GROWING NEW ORGANS

Researchers have taken the first steps toward creating semisynthetic, living organs that can be used as human replacement parts. **By David J. Mooney and Antonios G. Mikos**

EVERY DAY thousands of people of all ages are admitted to hospitals because of the malfunction of some vital organ. Because of a dearth of transplantable organs, many of these people will die. In perhaps the most dramatic example, the American Heart Association reports that only 2,300 of the 40,000 Americans who needed a new heart in 1997 got one. Lifesaving livers and kidneys likewise are scarce, as is skin for burn victims and others with wounds that fail to heal. It can sometimes be easier to repair a damaged automobile than the vehicle's driver because the former may be rebuilt using spare parts, a luxury that human beings simply have not enjoyed.

An exciting new strategy, however, is poised to revolutionize the treatment of patients who need new vital structures: the creation of man-made tissues or organs, known as neo-organs. In one scenario, a tissue engineer injects or places a given molecule, such as a growth factor, into a wound or an organ that requires regeneration. These molecules cause the patient's own cells to migrate into the wound site, turn into the right type of cell and regenerate the tissue. In the second, and more ambitious, procedure, the patient receives cells—either his or her own or those of a donor—that have been harvested previously and incorporated into three-dimensional scaffolds of biodegradable polymers, such as those used to make dissolvable sutures. The entire structure of cells and scaffolding is transplanted into the wound site, where the cells replicate, reorganize and form new tissue. At the same time, the artificial polymers break down, leaving only a completely natural final product in the body—a neo-organ. The creation of neo-organs applies the basic knowledge gained in biology over the past few decades to the problems of tissue and organ reconstruction, just as advances in materials science make possible entirely new types of architectural design.

Science-fiction fans are often confronted with the concept of tissue engineering. Various television programs and movies have pictured individual organs or whole people (or aliens) growing from a few isolated cells in a vat of some powerful nutrient. Tissue engineering does not yet rival these fictional presentations, but a glimpse of the future has already arrived. The creation of tissue for medical use is already a fact, to a limited extent, in hospitals across the U.S. These groundbreaking applications involve fabricated skin, cartilage, bone, ligament and tendon and make musings of "off-the-shelf" whole organs seem less than far-fetched.

Indeed, evidence abounds that it is at least theoretically possible to engineer large, complex organs such as livers, kidneys, breasts, bladders and intestines, all of which include many different kinds of cells. The proof can be found in any expectant mother's womb, where a small group of undifferentiated cells finds the way to develop into a complex individual with multiple organs and tissues with vastly different properties and functions. Barring any unforeseen impediments, teasing out the details of the process by which a liver becomes a liver, or a lung a lung, will eventually allow researchers to replicate that process.

A PINCH OF PROTEIN

Cells behave in predictable ways when exposed to particular biochemical factors. In the simpler technique for growing new tissue, the engineer exposes a wound or damaged organ to factors that act as proponents of healing or regeneration. This concept is based on two key observations, in bones and in blood vessels.

In 1965 Marshall R. Urist of the University of California at Los Angeles demonstrated that new, bony tissue would form in animals that received implants of powdered bone. His observation led to the isolation of the specific proteins (the bone morphogenetic proteins, or BMPs) responsible for this activity and to the determination of the DNA sequences of the relevant genes. A number of biotechnology companies subsequently began to produce large quantities of recombinant human BMPs; the genes

GRANT JERDING

coding for BMPs were inserted into mammalian cell lines that then produced the proteins.

Various clinical trials are under way to test the ability of these bone growth promoters to regenerate bony tissue. Applications of this approach that are currently being tested include healing acute bone fractures caused by accidents and boosting the regeneration of diseased periodontal tissues. Creative BioMolecules in Hopkinton, Mass., recently completed clinical trials showing that BMP-7 does indeed help heal severe bone fractures. This trial followed 122 patients with leg fractures in which the sections failed to rejoin after nine months. Patients whose healing was encouraged by BMP-7 did as well as those who received a surgical graft of bone harvested from another part of their body.

A critical challenge in engineering neo-organs is feeding each and every cell. Tissues more than a few millimeters thick require blood vessels to grow into them and supply the necessary nutrients. Fortunately, investigations by Judah Folkman have shown that cells already in the body can be coaxed into producing new blood vessels. Folkman, a cancer researcher at Harvard Medical School's Children's Hospital, recognized this possibility almost three decades ago in studies aimed, ironically, at the prevention of cellular growth in the form of cancerous tumors.

