

MES *in* NANO- MEDICINE

In the mechanics of medical treatment, nanotechnology has lived up to much of its early promise.

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Twelve years ago, *Mechanical Engineering* magazine featured an article titled “The Great Out of the Small” that introduced the potential of the then relatively new and uncharted world of nano-engineering. The article speculated on how nanotechnology might transform the aerospace industry, on how some of these breakthroughs might come from emulating nano-scale systems in nature,

and on how these breakthroughs might come back to change the ways we engineer treatments to disease and disability.

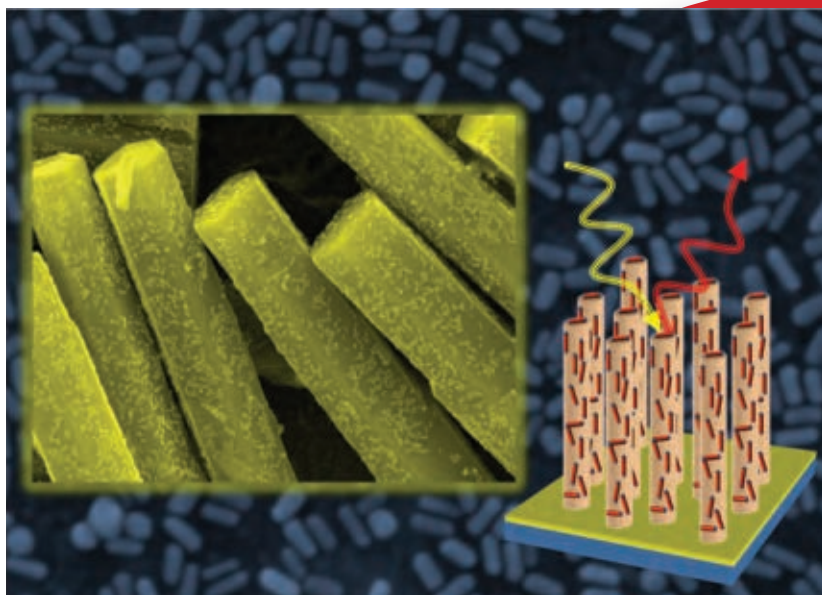
While the field has been slower than the article predicted in developing nano-engineered structural materials, we are now far ahead in nano-engineered cures, and a new nano-engineering toolbox has become standard for mechanical engineers of the 'teens. ASME and its Bioengineering Division are at the center of the application of this toolbox to problems of disease and disability.

Nano-engineered solutions now exist for a range of medical diagnostics, therapeutics, and imaging, and are at the core of many of the current generation of regenerative medicine and tissue engineering strategies. The technol-

ogy is easily detected and very sensitive to the nanostructure's local environment. The field is called plasmonics, and it is a rapidly emerging area in the broader field of nanophotonics. The trick is to manipulate light with nanostructures that are smaller than the wavelength of the photons themselves.

The basic science is straightforward. The electronic properties of a nanomaterial surface are different from those of a bulk solid, and mechanical engineers have manipulated the resulting nanomaterial surface usefully for decades. Although nanoparticles by definition are too small to be seen using light in the visible range—the optical resolution limit is about 200 nm—these nanomaterial surfaces can interact with the electromagnetic wave of a

COURTESY OF SOFT NANOMATERIALS LABORATORY AT WASHINGTON UNIVERSITY, IN ST. LOUIS



A demonstration of 3-D surface enhanced Raman scattering based on vertically aligned zinc oxide wires uniformly decorated with gold nanorods. Owing to the large increase in the surface area and hence the plasmonic nanostructures, sub-picomolar concentrations of a model analyte (1,4-benzenedithiol) were detected. The platform demonstrated is highly promising for sensitive label-free detection of bioanalytes (e.g., proteins, enzymes).

From: "Nanorod Decorated Nanowires as Highly Efficient SERS-active Hybrids" Kattumenu, R.; Lee, C. H.; Tian, L.; McConney, M. E.; Singamaneni, S. J. *Mater. Chem.*, 2011, 21, 15218.

ogy is emerging now to understand pathologies by modeling across scales, relating the basic mechanics of proteins and the peptides that compose them to the functionality and development of tissues and organs. We describe here a few examples of very large successes in the manipulation and prediction of the very small.

