Science & Society

EMBO reports

Ethical rejections of xenotransplantation?

The potential and challenges of using human-pig chimeras to create organs for transplantation

John D Loike[1] & Alan Kadish[2]

Gene editing and stem cell biotechnologies are being applied to create chimeric animals with human organs, which could alleviate the current shortage of human organs for transplantation. While the medical benefits of xenotransplantation appear to be significant, we discuss specific ethical, medical, cultural, and financial challenges and potential solutions that need to be addressed before clinical trials using organs from human-animal chimeras should begin.

"The use of animal organs to replace defective organs in human patients dates back at least to 1838 when the first pig-to-human corneal transplant was performed."

Organ transplantation has a long history. The use of animal organs to replace defective organs in human patients dates back at least to 1838 when the first pig-to-human corneal transplant was performed. The first human skin transplant was done in 1869, the first successful kidney transplant between fraternal twins took place in 1954, and the first heart transplant was performed in 1967. Aortic valve replacement with a porcine heart valve was first reported 2 years later in 1969 and continues to be used to this day. Notwithstanding, of the clinical successes, organ transplantation has been held back by a huge shortage of people willing to donate their organs. Currently, more than 115,000 people are organ transplant candidates in the USA; 75,000 of these are on the national organ waiting list (https://optn.transplant.hrsa.gov). About 25% of patients on the list die before an organ becomes available.

Xenotransplantation—using organs from animals for transplantation—has great potential to alleviate this organ shortage. However, tissue rejection and infections have greatly limited the effectiveness of cross-species transplantations. Recent advances in biotechnology, including gene editing and stem cell science, may provide the key elements needed to create animals that grow human organs for transplantation. Research is currently focusing on growing human hearts, kidneys, lungs, and livers in genetically altered pigs and sheep. Since heart transplantation is the most costly and ethically challenging procedure, we focus our discussion on the medical, financial, and ethical challenges associated with human-pig heart xenotransplantation.

Biotechnologies of xenotransplantation

Not all animals are suitable for generating organs for human transplantation because of their different size, life expectancy, hormonal environment, body temperature, the risk of infections, and immunological tissue rejection. So far, pigs have been the most common source of organs as they hold several advantages for xenotransplantation: the similar size of pig organs to human organs; the ease with which it is possible to clone and genetically modify pigs; the large number of progeny; and the fact that pigs have a relatively short reproduction time and require only about 6 months to grow a sufficiently large and transplantable organ. This does not however solve the problems of infections or tissue rejection—most patients with a porcine transplant need to take immunosuppressive medication.

"So far, pigs have been the most common source of organs as they hold several advantages for xenotransplantation..."

Groundbreaking advances in stem cell and gene editing (CRISPR/Cas9) technologies now enable scientists to efficiently address these two problems and could thereby bring the scientific community closer to developing human organs in a non-human animal. Basically, it would involve seeding a pig blastocyst or embryo with human stem cells that would eventually grow into the desired organ. There are at least four specific challenges that need to be resolved before this can become a reliable source of human organs [1]. First, master gene regulators of organ development in pigs need to be identified to prevent the development of the organ that is to be grown from the human cells. Second, scientists must perfect the reprogramming of human pluripotent stem cells obtained from patients. These stem cells will then be injected into the genetically altered pig embryo to fill the empty organ niche left by gene deletion and differentiate into distinct organ-specific cell lineages. Third, gene editing is needed to eliminate immunogenic galactosyl moieties from the surfaces of pig cells to ensure that the human organs will not be rejected by the patient's immune system. Fourth, gene editing would also be needed to delete dozens of oncogenic porcine retroviruses that could trigger malignancies or zoonotic infections in the transplant recipients. The intersection and combination of these emerging technologies,

1 Professor of Biology at Touro College, New York, NY, USA. E-mail: jdl5@cumc.columbia.edu
2 President of the Touro College and University System, New York, NY, USA
DOI 10.15252/embr.201846337 | EMBO Reports (2018) 19: e46337 | Published online 10 July 2018

sometimes referred to as blastocyst complementation, makes it feasible to create patient-specific transplantable organs in pigs.

Growing a genetically unaltered adult pig from an embryo requires a minimum of 6 months. This is important because the average waiting times for traditional human organ transplants vary from 191 days for heart transplants to 679 days for kidney transplants—longer than it would take to grow a human organ in a pig. We do not know yet the time needed to apply CRISPR and stem cell technology to generate a human organ in these animals. But even if this would add significantly to the 6 months, xenotransplantation still has at least the potential to provide more patients with organs and to eliminate the costs and side effects of immunosuppressive drugs.

