

The Path to Printed Body Parts

Prachi Patel

Scientists are making steady progress toward 3-D printed tissues and organs.

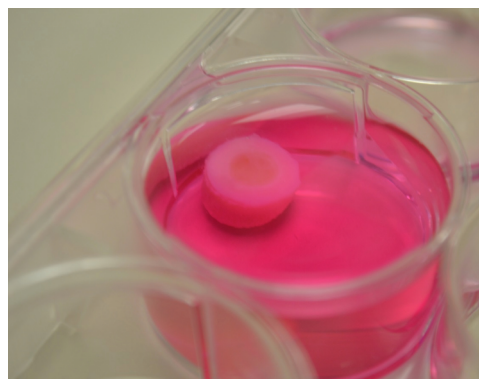
For patients in need of an organ transplant, the odds are bleak. Only around 30,000 people got transplants in the U.S. last year, while more than 8,000 died waiting, according to the United Network for Organ Sharing. In the U.S., 120,000 people are on the waitlist for organs; China's list contains 1.5 million names. The World Health Organization estimates that the black market created by this soaring demand results in 10,000 illegal transplants each year.

What if doctors could order an inexpensive, custom-made liver or heart to replace a failing one? Some scientists believe that could be possible with 3-D printing. Advances in 3-D printing technology since its debut in the 1980s have led to its use today for making everything from toys to wind turbines. Printed prosthetics and bone implants are already possible; living body parts, some say, will soon follow. But making a functioning human organ entails developing the right types of cell-loaded inks, laying them down in intricate patterns, and getting nutrients to the newly formed tissues—challenges for chemists and materials scientists.

The company [Organovo](#) has already printed layers of bioinks containing living cells to create slivers of liver tissue for chemical and drug testing. They began offering kidney tissue this month and are now working on printed skin. [Sharon Presnell](#), Organovo's chief scientific officer, says that it should be possible to print tissue patches to repair failing human organs within the decade. And in November 2015, one company in Russia reported printing and testing a functional thyroid gland in living mice.

"Ultimately people will tackle the 'o' word," says [Jennifer A. Lewis](#), a materials scientist at Harvard University, referring to whole, fully functional human organs like kidneys and livers that could save thousands of lives and millions in healthcare costs.

Although human tests are far away, the idea is to use cells harvested from the patient so the tissues are accepted by the immune system. Whether or not these body parts look like the real thing, they would do the same job. And 3-D printing



A patch of printed cartilage sits atop a bone plug in nutrient media. Credit: Ozbolat Lab/Penn State.

would allow them to be printed on-demand, in hours, and for a low cost.

"There has been incredible progress in the past decade, and when I translate to the next 10, 15 years, I envision that we should be able to print large-scale, complex organs", says [Ibrahim Ozbolat](#), a professor of engineering science and mechanics at Pennsylvania State University. "I'm not saying they will be transplanted in humans, but we'll be making them."

The Fine Print

Printing tissue—let alone an organ—is, not surprisingly, much more complicated than printing car parts or toys. Tissues are made of millions of specialized cells arranged in highly organized architectures and embedded in an extracellular matrix of fibrous proteins and carbohydrate polymers. All tissues except cartilage are also laced with an intricate network of blood vessels that deliver oxygen to keep cells alive.

Organs, meanwhile, are ensembles of multiple tissues with varying complexity. Flat, layered organs like skin and cartilage are less challenging to construct than hollow organs such as the stomach or bladder. Solid organs like the kidney, liver, and heart are trickiest because of their complicated 3-D geometry.

The simpler structures of cartilage, blood vessels, and windpipes made them early targets for tissue engineering—culturing cells within biocompatible scaffolds—even before

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3-D printing came on the scene. But 3-D printing really shines at constructing the miniscule geometries of complex tissue because of its computer-aided process: Software drives the printer to stack cells in a predesigned pattern of nearly limitless complexity. It can produce tissue in hours and make hundreds of samples that are exactly alike. “You put cells where you want them to be rather than waiting for them to pile up and form a structure”, Presnell says.

Brewing the Right Ink

Thomas Boland, a bioengineer at the University of Texas, El Paso, pioneered bioprinting in 2000 when he used a Hewlett-Packard inkjet printer to print a bioink made of living bovine cells suspended in cell-culture medium. But since those early days, 3-D bioprinting research has tackled increasingly difficult problems with different approaches.

