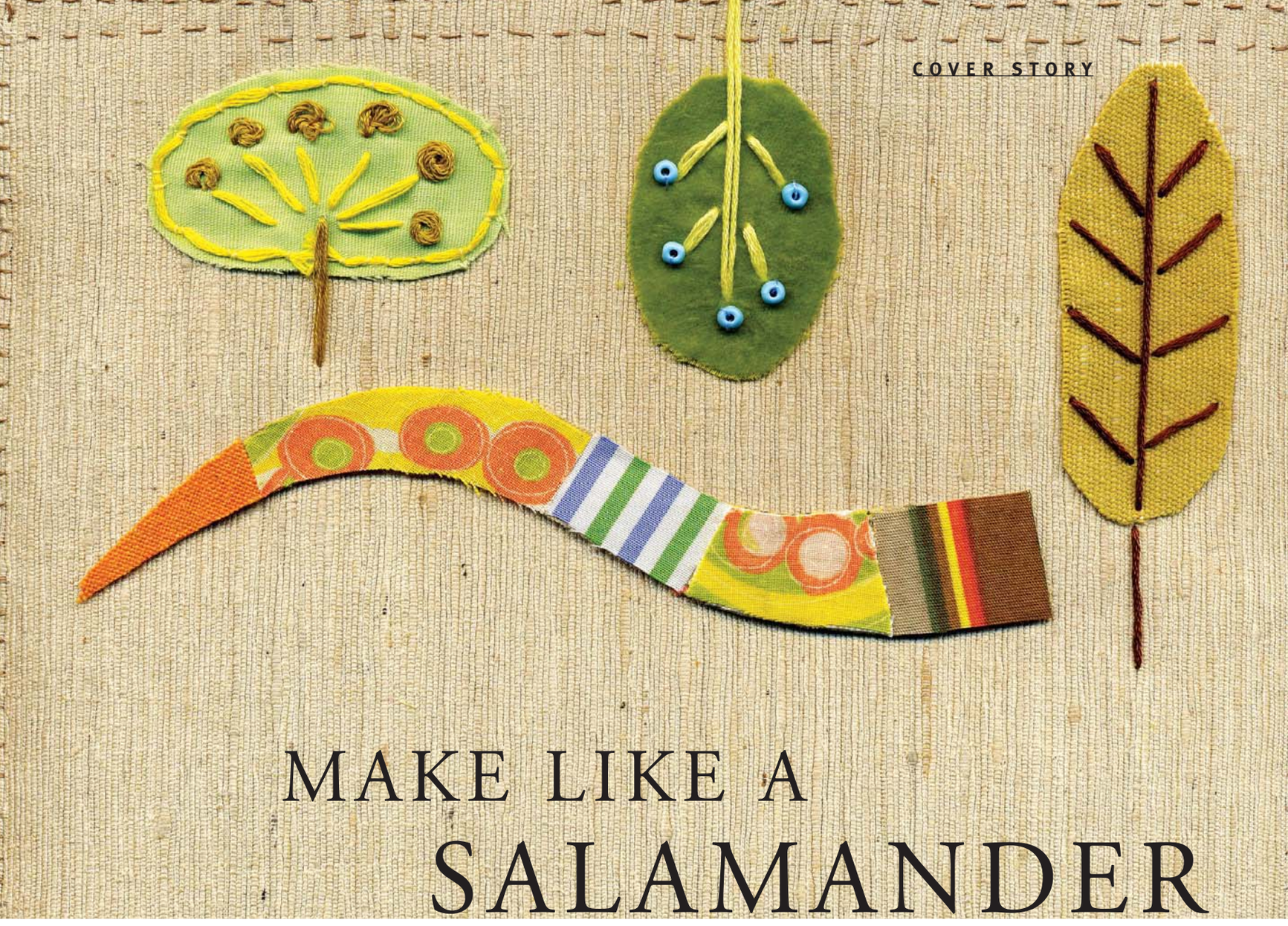


MAKE LIKE A SALAMANDER

Salamanders and newts do it, so why not us? Pitt's Stephen Badylak coordinates a team that hopes to learn how to regrow a mammalian digit. They believe these amphibians can show them how.

LIMBS ON DEMAND?