Scientific, medical, and financial issues

Over the past few years, various proof-of-principle studies have demonstrated the possibility of generating targeted organ chimeras using blastocyst-complementation strategies. In 2017, scientists successfully produced a rat pancreas in a mouse [2]. Another 2017 study showed that injecting murine iPS stem cells into rat blastocysts generated a functional mouse–rat chimeric pancreas, which, after it was transplanted into a mouse engineered to model diabetes, maintained the rodent's blood-glucose levels for more than a year [3]. Researchers at the Salk Institute have already grown human tissue inside a pig embryo [4]. Blastocyst-complementation strategy has also produced organs such as the kidney and liver in rodents. A team at Emory University announced in 2017 that a kidney from a genetically engineered pig was transplanted into a rhesus monkey and sustained that monkey for more than 400 days before being rejected, breaking the record by more than 250 days [5].

However, there are several medical and scientific barriers that need to be overcome before xenotransplantation can enter human trials. To prevent tissue rejection, the transplanted organ has to contain at least 90% human cells, which will make it necessary to generate a human organ with a human vascular system. Second, we do not know how many pig chimeras will be needed to ensure that a proper patient-specific organ is obtained for transplantation. Finally, heart transplant candidates require intense medical care and usually receive a transplant within 6 months. Will it be possible to generate a human heart in human-pig chimeras within 6 months or will more time be necessary that may place these patients at a higher mortality risk while waiting for the donor heart?

> *"... xenotransplantation may provide huge benefits for organ transplant recipients beyond just closing the gap between patients on the waiting list and the availability of suitable immune-compatible organs."*

Assuming that these barriers can be overcome, xenotransplantation may provide huge benefits for organ transplant recipients beyond just closing the gap between patients on the waiting list and the availability of suitable immune-compatible organs. In fact, the number of donors is not increasing as rapidly as the number of people in need of transplants. The yearly increase in the number of heart transplant candidates, for example, has grown by 34% since 2003. In addition, only 27% of hearts from donors after brain stem death are eventually accepted for transplantation. Thus, about 43% of patients on the heart transplant waiting list either die or become too sick to be suitable for a heart transplant. Moreover, since it is extremely rare to find a donor and recipient who share the same genetic histocompatibility antigens, most patients need to take anti-rejection drugs for the rest of their life in order to prevent tissue rejection. The use of patient-specific organs from pig chimeras may prevent or reduce the need to take this medication and may dramatically reduce or even eliminate the need to recruit organ donors.

Heart xenotransplants as compared to other transplantable organs, such as kidneys, have unique medical challenges. Most prospective heart recipients, for example, must be younger than 65 years. In contrast, more than 22% of kidney transplant recipients were older than 65 in the 2017. Theoretically, xenotransplantation may thus eliminate any age barrier for patients. In addition, obtaining a human heart from a brain-dead donor after severe trauma has numerous adverse effects on those transplantable organs that may lead to serious side effects. In the case of the xenotransplantation of pig organs, this could be avoided as organs would be excised from a healthy pig under anesthesia.

Financial considerations are a critical factor for basic and clinical research on human-pig xenotransplantation. *Fortune* Magazine estimated that the average cost of a heart transplant is about US $1.4 million, 15% of which are the costs of anti-rejection medications [6]. The bulk of the costs are admission to the hospital and surgery (70% of total costs) and treatments for the first 6 months after transplants (14% of total costs; http://smallbeats.childrensomaha.org/much-heart-transplant-cost/). Currently, it is difficult to estimate the potential cost savings of xenotransplantation, because we do not know how much it will cost to generate a human heart in a genetically altered pig. At the very least, xenotransplantation may either eliminate or reduce the costs of anti-rejection medications, which amount to an average sum of US$17,000 per patient in the USA, where Medicare covers only the first 3 years after transplantation (http://centerforhealthreporting.org/article/medicare-limits-anti-rejection-drugs). In addition, these drugs can have serious side effects, including kidney toxicity, neurotoxicity, hypertension, gout, and skin cancer. If xenotransplantation only reduced the need for anti-rejection drugs, it would not amount to huge savings for patients and insurance companies. However, if it also reduced the post-surgery side effects of traditional heart transplants, the financial and health benefits to the recipients would be significant.

