Less is more in Medicine

SOPHISTICATED FORMS OF NANOTECHNOLOGY WILL FIND SOME OF THEIR FIRST REAL-WORLD APPLICATIONS IN BIOMEDICAL RESEARCH, DISEASE DIAGNOSIS AND, POSSIBLY, THERAPY

BY A. PAUL ALIVISATOS

The 1966 film *Fantastic Voyage* treated moviegoers to a bold vision of nanotechnology applied to medicine: through mysterious means, an intrepid team of doctors and their high-tech submarine were shrunk to minute size so that they could travel through the bloodstream of an injured patient and remove a life-threatening blood clot in his brain. In the past 35 years, great strides have been made in fabricating complex devices at ever smaller scales, leading some people to believe that such forms of medical intervention are possible and that tiny robots will soon be coursing through everyone's veins. Indeed, in some circles the idea is taken so seriously that worries have emerged about the dark side of such technology: Could self-replicating nanometer-scale automatons run amok and destroy the entire biological world?

In my view, shared by most investigators, such thoughts belong squarely in the realm of science fiction. Still, nanotechnology can potentially enhance biomedical research tools—for example, by providing new kinds of labels for experiments done to discover drugs or to reveal which sets of genes are active in cells under various conditions. Nanoscale devices could, moreover, play a part in quick diagnostic screens and in genetic tests, such as those meant to determine a person's susceptibility to different disorders or to reveal which specific genes are mutated in a patient's cancer. Investigators are also studying them as improved contrast agents for noninvasive imaging and as drug-delivery vehicles. The emerging technologies may not be as photogenic as a platelet-size Raquel Welch blasting away at a clot with a laser beam, but they are every bit as dramatic because, in contrast, the benefits they offer to patients and researchers are real.

HIGHLY MAGNIFIED VIALS contain solutions of quantum dots—semiconductor nanocrystals—of specific sizes. The precise size of a quantum dot determines the color it emits after exposure to light. By attaching different sizes of dots to different biological molecules, investigators can track the activities of many tagged molecules simultaneously.

The technologies may not be as photogenic as a minute Raquel Welch blasting away at a clot, but they are just as dramatic because their benefits are real.

How exactly can nanotechnology do all these things? The answer hinges on one's definitions. All of biology is arguably a form of nanotechnology. After all, even the most complicated creature is made up of tiny cells, which themselves are constructed of nanoscale building blocks: proteins, lipids, nucleic acids and other complex biological molecules. But by convention the term "nanotechnology" is usually restricted to artificial constructions made, say, from semiconductors, metals, plastic or glass. A few inorganic structures of nanometer scale—minute crystals, for instance—have already been commercialized, notably as contrast agents.

Magnetic Attraction

NATURE ITSELF provides a beautiful illustration of the usefulness of such inorganic crystals in a biological context: humble magnetotactic (magnetic-sensing) bacteria. Such organisms, which live in bodies of water and their muddy bottoms, thrive only at one depth in the water or sediment. Above this position, oxygen is too abundant for their liking; below, too scarce. A bacterium that drifts away from the right level must swim back, and so, like many of its cousins, the microbe wields a whiplike tail for propulsion. But how does the buoyant cell tell up from down when gravity has essentially no effect on it?

The answer is that this bacterium has fixed within it a chain of about 20 magnetic crystals that are each between 35 and 120 nanometers in diameter. Together these crystals constitute a miniature compass. Because the magnetic field of the earth is inclined in most places (it points not only north but also downward in the Northern Hemisphere and upward in the Southern), a magnetotactic bacterium can follow a magnetic field line up or down to its desired destination.

This compass is a marvel of natural nanoscale engineering. For one, it is made of the perfect material—either magnetite or greigite, both highly magnetic iron minerals. The use of multiple crystals is also no accident. At very small scales, the larger a magnetic particle is, the longer it stays magnetized. But if the particle becomes too large, it will spontaneously form two separate magnetic domains with oppositely directed magnetizations. Such a crystal has little overall magnetization and does not make for a very effective compass needle. By building its compass out of crystals that are of just the right size to exist as stable, single magnetic domains, the bacterium makes the best use of every bit of iron it lays down. Interestingly, when people design media for hard-disk storage, they follow exactly the same strategy, using magnetic nanocrystals that are of the proper size to be both stable and strong.

