

These tiny (the smallest is about 5 millimeters) bioactive scaffolds were created by Rocky Tuan using a 3-D "printer," a mixture of chemicals, and light. (The blue tint was added to enhance visual impact.) Though here they look a lot like something you might drop into a cocktail, they aren't for cooling; the material was designed to form the foundation of new tissue and organs.

COURTESY ROCKY TUAN AND HANG LIN

WITH A LITTLE HELP FROM BIOMATERIALS

BY JOE MIKSCH

HEAL THYSELF



Things fall apart …

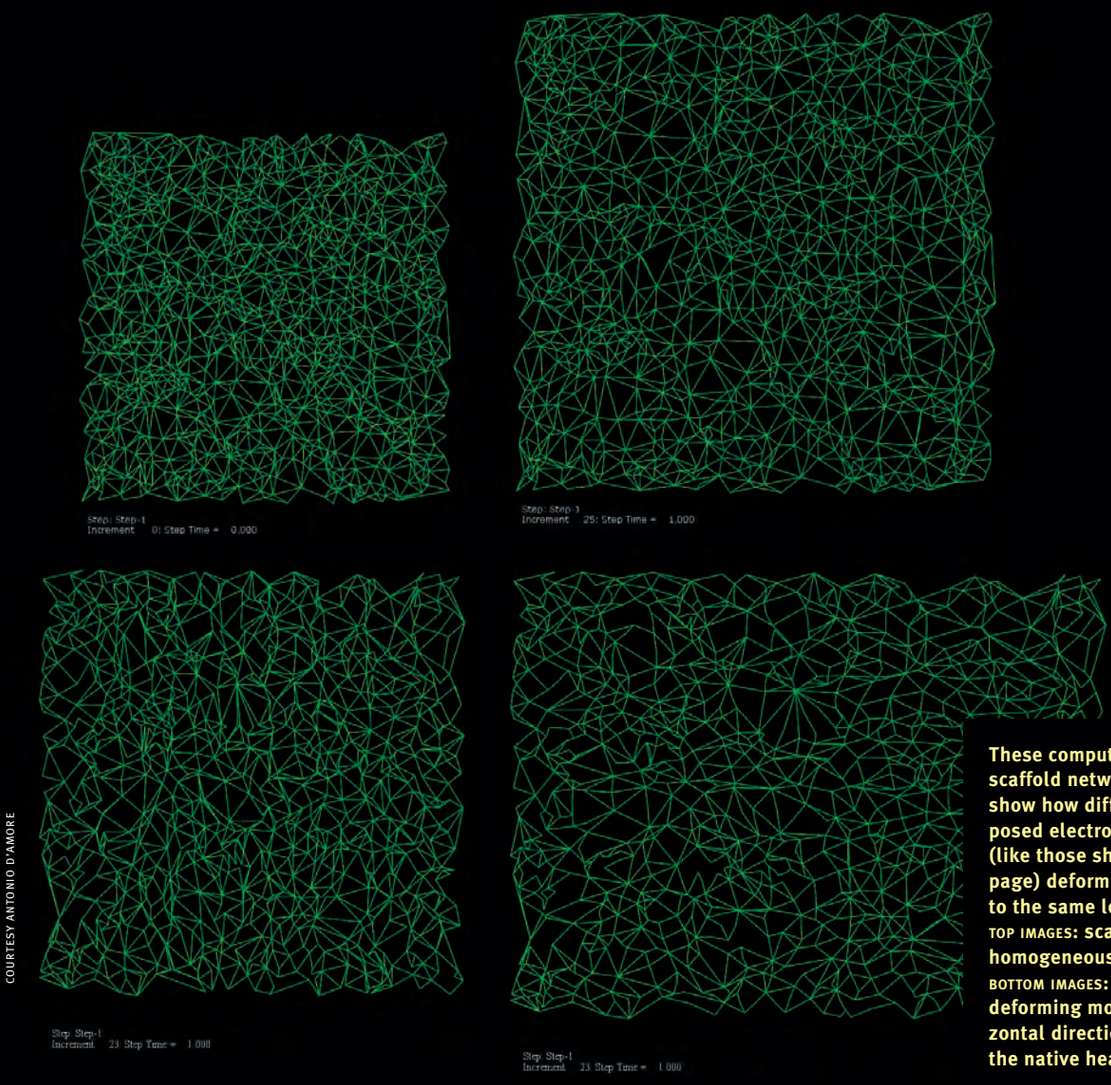
So wrote W.B. Yeats in "The Second Coming." It's safe to say he wasn't talking about the human body, but it's an apt metaphor. Things do fall apart. From injury, from illness, from inborn defects. It's rough out there (and in there, for that matter) for the corporeal being.

