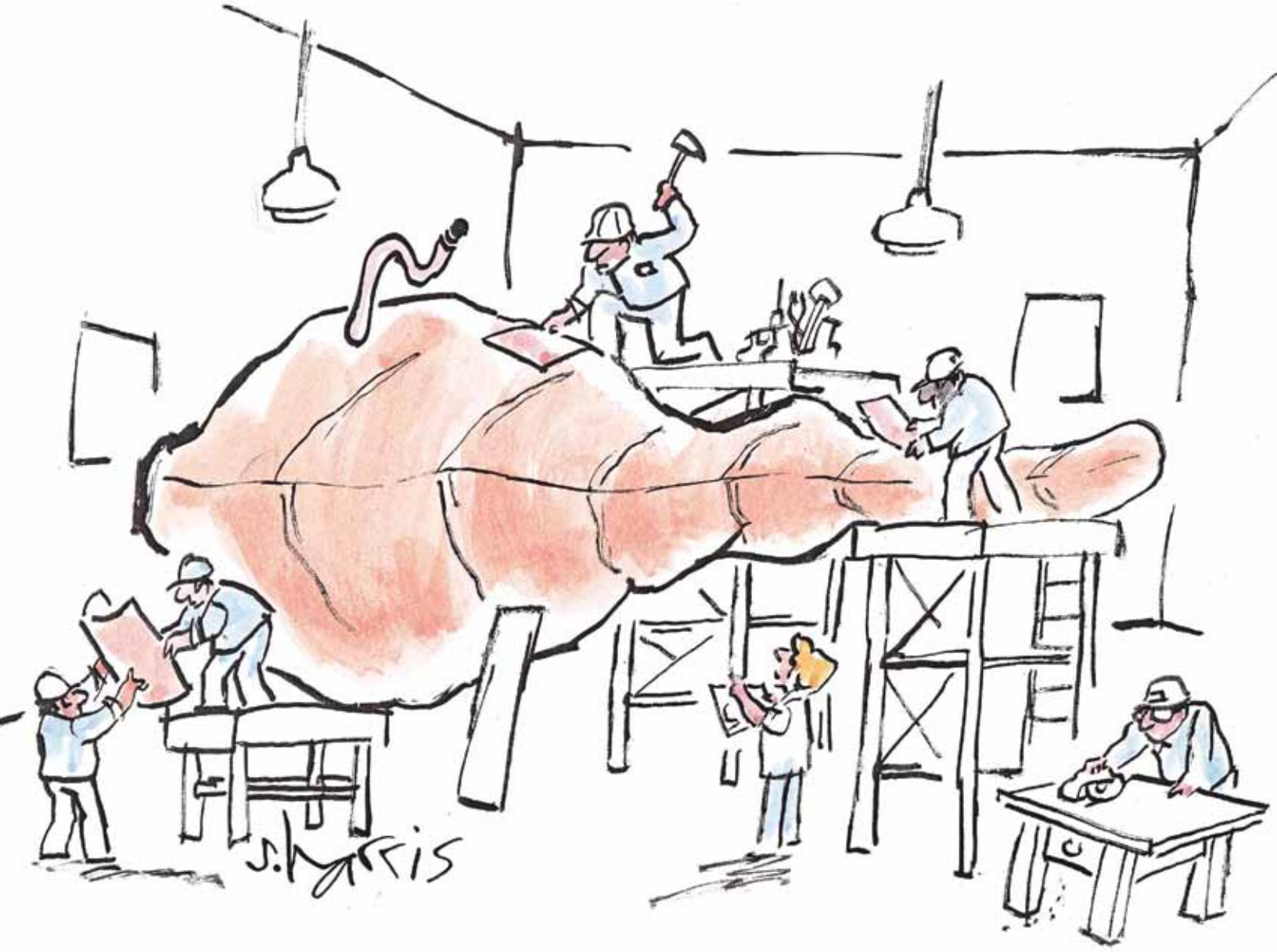
Some Assembly

By Kathy Svitil



We're a long way from transplantable organs grown to order, but Caltech chemists are developing some of the tools we will need to make that happen.