NANOSTRUCTURES, PHOTONS, AND PLASMONS

One of the major new tools in the modern engineer's toolkit is the ability to design, build, and deploy nanostructures that take energy from passing photons in a way that

passing quantum of light, or photon.

This interaction is what generates the visible part of the electromagnetic spectrum for noble metals such as gold, silver, and copper, and is the source of the bright colors of these metal nanostructures. This interaction is also known as localized surface plasmon resonance (LSPR).

What can we do with this? Since LSPR is an interaction of a photon or quantum of light and the nanomaterial surface, it is extremely sensitive to changes in the electromagnetic environment around nanomaterials. This renders it an attractive transduction platform for chemical and biological sensing, sensitive enough to differenti-

ate various inert gases, probe the conformational changes of individual proteins, detect single biomolecule binding events, monitor the kinetics of catalytic activity of a single nanoparticle, and even optically detect single electrons. LSPR of metal nanostructures is being investigated for numerous biomedical applications including label-free biosensors, bioimaging, and therapy.

One compelling example comes from the laboratory of ASME Bioengineering Division member Srikanth Singamaneni at Washington University in St. Louis. His research team has demonstrated an engineered nanostructure that can provide an easily read signal to detect sub-picomolar concentrations of a molecule, i.e., identify the presence of one molecule in a billion. The three-dimensional surface enhanced Raman scattering (SERS) system shown on the preceding page consisted of vertical zinc oxide wires coated with gold nanorods.

ZnO has unique optical and electrical properties, which make it a commonly used metal oxide semiconductor. The use of gold nanorods, as opposed to spherical gold nanoparticles, was to take advantage of the sharp corners and anisotropic shape of gold nanorods that lead to a lightning rod or antenna effect and consequently to an increase in the sensitivity of the SERS system. The dramatic increase (about 3,000 times) measured in the SERS intensity of the new 3-D system over a traditional 2-D system is postulated to be due to the increase (about 40 times) in the number of gold nanorods attached to the ZnO wires that are within the incident laser footprint (laser spot size of about 530 nm and a focal depth of 2.2 μm) of the 3-D system when compared to the 2-D system using a flat silicon substrate. Basically, the 3-D SERS system has more gold nanorods within the incident laser and hence, higher SERS sensitivity.

Obviously, an ability to detect sub-picomolar concentrations of a molecule has a wide variety of practical applications, including detection of high explosives and other threat chemicals. The technology could also be used to non-invasively probe and monitor the differentiation of stem cells.

SEARCHING AND DESTROYING

The technology not only to create but also to deliver and selectively control nanoparticles is a grand challenge in nanomedicine. The article that appeared in *ME* twelve years ago included the picture of a phage-like nano-robot attacking a virus floating through the blood stream, and this is just as unlikely today as it was in November 2000. However, while the prospect of automatons patrolling our capillaries is still

far away, much more exquisite control of nanoparticles is already under development.

Nanoparticles can be designed to enter several of the body's circulatory systems and circulate through the body until they adhere to specific proteins or are cleared by the liver. Once they are in place, the goal is to activate the particles in the vicinity of a disease site to perform certain therapeutic functions such as cleavage of undesired proteins, delivery of regulators of specific genes, or thermal ablation of undesired cells or tissues.

An important class of approaches in this regard involves particles that can be turned on selectively by a laser. The mechanism for this is, once again, surface plasmon resonance.

Nanoparticles can be developed to absorb energy with high efficiency from photons of certain frequency ranges.

Our bodies are particularly transparent to photons in the near infrared regime (wavelengths of 750-1,400 nm), meaning that large fluxes of photons in this regime can be applied to the body with relatively little absorption and heating of tissues. However, nanoparticles in this range can be engineered to capture these photons and convert them to heat, with a broad range of effects.