Artificial magnetic crystals of similar dimension might soon serve biomedical research in a novel way. Two groups, one in Germany and the other at my institution, the University of California at Berkeley, are exploring the use of magnetic nanoparticles to detect particular biological entities, such as microorganisms that cause disease.

Their method, like many of the techniques applied today, requires suitable antibodies, which bind to specific targets. The magnetic particles are affixed, as labels, to selected antibody molecules, which are then applied to the sample under study. To detect whether the antibodies have latched onto their target, an investigator applies a strong magnetic field (which temporarily magnetizes the particles) and then examines the specimen with a sensitive instrument capable of detecting the weak magnetic fields emanating from the probes. Labeled antibodies that have not docked to the sample tumble about so rapidly in solution that they give off no magnetic signal. Bound antibodies, however, are unable to rotate, and together their magnetic tags generate a readily detectable magnetic field.

Because the unbound probes produce no signal, this approach does away with the time-consuming washing steps usually required of such assays. The sensitivity demonstrated with this experimental technique is already better than with standard methods, and anticipated improvements in the apparatus should soon boost sensitivity by a factor of several hundred.

Despite these advantages, the magnetic method probably will not completely replace the widespread practice of labeling probes with a fluorescent tag, typically an organic molecule that glows with a characteristic hue when it is energized by light of a particular color. Colors are very useful in various diagnostic and research procedures, such as when more than one probe needs to be tracked.

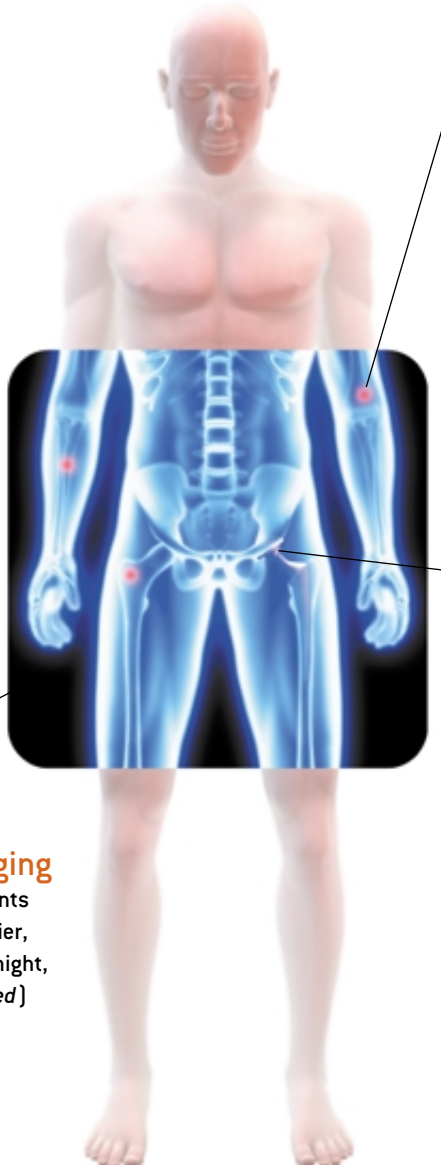
Overview/*Nanomedicine*

- Nanometer-scale objects made of inorganic materials can serve in biomedical research, disease diagnosis and even therapy.
- Biological tests measuring the presence or activity of selected substances become quicker, more sensitive and more flexible when certain nanoscale particles are put to work as tags, or labels.
- Nanoparticles could be used to deliver drugs just where they are needed, avoiding the harmful side effects that so often result from potent medicines.
- Artificial nanoscale building blocks may one day be used to help repair such tissues as skin, cartilage and bone—and they may even help patients to regenerate organs.

