Designer Tissues Take Hold

By engineering polymers that attract and bind liver, nerve, and other cells, scientists are beginning to create artificial organs that are biologically "real"

There's nothing simple about the liver. The organ's many different cell types are arranged in precise three-dimensional patterns to filter toxins from the blood, convert nutrients into forms usable by body tissues, and perform a broad range of other functions. Now scientists are taking basic steps toward growing this complicated organ in the lab.

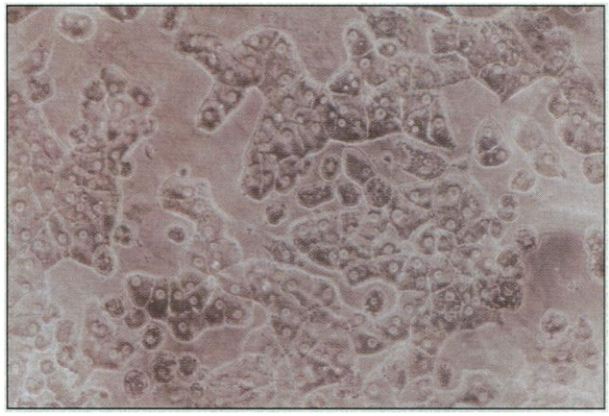
Tissue engineering, as this field is known, has been around for a little more than a decade. Its researchers have already developed laboratory-grown versions of tissues such as skin and cartilage that are now being tested as replacements for damaged tissues. These are, however, comparatively simple body parts, consisting of one or two cell types layered on a synthetic polymer scaffold or a mesh made of the structural protein collagen (see box).

A lab-grown liver, however, calls for a new level of sophistication and control: A variety of cells need to be arranged and grown in particular orientations. That means a scaffold with a high degree of selectivity, something polymer and collagen scaffolds lack. But by attaching specific cellular recognition molecules, such as protein fragments, to synthetic scaffolds, biologists and chemists have been able to demonstrate that such feats are indeed possible.

Linda Griffith-Cima, a chemical engineer at the Massachusetts Institute of Technology (MIT), and her colleagues have designed a scaffold that attracts liver cells called hepatocytes while rejecting other cell types. Scientists are using similar hybrid technology to direct the growth of nerve cells on biocompatible materials in hopes of eventually repairing damaged nerves, and to create synthetic blood vessels lined with cells that minimize the formation of dangerous blockages.

Although the work on these hybrid scaffolds is still of a preliminary nature, "it's definitely where the field is going," says David Mooney, an assistant professor of chemical engineering and dentistry at the University of Michigan. The dual nature of the devices is allowing researchers to take advantage of the ability to precisely control the structure of synthetic materials while at the same time camouflaging their foreign nature, minimizing conflicts with the body's immune system. "We not only remove the foreign surface, but we replace it with one that promotes directed growth of the tissue we're interested in," says David Clapper, a cell biologist at BSI Corp., an Eden Prairie, Minnesota–based company working to improve the performance of blood vessels and other implants.

Although the destination is exciting, the field still has to negotiate some bumps before it gets there. One possible barrier, points out Martin Yarmush, a bioengineer at Rutgers University in New Jersey, is that once cells attach themselves to a matrix, hybrid or otherwise, they begin to excrete proteins which may interfere with the carefully laid plans by binding unwanted cells in that region.



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Liver in a lab. Researchers attached cellular recognition molecules to this polymer mesh, selectively binding these liver cells, known as hepatocytes, which remove damaged proteins from the blood.

Liver under construction

Selection of specific cell types is a crucial issue in tissue design. Natural and synthetic scaffolds for body tissues, by themselves, are not terribly picky hosts. "Synthetic scaffolding materials are easy and cheap to make and form into a desired structure," says Mooney. But they typically provide holdfasts for many different types of cells, he adds; collagen is similarly undiscriminating. And, notes Mooney, collagen is "hard to isolate in large quantities." So researchers have tried to take advantage of the mass-production capacity of synthetics, but modify them with a biological ability to select specific cell types.

The efforts to bind liver cells illustrate the potential power of such a combination. Thus far, most researchers have been trying to design polymers to selectively bind hepatocytes, the most common and important cell type in the liver. These cells carry out more metabolic functions than any other group of cells in the body. One of their duties is to remove damaged proteins from the blood. The cells can do this because they recognize specific carbohydrate sequences, attached to these proteins, that mark these complexes as damaged goods. In the late 1970s Paul Weigel and his colleagues at Johns Hopkins University made synthetic versions of the carbohydrate sequences and used them as lures for the cells: The scientists attached the carbohydrates to a polymer surface made from polyacrylamide, and saw that the hepatocytes engulfed the lures and remained stuck to the polymer surface.

