Replacement Parts

To cope with a growing shortage of hearts, livers, and lungs suitable for transplant, some scientists are genetically engineering pigs, while others are growing organs in the lab.

By Ed Yong | August 1, 2012

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For Joseph Vacanti, the quest to build new organs began after watching the death of yet another child. In 1983, the young surgeon was put in charge of a liver transplantation program at Boston Children's Hospital in Massachusetts. His first operation was a success, but other patients died without ever being touched by a scalpel. "In the mid-80s, kids who were waiting for organs had to wait for a child of the same size to die," says Vacanti. "Many patients became sicker and sicker before my eyes, and I couldn't provide them with what they needed. We had the team, the expertise, and the intensive care units. We knew how to do it. But we had to wait."

On the other side of the Atlantic, David Cooper was having the same problem. Having taken part in the first successful series of heart transplants in the United Kingdom, he had moved to South Africa to run a transplantation program at the University of Cape Town Medical School. At the time, people had a 50/50 chance of surviving such a procedure, but Cooper recalls that most of his patients were killed by a lengthy wait. "We just didn't have enough donors," he says.

Today, the organ shortage is an even bigger problem than it was in the 1980s. In the United States alone, more than 114,000 people are on transplant lists, waiting for an act of tragedy or charity. Meanwhile, just 14,000 deceased and living donors give up organs for transplants each year. The supply has stagnated despite well-funded attempts to encourage donations, and demand is growing, especially as the organs of a longer-lived population wear out.

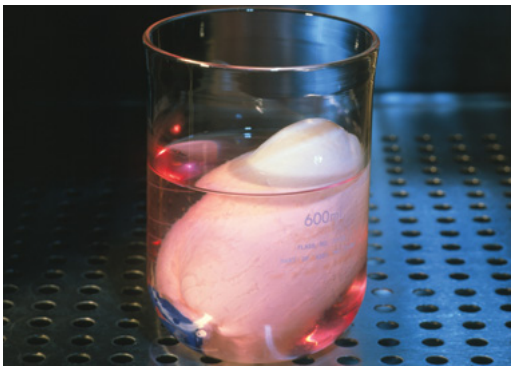
Faced with this common problem, Vacanti andCooper have championed very different solutions.Cooper thinks that the best hope of providingmore organs lies in xenotransplantation—the actof replacing a human organ with an animal one.From his time in Cape Town to his currentposition at the University of Pittsburgh, he hasbeen trying to solve the many problems thatoccur when pig organs enter human bodies, fromimmune rejection to blood clots. Vacanti, now atMassachusetts General Hospital, has instead been developing technology to create genetically tailored organs out of a patient's own cells, abolishing compatibility issues. "I said to myself: why can't we just make an organ?" he recalls.

In the race to solve the organ shortage, xenotransplantation is like the slow and steady tortoise, still taking small steps after a long run-up, while organ engineering is more like a sprinting hare, racing towards a still-distant finish line. Most of those betting on the race are backing the hare. Industry support has dried up for xenotransplantation after years of slow progress, leaving public funders to pick up the expensive tab. Stem cells, meanwhile, continue to draw attention and investment. But both fields have made important advances in recent years, and the likely winner of their race—or whether it will result in a draw—is far from clear.

Pigs might fly

Pigs could provide all the organs that we need. They are the right size, and we already have the infrastructure to breed them in large numbers. For decades, people have been fitted with heart valves from pigs, and diabetics injected themselves with pig insulin before we learned how to synthesize the human version of the hormone. Whole-organ transplants, however, are another matter.

The human immune system does not take kindly to the presence of a pig organ. A ready-made armada of antibodies recognizes a sugar molecule called alpha-1,3-galactose (a-gal), which coats the surface of pig blood vessels but is absent from human tissues. The antibodies activate a squad of proteins that make up the complement system, which punches holes in the membranes of the foreign cells on contact. "When I started in the field around 15 years ago, if you put a pig organ into a primate, it was lost in a matter of minutes," says David Sachs, an immunologist at Massachusetts General Hospital.



