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NEW LIFE FOR PIG ORGANS

Gene-editing technologies have breathed life into the languishing field of xenotransplantation.

BY SARA REARDON

Pale on its bed of crushed ice, the lung looks like offal from a butcher's counter. Just six hours ago, surgeons at the University of Maryland's medical school in Baltimore removed it from a hefty adult pig and, with any luck, it will soon be coaxed back to life, turning a rich red and resuming its work in the chest of a six-year-old baboon.

An assistant brings the lung to Lars Burdorf and his fellow surgeons, who currently have their hands in the baboon's splayed chest. The team then begins the painstaking process of connecting the organ to the baboon's windpipe and stitching together the appropriate arteries and blood vessels. But this 5-hour, US$50,000 operation is just one data point in a much longer experiment — one that involves dozens of labs and decades of immunological research and genetic engineering to produce a steady and safe source of organs for human transplantation. If the baboon's immune system tolerates this replacement lung, it will be a sign that the team is on the right track.

Robin Pierson heads the Maryland lab, which has performed about 50 pig-to-primate transplants like this one to test different combinations of genetic modifications in the pig and immune-suppressing drugs in the primate. Even so, the team has not had a primate survive for longer than a few days. The complexities of the immune system and the possibility of infection by pig viruses are formidable and drove large companies out of the field in the early 2000s.

That trend may now be reversing, thanks to improved immunosuppressant drugs and advances in genome-editing technologies such as CRISPR/Cas9. These techniques allow scientists to edit pig genes, which could cause rejection or infection, much more quickly and accurately than has been possible in the past. In October, eGenesis, a life-sciences company in Boston, Massachusetts, announced that it had edited the pig genome in 62 places at once.

Some researchers now expect to see human trials with solid organs such as kidneys from genetically modified pigs within the next few years (see 'Choice cuts'). United Therapeutics,

Surgeons prepare a genetically modified pig lung for transplantation experiments.

a biotechnology company in Silver Spring, Maryland, has spent $100 million in the past year to speed up the process of making transgenic pigs for lung transplants — the first major industry investment in more than a decade. It says that it wants pig lungs in clinical trials by 2020. But others think that the timeline is unrealistic, not least because regulators are uneasy about safety and the risk of pig organs transmitting diseases to immunosuppressed humans.

"I think we're getting closer, in terms of science," says transplant surgeon Jeremy Chapman of the University of Sydney's Westmead Hospital in Australia. "But I'm not yet convinced we've surpassed all the critical issues that are ahead of us. Xenotransplantation has had a long enduring reality that every time we knock down a barrier, there's another one just a few steps on."

LONG HISTORY

Surgeons have been attempting to put baboon and chimpanzee kidneys into humans since at least the 1960s. They had little success — patients died within a few months, usually because the immune system attacked and rejected the organ. But the idea of xenotransplantation persisted. It could, proponents say, help to save the lives of the tens of thousands of people around the world who die each year while waiting for a suitable human donor. And having a steady supply of farm-grown organs would allow doctors to place recipients on immunosuppressant drugs days ahead of surgery, which should improve survival rates.

When details about why non-human organs are rejected began to emerge in the 1990s, the transplantation field was ready to listen. In 1993, surgeon David Cooper of the University of Pittsburgh in Pennsylvania and his colleagues discovered that most of the human immune reaction was directed at a single pig antigen: a sugar molecule called α-1,3-galactose, or α-gal, on cell surfaces that can cause organ rejection within minutes[1]. An enzyme called α-1,3-galactosyltransferase is necessary for producing this sugar, and knocking out the gene that produces the enzyme should temper the reaction.

This discovery and other advances in transplantation medicine made the problem seem more tractable to big pharmaceutical companies. In 1996, Novartis in Basel, Switzerland, began to invest heavily in xenotransplantation research, says Geoffrey MacKay, who was the firm's business director for transplants and immunology at the time and oversaw the xenotransplantation effort. "They wanted to not only put a dent into the organ shortage but really solve it via transgenic pigs." MacKay is currently interim chief executive at eGenesis.

Novartis initially planned to spend more than $1 billion on xenotransplantation, including both scientific research and planning the infrastructure that would be needed to grow pigs in germ-free facilities around the world. Other companies put some skin in the game, including Boston-based Genzyme and PPL Therapeutics, the British company that collaborated in the creation of Dolly, the first cloned sheep. Regulators such as the US Food and Drug Administration (FDA) began to draw up the guidance and standards that companies would need to meet before the technology could be moved into people.

But the immune system turned out to be much more complex than anticipated, and baboons that received pig organs never survived longer than a few weeks, even when the researchers were able to suppress α-gal production with drugs. A second major concern, especially to regulators, was the risk of infection. Even if pigs could be kept entirely sterile, the pig genome is sprinkled with dozens of dormant porcine endogenous retroviruses (PERVs), and studies conflicted as to whether these could become active in humans.

