waited to see if mice missing both cryptochromes could adapt to light.

In *Nature* this week, Jan Hoeijmakers at Erasmus University in Rotterdam, Netherlands, Akira Yasui of Tohoku University in Sendai, Japan, and their co-workers report the first tests on such mice. But instead of providing an answer about light response, the results delivered a surprise: The mice have no clock. Under conditions of 12 hours of light followed by 12 hours of dark, they act like normal mice, running in their exercise wheels in the dark and sleeping when it is light. But in constant darkness, when the clock would normally maintain the alternating cycles, their behavior loses that pattern; they run on and off around the clock.

Those results suggest the animals' clocks fail in constant darkness. But further tests show they actually have no clock at all. When normal animals are subjected to a new light-dark pattern, they begin to adapt their clocks, a slow process as any jet-lagged traveler knows. But the mutant mice instantly adjust to any light pattern; they run when the lights go out and stop when they come on. That, says clock researcher Jeff Hall of Brandeis University in Waltham, Massachusetts, is the kind of behavior observed in clockless animals. Without a clock to control their behavior, it is driven directly by the light. Sancar and Takahashi, working with Takeshi Todo of Kyoto University in Japan, have also made double cryptochrome knockout mice and have preliminary results similar to those of the Dutch-Japanese team.

Slight abnormalities in *cry2* mutant mice that Sancar and Takahashi reported last fall suggested that *cry2* might play a central role in the clock, but most researchers were surprised to learn it is essential for the clock to work. That creates a new mystery: What is the cryptochrome doing in the clock? But it does little to solve the old puzzle of whether cryptochrome transmits light signals.

"Perhaps both functions, the clock and the light input, are being taken out" in the double mutants, says Takahashi. Ironically, the lack of a clock in the mutant mice makes it hard to test that hypothesis; one can't measure the effect of light on a nonexistent clock. But Kay notes that even if the clock is disabled, some of its molecular parts remain and should be able to respond to light. He suggests the authors check the behavior of those proteins to see if a light signal is getting through, something both groups plan to do. Wherever the search for the circadian photopigment leads as it moves beyond the rods and cones, one thing is for sure: Cryptochrome has guaranteed itself a place in the story of the circadian clock. **–MARCIA BARINAGA**

TISSUE ENGINEERING

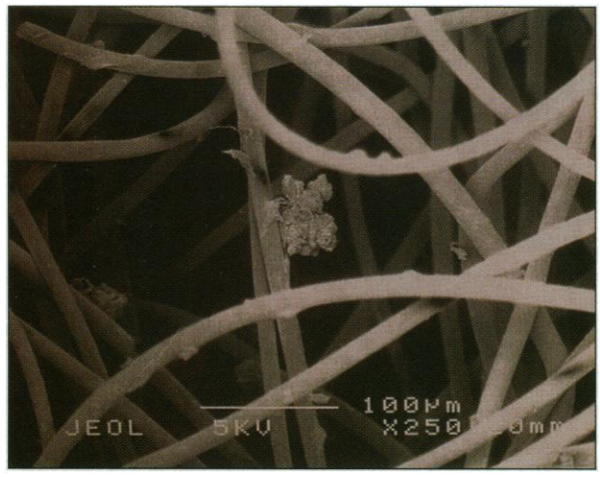
Lab-Grown Organs Begin To Take Shape

With the need for transplant organs growing, researchers are making progress toward developing them, using cultured cells and special polymers

Call it the seaweed that's changing medicine. On a balmy summer afternoon in 1986, surgeon Joseph Vacanti of Harvard Medical School in Boston was sitting on a stone breakwater near his Cape Cod vacation house watching his four children play on the beach. He and biomedical engineer Robert Langer of the Massachusetts Institute of Technology (MIT) had been trying for more than a year to devise new ways to grow thick layers of tissues in the laboratory—a first step toward their long-term goal of growing replacements for damaged tissues and organs.

