Lijie Grace Zhang

S

She wants to build a beating human heart. Not just a heart-shaped blob of cells and not an organ that beats to an off-kilter cadence. She wants to make from scratch—using something not far removed from an inkjet printer—a heart that surgeons can transplant into a person's body to keep them alive. This heart, Lijie Grace Zhang imagines, could be personalized with a patient's own cells and be created on-demand by a 3D printer in a matter of hours to treat heart failure.

Zhang, an associate professor in the School of Engineering and Applied Science, admits the goal of a printed, transplantable heart is likely decades away. But as she works toward it, the smaller feats along the way carry their own potential to revolutionize medicine. Zhang's lab is among just a few in the country using a cutting-edge 4D bioprinting approach to create moving, responsive shapes that can carry out myriad tasks in the human body. Her approach might allow engineered blood vessels implanted into a child to grow as they age, or a drug-delivery device to open like a blooming flower when it reaches the right organ.

In 3D printing, three-dimensional solid objects are created, layer by layer, based on a design plugged into a computer. In place of standard ink, a 3D printer can extrude a variety of materials, from plastics and resins to foodstuff— chefs have used 3D printing to concoct uniquely shaped candies and pastas. And when scientists discovered that living cells can survive the trip through a 3D printer, they began printing layers of cells in the shapes of organs—heart cells in the exact shape of a human heart and liver cells in the shape of a liver, for instance.

"People have bioprinted hearts already," Zhang says. "But the problem is functional: How can we make that 3D heart work? How can we make it beat?"

To make these inert organs more versatile and useful, Zhang's team is adding a new dimension to bioprinting—a fourth dimension: time, or more precisely, the ability to change over time, shifting shape or rearranging molecules.

By printing in 4D with unique nanomaterials, the scientists can design shapes they're able to control using external forces—light or heat, for instance. Then human cells can be layered onto that shape-changing scaffold.

WILLIAM ATKINS

BEFORE 2010, when she became a GW faculty member, Zhang had never used a 3D printer. She studied chemical engineering in China, then flew halfway around the world for a graduate program at Brown University in Rhode Island, where—despite never having worked with living cells before—she joined a biomedical engineering lab in which culturing cells was an everyday task. By the time she arrived at GW, she had jumped around two more times, with postdoctoral positions at Rice University and Harvard, developing nanomaterials for cartilage and bone regeneration.

At GW, Zhang became part of the Department for Mechanical & Aerospace Engineering, another unlikely move—they'd been looking for expertise in biomaterials, she says—that shaped the arc of her research ever since.

"I was suddenly surrounded by these mechanical engineering students with very different backgrounds than my own," Zhang says. "They excelled in mathematics and design and modeling. They already had expertise in using 3D printing to make device prototypes."

For Zhang, it was more intriguing than intimidating. She immediately saw the promise in applying the new 3D technology to tissue engineering—she could take material similar to the injectable bone substitutes she'd engineered, for instance, and print it in the shape of a bone to create entirely new types of artificial joints and prosthetics. So Zhang's inventive students built a custom 3D printer (and then another and another) for her lab. And as she forged ahead into 3D—and then 4D—printing, Zhang branched out from bones and cartilage.

"She tells everyone that they can pick any organ they want to study and she'll support them," says postdoctoral fellow Timothy Esworthy. "So we have people join the lab and get really excited about new organs; livers and muscles and hearts and brains."

That diversity, and the knack Zhang has for bringing together expertise in so many areas, is part of why the Zhang lab has gained a reputation as a hot spot of innovation.

"I'd certainly describe her as a top-caliber researcher," says Michael McAlpine of the University of Minnesota, whose 3D and 4D bioprinting work overlaps with Zhang's in many areas. "She's brought a lot of high-quality work to the field."

TODAY, the closest thing that the Zhang lab has made to a beating heart is a thin patch just half a centimeter in diameter. It contains a mixture of stem cells isolated from human bone marrow and, despite its diminutive size, could someday change the way people recover from heart attacks. Other researchers have tried injecting similar mixtures of stem cells into injured hearts, but the very power of the organ keeps that approach from being successful—as the heart beats, even weakly, it pushes cells out to the rest of the body instead of keeping their healing power close. But a bioprinted patch, Zhang has shown, can hold the cells in place on the heart as they form new tissue.