IMAGE BY FELICE FRANKEL, WITH TECHNICAL HELP FROM K. F. JENSEN, M. G. BAWENDI, C. MURRAY, C. KAGAN, B. DABBOUSI, J. RODRIGUEZ-VIEGO *M.I.T.* *(page 66)*

A GRAND PLAN FOR MEDICINE

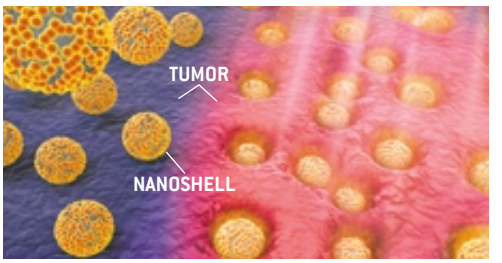
The National Nanotechnology Initiative includes among its goals, or "grand challenges," a host of futuristic improvements in the detection, diagnosis and treatment of disease. Some are depicted here. The goals, many of which are far from being realized, also feature new aids for vision and hearing, rapid tests for detecting disease susceptibility and responses to drugs, and tiny devices able to find problems—such as incipient tumors, infections or heart problems—and to relay the information to an external receiver or fix them on the spot.



1 GOAL: Improved Imaging

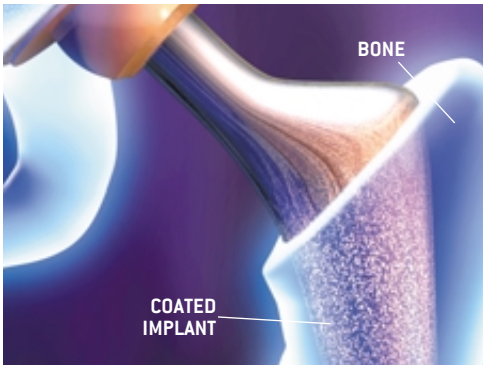
Improved or new contrast agents would detect problems at earlier, more treatable stages. They might, for instance, reveal tumors (*red*) only a few cells in size.

2 GOAL: New Ways to Treat Disease



Nanoparticles would deliver treatments to specifically targeted sites, including places that standard drugs do not reach easily. For example, gold nanoshells (*spheres*) that were targeted to tumors might, when hit by infrared light, heat up enough to destroy the growths.

3 GOAL: Superior Implants



Nanometer-scale modifications of implant surfaces would improve implant durability and biocompatibility. For instance, an artificial hip coated with nanoparticles might bond to the surrounding bone more tightly than usual, thus avoiding loosening.

JEFF JOHNSON *Hybrid Medical Animation*

The world of modern electronics is also full of light-emitting materials. Every CD player, for instance, reads the disc with light from a solid-state laser diode, which is made of an inorganic semiconductor. Imagine carving out a vanishingly small piece of that material, a scoop the size of a protein molecule. The result is a semiconductor nanocrystal, or, in the talk of the trade, a "quantum dot." Like nanoscale magnetic crystals, these minuscule dots have much to offer biomedical researchers.

As the name suggests, quantum dots owe their special properties to the weird rules of quantum mechanics, the same rules that restrict the electrons in atoms to certain discrete energy levels. An organic dye molecule absorbs only photons of light with just the right energy to lift its electrons from their quiescent state to one of the higher levels available to them. That is, the incident light must be exactly the right wavelength, or color, to do the job. The molecule will subsequently emit a photon when the electron falls back to a lower energy level. This phenomenon is quite different from what happens in bulk semiconductors, which allow electrons to occupy two broad bands of energy. Such materials can absorb photons in a broad range of colors (all those that have enough energy to bridge the gap between these two bands),

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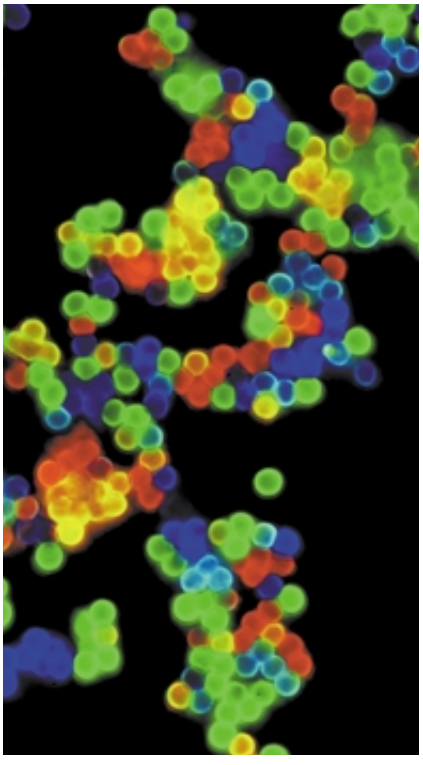
For therapy, one might encapsulate drugs within nanometer-scale packages that control the medicines' release in sophisticated ways.