ARTIFICIAL BLADDER: Simple structures like bladders are already being grown over biodegradable polymer scaffolds. The one pictured here was made by a Anthony Atala's team at the Wake Forest Institute for Regenerative Medicine in Winston-Salem, North Carolina.

Credit: Photo Researchers, Sam Ogden

Cooper first discovered the a-gal problem in 1992, but it took him until 2003 to fix it. He and others engineered pigs without the a-1,3- galactosyltransferase gene that produces the a-gal residues. In addition, the pigs carry human cell-membrane proteins such as CD55 and CD46 that prevent the host's complement system from assembling and attacking the foreign cells. "It took 10 years for those pigs to become available, but they made a big difference," says Cooper.

The organs from the genetically modified pigs were no longer instantly rejected when tested in nonhuman primates, but subtler problems appeared. "It's like an onion," says Sachs. "You take away a layer and you get another." While the body's first-line

defense against foreign tissues was stymied, over time host surveyor cells that sample proteins on the pig organ would recognize it as foreign and initiate the second line of immune attack—the production of anti-pig antibodies that trigger immune rejection after days or weeks. In addition, researchers discovered incompatibilities between the pig and primate versions of the proteins that control the clotting process. As a result, some blood vessels become clogged with clots, while others bleed uncontrollably.

Researchers in the field are confident that these problems can be overcome by using immunosuppressive drugs, and by further tweaking the genes of the *gal-less* pigs. Cooper, for example, has just bred swine that have the human THBD gene, which prevents clotting. Their hearts have been transplanted into baboons, and the monkeys are "doing particularly well," he says.

Even if the rejection problems are solved, it is unclear whether pig organs will work properly in human hosts, who have longer lives and different physiologies. Cooper says, "No one's had organs functioning long enough to gather much data." The short-term results suggest that hearts and kidneys do their job well enough, but Peter Cowan, an immunologist at St. Vincent's Hospital in Melbourne, Australia, says that liver transplants present a much trickier problem. "The liver makes so many proteins and hormones, and many of them that work in the pig probably won't work in humans," he says. "It's not a long-term proposition."

There are also concerns that pig organs will spread animal diseases to their recipients. But surgeon Jeffrey Platt at the University of Michigan argues that the risks are no greater than for any other form of animal contact, especially since genetically modified pigs are housed in pristine conditions and regularly checked for infections. "Swine flu would be no more or less likely in a society that carried out xenografts," he says. But pig genomes also contain the remnants of viruses that stowed away millions of years ago. These porcine endogenous retroviruses (PERVs) are impossible to eradicate, and the worry is that they might become activated in a new host, as researchers have observed with other endogenous retroviruses. "The virologists I've spoken to don't seem to be that concerned," says Cooper. "When we transplant pig organs to baboons, we haven't detected the transfer of the [PERVs], even when the baboons are immunosuppressed."

Despite these concerns, transplants of cells and tissues from pigs may be on the horizon, including corneal transplants or neuronal transplants for Parkinson's disease. In New Zealand, John Baker from Middlemore Hospital is leading a clinical trial in which 14 diabetic patients have been implanted with pig pancreatic islets. The islets are encased in microcapsules that keep the cells of the host immune system out, but allow passage of nutrients and insulin. "That might give the field a bit of a boost. We need some positive results," says Cowan.

Whole organ transplants are further in the future. Since the 1900s, a few surgeons have tried transplanting kidneys, livers, and hearts from pigs, baboons, and chimps into desperate patients with no other options. None of these operations were successful. Today, pig lungs and livers are still lucky to last for a week in a baboon, although kidneys have survived for 3 months and non-life-supporting hearts (beating in the recipient's abdomen to test for rejection) for eight. "We still haven't had any long-term survivors," says Sachs. "We need to get to years before we have any clinical applicability, because nobody wants a heart for just a few months."

However, short-term transplants may provide opportunities for xenotransplantation to prove its worth. If a patient is dying of liver failure, a pig organ could buy her precious time while she waits for a human donor. Because the liver is only in place a short while, the problem of the organ producing pig enzymes rather than human ones isn't likely to be an issue. "Even if it just functions for a few weeks, it could be a bridge to a human transplant and give us valuable experience about whether this works as well in a patient as in a baboon," says Cooper.