BY JOE MIKSCH

You really don't need to think much about this one: A pig's bladder is a very good thing to have … if you're a pig.

But if you've amputated the tip of your pointer finger above the first joint while handling a model airplane as its propeller was still spinning, perhaps the organ isn't the first thing to look for in your medicine chest.

However, when a Cincinnati man in his 60s lost the pointy part of his pointer in the manner described above, he sought out the University of Pittsburgh's Stephen Badylak,

ILLUSTRATION | CATHERINE LAZURE

understanding that, in his particular case, one might come in handy. One of Badylak's colleagues had told the man that the doctor had done impressive feats with pig tissue.

Badylak is a research professor in the Department of Surgery with a secondary appointment in bioengineering at Pitt. He has a PhD, a DVM, and an MD and directs the Center for Pre-Clinical Tissue Engineering in the McGowan Institute for Regenerative Medicine.

Badylak placed a piece of biologic scaffolding—derived from a pig bladder by stripping the organ of cells—on the injured finger. A similar and commercially available material has been used in half a million patients to regenerate tissue, including those who would like to regrow rotator cuffs, Achilles tendons, and other soft tissues. Could Badylak's experimental scaffold cultivate an entire tip of a finger? He didn't know.

Six weeks later, the man who once faced deformity was made whole again. Molecules within the scaffolding drew stem cells to the amputation site. The cells differentiated and multiplied, and the fingertip—bone, blood vessels, nerves, skin, fingernail—grew back. It looked perfect and was perfectly functional.

The fingertip—bone, blood vessels, nerves, skin, fingernail—grew back. It looked perfect and was perfectly functional.

Then Badylak did the same for a Boston man in his 70s who nipped a fingertip with a band saw.

This is the stuff of salamanders and newts, a pair of amphibians who have the ability to regenerate lost limbs. When it happens in a person with the aid of the structural material that helps support and organize cells in a pig's urinary bladder, it's the stuff of science fiction.

"The chances of this happening," Badylak says, pausing, "I mean, it's really rare. I'm not saying it's impossible, but nobody would have predicted it [working]. Now, it didn't go across a joint, so we didn't regrow a joint, and that's going to be a real challenge."

It's a challenge Badylak, with five other scientists from across the United States, has accepted. The group is equipped with a $3.7-million, 12-month grant from the Department of Defense's Defense Advanced Research Projects Agency (DARPA). The researchers hope their efforts will result in a mouse regenerating a functional digit—much in the same manner a salamander or newt regenerates a limb. The grant could be worth up to $15 million throughout four years.

Badylak's former boss, retired Purdue University President Steven Beering (MD '58), believes the group has an able coordinator. Beering is a member of the University of Pittsburgh Board of Trustees, and was recently appointed chair of the National Science Board, which serves as the science adviser to the president and Congress and oversees the National Science Foundation. He says that Badylak is "the most innovative researcher I've ever met. I'm a great admirer of his work and his research."

Badylak began meeting with team members—experts in places as far-flung as Lowell, Mass., and Salt Lake City, Utah—two years before they received any funding.

"We've got two developmental biologists, a great classical cell biologist, an excellent molecular biologist, and an immunologist who's spent her life understanding the role of the immune system in regeneration," he says.

"If we're smart enough, we'll combine the right talents and discover things we never would have discovered on our own."

Hans-Georg Simon is a developmental biologist, an assistant professor of pediatrics at Children's Memorial Research Center and Northwestern University in Chicago, and a member of the DARPA team. He expects the team's diversity will result in "great quantum synergy."

So where does the A team begin? With the salamander.

One day, a salamander—let's call him Jimbo—is minding his own business near a little stream. Nice and pastoral. Calm. But danger lurks in the weeds. A garter snake attacks, lunging at our little mudpuppy. Jimbo's leg gets trapped, then the bone snaps, the muscle and skin tear off, and he scurries to safety on three legs.

A couple of months later, a four-legged salamander is lolling about near the same stream. Looks a bit familiar, no? Well, he should. That's Jimbo with a full complement of legs. How the heck did that happen?

"Salamanders are champions of regenerative ability," says Simon.

When a salamander loses a limb, molecular signals compel the surrounding tissue to produce cells that divide and accumulate at the wound site. The resulting cell mass, called a blastema, specializes as it matures, forming all the necessary components of the missing limb. Here, an adult human would form a scar.