But even though they were using the latest in cell-friendly, biodegradable polymers as scaffolds to support the growing tissue, the thickest slices they could grow were thinner than a dime—not much use for building complex three-dimensional organs like livers, kidneys, or hearts. The problem, Vacanti realized, was that as the tissues thickened, the interior cells couldn't take in enough nutrients and oxygen or get rid of sufficient carbon dioxide to continue growing.

Then, as Vacanti gazed into the water, inspiration struck. He spotted a seaweed waving its branches, silently soaking up nutrients from the water around it. He immediately made the connection: Branching is nature's way of maximizing surface area to supply thick tissues with nutrients, and polymer materials that branch, rather than being completely solid, would be porous enough to support growing tissue in the lab. Vacanti raced up the road to a pay phone to call Langer. "He asked if we could design [biodegradable] polymers that had a branching structure," Langer recalls. "I said, 'Well, we could probably do that,' and we tried and we did."



Web site for cells. The micrograph shows smooth muscle cells growing in a porous polymer used for tissue engineering.

CREDIT: NIKLASON *ET AL.*

Thirteen years later, branched biodegradable plastics and related sponge-shaped plastics undergird tissues growing in dozens of laboratories around the world. Some of the simpler of these tissues, including skin and cartilage, have already made it to the clinic or are on their way (see sidebar). But, fueled by recent advances in polymer chemistry, in the design of the bioreactors that incubate the tissues, and in the understanding of basic cell and tissue biology, researchers are also beginning to grow organs with more complex architectures.

Two months ago, for example, a team at Harvard Medical School reported in *Nature Biotechnology* that it had used tissue engineering to produce new urinary bladders that appeared to work normally in dogs. And on page 489, Langer, anesthesiologist and biomedical engineer Laura Niklason of Duke University in Durham, North Carolina, and their colleagues report growing functioning pig arteries. Less advanced, but showing progress, are efforts to engineer tissues to fill in for failing hearts, livers, and kidneys—organs for which the demand for transplants far outstrips supply.

"I'm very excited about it all," says biomedical engineer Michael Sefton of the University of Toronto. "Things are going much better than I expected." Indeed, he has organized more than 25 leading tissue engineers into an informal interna-

tional network called the Life Initiative. Their goal? To raise more than $1 billion from industry, government, and private foundations for a decade-long effort to grow livers, kidneys, and hearts (*Science*, 12 June 1998, p. 1681).

Tissue in three dimensions

The first bioengineered internal organ to reach the clinic may be the bladder, produced by surgeon Anthony Atala of Harvard Medical School and his colleagues. The team faced several challenges during its 9-year quest to grow a working urinary bladder in the laboratory, Atala says. First, the researchers had to isolate the necessary cells and coax them to grow in culture dishes. They had little trouble in harvesting from dog bladders the smooth muscle cells that would form the bladder's outer surface and getting them to grow. But growing the specialized epithelial cells called urothelial cells that would line the inside surface of the organ was another matter.

The main problem was that in culture, the urothelial cells, taken from the same bladder samples, tended to revert to less specialized, primitive forms that would function poorly, allowing urine to leak into bladder tissue, which could cause scarring and pain. But with the right culture conditions and combination of growth factors, the team was eventually able to steer urothelial cells toward their mature state. "It's important to get the cells to differentiate and to stop differentiating; to grow and to stop growing," Atala says.

The researchers also had to find the right polymer scaffolding on which to grow the cells. The polymers had to be elastic enough to give the cells a lifelike mechanical environment, sufficiently porous to allow the fluids bathing the growing tissues to deliver nutrients and flush away cellular wastes, and capable of degrading as the tissue developed —but not so fast that it dissolved before the cells had time to grow into it. Eventually, the Harvard team settled on a branched, porous version of a polymer called polyglycolic acid that Langer had produced. They set the polymer in a bladder-shaped mold and coated it with a second polymer called polylactide-coglycolide.

