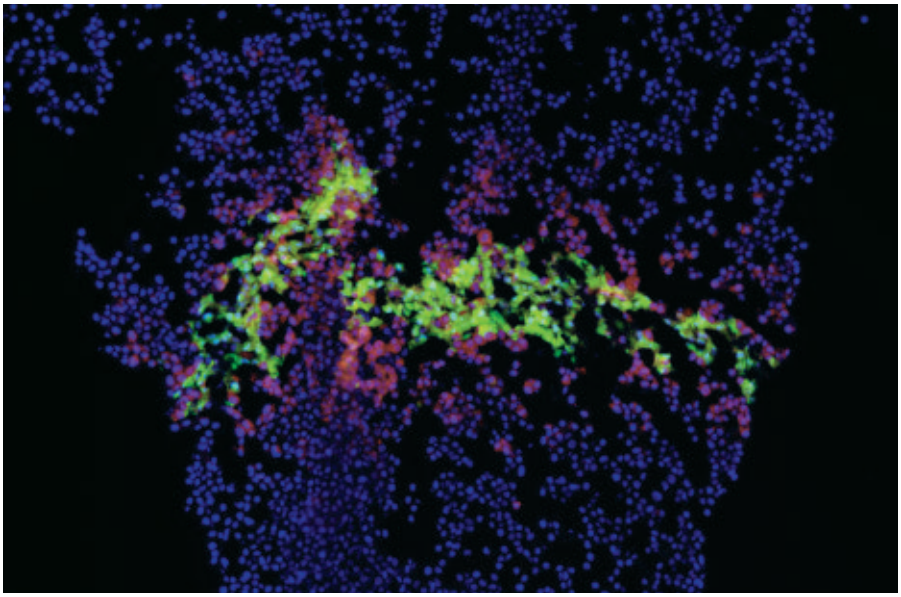
ORGANOVO



LIVER DELIVERY Organovo's 3-D printed liver tissue contains three different types of cells. The dominant cells, stained blue, are roughly 20 µm in diameter.

PRINT YOUR HEART OUT

3-D BIOPRINTING can already create living tissue, but it's unclear whether it will ever replicate organs

MATT DAVENPORT, C&EN WASHINGTON

IT WAS AN HONEST QUESTION. But the way Stuart K. Williams asked it sounded like the prelude to a wager: Which organ will researchers first replicate with three-dimensional bioprinting?

Williams, the director of the Bioficial Organs Program at the University of Louisville, posed the question to Gabor Forgacs of the University of Missouri at last month's Select Biosciences Tissue Engineering & Bioprinting Conference in Boston. Forgacs, having just delivered the keynote speech, mulled the question over.

Some believe 3-D printers will one day create viable organ transplants using a patient's own cells. This would alleviate complications that arise when a patient's immune system rejects a donor organ. And it would put an end to growing transplant wait lists. For every organ donor in 2012, there were more than eight patients on the transplant wait list, according to the U.S. Department of Health & Human Services.

Williams's question hung in the air for a moment. The conference hall overlooking the Charles River was packed even though the latest in a series of record-setting snowstorms kept many would-be attendees away. The crowd waited silently for Forgacs's answer, but everyone there had an inkling of what it would be.

Forgacs, a pioneer in bioengineering who's printed 3-D structures with "inks" made of living cells, hedged the question, reminding the audience of comments he made during his talk. "Everybody's dream is the 3-D printed organ. Are we ever going to get there?" he asked himself. "I'm not so sure."

Bioprinting's more immediate impact will be in making small patches of tissue for screening drugs or for better understanding biology, Forgacs said. Before researchers can even hope to tackle the far more complex problem of printing an entire organ, he added, they will need to confront some daunting challenges, such as figuring out how to print blood vessels capable of supplying artificial organs with essential nutrients.

These challenges influence the decisions researchers make in every phase of the printing process: from concocting a suitable bioink to printing the ink to goading the printed cells to act like an organ. This last bit, Forgacs said, is the most important and most difficult challenge.

He's not convinced that researchers will ever duplicate an organ with bioprinting, but he doesn't believe that they should try to copy organs exactly. "There's no reason we can't make something that functions exactly the same, if not better, than the natural organ," Forgacs told C&EN. The day when an improved heart or liver can be printed on demand is several decades away, but Forgacs is optimistic it's coming. "We are fantastic engineers."

RESEARCHERS' ENGINEERING ingenuity is evidenced by how far bioprinting has come since its birth about 15 years ago. It's tough to pin down an exact starting point for the field, but many researchers point to the early-2000s work of Thomas Boland, who was then working as a bioengineer at Clemson University.

Boland swapped out the contents of an ink-jet printer cartridge for a bioink containing bovine cells suspended in a mixture of serum and cell-culture medium. After installing the cartridge in a modified Hewlett-Packard desktop printer, his team printed a 2-D pattern of the ink on a biopaper—a substrate that makes cells feel more at home outside the body. In this case, the biopaper was a gelatinous mixture of collagen and a protein matrix to help anchor cells. Within a few years, the ink-jet technology could print stacks of these cellular patterns to make 3-D structures.

