PROGRESS IN TISSUE

By Ali Khademhosseini, Joseph P. Vacanti and Robert Langer

Pioneers in building living tissue report important advances over the past decade

KEY CONCEPTS

- Efforts to build living tissue replacements have progressed over the past decade, and some simple engineered tissues are already used in humans.
- Advances have come from a greater understanding of cell behavior and sophisticated new building materials.
- More tissue-engineered products are close to commercial readiness but must undergo the complex regulatory scrutiny given to living materials.

—The Editors

When two of us (Langer and Vacanti) last wrote in this magazine 10 years ago about prospects for tissue engineering, the very idea that living flesh could be "constructed" by following engineering principles and combining nonliving materials with cells sounded fantastical to many. Yet the need for such transplantable human tissues to replace, restore or enhance organ function was, and remains, urgent. Today nearly 50 million people in the U.S. are alive because of various forms of artificial organ therapy, and one in every five people older than 65 in developed nations is very likely to benefit from organ replacement technology during the remainder of their lives.

Current technologies for organ substitution, such as whole-organ transplants and kidney dialysis machines, have saved many lives, but they are imperfect solutions that come with heavy burdens for patients. Engineered biological tissues are customizable and immune-compatible and can therefore potentially make a significant difference in the lives of people with failing organs. They can fill other human needs as well, for example, serving as "organs on a chip" for testing the toxicity of candidate drugs.

Engineered tissues can take many forms, from aggregations or thin sheets of cells to thick constructs of complex tissue and, the ultimate engineering challenge, an entire functioning organ. Since we initially presented the obstacles involved in creating these implantable tissues [see "Tissue Engineering: The Challenges Ahead," by Robert S. Langer and Joseph P. Vacanti; Scientific American, April 1999], scientists have made considerable progress. Products such as skin substitutes and cartilage replacements have already helped thousands of patients. Artificial tissues such as bladder, cornea, bronchial tubes and blood vessels are in clinical trials. And laboratory work on building more complex tissue structures is producing encouraging results.

Although some of the obstacles we described 10 years ago remain, significant advances over the past decade have come from new insights into the way the body naturally builds tissues, during both embryonic development and natural wound healing. And engineering approaches to assembling tissue structures have become more sophisticated, as have the chemical, biological and mechanical properties of the materials available for the task. As a result, the field is coming of age, and tissue-engineered products are increasingly a realistic option for medical treatment.

