Organs on Demand

3-D printing has made inroads in the clinic, but constructing functional complex organs still faces major hurdles.

By Kate Yandell | September 1, 2013

RENAL RECONSTRUCTION: Wake Forest postdoctoral fellow Hyun-Wook Kang operates a 3-D printer that is making a kidney prototype with cells and biomaterials.
IMAGE COURTESY OF WAKE FOREST INSTITUTE FOR REGENERATIVE MEDICINE

On a stage in front of an audience of thousands, a futuristic-looking machine squirted gel from a nozzle. Layer by layer, it built up the material, shaping it into a curved, pink, kidney-shape structure based on a medical CT scan of a real organ.

It was 2011, and Anthony Atala, director of the Wake Forest Institute for Regenerative Medicine, was demonstrating his progress in using three-dimensional (3-D) printing to make a kidney during his TED Talk. Like a TV chef pulling a previously baked casserole from the oven, Atala soon held a bean-shape object in his gloved hands. "Here it is," he said. "You can actually see the kidney as it was printed earlier today." The audience erupted into cheers.

But Atala had not made a functional human kidney, as he at times seemed to imply and as the Agence France-Presse reported in a widely disseminated article. "A surgeon specializing in regenerative medicine . . . 'printed' a real kidney using a machine that eliminates the need for donors when it comes to organ transplants," it read.

Wake Forest quickly tried to stem the misleading coverage. "Reports in the media that Dr. Anthony Atala printed a real kidney at the TED conference in Long Beach, Calif., are completely inaccurate," stated a press release issued by the university following the media coverage. Rather, Atala had printed only a kidney-shape "mold" made of biocompatible materials combined with cells. The prototype, as Atala calls it, lacked the kidney's intricate inner structures, such as the fine networks of vessels called glomeruli that allow the organ to filter waste materials from the blood. The prototype could not have functioned as a real organ and thus was not ready for transplantation prime time; that may only be possible "many years from now," cautioned the press release.

BUILDING BODY PARTS: Anthony Atala has printed a kidney prototype (left) and biomaterial scaffolds for growing new ears (top right) and finger bones (bottom).
IMAGE COURTESY OF WAKE FOREST INSTITUTE FOR REGENERATIVE MEDICINE

Atala's kidney prototype represents both the promise of 3-D printing in a medical context and the hurdles that tissue engineers have yet to clear. With recent technological advances, using 3-D

printing to shape gels embedded with living cells into the general form of organs has become a relatively achievable task. Printing a liver or a kidney that functions in the same way and with the same efficiency as a real organ, however, is a different story. The most formidable obstacle standing in the way of functioning 3-D–printed organs is the difficulty of replicating the branching networks of veins, arteries, and capillaries that nourish the body's tissues and filter out waste. In most organs, cells must be within 150 to 200 microns—the width of a few human hairs—of the nearest capillary to survive.

As researchers modify and build devices that print with ever greater precision, and invent new biomaterials to serve as ink for these machines, they have been able to make substantial progress on printing ears, spinal discs, heart valves, and bone, which are moving towards the clinic. (See illustration below.) Similarly, simple engineered tissues, such as tracheas and bladders made from cells seeded onto biocompatible scaffolds and created without the use of 3-D printing, have already been inserted into patients. But these tissues have thrived only because they are thin enough not to require extensive infiltration by blood vessels.

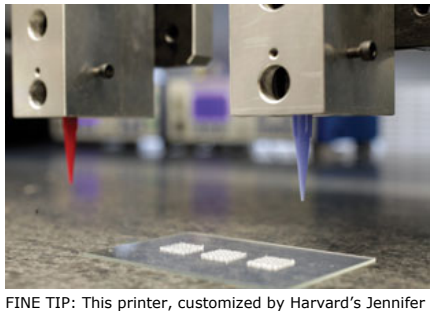
"[Vascularization has] been something that's been worked on for 20 years," says Jennifer Lewis, a materials engineer at Harvard University who is designing printers she hopes will produce vascularized tissues. "It's plagued a number of advances."

Achieving vascularization may be the biggest challenge that faces researchers attempting to 3-D print organs, but 3-D printing could also be the very technology to solve this problem. Researchers are harnessing 3-D printers to build tiny, hierarchical networks of blood vessels to supply increasingly complex 3-D–printed organs with blood.

"For me the holy grail of tissue engineering is to fabricate tissues with their own vascular network," says Jason Spector, an associate professor of plastic surgery at Weill Cornell Medical College, who is working on printing ears and other tissues. "Once you can make that, everything else is cake."

