printed life

The 3-D printing of living organs for transplant isn't far-fetched; it's almost here.

By Jean Thilmany

Its developers spent nearly 30 years working on the artificial heart before Barney Clark received the Jarvik 7 in 1982. There are those today who predict it will be about two more decades—give or take—before technology will be able to print the first biocompatible heart for implant.

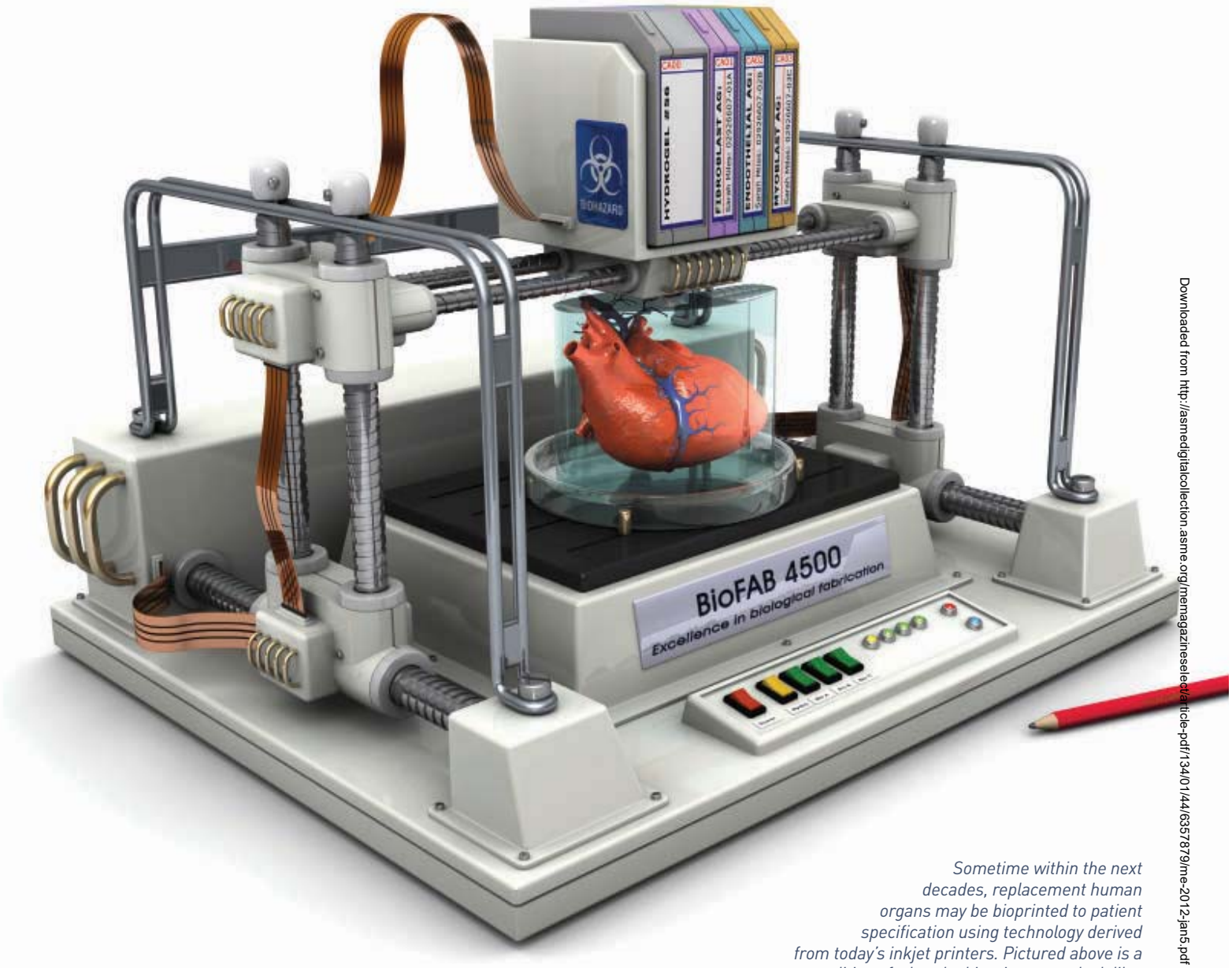
Such a heart will be a fully functioning, three-dimensional body part, according to Ibrahim Ozbolat, assistant professor of mechanical and industrial engineering at the University of Iowa.

