Xeno's paradox

Why pig cells are better for tissue transplants than human cells

There has always been a serious shortage of transplant organs for use in patients with critical organ failure as the result of accident or disease. As such, the idea of transplanting animal organs into humans is almost a century old, having first been advanced in 1906 by the French surgeon Mathieu Jaboulay (1860–1913), who implanted a pig's kidney into one woman and a goat's liver into another, neither of whom survived. However, although xenotransplantation has promised to improve the provision of organs and tissues for sufferers from various otherwise terminal conditions, it has yet to find its way into clinical use. In fact, the 1990s seemed to sound a death knell for xenotransplantation, as growing fears that xenografts could be a source of potentially lethal zoonoses resulted in the mass withdrawal of the pharmaceutical industry from the research; a blow that came on top of the already seemingly intractable problem of immunological rejection of organ grafts.

Against all odds, however, xenotransplantation research lived on in several countries, ultimately allowing the development of new techniques to address many of the problems, as well as fostering the conviction that the zoonosis threat might have been overstated. In any case, the chronic shortage of human organ donors continues, which means that, on average, about 60% of patients waiting for a transplant die before an organ becomes available. More recently, it has also become clear that it is going to take some time before artificial organs can be developed for use in the clinic. "Animal organs are, in my view, much further along than human-made equivalents in being able to replace end-stage failed organs," commented Megan Sykes, Associate Director of the Transplantation Biology Research Centre at Massachusetts General Hospital (Boston, MA, USA). "The total artificial heart is farthest along, but is still far from being appropriate for widespread use and quite possibly never will be. It remains to be seen whether we will ever develop clinically applicable ways of generating organs from stem cells."

Yet, as Sykes pointed out, any transplantation successes with stem cells are likely to be at the tissue level rather than the replacement of whole organs, though this is also the case for xenotransplantation. However, xenotransplantation is already well ahead in this regard, particularly in the use of pig pancreatic islet cells to treat people with type I diabetes, who cannot produce insulin as their own Islets of Langerhans have been destroyed by autoimmune processes.

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One of the leading proponents of pig islet transplantation is David Cooper, Professor of Surgery at the University of Pittsburgh School of Medicine (PA, USA). Originally from London, UK, Cooper was part of Christiaan Barnard's pioneering heart transplant team in South Africa in the 1960s, when he was a young cardiac surgeon. He eventually gave up heart surgery in the mid-1990s out of frustration over the continuing shortage of donors, and took up xenotransplantation research in the belief that it would ultimately lead to a sustainable supply of healthy and reliable organs. "I admit I didn't expect it would take so long, but I think we're getting there now," Cooper said. Although he concedes that xeno-heart transplants are still some way off, he is convinced that his research on islet transplantation will soon provide a major breakthrough and will both save and improve the quality of many lives.

Patients with type I diabetes have two options for controlling their blood glucose levels: they can either monitor their blood sugar and inject themselves with insulin—so-called exogenous insulin therapy—or they can be given replacement islet cells. Yet, both approaches have limitations. The Diabetes Control and Complications

Trial, which ran from 1983 to 1993 and was funded by the National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, MD, USA; <http://diabetes.niddk.nih.gov/dm/pubs/control/>), showed that patients using exogenous insulin therapy to maintain their blood glucose levels still suffered an increased risk of hypoglycaemia, or low blood glucose. The alternative—the use of a human pancreas or islet cells in transplantation—requires the use of anti-rejection drugs that have their own consequences, and is severely hampered by the shortage of human organ donors. "There have been about 1,000 transplants worldwide over the last five years, and yet there are 2.5 million type I diabetes sufferers in the US alone," Cooper noted. "The situation is even worse than it sounds because we often need two or three pancreases per recipient."

These figures highlight the need for alternatives, and thus the potential of pig islets with growing evidence that they will work. In fact, pig islets might already have been put into routine transplant use, were it not for pig endogenous retrovirus (PERV). Endogenous retroviruses were first discovered in pig kidney cells in 1971 (Armstrong *et al*, 1971), and are now known to inhabit the genomes of most, if not all, mammalian species. Although they have long lost their virulence in the host through countless mutations, they have the potential to become infectious again through recombination (Löwer, 1999). Therefore, the 1997 discovery that PERV can be transmitted to a human cell *in vitro* (Patience *et al*, 1997) led to a degree of panic in the xenotransplantation community, resulting in a withdrawal from the field by pharmaceutical companies.