but they emit light only at one specific wavelength, corresponding to the band-gap energy. Quantum dots are an intermediate case. Like bulk semiconductors, they absorb photons of all energies above the threshold of the band gap. But the wavelength of light a quantum dot emits—its color—depends very strongly on the dot's size. Hence, a single type of semiconducting material can yield an entire family of distinctly colored labels.

Physicists first studied quantum dots in the 1970s, thinking that they might one day fashion new electronic or optical devices. Few of the pioneering investigators had any idea that these objects could help diagnose disease or discover new drugs. And none of them would have dreamed that the first real-world applications of quantum dots would be in biology and medicine. Making quantum dots that would function properly in biological systems did indeed require years of research, but they are now a reality.



LATEX BEADS filled with quantum dots of single colors glow at nearly the same wavelengths as the dots themselves. Researchers have also loaded selections of different dots into single beads. Their aim is to create a huge variety of distinct labels for biological tests. (See also "Nano Bar Codes" in the box on the opposite page.)

SHUMING NIE *Indiana University*

The Rainbow Coalition

THE COMPANY LEADING the push to commercialize this technology, Quantum Dot Corporation, has licensed techniques developed in my lab and at the Massachusetts Institute of Technology, Indiana University, Lawrence Berkeley National Laboratory and the University of Melbourne in Australia. I helped to found this company, so my assessments may be biased, but I view the prospects for quantum dots as, well, bright.

Semiconductor nanocrystals have several advantages over conventional dye molecules. Small inorganic crystals can withstand significantly more cycles of excitation and light emission than can typical organic molecules, which soon decompose. And this stability allows investigators to track the goings-on in cells and tissues for longer intervals than can now be achieved. But the greatest benefit semiconductor nanocrystals offer is less subtle—they come in more colors.

Biological systems are very complex, and frequently several components must be observed simultaneously. Such tracking is difficult to achieve, because each organic dye must be excited with a different wavelength of light. But quantum dots make it possible to tag a variety of biological molecules, each with a crystal of a different size (and hence color). And because all of these crystals can be energized with a single light source, they can all be monitored at once.

This approach is being pursued actively, but quantum dots offer even more interesting possibilities. Imagine a small latex bead filled with a combination of quantum dots. The bead could, for instance, contain five different sizes of dots, or five colors, in a variety of concentrations. After the bead is illuminated, it will give off light, which when spread out by a prism will produce five distinct spectral lines with prescribed intensities—a spectral bar code, if you like. Such beads allow for an enormous number of distinct labels (billions, potentially), each of which could be attached, say, to DNA molecules composed of different sequences of genetic building blocks.

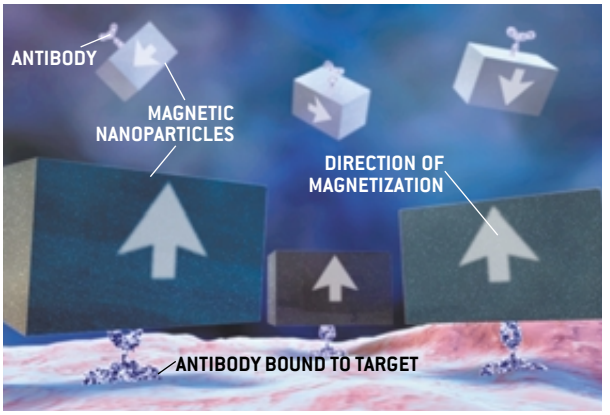
With these kinds of beads, technicians could easily compare the genetic material in a sample against a library of known DNA sequences, as might be done if an investigator wanted to find out which genes were active in certain cells or tissues. They would simply expose the sample to the full beaded library and read the spectral bar codes of the library DNAs that bind to sequences in the sample. Because binding takes place only when genetic sequences match closely (or more precisely, when one sequence complements the other), the results would immediately reveal the nature of the genetic material in the sample.