Although successful at binding hepatocytes, polyacrylamide isn't a viable scaffold for tissue engineering, because the material provokes a strong immune response in the body. So more recently Griffith-Cima and her colleagues decided to try attaching the same carbohydrates to a more biocompatible synthetic surface. But they had to choose their material carefully, as many synthetics, such as polypropylene, can be tolerated by the body but quickly become coated with proteins which attract all kinds of cells—exactly what the scientists didn't want.

The researchers decided to build their scaffold with a meshed, water-filled network of polyethylene oxide, or PEO, which is resistant to protein adsorption. PEO molecules are star-shaped, with several arms emanating from a central core. When linked in a network in a water-based solution, the end of each arm floats free.

These ends contain reactive hydroxyl groups, to which the researchers attached carbohydrate molecules as lures for hepatocytes. Griffith-Cima and her colleagues report in the journal *Biomaterials* (in press) that when they added rat hepatocytes, the cells went right for the lures and ended up bound to the polymer mesh. But other cells, such as fibroblasts, failed to bind to the mesh when Griffith-Cima's group added them to the solution. "[Griffith-Cima] has solved one of the big problems—getting a receptor that is unique for hepatocytes," says Kevin Healey, a biomedical engineer at the Chicago campus of Northwestern University.

The next step is to build cell-specific scaffolds in three dimensions. A normal liver,

Out of the Lab, Into the Body

Although efforts to make hybrid cellular scaffolds for tissue engineering have barely begun (see main text), projects that use natural or synthetic matrix material alone—or nothing at all—have forged further along. Attempts to engineer structural tissues, such as skin and cartilage, have scored the greatest successes, with several new products on or nearing the market.

During one recent study, a Swedish team engineered a patient's own cartilage-producing cells, or chondrocytes, to treat damaged knee cartilage. Joint cartilage normally does not regenerate in the body; hence, damage from injury or illness tends to become more severe over time. More than 500,000 patients undergo surgery in the United States each year to alleviate the pain and restricted movement that accompany cartilage damage. But such procedures typically provide only short-lived symptomatic relief.

So researchers at the University of Göteborg and Sahlgrenska University Hospital in Sweden extracted chondrocytes from 23 patients, multiplied the cells in a bioreactor, and implanted the new tissue in the patients' damaged knee joints. In the 6 October 1994 issue of the *New England Journal of Medicine*, the researchers, led by Lars Peterson and Anders Lindahl, reported "good to excellent" results for 14 out of 16 patients in which the lab-grown cartilage was implanted to repair damage at the upper end of the joint; the new cartilage cells displayed the consistency of healthy cartilage. The other seven patients, treated to repair defects in the cartilage surrounding the kneecap, fared less well, in part because cartilage there experiences greater mechanical stress, says Lindahl.

This technique is somewhat time-consuming, however: It took the Swedish team a few weeks to collect, grow, and transplant these cells. So other researchers want to develop engineered tissues that are more ready-made. For instance, a team at Organogenesis, a Massachusetts-based biotech company, has had success growing sheets of artificial skin on natural matrix materials that form ready-to-use grafts. This May, they finished a clinical trial of skin grown on collagen, a natural material in the extracellular matrix that binds cells to form tissues. The Organogenesis team reported that the engineered skin was 60% better than conventional bandaging treatments at healing venous ulcers, skin lesions that typically affect the elderly.

ORGANOGENESIS

Ready to wear. Lab-grown skin such as this patch, grown on a collagen scaffold, has proven successful in clinical trials.

To grow this skin, a team led by cell biologist Nancy Parenteau multiplied cells from neonatal foreskin tissue donated after circumcisions. Parenteau's team uses two cell types that don't seem to trigger an immune reaction in the graft. The first type, fibroblasts, make up the "dermal" or underlying skin layer. The second, keratinocytes, constitute the top or epidermal covering. The team first seeds fibroblasts on collagen purified from bovine tendons, and then after about a week adds keratinocytes. Another 2 weeks of incubation yields a replacement skin ready for transplant. Over a period of weeks, successive layers of lab-grown skin are placed over a patient's ulcerated skin, and blood vessels grow into and sustain the new tissue. After 3 months, Parenteau says, none of the implanted skin has shown signs of rejection. Another approach—developed by Advanced Tissue Sciences in La Jolla, California—grows skin cells on a biodegradable polymer mesh; it has also fared well in early human trials.