By pulsing a laser with a prescribed dose of energy, the particles can modulate water in their vicinity, with effects ranging from melting of ice in cryotherapy to denaturing proteins. With even higher laser power, tissues can be strained significantly by the expansion of the nanoparticles, or shocked with mechanical waves resulting from rapid formation of water vapor bubbles. Still higher laser power can generate plasma in the vicinity of the particle, or melt, vaporize, or fragment it.

What can an engineer do with this toolkit? An important goal is to destroy tumors, particularly invasive tumors, and this entire range of possibilities is useful to the physician. Consider, for example, astrocytic tumors of the central nervous system, the most common primary brain tumors. They are highly invasive in adults with very poor outcomes even following surgical resection.

The challenge is that astrocytic tumors are highly diffuse, with cancerous cells crawling away from the central tumor mass and invading healthy brain tissue to the extent that they cannot all be found and removed surgically.

The entire range of therapies is useful in treating a single disease in this case. For regions of the brain with diffuse populations of cancerous cells, milder doses of energy absorbed by nanoparticles are desirable to release and activate toxins bound to them within cancerous cells, or to disrupt their membranes. For denser regions of cancer-

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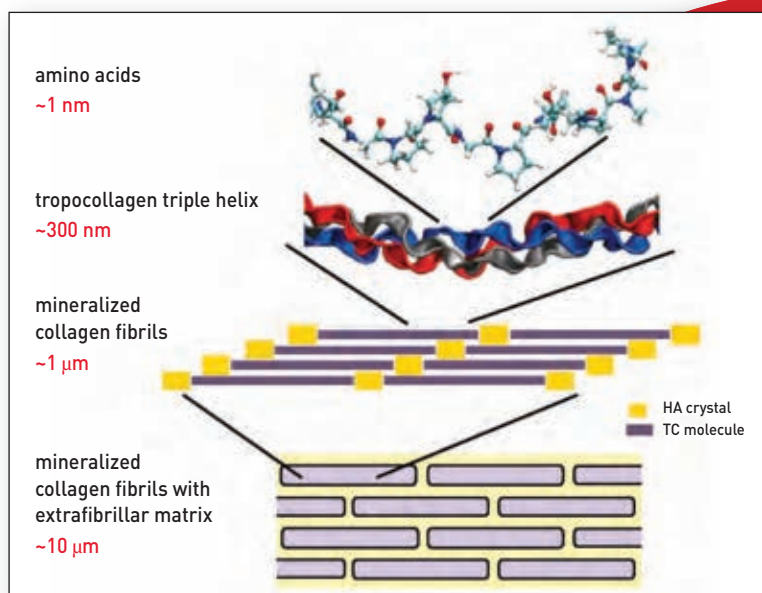
ous cells, higher doses of heating are desired with the goal of simply ablating the tissue, or alternatively, lower doses can be useful in recovery following cryoablation therapy.

Use of nanoparticles to enhance a primary treatment is called adjuvant therapy. Effective implementation of these therapies requires not only mechanical engineering design and analysis that predict outcome in terms of fundamental engineering principles, but also carefully designed engineering experimentation.

A challenge that occurs in thermal therapies designed to destroy diseased tissues is that our cells and tissues are

broad range of thermal ablation procedures. One of the goals is to deliver to a tumor a preconditioning agent that makes the tumor tissue especially susceptible to thermal ablation therapy.

One such agent is a small signaling molecule known as tumor necrosis factor- α . A significant challenge is that these molecules are toxic to the entire body, not just to tumor cells, and careful mechanistic and engineering analysis of transport within the body is needed to devise schemes that deliver the toxins predominantly to the tumor site.



Geometry of the nanostructure of bone, showing the various scales. In the structural response of bone, which is largely a composite of collagen and a mineral phase, the toughening mechanisms begin at the smallest scale. A triple-helix protein known as tropocollagen (hundreds of nanometers long) toughens through a mechanism of unfolding and crosslinking with neighbors. The protein then extends across multiple scales to include sliding interactions with mineral, and between mineralized bundles known as fibrils and fibers.