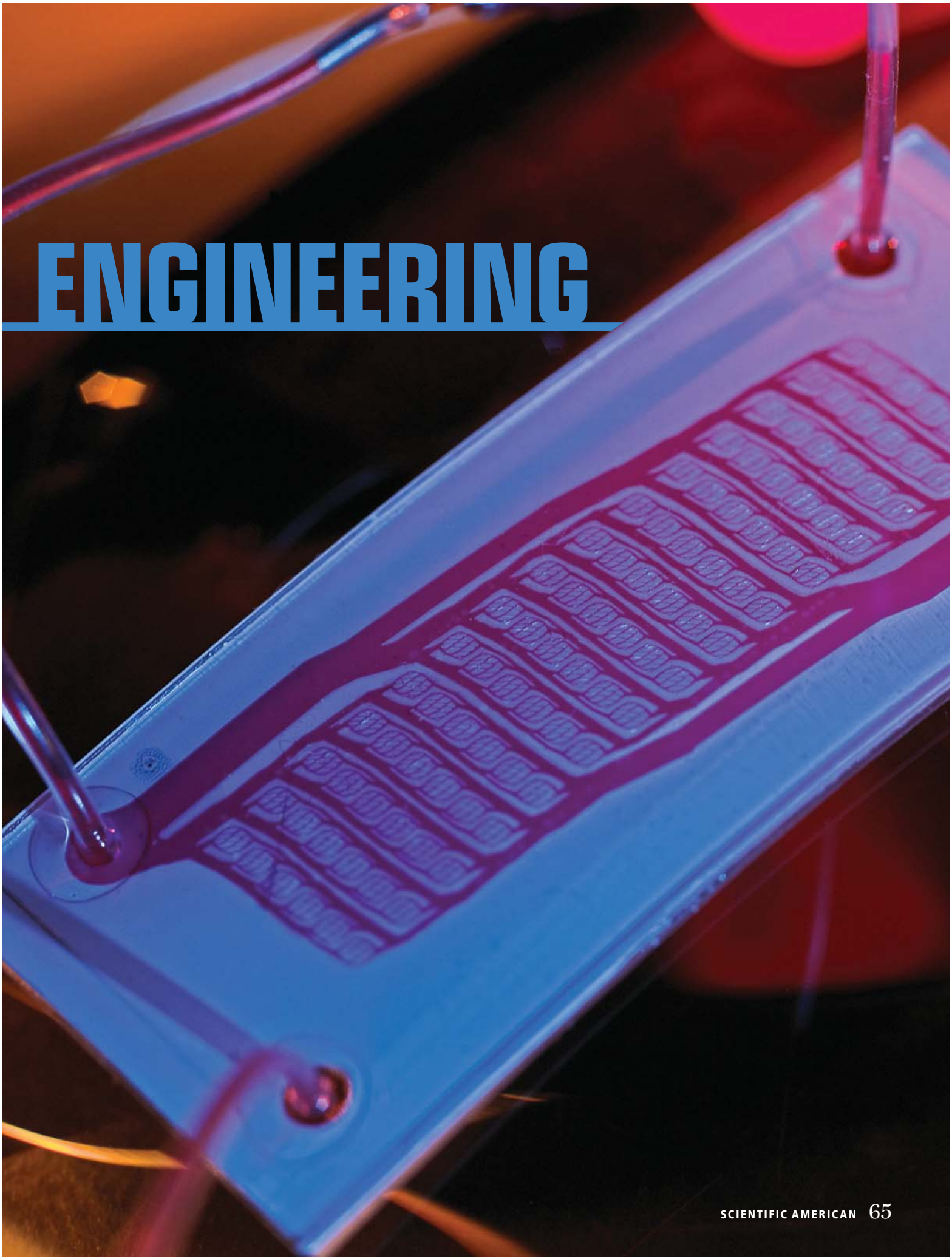
Delivering Life's Blood

One reason that tissues such as skin and cartilage were among the first to be ready for human testing is that they do not require extensive internal vasculature. But most tissues do, and

TISSUE CULTURE DEVICE containing microfabricated "blood vessels" is one of the advances made possible by novel materials and technologies available to tissue engineers. A membrane containing nanoscale pores separates the artificial vessels from a layer of liver cells.

"MICROFABRICATION OF THREE-DIMENSIONAL ENGINEERED SCAFFOLDS," BY JEFFREY T. BORENSTEIN, ELI J. WEINBERG, BRIAN K. ORRICK, CATHRYN SUNDBACK, MOHAMMAD R. KAAZEMPUR-MOFRAD AND JOSEPH P. VACANTI, IN *TISSUE ENGINEERING*, VOL. 13, NO. 8, 2007

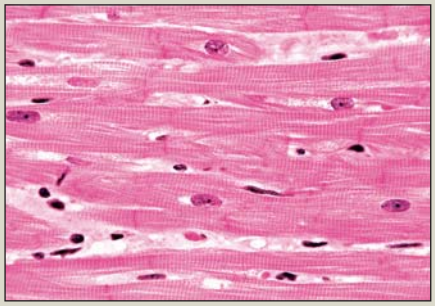
ENGINEERING



[THE GOAL]

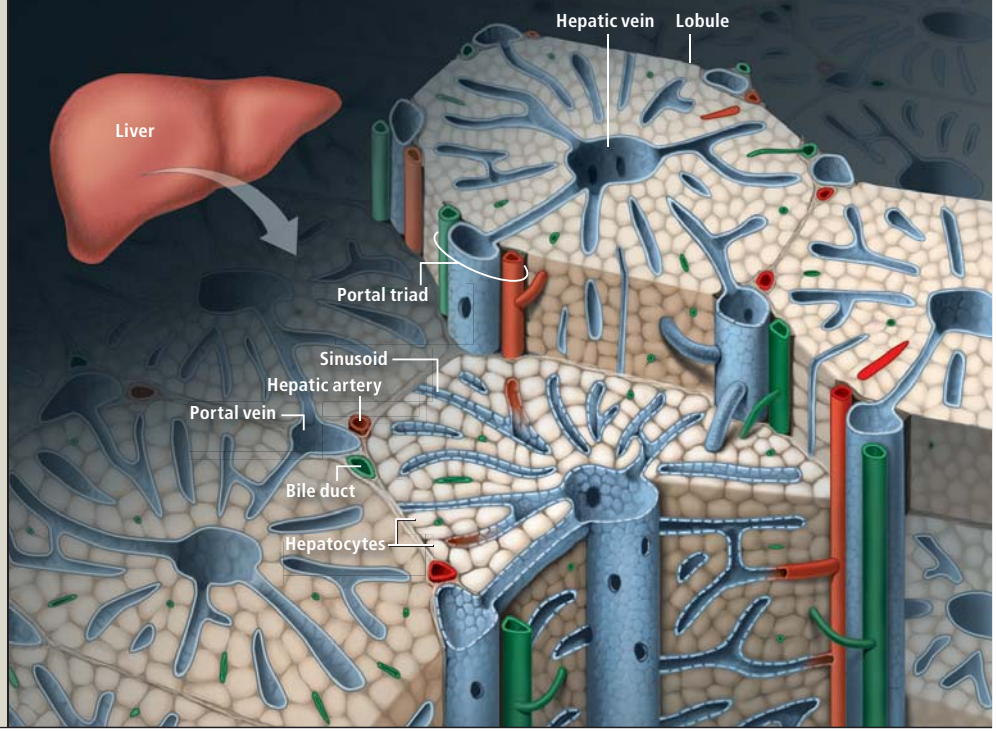
COPYING NATURE'S ARCHITECTURE

The health and functioning of a natural tissue depend closely on its internal structure. Tissues are made up of multiple cell types that work together to accomplish an organ's task—in the case of the liver (*right*), that is mainly to act as a giant blood filter, whereas heart tissue (*below*) forms a muscular pump. Because cues exchanged between cells and their surroundings are critical to a tissue's development and maintenance as well as its work, the engineer's challenge in building a replacement tissue is to mimic the organ's complex natural organization using a mixture of engineered materials and living cells.