We try to fix things, then. Artificial joints, surgical mesh to help heal abdominal damage… In many cases, such interventions work quite well. But there are physical problems that require a more delicate touch. The injured heart. A ruined nerve. Can these things be fixed with "fakery," with something not human placed inside a human? The answer is: It's possible, with the help of nature.

Investigators at the University of Pittsburgh are developing various scaffolds to help with healing, even with regrowth. The key, in the minds of these bioengineers (all the players in this story are School of Medicine appointees who are also members of Pitt's Swanson School of Engineering's mega-multidisciplinary Department of Bioengineering) is to seed such temporary supports with stem cells or growth factors, which focus the body's healing powers on the site of insult or injury. And it's working, showing promise in several concurrent animal studies. The concepts here are not new: *Science* swooned over the nearing prospect of "off-the-shelf replacement parts for the human body" in a 2002 special issue. We didn't get there in the past decade and maybe we won't in the next, but observers say we've made some big leaps forward.

Physician, heal thyself? More like, *Body, heal thyself.*

COURTESY OF ROCKY TUAN AND HANG LIN.



COURTESY ANTONIO D'AMORE

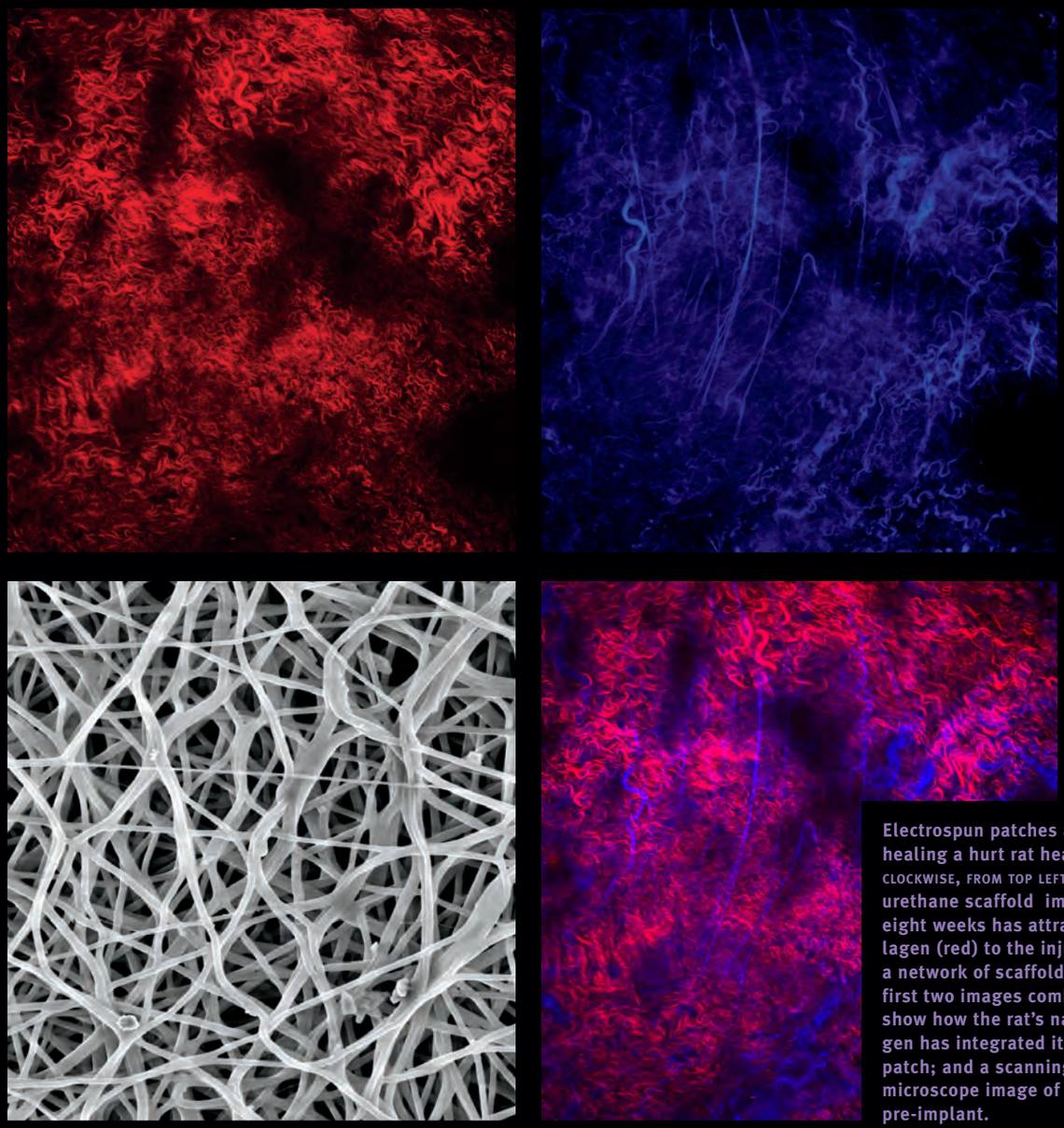
These computer-generated scaffold network models show how differently composed electrospun scaffolds (like those shown on the next page) deform when subjected to the same level of force.
TOP IMAGES: scaffold deforming homogeneously.
BOTTOM IMAGES: scaffold deforming mostly in the horizontal direction, mimicking the native heart wall tissue.

