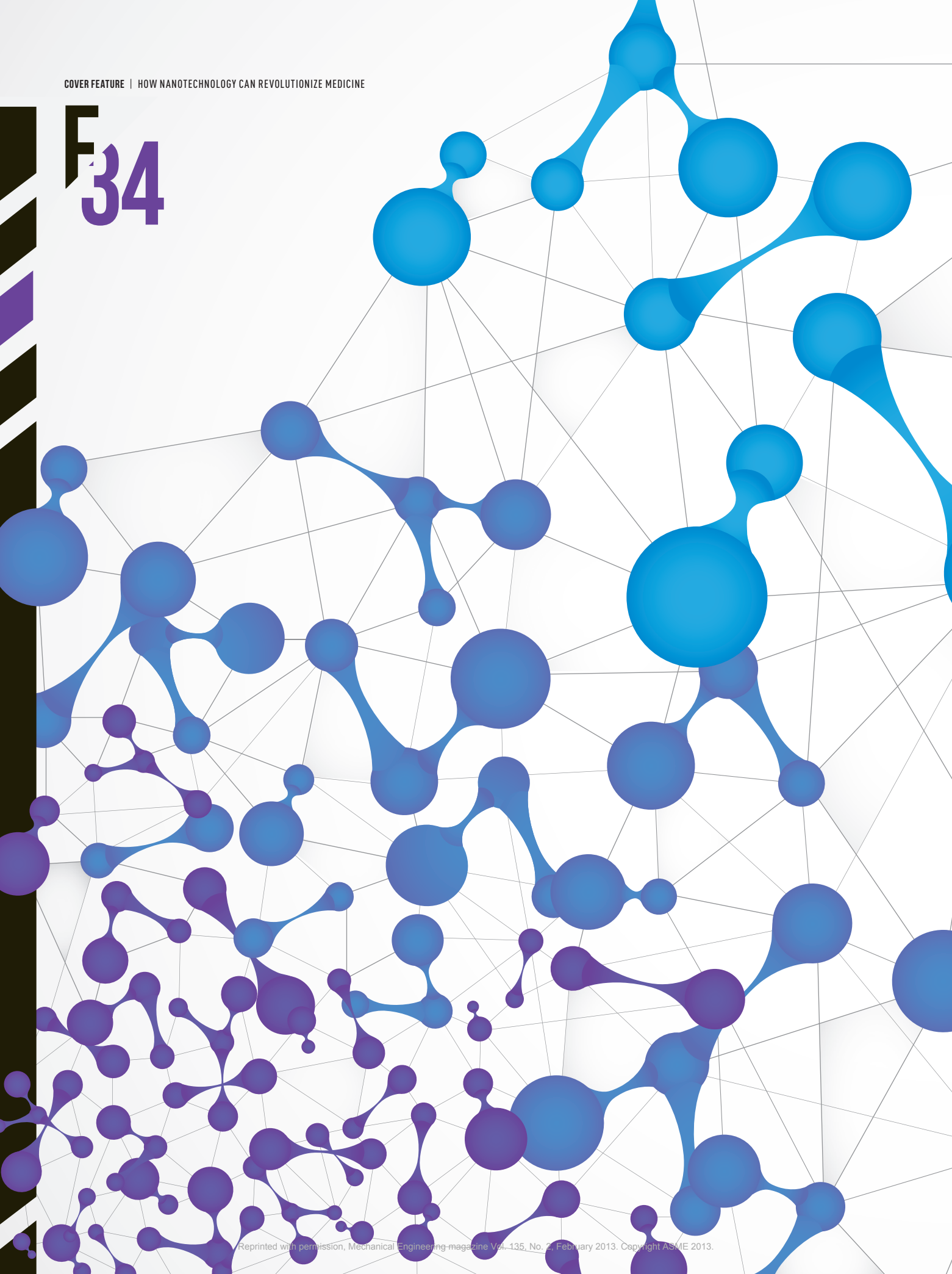


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BY ROHIT KARNIK &amp; ROBERT S. LANGER

# REBUILDING OURSELVES

## USHERING IN AN AGE OF SYNTHETIC ORGANS AND TARGETED MEDICINE.

**A**n American male born in 1850 could expect to live to be 38. An American female had a life expectancy of 41. Today, someone who died that young would seem to have had been cut down in middle age, and it would be true: The life expectancy for American men and women has doubled. People in developing and least developed nations also have added to their expected lifespans—extending them by nearly 25 years since 1950.