▲ The heart is made up of long fibrous muscle cells, wrapped in collagen sheaths and interwoven with blood vessels. Collagen also connects the muscle bundles end to end and conducts the neural signals that control their contractions. The shape and orientation of muscle cells within heart tissue are therefore critical to their electrical and mechanical properties.

▼ **A human liver is organized into roughly hexagonal columns called lobules, each containing spongy tissue radiating around a central hepatic vein. At the corners of each lobule are the so-called portal triads consisting of the hepatic artery, bile duct and portal vein. Blood from both the hepatic vein and hepatic artery percolates through the lobule's rows of cells (*hepatocytes*), which are interleaved with endothelial cells that form broad capillaries known as sinusoids. The liver's repeating lobule structure maximizes blood delivery to the hepatocytes, which extract and break down nutrients and toxins.**



the difficulty of providing a blood supply has always limited the size of engineered tissues. Consequently, many scientists are focusing on designing blood vessels and incorporating them in engineered tissues.

Any tissue that is more than a few 100 microns thick needs a vascular system because every cell in a tissue needs to be close enough to capillaries to absorb the oxygen and nutrients that diffuse constantly out of those tiny vessels. When deprived of these fuels, cells quickly become irreparably damaged.

In the past few years a number of new approaches to building blood vessels—both outside tissues and within them—have been devised. Many techniques rely on an improved understanding of the environmental needs of endothelial cells (which form capillaries and line larger vessels), as well as an advanced ability to sculpt materials at extremely small scales. For example, when endothelial cells are laid on a bed of scaffolding material whose surface is patterned with nanoscale grooves—1,000th the diameter of a human hair—they are encouraged to form a network of capillarylike tubes [*see box on page 69*]. The grooves mimic the texture of body tissues that endothelial cells rest against while forming natural blood vessels, thus providing an important environmental signal.

The difficulty of providing a blood supply has always limited the size of engineered tissues.

Microfabrication, the set of techniques used to etch microelectronics chips for computers and mobile phones, has also been employed to make capillary networks. Vacanti, with Jeffrey T. Borenstein of the Draper Laboratory in Cambridge, Mass., has generated arrays of microchannels to mimic tissue capillary networks directly within degradable polymer scaffolds, for instance. Inside these channels, endothelial cells can be cultured to form blood vessels while also acting as a natural barrier that minimizes the fouling effect of blood on the scaffold materials. An alternative is to use a membrane filter to separate the blood-carrying channels from the functional cells in a tissue construct [*see illustration on preceding page and box on page 71*].

Another method for keeping cells and blood

INNERSPACE IMAGING *Photo Researchers, Inc. (heart muscle cells)*; JEN CHRISTIANSEN *(liver diagram)*

separate but close enough to exchange a variety of molecules is to suspend them within hydrogels, which are gelatinlike materials made from hydrated networks of polymers. Hydrogels chemically resemble the natural matrix that surrounds all cells within tissues. The functional cells can be encapsulated inside the material, and channels running through the gel can be lined with endothelial cells to engineer tissuelike structures with a protovasculature.

Research from the laboratories of Laura Niklason of Yale University and Langer has shown that larger blood vessels can be generated by exposing scaffolds seeded with smooth muscle cells and endothelial cells to pulsating conditions inside a bioreactor. Arteries made in this environment, which is designed to simulate the flow of blood through vessels in the body, are mechanically robust and remain functional after being transplanted into animals. In addition to enabling tissue engineers to incorporate such vessels into larger constructs, the engineered tubes by themselves may provide grafts for bypass surgery in patients with atherosclerosis.

Although the ability to engineer capillarylike structures and larger blood vessels outside the body is a significant breakthrough, a working engineered tissue implant will have to connect quickly with the recipient's own blood supply if the construct is to survive. Coaxing the body to form new vasculature is therefore an equally important aspect of this work. David Mooney of Harvard University, for example, has demonstrated that the controlled release of chemical growth factors from polymeric beads or from scaffold material itself can promote the formation of blood vessels that penetrate implanted tissue constructs.

Pervasis Therapeutics, with which Langer and Vacanti are affiliated, is conducting advanced clinical trials in which a variation of this principle is applied to healing a vascular injury. A three-dimensional scaffold containing smooth muscle and endothelial cells is transplanted adjacent to the site of the injury to provide growth-stimulating signals and to promote natural rebuilding of the damaged blood vessel [*see bottom photograph at right*].

Despite these advances, a number of challenges still remain in making large vascularized tissues and vascular grafts, and scientists have not yet completely solved this problem. New blood vessels grow and penetrate an implanted tissue construct slowly, causing many of the construct's cells to die for lack of a blood supply immediately after implantation. For this reason, tissue-engineering approaches that include a vascular system prefabricated within the tissue construct are very likely to be necessary for large transplants. Such prefabricated vessels may also be combined with controlled release of blood vessel–recruiting growth factors to induce further growth of the construct's vessels.