And it will be printed in the same way prototypes and one-of-a-kind parts are printed today. With one crucial difference: The human organ will be alive, having been seeded with cells from the transplant recipient's body, Ozbolat said.

But even 20 years down the road, printed, living organs capable of being transplanted into a human won't be feasible without concomitant stem cell research, he added.

Ozbolat is among a loose collection of researchers organizing around bioprinting, the potential to print replacement human organs grown in a self-organized way—that is, without the tissue scaffolding traditionally used now to grow biological tissue in two or three dimensions.

Jean Thilmany is associate editor.



Sometime within the next decades, replacement human organs may be bioprinted to patient specification using technology derived from today's inkjet printers. Pictured above is a rendition of what the bioprinter may look like.

IMAGE © CHRISTOPHER BARNATT, EXPLAININGTHEFUTURE.COM

Today, artificial or replacement tissue is commonly grown on collagen scaffolds that contain biological starter cells. The end goal here is the growing of a biocompatible piece of tissue to repair or replace a patient's own damaged body part, such as bone, cartilage, blood vessels, or skin.

Tomorrow's method is bioprinting, which will eventually take tissue engineers well beyond the realm of growing replacement tissue and into printing replacement organs, said Hod Lipson, associate professor of mechanical and aerospace engineering and computer and information science at Cornell University.

"Bioprinting is where some exciting things are happening," Lipson said. "Ultimately, we'll move away from replacement parts made from engineered material and go to living implants made out of your own cells that are alive, have no rejection issues, and last longer."

An example of an engineered material might include a titanium implant that replaces a worn jaw or hip joint, he said.

The method is much the same as additive manufacturing, in which a printer deposits a material, layer by layer, until a three dimensional object is made. For bioprinting, the material used is likely to be living cells taken directly from the patient's body and infused into an ink or gel to keep them alive, Lipson said.

After printing, the material is incubated in a cell culture that mimics human body conditions until it fuses or becomes otherwise usable for implant, he said.

Lipson himself is one of the researchers on this path. He's

working to perfect a bioprinter and a bioprinting process. The field is also being driven forward by researchers at the University of South Carolina, Wake Forest University, and the University of Missouri, among other sites.

And it's already being commercialized. In a March 2008 report in the journal *Tissue Engineering*, Gabor Forgacs, then part of the tissue-engineering group at the University of Missouri, and his team said they'd succeeded in printing functioning blood vessels and cardiac tissue. The group printed cardiac and endothelial cells, which fused into a tissue after 70 hours of incubation and began beating in time like regular heart tissue after 90 hours.

Since then, Forgacs has moved on to become chief scientist at Organovo in San Diego, where he has plans to commercialize the NovoGen bioprinting platform. Forgacs has said the company will test printed blood vessels in animals with the plan eventually to use the vessels for human heart bypass surgery.

new knee in 3-D

But Organovo's announcement doesn't mean bioprinting is ready for prime time, Lipson said. Many hurdles need to be cleared before a printed organ can be implanted into a human patient.

"We're developing the printing technology and we can make these tissue constructs, but how long before you can implant them is complicated because they must go through FDA testing," Lipson said. Meanwhile, Lipson works in conjunction with two other Cornell professors: Larry Bonassar, associate professor of biomedical engineering and associate professor of mechanical and aerospace engineering, and Jonathan Butcher, assistant professor of biomedical engineering. Butcher's team is bioprinting heart valves.

