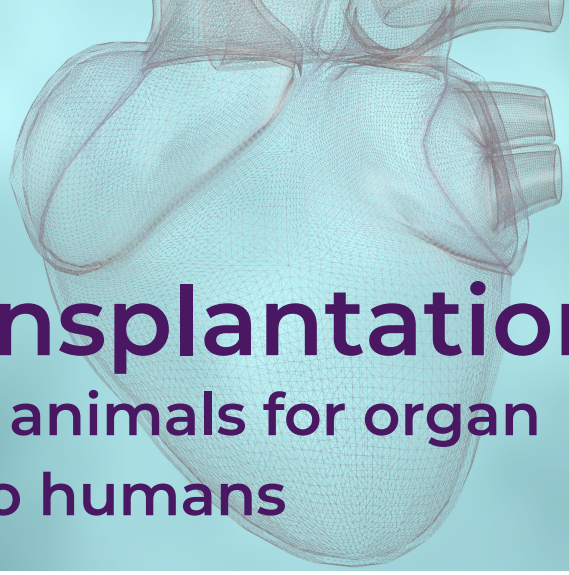

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Xenotransplantation:

Gene editing animals for organ transplants to humans

After decades of investigation, the recent transplantation of genetically modified pig kidneys and hearts into humans mark a significant breakthrough in the use of xenotransplantation to save human lives. These achievements have attracted attention worldwide, with the hope that xenotransplantation might soon become a viable option to alleviate human donor organ shortages.

Summary

- The gap between donor organ demand and supply is increasing
- Research efforts have resulted in successful preclinical trials of pig to non-human primate transplants, pig kidney and heart transplants to brain-dead patients, and a pig heart transplantation to a living heart failure patient
- A main contributor to this success has been the genetic engineering of the donor animal
- Rejection by the transplant recipient remains an issue
- Clinical trials for pig kidney and heart to human transplants are being considered
- Concerns remain that transfer of pathogens between animals and humans as a result of a xenotransplant could pose a problem

What is it and what is it for

Xenotransplantation is the transplantation of viable cells, organs, or tissues between species. There are long waiting lists for donor organs, and the gap between organ demand and supply continues to increase. The criteria to be included on an organ transplant waiting list is extremely stringent, and it is likely that many more people would benefit from a transplant were they available. Solutions for this shortage are being investigated through activities in different research areas, including xenotransplantation. Scientific advances, particularly in genetic engineering of the pig, mean that xenotransplantation could potentially alleviate human organ shortages.

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Xenotransplantation

Scientific status

A significant contributor to recent advances is the improvement of genetic engineering techniques, particularly gene editing using CRISPR, the focus of this briefing. Other contributing developments include:

- Development of cloning techniques and associated embryology techniques
- Improvement in immune suppressant drugs
- Advances in organ transportation by perfusion preservation
- Improved husbandry and veterinary care of animals
- Availability of genomic sequencing technologies and detailed annotated databases

Pigs have become the most commonly used animal for xenotransplantation of organs into humans. Their early sexual maturity (5 months), short gestation periods (3.5 months), large litters (5-10 piglets) and suitable organ size, offer advantages over other animals. Pig tissues (such as routinely used heart valves) have been successfully transplanted into people. Despite these benefits, rejection has been, and still is, a critical barrier to be overcome. Advances in genetic engineering could provide a solution to this particular problem.

Extensive genetic engineering

The body's strong immune defence system does not like foreign tissue, which it can identify by antigens (protein markers) attached to cells and will target for destruction. Immune rejection of foreign antigens, for example an organ from a different species, is the biggest barrier to xenotransplantation. There are several antigens expressed in the pig. The first major gene editing advancement was the removal ('knock out') of a number of antigens from the pig genome, reducing the likelihood of rejection.

Scientists have also had successes in making pig organs more human-like by introducing human genes into the pig's genome. These 'knock-ins' or transgenes result in human antigens and immune cells being expressed in the pig, increasing the likelihood that the human immune system will accept a pig donor organ. Prior to the advent of CRISPR gene editing technology, it could take three years to generate a pig with a single modification. However, CRISPR allows for multiple gene alterations to be done in no more than half that time [1].

To better control the immune response, more than 40 genetic modifications in pigs have been attempted, either individually or in combination. Different immune responses that contribute to various forms of rejection, as well as healing, are being targeted [1]. For example, the pig heart that was transplanted into a human had a set of ten gene modifications. The optimal combination of gene edits and immunosuppressant drugs has yet to be determined.