Semiconductor quantum dots should soon serve biomedical researchers in this way, but they are not the only nanostructures useful for optically sensing the genetic composition of biological specimens. Another example emerges from the work of Chad A. Mirkin and Robert L. Letsinger of Northwestern University, who recently developed an ingenious method to test for the presence of a specific genetic sequence in solution. Their scheme employs 13-nanometer gold particles studded with DNA.

The trick here is to use two sets of gold particles. The first set carries DNA that binds to one half of the target sequence; the second set carries DNA that binds to the other half. DNA with the complete target sequence readily attaches to both types of particles, linking them together. Because each particle has mul-

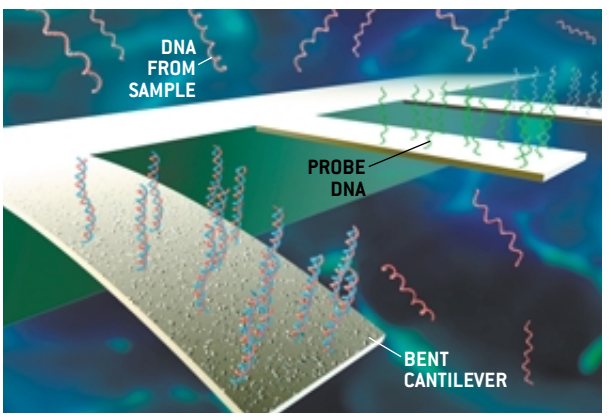
BIO-NANOTECH IN ACTION

The items here could one day enhance the speed and power of biomedical tests, such as those used to screen small samples of material for the presence of particular genetic sequences. For clarity, the images have not been drawn to scale.



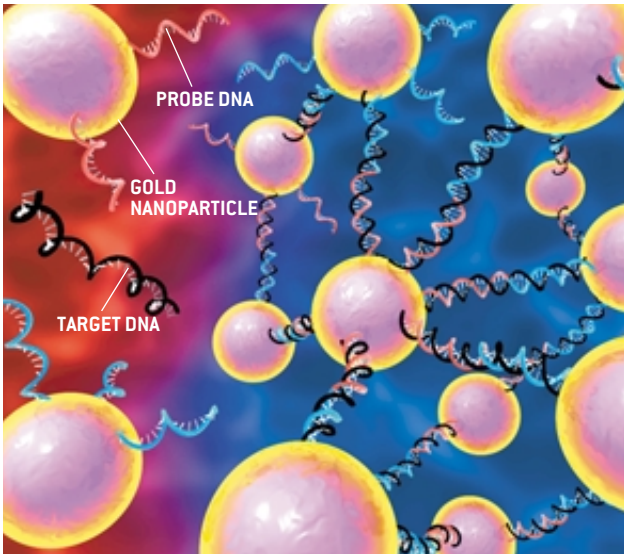
MAGNETIC TAGS

Many tests reveal the presence of a molecule or disease-causing organism by detecting the binding of an antibody to that target. When antibodies labeled with magnetic nanoparticles bind to their target on a surface (*foreground*), brief exposure to a magnetic field causes these probes collectively to give off a strong magnetic signal. Meanwhile unbound antibodies tumble about in all directions, producing no net signal. This last property makes it possible to read the results without first washing away any probes that fail to find their target.