Ethical concerns

Xenotransplantation also raises several ethical, cultural, and religious concerns that need to be addressed. First, many Islamic cultures do not allow the use of porcine organs, such as heart valves, to replace defective human organs. However, this issue might be mitigated by the views of several Islamic scholars who have allowed organ transplants from pigs because the patients would die without these transplants. Thus, saving human lives overrides the prohibition of using a porcine organ for transplantation [7]. For Jewish patients, porcine products

cannot be used as food, but can be used as organ transplants [8]. Moreover, Judaism promotes xenotransplantation to prolong or save the life of a human being who is ill or dying from organ failure. Catholic ethicists in Canada and the USA also favor xenotransplantation as long as the technology preserves human identity and precludes the use of aborted embryos as a source of human stem cells [7]. These ethicists emphasize that pluripotent and not embryonic stem cells must be used in these protocols. However, many religious ethicists stress that these protocols should not lead to mixing human and animal brain or gonadal cells, as these are seen as unique to human identity. Underscoring these permissive attitudes on xenotransplantation lies the ethical principle of autonomy that dictates that physicians must inform all patients from any religion about the source of the organ to be transplanted.

"Currently, it is difficult to estimate the potential cost savings of xenotransplantation, because we do not know how much it will cost to generate a human heart in a genetically altered pig."

A second ethical concern related to stem cell-based technologies is the principle of non-maleficence ("do no harm") as the transplanted stem cells may transform into cancer cells in the recipients. This concern is based on studies in animals showing that implanted embryonic and iPS stem cells can transform into teratomas and cause genetic instability. Once again, the fear that the transplanted organ can turn cancerous may be premature and more scientific validations in humans must be done. There is one 2015 study, for example, showing clinical benefits of using iPS stem cells to reformat the bone marrow of patients with multiple sclerosis in order to permanently eliminate lymphocyte clones that destroy tissue in this disease [9]. One outcome of this study is that the patients who received this stem cell therapy showed no evidence that the transplanted stem cells became tumorigenic.

A third ethical concern is the fear of a slippery slope. Will these technologies promote using CRISPR technologies on healthy human embryos to create designer babies for behavioral or cosmetic enhancements? While, in theory, the fear of a slippery slope is real, in practical terms, its implementation is not common. As far as we know, *in vitro* fertilization (IVF), starting in 1978, and pre-implantation genetic diagnosis (PGD), which became available in the early 1980s has been applied almost exclusively to generating healthy babies and not for eugenic purposes. Today, almost 6 million babies have been born via IVF and PGD has been successfully used to create thousands of healthy babies from parents who are either infertile or carry genes for serious inheritable diseases that can be passed down to their children. We believe that sometimes society exhibits a moral compass to prevent the use of these technologies for unethical purposes, thus mitigating the slippery slope argument.

The fourth ethical concern regards respecting both animal rights and human dignity, and it is the most difficult to address. It concerns the possibility that non-directed human iPS cells transplanted into genetically altered pig embryos will migrate to the animal's brain and alter its behavior or cognitive state. There may be a scientific benefit in researching the impact of incorporating human brain cells into animals to better understand the mechanisms underlying human neurological diseases such as Alzheimer's and Parkinson's disease. However, there is no consensus on accurately assessing what it means to possess a human-like cognitive state. There are many attributes to personhood including intelligence, capacity to be autonomous, capacity to communicate, and self-awareness. In fact, the US National Institutes of Health has previously refused to support research using human-animal chimeras for transplantation because of this ethical concern and because society does not have a consensus view on how to define personhood. For example, should personhood be defined as the percent of human brain cells expressed in a human-animal chimera or should it be assessed using psychological or cognitive learning evaluations?

In order to gain insight into this ethical dilemma, it is important to clarify that the term "human" is a biological term, which refers to all members of the species *Homo sapiens*. In contrast, the term "person" is a normative—legal, moral, or ethical—term, which refers to a moral and/or legal status that creatures or other bearers of human-like capacities can share with normal humans. Thus, even if human stem cells were incorporated into the pig's brain and enhanced their cognitive state, these animals would still not be human. Thus, we believe that the term "humanizing a pig" associated with xenotransplantation is an ethical paradox that will confuse and may even frighten the public from accepting xenotransplantation. We therefore advocate that such a term should not be used in describing this technology.