One approach involves concocting a bioink that not only delivers cells but also provides the extracellular, matrixlike scaffold that tissues need for structure. Because skin and liver cells, for instance, have different physical properties and nutritional needs, inks have to be developed specifically for the tissue you want to print, says Paul Gatenholm, a professor of biopolymer technology at Chalmers University of Technology and director of the university’s 3-D Bioprinting Center.

In addition, an ink needs the right balance of flow properties and viscosity—typically similar to toothpaste—so it can be extruded from nozzles but maintains its shape. It also needs biomolecules and chemicals that allow cells to migrate, attach, communicate with each other, and proliferate after printing, Gatenholm says. What’s more, he adds, “it has to be reproducible and storable. So it’s getting more and more complicated to design and prepare bioinks.”

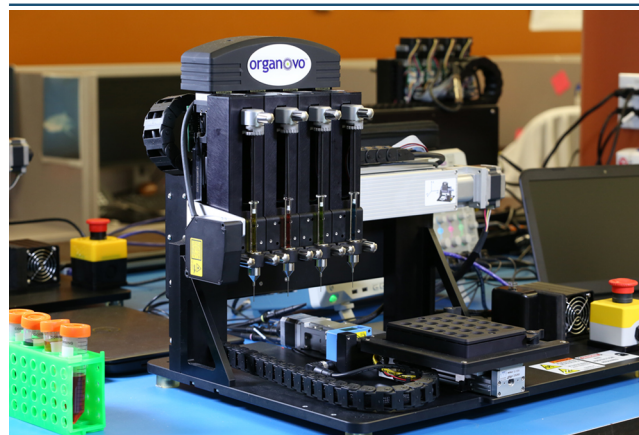
Most researchers use a hydrogel base for the ink and then add biomolecules to the mix that match the tissue they are looking to make. Skin and lung tissue, for instance, needs collagen, elastin, laminin, and hyaluronic acid.

Gatenholm’s team has had success making printed cartilage that grew and produced new cartilage in living mice for two months—a promising advance that could help plastic surgeons repair ears and noses, he says. The ink is based on alginate polymer extracted from brown algae and cellulose fibrils synthesized by engineered bacteria.

The Skinny on Skin

Gatenholm is also working on printed skin—and he’s not alone. Artificial skin has become the biggest area of research in the bioprinting industry. Organovo—the first publicly traded 3-D bioprinting company—is working with French cosmetics giant L’Oreal on printed skin while

Germany-based BASF has launched a joint project with French startup Poietis. Proctor & Gamble has initiated a five-year, \$60 million program and asked for research proposals on 3-D printed skin from Singapore’s Agency for Science, Technology, & Research.



Organovo’s bioprinter has multiple printer heads that squeeze out cellular raw materials and proprietary bioinks (shown here with colored liquids in the reservoirs). Credit: Organovo.

The goal of all these projects is as an alternative to animals for drug and cosmetics testing. In Europe, cosmetic companies are prohibited from testing products on animals. Most cosmetics and pharmaceutical companies today use artificial skin grown with standard tissue engineering. But these constructs are simple. Organovo hopes to print more realistic skin containing, say, pigmentation and oil and sweat glands so that printed skin could speed up product testing on different skin types. The push in skin printing research could make skin the first bioprinted organ available for transplant, which could be a boon for burn victims.

Skip the Scaffold

Organovo and a handful of researchers pride themselves in using a “scaffold-free” printing process. Scaffolds can interfere with cell–cell interactions or degrade into products that are toxic to cells. Ideally, cells would secrete their own extracellular matrix to hold themselves together, more closely mimicking living tissue, Presnell says.

Penn State’s Ozbolat and colleagues achieve this by injecting 700- μ m-wide permeable tubes of alginate with cartilage cells that grow and stick to each other. The resulting cartilage strands are centimeters long and can be bioprinted as a solid ink, creating square centimeters of cartilage that look and feel just like the real thing.