Required

DAVID TIRRELL

At any given moment, more than 100,000 people in the United States are awaiting organ transplants. Although thousands of transplants are performed each year, thousands of other people die because there just aren't enough donated organs. One solution? *Making organs*, from scratch. Imagine a big assembly line, churning out kidney after kidney after kidney.

Science fiction? Of course. But through the development of synthetic tissuelike biomaterials, "artificial" proteins with programmable properties, and methods for the pinpoint placement of cells, Caltech chemists are inching us closer to the elusive goal of made-to-order organs.

In truth, artificial organs have been in use for decades. Back in 1982, for example, the first Jarvik-7 replaced the ailing heart of a Seattle dentist, who lived for 112 days (albeit hooked up to machines); artificial ears, a.k.a. cochlear implants, are now commonplace. But the ersatz organs available today are far from perfect substitutes—quite understandably, because human tissues are exceedingly complex, with a daunting variety of strategically placed cells and a complicated infrastructure of nerves and blood vessels. This architecture has, so far, proven impossible to duplicate; indeed, the bladder—essentially a balloon of soft, stretchy tissue—remains the only *living* lab-grown replacement organ yet developed.

A crucial first step in building artificial organs that are more lifelike is creating lifelike artificial tissues. At Caltech, such materials are the purview of chemist David Tirrell. Admittedly, Tirrell's work is not focused explicitly on making such tissues, and he offers no claims that it ever will: "It may be that in 100 years, something we're involved in now may lead to artificial tissue," he cautions.

What he *does* do is far more basic: He invents proteins made with amino acids not found in nature that function in ways that normal proteins do not. In these artificial proteins, as in natural ones, the sequence of amino acids within the molecule determines how it contorts itself into a three-dimensional shape, and that shape in turn determines the protein's function. But the sequence of a protein also determines how it behaves en masse, when surrounded by countless numbers of its fellows.

And that behavior matters if you're trying to build an artificial tissue, which needs to include not just the cells but the scaffold on which they hang. That framework—constructed mainly out of protein molecules—is called the extracellular matrix, or ECM. Tirrell builds artificial ECMs to order, controlling their properties by monkeying with the genetic blueprints of their constituent proteins.

When you design these genes, he says, "you have to think not only about the protein itself but about the behavior of the material that results—is it stiff, is it loose, what other properties does it have?" For example, say you want to build an artificial tissue composed of liver cells, or from the insulin-producing beta cells of the pancreas. An ECM—artificial or otherwise—is an elastic, but solid, gel. When the ECM grows up with the organ, getting the cells inside it is not a problem because they are already there, but otherwise "it's hard to get cells into an elastic solid," Tirrell says. "If you design your gel so that it's initially a liquid, you can distribute the cells within it." Then, once the cells are properly distributed—which can be as simple as stirring the mixture—you add another protein to the gel to make it harden like Jell-O in a refrigerator.

Tirrell, working with bioengineering grad student Alborz Mahdavi and and with H. Teresa Ku of the City of Hope, recently spiked one of his ECMs with pancreatic stem cells. Nestled within the matrix, the cells flourished—and, Ku says, differentiated into endocrine cells, as hoped. "We are planning to transplant the cell colonies into mice in the near future to see whether these cells will secrete insulin and correct diabetes in a mouse model," she says.

The usual way to build a "simple" artificial organ starts with a prefab scaffolding constructed of a porous, biocompatible material such as a water-swollen gel—or "hydrogel"—made from polyethylene glycol. (PEG, as it is known in the outside world, is a common ingredient in skin cream, shampoo, and toothpaste.) The gel is molded into the rough shape of the organ and seeded with cells. The challenge, of course, is steering all of the various cell types to their proper locations—including the cells that form blood vessels. Without blood flow, after all, any tissue will die.