Folkman perceived that developing tumors need to grow their own blood vessels to supply themselves with nutrients. In 1972 he proposed that specific molecules could be used to inhibit such vessel growth, or angiogenesis, and perhaps starve tumors. (This avenue of attack against cancer became a major news story in 1998.) Realizing that other molecules would undoubtedly abet angiogenesis, he and others have subsequently identified a number of factors in each category.

It is theoretically possible to **engineer organs** such as **livers, kidneys, breasts** and **intestines.**

That work is now being exploited by tissue engineers. Many angiogenesis-stimulating molecules are commercially available in recombinant form, and animal studies have shown that such molecules promote the growth of new blood vessels that bypass blockages in, for example, the coronary artery. Small-scale trials are also under way to test this approach in the treatment of similar conditions in human subjects.

Scientists must surmount a few obstacles, however, before drugs that promote tissue and organ formation become commonplace. To date, only the factors responsible for bone and blood vessel growth have been characterized. To regenerate other organs, such as a liver, for example, the specific molecules for their development must be identified and produced reliably.

An additional, practical issue is how best to administer the substances that would shape organ regeneration. Researchers must an-

The human body may be more than a sum of parts, but replacing failing parts should help to extend and improve life.

PRASAD SHASTRI AND IVAN MARTIN *Massachusetts Institute of Technology*

Synthetic polymer scaffold in the shape of a nose (*left*) is "seeded" with cells called chondrocytes that replace the polymer with cartilage over time (*right*) to make a suitable implant.

swer these questions: What specific concentrations of the molecules are needed for the desired effect? How long should the cells be exposed? How long will the factors be active in the body? Certainly multiple factors will be needed for complex organs, but when exactly in the development of the organ does one factor need to replace another? Controlled drug-delivery technology such as transdermal patches developed by the pharmaceutical industry will surely aid efforts to resolve these concerns.

In particular, injectable polymers may facilitate the delivery of bioactive molecules where they are needed, with minimal surgical intervention. Michael J. Yaszemski of the Mayo Clinic, Alan W. Yasko of the M. D. Anderson Cancer Center in Houston and one of us (Mikos) are developing new injectable biodegradable polymers for orthopedic applications. The polymers are moldable, so they can fill irregularly shaped defects, and they harden in 10 to 15 minutes to provide the reconstructed skeletal region with mechanical properties similar to those of the bone they replace. These polymers subsequently degrade in a controlled fashion, over a period of weeks to months, and newly grown bone fills the site.

We have also been studying the potential of injectable, biodegradable hydrogels—gelatinlike, water-filled polymers—for treating dental defects, such as poor bonding between teeth and the underlying bone, through guided bone regeneration. The hydrogels incorporate molecules that both modulate cellular function and induce bone formation; they provide a scaffold on which new bone can grow, and they minimize the formation of scar tissue within the regenerated region.

An intriguing variation of more conventional drug delivery has been pioneered by Jeffrey F. Bonadio, Steven A. Goldstein and their co-workers at the University of Michigan. (Bonadio is now at Selective Genetics in San Diego.) Their approach combines the concepts of gene therapy and tissue engineering. Instead of administering growth factors directly, they insert genes that encode those molecules. The genes are part of a plasmid, a circular piece of DNA constructed for this purpose. The surrounding cells take up the DNA and treat it as their own. They turn into tiny factories, churning out the factors coded for by the plasmid. Because the inserted DNA is free-floating, rather than incorporated into the cells' own DNA, it eventually degrades and the product ceases to be synthesized. Plasmid inserts have successfully promoted bone regrowth in animals; the duration of their effects is still being investigated.

One of us (Mooney), along with Lonnie D. Shea and our other aforementioned Michigan colleagues, recently demonstrated with animals that three-dimensional biodegradable polymers spiked with plasmids will release that DNA over extended periods and simultaneously serve as a scaffold for new tissue formation. The DNA finds its way into adjacent cells as they migrate into the polymer scaffold. The cells then express the desired proteins. This technique makes it possible to control tissue formation more precisely; physicians might one day be able to manage the dose and time course of molecule production by the cells that take up the DNA and deliver multiple genes at various times to promote tissue formation in discrete stages.