From: "Molecular Nanomechanics of Nascent Bone: Fibrillar Toughening by Mineralization" Buehler, M.J. Nanotechnology, 2007, 18, 295102.

remarkably resilient to heating and cooling. As anyone who has had to try several times to freeze off a wart knows, even severe cryodestructive therapies do not always achieve the desired results.

For a wart, the fix is straightforward and there is little cost. In the treatment of cancerous cells, however, allowing even a few cells to elude treatment can be fatal. One of the authors of this article, Ram V. Devireddy, searches at the Bioengineering Laboratory at Louisiana State University in Baton Rouge for agents that can enhance or alleviate the effect of freezing on induced cell self-destruction (apoptosis). For example, commercially available 1.4 nm diameter gold nanoparticles attached covalently to palmitic acid were found to increase the apoptotic response of a certain type of cancerous cell (HeLa cells) following freezing.

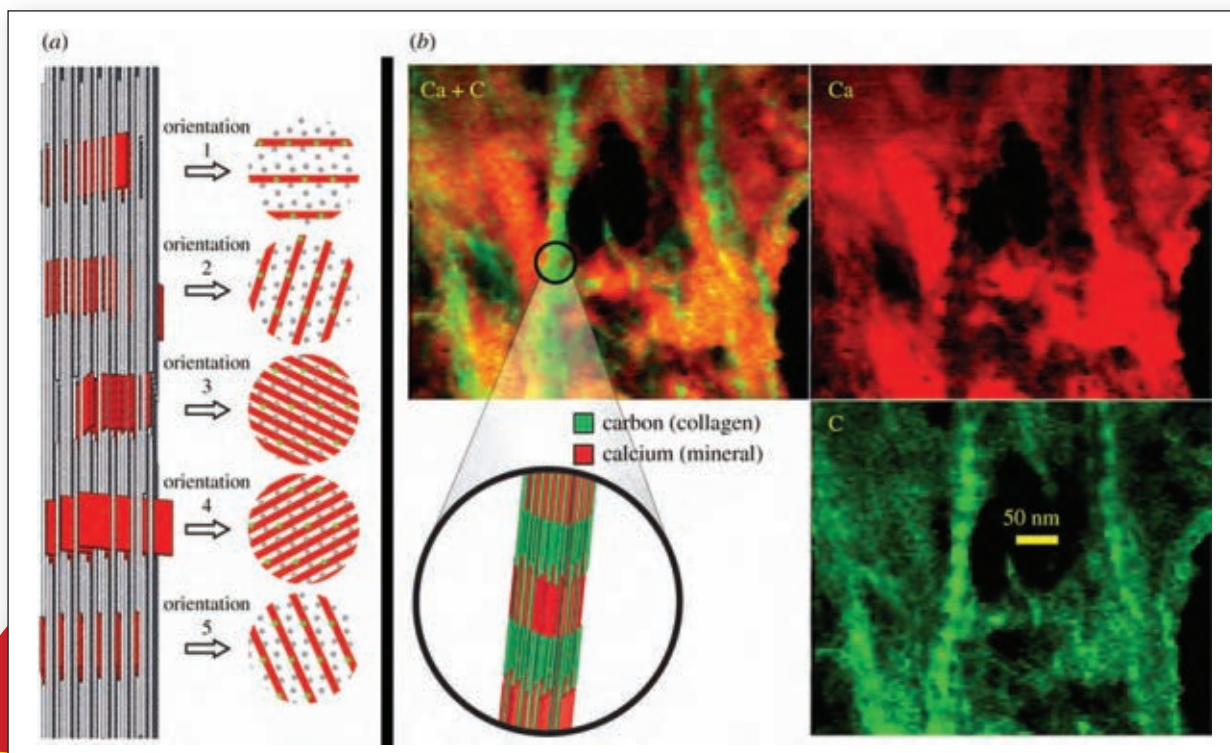
The current ASME Bioengineering Division chair, John Bischof, heads the BioHeat and Mass Transfer Laboratory at the University of Minnesota in Minneapolis, which focuses on nanoparticle-based adjuvant therapies for a

The strategy involves straightforward plumbing: many tumors have leaky ("fenestrated") vasculature, and Bischof's lab uses colloidal gold nanoparticles specifically designed to sequester these small molecules, and to leak through and accumulate preferentially in tumors.