Step by slow step, xenotransplantation is inching towards clinical use. But for some, progress has been frustratingly slow. "If you told me 10 years ago that we wouldn't be doing it by now, I'd have been surprised," says Sachs. "I won't speculate anymore." Modifying pigs is laborious and costly work. "You've got to introduce the genes, wait till animals are born, and breed them," says Platt. "It's extraordinarily expensive to do the kinds of research it would take to make incremental advances." Every new altered gene ramps up the technical challenges, costs, and regulatory hurdles—obstacles that Platt says have stifled the field and put off interested scientists.

The ideal scaffold

While some scientists struggle to get human bodies to accept pig organs, others are attempting the more ambitious feat of engineering bespoke human organs from scratch. Such organs, grown from a patient's own cells, should avoid the problems of immune rejection that plague the field of xenotransplantation. "Cartilage, skin, and bone are already on the market. Blood vessels are in clinical trials. The progress has been really gratifying," says Laura Niklason of Yale University.

These tissues—either flat planes or hollowtubes—are relatively simple to produce, andconsist of a small number of cell types. Solidorgans, such as the lungs, heart, liver, andkidneys, pose a greater challenge. They arebigger, they contain dozens of cell types, andthey have a complex architecture and anextensive network of the most essentialcomponent: blood vessels. "Every cell needs toeat and breathe, and each one needs to be closeto a source of nutrition and oxygen," saysVacanti. Still, he is optimistic that it should be possible to engineer even these complex organs. "People differ about whether it'll be achieved in 5 or 100 years, but most people in the field believe that it's a realistic goal," he says.

In 2008, Harald Ott of Massachusetts General Hospital and Doris Taylor of the University of Minnesota dramatically demonstrated the potential of organ engineering by growing a beating heart in the laboratory. As physician-scientists, the two often see patients in dire need of transplantation. They started by using detergents to strip the cells from the hearts of dead rats, leaving behind the extracellular matrix—a white, ghostly, heart-shaped frame of connective proteins like collagen and laminin. Ott and Taylor used this matrix as a scaffold. They seeded it with cells from newborn rats and incubated it in a bioreactor—a vat that provides cells with the right nutrients, and simulates blood flow. After 4 days, the muscles of the newly formed heart began contracting. After 8 days, it started to beat.

This laborious technique, known as whole organ decellularization, is like knocking down a house's walls to reveal its frame, only to replaster it again. It works because the frame is perfect—it retains the complicated three-dimensional architecture of the organ, including the branching network of blood vessels that provide the cells with nutrients and oxygen. It also preserves the array of complex sugars and growth factors that covers the matrix and

provides signposts for growing cells, nudging them into the right shapes and structures. “The matrix really is smart,” says Taylor. “If we put human cells on human heart matrix, they organise in remarkable ways. We can spend the next 20 years trying to understand what’s in a natural matrix and recreate that, or we can take advantage of the fact that nature’s put it together perfectly.”

Ott and Taylor’s groundbreaking feat has since been duplicated for several other organs, including livers, lungs, and kidneys. Rodent versions of all have been grown in labs, and some have been successfully transplanted into animals. Recellularized organs have even found their way into human patients.

Between 2008 and 2011, Paolo Macchiarini from the Karolinska Institute in Sweden fitted nine people with new tracheas, built from their own cells grown on decellularized scaffolds. Most of these operations were successful (although three of the scaffolds partially collapsed for unknown reasons after implantation). Decellularization has one big drawback: it still depends on having an existing organ, either from a donor or an animal. Frustrated by the wait, Macchiarini tried a different approach. In March 2011, he transplanted the first trachea built on an artificial, synthetic polymer scaffold. His patient, an Eritrean man named Andemariam Teklesenbet Beyene, had advanced tracheal cancer and had been given 6 months to live. “He’s now doing well. He’s employed, and his family have come over from Eritrea. He has no need for immunosuppression and doesn’t take any drugs at all,” says Macchiarini. A few months later, he treated a second patient—an American named Christopher Lyles—in the same way, although Lyles later died for reasons unrelated to the transplantation.