A method developed by grad students Udi and Ophir Vermesh (both PhD '11) and their colleagues in the laboratory of chemist Jim Heath may meet this challenge. By pressing a silicone template containing microfluidic channels against a microscope slide, a gridwork of anchor points is created on the glass. Each anchor can be tailored to adhere to one specific cell type, allowing individual cells to be placed in prescribed locations just a few millionths of a meter apart. "We then encase the patterned cells in a hydrogel matrix and stack these hydrogel sheets to form 3-D tissue constructs," explains

JULIA KORNFIELD

Ophir, who is now in his fourth year of medical school at UCLA as part of a joint MD/PhD program.

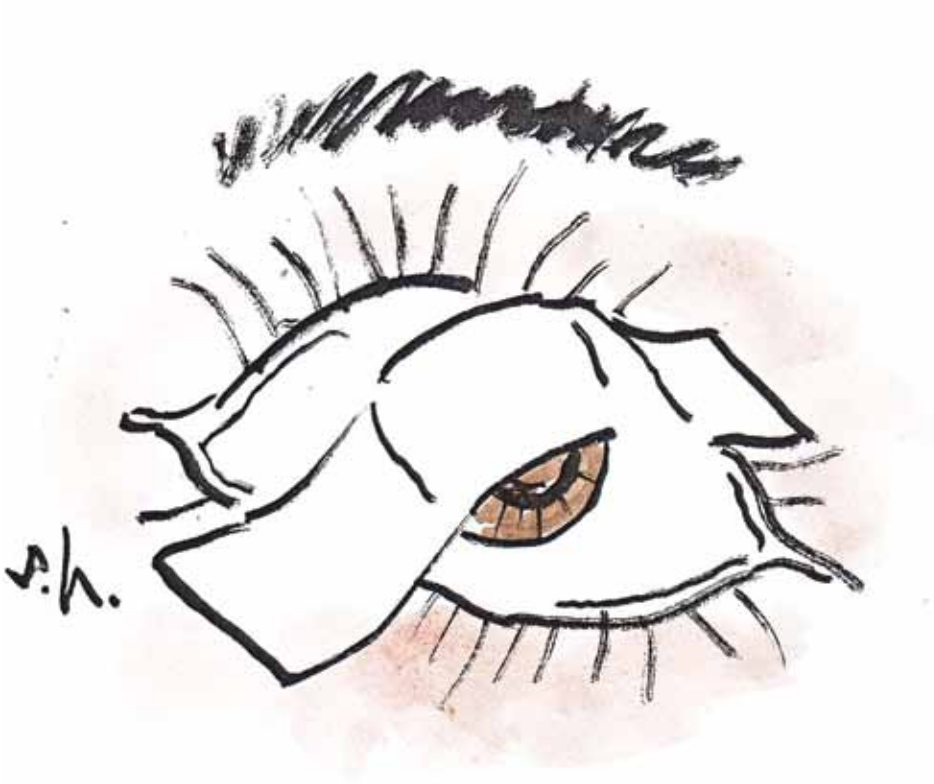
The team tested the process by building very rudimentary islets of Langerhans—as the insulin-producing parts of the pancreas are known—by setting out neat rows of insulin-secreting beta cells alternating with equally neat rows of non-insulin-secreting alpha cells. These cells were actually much better organized than absolutely necessary: in rats and mice, the beta cells form clusters encircled by the alpha cells, whereas in humans the alpha and beta cells are just sort of scattered willy-nilly, surrounded by blood vessels. And although random clumps of cells aren't prohibitively difficult to make, making blood vessels is another issue. To do so, you'd have to snake tunnels, constructed out of the appropriate cells, through your matrix, which means accurately creating and lining up *holes* in the stacked 2-D sheets. "We're still trying to figure that part out," Vermesh admits.