STRUCTURAL PROBLEMS AT THE NANOSCALE

Beyond optical and thermal properties, the body is full of structural problems at the nanoscale. Research is advancing to the point at which meaningful analysis of protein structures and their mechanics can be made, and in some instances progress is now being made in understanding disease mechanisms from a very fundamental standpoint.

Proteins in the body are structures—floppy, vibrating structures, but structures nonetheless. Their function depends upon their structure, and upon the landscape of attractive and repulsive forces that their structure provides. Understanding the folding of proteins and the resulting electronic structure has been a major focus of structural



A recent study by mechanical engineers shed light on the distribution of calcium, carbon, and collagen in bone, a hitherto unknown property. (a) In the cross-sectional views, gray circles represent collagen molecules in the gap channel and green circles represent the projected location of collagen molecules that are present in the overlap space, but are not present in the gap channel. For any given gap channel, there are five possible orientations in which calcium-rich mineral particles (red) can be virtually inserted. For illustration purposes, these five insertion orientations are shown. This is not necessarily the periodic pattern of these orientations in bone, which is unknown. (b) The images on the top show carbon-rich regions (i.e. collagen) in green and calcium-rich regions (i.e. mineral) in red. A three-dimensional structural model of a mineralized fibril is shown in the middle bottom position of the panel. Mechanical engineers will recognize the plot from a standard CAD package, which was an essential component of this analysis.

From: "The Nanometre-Scale Physiology of Bone: Steric Modelling and Scanning Transmission Electron Microscopy of Collagen-Mineral Structure" Alexander, B.; Daulton, T.L.; Genin, G.M.; Lipner, J.; Pasteris, J.D.; Wopenka, B.; Thomopoulos, S. *J. Roy. Soc. Interface.*, 2012, doi: 10.1098/rsif.2011.0880.

Also, see www.youtube.com/watch?v=Ec8697M206s

biologists for decades, and, although much debate exists, a great number of established protocols and commercial simulation packages can be found. Many proteins in the body such as actin and collagen serve structural and mechanical roles, meaning that the boundary conditions in such simulations need the careful attention of mechanical engineers.

This is an area in which mechanical engineers have made much progress over the past decade, thanks in large part to ASME member Markus Buehler and his co-workers. Buehler's Laboratory for Atomistic and Molecular

Mechanics at Massachusetts Institute of Technology in Cambridge has developed tools to perform virtual stress-strain tests on models of proteins, and has opened up a window into the ways that tissues respond to stress and develop toughness over multiple spatial hierarchies. The ability to understand specific diseases such as osteogenesis imperfecta (brittle bone disease) based upon such fundamental analyses has been demonstrated by ASME member Sandra Shefelbine of Imperial College London in collaboration with the Buehler group.

Many of the open problems in these areas are familiar

to those of us trained in classic engineering materials and their composites. Composite materials present properties that depend critically upon the distribution of phases, but these distributions are tremendously challenging to map out in biological systems.

Biological tissues change fundamentally in the testing environments that are most conducive to nanoscale characterization, such as the hard vacuum of a tunneling electron microscope, and therefore must be processed heavily before characterization.

Bone, for instance, consists of mineral and collagen. Several fundamental questions persist about the nature of bone, including how much mineral exists per unit volume, where the mineral lies, how stiff it is, and how it interacts mechanically with collagen.

Mechanical engineers are taking important steps forward in characterization at this scale. For example, one of the authors of this article, Guy M. Genin, and his colleague, ASME member Stavros Thomopoulos at Washington University in St. Louis, recently published as part of a cross-disciplinary team an electron microscopy analysis that put to rest a longstanding debate on where mineral lies within bone, and shed light on how much there might be.