Macchiarini now has approval from the US Food and Drug Administration to perform these transplants in the United States on a compassionate basis, for patients who have no other options. “The final organ will never ever be as beautifully perfect as a natural organ,” says Macchiarini, “but the difference is that you don’t need a donation. It can be offered to a patient in need within days or weeks.” By contrast, even if a donor is found, a simple trachea can take a few months to regrow using a decellularized scaffold. Other scientists have enjoyed similar success with other organs. In 1999, Anthony Atala of the Wake Forest Institute for Regenerative Medicine grew bladders using artificial scaffolds, and transplanted them into seven children with spina bifida. By 2006, all the children had gained better urinary control. Atala has just completed Phase II trials of his artificial bladders.

To Vacanti, artificial scaffolds are the future of organ engineering, and the only way in which organs for transplantation could be mass-produced. “You should be able to make them on demand, with low-cost materials and manufacturing technologies,” he says. That is relatively simple for organs like tracheas or bladders, which are just hollow tubes or sacs. Even though it is far more difficult for the lung or liver, which have complicated structures, Vacanti thinks it will be possible to simulate their architecture with computer models, and fabricate them with modern printing technology. (See “3-D Printing,” *The Scientist,* July 2012.) “They obey very ordered rules, so you can reduce it down to a series of algorithms, which can help you design them,” he says. But Taylor says that even if the architecture is correct, the scaffold would still need to contain the right surface molecules to guide the growth of any added cells. “It seems a bit of an overkill when nature has already done the work for us,” she says.

Whether the scaffold is natural or artificial, clinicians need to seed it with patient’s cells. For bladders or tracheas, it is enough to collect these from a small biopsy. That will not work if the organ is diseased, or if it’s a complex structure of multiple tissue types, or, as in the heart, if its cells are naturally reluctant to divide. In such cases, clinicians will need either stem cells, which can divide and differentiate into any cell type, or progenitor cells that are restricted to specific organs. Since 2006, one source of stem cells has been adult tissues, which scientists can now reprogram back into a stem-cell like state using just a handful of genes. These induced pluripotent stem cells or iPSCs, could then be coaxed to develop into a tissue of choice. “For me, the cells have always been the most difficult part,” says Vacanti, “and I’d say the iPSCs are the ideal solution.”

The finish line

Xenotransplantation and organ engineering offer different solutions to the organ crisis, but they share similarities. After decades of research, both fields are in the middle of important clinical trials involving simpler tissues and organs, but complex ones like lungs or liver remain a distant goal. “I think we’re still 2 decades away from something that’s clinically realizable,” says Niklason.

Xenotransplants will always have to deal with an immune clash of some degree, so growing an organ that is perfectly matched to a patient would be preferable. The question is whether tissue-engineering technologies will reach that point before genetic engineering enables the first transgenic pig hearts or kidneys to be successfully installed in patients. Sachs says, "I consider xenotransplantation still the nearest-term, best hope for solving the organ shortage, but in the long run, I think tissue engineering will replace it."

There is also the matter of scale. Platt thinks that organ engineering is too costly to meet the needs of everyone waiting for a transplant. "You'd have to turn over the entire GDP of a country to accomplish that," he says. On the other hand, "I could get a pig for a couple of hundred dollars." But Macchiarini argues that organ engineering is in its infancy, and every advance improves efficiency and lowers cost. "What we did in 2008 in 6 months, we can now do in a few weeks," he says. "We do care about getting this to every patient." Vacanti adds that mass-producing artificial scaffolds will make organ engineering even more cost-effective. "When you scale them up, the bulk materials and manufacturing tech are extremely cheap," he says. "I think it's going to be cheaper than growing lots of pigs."

For Taylor, it does not matter which technique comes out on top. The goal is, and has always been, to help people. "I'm not afraid to build hybrid approaches and work together to solve the organ shortage, rather than in competition," she says. "I don't want to look in the face of a mother or father and say, 'Sorry, we just don't have an organ.'"

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