A person with a failing vital organ usually has only one treatment option: get a new one. But right now, that often means joining a long waiting list and hoping for the best. Moving up that list means someone ahead of you gets taken off it – either through their death, donation from a living donor, or the death of a healthy person who donates to them the needed organ.

In the US, an average of 79 people receive transplants every day, and 18 die as a result of a shortage of appropriate organs. Even those who make it to the top of the list are not guaranteed a perfect match; following transplantation, a person’s immune system may reject the very thing that can keep their body alive.

However, a few decades from now, getting a new organ may be a much less risky endeavour. Future generations may look back on the complications of today as long-gone artefacts that vanished somewhere in the middle of the 21st century. That is because, thanks to the marriage of biology and physics in the field of tissue engineering, patients in need of a transplant may soon not need to rely on inheriting viable organs from recently deceased donors. They may just press “print”.

Patients requiring an organ transplant may one day no longer have to wait for a matching donor. As Stephen Ornes explains, researchers are making progress towards creating human organs with techniques such as 3D printing, using the patient’s own cells for ink

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Just press print

Wake Forest Baptist Medical Center
Scaffolding and beyond

The pursuit of a more perfect printed organ has inspired researchers to come up with some novel ideas. Three fundamental ingredients are needed for the organ, says Jennifer Lewis, an engineer at Harvard University in Massachusetts who is revolutionizing the field of biological “inks” for these printers. First, the printed organ needs structure, just as in a natural organ where the extracellular matrix — a mixture of proteins and molecules — holds the small and deformable cells together in one place. Second, the printed organ must be populated with the many types of living cell that communicate with each other and keep the organ running. Third, a printed organ needs a vascular network of blood vessels that deliver nutrients to the cells. “If you don’t have blood flowing to deliver oxygen and nutrients, and ways to carry away waste, the cells will die,” explains Lewis.

An early milestone in organ generation came in 1999 when physician Anthony Atala, from Wake Forest Baptist Medical Center’s Institute for Regenerative Medicine in North Carolina, began building new bladders for young patients suffering from spina bifida. Babies born with the most common type of this congenital disorder often have severe nerve damage, as the vertebrae fail to fuse completely and leave the spinal cord partially exposed. The nerve damage can cause bladders to work improperly in these patients, who may undergo drug treatments or surgeries to repair or replace the damaged organ. In the past, surgeons have managed to fashion new bladders from synthetic materials, and reconstructed new ones from excised segments of the bowel, but both approaches came with the risk of serious complications. Atala wanted to offer these patients another option: a new bladder grown from their own cells.

Atala’s approach was to first grow a colony of cells from a stamp-sized biopsy of the bladder. He then “seeded” these newly grown cells on a scaffold in the shape of the patient’s own bladder, which was made of biodegradable materials and would dissolve in the body over time. The cells multiplied and soon covered the scaffold, and Atala implanted the newly grown organ. Blood vessels began to grow, delivering nutrients to the new organ, and seven years later, in 2006, Atala reported that all of his patients – the first recipients of lab-grown organs – remained healthy (Lancet 367 1241).

Using a scaffold to grow cells may also work for tracheas: in 2006 Martin Birchall, an academic otolaryngologist specializing in disorders of voice, swallowing and breathing from University College London, used a donor’s trachea as a scaffold on which to build a new one. He did this by rinsing the donor’s cells from the organ, leaving only the collagen structure, which he then repopulated with cells from a patient who needed a new trachea. This process is called decellularization and recellularization. Ciaran Finn-Lynch, a boy from Northern Ireland who was 11 when he became the first patient with a trachea grown this way, is reportedly doing well three years on. But that does not mean the procedure is a bona fide success.

The process of decellularization and recellulariza-
tion is still in its early days, says Jayasinghe, noting that residual DNA from the donor may still remain in the scaffold and contaminate the patient’s cells. Another drawback is that – as with transplantation – a patient must wait until a donor dies. An artificial trachea built from a synthetic scaffold and seeded with the patient’s cells would do away with these concerns, and in April a two-year-old girl in Illinois became the youngest recipient of such a trachea. Sadly, the girl died in July – not because the trachea failed, but from complications of surgery.

While bladders and tracheas are fairly simple organs, when it comes to more complex organs such as the heart or pancreas, which have a greater variety of cell types, things start to get complicated. “When we look at internal organs, complex in functionality and geometry, scaffolding doesn’t work,” says Ibrahim Ozbolat, a mechanical engineer at the University of Iowa. “We can manufacture some scaffold, then we can seed the cells, but when we try to make something [more complex] the cells inside the scaffold don’t receive enough nutrients and oxygen.”

