Prints of pieces

3D bioprinting allows researchers to construct increasingly elaborate living structures. A new generation of accessible tools has opened the field to many more labs.

By Alan Dove

In 1987, American inventor Chuck Hull built the first successful 3D printer, a robot that could extrude material in layers, slowly turning a digital blueprint file into a physical object. Over the next 30 years, Hull and other engineers refined the concept, and today 3D printing has become one of the core technologies of modern design and manufacturing. It’s ideal for creating one or a few copies of a complex shape, such as a product prototype.

Custom-built, complex shapes are also common in biology, leading many biomedical researchers to ask whether it’s possible to print working tissues and organs. Beginning with repurposed inkjet printers in the early 2000s, bioprinting pioneers have since moved on to 3D printing, yielding increasingly complex living structures. At the same time, cell biologists have refined techniques for creating induced pluripotent stem cells (iPSCs).

By combining these two technologies, future physicians might bioprint replacement tissues and organs from a patient’s own cells—eliminating the need to find donors and the risk of immunological rejection. Researchers still have to solve some tricky problems to get bioprinting into the clinic, especially for complex organ replacement, but a series of recent developments and the availability of relatively cheap, user-friendly bioprinters has made the technique more accessible than ever.

Organoids keep the beat

In a typical bioprinting experiment, scientists employ a specialized 3D printer that extrudes bioinks, consisting of extracellular matrix materials or live cells. The printer converts a 3D computer model into a printed shape. That was the approach Jennifer Lewis, professor of engineering and applied sciences at Harvard University in Cambridge, Massachusetts, first used when she set up her own bioprinting lab.

“Our focus initially was to create vascularized human tissues by fully printing everything ... building up multicellular constructs that had vascular networks,” says Lewis. Printing the cells and extracellular matrix together allowed her team to control the structure precisely, but Lewis realized that the technique couldn’t build the smallest structures inside complex organs. “The sort of 1-micron to 10-micron scales [we are looking for], which are the sizes of the cells themselves, would be very hard to do by bioprinting alone,” she says.

Meanwhile, stem cell researchers were making major strides in growing organoids, small organlike structures that self-assemble from partially differentiated stem cells. Lewis decided to combine the two methods—using 3D printing to lay down a vascular structure, then seeding it with stem cells stimulated to form appropriate tissue types for the organ.

To test the new approach, Lewis’s team focused on heart tissue. Hearts are a natural choice for bioprinting proof-of-concept experiments. They have fewer cell types than some organs, and provide clear visual evidence of whether the cells are functioning properly, as cardiomyocytes spontaneously start beating when they’ve formed working tissue.

Using their hybrid printing approach, the investigators built complex sections of vascularized heart tissue, seeded with iPSCs that were stimulated to form cardiac cells. It worked. “They go from basically an asynchronous beating, where each individual organoid is beating at a different rate; and as they fuse together, they start to beat together, they synchronize, and then the whole tissue is contracting at the same time,” says Lewis.

While the result is impressive, Lewis cautions that it’s still several steps removed from building a functional heart. One problem is that the organoids remain immature, with contractions that are much weaker than...
those of an adult heart. Making a working heart will require more postprocessing to increase the tissue’s strength, and more advances in stem cell biology to improve the cells’ maturation. It will also entail printing a full organ, something Lewis is reluctant to do at this point. “We purposely didn’t even go anywhere near to a construct that looked like a heart,” says Lewis, adding that she didn’t want to create false hope with images of a complete but nonfunctional organ.

**A patch for broken hearts**

Despite the fact that they are not yet prepared to build complete hearts, researchers are nevertheless exploring ways to get bioprinting into the clinic. Tal Dvir, professor of molecular microbiology and biotechnology at *Tel Aviv University* in Tel Aviv, Israel, has been copying patients’ cardiac blood vessels. “We started with printing cardiac patches with blood vessels, and what we’ve initially done has been to look at the CT [computed tomography] images of patients, at the blood vessels in the left ventricle, which is the ventricle or the piece of tissue we need to replace,” says Dvir.