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The challenges proved too daunting, and in the early 2000s Novartis killed its xenotransplantation programme, reshuffling or laying off its researchers. Other companies followed suit. It became, Pierson says, "the third rail of biotech to discuss xenotransplantation as a business plan".

For the next ten years, the business side of the field went dark, at least as far as solid-organ transplants were concerned. Meanwhile, a few research teams and start-up companies began pursuing pig tissue transplants: a much simpler goal than using solid organs because the immune response is not as severe. In April, Chinese regulators approved the use of pig corneas from which all the cells have been removed[2]. Also on the near horizon are pig insulin-producing islet cells that might be transplanted into people with diabetes.

The first commercially available islets are likely to come from technology designed by Living Cell Technologies (LCT), a biotech company based in Auckland, New Zealand, that has developed a process to encapsulate pig islet cells in a gelatinous 'dewdrop' that protects them from the human immune system. The product, called DIABECELL, is currently in late-stage clinical trials in several countries. Patients implanted with the cells have survived more than nine years without evidence of immune rejection or infection[3].

"I think people are coming around to look at xenotransplantation in a more-favourable light knowing that we have strong safety data," says LCT research lead Jackie Lee. Diatranz Otsuka Limited, in Auckland, is now running the programme.

Solid organs still pose a challenge. The handful of researchers who have continued to work with them have solved some of the problems that vexed Novartis, such as identifying other key pig antigens and the correct combinations of immunosuppressant drugs. But different organs have different problems: kidneys may be safer than hearts, for instance. Lungs, as Pierson's team has discovered, are extremely difficult to transplant, because they have extensive networks of blood vessels, which provides more opportunities for primate blood to meet pig proteins and to coagulate. Pierson's current trials use lungs from an α-gal-knockout pig that includes five human genes. The baboon is treated with a combination of four immunosuppressant drugs.

Most US researchers, including Pierson and Cooper, have relied on pigs made by a regenerative-medicine company called Revivicor in Blacksburg, Virginia, that spun-out from PPL Therapeutics. In 2003, Revivicor co-founder David Ayares and his colleagues created the first cloned pig genetically modified to delete α-gal[4]. The company has since been experimenting with altering other protein antigens that trigger the immune system or cause human blood to coagulate.

These modifications have greatly lengthened the time that an organ can survive in a baboon. In one trial, surgeon Muhammad Mohiuddin at the National Heart, Lung, and Blood Institute in Bethesda, Maryland, and his colleagues took the heart from an α-gal-free pig that had two human genes that protect from coagulation and sewed it into the abdomen of a baboon[5]. The organ did not replace the baboon's heart, but the animal lived with the implant for two and a half years.

Mohiuddin says that the group is now attempting a 'life-supporting' transplant by replacing the baboon's heart with a pig heart. The longest life-supporting transplant was published in June[6], when Cooper's group announced that a kidney transplant from a Revivicor pig with six modified genes supported a baboon for 136 days.

GENERATION GAME

But the process is slow, Cooper says. It generally takes several generations of breeding to knock out both copies of just one given gene in a pig. Deleting multiple genes or swapping them for their human counterparts takes many more generations, because every litter contains pigs with different combinations of the modified genes.

That is why so many are excited about precise genome-editing tools such as

CRISPR/Cas9, which can precisely cut both copies of a gene — or genes — straight from a pig embryo in one go. "Our first [α-] gal-knockout pig took three full years," says Joseph Tector, a transplant surgeon at Indiana University in Indianapolis. "Now we can make a new pig from scratch in 150 days." His group recently used CRISPR to knock out two pig genes simultaneously[7]. The researchers are now beginning to transplant CRISPR-modified pig organs into macaques, one of which has survived for more than three months.

Eventually, gene editing might even eliminate the need for immunosuppression, says Bernhard Hering, a transplant surgeon at the University of Minnesota in Minneapolis. His group is using CRISPR to create pig islets that could be transplanted without the need for drugs. Partly because of LCT's success with encapsulated islets, many are hopeful that islet cells will be the first genetically modified tissue to make it into clinical trials, paving the regulatory pathway for the more-difficult organs. A non-profit organization has built a germ-free facility in which to raise Hering's pigs.

TECHNOLOGY REVIVAL

The gene-editing advances have brought new investment into the field. In 2011, United Therapeutics acquired Revivicor for about $8 million and announced an ambitious plan to start clinical trials of gene-edited pig lungs by the end of the decade. The company's co-chief executive, Martine Rothblatt, secured land in North Carolina for a farm that could produce 1,000 pig organs per year and says she expects to break ground by 2017. The facility's elaborate plans include solar panels and helicopter landing pads to help speed fresh organs to those in need.

In 2014, United Therapeutics formed a $50-million partnership with the biotech firm Synthetic Genomics (SGI) in La Jolla, California, founded by genome-sequencing pioneer Craig Venter. Rather than simply knocking out antigens, SGI is also engineering tissues that sidestep rejection in a different way — such as pig cells that produce surface receptors that act as 'molecular sponges' and sop up human immune signalling factors that would otherwise attack the organ. CRISPR and other methods also allow the researchers to make tweaks such as lowering a gene's expression rather than deleting it completely, says Sean Stevens, head of SGI's mammalian synthetic-biology group. In September, United Therapeutics committed another $50 million.