Because integrating the engineered vasculatures and those of the host is also critical, researchers need a better understanding of the cross talk between the host tissue cells and implanted cells to foster their connection. This need to decipher more of the signals that cells exchange with one another and with their environments also extends to other aspects of building a successful tissue implant, such as selecting the best biological raw materials.

Suitable Cells

In most situations, building an implantable tissue from a patient's own cells would be ideal because they are compatible with that person's immune system. Realistically, such implants might also face fewer regulatory hurdles because the material is derived from the patient's own body. The ability of normal cells to multiply in culture is limited, however, making it difficult to generate sufficient tissue for an implant. So-called adult stem cells from the patient's body or from a donor are somewhat more prolific, and they can be isolated from many sources, including blood, bone, muscle, blood vessels, skin, hair follicles, intestine, brain and liver.

Adult stem cells—which occur in adult tissues and are able to give rise to a variety of cell types characteristic of their native tissue—are difficult to identify, however, because they do not look very different from regular cells. Scientists therefore must look for distinctive surface proteins that serve as molecular markers to flag stem cells. The identification of additional markers would make it considerably easier to work with adult stem cells in tissue-engineering applications. Fortunately, over the past few years a number of major advances have been made, including development of novel methods of isolating the cells and inducing them to proliferate and to differentiate into various tissue types in culture.

Notably, Christopher Chen and Dennis Discher, both at the University of Pennsylvania, have demonstrated that mesenchymal stem cells, which are typically derived from muscle, bone or fat, will respond to mechanical cues from their surroundings. They have been shown to

NO LONGER ON THE HORIZON

A number of products based on tissue-engineering principles are already being used to treat patients, in clinical trials or as FDA-approved therapies. Examples include simple skin and cartilage, as well as a patch designed to speed tissue healing.

SKIN: Epicel, a permanent replacement epidermis, is grown from the patient's own skin cells and intended to treat burns.

CARTILAGE: Carticel, one of the first cell-based treatments to be marketed, is an injectable suspension of cartilage-repairing chondrocytes derived from the patient and cultured with growth-promoting factors.

VESSEL PATCH: Vascugel, currently in clinical trials, is a construct made of donor endothelial cells and designed to be placed on top of an injured blood vessel. The healthy patch cells send signals to cells within the damaged vessel that promote healing and reduce inflammation and scarring.

COURTESY OF GENZYME CORPORATION (*Epicel* and *Carticel*); COURTESY OF JACK HARVEY *Pervasis Therapeutics, Inc. (Vascugel)*

differentiate into the tissue that most closely resembles the stiffness of the substrate material on which they are growing. Other researchers have also shown that chemical signals from the substrate and surrounding environment are important for directing the differentiation of adult stem cells into one tissue type or another. Scientists disagree, though, about whether adult stem cells are able to give rise to cells outside their own tissue family—for instance, whether a mesenchymal stem cell could generate liver cells.

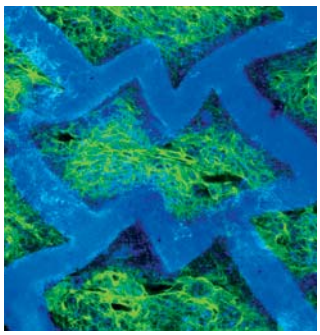
In contrast to adult stem cells, embryonic stem (ES) cells are easy to expand in culture and can differentiate into all the cell types of the human body. Langer, along with Shulamit Levenberg of the Technion-Israel Institute of Technology in Haifa and her colleagues, has demonstrated that ES cells can even be made to differentiate into a desired tissue type right on tissue-engineering scaffolds. This capability suggests the potential to make 3-D tissues on scaffolds directly from differentiating ES cells. These cells do present various challenges, however.

Directing the uniform differentiation of ES cells into the desired cell types is still quite difficult. In attempts to mimic the complex natural microenvironment of ES cells and to optimize their differentiation, investigators are testing many conditions simultaneously to find the right combination of cues from different materials and matrix chemicals. They are also screening various small molecules as well as signaling proteins to identify factors that control "stemness"—the cells' ability to give rise to differentiated progeny while remaining undifferentiated themselves, ready to produce more new cells as needed.

Those insights could also be applied to producing cells with the capabilities of embryonic cells but fewer of the drawbacks. Beyond the difficulties just outlined, scientists are still unable to predict the behavior of transplanted stem cells in patients. Undifferentiated ES cells can form tumors, for instance, creating a risk of cancer if the cells are not all successfully differentiated before transplantation. In addition, researchers have been making efforts to address the ethical issues associated with deriving ES cells from human embryos by exploring approaches to producing ES-like cells from nonembryonic sources.

In the past couple of years remarkable progress has been made in producing ES-like cells from regular adult body tissue, such as skin cells. These altered cells, known as induced pluripotent stem (iPS) cells, are emerging as an exciting alternative to ES cells as a renewable resource for tissue engineering. In 2007 Shiro Yamanaka, then at Kyoto University, and James A. Thomson of the University of Wisconsin–Madison first showed that cells of adult tissue can be transformed to a primitive iPS state by reactivating a number of genetic pathways that are believed to underlie stemness.

Tissue engineers are looking to other fields for insights, including studies of developing tissues and regenerating wounds.