A DASH OF CELLS

Promoting tissue and organ development via growth factors is obviously a considerable step forward. But it pales in comparison to the ultimate goal of the tissue engineer: the creation from scratch of whole neo-organs. Science fiction's conception of prefabricated "spare parts" is slowly taking shape in the efforts to transplant cells directly to the body that will then develop into the proper bodily component. The best way to sprout organs and tissues is still to rely on the body's own biochemical wisdom; the appropriate cells are transferred, in a three-dimensional matrix, to the desired site, and growth unfolds within the person or organism rather than in an external, artificial environment. This approach, pioneered by Ioannis V. Yannas, Eugene Bell and Robert S. Langer of the Massachusetts Institute of Technology, Joseph P. Vacanti of Harvard Medical School and others in the 1970s and 1980s, is now actually in use in some patients, notably those with skin wounds or cartilage damage.

The usual procedure entails the multiplication of isolated cells in culture. These cells are then used to seed a matrix, typically one consisting of synthetic polymers or collagen, the protein that forms the natural support scaffolding of most tissues. In addition to merely delivering the cells, the matrix both creates and main-

Sufficient knowledge of how **organs naturally develop** should eventually make true **"off-the-shelf" organs** a reality.

ADVANCED TISSUE SCIENCES, INC.

Cartilaginous ear awaits a useful incarnation as a replacement body part. An ear-shaped polymer mold and cartilage-secreting cells enabled researchers to produce the "bioartificial" structure in the laboratory.

tains a space for the formation of the tissue and guides its structural development. Once the developmental rules for a given organ or tissue are known, any of those entities could theoretically be grown from a sample of starter cells. (A sufficient understanding of the developmental pathways should eventually allow the transfer of this procedure from the body to the laboratory, making true off-the-shelf organs possible. A surgeon could implant these immediately in an emergency situation—an appealing notion, because failing organs can quickly lead to death—instead of waiting weeks or months to grow a new organ in the laboratory or to use growth factors to induce the patient's own body to grow the tissues.)

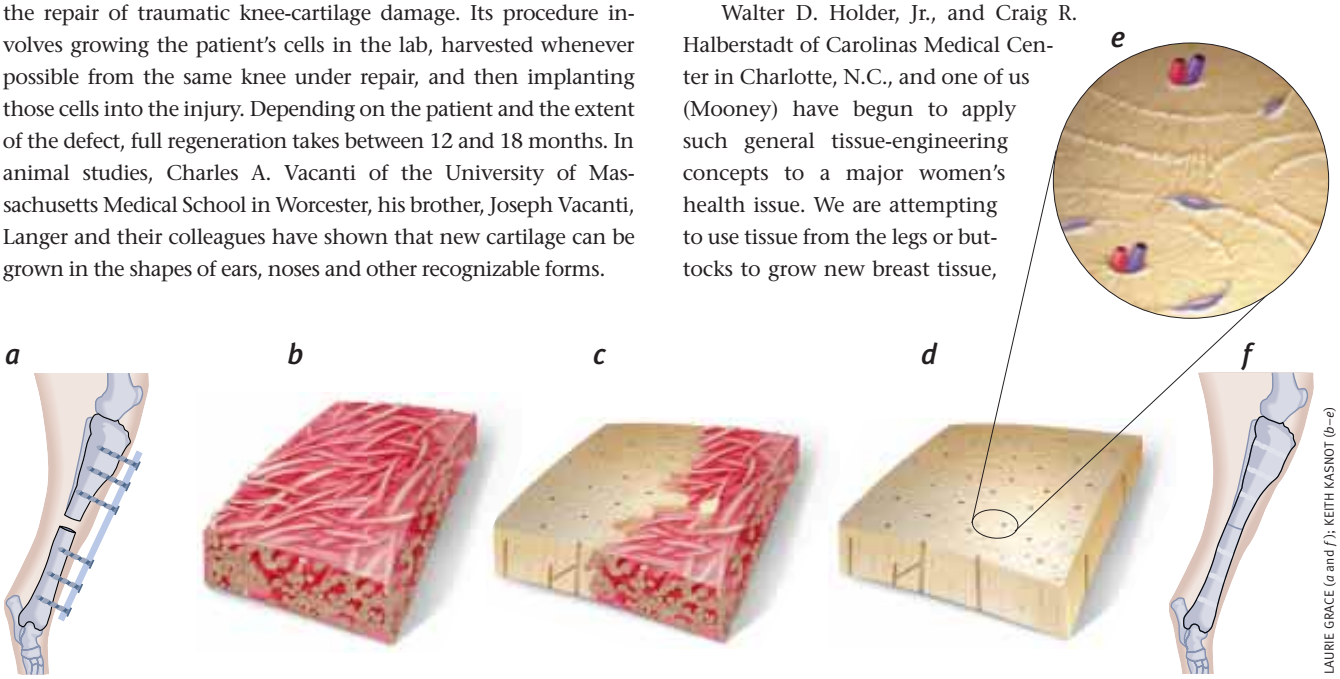
In the case of skin, the future is here. The U.S. Food and Drug Administration has already approved a living skin product—and others are now in the regulatory pipeline. The need for skin is acute: every year 600,000 Americans suffer from diabetic ulcers, which are particularly difficult to heal; another 600,000 have skin removed to treat skin cancer; and between 10,000 and 15,000 undergo skin grafts to treat severe burns.