Other tissues being explored by these biological engineers include bone, tendon, intestine, heart valves, bone marrow, and trachea. Researchers are also working to implant insulin-producing cells into diabetics to help these patients regulate their blood sugar without the need for regular injections of the hormone, and to implant dopamine-producing cells into Parkinson's disease victims to treat the symptoms of this neurological disorder. Says David Mooney, a tissue engineer at the University of Michigan: "The range of applications people are looking at is rather immense."

–R.F.S.

which has the largest volume of any organ in the body, consists of a mass of cells tunneled through with blood vessels. The organ's size enables it to filter toxins continuously from the large volume of blood in the body as well as perform its myriad of other functions. To do so, however, the variety of liver cells have to be precisely interspersed. Griffith-Cima's group is taking on these 3D challenges as well. The researchers recently devised a way to use computer-controlled printing techniques to lay down polymers known as polylactic acid (PLA) in specific patterns, one paper-thin layer at a time. That allows them to build up a porous 3D scaffold with a precisely controlled architecture.

The researchers are now working to attach PEO molecules (with the carbohydrate lures) to their PLA scaffolds. And over the next couple of years, says Griffith-Cima, they hope to attach other recognition molecules, such as antibodies, to the PEO arms in order to fix liver cells called bile duct cells. They also plan to use the amino acid sequence arginine, glutamic acid, aspartic acid, and valine—known as the REDV sequence—to specifically attract endothelial cells. Eventually, with the proper lures, they hope to fix the right mix of cells for a functioning liver.

Gathering nerve

Hybrid scaffolds are also useful because they can influence the direction of cell growth. At the State University of New York, Buffalo, Joseph Gardella and his colleagues are using the technique on nerve cells. Ultimately, they and other researchers hope to direct growing neurites—the cell arms that carry nerve impulses—across gaps caused by accidents or illness in the central and peripheral nervous systems. One possibility now being explored by Gardella and his colleagues involves organizing cellular adhesion molecules on a polymer surface.

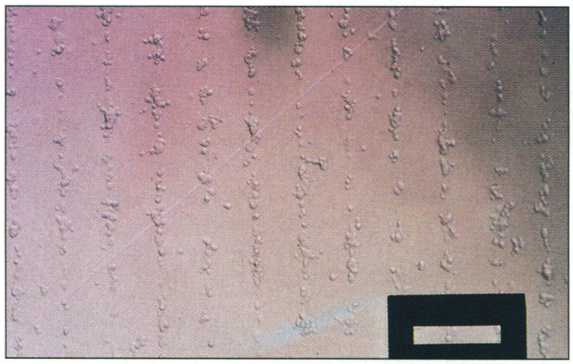
Gardella, John Ranieri—who recently moved from Brown University to CarboMedics in Austin, Texas—and a group of Swiss colleagues led by Patrick Aebischer at the Lausanne University Medical School and Hans Mathieu at the Ecole Polytechnique Fédérale de Lausanne, start with a Teflon fabric mesh. Teflon is considered safe for a variety of biological implants. The researchers then prepare a pattern on it to guide neurites, using a segment of the extracellular matrix protein laminin, which binds to nerve cells, as that guide. They begin by covering the polymer mesh with a mask made of nickel with tiny slits cut in the metal. The researchers then expose the nickel-masked Teflon to a hot ionized gas, which penetrates the slits in the nickel and converts fluorine atoms in the fabric to reactive hydroxyl groups. These

hydroxyl groups then act as links to which the researchers attach peptide sequences—known as YIGSR sequences—from laminin.

In the December issue of the *International Journal of Developmental Neuroscience*, the researchers reported that when they placed mouse nerve cells on their scaffold, the cells bound selectively to regions with the YIGSR-modified Teflon. Moreover, when new neurite branches grew from these cells, they followed the patterned surface. "This is a critical demonstration that you can pattern polymers and nerves will follow the patterns," says MIT's Christine Schmidt, who is researching directed nerve-cell growth. Now the researchers are working to roll their modified fabrics into tubes that can be wrapped around damaged nerves in the body.

Designer liners

Teflon has a long history as another type of implant: artificial blood vessels. But here its history is somewhat spotty. Although the synthetic works well on large-diameter vessels—wider than 6 mm—smaller vessels develop problems. These vessels typically clog up within 2 years after implantation—platelets and smooth muscle cells in the blood begin sticking to the surface of the polymer mesh, occluding the opening. This wouldn't happen if the implant walls more closely resembled natural blood vessels, so scientists are re-engineering them to do just that.