Although he stresses that the method is still far from ready for use in humans, Dvir’s team has managed to bioprint copies of left ventricles and seed them with patient-derived iPSCs. To support the cells, he uses a hydrogel that’s also customized to the patient’s own immunological profile, so that it won’t stimulate an immune response when transplanted. In their most recent project, Dvir and colleagues scaled the process up to print a full heart-shaped structure. “I need to emphasize that it’s a very basic heart, and now we’re working on making it a little bit more advanced—more mature, with more blood vessels,” says Dvir.

Just as Lewis’s team did, Dvir’s lab is struggling with the relative weakness of the iPSC-derived cardiac cells. In addition to strengthening the bioprinted heart muscle, researchers are also pondering ways to keep the completed organ alive long enough to transplant. “When you’re talking about such thick and volumetric organs, you cannot culture these cells normally in a regular incubator, because ... oxygen cannot penetrate into the tissue, and most of the organ will die,” says Dvir. He adds that specialized bioreactors will have to provide something akin to circulating blood to support full-scale organs. Bioprinted hearts will also need electrical components, such as a pacemaker and specialized signaling cells.

Despite the complexity of the structures his lab is now printing, Dvir uses off-the-shelf equipment and supplies that other researchers could easily acquire. His bioprinter is a standard model from *regenHU* in Villaz-Saint-Pierre, Switzerland. “The most important thing is not the printer itself, [but] the biomaterial in which the cells are printed,” says Dvir. He encourages scientists who want to get into bioprinting to pay close attention to the properties of the biomaterials that they intend to use.

**The prints and the paupers**

Just a few years ago, research-grade bioprinters were expensive, finicky devices that only the best-funded labs could afford. Now, though, several companies offer relatively affordable, user-friendly units with advanced capabilities, such as the *regenHU* system Dvir uses. Indeed, bioprinter prices have plummeted recently. In 2016, it wasn’t unusual for researchers to spend USD 200,000 or more for a bioprinter. That was the year *Cellink* in Boston, Massachusetts, introduced an entry-level bioprinter for USD 5,000. There was a minor catch: That price was only available to labs willing and able to collaborate on the device’s development. “With that feedback we could develop our flagship system, called the Bio X, [which] we sell for USD 39,000 in most areas of the world,” says Erik Gatenholm, the company’s CEO and founder. Gatenholm adds that the Bio X is now the best-selling bioprinter globally.

Though it’s priced at a level that many academic labs and small biopharmaceutical startups can afford, Gatenholm says the Bio X is also versatile and user-friendly. “You have the ability to exchange the print heads depending on which material you work with,” he says. Different heads accommodate printing with thermoplastics, collagen, cells, and tiny droplets of liquids such as ink. Cellink has been pleasantly surprised by the ways some customers have used the system. “You obviously want to solve the biggest [bioprinting] problems, which must be hearts and kidneys and livers, but some of the really cool applications that we’ve seen come very far are, for instance, printing corneas,” says Gatenholm. That work, done by Che Connon, professor of tissue engineering at *Newcastle University* in Newcastle, United Kingdom, could eventually lead to new treatments for the estimated 15 million people worldwide awaiting cornea transplants. “The human cornea market is a USD 1 billion industry, but it’s not something we typically talk about,” says Gatenholm.

The potential for such clinical breakthroughs has driven much of the interest in bioprinting by pharmaceutical companies, but the technology still has to clear some major hurdles to reach patients. *Organovo* in San Diego, California, has long been viewed as a leader in the development of clinical bioprinting. However, the company announced last August that it was suspending its lead liver bioprinting program and restructuring.

**Getting ink done**

Despite the grim news from Organovo, researchers working on more fundamental projects in academic and industry labs continue to flock to bioprinting. “We’ve seen a lot of different applications from our customers; there are people working with everything from food to in vitro tissue models for high-throughput drug screening,” says Taciana Pereira, vice president of Life Science Solutions at *Allevi* in Philadelphia, Pennsylvania.