One reason for the increase came from advances in medical science and technology. In 1850, for instance, scientists were still debating whether microorganisms caused disease. Once accepted, germ theory gave researchers and physicians a new way to combat ill-

nesses. Practical applications included sophisticated vaccines and antibiotics, and such simple measures as washing hands and instruments before surgery and rigorously segregating drinking and waste water.

Today, we are in the midst of another explosion in medical technology. From powerful antivirals and chemotherapeutics to robotic surgery and genomic medicine, we are developing new ways to fight disease, carry out surgical procedures, and even transplant organs. Yet despite these advances—and sometimes because of them—we face new challenges, ranging from antibiotic-resistant bacteria to long waiting lists for scarce donor organs.

We are slowly disentangling the complex processes of life—such as why some cells form a liver and others a heart, and how cells transport some molecules (but not others) into their interior—and starting to learn practical ways to apply this knowledge. Nearly all our bodies' biochemical processes occur at the molecular level. Nanotechnology gives us a set of tools to influence and even control these interactions. For example, nanotechnology is already being used to create systems that seek out and attack the weaknesses of cancer and infections. In the lab today, we are developing nano-enabled systems that target only tumors, so we can potentially deliver medicine only where needed.

Nanotechnology also has the potential to provide many of the tools needed to create artificial organs. Over the past 15 years, researchers have made

## WE ARE STILL A LONG WAY FROM CREATING VIABLE REPLACEMENT TISSUES AND ORGANS. YET WE CAN STILL REAP SIGNIFICANT REWARDS EVEN FROM IMPERFECTLY GROWN ORGANS BY USING THEM TO TEST NEW MEDICINES.

enormous headway in growing pumping heart muscles and sections of functional kidneys, livers, and cartilage. Instead of waiting for transplants, physicians in the future may generate entire organs from patients' own healthy cells. The ability to regenerate body tissues and organs using a patient's own cells would also eliminate the need for a lifetime of medication to keep the body from rejecting the transplant.

### **SCAFFOLDS, TISSUES, AND ORGANS**

Building tissues and organs is an enormously complex task. For decades, researchers tried and failed to grow even the simplest functioning organs in Petri dishes. When injected in animals, cells nearly always failed to form tissue.

This problem has begun to yield to an onslaught of basic research. To greatly simplify, one approach is to start with cells, including stem cells, which have not yet begun to develop into tissue,

assemble them close together in three-dimensional structures, and prompt them with the right physical and chemical cues to develop properly. Researchers have made enormous progress over the past decade. They have, for example, produced neurons with axons and dendrites, elongated heart tissue that contracts, and functional liver tissue—all outside the body.

While researchers have generated thin sections of various tissues, replicating complex functional 3-D organs with specialized features and blood vessels to supply nutrients is still beyond our reach.

We face a number of basic challenges. We need to understand fully how cells differentiate into specialized cells and then organize themselves into the complex structures needed to carry out organ functions. We need to understand how mechanical and chemical stimuli in the environment around the cell influence the complex processes occurring within it. And we need to build on this knowledge to engineer 3-D structures that will enable cells to form tissue outside the body.

These 3-D structures, or scaffolds, combine multiple length scales with complex chemical signaling agents. They consist of intricately woven protein fibers (10 to 500 nanometers in diameter) that form a graded array of pores. Their surfaces are dotted with adhesive proteins to bind cells to them, and growth factors to prompt cell growth, shape, migration, and differentiation.

Reproducing natural scaffolds is not a simple task. One approach involves electrospinning. It uses an electric field to form a porous yet interconnected matrix of polymeric fibers. Researchers then add a combination of growth factors, enzymes, drugs, DNA, and RNA to signal the cells that populate the matrix.

Another approach is to create scaffolds through molecular self-assembly. This involves synthesizing molecules with different polarities or solubilities on opposite ends. Under the proper conditions, the molecules will form ordered structures spontaneously, the way a group of rod magnets will try to align north-to-south when placed on a table.