In the next step, the researchers applied the urothelial cells to the inner surface of the polymer bladders and the smooth muscle cells to the outer surface, and then nurtured the synthetic organs for 7 days in a sterile nutrient broth. At that point, they surgically replaced the bladders of six beagles with the lab-grown bladders. Within 3 months, the polymers had disappeared from the tissues, the dogs' bodies had supplied the bladders with blood vessels, and the neobladders held just as much urine as normal bladders and maintained a normal bladder shape. In addition, the bladders worked and even developed innervation. "The dogs empty by themselves," Atala says. Indeed, says bioengineer Antonios Mikos of Rice University, the Harvard group's success at engineering the bladder "proves the feasibility of engineering other organs using cocultures of cells and synthetic, biodegradable organ scaffolds."

The Harvard group has since gone on to grow human bladders in the laboratory and is now seeking regulatory approval to start clinical trials. Lab-grown bladders could serve as replacement organs in some of the hundreds of thousands of people whose bladders have been damaged by accidents, chronic infections, bladder cancer, or congenital birth defects, Atala says.

Building blood vessels

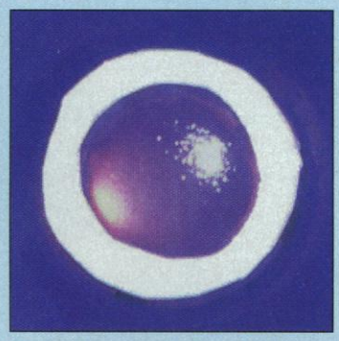
The work of the Niklason-Langer team in creating artificial arteries looks equally promising. Blood vessels have been engineered before using other techniques but with only mixed success. For example, cell biologist François Auger and colleagues at Laval University in Québec City, Québec,

CREDIT: LOEX RESEARCH LABORATORY/LAVAL UNIVERSITY

From the Lab to the Clinic

Even as tissue engineers work to produce whole organs such as bladders and livers (see main text), lab-grown versions of more than a dozen different tissues, ranging from skin and cartilage to heart valves and corneas, are either in the clinic or under development. Here's a sample:

• *Approved for clinical use.* The first engineered tissues to hit the market have been skin and cartilage products. In 1997, the U.S. Food and Drug Administration approved TransCyte, a skin replacement made by Advanced Tissue Sciences Inc. of La Jolla, California. Consisting of cells from the inner, or dermal, skin layer grown on a biodegradable polymer, TransCyte can serve as a temporary wound cover for some of the more than 30,000 patients hospitalized each year in the United States with second- and third-degree burns. Another skin product, Apligraf, which is made by Organogenesis Inc. of Canton, Massachusetts, and consists of both the dermal and epidermal skin layers, was approved last year in the United States and Canada to treat leg ulcers that don't spontaneously heal.



Ready for transplant? This human cornea tissue, which is about 2.5 centimeters across, was grown in the lab.

One cartilage product has also won regulatory approval. This is Carticel, made by Genzyme Corp. of Cambridge, Massachusetts, to replace damaged knee cartilage. Genzyme takes cartilage-forming cells, called chondrocytes, from cartilage snipped from the patient and grows them in a degradable matrix. The surgeon can then cut out the damaged cartilage and replace it with this new tissue.

• *In clinical trials.* Reprogenesis Inc. in Cambridge, Massachusetts, has a different sort of cartilage product in advanced clinical trials. Consisting of chondrocytes growing in a polymer called a hydrogel, which hardens when injected into the body, it's intended for replacing defective bladder valves in children with vesicourital reflux, which causes urine to flow from the bladder to the kidney, and in women with urinary stress incontinence, in which patients urinate when they cough or sneeze. Other products in or nearing clinical trials include Dermagraft, from Advanced Tissue Sciences, a variation on TransCyte designed to treat difficult-to-heal foot diabetic ulcers, and Vitrix, a connective tissue product from Organogenesis consisting of fibroblasts and collagen, which helps deep wounds heal without scarring.