Boland's experiments would essentially define the criteria needed for a method to truly be considered bioprinting. First, the bioink must contain cells. Metals, plastics, and ceramics have been printed without cells to repair or replace biological structures such as teeth, windpipes, and skulls. Many consider these uses to be examples

"There's no reason we can't make something that functions exactly the same, if not better, than the natural organ."

of conventional 3-D printing with biological applications rather than bioprinting.

Second, the bioprinter must be able to pattern a user-defined 3-D structure on demand. This means that organs made from cells cultured in molds don't get the "bioprinted" label. This method has been used by researchers at Wake Forest University to produce bladders for transplantation.

And finally, the cells must survive the printing process and remain viable. In other words, a printer shouldn't murder cells with heat, laser light, or mechanical stress. Cells also need a print medium that fosters a nurturing biological environment, which can be provided by the bioink, the biopaper, or some combination thereof.

Since Boland's ink-jet innovation, researchers have developed a variety of inks, papers, and printers that work together to satisfy the basic requirements of bioprinting. Some of these products have even been commercialized.

There are currently more than a dozen 3-D bioprinting companies, according to a list provided by Select Biosciences. Organovo, a company founded in 2007 based on technology developed by Forgacs, is the most notable among these, according to many in the field.

In November of last year, Organovo started selling a 3-D bioprinted liver tissue called exVive3D. The tissue accurately predicts human response to drugs that are toxic to the liver, according to the company's chief executive officer, Keith Murphy.

Pharmaceutical companies could thus use the tissue to test drugs at a stage between preclinical animal trials and clinical human trials. Catching adverse effects in human tissue before moving a drug into clinical trials would not only better protect patients but also save companies time and money in drug development.

So far, the response to the exVive product has been good, Murphy said. The printed tissue accounted for nearly $140,000 of Organovo's revenue between its November launch date and the end of the calendar year, according to the company's most recent quarterly report.

But to get from small tissue swatches—exVive is a 3-mm-square patch that's thinner than the ink trail left by most ballpoint pens—to organ-sized structures, researchers still have a ways to go.

ONE OF THE BIGGEST obstacles researchers have to overcome is figuring out how to create 3-D printed blood vessels, said Yong Huang, a mechanical engineer at the University of Florida. He didn't make the trip to Boston—he spoke to C&EN from the warmer climes of Gainesville—but his sentiment was echoed by many speakers at the Select Biosciences conference.

To print any tissue thicker than a few hundred micrometers, researchers will need to be able to create vasculature networks that mimic what's found in nature. Blood vessels within the body penetrate tissue like roots through soil to deliver oxygen and nutrients to cells while also carrying away cellular waste. Without vasculature, a cell is doomed if it can't get its nutrients by diffusion.

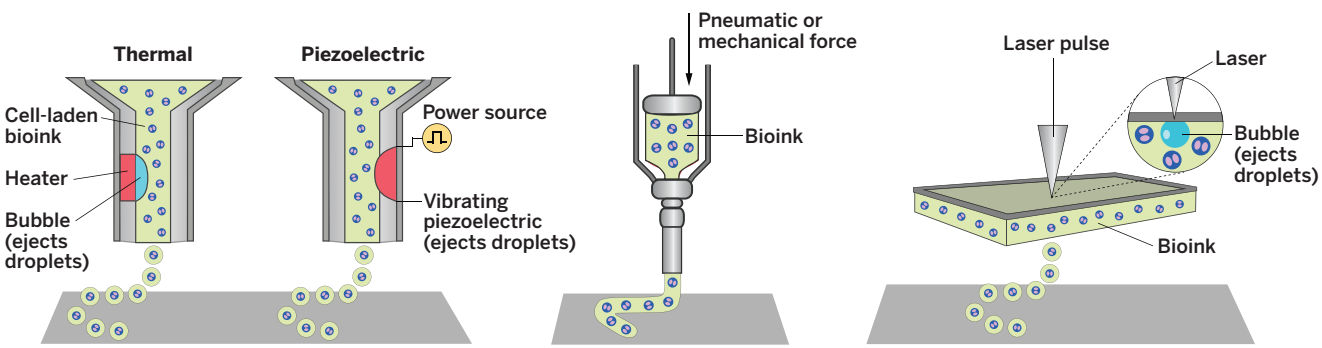
Researchers have already bioprinted blood vessels, but these are typically homogeneous and often highly symmetric cylinders, Huang said. Real vasculature includes heterogeneous constructs that bend, branch, and vary in diameter. Scientists can't readily replicate that complexity by relying on simple cylinders, he added.

To create vasculature with more complex geometries, Huang's team used an ink-jet-style printer to deposit drops of a cell-laden sodium alginate ink into a calcium chloride solution. The alginate, a polysaccharide derived from brown algae, cross-links and becomes a gel when it encounters calcium. Researchers could thus deposit their faux blood vessels one layer at a time, building in the branches and bifurcations needed to mimic real vasculature (*Biotechnol. Bioeng.* 2015, DOI: 10.1002/bit.25501).