With this structural information, it is now possible to replicate the mechanics of a mineralized or partly mineralized tissue system to provide stability following surgery by utilizing electrospun nanofibrous systems. These electrospun systems consisting of fibers of nanoscale diameter but centimeters in length can be generated and mineralized for use as surgical grafts. These systems provide an environment for living cells that is conducive to regrowth of natural tissue. The subject of characterizing and replicating these optimal environmental conditions for living cells is the focus of a large effort in the mechanical engineering community.

WHERE WILL MECHANICAL ENGINEERS TAKE THE FIELD?

The tools of nanotechnology have enabled mechanical engineers to engineer the beginnings of an entirely new generation of cures and therapies, and this article has discussed just a sample. Where will mechanical engineers take the field next?

We certainly have ideas, and they are well represented by ASME membership's efforts. To serve as a forum for discussion of these advances ASME is recommissioning the *Journal of Nanotechnology in Engineering and Medicine*. ASME also hosts two major conferences: the Bioengineering Division's annual Summer Bioengineering Conference and the International Congress on NanoEngineering in Medicine and Biology.

It's too late to submit an abstract for the 2012 Bioengineering Conference (www.asmeconferences.org/SBC2012), which will be held June 20-23 in Fajardo, Puerto Rico, but might not be too late to attend. The 2013 conference will be held in Sunriver Resort in Oregon, and the deadline for abstracts is in January 2013. The program adapts constantly to breaking advances, and will include in 2012, for example, the first ever session on Microsystems and Genetic Regulation in Biological Machines.

The 2013 Congress on NanoEngineering in Medicine and Biology (www.asmeconferences.org/NEMB2013) will be held in Boston in February 2013. Deadline for abstracts is Oct. 15, 2012. The 2013 Congress on NanoEngineering will focus on the integration of engineering, materials science, and nanotechnology in addressing fundamental problems in biology and medicine, and in developing devices, materials, and methods for the early detection, imaging of pathological and physiological mechanisms, and treatment of disease.

Anyone joining us at these conferences is invited to bring a virus-killing nano-automaton. ■

TO EXPLORE FARTHER

The information used in this article was drawn from a variety of publications, including the following:

"Surface Plasmon Resonance Sensors: Review." J. Homola, S.S. Yee, and G. Gauglitz. *Sensors and Actuators B: Chemical*, Vol. 54, pp. 3-15 (1999).

"Surface Plasmon Subwavelength Optics." W.L. Barnes, A. Dereux, and T.W. Ebbesen. *Nature*, Vol. 424, pp. 824-830 (2003).

Plasmonics: Fundamentals and Applications. S.A. Maier. Springer, ISBN: 0387331506 (2007).

"Biosensing With Plasmonic Nanosensors." J.N. Anker, W.P. Hall, O.

Lyandres, N.C. Shah, J. Zhao, R.P. Van Duyne. *Nature Materials*, Vol. 7, pp. 442-453 (2008).

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"LSPR-Based Nanobiosensors." B. Sepulveda, P.C. Angelome, L.M. Lechuga, L.M. Liz-Marzán. *Nano Today*, Vol. 4, pp. 244-251 (2009).

"Nanofibrous Biologic Laminates Replicate the Form and Function of the Annulus Fibrosus." N.L. Nerurkar, B.M. Baker, S. Sen, E.E. Wible, D.M. Elliott, and R.L.

Mauck. *Nature Materials*, Vol. 8, pp. 986-992 (2009).

"Localized Surface Plasmon Resonance Sensors." K.M. Mayer and J.H. Hafner. *Chemical Reviews*, Vol. 111, pp. 3828-3857 (2011).

"Beating Cancer in Multiple Ways Using Nanogold." E.C. Dreaden, M.A. Mackey, X. Huang, B. Kang, and M.A. El-Sayed. *Chemical Society Reviews*, Vol. 40, pp. 3391-3404 (2011).

"Structural and Mechanical Differences Between Collagen and Homo- and Heterotrimers." S.W. Chang, S.J. Shefelbine, and M.J. Buehler. *Biophysical Journal*, Vol. 102, pp. 640-648 (2012).