Peter Cowan, an immunologist at St Vincent's Hospital in Melbourne, Australia, is taking a different approach. His group has made pigs that generate antibodies against human immune cells. In their design, the antibodies would be made only by transplanted liver cells, ensuring that the immune system is suppressed just around the organ.

eGenesis was founded in April by bioengineer Luhan Yang and geneticist George Church of the Wyss Institute and Harvard University in Cambridge, Massachusetts. MacKay says that the firm plans to begin transplanting organs into primates next year. To that end, Church says that the company has made embryos that have more than 20 genetic alterations to cell-surface antigens and other factors and is ready to implant the embryos into female pigs. One of its first publications used CRISPR to inactivate 62 occurrences of PERV genes in pig kidney cells[8]. The researchers have since transferred the cells' nuclei into pig embryos.

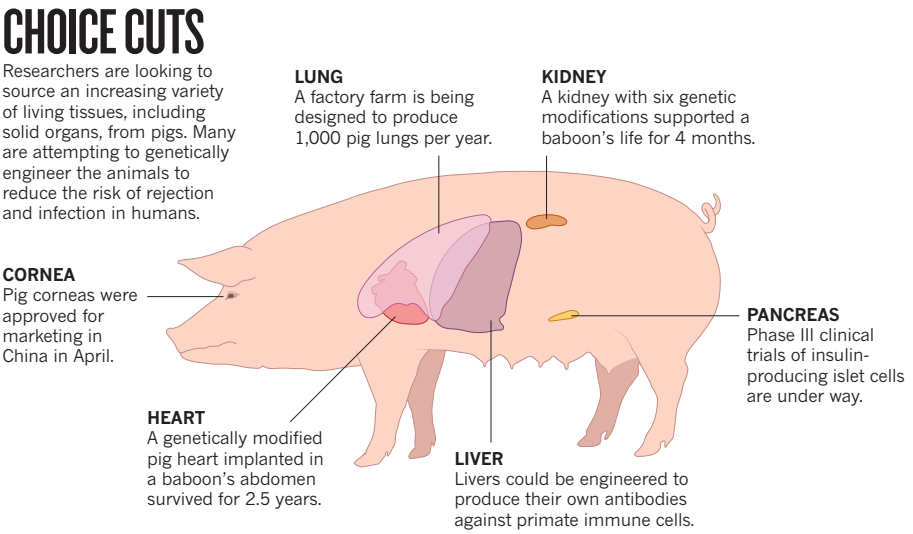
Incidentally, few researchers in the field see the PERV problem as a major safety concern. The virus replicates poorly in human tissues and the risk of spreading it is virtually non-existent, says Jay Fishman, an infectious-disease specialist at Massachusetts General Hospital in Boston. He says that researchers have tracked dozens of people who received unregulated porcine skin grafts, and none seems to have developed disease.

But dealing with PERVs may be a regulatory necessity. The FDA said in an e-mail to *Nature* that it is still concerned about the possibility of disease caused by PERVs. There are other pathogens to worry about, too. Most major epidemics start with an animal pathogen that jumps to humans, warns Peter Collignon, an infectious-disease scientist at the Australian National University in Canberra. "If you want to do the perfect experiment for finding new novel viruses and letting them multiply, this is it."

Unless xenotransplants are proved to be extremely safe, the FDA suggests that they be limited to people with life-threatening conditions who have no other options. It will be even harder to get organs from genetically modified pigs to market, the agency says, because regulators must approve both the genetic construct used to make the animal and the organ itself.

Even if safety can be assured, questions remain about whether pig organs would work correctly in their new home, Chapman says. It is unclear whether a pig kidney would, for instance, respond to the human hormones that regulate urination, or whether proteins produced by a pig liver would interact correctly with human systems. And because pigs live for only about ten years, their organs might not survive a human lifetime. Even using a xenotransplant as a 'bridge' until a suitable human donor is found will be difficult. After a heart transplant, for instance, fibrous tissue forms around the new organ, making second transplants very difficult, Chapman says.

Given the long list of known hurdles, the surprise setbacks that researchers encounter along the way can be particularly disheartening. About half an hour after its surgery at the University of Maryland, the baboon with a pig's lung woke up in a cage wearing a small vest that monitored its vital signs. The lung functioned well overnight and was even able to provide enough oxygen to the animal when blood flow to its other lung was temporarily blocked. But the next day, the animal became ill and had to be killed. That was unexpected, Pierson says, because the pig's multiple genetic modifications seem to have worked well with the baboon's immune system. A post-mortem examination revealed that fluid had accumulated in the lung and the organ had developed blood clots. Like so many other aspects of xenotransplantation, Pierson says, "this is a problem that we are still learning about". ■



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