HONEYCOMB SCAFFOLD design (*blue*) guides the alignment of rat heart cells, whose contractile fibers are stained green above. Human heart muscle must contract and dilate some 300 million times over an average life span without tiring. To replicate mechanical cues that enhance the cells' contractile ability, Lisa E. Freed and George C. Engelmayr, Jr., both at the Massachusetts Institute of Technology, designed their scaffold to have accordianlike flexibility. They used a laser to cut the honeycombed pores in "biorubber," a unique elastic material developed by Yadong Wang and Robert Langer.

Reintroducing as few as four master regulatory genes into adult skin cells, for instance, caused the cells to revert to a primitive embryonic cell type. The early experiments used a virus to insert those genes into the cells, a technique that would be too dangerous to use in tissues destined for patients. More recent research has shown that a safer nonviral technique can be adapted to activate the same repertoire of stemness genes and even that activation of just a single regulatory gene may be sufficient. The rapid progress in this area has tissue engineers hopeful that soon a patient's own cells, endowed with ES cell capabilities, could become the ideal material for building tissue constructs. And even as we experiment with these different cell types, tissue engineers are also refining our building methods.

Architectural Advances

A decade ago researchers assumed that cells are smart: if we put the correct cell types in proximity to one another, they would "figure out" what to do to form their native tissues. To some degree, this approach is effective, but we now have a greater appreciation of the intricacy of signals exchanged among cells and their surroundings during organ and tissue development as well as during normal functioning, and we know the importance of providing a tailored environment in our constructs.

Further, every tissue in the body performs specific tasks that engineered replacements must be able to perform, and we are learning that replicating the underlying biology of the tissue in question as closely as possible is critical to generating tissues that can carry out their intended functions. In more complex organs, multiple cell types work in concert—in the liver, for instance, the cells' jobs include detoxification and nutrient breakdown. Thus, the microarchitecture of tissues and the positioning of cells relative to one another must be re-created in tissue-engineered constructs to reproduce the desired functionality.

Early tissue-engineering work used scaffolds made from assorted materials to try to replicate the 3-D shape of the tissue as well as crudely approximate this spatial cell organization. A number of advances in the past few years have en-

hanced the level of complexity of engineered tissues and reproduced the tissue environment more closely. For example, scaffolds have been made by removing all the cells from natural tissues, leaving only connective fibers. These shells can be used to grow engineered tissues that recreate a significant amount of the function of the original tissue. In one particularly impressive study, decellularized rodent heart scaffolds that were seeded with cardiac and endothelial cells produced cardiac muscle fibers and vascular structures that grew into a beating heart.

Assorted "printing" technologies can also be used to arrange cells precisely. By modifying standard ink-jet printers, engineers can dispense cells themselves or scaffold materials to generate tissues or frameworks onto which cells can be seeded. Mimicking the tissue's natural topography also helps to guide the cells, and another technology borrowed from the engineering world, electrospinning, can produce scaffolds that resemble the texture of natural tissue matrix. Very thin polymer fibers are spun to form weblike scaffolds, which provide cells with a more natural 3-D environment, and the chemical and mechanical features of the polymer materials can be finely manipulated. David Kaplan of Tufts University has fashioned similar scaffolds from silk materials that resemble spider webs to generate ligaments and bone tissues.

Because the biological, chemical and mechanical properties of hydrogels can be readily manipulated, the gels are proving useful for supporting and encasing cells while enhancing the function of the resulting tissues. Hydrogels containing live cells can be "printed" or otherwise arranged and layered to delineate correct tissue structure. One of us (Khademhosseini) has shown, for example, that hydrogel-encased cell aggregates can be molded into any number of complementary shapes [*see box on next page*], then pooled together to self-organize into a larger complex pattern. This technique could be used to replicate the natural organization of cells in a tissue such as the liver, which is made up of hexagonal structures that each contain toxin-filtering cells surrounding a central blood vessel.

Some gels are designed so that their polymers link together in response to ultraviolet light, making it possible to sculpt the desired construct shape and then solidify it by exposing all or parts of the construct to light. Kristi Anseth of the University of Colorado at Boulder and Jennifer Elisseeff of Johns Hopkins University have generated cartilage and bone tissue using such pho-

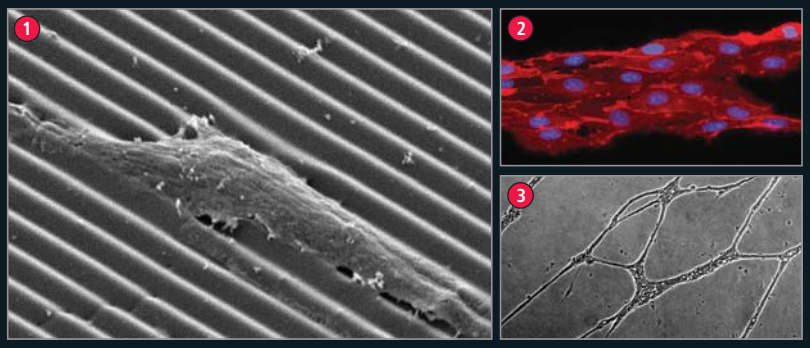
[TECHNIQUES]

Building Blood Vessels

Living tissues quickly starve without the oxygen and nutrients delivered to cells by blood, so an engineered tissue construct that is more than a few cells thick usually requires an integrated vasculature. Endothelial cells form tiny capillaries and the interior lining of larger vessels within natural tissues, but coaxing endothelial cells to build a vascular network that penetrates an engineered tissue has been a major challenge. Nanoscale and microfabrication technologies borrowed from other fields, such as the semiconductor industry, are now allowing tissue engineers to control the cells' behavior and placement with unprecedented precision.