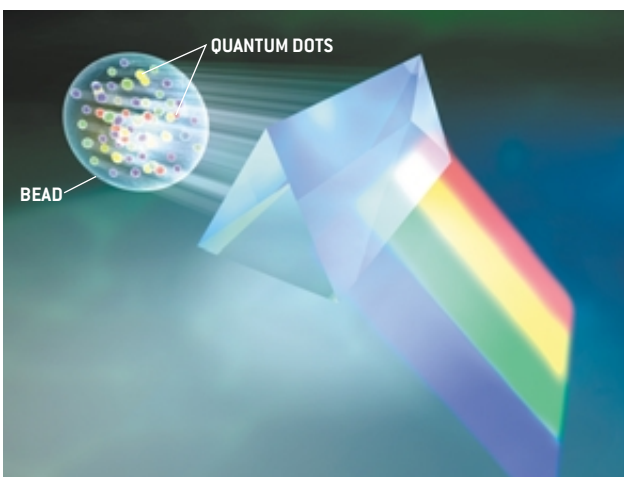
CLEVER CANTILEVERS

Biological samples can be screened for the presence of particular genetic sequences using small beams (cantilevers) of the type employed in atomic force microscopes. The surface of each cantilever is coated with DNA able to bind to one particular target sequence. A sample is then applied to the beams. Binding induces a surface stress, which bends the affected beams by nanometers—not much, but enough to reveal that the bent beams found their specific targets in a sample.



GOLD PARTICLES

Gold nanoparticles studded with short segments of DNA could form the basis of an easy-to-read test for the presence of a genetic sequence (*black*) in a sample under study. DNA complementary to half of such a sequence (*red*) is attached to one set of particles in solution, and DNA complementary to the other half (*blue*) is attached to a second set of particles. If the sequence of interest is present in the sample, it will bind to the DNA tentacles on both sets of spheres, trapping the balls in a dense web. This agglomeration will cause the solution to change color (*from red to blue*).



NANO BAR CODES

Latex beads filled with several colors of nanoscale semiconductors known as quantum dots can potentially serve as unique labels for any number of different probes. In response to light, the beads would identify themselves (and, thus, their linked probes) by emitting light that separates into a distinctive spectrum of colors and intensities—a kind of spectral bar code.

JEFF JOHNSON *Hybrid Medical Animation*

tiple DNA tentacles, bits of genetic material carrying the target sequence can glue many particles together. And when these gold specks aggregate, their optical properties shift markedly, changing the test solution from red to blue. Because the outcome of the test is easy to see without any instrumentation at all, such a system might be particularly useful for home DNA testing.

Feeling the Force

NO DISCUSSION of bio-nanotechnology would be complete without at least a brief mention of one of the hottest instruments in science today—the atomic force microscope. Such devices probe materials in the same way an old-fashioned phonograph reads the grooves in a record: by dragging a sharp point over the surface and detecting the resulting deflections. The tip of an atomic force microscope is, however, much finer than a phonograph needle, so it can sense far smaller structures. Regrettably, fabricating tips that are both fine and sturdy for these microscopes has proved to be quite difficult.

The solution appeared in 1996, when workers at Rice University affixed a slender carbon nanotube to the tip of an atomic force microscope, making it possible to probe samples just a few nanometers in size. In 1998 Charles M. Lieber and his co-workers at Harvard University applied this approach to probing biomolecules, providing a very high resolution means to explore complex biological molecules and their interactions at the most basic level.

But atomic force microscopy may soon be applied to more than just making fundamental scientific measurements. Last year James K. Gimzewski, then at the IBM Zurich Research Laboratory, showed with collaborators at IBM and the University of Basel that an array of micron-scale arms, or cantilevers, much like the ones employed in atomic force microscopes, could be used to screen samples for the presence of certain genetic sequences. They attached short strands of DNA to the tops of the cantilevers. When genetic material carrying a complementary sequence binds to the anchored strands, it induces a surface stress, which bends the cantilevers subtly—by just nanometers—but enough to be detected. By fabricating devices with many cantilevers and coating each with a different type of DNA, researchers should be able to test a biological sample rapidly for the presence of specific genetic sequences (as is now done routinely with gene chips) by nanomechanical means without the need for labeling.

This example, like the others described earlier, illustrates that the connections between nanotechnology and the practice of medicine are often indirect, in that much of the new work promises only better research tools or aids to diagnosis. But in some cases, nanoobjects being developed may themselves prove useful for therapy. One might, for instance, encapsulate drugs within nanometer-scale packages that control the medicines' release in sophisticated ways.

Consider a class of artificial molecules called organic dendrimers. Two decades ago Donald A. Tomalia of the Michigan Molecular Institute in Midland fashioned the first of these intriguing structures. A dendrimer molecule branches successively from inside to outside. Its shape resembles what one would get by taking many sprigs from a tree and poking them into a foam ball so that they shot out in every direction. Dendrimers are globular molecules about the size of a typical protein, but they do not come apart or unfold as easily as proteins do, because they are held together with stronger chemical bonds.