The engineers use live cells taken directly from the patient's body part to be replaced. So bioprinted knee cartilage would begin with cells taken from either of a patient's knees. They place the cells in a special ink they've developed.

"The printer is pretty straightforward but the challenge is making the inks," Lipson said. "You want to make an ink that the cells are happy in, that doesn't kill the cells, but is stiff enough the printer can make a 3-D object that can hold its shape.

"The printer deposits this ink to gradually create a 3-D tissue construct; we incubate this item, and if successful, you have a 3-D tissue with those live cells in it," Lipson said. "It's a, quote-unquote, replacement part."

Lipson's group has already successfully printed meniscus knee cartilage. The cells are printed in the C-shape of the knee and then incubated in a cell culture until collagen, the tough fibrous protein that holds the meniscus together, forms around them.

"Meniscus is tricky because it has to bear a lot of load," Lipson said. "But we are starting with these simpler tissues that don't have a lot of vasculature."

Printing tissue that contains many different types of cells is still quite a ways out due to cell and printer limitations, he said. First, cells must be strong enough, like meniscus cells, to withstand the printing process. Next, they should be all or most of a type.

Complex organs—such as the liver—fed by small blood vessels will be difficult to print in a single process as the printer must be able to print several types of cells in exactly the right location. How to pump blood through tiny, printed capillaries to all parts of the organ is a separate problem.

But Lipson was drawn to the bioprinting field in the first place thanks to his work in multimaterial printing. He uses a multimaterial printer to create small, 3-D robots that contain wires, actuators, and batteries made of different materials. The robots are printed in a single process.

"The printer head for both multimaterial and bioprinting contains multiple nozzles with different materials coming from each nozzle, the same way a color printer contains different cartridges, each printing a different color," he said. "For bioprinting, each nozzle has ink coming out with different cell types within the ink. They work together to print a part.

"If you're doing multimaterial printing, it's natural to try to print biological material," Lipson added. "The method is the same but the materials are different."

there will be blood

Meanwhile, halfway across the nation, Ozbolat is working in the University of Iowa Biomanufacturing Laboratory at the Center for Computer-aided Design to create microfluidic vessel-like chambers that will one day stand in for the capillaries and vessels that pump blood through complex printed organs to supply nutrients and oxygen.

"Today, you basically print cells and build a 3-D tissue culture, but the bottom layer will die because cells at that layer have no contact with air and with nutrients normally transported to the bottom cell line," he said. "Vessel-like channels are necessary so cells can get the nutrients from the body and oxygen."

Using a coaxial-nozzle printer, which allows one material to be printed around another, he and his team now print hollow filaments.

They plan to eventually print a filament with a hollow core mimicking blood vessels. This will be the essence of the microfluidic chambers that will feed the organ. Those channels will then be connected to a tiny, implantable pump that will pump nutrients through the channels and to the cells of the organ.

The filament must be permeable to properly diffuse the nutrients, mimicking the role of the capillaries in the human body, Ozbolat said.

"A natural organ contains blood vessels that the blood can flow through but not out," he said. "Then it flows to the small capillaries, which have contact with cells, and the nutrients and oxygen diffuse out to the cells."

Today's printed vessel-like structures, such as the blood vessels printed by the Medical University of South Carolina

team, are around 400-500 micrometers. Printed capillaries that feed a large, complex organ would need to be on the nanoscale, Ozbolat said.

"It's difficult to get to that scale now, so we need these permeable microfluidic channels," he said.

inside the incubator

So if all research aligns and 3-D organ printing becomes a reality how exactly will it work?

Likely a physician or technician would first take a 3-D CT or MRI scan of the body part in need of aid, Lipson said. The image would then be digitized in a CAD system so the replacement organ, or tissue, exists as a CAD model.

On the other hand, physicians could use the CAD system to directly create a design to be used for surgical practice or planning rather than for direct transplant, Lipson said. The design could be biologically printed in three dimensions.