The next tissue to be widely used in humans will most likely be cartilage for orthopedic, craniofacial and urological applications. Currently available cartilage is insufficient for the half a million operations annually in the U.S. that repair damaged joints and for the additional 28,000 face and head reconstructive surgeries. Cartilage, which has low nutrient needs, does not require growth of new blood vessels—an advantage for its straightforward development as an engineered tissue.

Genzyme Tissue Repair in Cambridge, Mass., has received FDA approval to engineer tissues derived from a patient's own cells for the repair of traumatic knee-cartilage damage. Its procedure involves growing the patient's cells in the lab, harvested whenever possible from the same knee under repair, and then implanting those cells into the injury. Depending on the patient and the extent of the defect, full regeneration takes between 12 and 18 months. In animal studies, Charles A. Vacanti of the University of Massachusetts Medical School in Worcester, his brother, Joseph Vacanti, Langer and their colleagues have shown that new cartilage can be grown in the shapes of ears, noses and other recognizable forms.

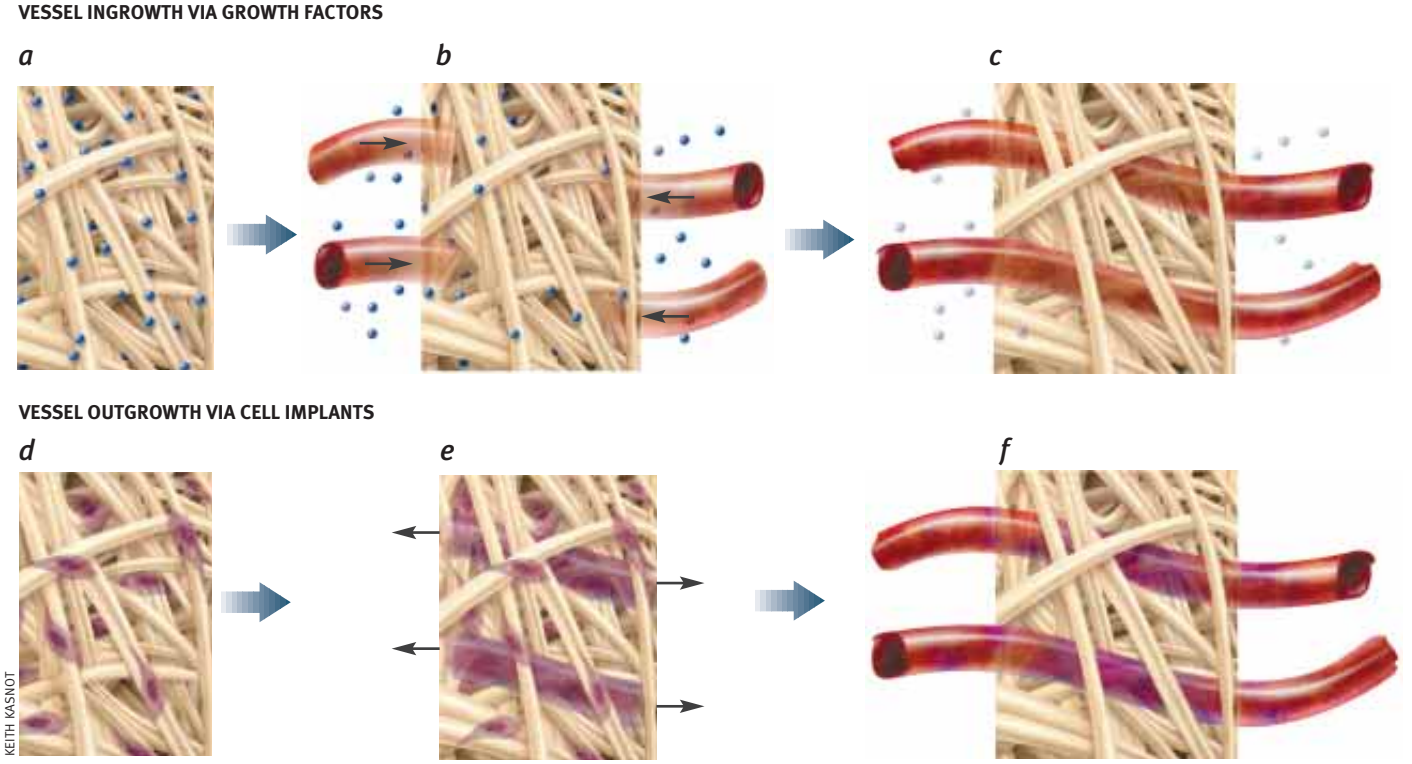
The relative ease of growing cartilage has led Anthony J. Atala of Harvard Medical School's Children's Hospital to develop a novel approach for treating urological disorders such as incontinence. Reprogenesis in Cambridge, Mass., which supports Atala's research, is testing whether cartilage cells can be removed from patients, multiplied in the laboratory and used to add bulk to the urethra or ureters to alleviate urinary incontinence in adults and bladder reflux in children. These conditions are often caused by a lack of muscle tone that allows urine to flow forward unexpectedly or, in the childhood syndrome, to back up. Currently patients with severe incontinence or bladder reflux may undergo various procedures, including complex surgery. Adults sometimes receive collagen that provides the same bulk as the cartilage implant, but collagen eventually degrades. The new approach involves minimally invasive surgery to deliver the cells and grow the new tissue.