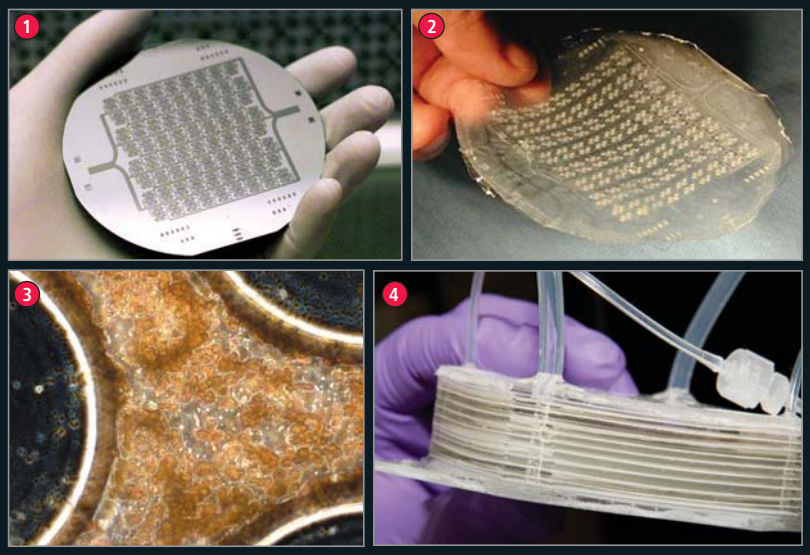
NANOPATTERNED SURFACE

Cells respond to chemical signals from their neighbors and from the supportive extracellular matrix around them, but cells also respond to mechanical cues from the shape and texture of the surface on which they are growing. Grooves 600 nanometers (nm) deep and 1,200 nm wide mimic the natural matrix topography of certain tissues and provide endothelial cells with mechanical signals that affect the cells' shape and rate of migration and proliferation (*1*). After growing on the nanopatterned surface for six days, the cells multiply and align themselves in the direction of the grooves (*2*) and form a network of capillarylike tubes (*3*).



MICROFABRICATION

To control the vasculature pattern within an implantable liver-assisting device, tissue engineers etch the desired blood vessel arrangement into a silicon mold (*1*). A biocompatible polymer scaffold cast from the silicon template (*2*) is then seeded with endothelial cells to coat the artificial vessel walls. Liver cells are cultured in the channels of similar scaffolds (*3*). Alternating layers of scaffolds containing "blood vessels" or liver cell cultures are stacked, with a nanoporous membrane between each layer, so that the liver cells are always close to the blood supply (*4*). The resulting hybrid tissue device, which has been tested in animals, is intended to serve as a bridge for patients awaiting a liver transplant.



COURTESY OF DRAPER LABORATORY (*1, top,* and *1 and 4, bottom*); "ENHANCEMENT OF IN VITRO CAPILLARY TUBE FORMATION BY SUBSTRATE NANOTOPOGRAPHY," BY C. J. BETTINGER, Z. ZHANG, S. GERECHT, J. T. BORENSTEIN AND R. LANGER, IN *ADVANCED MATERIALS*, VOL. 20, 2008 (*2 and 3, top*); "MICROSCALE TECHNOLOGIES FOR TISSUE ENGINEERING AND BIOLOGY," BY A. KHADEMHOSSEINI, R. LANGER, J. T. BORENSTEIN AND J. P. VACANTI, IN *PNAS*, VOL. 103, NO. 8; FEBRUARY 21, 2006. COPYRIGHT NATIONAL ACADEMY OF SCIENCES, U.S.A. (*2, bottom*); "THREE-DIMENSIONAL MICROFLUIDIC TISSUE-ENGINEERING SCAFFOLDS USING A FLEXIBLE BIODEGRADABLE POLYMER," BY C. J. BETTINGER, E. J. WEINBERG, K. M. KULIG, J. P. VACANTI, Y. WANG, J. T. BORENSTEIN AND R. LANGER, IN *ADVANCED MATERIALS*, VOL. 18; 2006 (*3, bottom*)

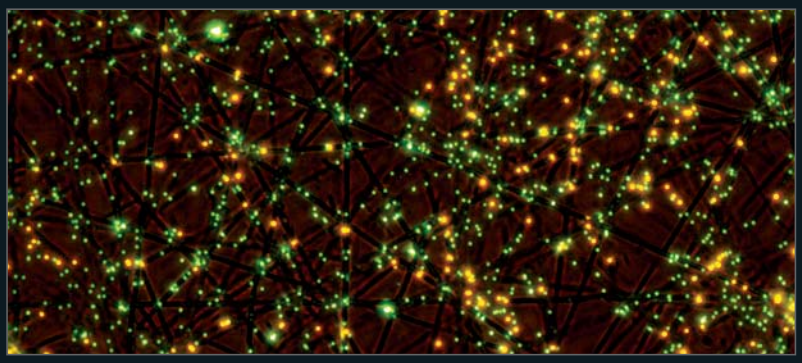
[TECHNIQUES]

Advanced Building Materials

Engineers want to reproduce the internal structure of a natural tissue as closely as possible because cells depend on environmental cues to maintain themselves and to do their jobs. New techniques and materials are giving tissue engineers finer control and faster methods of creating cell constructs designed to grow into a functioning implant.