• *In the pipeline.* Still in lab studies are a host of additional products. For example, bioengineer Antonios Mikos of Rice University and his colleagues have recently developed an injectable polypropylene-fumarate copolymer that hardens quickly in the body and provides a surface that guides severed long bones to regenerate in rats and goats. Joseph Vacanti of Harvard Medical School in Boston and his colleagues have recently used a polymer matrix to grow lengths of replacement intestines in rats, which they then attached successfully to the animal's gut. Also in the works are the first lab-grown human cornea, from François Auger's team at Laval University in Québec City; a portion of the heart's pulmonary valve, grown by pediatric cardiovascular surgeon John Mayer of Harvard Medical School and his colleagues; and soft tissue engineered by David Mooney of the University of Michigan, Ann Arbor, together with colleagues at the Carolinas Medical Center in Charlotte, North Carolina, as a potential replacement for breast tissue removed during mastectomies. **–D.F.**

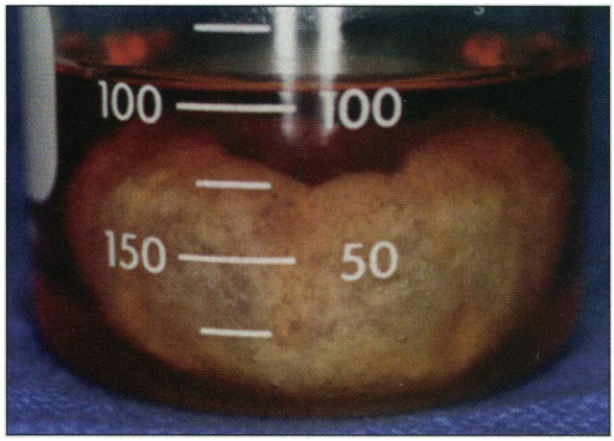
reported in the January 1998 issue of *The FASEB Journal* that they had assembled blood vessels from smooth muscle cells and endothelial cells from umbilical cords, and fibroblasts from adult human skin.

They did this by growing the smooth muscle and fibroblast cells as sheets, without a polymer support, in separate culture flasks, then wrapping them, in successive layers, around a pipette, "like a cinnamon bun," as Auger puts it. After lining these artificial blood vessels with endothelial cells, which form the internal surface of natural blood vessels, the Laval team showed that they held up under arterial pressures and could be stitched into dogs. But blood leaked between the sheets of cells, and many of the vessels plugged up with blood clots within a week. Auger is pleased, though, with these still-early results. "We wanted to see if the plumbing held, and it did," he says.

Rather than stacking sheets of tissue, the MIT team grew its blood vessels on tubes of polyglycolic acid, under conditions Niklason designed to mimic those encountered by a newly formed artery in the body. First, co-author Jinming Gao, now of Case Western Reserve University in Cleveland, partially hydrolyzed the polymer with sodium hydroxide. This creates water-loving hydroxyl groups that enable more cells to attach. Then, Niklason drizzled smooth muscle cells from cows onto the tube-shaped scaffold and inserted a pliable piece of silicon into the interior. The tubing was hooked up to a pump, which pulsed 165 times a minute to mimic the pulsing pressure on a developing embryonic artery. After 8 weeks, Niklason coated the inside of the vessels with endothelial cells. "To me the most novel thing [about this study] is the idea of using a bioreactor that beats like a heart," Langer says.

The pulsing made the tissue stronger because, Niklason says, it increased the cells' production of collagen, a tough protein found in connective tissue. In any event, the pulsed blood vessels had walls that were twice as thick as nonpulsed vessels. They could also withstand sutures without tearing, although not as well as natural arteries, and they contracted in response to the same chemical signals that normally spur arterial contractions. The pulsed vessels also worked in animals. When the researchers took cells from tiny arterial biopsies of miniature pigs, grew vessels, and used them to replace an artery in the same animals' legs, the engineered arteries lasted more than 3 weeks without clogging, whereas arteries grown without pulsing clogged.