Walter D. Holder, Jr., and Craig R. Halberstadt of Carolinas Medical Center in Charlotte, N.C., and one of us (Mooney) have begun to apply such general tissue-engineering concepts to a major women's health issue. We are attempting to use tissue from the legs or buttocks to grow new breast tissue,



LAURIE GRACE (*a* and *f*); KEITH KASNOT (*b*–*e*)

New bone grows to fill a space between two bone segments. A dog leg bone with a missing section is held in place with braces (*a*). A polymer scaffold primed with bone growth–promoting proteins (*b*) fills in the gap. The scaffold is slowly infiltrated by new bone (*c*) and ultimately gets completely replaced (*d*). The cells (*e*) have their own blood supply (*red and blue vessels*). After several months the leg bone has healed completely (*f*).



Vascularization of new, implanted tissue can be accomplished in two ways. Vessels from the surrounding tissue can be induced to infiltrate the tissue implant. Such vessel growth is promoted by including growth factors (*blue dots*) in the polymer scaffold of the insert (*a*). These factors diffuse into the local environment, where they encourage existing blood vessels to grow into the polymer (*b*). Ultimately, cells growing in from both sides knit together to form a continuous blood vessel (*c*). Vessels may also grow from within a polymer scaffold if that scaffold is seeded (*d*) with endothelial cells (*purple*). The cells will proliferate within the polymer matrix and grow outward toward the natural tissue (*e*). These new vessels combine with existing blood vessels (*red*) to create a continuous vessel (*f*).

to replace that removed in mastectomies or lumpectomies. We propose to take a biopsy of the patient's tissue, isolate cells from this biopsy and multiply these cells outside the body. The woman's own cells would then be returned to her in a biodegradable polymer matrix. Back in the body, cell growth and the deterioration of the matrix would lead to the formation of completely new, natural tissue. This process would create only a soft-tissue mass, not the complex system of numerous cell types that makes up a true breast. Nevertheless, it could provide an alternative to current breast prostheses or implants.