Before this, several small clinical trials of pig islet transplants had been undertaken. A Swedish study conducted in the early 1990s was the first to report pig islet transplantation into immunosuppressed patients who had received kidney allotransplants—that is, from humans—but who also suffered from type I diabetes (Groth *et al*, 1994). Yet, according to Cooper, none of these studies cured the diabetes; patients still needed insulin injections.

But, Cooper insists that pig islets and other transplanted xeno-organs might eventually prove to be more successful and reliable than donated human ones. First, the animal donors are bred specifically for the task, which means that they yield healthy organs harvested at the optimum time; by contrast, human organs frequently come from people who are beyond their prime and often suffering from undiagnosed infections. "Increasingly, human organs are causing complications resulting from infections the donor happened to have," Cooper said. The most compelling argument, however, is that human organs—with a few exceptions, such as kidneys donated by relatives—are harvested from brain-dead cadavers. "I did a great deal of work in Cape Town looking at the effect of brain death on organs," Cooper commented. "It has many detrimental effects: first, the blood pressure goes very high, causing structural damage to the heart and kidney; secondly, brain death causes

metabolic changes [as] the body relies on energy reserves in the absence of oxygen. The result is that the organs often do not work very well, or not at all."

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Another point in favour of pig islets is that they might be resistant to the autoimmune attack that destroys human islets and causes type I diabetes in the first place, according to Bernhard Hering, Professor of Surgery at the University of Minnesota (Minneapolis, MN, USA) and Scientific Director of the Diabetes Institute for Immunology and Transplantation at the university. "Experimental studies suggest that porcine islet xenografts may be resistant to destruction by recurrent autoimmunity," he said.

Meanwhile, concern over the zoonosis threat posed by PERV has been declining, according to Henk-Jan Schuurman, CEO of the Spring Point Project; a non-profit organization based in Minneapolis (MN, USA) that relies on charitable donations to rear pig herds for use as islet donors. "Research during the last decade has shown that it is highly unlikely that porcine viruses could cause diseases similar to the HIV/AIDS epidemic, and that a potential cross-species transmission of PERV appears manageable," Schuurman said. "First, there is no disease associated with PERV in pigs. Second, in the *in vitro* cell co-culture study, only a selected small group of human cells seemed susceptible to transmission, while the majority of human cells or cell lines were not susceptible. Third, in the *in vitro* co-culture model, it was shown that PERV genomic segments, on infection of a human cell, do not recombine with human endogenous retroviral segments, so there is no

formation of new pathogenic (humanized) viruses; and fourth, it proved impossible to establish an animal model of cross-species PERV transmission (and disease)." Extensive investigations into humans and animals that had been exposed to living porcine cells, tissues or organs, also did not reveal any indication of pig-to-other species transmission of PERV, Schuurman added. "We seem to be in a very advantageous situation as the pig appears to be low in endogenous retrovirus content."

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Schuurman agreed, however, that regulatory authorities are right to remain concerned, given that some human-derived cells are susceptible to cross-species transmission by PERV. More generally, xenotransplantation will always pose some risk of transmitting a zoonosis, and no amount of research can eliminate this possibility entirely. The regulatory assessment of xenotransplantation applications will therefore always need an additional assurance stage, given that the risks of zoonoses are not confined to the patient, but extend to society as a whole. This will require firm evidence of the significant benefits of xenotransplantation to society to offset the additional risks. Meanwhile, according to Hering, more pre-clinical trials in monkeys are needed to establish that the benefits are indeed real. "Compelling efficacy data will go a long way," he said. "A high expectation of these benefits calls for their previous documentation in a pre-clinical study that mimics the intended clinical scenario."

According to Cooper, recent evidence from primate studies suggests that this high-benefit threshold will soon be reached in the case of pig islet transplantation: "Massimo Trucco's group at the Children's Hospital of Pittsburgh (PA, USA) has been putting genetically engineered pig islets into diabetic monkeys, which has resulted in complete control of their diabetes for over a year with no insulin required." Cooper added that this work could soon translate into a human clinical trial, although an alternative immunosuppressive drug will have to be used to gain regulatory approval.