"Surgeons could practice taking a tumor out in a complex environment," Lipson said. "Right now there's no real way to practice that because they're unlikely to find a cadaver with the right kind of pathologies to train on."

They could also practice surgically on a bioprinted CAD model before eventually performing the same operation on the patient, he added.

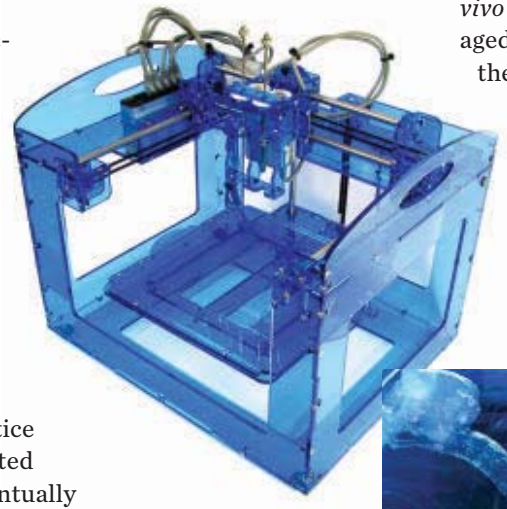
A robot working within an incubator that mimics human body conditions would operate the actual printer, Ozbolat said. The incubator ensures cells remain alive and rapidly regenerate.

"The robot would have two or three arms. One will be printing the microfluidics channel at the same time a second will be printing the cells," he said. The two processes would work in tandem.

"The microfluidics material will provide for some mechanical support and then we definitely need to wait for cell fusion," Ozbolat added. "Then the cells will generate the tissue within the incubator."

But even if organs can be successfully printed within the incubator, researchers still don't know if they can function as stand-ins for their human counterparts, he added.

"If you print a heart, will it have the same physiological properties as a human heart? Will it contract and beat at the same rate?" Ozbolat asked.



Hod Lipson of Cornell University has demonstrated the printing of an artificial ear. The process starts with a scan of a real ear and prints the shape using a silicon gel.

"And then once it's printed, there's the rejection issue."

Without advancement in stem cell technology, organ printing will be difficult. A printed organ is typically composed of many different types of cells, he said. To mitigate rejection, doctors will use the recipient's stem cells to seed the bioprinter. And those cells will need to be differentiated into the individual cell types included within the organ, Ozbolat said.

"It's easy now to differentiate stem cells into heart cells, but not so easy for relatively complex organs like a kidney, which consists of several different tissue types and a structure itself that's very complex," he said.

made in the body

Technologists are already looking at even more complex bioprinting scenarios, Ozbolat said.

"Right now it's pure science fiction; a 3-D bioprinter *in vivo* that can directly print cells and tissues on the damaged area of the organ isn't feasible until around 2030, if then," he said.

For those not used to throwing around medical terminology, *in vivo* means the printer will be doing its tissue-making work inside the body, while the patient is under anesthesia.

Ozbolat points out that an organ couldn't be wholly printed *in vivo*, as the organ will be connected to other living tissues inside the body. A biologically replicated heart, for example, needs a connection to the aorta and other major blood vessels and those vessels couldn't feasibly be tamped off as the organ was being printed, then surgically connected.

"Once you print an organ, you need to make sure it keeps its mechanical integrity until the cells fuse and regenerate the organ," he added. "You need surgical intervention for this and you can't keep the patient under for a couple of days, so it's science fiction."

But a bioprinter could work *in vivo* to replace a portion of a damaged organ, he said. The bioprinter would essentially act as a robotic printer in this instance, working inside the body just as robotic surgery does today, he said.

Chris Barnatt, creator of the Web site ExplainingTheFuture.com, posits that bioprinters of the future could have place in cosmetic applications. Face printers, for example, could evaporate existing flesh and simultaneously replace it with new cells to exact patient specification, Barnatt wrote on his Web page devoted to bioprinting.

Speculation aside, the era of the printed organ is upon and scientists are teaming to inch toward the finish line. ■