Optimism for the growth of large neo-organs of one or more cell types has been fueled by success in animal models of human diseases. Mikos has demonstrated that new bone tissue can be grown by transplanting cells taken from bone marrow and growing them on biodegradable polymers. Transplantation of cells to skeletal defects makes it possible for cells to produce factors locally, offering a new means of delivery for growth-promoting drugs.

RECIPES FOR THE FUTURE

In any system, size imposes new demands. As previously noted, tissues of any substantial size need a blood supply. To address that requirement, engineers may need to transplant the right cell types together with drugs that spur angiogenesis. Molecules that promote blood vessel growth could be included in the polymers used as transplant scaffolds. Alternatively, we and others have proposed that it may be possible to create a blood vessel network within an engineered organ prior to transplantation by incorporating cells that will become blood vessels within the scaffold matrix. Such engineered blood vessels would then need only to connect to surrounding vessels for the engineered tissue to develop a blood supply.

In collaboration with Peter J. Polverini of Michigan, Mooney has shown that transplanted blood vessel cells will indeed form such connections and that the new vessels are a blend of both implanted and host cells. But this technique might not work when transplanting engineered tissue into a site where blood vessels have been damaged by cancer therapy or trauma. In such situations, it may be necessary to propagate the tissue first at another site in the body where blood vessels can more readily grow into the new structure. Mikos collaborates with Michael J. Miller of the M. D. Anderson Cancer Center to fabricate vascularized bone for reconstructive surgery using this approach. A jawbone, for instance, could be grown connected to a well-vascularized hipbone for an oral cancer patient who has received radiation treatments around the mouth that damaged the blood supply to the jawbone.

On another front, engineered tissues typically use biomaterials

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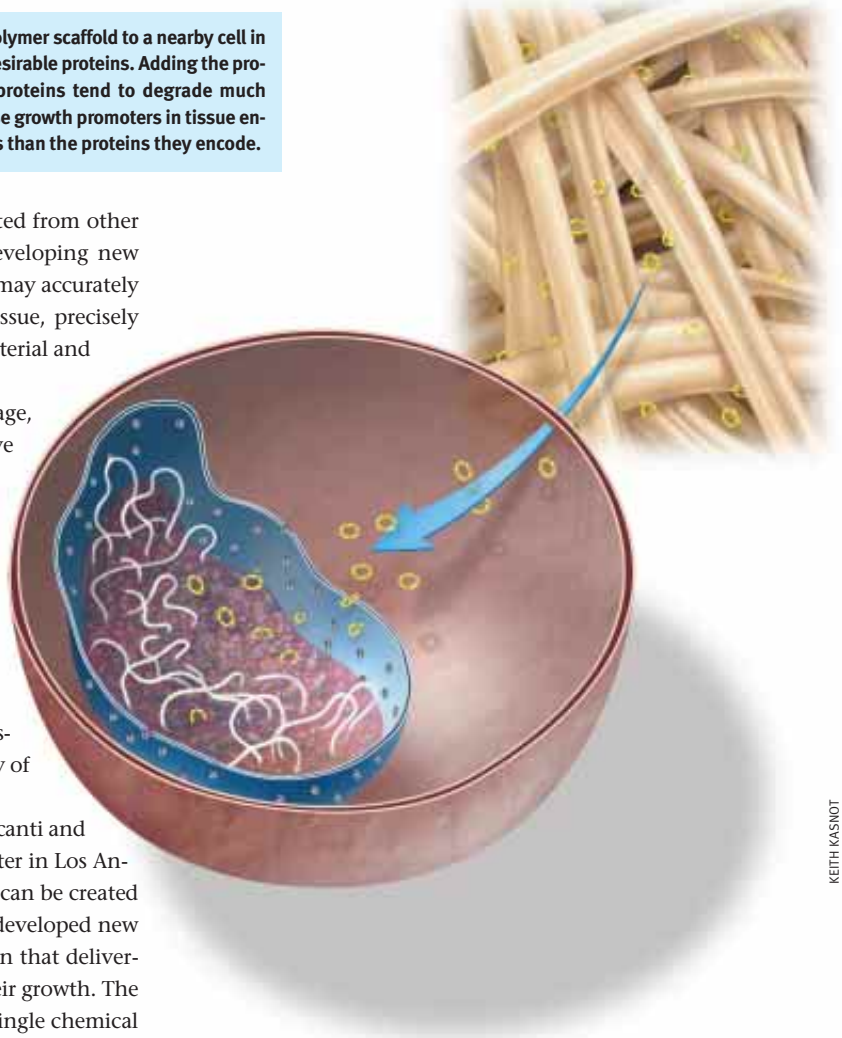