Using self-assembly, researchers have formed peptide-based 3-D gels and scaffolds with fiber



*This engineered nanoparticle encapsulates drugs within controlled released polymers that are coated with a stealth layer to sneak by the immune system.*

diameters as small as 10 nanometers and pore sizes ranging from 5 to 200 nanometers, far more graded than those produced by electrospinning. Cells can also be embedded in these gels during the fabrication process, which is bringing us closer to realizing complex synthetic tissue structures.

Scaffolds provide the 3-D structure needed to keep cells close to one another. They also hold the adhesive molecules needed to bind the cells to the scaffold and can provide cues that guide cell development. For example, adding neural growth factors to a self-assembled scaffold causes neural progenitor cells to differentiate rapidly into neurons rather than other types of cells.

The topography of the scaffold also influences cell development. On smooth surfaces, for example, epithelial cells grow rounded. On surfaces with nanoscale grooves and ridges, they stretch and elongate along the grooves. Many researchers believe the patterns generate anisotropic stresses in cells that cause elongation.

Scaffolds are the building blocks of tissue, but each type of tissue requires a scaffold engineered to its own individual requirements. The heart, for example, needs a dense, elastic scaffold that forces cells to elongate and couple mechanically with one another. Without it, they will not produce aligned contractions.

Bone, on the other hand, needs a composite scaffold that includes nanocrystals of hydroxyapatite, a calcium mineral, in a matrix of proteins. Shape counts. Scaffolds with needle-shaped hydroxyapatite crystals promote bone cell differentiation better than those with cylindrical or spherical crystals.

In addition to scaffold topography and chemistry, tissues present other challenges. For example, liver, pancreas, and kidney tissues secrete, absorb, and transport biochemicals. This requires a complex structure where cells face blood vessels on one side and ducts that deliver those chemicals on the other. While the polarized cells will self-organize to form ducts, it is difficult to get them to mimic the function of natural organs.

Nanotechnology can also help us alter natural designs. For example, in the laboratory, researchers have

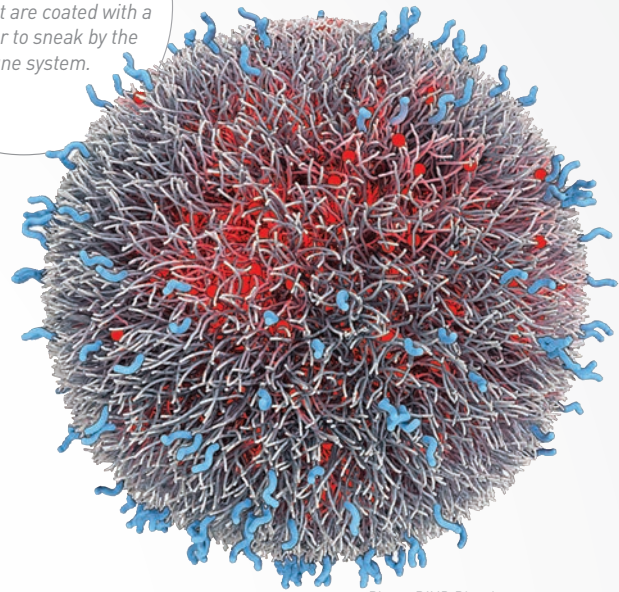


Photo: BIND Biosciences

shown that it may be possible to compensate for the limitations of human-made tissues by adding carbon nanotubes. They act like a reinforcement to give synthetic tissue the strength, stiffness, and viscoelastic performance of natural membranes. Carbon nanotubes have been shown to improve the electrical responsiveness of artificially grown neurons. We can potentially use electrically or optically active nanostructures to trigger certain processes or measure how tissues develop.

We are yet a long way from creating viable replacement tissues and organs. Still, we can reap significant rewards even from imperfectly grown organs by using them to test new medicines, because they go a long way beyond Petri-dish grown cells in mimicking the body's environment.

Many medicines look promising in Petri dishes but prove ineffective or too toxic in animals and humans. This is because we test them on individual cells, rather than on the complex living system of cells that make up tissues and organs in our bodies.

