way."

Polytherapy. Because of multiple, often redundant, pathways, biological systems are not inherently monotherapeutically oriented, said Dr. Zarbin. Nanomedicine affords the opportunity to get away from monotherapy with tools such as dendrimers—artificial, repeatedly branching molecules of various molecular weights. They would be characterized by a high degree of symmetry with lots of surface area to carry payloads, respond to multiple triggers and perform multiple functions.

Customization. "I'm most excited about the possibility of manufacturing drugs-to-order inside living cells, and that's where the molecular biosensor switch comes in," said Dr. Leary. "If we introduce a therapeutic gene-manufacturing template and upstream molecular biosensor feedback control switches, we can deliver exactly the right amount of drugs to these cells because we can literally manufacture according to order."[6]

Nanosurgery. Surgery in the nano age will also change in fundamental ways.

Dr. Zarbin described the Jules Verne-esque idea of machines and motors that are nanoscale. To treat elevated IOP, for example, it might become possible to use tiny motors to pump the aqueous out, he said, or to use the molecular equivalent of tiny brushes to scrub out the trabecular meshwork and improve the outflow. Or, if you could move instruments with the delicacy required, you might also be able to solve the problem of retroretinal gliosis following retinal detachment.

"If you could operate in the subretinal space with nanovideo cameras and nanoinstruments, you would now become a *real* microsurgeon," said Dr. Zarbin, adding that any type of anterior chamber surgery might be a good candidate due to relatively simple visualization and spatial orientation. "If we can make ourselves small enough or, conversely, the surgical field we're working in big enough, we can turn what appear to be biological problems into mechanical ones," said Dr. Zarbin.

Nanoengineering. Replacement of tissues and the construction of scaffolds that can allow the body to heal itself are two main techniques of nanoengineering, said Dr. Ritch. For example, it's theoretically possible to make an artificial trabecular meshwork and then seed it with stem cells to regenerate a functional meshwork.

Christopher J. Murphy, DVM, PhD, professor of comparative ophthalmology at the University of Wisconsin, Madison, and Paul Nealey, PhD, professor of chemical and biological engineering there, are exploring the topographic features of the cornea's basement membrane. They've found rich and complex three-dimensional matrices that have topography on the nanoscale.

"It's a feltlike architecture with narrow woven fibers, pores and elevations that are on a scale such that every single cell that interfaces with the basement membrane interacts with thousands of these features," said Dr. Murphy, adding that this seems to be a conserved architecture across species and anatomic sites.

He and Dr. Nealey have learned that topographic cueing fundamentally changes how a cell behaves as it approaches the biomimetic nanoscale range; cells behave differently on topographically patterned surfaces than they do on flat surfaces. "Nanoscale topography will increase adhesion, decrease proliferation, cause cells to align and, depending upon the cell type, align to different degrees," said Dr. Murphy. "They migrate differently and you can modulate differentiation."

This may have broad implications for lab experiments uniformly carried out on flat surfaces, said Dr. Murphy, since cells in real life exist on topographically patterned surfaces. Perhaps, for example, this could explain why in vivo results often inexplicably fail to match those found in vitro. Knowledge about how to modulate adhesions, proliferation, migration and differentiation may also have immediate relevance to emerging strategies in cell tissue and stem cell engineering, as well as the design of implantable prosthetic devices, such as the artificial cornea that Drs. Murphy and Nealey are hoping to develop.

The remaining challenges include understanding intracellular signaling, designing materials and creating the curved surfaces with nanoscale architecture on them, said Dr. Murphy. These are no simple matters with such complex structures. The cornea, for example, may require different topography on the periphery than it does near its center.

Top

The NIH Nano Initiative
In the fall of 2006, the National Institutes of Health completed formation of a national network of eight Nanomedicine Development Centers (NDCs). Funding for the centers is $24 million over 2008 and 2009. Staffed by multidisciplinary biomedical scientific teams, these collaborative centers will serve as the centerpiece of the NIH Roadmap Nanomedicine Initiative. The goal of early research at the NDCs is to gather extensive information about the physical properties of intracellular structures. This will help scientists better understand how biology's molecular machines are built so they can engineer molecular structures, assemblies and organelles for treating diseases or damaged cells and tissues. Knowledge gained can lead to development of new tools that are functional at the nanoscale. In addition, the NDCs are charged with training the next generation of students in the emerging field of nanomedicine. Richard S. Fisher, PhD, is the nanomedicine program director at the National Eye Institute, which has a leadership role in implementing the NIH Roadmap Nanomedicine Initiative. "My greatest hope is that the multidisciplinary approach—which includes clinical and engineering input at very early stages of projects—eventually leads to highly specific and efficacious treatments of disease," said Dr. Fisher. "While we envision this requiring 10 years or more, our greatest hope is that it occurs sooner and sets the stage for new thinking on how to proceed from the bench to bedside."

Top

Nanoneurology

Neural engineering is another burgeoning field in nanomedicine that will have consequences for ophthalmology.

Let there be light. Richard Kramer, PhD, associate professor of neurobiology at the University of California, Berkeley, is one of several scientists pushing the envelope in this field. With the help of collaborators Ehud Y. Isacoff, PhD, professor of molecular and cell biology, and Dirk Trauner, PhD, professor of chemistry, Dr. Kramer has used a synthetic chemical called a photoswitch to make neurons sensitive to light in rats with dead rods and cones.[7] "This is a photoisomerizable small molecule that you apply and it covalently attaches to ion channels from the outside of cells, including neurons," he said. "If we inject this into the eye, we can get it to attach to retinal ganglion cells and they become sensitive to light," which may make it possible for them to take over some of the functions of dead photoreceptors.