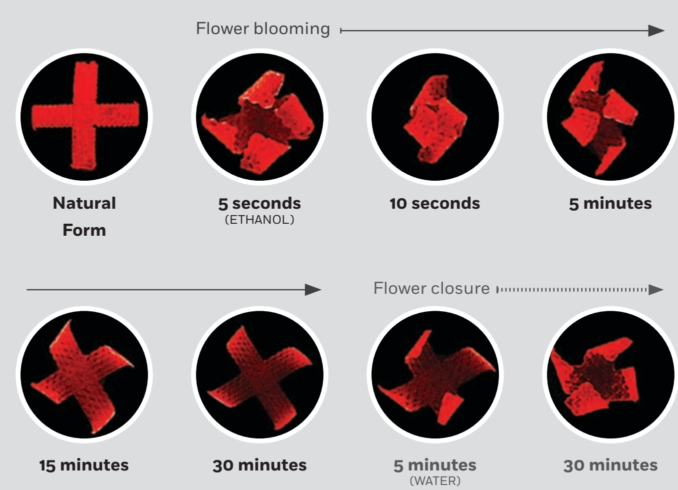
A perfect scaffold for harnessing stem cells and encouraging them to grow into mature heart tissue has two qualities that are hard to achieve: tiny—really tiny—grooves and the ability to bend and move.

The grooves, which must be mere fractions of a millimeter wide, help align cells so they can form a continuous, unbroken sheet. And the motion— ideally close to that of a beating heart—helps coax the stem cells to become heart cells rather than

"WITH 4D PRINTING, WE CAN NOT ONLY SPECIFY HOW CELLS ARE PLACED TO MIMIC THE ACTUAL CIRCUITRY OF THE BRAIN, WE CAN NOW CONTROL THIS SHAPE CHANGE AS WELL."

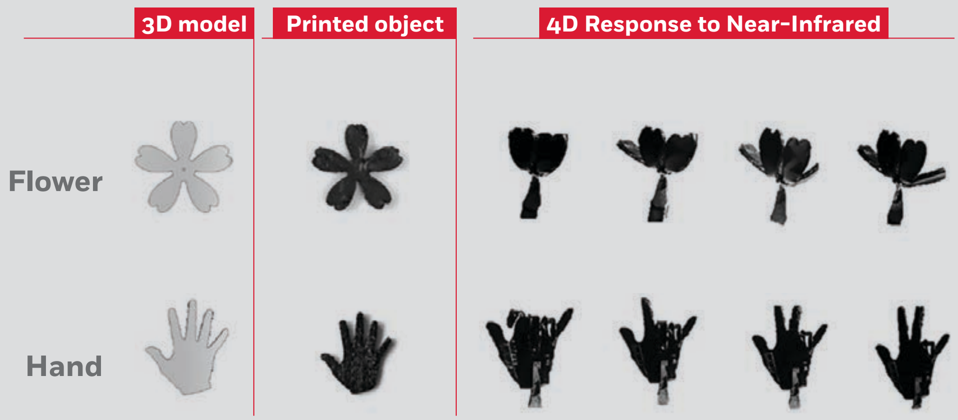
FROM FLAT TO FLOWER BUD

Zhang and her colleagues demonstrate the reversible, shape-changing effect of their process with this 4D-printed structure that opens and closes like a flower, published in a cover article last year in the journal *Advanced Biosystems*. The structure is shown first in its natural form, then scrunching and slowly flattening as it's immersed in ethanol, then gradually recoiling again in response to immersion in water.



A SAFE, NIMBLE APPROACH

This year in the journal *Nano Research,* Zhang's team demonstrated a 4D-printing nanomaterial that could be controlled using near-infrared light, which would be safe for human tissue—unlike the use of temperature, solvents or ultraviolet light—and brings the research a step closer toward reality.



other kinds of cells.