Although Huang said he is excited by the results, he is keenly aware of the lingering questions. In particular, he wonders whether the printed blood vessels will actually act like blood vessels when implanted inside living tissue.

"Do the cells behave like we want them to? We don't know yet," Huang said. "You want to build a house brick by brick? That's fine. But when you put living tissue together cell by cell, you don't know how it will work."

The biggest challenge in 3-D bioprinting is getting printed cells to mature into functional, living tissue, Huang added. Overcoming that challenge will require an army of engineers, biologists, clinicians, and many others.



TOOLS OF THE TRADE Researchers have developed multiple strategies to print tissue from the bottom up.

ADAPTED FROM *NAT. BIOTECHNOL. & J. MANUF. SCI. ENG.*

& MORE ONLINE Visit http://cenm.ag/3dbio to watch the Lewis lab 3-D print cells and blood vessels.

Jennifer A. Lewis of Harvard University has adopted a different approach to the vasculature problem. Her group is using a sacrificial ink to print smaller channels, tens to hundreds of micrometers in diameter. The researchers extrude the ink as a gel, squeezing it out of their printer's nozzle like toothpaste. But the ink vanishes from the final product, leaving behind channels that can help keep printed cells alive, she told the audience at the Select Biosciences meeting.

The sacrificial ink, also called a fugitive ink, is based on a block copolymer known as Pluronic F127. The team can deposit this fugitive ink adjacent to cell-laden bioink. Both inks get encapsulated within a material that simulates the extracellular matrix, a natural, biological support system for cells. The materials are all gels at room temperature—both the bioink and the matrix make use of gelatin methacrylate.

But the Pluronic F127 ink has an unusual property. It actually liquefies as it cools from room temperature down to around 4 °C. Once researchers have finished printing, they can simply cool their part to flush away the fugitive ink, while the cells and matrix stay put.

The team then injects endothelial cells into the empty channels the fugitive ink leaves behind. The cells latch onto the matrix and begin forming open vessels (*Adv. Mater.* 2014, DOI: 10.1002/adma.201305506).

Although this strategy doesn't directly produce vascular networks that look like the ones found in real circulatory systems, Lewis said it may not need to. The Harvard team hopes to print the vascular highways, then let the cells build the branching streets and alleys themselves.

"Ultimately, you want the cells and the biology to take over," she said. "But we don't know exactly how much programmed structure we have to print before we can rely on biology."

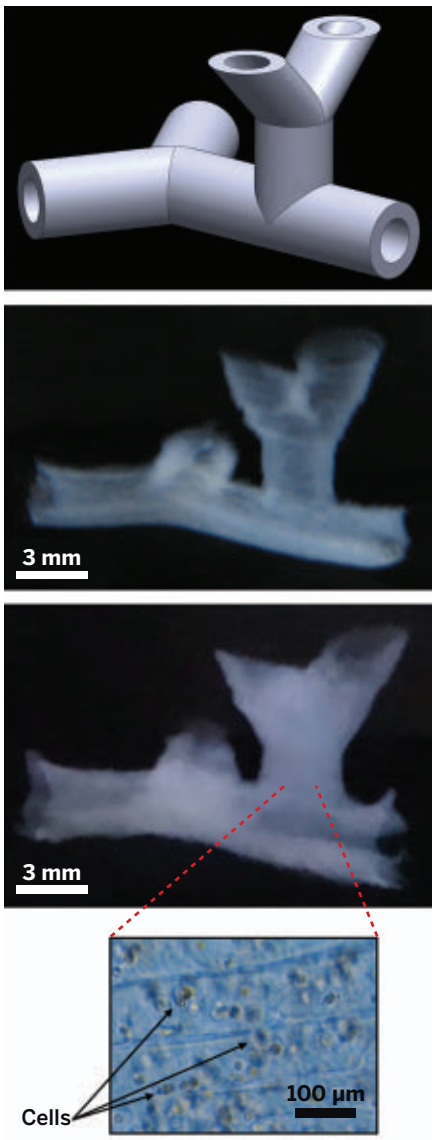
Despite all the questions surrounding the future of bioprinting, it's easy to get drawn back to the one asked by Williams at the beginning of the conference: What will be the first 3-D printed organ? That it's so boldly posed—there are no qualifiers or conditions—seems to invite at least a little brazen speculation.

Yet Williams himself had a rather sober response. He, like Forgacs, believes that it will be a long time before we see a full organ, but we might see small tissue implants first, probably in cases where a current implantation method leaves something to be desired. For this reason, he suspects 3-D bioprinting might find a niche in replacing facial or breast tissue lost during cancer-removing surgeries.

But Williams hopes bioprinting's reach doesn't end there. He also agrees with Forgacs that researchers can design better organs than what humans currently work with. It will take a lot of time and scientists crossing disciplinary lines to reach that goal, he said, but it's certainly possible. "We can build new organs that are better than the biological ones," he said. "That's what makes this whole thing so much fun." ■

TUBULAR A computer model (top) guides the 3-D printing of vasculature with cell-free (middle) and cell-laden ink (below).

BIOTECHNOL. BIOENG.



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