Like the lush canopies of mature trees, dendrimers contain voids. That is, they have an enormous amount of internal surface area. Interestingly, they can be tailored to have a range of different cavity sizes—spaces that are just perfect for holding therapeutic agents. Dendrimers can also be engineered to transport DNA into cells for gene therapy, and they might work more safely than the other leading method: genetically modified viruses.

Other types of nanostructures pos-

Petite Plumbing Jobs

Microfluidics enhances biomedical research

Most of the nanotechnologies now being developed for biomedical use take the form of minute objects immersed in comparatively large quantities of fluid, be it water, blood or a complex experimental concoction. But investigators are also building devices to manipulate tiny amounts of such liquids. These so-called microfluidic systems pump solutions through narrow channels, controlling the flow with diminutive valves and intense electric fields.

The ability to handle vanishingly small quantities of a solution in this way allows biomedical researchers to carry out many different experiments on what might be only a modest amount of sample—and to do so in an efficient manner, with hundreds of tests being performed, say, on the surface of a single glass slide. Microfluidic devices also offer researchers the means to carry out experiments that could not otherwise be done; for example, to deliver test solutions of specific compositions to different parts of a cell under study.

Although many of the components being created for these systems are considerably larger than a micron, some experimental devices include nanoscale dimensions. Notably, Harold G. Craighead's team at Cornell University has devised methods for sorting different sizes of DNA fragments in water according to how fast the fragments traverse passages measuring 100 nanometers across or travel through microchannels that repeatedly narrow to a depth of 75 to 100 nanometers. These or other nanofluidic devices could potentially increase the speed and reduce the costs of separating DNA molecules for sequencing and could in theory be adapted for separating proteins or other molecules. —*A.P.A.*

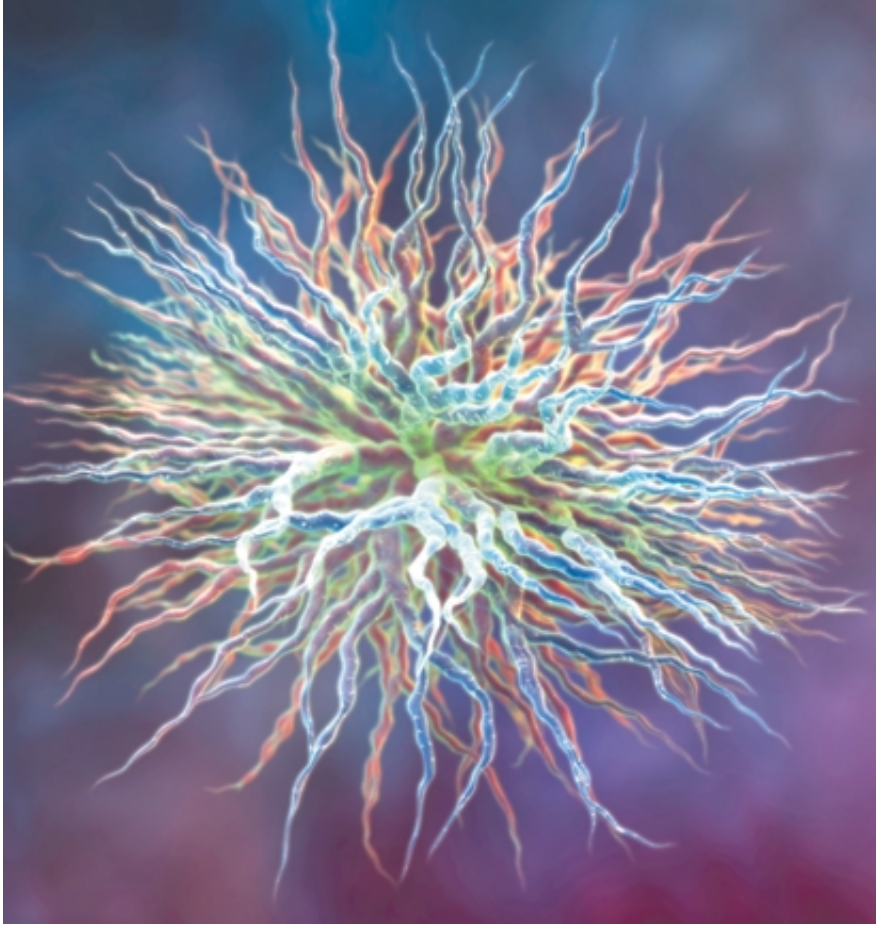
sess high surface area, and these too may prove useful for delivering drugs where they are needed. But dendrimers offer the greatest degree of control and flexibility. It may be possible to design dendrimers that spontaneously swell and liberate their contents only when the appropriate trigger molecules are present. This ability would allow a custom-made dendrimer to release its load of drugs in just the tissues or organs needing treatment.