A NEW EAR AT KINKO'S?

What you see on the previous page is not a pile of ice cubes. It's a product of chemistry and visible light that could create a sea change in regenerative medicine.

About a year ago, Rocky Tuan, with the aid of Pitt's Clinical and Translational Science Institute, acquired a 3-D printer. Tuan is a PhD and director of the Center for Cellular and Molecular Engineering in Pitt's Department of Orthopaedic Surgery, as well as director of Pitt's Center for Military Medicine Research. To create this extraordinary material, Tuan starts with a liquid made from a photoinitiator (a compound that decomposes into free radicals when exposed to light; it's necessary for the production of both smog and plastics) and a form of polyethylene glycol (a component of the polymerization process, which creates three-dimensional chemical networks) or gelatin. Tuan uses the "printer" to pass visible light through the material. As the light passes through, layer by layer, the liquid solidifies into shapes as small as 5 millimeters high.

So, that's nifty, being able to make biodegradable, body-friendly solids—down to a micron scale—out of liquid and light. (Tuan says the machine can replicate any body structure, working off computer-aided drafting software.) And the end product needn't be solid; it can have a grid-like internal architecture mimicking that of the tissue it is intended to replace. The truly exciting part is what Tuan includes in his primordial soup: stem cells. By adjusting the density of the liquid, Tuan can ensure that the cells will be evenly distributed throughout the resulting solid. And, most importantly, the cells live. More than 90 percent of them remain viable a week after being polymerized. ("We have an interior hole in here, and it's 50 microns," Tuan says, pointing to an image of his non-ice cubes. "A cell is about 10 microns; so we can have a cell and a bunch of his little [cell] buddies living in there.") If things work as Tuan expects, these stem cells will grow up to become tissue, and the scaffold will melt away. *And, here, Mr. Van Gogh, is your new ear.*