From playthings to medical devices

These days, 3-D printing, which has been around since the 1980s, calls to mind baubles such as iPhone cases, high-fashion shoes, personalized sex toys, and even working guns. There's a growing market for personal printers—relatively inexpensive machines that print at fairly low resolution, often using proprietary polymers—for producing such items at home.



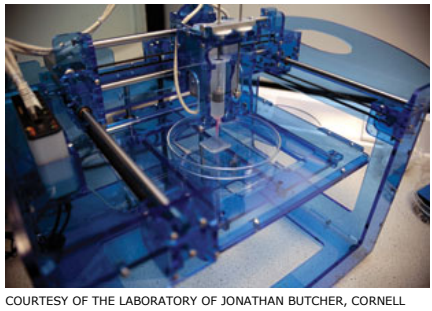
FINE TIP: This printer, customized by Harvard's Jennifer Lewis, can be used to print structures ranging from scaffolding for cell and tissue culture (as shown) to microvascular templates for blood vessel growth.
COURTESY OF JENNIFER LEWIS

But playthings, accessories, and weapons aside, 3-D printing has also made incursions into the medical device business. The 3-D–printing industry brought in $2.204 billion in 2012, $361 million of which was revenue from 3-D printing for medical and dental uses, according to the 2013 Wohlers Report. And researchers are now testing the feasibility of using printers to create patient-specific tissues and organs that may one day be used to supplement scarce donor body parts.

SUPPORT STRUCTURE: A 3-D–printed tracheal splint made of bioresorbable polycaprolactone is designed to fit around an infant's collapsing airway.
COURTESY OF JENNIFER LEWIS

Medical 3-D printing takes advantage of two major printer types. One type, used by Atala to print kidney prototypes, extrudes a pliable material, often a melted polymer or a gel, through a nozzle, building up the desired shape layer by layer according to a computerized blueprint. The second type of printer operates by shooting a laser or a binding material at a bed of powder and solidifying it in a highly specific pattern. As the laser or binding agent moves through the powder, layer by layer, it builds a solid structure embedded in powder, which is dusted off when the job is done. The powder can be a polymer, or it can be metal particles, useful for creating implants such as hip joints.

Physicians already rely on 3-D–printed hearing aids, cups for hip implants, dental crowns and bridges, and now cranial implants, modeled on scans of patients' bodies. Researchers even printed a customized titanium lower-jaw bone last year for a patient in the Netherlands. There is also a booming business in 3-D–printed surgical guides—implements somewhat like draftsman's rulers and compasses—which doctors place over surgical sites to guide their drills and knives as they bore and cut into flesh and bone.



COURTESY OF THE LABORATORY OF JONATHAN BUTCHER, CORNELL UNIVERSITY

Physicians also order 3-D–printed plastic replicas based on scans of patients' actual body parts—a hip joint that needs replacing, for example, or a patient's abdominal circulatory system—to practice upcoming surgeries using realistic models. The Mayo Clinic orthopedics department in Arizona

found custom surgical models so helpful, doctors there decided to purchase their very own 3-D printer last summer.

And with the success of 3-D–printed implants, it did not take long for tissue engineers to decide they could adapt 3-D printers to extrude biologically compatible scaffolds and cells to construct whole organs. In printing his kidney-shape structure at the TED conference, Atala was demonstrating the technology's ability to print a complex structure that was a hybrid between an implant and an organ: a scaffold made from a biocompatible and bioresorbable gel mixed with living cells that could conceivably, if placed in the proper environment, grow into living tissue.

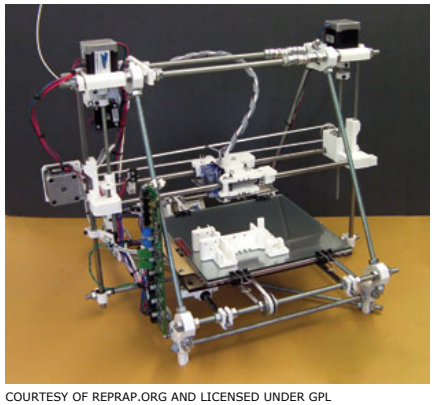
FAB MACHINE: University of Nottingham tissue engineer Kevin Shakesheff uses a Fab@Home printer (top) to engineer bone. Others have used the open-source printer for printing heart valves (bottom), ears, and more.
COURTESY OF THE LABORATORY OF JONATHAN BUTCHER, CORNELL UNIVERSITY

"The human body has tissues that are very highly structured," says Kevin Shakesheff, a tissue engineer at the University of Nottingham who is working on printing bone. "Their actual architecture is essential to how the tissue works. The level of control that the human body has is something we can now replicate with 3-D printing."