The experiment was merely intended to demonstrate the ability to control where specific cells went, so "we didn't place much emphasis on incorporating ECM proteins into our hydrogels," Vermesh says. "But the types of proteins found in an ECM play an important role in keeping cells alive in real tissues. So while these cells survived for a time, I would expect they would need those ECM proteins in their immediate surroundings, especially in an implantable device."

ECM proteins are also key to a different implantable material that Tirrell, chemist Robert Grubbs, chemical engineer Julia Kornfield (BS '83, MS '85), and collaborators at UC San Francisco have developed for healing damaged corneas.

The cornea is the eye's half-millimeter-thick outer "skin." It's a complex sandwich whose filling contains three collagen-rich layers, where long fibrils of collagen are placed so precisely that the resulting structure is as transparent as a perfect piece of glass.

If these fibrils' orientation gets disrupted by a scratch or infection, the glass can turn cloudy, resulting in corneal blindness—some 2,000,000 cases worldwide. (Cataracts, the most common cause of blindness, are caused by cloudiness of the lens—the light-focusing crystal located deeper within



the eye.) "We want to understand how we can guide wound healing so that it does *not* lead to scarring and blindness," Kornfield says.

Corneal scarring results from the body's overly enthusiastic effort to slap a Band-Aid on a wound. Your system's first responders at the scene of any injury are the constantly circulating platelets in the bloodstream. They set up a triage center by forming a makeshift ECM, upon which the other incoming cells on the emergency team alight so they can fix things. Soon, fresh collagen fibers are being plastered down, mending the damaged area. "The healing response is designed to close a wound as quickly as possible," Kornfield says. "It doesn't have to be pretty, just get it closed."

And, indeed, the result is *not* pretty, because the patch's fibers lack the regimented organization of their neighbors. Instead, they are strewn haphazardly, like a microscopic crazy quilt. And instead of transmitting light unscathed, the patch scatters it. The cornea takes on a milky hue, and vision is obscured.

"What we need to do," Kornfield says, "is tell the system how to come in and clean up the mess without making a mess." The way to do that, she and her colleagues have found, is with an implant somewhat akin to a contact lens, but made of Tirrell's specially crafted gel. In addition to the ECM proteins that form the gel itself, the implant contains signaling molecules that slow the triage process and buy valuable time for the body to lay down collagen fibers the *right* way. "We need to get in there within a few hours to prevent the wrong stuff from laying down," she continues.

"We're very enthusiastic about the cornea work," Kornfield says, "but my hope is that we can create a gel that could prevent *any* kind of scar," anywhere in the body.

The idea is not far-fetched, she argues. Because it has to be transparent, "the precision with which the cornea has to be built is extreme," she says. "If we understood *that*, and could keep that process from going wrong, it would be easier to promote healing without scarring in other tissues, including the skin." Surgical incisions could someday be closed without a trace—but also wounds caused by accidents, say, or even burns.

> Although random clumps of cells aren't prohibitively difficult to make, making blood vessels is another issue. To do so, you'd have to snake tunnels, constructed out of the appropriate cells, through your matrix, which means accurately creating and lining up *holes* in the stacked 2-D sheets.

This type of carefully orchestrated repair would likely take longer than the natural healing process, but, Kornfield says, the results "would be *perfect*"—and that, she adds, is worth the wait. E&S

David Tirrell is the Ross McCollum–William H. Corcoran Professor and professor of chemistry and chemical engineering. Research on artificial ECM proteins has been funded by the National Institutes of Health and by Caltech's Jacobs Institute for Molecular Engineering for Medicine.

Jim Heath is the Elizabeth W. Gilloon Professor and professor of chemistry. The cell-patterning project was funded by the National Cancer Institute, the Ivy Foundation, and the Grand Duchy of Luxembourg.

Nobel Laureate Robert Grubbs is the Victor and Elizabeth Atkins Professor of Chemistry.

Julia Kornfield is a professor of chemical engineering. Her research on corneal wound healing is also sponsored by the Jacobs Institute for Molecular Engineering for Medicine.