Indeed, immune suppression continues to be a hurdle for all types of transplantation, as rejection can result from four types of immune response: hyperacute rejection, acute vascular rejection, cellular rejection and chronic rejection. Acute vascular rejection is only relevant to xenographs, whereas the other three also affect transplants from the same species. The first two categories—hyperacute and acute—are both humoral responses, in which antibodies attach to foreign tissue and mark it for destruction. As its name implies, hyperacute is the most rapid and violent form of rejection: it occurs within minutes of the transplantation as host antibodies bind to the donor tissue endothelium—the blood vessel lining—where they cause inflammation and thrombosis followed by necrosis. Acute vascular rejection, which only operates against xenographs, usually occurs within a few days; it is more complex and, unlike the hyperacute response, involves the synthesis of new proteins—hence the delay. Although it is not fully understood, it seems to be driven by the interaction between host antibodies, macrophages and platelets.

Cellular rejection involves natural killer cells and T cells that recognize surface antigens on the foreign tissue. Chronic rejection is the least understood; it is slow and progressive, and affects transplants that survive the other forms of rejection. The main mechanism involves arteriosclerosis, which is probably caused by lymphocytes that are activated over time by the antigens in the vessel walls of the transplants. It is because of chronic rejection that transplanted human organs eventually need to be replaced.

Although many believe that xenographs trigger stronger immune responses than grafts of human origin, this view is not helpful in understanding or solving the problems, according to Hering: "In my opinion, the immune response to islet cell xenografts is qualitatively different, but not necessarily stronger than the immune response to islet allografts."

Schuurman pointed out that there should also be a distinction made between transplants of solid organs, such as hearts, and groups of cells, such as islets, which come without blood vessels. "For solid organ transplants, the antibody-mediated rejection reaction is the main and first component, and for a cell transplant like islets, a cell-mediated reaction showing similarities with the rejection in allotransplantation is most important,"

Schuurman said. "In the experimental animal models, both types of rejection (antibody-mediated and cellular) require different types of immune suppressors/modulators to prevent xenograft rejection than they do to prevent allograft rejection."

This implies that the current range of immunosuppressants, which have been developed for allotransplantation, are not necessarily suitable for xenotransplantation. However, xenotransplantation does have the big advantage that the donor animal can be genetically engineered to produce tissue and organs that minimize the host immune response, either by expressing relevant human proteins or by avoiding the expression of enzymes that trigger rejection mechanisms. For example, pigs with the α -1,3-galactosyltransferase gene knocked out do not produce this enzyme, which is the main antigen that causes antibody-mediated rejection (LaTemple & Galili, 1998). The same pigs can also be engineered to express regulators of the human complement system—a part of the innate immune systems—that inhibit the complement cascade (Huang *et al*, 2001).

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Other solutions are also needed, in particular, to overcome cellular rejection. Haematopoietic chimerism, for example, involves the introduction of stem cells from the donor animal into the bone marrow of the recipient before transplantation of the organ. The donor stem cells associate with the host stem cells and differentiate into various lineages through the process of haematopoiesis, which is the production of mature blood cells. This leads to the generation of T cells that are reactive against both donor and host tissue, and are therefore eliminated by apoptosis because they would attack the host itself (Dooldeniya & Warrens, 2003). When the xenograft is subsequently transplanted, the host fails to produce T cells that attack it because such cells are already recognized as self-attacking by the host.

Although more work remains to be done, the progress made so far is encouraging optimism in some

human transplant surgeons. "If readily available and usable without huge complications, pig hearts would make a big difference," commented John Dark, consultant cardiothoracic surgeon at the Freeman Hospital in Newcastle, UK. "We estimate about 10 patients per million would benefit from heart transplantation with current acceptance criteria. In the UK we do only two per million. There are thousands more, particularly elderly patients with bad heart failure, who might benefit." Dark also considers that pig pancreatic islet transplants will be successful much sooner than whole-organ xenotransplants, in large part because they avoid the humoral rejection problems associated with vascular tissue, and because pig and human insulin differ in just one amino acid.

Over nearly 100 years of research, xenotransplantation has seen many ups and downs, and it remains to be seen whether pigs or other animals will become a source for organs to replace those damaged by disease in humans. The scientific, technical and moral issues are complex, yet research proceeds and the knowledge generated should help researchers to overcome the various hurdles towards the eventual clinical use of xenotransplants.

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