In 2012, the National Institutes of Health and the Defense Advanced Research Projects Agency launched a \$132 million program to create a human-on-a-chip, collections of tissues that mimic interdependent organ behavior reasonably well and react more naturally when testing drugs for efficacy and toxicity. This could improve initial drug screening, reduce the cost of prolonged animal testing, and speed the development of new medications.

#### NANO DESIGN OF MEDICINE

If tissue engineering represents the promise of the future, then nanomedicine is the emerging reality of the present. Already, more than 50 pharmaceutical companies in the United States are de-



# REBUILDING OURSELVES

veloping nano-enabled medicines to treat, image, or diagnose cancer, and the number is growing rapidly. Nanotherapeutics to treat pain and infectious diseases are under development as well.

Nanotechnology is well-suited for delivering medications. As the famous physician, Paracelsus, noted 500 years ago, all medicines are poisons in high enough doses. Cancer medications, for example, may destroy tumors, but they kill healthy cells as well. This is why chemotherapy often produces such devastating side effects. An ideal drug would target only the diseased organ or tissue in steady, sustained doses.

Nanomaterials promise a combination of approaches that may overcome some of these limitations on drug delivery. Researchers began applying nanotechnology to these problems in a simple way more than 25 years ago. The first clinically used nanoscale systems used liposomes, small spheres made from natural fatty acids, to encapsulate cancer medicines. Doxil, a liposome carrying the chemotherapeutic doxorubicin, became the first nanocarrier approved by U.S. Food and Drug Administration in 1995 for treatment of Kaposi's sarcoma.

Liposomes measure from tens of nanometers to roughly 100 nanometers in diameter, large enough to avoid clearance by the kidneys but small enough to avoid drawing too much attention from the body's immune system. They tend to remain in the blood stream until they slip through the leaky blood vessels that usually surround tumors. Since tumors have no way to remove liposomes effectively, they stay there until they release medication that the tumor cells will absorb.

Another notable nanomedicine is Abraxane by Abraxis BioScience (which has been acquired by Celgene) and approved by the U.S. Food and Drug Administration in 2005 to treat metastatic breast cancer. It consists of an anticancer drug, paclitaxel, which could not be used without a solvent that produces severe reactions in some patients. Abraxane eliminates the solvent by coating paclitaxel with albumin, a common protein that is soluble in water. The carrier enables paclitaxel to flow through the bloodstream and accumulate in the tumor.

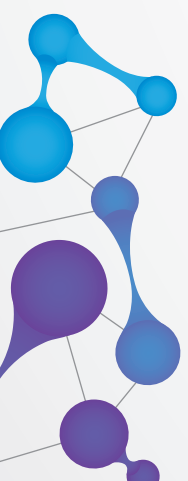
Doxil and Abraxane reduce side effects by encapsulating toxic drugs until they reach their destination, where they accumulate passively. Next-generation nanosystems take this one step further, peppering drug carrier surfaces with targeting ligands, chemical groups that bind to receptors that are found most frequently on tumor surfaces. Recently, BIND Biosciences performed Phase I clinical

trials of the first such targeted nanoparticle drug carrier to treat prostate cancer that uses this approach to deliver the drug preferentially to tumors.