Other drug-delivery vehicles on the horizon include hollow polymer capsules under study by Helmuth Möhwald of the Max Planck Institute of Colloids and Interfaces in Golm, Germany. In response to certain signals, these capsules swell or compress to release drugs. Also intriguing are so-called nanoshells, recently invented by the researchers at Rice.

Nanoshells are extremely small beads of glass coated with gold. They can be fashioned to absorb light of almost any wavelength, but nanoshells that capture energy in the near-infrared are of most interest because these wavelengths easily penetrate several centimeters of tissue. Nanoshells injected into the body can therefore be heated from the outside using a strong infrared source. Such a nanoshell could be made to deliver drug molecules at specific times by attaching it to a capsule made of a heat-sensitive polymer. The capsule would release its contents only when gentle heating of the attached nanoshell caused it to deform.

A more dramatic application envisioned for nanoshells is in cancer therapy. The idea is to link the gold-plated spheres to antibodies that bind specifically to tumor cells. Heating the nanoshells sufficiently would in theory destroy the cancerous cells, while leaving nearby tissue unharmed.

It is, of course, difficult to know for certain whether nanoshells will ultimately fulfill their promise. The same can be said for the myriad other minuscule devices being developed for medical use—among them, one-nanometer buckyballs made from just a few dozen carbon atoms. Yet it seems likely that some of the objects being investigated today will be serving doctors in the near future.



ORGANIC DENDRIMER, shown in an artist's conception, could be roughly the size of a protein molecule. Dendrimers harbor many internal cavities and are being eyed as drug-delivery vehicles.

Even more exciting is the prospect that physicians will make use of nanoscale building blocks to form larger structures, thereby mimicking the natural processes of biology. Such materials might eventually serve to repair damaged tissues. Research on these bold strategies is just beginning, but at least one enterprise already shows that the notion has merit: building scaffoldings on which to grow bone. Samuel I. Stupp of Northwestern is pioneering this approach using synthetic molecules that combine into fibers to which bone cells have a strong tendency to adhere.

What other marvels might the future hold? Although the means to achieve them are far from clear, sober nanotechnologists have stated some truly ambitious goals. One of the "grand challenges" of the National Nanotechnology Initiative is to find ways to detect cancerous tumors that are a mere few cells in size. Researchers also hope eventually to develop ways to regenerate not just bone or cartilage or skin but also more complex organs, using artificial scaffoldings that can guide the activity of seeded cells and can even direct the growth of a variety of cell types. Replacing hearts or kidneys or livers in this way might not match the fictional technology of *Fantastic Voyage*, but the thought that such medical therapies might actually become available in the not so distant future is still fantastically exciting. SA

MORE TO EXPLORE

Ultrasensitive Magnetic Biosensor for Homogeneous Immunoassay. Y. R. Chemla, H. L. Grossman, Y. Poon, R. McDermott, R. Stevens, M. D. Alper and J. Clarke in *Proceedings of the National Academy of Sciences USA,* Vol. 97, No. 27, pages 14268–14272; December 19, 2000.

The author's Web site is at www.cchem.berkeley.edu/~pagrp/

Information on using gold nanoparticles for DNA testing is available at www.chem.nwu.edu/~mkngrp/dnasubgr.html

Information on nanoshells is available at www.ece.rice.edu/~halas/

Information about quantum dots and their use in biomedicine is available at www.qdots.com

JEFF JOHNSON *Hybrid Medical Animation*