"Salamanders replace lost structures without forming a scar," Simon says, "but the process is slow. Our wounds heal fast, and the expense is a scar."

"That's the default healing mechanism in adults," says Badylak. "Fetuses don't form scar tissue; they regenerate."

Likewise, doctors can perform heart surgery on fetuses in utero without risking loss of function because of scarring. Badylak thinks adult humans lose their regenerative potential in maturity because the genes that govern the process in fetuses and infants have been turned off.

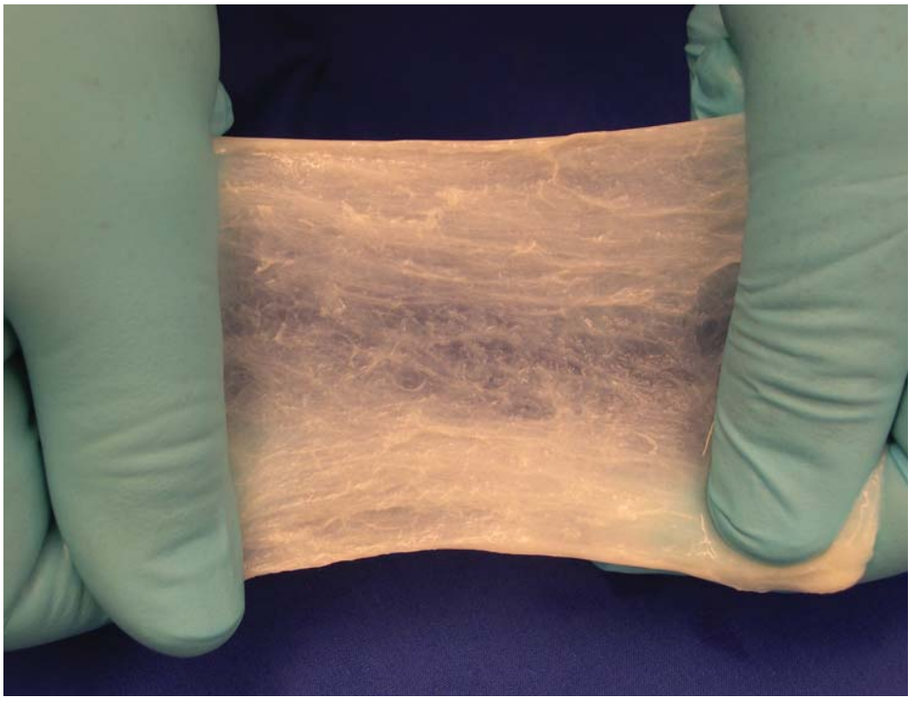
Adult humans do have some regenerative abilities, producing red blood cells, liver cells, and skin cells continually. Compared to a fetus or a salamander, though, this is bush league.

"We're asking ourselves what genes get turned on and off differently between a newborn and an adult," Badylak says. "Is it possible to reverse the gene expression pattern to that of a fetus, at least temporarily and at the site that you're interested in? If we can do that, maybe we can reprogram the local cells that we get [at an amputation site] to think that they need to form a new limb."

Simon says that understanding the regenerative ability of animals such as the salamander is key to tapping into human regenerative potential.

Cells that form a blastema, he says, seem to have control over what they become. They are aware of where they are in the sense that they can tell if the salamander lost his front leg or back. They can tell whether the amputation is near the shoulder or foot. They know the structure of what it is they are to replace.







GLOVED HAND WITH SCAFFOLD: COURTESY J. MURPHY. JUNCTION DEVICE: COURTESY T. GILBERT.

ABOVE: Stretched between two gloved hands, this piece of biologic scaffolding, derived from a pig's bladder, is ready to be used as a kind of trellis upon which new tissue can grow. LEFT: The upside-down funnel is a similar piece of scaffolding built to help the body repair the junction between the esophagus and the stomach.

"They never make anything extra, and they never leave anything out," says Simon.

"We're trying to follow nature," he adds. "Nature has millions and millions of years of experience. And us human beings, what do we have comparatively? Nothing." People are just starting to understand the mechanisms behind regeneration, he notes.