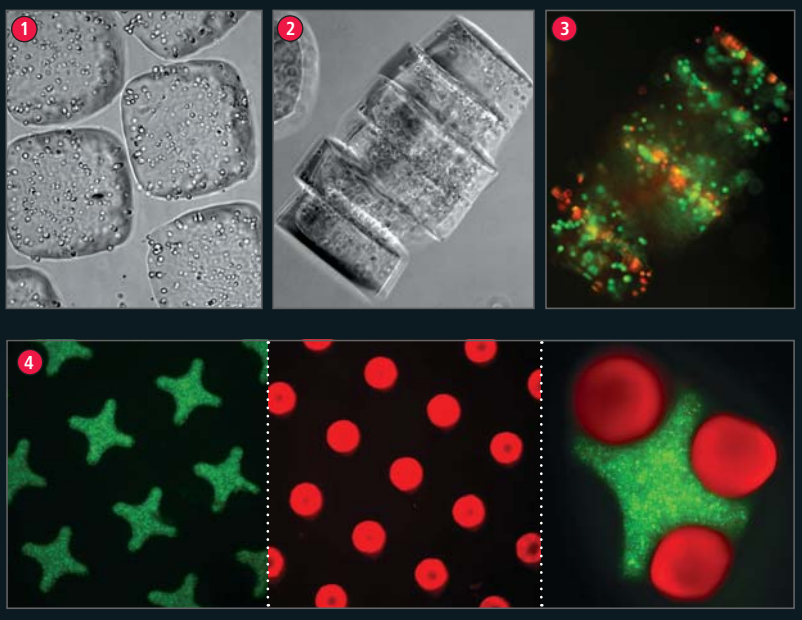
ELECTROSPINNING FIBERS

A manufacturing technique for producing ultrafine fibers—nanometers or microns in diameter—from liquids and other substances has recently been adapted to produce meshlike cell scaffolds. This type of surface maximizes the space available for cells and the cells' contact with the scaffolding material itself—which mimics the texture of extracellular matrix and can be imbued with growth-promoting chemicals. Such scaffolds have been made from silk and a variety of polymers. Living cells can even be incorporated within the fibers themselves (*green dots*) to ensure they are evenly distributed throughout the scaffold.



ASSEMBLING HYDROGELS

Suspending living cells within polymer hydrogels allows tissue engineers to create cell arrangements that mimic natural tissue structure. Polymer molecules link to one another in response to ultraviolet light, causing the gel to stiffen enough to be sculpted into building blocks and assembled into larger patterns. A method for producing self-assembling hydrogel-cell blocks begins with a hydrophilic (water-loving) gel formulation that is laden with live cells and made into cubes using photolithography (*1*). When the blocks are suspended in oil and agitated, the hydrophilic units are drawn to one another, forming larger aggregates that can be stabilized by a second cross-linking light exposure (*2*). Cells (*green*) remain viable within the blocks (*3*). Dye-containing blocks illustrate how hydrogel units carrying different types of cells could be shaped to self-assemble into larger constructs mirroring natural tissue structures such as liver sinusoids (*4*).



tocrosslinkable hydrogels. Gels can also be imbued with a number of signaling molecules to promote tissue growth or differentiation. Samuel Stupp of Northwestern University has shown that neural stem cells differentiate into neurons within a hydrogel that incorporates small proteins that act as environmental signals directing the cells' behavior. Helen M. Blau of Stanford University has also used hydrogels containing extracellular matrix components to control and study the properties of individual stem cells.

Finally, nanotechnology has been enlisted to generate engineered sheets of cells suitable for transplantation. Teruo Okano of Tokyo Women's Medical University has produced surfaces coated with a temperature-responsive polymer that swells as the temperature is lowered from 37 to 20 degrees Celsius. Cells are first induced to form a single layer on these nanoengineered surfaces, then the temperature is lowered to swell the underlying substrate and detach the intact cell sheet. These cell sheets, which contain appropriate cell-secreted matrix molecules can then be stacked or rolled to build larger tissue constructs.

Although these advances have made a significant improvement in the range and diversity of scaffolds that can be generated, challenges persist in this area as well. One difficulty is a lack of knowledge of the concentrations and combinations of growth factors and extracellular molecules that are present at specific stages of development and wound healing in various tissues. A better understanding of these design parameters is needed to engineer tissues that mimic the body's own healing and development. Thus, tissue engineers are looking to other fields for insights, including studies of gene and protein interactions in developing tissues and regenerating wounds. Incorporating these findings with advanced culture systems is helping us to better control the responses of cells outside the body, but more progress is needed.