"... we believe that the term "humanizing a pig" associated with xenotransplantation is an ethical paradox that will confuse and may even frighten the public from accepting xenotransplantation"

Some ethicists argue that human cognition should not be the sole determinant of personhood since individuals born with severe cognitive abilities are clearly human, emphasizing that being born from a person is a primary determinant of personhood. Others argue that society must better respect higher forms of animals, such as non-human primates because they possess human-like characteristics. This type of argument opens the door to banning the transplantation of human brain cells into animals that may enhance the animal's cognitive development. Before we can mitigate this ethical concern as it may apply to human-pig xenotransplantation, it will be important to first validate whether random human stem cells in pigs will embed and differentiate into neurons or sex organs. We advocate that it is justified to conduct more preliminary experiments in human-animal chimeras to assess whether human stem cells will implant into the animal's brain and whether these cells will enhance the cognitive state of the animal. Only then can we consider how to better evaluate "human cognition" and assess its ethical concerns to determine whether the potential life-saving technologies trump the ethical concerns concerning personhood in animals.

"... all ethical discussions on xenotransplantation should go hand in hand with further bioethical, physiological and psychological analyses of stem cell and CRISPR-based biotechnologies in organ transplantation..."

A related ethical challenge is whether creating such human-pig chimeras violates animal rights. Transforming animals into human organ donors necessitates experimentation, procedures and genetic modification that might be harmful, deleterious and distressful to the animals. Interestingly, a 2016 survey conducted in Japan of both the general public and researchers showed that more than 60% of the public and 83.8% of researchers supported the creation of human-pig chimeras and 81.0% of the public and 92.4% of researchers supported research on human-pig chimeric embryos [10]. Most likely, the potential that xenograft technology can help save human life may justify the use of animals in this venture.

Conclusions

Human-pig xenotransplantation research is only in its beginning stages. This innovative technology has the potential to offer transplant recipients hearts and other organs without the need to take anti-rejection drugs and should dramatically reduce the need for human organ donors. However, all ethical discussions on xenotransplantation should go hand in hand with further bioethical, physiological, and psychological analyses of stem cell and CRISPR-based biotechnologies in organ transplantation to assess the effects of implanting human stem cells in the brains or gonads of the pig. It is society's responsibility to maintain its moral compass and to utilize the best available experimental methodologies to assess the scientific premises of these ethical concerns. Real concerns should be dealt with transparently, but fear should not impede medical progress in the development of xenotransplantation for human organ transplants.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Oldani G, Peloso A, Lacotte S, Meier R, Toso C (2017) Xenogeneic chimera—Generated by blastocyst complementation—As a potential unlimited source of recipient-tailored organs. *Xenotransplantation* 24: e12327
2. Liu Z, Hu W, He T, Dai Y, Hara H, Bottino R, Cooper DKC, Mou L (2017) Pig-to-primate islet xenotransplantation: past, present, and future. *Cell Transplant* 26: 925 – 947
3. Yamaguchi T, Sato H, Kato-Itoh M, Goto T, Hara H, Sanbo M, Mizuno N, Kobayashi T, Yanagida A, Umino A *et al* (2017) Interspecies organogenesis generates autologous functional islets. *Nature* 542: 191
4. Wu J, Platero-Luengo A, Sakurai M, Sugawara A, Gil MA, Yamauchi T, Suzuki K, Bogliotti YS, Cuello C, Morales Valencia M *et al* (2017) Interspecies chimerism with mammalian pluripotent stem cells. *Cell* 168: 473 – 486
5. Iwase H, Hara H, Ezzelarab M, Li T, Zhang Z, Gao B, Liu H, Long C, Wang Y, Cassano A *et al* (2017) Immunological and physiological observations in baboons with life-supporting genetically engineered pig kidney grafts. *Xenotransplantation* 24: e12293
6. Rapp N, Vandermay A (2017) *Here's what every organ in the body would cost to transplant*. Fortune, Sep 14.
7. Paris W, Seidler RJH, FitzGerald K, Padela AI, Cozzi E, Cooper DK (2018) Jewish, Christian and Muslim theological perspectives about xenotransplantation. *Xenotransplantation* 25: e12400
8. Rosner F (1999) Pig organs for transplantation into humans: a Jewish view. *Mount Sinai J Med* 66: 314 – 319
9. Nash RA, Hutton GJ, Racke MK, Popat U, Devine SM, Griffith LM, Muraro PA, Openshaw H, Sayre PH, Stüve O *et al* (2015) High-dose immunosuppressive therapy and autologous hematopoietic cell transplantation for relapsing-remitting multiple sclerosis (HALT-MS): a 3-year interim report. *JAMA Neurol* 72: 159 – 169
10. Sawai T, Hatta T, Fujita M (2017) Public attitudes in Japan towards human–animal chimeric embryo research using human induced pluripotent stem cells. *Regen Med* 12: 233 – 248