Dr. Kramer is currently working on a compound that doesn't require a foreign gene to make cells light sensitive. Funded by the National Institutes of Health Roadmap Nanomedicine Initiative (see "The NIH Nano-Initiative"), Dr. Kramer said these tools may become indispensable for understanding how things work in complex tissues. "In the nervous system, you're dealing with this bewildering array of many different kinds of cells, all interspersed. It's a daunting job to understand with electrodes, which only allow you to interrogate one cell at a time." Besides working to restore optically active retinal cells, Dr. Kramer sees many other potential applications for these photoswitches. Just one example, he said, is the activation of drugs, such as a light-sensitive local anesthetic that you can turn on and off with the flash of a light.

Mimicking neurons. Known for engineering and fabricating the first nanobiochemical motor system, Dr. Montemagno and his team are now working on building an artificial neuron system. "We're figuring out how to make a gellike material that will function similarly to a neuron," said Dr. Montemagno. "One portion of a nerve fires, and we're able to bridge it with material that takes the signal and amplifies and propagates it. We see the promise of this among many things, for example, providing support for the optic nerve and for people with degenerative neural diseases."

Knitting neurons. Rutledge Ellis-Behnke, PhD, principal investigator in the brain and cognitive sciences department at the Massachusetts Institute of Technology and associate professor in the department of anatomy at the University of Hong Kong, is known for his work in neural regeneration of the optic nerve using a self-assembling peptide nanofiber scaffold, shaped like miniature combs. "One of the things nanotechnology lets us do is build very small, discrete structures that will allow for cells to migrate in and out, and that will change the environment to allow normal processes to happen," said Dr. Ellis-Behnke, who compares this to the way skin cells migrate in during the healing process.

In experiments with hamsters, Dr. Ellis-Behnke cut the optic nerve, then inserted a scaffold solution—developed by Shuguang Zhang, PhD—that assembled into a gel. In a process dubbed "nano neuro knitting," tissue reconstituted into its original form without scarring.[8] "By creating a permissive environment, we observed healing of the brain in the adult animal, axons growing back through the center of the cut, and we had return to functional vision," said Dr. Ellis-Behnke. Though this result was expected in young hamsters, in adult hamsters, it was startling. The researchers found the "axon sprouting process" had a window of plasticity. "If you understand exactly when that is, you can use it to your advantage to maximize reconnection and rewiring," he said.

He envisions a variety of potential applications: To replace lost retinal ganglion cells, to reinflate the eye after a penetrating wound or to replace whole systems in the eye. "Typically what you see in most diseases, especially in the eye, is not a massive catastrophic event, unless it's a physical injury," said Dr. Ellis-Behnke. "Mostly it's a slight change—a slight change in pressure, a slight increase in growth of blood vessels, a minute disconnection of retinal ganglion cells, a bit of dysfunction due to diabetes." Dr. Ellis-Behnke hopes his work and that of others in the field will be a way to nudge the system in the other direction, thereby preventing the cascade toward disease and disability. "Can we push the system back far enough so it can repair itself? It may sound a little radical, but I think that's the promise of some of this work."

If Dr. Ellis-Behnke and colleagues are on the right track, nanomedicine will have a megafuture.

____

1 Zhang, S. *Biotechnol Adv* 2002;20:321–339.
2 Hall, J. S. *Nanofuture: What's Next for Nanotechnology* (New York: Prometheus Books, 2005).
3 Purdue Weldon School of Biomedical Engineering Web site: www.engineering.purdue.edu/BME/Research
4 Prow, T. et al. *Mol Vis* 2006;12:616–625.
5 Prow, T. et al. *J Mol Histol* 2004;35:555–564.
6 Seale, M. M. et al. *Proc of SPIE* 2007;6430:1–7.
7 University of California, Berkeley, Web site: www.berkeley.edu/news/media/releases/2006/10/31_photoswitch.shtml
8 Ellis-Behnke, R. G. et al. *Proc Natl Acad Sci* 2006;103(19):7530.

____

Top

MEET THE EXPERTS

Rutledge Ellis-Behnke, PhD
Principal investigator, brain and cognitive sciences depart- ment, Massachusetts Institute of Technology; associate professor, department of anatomy, University of Hong Kong.

Richard S. Fisher, PhD
Nanomedicine program director, National Eye Institute.

Richard Kramer, PhD
Associate professor of neurobiology, University of California, Berkeley.

James F. Leary, PhD, SVM
Professor of nanomedicine, basic medical sciences and biomedical engineering, Purdue University School of Veterinary Medicine.

Carlo D. Montemagno, PhD
Dean of the College of Engineering, University of Cincinnati.

Christopher J. Murphy, DVM, PhD
Professor of comparative ophthalmology, University of Wisconsin, Madison.

Robert Ritch, MD
Professor of clinical ophthalmology, New York Medical College; surgeon director and chief, glaucoma services, The New York Eye & Ear Infirmary.

Marco A. Zarbin, MD, PhD
Professor and chairman, Institute of Ophthalmology and Visual Science, University of Medicine and Dentistry of New Jersey.

None of the experts report related financial interests.

Top