To build a patch with those qualities, Zhang starts with an ink developed by her lab in 2016 called smart soybean oil epoxidized acrylate, or SOEA. It's considered safe in the human body and, importantly, it turns from a thick liquid into a solid in response to light from a laser. That means it can be used in a type of 3D printing called photolithography. In that type of printing, rather than extrude an ink directly, the computer-controlled printer directs a laser in precise patterns into a vat of liquid resin, such as SOEA. As the laser moves, it solidifies section after section of the resin, creating a three-dimensional solid object that emerges from the liquid.

SOEA has another property that makes it ideal for 4D printing: When a solid, printed SOEA scaffold is cooled, the temperature change affects the way its molecules link together, pulling them taut and flattening the shape. But when it's warmed again, the scaffold spontaneously curls up, like a roly-poly bug hiding from danger. While there are other photolithography inks that can change shape, few are safe in humans and other animals.

Making a cardiac patch using photolithography and SOEA gives it the ability to flex, but the technique isn't ideal for creating micro-sized grooves. For that, Zhang and her colleagues use a second type of 3D printing—stereolithography, which uses a more precise laser beam of a different intensity to form channels in the patch after it's been formed by photolithography.

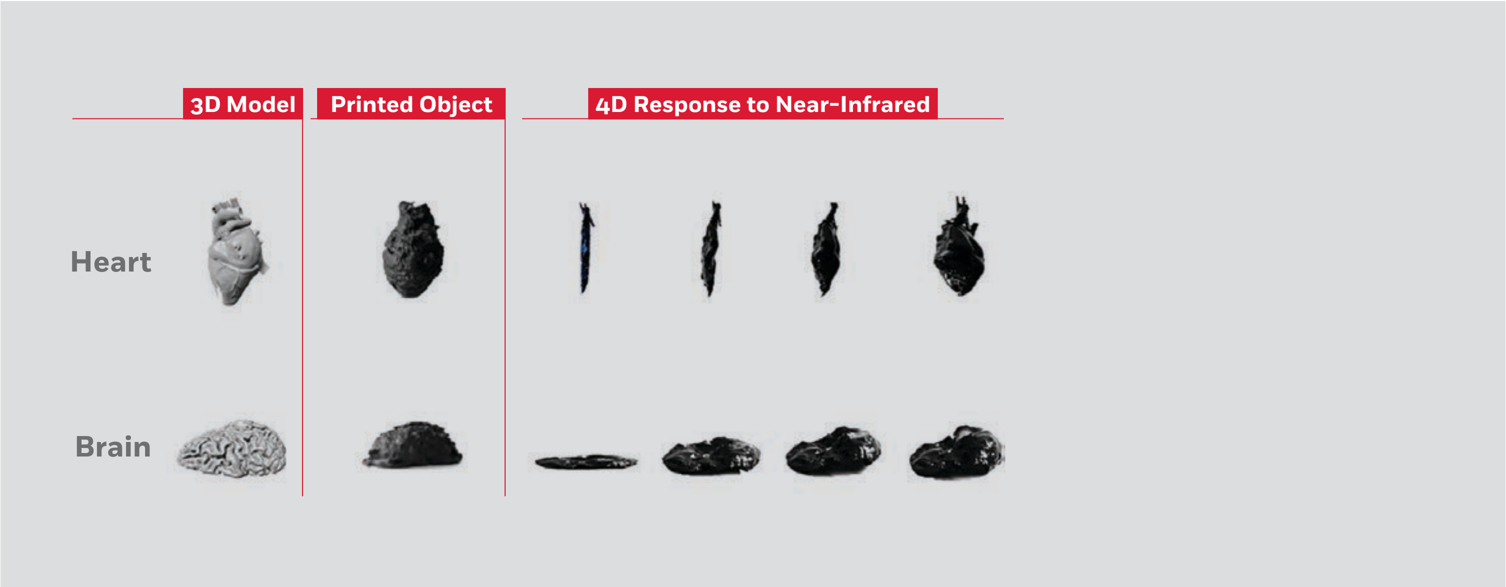
In 2018, Zhang reported in the journal *Biofabrication* that stem cells grown on these double-printed 4D cardiac patches are more efficient at maturing into heart cells than those grown on other types of scaffolds. Her lab has already tested one 3D-printed patch—although not the bendable, SOEA version— in mice with heart injuries similar to those seen in the aftermath of a heart attack, and the patch helped the mice recover normal heart function.