Indeed, researchers are hoping to introduce even more 3-D–printed tissues into the clinic and into patient's bodies in the coming decade. For instance, 3-D–printed vertebral discs and small pieces of bone are being tested in animals, while ears, heart valves, and more are being printed in the lab. But one physiological fact continues to stand in the way of a true 3-D printing revolution that could potentially save thousands of lives by stocking operating rooms with a steady supply of replacement body parts: the complexity of the vascular system that supplies organs with blood.
"You could print things up that look exactly like a tissue or an organ if you have a CT scan or MRI-derived data, but unless you can hook it to [the body's] blood supply . . . it will die," says Spector.

Blood Trouble**Be**

Though supplying blood to 3-D–printed organs is the major stumbling block preventing such implants from becoming a reality, capillaries can, to some extent, branch out from already-existing blood vessels and into transplanted tissues on their own. Researchers have seen signs of spontaneous vascularization in small areas of engineered tissue, such as in healed rat bone defects around 3 millimeters in diameter.[7]



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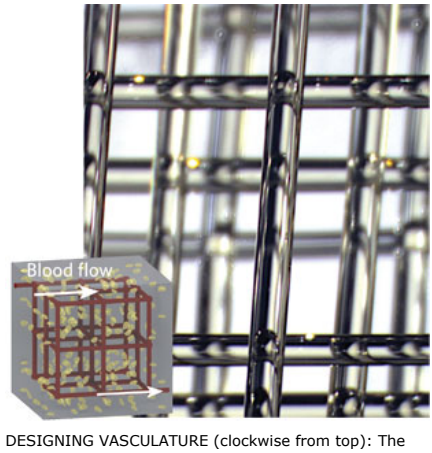
But can undirected capillary growth provide the hierarchical, all-penetrating networks that complex tissues and organs depend on? Spector says it's not likely. He deals with microvascular networks in his medical practice, hooking tissue grafts into the intricate vascular plumbing of their new hosts, and he has come to realize how difficult it is to achieve the level of vascularization needed for a transplanted tissue to thrive. "I have yet to see anything close to [an engineered solid organ] that will survive in a real clinical situation," he says.

Rather than letting blood vessels spontaneously branch off and expand into engineered tissues, some researchers are crafting templates for more orderly vascular growth. The idea is to create hierarchical microvascular networks that will guide the endothelial cells that line blood vessels to form tubes along predetermined courses. Some see 3-D printing as the best way to accomplish that goal.

Printing such tiny negative spaces, however, is easier said than done. Capillaries can be as small as a few microns in diameter. Even with high-resolution printers, such tiny vascular structures would likely collapse, especially when printed into a soft, biocompatible gel.

Harvard's Lewis, who serves as the university's Hansjörg Wyss Professor of Biologically Inspired Engineering, is interrogating this problem using a customized, high-resolution 3-D printer that can form microchannels in biocompatible gels. "We can print hydrogel materials down at the micron-length scale, smaller than other groups can print

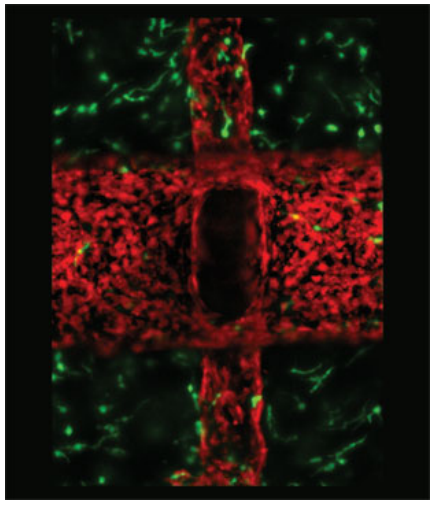


DESIGNING VASCULATURE (clockwise from top): The RepRap Prusa Mendel, an open-source printer that Rice University's Jordan Miller modified to print vascular templates for engineered tissues; a 3-D–printed carbohydrate template for vascular channels, which will be dissolved after it is encapsulated in cells and biomaterials, leaving vascular channels in living tissue; a diagram shows vascular channels encapsulated in a cell-filled biocompatible gel.
COURTESY OF REPRAP.ORG AND LICENSED UNDER GPL

anything," Lewis says. The smallest microvascular channels her group has been able to print are around 10 microns in diameter.