COURTESY ANTONIO D'AMORE

Electrospun patches assist in healing a hurt rat heart. CLOCKWISE, FROM TOP LEFT: **The polyurethane scaffold implanted for eight weeks has attracted collagen (red) to the injured area; a network of scaffold fibers; the first two images combined to show how the rat's native collagen has integrated itself into the patch; and a scanning electron microscope image of the patch pre-implant.**

TAKEN FOR A SPIN

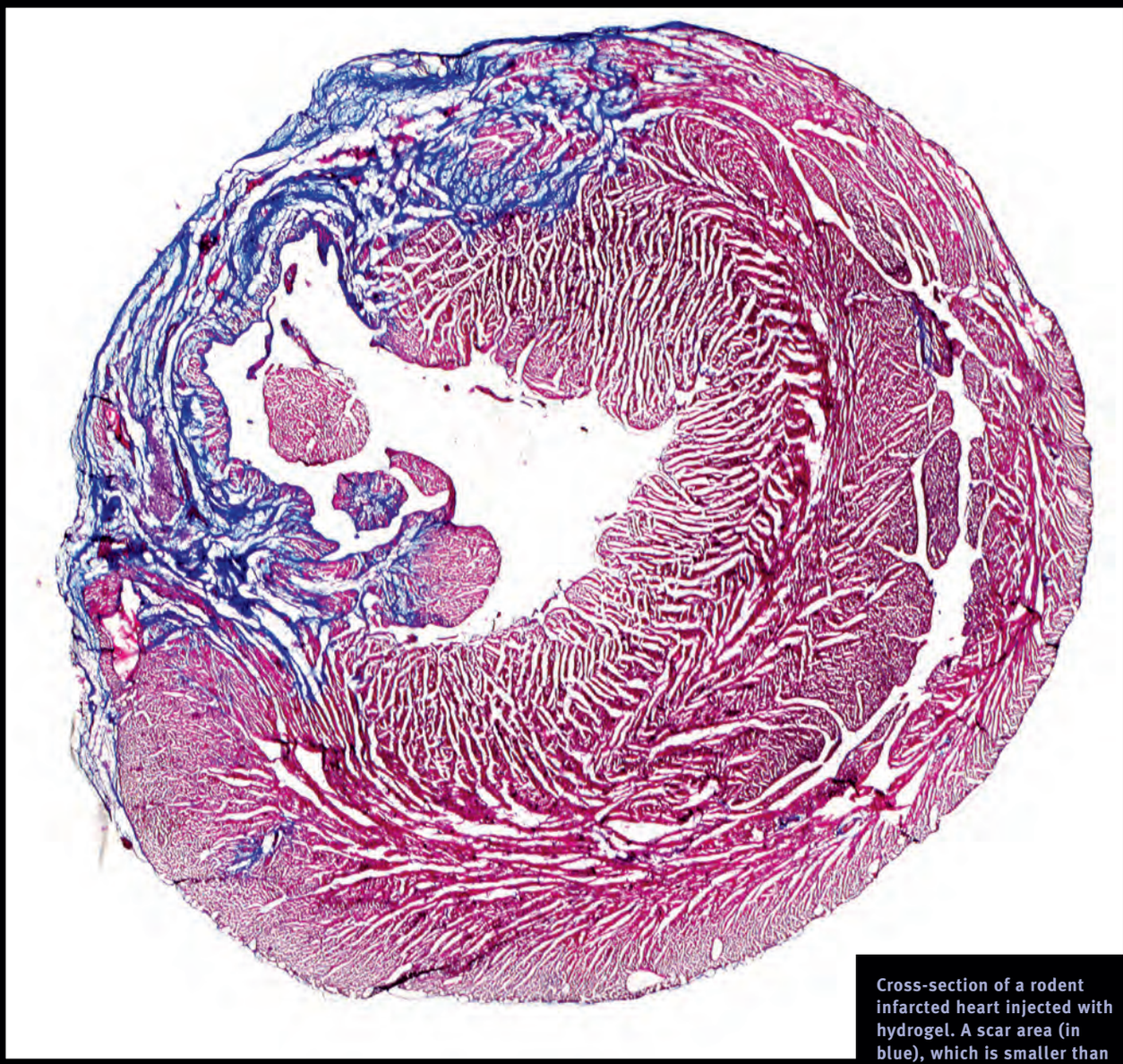
If your abdominal wall became perforated, a surgeon might implant a mesh to reinforce the damaged area. This works, but it can also lead to complications such as a fistula or infection. Pitt's William Wagner, a PhD, director of the McGowan Institute, and professor of surgery and chemical engineering, as well as bioengineering, thinks we can do better.