Like Cellink, Allevi focuses on making affordable, user-friendly bioprinters. The company offers four models, numbered 1, 2, 3, and 6. “The model number corresponds to the number of extrusion heads on the printer,” explains Pereira. Prices range from USD 20,000 for a base model Allevi 1 to about USD 100,000 for a top-of-the-line Allevi 6. The company also sells a range of bioinks and reagents.

“We have a very easy-to-use software so that anyone can start printing from minute one of logging in,” says Pereira, adding that “users can just select the shapes they want to print, and they don’t have to work with the back-end code to get the structure they want out of biomaterials.” Advanced users can still delve into the code for more control over the process. Regardless of their level of expertise, most scientists will appreciate features such as wireless connectivity, which allows the bioprinter to be placed on a bench or in a containment cabinet without running additional cables.

Investigators planning to buy one of Allevi’s printers should think carefully about their current and future needs, as the systems have swappable extrusion heads, but only for the original number of heads on the system. The single-head model, for example, cannot be expanded into a two-headed model, so labs stepping up to more complex printing will have to buy a new model.
Bioprinting is currently used to print tissues and organs for research. Its ultimate goal is to make these products ready for transplantation into patients.

**Featured participants**

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Machine. That said, Pereira explains that the company is willing to negotiate with existing customers to keep upgrade costs down.

With multiple companies offering bioprinters with similar capabilities, researchers should shop around before buying. “You want to make sure that a bioprinter will let you print [both] simple and complex structures, as well as go into the code as you acquire expertise. You will also want to make sure that the company you’re choosing is looking to enable you to do what’s most relevant to your work,” says Pereira.

**Houston, we have a printer**

As commercially built bioprinters become more common in labs around the world, at least one company is already pushing them into another frontier. In July 2019, the BioFabrication Facility (BFF) arrived for installation on the International Space Station, and the first experiments on this orbiting bioprinter began in August. It marked a literal high point in an effort that began more than a decade ago.

After his daughter was born with a diaphragmatic hernia requiring the removal of one of her lungs, laser engineer and nScrypt CEO Ken Church became interested in bioprinting organs. He quickly learned that it would take a multidisciplinary team. “Biologists are fundamentally good at … keeping cells alive, and engineers are very creative but we kill everything we touch,” says Church. Working closely with biologists through numerous design iterations, nScrypt eventually developed working bioprinters that could produce complex tissues.

Now, they’re confronting the same problems facing others trying to build full-size organs. “We can print tissues really well … but we were struggling getting past a certain thickness, [because] waste can’t come out, oxygen can’t get in, and nutrients can’t get in,” says Church. While biologists such as Lewis and Dvir are trying to solve that problem by building internal blood vessels into the printed organs, Church says his team took a different approach: “What if we lowered the viscosity a little bit and allowed those cells to move more freely?”

Gravity will collapse a less viscous structure, so nScrypt decided to take their printer into space. To do that, they collaborated with veteran aerospace company Techshot in Greenville, Indiana, which has extensive experience preparing payloads for orbit. Initial experiments aboard NASA’s microgravity-testing “vomit comet” aircraft showed that the nScrypt printer could produce a heart valve with a low-viscosity matrix. The BFF uses a version of the same printer that is ruggedized and adapted for space use, and Church is optimistic about its potential.

The modifications that made the nScrypt bioprinter capable of surviving in space also make it suitable for other hostile environments. “The same sort of configuration that we sent into space, we sent to Africa with the [U.S.] Army,” says Church. That system has since printed custom antibiotic-impregnated bandages there.

Regardless of the type of bioprinting they’re doing, experts in the field are guardedly optimistic about its future. “There’s a lot of exciting things going on in bioprinting, as long as the field doesn’t overshoot itself, really good stuff will come out in the next three to five years,” says Lewis.

**References**


Alan Dove is a science writer and editor based in Massachusetts.