To solve the problem of collapsing channels, she prints them in "fugitive ink"—a substance designed to melt away after forming the channel's pattern. For her fugitive ink, Lewis settled on Pluronic F127, a gel often used in eyeglass lens cleaner and cosmetics. Pluronic F127 is made up of three parts—the two poles of the molecule are hydrophilic while the middle segment is hydrophobic. It also has an unusual property. "Most materials, when you cool them down, they solidify," says Lewis. "This material liquefies when you cool it down."

Lewis also used Pluronic F127 as the matrix into which she prints the channels, but she modified the matrix molecules so that they polymerize, and thus solidify, in the presence of UV light. This allows her to firm up the matrix before cooling the gel so that the fugitive ink melts away. Taking advantage of her printer's fine-tipped nozzle, she printed a capillary network of fluorescently labeled fugitive ink into the Jello-like matrix.8 "We were able to show for the first time a way to pattern hydrogels with these vascular channels," she says.



PRINTED VESSELS: Endothelial cells (red) line the walls of Miller's 3-D–printed vascular structures.
COURTESY OF JORDAN MILLER

The next step, Lewis says, is to take advantage of the self-organizing quality of endothelial cells in her own 3-D–printed constructs, seeding her printed vascular structures with these blood vessel–lining cells. (See "Crowd Control," The Scientist, July 2013, for a more in-depth look at how endothelial cells coordinate such behavior.) She will rely on the tendency of the finest capillaries to grow spontaneously out of larger microvascular structures. "It's not trivial, but biology will work," she says. "Once you give [them] a reasonable environment, the cells are happy."

Pour Some SugarPo

Inspired by some of Lewis's early work with "fugitive inks," Jordan Miller, formerly a postdoc in Christopher Chen's lab at the University of Pennsylvania and now an assistant professor of bioengineering at Rice University, created his own technique for 3-D printing of vasculature-mimicking channels. Using a simple open-source 3-D printer, he constructed a carbohydrate lattice made from a combination of simple and complex sugars.

"A lot of Jennifer Lewis's work is very inspiring, but the machines she is using are very high-end," he says. "They have incredible precision, but they are not duplicable by anyone else."
Miller saw potential in the Frostruder, a printer originally used to extrude sugar frosting for printing fancy designs onto edible treats. With help from members of the RepRap community—a group founded in 2005 with the purpose of designing self-replicating, open-source printers—Miller adapted a RepRap printer to incorporate elements of the food printer's design and was soon able to print dissolvable lattices of carbohydrate filaments.

Miller decided to use a process called "3-D sacrificial molding" that is akin to the lost-wax method used by sculptors. His printer deposits filaments of carbohydrate on top of each other in sequence so they are self-supporting. Miller then covers the entire lattice structure in a protective layer of a biodegradable polymer. After pouring and crosslinking a cell-filled gel over the carbohydrate lattice, he dissolves away the lattice with an aqueous solution.9 (See photographs here.)

Miller's channels are not as small as Lewis's—his channels range from 150 microns to around a millimeter in diameter. However, when he and colleagues seeded his channels with endothelial cells, they lined the interiors of the channels and even began to penetrate the surrounding cell-gel mixture. Miller says he hopes that by guiding blood cells into the larger channels, he can set the stage for endothelial cells to spontaneously form their own capillary networks. "We may not have to print an entire capillary bed," Miller says.

Miller is also working on building more expensive, high-resolution printers in case the cells aren't capable of forming capillaries on their own. But, he says, it's possible that endothelial cells, if seeded into a predefined set of capillary channels, might not follow the planned architecture anyway. "If we had put them in a capillary bed initially, they would probably remodel it [based] on local needs."

Miller has successfully pumped human blood through his constructs in vitro, and he plans to cooperate with a surgeon to connect one of his printed tissues to the vascular system of a rat to see how long he can get blood to flow through his channels.

In addition to being relatively cheap, Miller's method is fast. "The big challenge in that field is [that] a lot of the interesting cell types we would want to build into large-scale tissues—things like liver cells—[are] not going to survive the several hours in the extruder nozzle, long enough to build something the size of the human liver," he says. Quickly pouring the cells and gels over the 3-D–printed lattice is easier on fragile cells than the arduous process of printing.