Organovo, meanwhile, uses a hydrogel that is engineered to break down and disappear within 24 h—just long enough for the skin cells printed within it to fuse, secrete extracellular matrix, and begin to grow—as real skin would do

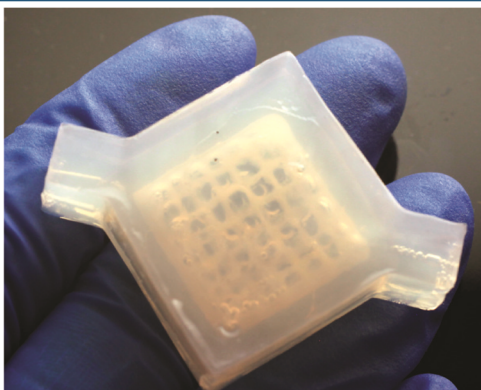
when a cut heals. By carefully optimizing the culture media, they have also developed inks containing only cells—truly scaffold-free.

On to Organs

Organovo's liver tissue comes in slivers less than 1 mm thick that can last for a few weeks. It has a 3-D architecture with multiple cell types and responds to drugs much like actual liver tissue would. But it's far from a real organ.

Above all, what's missing is blood supply. To print complex organs, researchers will have to figure out how to build a vascular network within organs to provide oxygen and nutrients to the tissues.

Harvard's Lewis has tackled this problem. Her method makes tissues more than a centimeter thick, laced with blood vessels. Her team first prints a 3-D grid made of layers of two different inks, both gels at room temperature, inside a printed silicone mold. One ink contains human stem cells, gelatin, and fibrinogen protein. The other is an ink based on a block copolymer that becomes a liquid when cooled down to 4 °C. Once the tissue sets, the researchers cool it and flush out this so-called fugitive ink so that it leaves behind hollow channels for blood vessels to grow. They then encapsulate the material in an extracellular matrix. Finally, they flow growth factors through the vascular channels to guide the stem cells to differentiate into bone marrow cells, and nutrients that allow the tissue to survive for more than 100 days. Lewis will soon test the printed tissue in animals to see if it is accepted by the immune system and gets perfused by blood flowing through the animal's vessels.



Inside a printed silicone mold, researchers print a 3-D lattice including channels for circulating nutrients that allow blood vessels to grow. Credit: Jennifer Lewis.

Anthony Atala and his colleagues at Wake Forest University take a different approach to make vascular channels. Instead of using fugitive ink, they program their printer software to leave microchannels in the tissue structure so nutrients and oxygen in the body can flow

through and blood vessels can eventually grow into the structure.

As they [reported earlier this year](#), the team implanted printed, human-ear-like structures and jawbone fragments under the skin of mice and rats. Two to five months later, native tissue and blood vessels had formed around the printed artificial tissues.

Likely the most astonishing—and eyebrow-raising—news in bioprinting came last year, when Russian company [3-D Bioprinting Solutions](#) reported printing the first fully functional vascularized organ, a mouse thyroid gland. The company's founder is [Vladimir A. Mironov](#), who worked on 3-D printing back in 2004 with Organovo cofounder [Gabor Forgacs](#) of the University of Missouri, Columbia. Mironov has since returned to his native Russia after being [suspended from his position](#) at the Medical University of South Carolina in 2011. The company hasn't published the work in a peer-reviewed journal, but has presented results at conferences. Mironov says that the glands partially restored the level of thyroid hormones in the animals.

Mironov is working with researchers in Finland to print kidneys. He says that printing a kidney that has the same minute details as the biological organ could be difficult. Instead, he says the first bioprinted kidney could be a much simpler structure that performs essential functions. Kidneys produce urine through three main steps: filtration, reabsorption, and secretion. "If you can even create filtration in an artificial kidney, that will be big", he says. "An organ transplant specialist told me that even if it's like a cube but provides the same basic functions, he would transplant it."

Meanwhile, Organovo is taking it a step at a time. The company is working on larger, thicker tissue chunks that can be stitched into weak organs to save lives. "The liver is a good example where you can effect a meaningful clinical outcome by replacing 10% or less of the organ", Presnell says.

When printed hearts and livers will find their way into humans is anyone's guess. But with rapid advances in 3-D printing, chemistry, and biomaterials, as well as stem cell research, to many in the field, the goal seems attainable. "When will a surgeon open a box and find a fully formed printed kidney?" muses Presnell. "I think I will see that in my lifetime."

Prachi Patel is a freelance contributor to [Chemical & Engineering News](#), the weekly newsmagazine of the American Chemical Society.