The results are "really astounding," says cardiovascular surgeon Timothy Gardner of the University of Pennsylvania School of Medicine, who is chair of the surgery council of the American Heart Association. "If this works out," he adds, "it will be a major development in cardiovascular surgery." In many of the nearly 600,000 coronary bypass operations performed each year in the United States, surgeons can use blood vessels from the patient's own leg or chest to replace clogged coronary arteries. But in some cases, as when the individual has already undergone one such operation and doesn't have any suitable blood vessels left, this may not be possible. And small-diameter synthetic arteries haven't worked because they tend to clog up.



Bladder in a beaker. Tissue-engineered bladders, such as this one, appear to function normally in dogs.

CREDIT: ANTHONY ATALA

Organizing organs

Although engineered bladders and arteries are a significant accomplishment, making these hollow structures, formed by relatively thin layers of cells, is easier than achieving another goal the tissue engineers have set for themselves: producing large internal organs like the liver and kidney that need complex networks of arteries, veins, and capillaries to carry blood to and from every cell. "Large, vascularized tissue is the Holy Grail of tissue engineering," Vacanti says.

There has been some progress, however. In two recent papers, one published in the *Annals of the New York Academy of Sciences* in December 1997 and the other in the July 1998 *Annals of Surgery*, Vacanti, bioengineer Linda Griffith of MIT, and their colleagues reported taking a step toward producing a synthetic liver. "We are fabricating tissue-engineered structures that will have their own blood supply," Vacanti says.

To do this, Vacanti, Griffith, and colleagues first created a plastic scaffold, made from polylactide and polyglycolide polymers, that contains channels that allow fluid to flow and capillaries to develop inside the tissue. The trick to making the scaffold was a method called three-dimensional printing (3DP), which was originally designed to make intricate molds for metal engine parts. By depositing successive thin layers of polymer, the technique creates a complex internal structure, including channels as narrow as 300 micrometers. When the researchers seeded the matrix with rat liver and endothelial cells and gently pumped growth medium through it for 5 weeks, the cells not only survived, but they rearranged themselves into microscopic structures resembling those in liver tissue. What's more, the liver cells grown inside the matrix produced the protein albumin, just as they do in a real liver.

But despite the successes so far, researchers will need to surmount some major challenges before tissue-engineered organs are routinely available. Besides a network of capillaries, arteries, and veins, most engineered organs will also need to be wired with nerves that regulate their function. Tissue-engineered organs "will do an OK job, but ultimately you'll need fine-tuning," says biomedical engineer Christine Schmidt of the University of Texas, Austin, who is designing new ways to regrow severed nerves.

Tissue engineers also need a reliable source of cells. Today, many of them are growing tissues using cells from an animal's or a patient's own body, but that can take weeks—time that some patients don't have. And engineered tissue from outside sources, although easier to come by, will run into the same problems that plague organ transplants. Unless scientists can prevent it, the immune system will reject the tissue as foreign.

Then comes the challenge of reliably manufacturing the tissue-engineered products, says Gail Naughton, president and chief operating officer of Advanced Tissue Sciences, a biotech firm in La Jolla, California, that is doing tissue engineering. That means that researchers will need to develop ways to test the products for sterility, mechanical strength, and function; ways to freeze the tissue and store it; and ways to scale up manufacturing to meet the tremendous demand. That goal has already been met for some skin and cartilage products. And researchers are optimistic that it can be achieved for internal organs as well. "I think that anyone in tissue engineering, myself included, believes that any tissue or organ can be grown outside the body," Naughton says.

–DAN FERBER

Dan Ferber is a writer in Urbana, Illinois.