Using a technique called electrospinning, Wagner and colleagues use a liquid polymer sprayed on a rotating spindle to create small sheets of biodegradable material that can be used to patch anything from injured abdomens to hurt heart walls. The chemistry of the polymeric solution can be adjusted to mimic the tensile strength of the tissue, and the fibers can be arranged to replicate other mechanical properties, such as the direction in which the tissue stretches. These sheets, when properly designed and properly appended to a weakened area, can serve as support while the injury heals naturally. A fine temporary crutch.

Now Wagner et al., with the help of Stephen Badylak (DVM, PhD, MD, and Pitt professor of surgery and deputy director of the McGowan Institute), plan to use their electrospun material to speed up healing. Badylak has created an extracellular matrix (a gel created from pig tissue that has been stripped of cells but which retains growth factors) that Wagner incorporates into the polymeric solution before spraying it onto the rotating mandrel. "So we get these biohybrid composites that have the mechanical qualities of the polymer, but also have tissue ingrowth that's much more rapid than if we didn't have [Badylak's] material," Wagner says.

Essentially, they've created a bioactive Band-Aid.

Wagner cautions that "it's not magic. [We're not] getting new, fully functional tissue. But it is an important step getting the cells to come in [to the site of the injury] rather than just 'walking off.' We're actively trying to figure out how to get more of a positive healing response with these kinds of materials."