"With the 4D patch, we'll be able to have these shape-changing effects that might move in conjunction with a beating heart," says Zhang. "So I think the success we've already seen with the 3D patch will be amplified with the 4D version."

A PRINTED OBJECT that can furl and unfurl—and at the same time act as a scaffold for living cells—has a plethora of other uses in the human body. For instance, Zhang is one of the only researchers in the U.S. applying 4D bioprinting technologies to brain tissue. In a developing embryo, the surface of the brain buckles and folds as it grows, eventually resulting in the organ's wrinkly texture. This folding signals neural stem cells to differentiate into mature brain cells. And folding is just the kind of motion that a printed nanomaterial can achieve.

"The brain is arguably the most complicated tissue that exists in the body," says Esworthy, who helps lead brain tissue projects in the Zhang lab. "But with 4D printing we can not only specify how cells are placed to mimic the actual circuitry of the brain, we can now control this shape change as well."

To that end, the researchers have 3D-printed a scaffold in a kinked shape that mimics the brain's folds. With heat, they can pull the resulting shape



flat to seed it with stem cells. Over the course of a few weeks, the scaffold begins to refold, eventually assuming its original shape. Cells proliferate and mature in the troughs of each kink—the same pattern seen in developing embryos. It's an approach that may improve methods for growing brain cells for research and clinical use.

To really effect change in the human body, though, researchers still need better ways to remotely, and quickly, control the 3D-printed organs.

Temperatures and solvents affect structural change slowly—over hours or days—and aren't ideal for use in the body, where temperatures and chemical conditions need to remain stable. The use of light is an option, and a number of other responsive materials use ultraviolet light to effect change over mere seconds, but it can damage human cells. So Esworthy, Zhang and others have developed a new nanomaterial, which they described this year in the journal *Nano Research*, that changes shape in response to infrared light instead, which Esworthy says is safe for human cells. Engineered neural tissue that's implanted in the brain would still be reachable by infrared, too, he says.

INSIDE THE NEXT DECADE, Zhang predicts the emergence of 4D-printed tissues that can help guide the treatment of diseases, albeit without being transplanted into a person. Organs-on-chips have already gained notoriety as tiny, often very simplified replicas of human organs that can be used to test the effects of drugs. With 4D printing, more complex replicas could be built from a patient's own cells to determine whether a particular drug works to treat conditions like heart disease or neurological, gastrointestinal or breathing disorders, Zhang says, or whether it might carry side effects.

"The future is now," says Esworthy. "We're really entering into the age of personalized medicine, and 4D tissue models like this could allow doctors to do more accurate drug testing so we're not just playing the game of prescribing one thing after another."

4D bioprinting, of course, is still very much in its infancy. 3D printers now appear in elementary schools, libraries and co-working spaces, and are being used to make everything from shoes and bicycles to robotic hands. But Zhang and McAlpine, of the University of Minnesota, were two of the first researchers to integrate 3D printing into tissue engineering less than a decade ago.

"At the time both our groups started in this field, there were not many people doing it," says McAlpine. "We got into it early and are both recognized as early pioneers. But now there's been an absolute explosion in interest. Things that were really novel a year or two ago are commonplace now."

There still are challenges. Zhang says she'd like to come up with more versatile inks that are safe for use in humans, and printers with better resolution are needed for more precise control over nanomaterials. And even if the devices that Zhang is producing now are small, and don't yet operate within the body, her ideas loom large.

"I think printed organs that we can fabricate and put in patients will be realized within our lifetimes," she says. "And it will be a significant milestone in human history." GW