KEITH KASNOT

Plasmids, circlets of DNA (*yellow*), find their way from a polymer scaffold to a nearby cell in the body, where they serve as the blueprints for making desirable proteins. Adding the proteins themselves would be less effective because the proteins tend to degrade much faster than the plasmids do. Researchers attempting to use growth promoters in tissue engineering may thus find it more reliable to insert plasmids than the proteins they encode.

that are available from nature or that can be adapted from other biomedical uses. We and others, however, are developing new biodegradable materials specific to this task. These may accurately determine the size and shape of an engineered tissue, precisely control the function of cells in contact with the material and degrade at rates that optimize tissue formation.

Structural tissues, such as skin, bone and cartilage, will most likely continue to dominate the first wave of success stories, thanks to their relative simplicity. The holy grail of tissue engineering, of course, remains complete internal organs. The liver, for example, performs many chemical reactions critical to life, and more than 30,000 people die every year because of liver failure. It has been recognized since at least the time of the ancient Greek legend of Prometheus that the liver has the unique potential to regenerate partially after injury, and tissue engineers are now trying to exploit this property of liver cells.

A number of investigators, including Joseph Vacanti and Achilles A. Demetriou of Cedars-Sinai Medical Center in Los Angeles, have demonstrated that new liverlike tissues can be created in animals from transplanted liver cells. We have developed new biomaterials for growing liverlike tissues and shown that delivering drugs to transplanted liver cells can increase their growth. The new tissues grown in all these studies can replace single chemical functions of the liver in animals, but the entire function of the organ has not yet been replicated.

H. David Humes of Michigan and Atala are using kidney cells to make neo-organs that possess the filtering capability of the kidney. In addition, recent animal studies by Joseph Vacanti's group have demonstrated that intestine can be grown—within the abdominal cavity—and then spliced into existing intestinal tissue. Human versions of these neointestines could be a boon to patients suffering from short-bowel syndrome, a condition caused by birth defects or trauma. This syndrome affects physical development because of digestion problems and insufficient nutrient intake. The only available treatment is an intestinal transplant, although few patients actually get one, again because of the extreme shortage of donated organs. Recently Atala has also demonstrated in animals that a complete bladder can be formed with this approach and used to replace the native bladder.

Even the heart is a target for regrowth. A group of scientists headed by Michael V. Sefton at the University of Toronto recently began an ambitious project to grow new hearts for the multitude of people who die from heart failure every year. It will very likely take scientists 10 to 20 years to learn how to grow an entire heart, but tissues such as heart valves and blood vessels may be available sooner. Indeed, several companies, including Advanced Tissue Sciences in La Jolla, Calif., and Organogenesis in Canton, Mass., are attempting to develop commercial processes for growing these tissues.

Prediction, especially in medicine, is fraught with peril. A safe way to prophesy the future of tissue engineering, however, may be to weigh how surprised workers in the field would be after being told of a particular hypothetical advance. Tell us that completely functional skin constructs will be available for most medical uses within five years, and we would consider that reasonable. Inform us that fully functional, implantable livers will be here in five years, and we would be quite incredulous. But tell us that this same liver will be here in, say, 30 years, and we might nod our heads in sanguine acceptance—it sounds possible. Ten millennia ago the development of agriculture freed humanity from a reliance on whatever sustenance nature was kind enough to provide. The development of tissue engineering should provide an analogous freedom from the limitations of the human body.

ABOUT THE AUTHORS

DAVID J. MOONEY and ANTONIOS G. MIKOS have collaborated for eight years. Mooney has been on the faculty at the University of Michigan since 1994, where he is associate professor of biologic and materials sciences and of chemical engineering. Mikos is associate professor of bioengineering and of chemical engineering at Rice University. This article also appeared in *Scientific American* in April 1999.