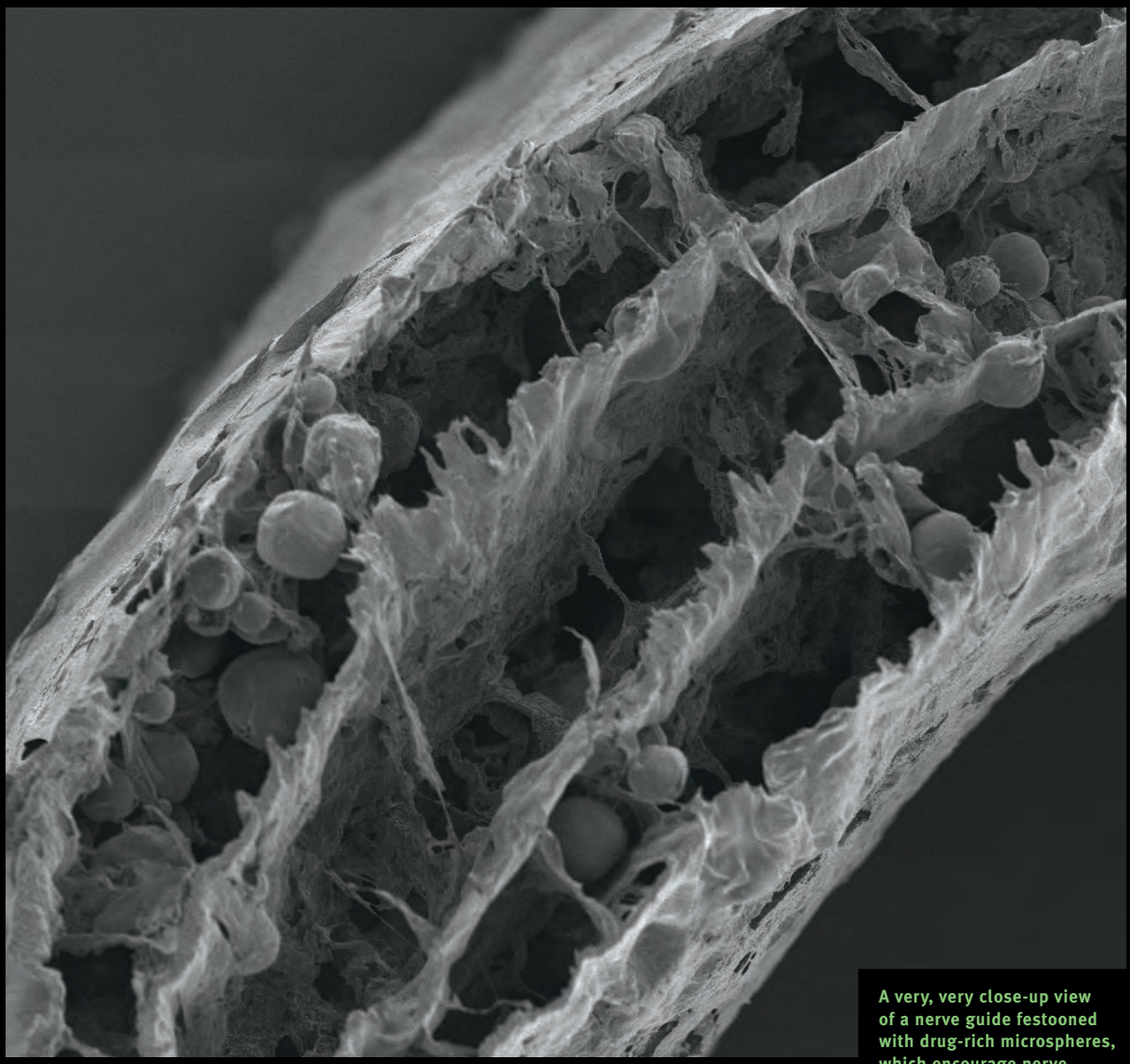
COURTESY WAGNER

Cross-section of a rodent infarcted heart injected with hydrogel. A scar area (in blue), which is smaller than the one observed in untreated animals, indicates early repair facilitated by hydrogel. The gel reduces stress on the heart while the body's own healing apparatus kicks into gear.

A GOO REVIEW

But maybe you don't want a bioactive Band-Aid. In some circumstances, perhaps it would be better to inject the material—replete with growth factors or stem cells—directly into the injured site.

A rat has a heart attack, damaging part of the heart wall. The wall is now thinned out, stretched, and weak; yet heart muscle ought to be strong and resilient. So researchers inject a gel into the heart to thicken it, to reduce the concentration of force on the damaged site, and lower the mechanical stress. Gel? Wouldn't that wash away? It would but for this: Wagner and his team have engineered the gel to be injectable through a small needle and then to solidify at body temperature into an elastic form. In time, as the heart heals, the chemistry of the material changes, allowing it to return to liquid form, still at body temperature, and wash away after doing its job as a structural support.



COURTESY MARRA

A very, very close-up view of a nerve guide festooned with drug-rich microspheres, which encourage nerve growth through the 5-centimeter conduit.

THE NERVE!

Injured peripheral nerves are treatable. One method, which has been tried with varying success since the 19th century, is the autologous nerve graft. This involves taking a nerve segment from another part of the body and inserting it into the lesion to reconstruct the damaged nerve. The technique can restore function (but only about 50 percent of it). It can also result in injury to the donor site or the formation of painful nerve scars called neuromas, among other complications. So, doctors went about creating artificial nerve conduits. But they only work on lesions less than 3 centimeters long.

Kacey Marra, PhD associate professor of plastic surgery in the School of Medicine and McGowan Institute member, has found that treating these scaffolds with a protein called glial cell line–derived neurotrophic factor (GDNF) may be the key to growing new, fully functional nerves that surpass the longstanding 3-centimeter barrier.

"In the walls of the guide [a 5-centimeter nerve conduit], we have microspheres—small, polymeric beads that deliver a drug, kind of like a gel-cap—and they release the drug for over 60 days," Marra says. The nerve endings, attracted by the GDNF, then grow to meet in the middle. "It works in the rat, and we're testing it in the nonhuman primate," Marra says. "If it works there without side effects, we're ready to go to humans."