Technical effects of gene editing also need to be investigated further, such as when unintended edits occur, or 'random' inserts, the potential impact of which is still unknown. The inclusion of additional quality control steps such as whole genome sequencing could minimise these effects by identifying any unintentional changes.

Managing infection risks

Zoonosis - the transfer of an infectious agent from the animal to the human [2] – is a serious concern in xenotransplantation, not only for the person receiving the organ, but also to those they come into contact with. In the case of a highly infectious pathogen, this could present a serious risk to public health.

Research to determine the risk of zoonosis continues. Strategies to eliminate, control or manage this potential threat are being explored. Transmission of bacteria, fungi, and parasites can largely be prevented with improved animal husbandry practices, including vaccination. Screening procedures to exclude potential pig and human pathogens from 'designated pathogen free' breeding pig colonies are also used. Genetic engineering may provide a solution, and CRISPR gene editing technology has been used to produce porcine endogenous retroviruses (PERV)-free pigs. In addition, various laboratory test assays that can detect infection in pigs and humans exist, but additional assays that can detect pig infections in humans are still needed.

Given the gaps in knowledge, carefully designed infectious disease protocols, laboratory practices, and surveillance strategies of source animals, recipients, and contacts are essential components to mitigate donor-derived infectious disease risk in xenotransplant clinical trials [3].

Regulatory frameworks

The World Health Organization (WHO) has outlined ten principles on xenotransplantation in the 2018 Changsha Communiqué [4], which also reflects the position of the International Xenotransplantation Association. Many countries recognise the importance of a regulatory framework for safe implementation and have regulations or guidelines for the clinical use of xenotransplantation or follow the FDA guidelines. They include essential principles and recommendations for the planning of future clinical trials.

China, one of the leaders in xenotransplantation, has a regulatory framework that it regularly reviews and updates. In China, all the cells, tissues, organs, and acellular products derived from pig origin are considered to be xenotransplantation products. In January 2022, the China Organ Transplantation Development Foundation convened a symposium where a formal consensus was reached outlining the expert opinions on scientific, regulatory, and ethical issues of clinical trials of xenotransplantation in China [5].

Xenotransplantation

Moving towards clinical trials

Pre-clinical trials of xenotransplantation are vital in providing the evidence required to demonstrate feasibility for clinical trials, i.e. before human trials can take place. They should demonstrate long term survival of the transplanted organ, fully characterise the transplanted organ function, demonstrate reproducibility, and predict safety and durable efficacy. Pre-clinical trials have been focused on pig to non-human primate transplants, and are showing success for kidney and heart xenotransplants.

Clinical trials in humans will raise many ethical questions, and there are calls for more discussion before such trials go ahead. Issues include selecting patients for trials, routine monitoring for possible infection, biobanking of samples and considering the societal risk from zoonoses.

For the purposes of infection control, counselling of prospective xenotransplant candidates, close contacts and primary caregivers will need discussions about factors including the potential lifelong risk of infections with unknown or novel pathogens; the potential need for life-long surveillance; and the importance of participation in recommended infection prevention measures, such as screening, vaccination, and adherence to immunosuppressive regimens.

Conclusion

The advancement of scientific technologies has made xenotransplantation a viable possibility. Due to the acute need for donor organs, there is a significant push to advance the technology further. Whilst this is an exciting area of development that could provide a solution to the shortage of organs for people in organ failure there are still technical challenges to overcome. Xenotransplantation therefore needs extensive further research including clinical trials before pig donor organs could become an option for clinical care. Appropriate regulatory and ethical oversight will also be important.

References

1. Yoon CH, Choi HJ, Kim MK. Corneal xenotransplantation: Where are we standing? Prog Retin Eye Res, 2021. 80: 100876.
2. Fishman JA. Infectious disease risks in xenotransplantation. American Journal of Transplantation, 2018. 18(8): 1857-1864.
3. Nellore A et al. Moving xenotransplantation from bench to bedside: Managing infectious risk. Transplant Infectious Disease, 2022.
4. Hawthorne WJ et al. Third WHO Global Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials, Changsha, Hunan, China December 12–14, 2018. Xenotransplantation, 2019. 26(2): e12513.
5. Huang J. Expert consensus on clinical trials of human xenotransplantation in China. Health Care Science, 2022. 1(1): 7-10.

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