A class of genes called TBX is linked to the blastema's special knowledge. In salamanders, these genes are, obviously, active. In mice and adult humans they are not. When a certain TBX gene malfunctions in a mouse, the mouse doesn't grow forelimbs. When it's mutated in the human genome, problems can range from missing thumbs to truncated, flipper-like arms.

But check that bit about mice being nonregenerative. One transgenic mouse surprised scientists by making itself whole again.

One of the ways scientists mark individual mice in the lab for identification is to pierce their ears. When small holes were punched in the ears of a breed known as MRL mice, DARPA team member Ellen Heber-Katz, a professor in the molecular and cellular oncogenesis program in the Wistar Institute in Philadelphia, noticed that the holes filled in.

"This isn't supposed to happen," Badylak says with a laugh.

But it did give the team an idea. "One of our approaches is to take a regenerating species [salamanders], a partially regenerating species [MRL mice], and a nonregenerating species [conventional mice] and look at common things among them and the different things."

Susan Braunhut's lab at the University of Massachusetts Lowell has taken a major role in sorting out the commonalities and differences. A professor of biological sciences, Braunhut is attempting to identify the biological factors, genes aside, that cause cells to participate in blastema formation.

Being able to harness such a molecule could translate to therapies that eliminate scarring as well as regenerate tissue.

Braunhut's work, Badylak says, is vital for the team to meet its first-year goal: DARPA has challenged the team to identify at least one molecule that shows bioactivity toward a progenitor cell, a cell that participates in blastema formation.

"We need to know the regulators of these processes," Badylak says. "We need to know what molecules recruit these cells as well as which ones regulate gene expression."

Braunhut has made inroads in this area on her own. Three years ago, she built a "smart" bandage. It's an extracellular matrix enriched with specific growth factors that speed healing in specific types of wounds. This accomplishment serendipitously led to her collaboration with Badylak.

"The work was funded by DARPA, and my program manager had been working with Steve," she says. "He knew about my basic research into matrix and growth factors and told me to call [Badylak]. He said we were meant to meet. We did, and by the time we left, we had agreed to collaborate."

The pair found out about a DARPA opportunity and began searching for other researchers to join them, including Braunhut's former postdoctoral advisor, Lorraine Gudas, Revlon Pharmaceutical Professor of Pharmacology and Toxicology and chair of pharmacology at Cornell University, and regenerative gene expert Shannon Odelberg, assistant professor of internal medicine and of neurobiology and anatomy at the University of Utah.

On their own dime, the six met in Washington, D.C., and began to pursue the DARPA money with tenacity. "When this project came, it resonated with this team," Braunhut says. The idea had been "cooking" for some time with each of them, she notes.

Wrapped up in Braunhut's work is another mystery to solve. Some members of the team have already identified possible factors that propel progenitor cells to wound sites. What they don't know is whether these cells are derived from the adjacent tissue at an amputation site or are drawn from bone marrow. Could be both, Badylak notes. And once all this is understood, there's still the question of how to stimulate a regenerative response rather than a scar tissue response.

In 12 months, the team is expected to isolate a molecule that causes cells to migrate to a wound site and cause those cells to multiply and differentiate. The mystery molecule also must be genetically related to a blastema cell. "That's a lot of work," Badylak says.

But Braunhut is ready to go with assays that will help distinguish molecules in Badylak's biologic scaffolding or in Simon's salamanders that beckon cells or tell them to commit to becoming nerve, bone, or muscle.

If the team meets with success here, the next goal is to form a blastema in a nonregenerative animal. From there, a functioning digit on a mouse. And, further down the line, this team or another group of scientists will attempt to regenerate a fully functional limb on an adult human being. Of course, doing all this requires understanding not just the individual pieces of the puzzle, but also how all the components fit together. Braunhut is humbled but unbowed:

"If five or 10 years ago you were to say we'd be able to get a very complex three-dimensional structure to regrow in an adult, I'd say we wouldn't be able to do it.

"Today I say we might be able to do it. We're at the edge of what's in the realm of possibility."

The other team members also talk with cautious optimism. The six senior investigators think that not only are they well prepared in their individual areas of expertise, they're getting the right kind of support from the right kind of funding agency.