The disadvantage is, of course, that the researchers can't control the exact placement of the cells. Someone planning to print using multiple cell types, for instance, might not want to pour them out over a lattice willy-nilly—although Miller says that cells are surprisingly good at organizing themselves even

when poured into a gel.

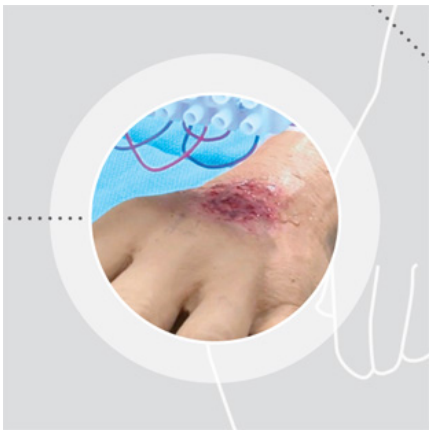
"I wouldn't say this is the end-all be-all solution to tissue engineering," says Miller, but "it's allowed us to take the next step." He has already shown, in versions of his constructs printed with rat liver cells or with human embryonic kidney cells, that the cells near the channels survive longer than the cells deeper in the gel, suggesting that the faux vasculature is doing its job. He says that even if his tissues are nowhere near ready for implantation into humans, at least he can now keep cells alive for longer in order to do in vitro experiments to understand better what they need to thrive over the long term.

Numerous other groups are also trying their hand at 3-D printing of vasculature, and should any of them prove successful, it would pave the way for tissue engineering on a grander scale than ever before. Growing and implanting larger swaths of bone or skin may become feasible, and producing more-complex organs like hearts or kidneys might become more realistic. "It's a pretty competitive landscape right now," says Lewis, who is continuing work with her Pluronic F127 fugitive ink, as well as experimenting with growing living cells in various kinds of extracellular matrix material. "In the next 2, 3, 4 years, you'll see lots of groups publishing on this idea—that they can create deterministic patterns of vascular growth," agrees Cornell biomedical engineer Lawrence Bonassar, who is working on his own version of microchannels.

"It's just a really fun technology," says Miller. "We've made thousands of these structures and every time they print it's just magic."

PRINTING LIFE

3-D printing allows tissue engineers to fabricate more-complex shapes and to more precisely mix materials than does the process of growing organs by seeding cells onto handmade scaffolds. Anthony Atala—whose artificial bladders, made by growing a patient's own urothelial and muscle cells on collagen and polyglycolic acid (PGA) scaffolds, are now in clinical trials—says he is working on bioprinting a multitude of tissues and organs, including muscles, bones, tracheas, ears, noses, and kidneys. His goal is to design bioprinters that can print usable engineered tissues at all levels of complexity. Many other researchers are also forging ahead with 3-D printing projects that may reach patients in the near future. Of the projects below, only the tracheal splint has thus far made it into a human patient, but some engineered tissues are now being tested in animals.



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References

1. A. Atala et al., "Tissue-engineered autologous bladders for patients needing cystoplasty," *The Lancet*, 367:1241-46, 2006.
2. A.J. Reiffel et al., "High-fidelity tissue engineering of patient-specific auricles for reconstruction of pediatric microtia and other auricular deformities," *PLOS ONE*, 8:e56506, 2013.
3. D.A. Zopf et al., "Bioresorbable airway splint created with a three-dimensional printer," *NEJM*, 368:2043-45, 2013.
4. B. Duan et al., "Stiffness and adhesivity control aortic valve interstitial cell behavior within hyaluronic acid based hydrogels," *Acta Biomaterialia*, 9:7640-50, 2013.
5. A. Skardal, "Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds," *Stem Cells Transl Med*, 1:792-802, 2012.
6. R.D. Bowles et al., "Tissue-engineered intervertebral discs produce new matrix, maintain disc height, and restore biomechanical function to the rodent spine," *PNAS*, 108:13106-11, 2011.
7. G. Fielding, S. Bose, "SiO2 and ZnO dopants in three-dimensional printed tricalcium phosphate scaffolds enhance osteogenesis and angiogenesis in vivo," *Acta Biomaterialia*, doi:10.1016/j.actbio.2013.07.009, 2013.
8. W. Wu et al., "Omnidirectional printing of 3-D microvascular networks," *Adv Mater*, 23:H178-83, 2011.
9. J.S. Miller et al., "Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues," *Nature Materials*, 11:768-74, 2012.

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