That is why researchers like Ozbolat want to print an organ from the bottom up, layer by layer, using a process called additive manufacturing. This is the concept behind most commercially available 3D printers, which employ nozzles with varying degrees of freedom to build each layer. A bioprinter would use a variety of “inks”, each including the different materials needed to build an organ – or a single liquid containing all the necessary ingredients.

**Inkjets and lasers**

A popular approach in additive biomanufacturing may look familiar: many organ printers under construction have their roots in inkjet printers, which began showing up in offices and next to personal computers in the 1980s. This technology uses narrow needles to deposit droplets of ink – about 50 µm across – on a piece of paper. Inkjet printers allow for precise control over the locations of the droplets and so give the user great flexibility.

A short leap in imagination – if not in practice – is to go from desktop inkjet printers to machines that print living cells. Put simplistically, one replaces the ink in the cartridges with cells. That leap became reality in 2003 when bioengineer Thomas Boland led a study showing that inkjet-printed cells were viable (2005 Biomaterials 26 93).

To print a heart, for example, one ink cartridge might contain the epithelial cells to line inner cavities; another cartridge may contain myocytes, the cells that form cardiac muscle tissue. Other cartridges may contain a material used to build the extracellular matrix, such as a hydrogel. Instead of printing a 2D image on a piece of paper, the printer would build a new heart slice by slice, placing each type of cell exactly where it needs to go as instructed by a 3D computer model.

Inkjet printing offers a user the ability to control the volume of the drops, the concentration of cells in the “ink”, the size of the nozzle and the size of printed tissue, notes Ozbolat in an article published in March (IEEE Trans. Biomed. Eng. 60 691).

One of the current obstacles to using inkjet printing, says Jayasinghe, is ensuring that cells survive the process. To get ink to go through the needle, many inkjet printers use a heating element to form a bubble in the ink, which exerts pressure and sends a droplet out through the needle bore. However, an inkjet needle that measures tens of microns in diameter is the same order of magnitude as the cells being printed, and in a 2012 review article Jayasinghe noted that living cells are often sheared by the needle bore as they pass through (Adv. Healthcare Mat. 1 27). Some researchers have reported in the literature that cells are not damaged by the process, but Jayasinghe remains sceptical of the methods used to measure cell viability, and hopes to see studies of the biological effects on the cells at the molecular level.

Lasers offer the potential to build yet another type of bioprinter that comes with unrivalled precision. In a laser direct-write, a laser pulse guides an individual cell from a source to a substrate. Laser-guided direct-writing systems can be used to print cell patterns with a high resolution, says Ozbolat, if the printing speed, laser energy and pulse frequency are optimized. In 2005 a team of biomedical engineers from the University of Minnesota used this approach to show how it can be used to print not only the organ cells themselves, but also a hydrogel scaffold – and perhaps even the blood vessels needed to keep the cells alive.

Given their high precision, lasers may offer the most benefit for printing the smallest structures in an organ, says Ozbolat. But the technology has a number of limitations. The cells may be damaged by heat from the laser or – as may be the case for inkjet printers – deformed by the process. In addition, laser light itself may damage the cells or affect the ability of the cells to crosslink in the final organ.

**Obstacles to printed organs**

Regardless of the method, one of the biggest barriers to printing usable organs right now is keeping the
Spun out The glowing dots are living cells within fibres of a scaffold created through cell electrospinning by Suwan Jayasinghe and colleagues at University College London. This method could be used to create new blood vessels for an organ.

cells alive after they have been printed. That means imparting blood vessels, big and small, into the printing process.

Ozbolat, in his lab in Iowa, thinks one way to tackle that problem is to build a printer with multiple arms. He says that current bioprinting technology takes so much time that cells may not survive the hours they spend in the precursor solution. Instead of using one nozzle depositing one cell at a time, he says the production time can be reduced if the printer can tackle multiple chores at once. “The main advantage of a multi-arm printer is the reduced time of manufacturing,” he says. “Of course, it also gives us the freedom to do more things such as print multiple cellular structures at a time.”

Currently, he and his collaborators are designing a new type of organ that, like the pancreas, can regulate insulin in the blood. To print the pancreatic organ, as he calls it, one arm is in charge of the vascular system and the other is in charge of the organ itself. This reduces the manufacturing time, and ensures the cells receive essential nutrients from the printed blood vessels. When it is completed, such an organ could be implanted anywhere in the body connected to the bloodstream.