Typically, Badylak says, once investigators receive grants—from, say, the National Institutes of Health—they've got funding for a finite number of years, do their work, publish what they can, and file periodic progress reports throughout.

DARPA, he says, is different: "They give you extremely challenging problems and say, 'If we take away all the barriers, if we eliminate money as a limiting factor, we expect productivity. That's why each of the principal investigators on this project [has] gotten more money for this grant than they've gotten for any single activity in their career.

"Basically, they're buying our attention."

The team holds an hour-long biweekly conference call during which every team member plays a part, even when traveling abroad. A DARPA officer—who's also a scientist—participates in each of these calls.

The calls help DARPA assess the team's progress. If the team doesn't meet its goals, it could lose funding.

"But it also means that they know what the roadblocks are, what problems we have, what accomplishments we make," Badylak says. "I've worked on DARPA projects before. If things don't progress, they say, 'What's the issue? How can we help?'"

A lot of good can come from the attempts, notes Simon: "Even if we fail to make a digit regrow in a mouse, we could revolutionize how we deal with wounds in the clinic. To achieve better wound healing, less scarring, would be a major advance in medicine."

When officials at the defense department found out about Badylak's success in using biologic scaffolding to regrow the fingertips of the men from Cincinnati and Boston, they wanted to see whether the technology could help wounded soldiers.

"We told them it's not ready for prime time," Badylak says.

But the DOD pressed, saying that any finger regrowth—even a couple of centimeters—could greatly improve a wounded soldier's quality of life.

"They're saying if you can get extension of what's left of a finger, the finger can appose [think a pinching motion] the thumb," Badylak says, allowing a wounded vet to perform simple tasks such as putting a car key in the ignition or picking up silverware.

From the Civil War through the first Gulf War, the ratio of injured to killed soldiers stood at about 2.5 to 1, according to Badylak. In Afghanistan and Iraq, the DOD reports, the ratio is more like 9 to 1.

Because of advances in body armor, Badylak says, "Soldiers can withstand impact that would have killed them before, and the army is saying, 'What are we going to do with all these injured? What can we do to improve their quality of life?'"

So now Badylak is the principal investigator in two DOD-funded clinical trials set to take place in San Antonio, Texas, before the year is out.

Between five and 10 soldiers at Brooke Army Medical Center will participate in a trial that will attempt to regrow portions of their fingers. Twenty others at the hospital will take part in a second trial examining the effectiveness of biologic scaffolding in regrowing skin.

Doctors will apply scaffolding to the donor sites of burn patients who have had skin grafts, with the hope of accelerating the healing process at that site.

Why not the burn site itself? The donor site is cleaner and easier to work with, notes Badylak.

"If this works, that will give us the confidence to go to the primary wound," he says.

Badylak's lab is a noisy place. More than two dozen people work there, which is part of the reason. Another is the perpetual hum coming from the convenience store–style cooler against the wall.

Inside it are two clear plastic containers. One sits on an orbital shaker, the other on a rocking platform. Pale blobs slosh about in each. The blobs going in circles are pig bladders, the ones rocking back and forth are dog pancreases.

"It's a little gross," Badylak admits. "The reason they're so white is that the tissue no longer has cells in it." The organs are being washed in a solution that's stripping them of everything but the matrix, the material that cells hold onto as they form tissue. It's this material that Badylak used to help regenerate the fingertips of the men hurt by the band saw and model plane.

Two aisles over from the cooler, instruments are at work screening for the molecules within the matrix that prompt the kind of biologic response that allowed the men's fingers to become whole again and may contribute to the DARPA project's goal of regenerating an entire digit.

Badylak rattles off the list of questions this screening process could help answer: Why do stem cells accumulate? Why does biologic scaffolding have antimicrobial and antibacterial properties? Why do cells proliferate in the presence of the matrix? "We're developing assays to measure all of those things—proliferation, chemoattraction," he says.

Later, Badylak talks more reflectively: "If we can understand how to regrow a digit, why can't we use those same principles to regrow a kidney for patients on dialysis? Or a piece of the heart that was damaged by a heart attack? Or how about a lung for patients with emphysema?"

"That's what's gotten me really excited," he says, running his finger along a section of scaffolding formed like a trachea. "We're going to learn principles that will make many things possible." ■