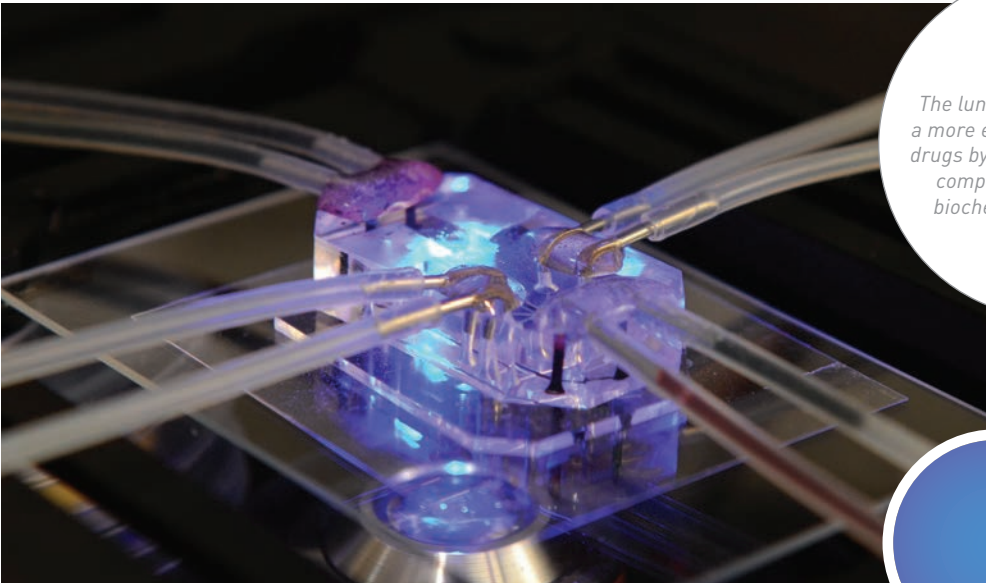
These rationally designed nanomedicines do a better job of encapsulating drugs, ranging from small molecules that would never otherwise pass through the kidneys to much larger peptides, proteins, DNA, and RNA. They can also encapsulate more than one medicine at a time, making treatments more synergistic.

Rationally designed nanocarriers can take advantage of size and shape. For example, spherical particles 100 to 200 nanometers in diameter tend to remain in circulation longest because they are not so easily filtered by the liver or spleen. And certain elongated nanocarriers do a good job navigating the body's immune system, slipping through narrow filters and pulling away from immune system cells the way a log catches a current as it floats down a rocky stream. They may also circulate in the bloodstream longer than spherical nanoparticles.

Moreover, certain types of cells have a preference for certain shapes, and ingest them more readily than others. These nanocarriers can also be coated with "stealth" layers, so white blood cells do not recognize and attack them. This also enables



## NEXT GENERATION NANO-SYSTEMS PEPPER DRUG CARRIER SURFACES WITH CHEMICAL GROUPS THAT TARGET AND BIND TO RECEPTORS THAT ARE FOUND MOST FREQUENTLY ON TUMOR SURFACES.



*The lung-on-a-chip provides a more efficient way to screen drugs by mimicking the body's complex mechanical and biochemical interactions.*

medications to remain in the bloodstream longer.

Nanotechnology also enables new types of therapies that do not use drugs. Instead, we can engineer targeted nanoparticles to absorb light or electromagnetic radiation, and locally heat tissue to kill it. Such particles could concentrate in diseased tissues, and could be activated without harming healthy tissues. This would enable physicians to treat infections and tumors that resist medication and are difficult to remove surgically.

Nanoparticles could also be used as diagnostics. For example, T2 Biosystems is commercializing a magnetic nanoparticle-based platform to rapidly diagnose bacterial and fungal infections without sample purification.

### REAPING THE REWARDS

Nanomedicine is rapidly moving into the mainstream and is poised to increasingly influence treatment of diseases such as cancer. The work of researchers has moved from laboratories to new startups and established pharmaceutical companies. And because concepts such as nanocarriers provide a flexible platform technology, they should prove easy to adapt to new breakthroughs as we unravel the biochemical secrets of our bodies.

In terms of tissue and organ engineering, we have come a long way from the days when we had little control over the growth and development of cells in Petri dishes. Today, outside the body, researchers can create heart muscle that contracts, fully differentiated nerve cells, and liver

tissues with complex structures.

To be sure, we have a long way to go before we can grow fully functional 3-D organs with blood vessels, ducts, and other specialized structures running through their interior, or

before we can send pharmaceuticals to kill individual disease-causing cells. But we expect to reap great rewards — and soon — as we apply nanotechnology to medicine. Nanocarriers have not yet achieved their full potential, but they are already prolonging lives and fighting infection and cancer. We may not need fully functional organ tissues to speed drug testing with organs on a chip.

Nature had billions of years to learn how to develop organs and the body's biochemical systems. But the tools of nanotechnology have given us the ability to begin to understand these designs, and the potential to adapt them to improving human life. And perhaps, one day in the not too distant future, no one will die waiting for an organ transplant. **ME**

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