Ozbolat can envision a bioprinter of the future that functions like a robot on a car assembly line: multiple arms, each with multiple degrees of freedom, will each print a certain type of cell or part of the organ – opening up the possibility of printing ever more complex organs.

Harvard’s Lewis and her collaborators are, meanwhile, tackling the problems of vasculature. Complex organs require blood vessels of various sizes and, accordingly, her efforts focus on coming up with ways to print these. Lewis is investigating new ways to print microvasculature networks – similar to what might be needed in a complex organ – in a hydrogel scaffolding that could be used to support an organ.

Her method involves using so-called “fugitive” inks, which are materials that can be used to form a mould, then dissolve once the structure is in place.

Christopher Chen, a bioengineer at the University of Pennsylvania, is working on a similar approach. As described in a paper published in 2012, he and his team created small cubes of liver tissue that were infused with their own network of blood vessels (Nature Mat. 11 768). The team first 3D-printed what would become the blood-vessel network using a sugar solution, surrounded the hardened sugar with cells and extracellular matrix, then dissolved the sugar, leaving an empty network that was perfused with blood.

Biological physicist Gabor Forgacs, who is at the University of Missouri, Columbia, co-founded the company Organovo in 2007, which sells bioprinters that use additive manufacturing to build tissues and blood vessels. These printers stack 2D layers of cell packets on top of each other. Once the printing is complete, the cells in different packets flow together and start communicating, and blend into a single organ. This approach takes advantage of the ability of cells to self-assemble into functional tissue.

At his lab in London, Jayasinghe is taking a dramatically different approach. He thinks the way to tackle all three necessary ingredients – the structure, the cells and the blood vessels – is to mix them together in a solution and use the mixture to print the organ directly. It is a simpler approach that requires only a single jet.

“We want to be able to deliver heart cells with pinpoint accuracy – not only heart cells, but all the cells in the human body,” he says. “It’s like taking a tissue, melting it down and then spraying it. It has all the constituents in native tissue and we package it as a liquid, then form it and make whatever we need.”

His approach, called bio-electrospaying, involves using an electric field formed between two electrodes: the charged needle of the sprayer and the ground electrode. Just as a high-voltage stun gun usually does not kill a person (because of the low current), the 30 kV potential of the electric field does not harm the cells. The electric field draws out the cellular mixture either as a diffuse spray to create sheets (electrospraying), or as a tiny fibre that can be spun to create blood vessels (electrospinning).

“We can spray or spin these cells to make beads, droplets, fibres or scaffolds,” says Jayasinghe, whose team has already used this approach to spray heart cells onto a damaged organ. They have shown that the heart can beat after being sprayed, and in animal models the spray has been shown to be safe and effective on animals with damaged hearts. Later this year, they hope to start recruiting for a phase I clinical trial, with the goal of soon being able to help humans mend a broken heart.

The future of bioprinting

which God forges Eve from one of Adam’s ribs – offers the first written reference to the idea. Indeed, in 1932 the future prime minister Winston Churchill wrote an article in Popular Mechanics in which he predicted that 50 years in the future, “we shall escape the absurdity of growing a whole chicken in order to eat the breast or wing, by growing these parts separately under a suitable medium”. (Churchill was referring to synthetically grown edible meat, another area where tissue engineering is taking off. In Missouri, Forgacs and his son Andras Forgacs are also working on tissue engineering at this front.)

The field has come a long way since then, with recent advances due in large part to a mechanical understanding of cells, tissues, and organs. But the future success of the field will heavily depend on what physicists and biologists learn about stem cells. These cells are undifferentiated, which means they have not yet developed the characteristics of a particular type of cell. Vital to the functioning of the body, stem cells can self-renew, which makes them important to the body’s ability to self-repair. There are many types of stem cell: those specific to a particular type of tissue; those that are found in an embryo and can become any one of a variety of cell types; and induced pluripotent cells, which began as specialized cells but were artificially coaxed – through gene expression – into becoming undifferentiated cells.

These cells, says Ozbolat, will be vital to printed organs. Stem cells are an appealing source for the “ink” of a bioprinter because they self-renew, so a small number of stem cells may be able to multiply and grow into different cells – and may be viewed as an unlimited source. But while the technology is promising, the reality is that researchers need to learn more about the differentiation process. An organ printed with a person’s own stem cells may still be rejected, especially if the stem cells change character during or after the printing process.

But step by step, and cell by cell, researchers are getting closer to the day when an ailing patient can be helped with one word: print.