Nerves grown in a row. *(Top)* By attaching cell adhesion molecules in patterns on a Teflon sheet, researchers have been able to bind nerve cells to the sheet (scale bar = 100 microns). Growing neurites from these cells *(right)* follow these patterns.

"We're trying to take surfaces that are recognized [by the body] as foreign and change them into something the body really likes," says BSI's Clapper. What the body likes in the case of blood vessel inner walls are endothelial cells, which normally create a slippery surface on those walls that prevents platelets and smooth muscle cells from adhering. The strategy Clapper and several other researchers are pursuing is to induce those cells to bind to the inner walls of polymer vessels.

Researchers have long known that extracellular matrix proteins such as fibronectin and laminin promote endothelial cell binding to different surfaces. Since the early 1980s researchers have identified a number of different peptide sequences of these proteins that are responsible for the adhesion. One, the REDV sequence, was singled out in 1986 by Martin Humphries and Kenneth Yamada at the National Institutes of Health in Bethesda, Maryland. And in 1991 Jeffrey Hubbell, a chemical engineer at the California Institute of Technology in Pasadena, showed that in vitro, the sequence enhances endothelial cell binding to the common graft polymers PTFE and polyethyleneterephthalate.

At the same time work has also progressed with polymers modified with other peptides. In the July issue of the journal *Heart Valve Disease*, researchers report the first in vivo results on a polymer coated with the RGD sequence, made up of arginine, glycine, and aspartic acid. Catherine Tweden and her colleagues at St. Jude Medical, an implant company in St. Paul, Minnesota, along with William Craig and collaborators at Integra Life Sciences in La Jolla, California, report that they implanted RGD-coated polymer patches in the aortas of dogs. After 33 weeks, endothelial cells covered 75% of the RGD coated patches, three times more area than was covered in controls without the peptide coating. The Integra researchers are now implanting RGD-coated synthetic vessels into animals to see if grafts show the same benefit.

The blood vessels, researchers hope, are harbingers of implants to come. Implant researchers are modifying surfaces of a host of other devices, including those for hip joints and breast and dental implants. But like the research on assembling cells into complex tissues, this work remains in its earliest stages. Most of the promising results come from lab studies of how cells interact with hybrid scaffolds. In large part, it remains to be seen how such materials will behave in the bodies of animals, let alone humans. And before that final step can be taken, researchers must convince health officials that any implanted material and its byproducts are safe. Says Rutger's Yarmush, "a lot of detailed work needs to be done."

–Robert F. Service

IMMUNOLOGY

How the Glucocorticoids Suppress Immunity

If you are diagnosed with a disorder caused by an overactive immune system, such as an allergic skin reaction or a serious inflammatory disease like rheumatoid arthritis, chances are your physician will prescribe one of a class of steroid drugs called glucocorticoids. But ask how these drugs suppress immune reactions, and you are likely to get a shrug. Even though they have been mainstays of clinical immunology for decades, researchers have had few clues about how the glucocorticoids suppress immune and inflammatory reactions—until now, that is.

Work described on pages 283 and 286 by two research teams, one led by Albert Baldwin of the University of North Carolina, Chapel Hill, and the other by Michael Karin of the University of California, San Diego, points to what could be a major immunosuppressive mechanism of the drugs. Researchers have known for several years that the drugs, which are derivatives of hormones whose effects include helping the body respond to stress, work by interfering with immune cells' ability to turn on many of the genes needed to mount effective immune responses. The new work suggests that a large part of this effect occurs because the drugs stimulate production of a protein called IκBα, which locks up a key activator of the genes known as NF-κB, so that it can't do its job. "We understood [the glucocorticoids'] end effects, but we didn't understand the path through which they work," says immunologist Jeffrey Leiden of the University of Chicago School of Medicine. These papers, he adds, provide "a simple and elegant explanation of at least one pathway."

The explanation they offer may do more than satisfy immunologists' curiosity about how the glucocorticoids suppress the immune system. Many of the conditions for which they are prescribed require long-term treatment, and that can lead to undesirable side effects, such as cataracts, weakened bones, and abnormal fat accumulation. "The glucocorticoids are a really fantastic development for treating many human diseases. The problem is the side effects," says Anthony Cerami of the Picower Institute in Manhasset, New York, whose team is also studying the drugs' mechanism of action. But if they do indeed work by inhibiting NF-κB activity, chemists might be able to design