Coming of Age

Despite the ongoing challenges we have described, engineered tissues are no longer a fantastical prospect. Simple manufactured tissues are already in clinical use, and this method of restoring or replacing biological function is now poised to become a viable therapy for millions of patients in need. As of late 2008, various tissue-engineered products generated annual sales of nearly $1.5 billion.

Those figures are all the more impressive in light of setbacks to the field that occurred short-

A FULL PIPELINE

At least 70 companies have developed or are developing implantable tissue-engineered products, meaning that they replace or restore human tissue function by combining engineering principles and materials with living cells. In many of the most commercially advanced products, those cells are supplied by the patient destined to receive the implant. These include a variety of cell-free scaffold materials intended to foster regeneration by the patient's own tissues, as well as cell grafts and aggregations. Whole tissues can include large engineered blood vessels and other implants that completely replace the patient's original tissue, as well as many types of complex skin used for patient grafts and, increasingly, for animal-free testing of chemicals.

IMPLANT TYPE	EXAMPLES OF COMPANIES AND STAGE OF PRODUCT DEVELOPMENT		
	Preclinical	Clinical Trials	Approved
Cell-free supports (Implantable or injectable scaffold materials and tissue matrix components)	3DM, Cardio, Cytomatrix, RegenTec, Regentis Biomaterials, Tepha	Celltrix, Forticell Bioscience, Kuros Biosurgery, Serica Technologies	Advanced Biopolymers, Baxter, Cook Biotech, Fidia, Imedex Biomateriaux, Integra, Johnson & Johnson, Lifecell, Medtronic, Orthovita, Pioneer Surgical Technology, ReGen Biologics, TEI Biosciences, Tissue Regeneration Therapeutics
Cell-based products (Encapsulated cells, single-cell-type aggregations or sheets, organ-assist devices)	BioEngine, Cerco Medical, GeneGrafts, MicroIslet	ArBlast, Excorp, HepaLife Technologies, Isolagen, LCT, Neurotech, Novocell, NsGene, Pervasis Therapeutics, TiGenix, Vital Therapies	Advanced BioHealing, Arthro Kinetics, Biotissue Technology, Cell Matrix, CellTran, Genzyme, Hybrid Organ, Interface Biotech, Organogenesis, SEWON Cellontech, Tetec, Vasotissue Technologies
Whole tissues (Blood vessels, cartilage, bone, bladder, heart muscle, complex skin)	Bio Nova, Humacyte	BioMimetic Therapeutics, Cytograft, Educell, Histogenics, Intercytex, ISTO, Tengion, Theregen	Euroderm, Japan Tissue Engineering, Karocell Tissue Engineering, MatTek, Skin Ethic Laboratories

ly after we last wrote for this magazine about the promise of tissue engineering. At the end of the 1990s and into the early 2000s, enthusiasm and investment were high, but with the burst of the Internet financial bubble, funding for biotechnology start-ups dwindled. Even companies with tissue-engineered products approved by the Food and Drug Administration had to restructure their business models, delaying introduction of their products to the market. Because engineered tissues are made from cells, biologically active chemicals and nonbiological scaffold materials, the constructs must undergo rigorous analysis by the FDA, which is costly and time-consuming. A lack of funding made conducting extensive clinical trials more difficult for companies. Ironically, the delay in commercializing some tissue-engineered products had an upside—it bought time for the science to mature and for business approaches to become more sophisticated.

There is still room for improvement. Obtaining FDA approval is still a major hurdle, in part because cells obtained from different people may not behave alike and because recipients can have varying responses to the same kind of implant. Such unpredictability can make it difficult for the FDA to determine that a given engineered construct is safe and effective. Further research is therefore important to measure and understand variations between individuals and to account for them in clinical trials that study tissue-engineered products. And future business models must include the extensive costs that will be associated with this work.

Still, armed with recent insights into how tissues develop and how the body repairs itself naturally, tissue engineers are now aiming to create second-generation products that are closer mechanically, chemically and functionally to their biological counterparts than ever before. Even in today's strained economic climate, we expect that research into nanotechnology, stem cell biology, systems biology and tissue engineering will soon converge to yield fresh ideas for devising the sophisticated organ substitutes needed by so many people today. ■

[THE AUTHORS]

Ali Khademhosseini is an assistant professor at Harvard-MIT's Division of Health Sciences and Technology and at Harvard Medical School. Since earning his Ph.D. under Langer's direction, Khademhosseini has focused his research on developing microscale and nanoscale technologies to control cellular behavior for tissue engineering and drug delivery. Joseph P. Vacanti is surgeon in chief at Massachusetts General Hospital for Children, a professor at Harvard Medical School, and deputy director of the Center for Regenerative Medicine at Massachusetts General Hospital. Robert Langer is an Institute Professor at the Massachusetts Institute of Technology and the most cited engineer in history. Langer and Vacanti pioneered tissue-engineering research together and wrote about their fledgling field in the September 1995 and April 1999 issues of *Scientific American.*

MORE TO EXPLORE

Bringing Safe and Effective Cell Therapies to the Bedside. Robert A. Preti in *Nature Biotechnology,* Vol. 23, No. 7, pages 801–804; July 2005.

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