Cross-Species Organ Transplants: Unlocking Solutions for Organ Failure using Porcine Organs

Kauana Magagnin de Novaes [a]

[a] Graduation in Biotechnology. Latin American Institute of Life and Nature Sciences. Federal University of Latin American Integration. Foz do Iguaçu, Brazil. kauana.magagnin@gmail.com

Abstract: Allotransplantation of organs has encountered a significant obstacle due to the inherent limitation in the availability of human donor organs. Given the associated morbidity, mortality, high costs, or the unavailability of supportive treatments, xenotransplantation emerges as a potential solution to tackle the severe scarcity of organ grafts. Over the last decade, research endeavours have concentrated on developing donor organs from pigs through the precise editing of various genes using genome editing technologies. However ethical and functional issues remain, some are broad issues that accompany the adoption of novel and expensive technologies, and some are unique to xenotransplantation. In this paper is possible to have an overview of the cross-species organ transplants and its advantages and challenges.
Keywords. Xenotransplantation, Porcine, Genetic engineering, Organ rejection, ethics

1. Introduction

The presence of genetically modified organisms (GMOs) in the lives of the population nowadays is undeniable. These organisms include plants, bacteria, viruses, and animals. GMOs are present in the food we eat, in the bloodstream in the form of vaccines, in medication, and now, even within or as organs. Transgenic animal technology represents one of the rapidly expanding fields in biotechnology. It involves the integration of foreign genetic material into an organism's genome, which can then be passed down and expressed in subsequent generations of genetically modified organisms [1]. GMO technologies find application in various fields, including medical and biotechnological research [2], disease models [3], agricultural sciences [4] and xenotransplantation, to name a few. Xenotransplantation refers to the transplantation of organs from one species to another [5].

In this light, xenotransplantation can be explained as any procedure that involves the transplantation, implantation, or infusion into a human recipient of either (a) live cells, tissues, or organs from a nonhuman animal source, or (b) human body fluids, cells, tissues, or organs that have had ex vivo contact with live nonhuman animal cells, tissues or organs (USFDA, 2021). The genetic alterations were intended to enhance the success of organ transplantation by reducing immune rejection, managing organ size, and regulating processes such as complement, coagulation, and inflammation [6].

On the other hand, allotransplantation is the transplantation of cells, tissues, or organs, sourced from a genetically non-identical member of the same species as the recipient [7]. However, xenotransplantation technology is being studied due to the necessity for a new supply of organs. There is a disparity between the patients in need of organs and the availability of organs within the system.

2. Applicability

Currently, xenotransplantation has utilized several large animals as potential donors, such as pigs, monkeys, chimpanzees, and baboons. Among these options, pigs stand out as the most suitable candidates for xenograft organ donation due to their organ size, physiological metabolism, and immune system closely resembling those of humans (4). And more, pigs have a lower risk of zoonosis than non-human primates and they can be reared under specific pathogen free (SPF) housing conditions further reducing risk of infections (5)

Currently, xenotransplantation has utilized several large animals as potential donors, such as pigs, monkeys, chimpanzees, and baboons. Among these options, pigs stand out as the most suitable candidates for xenograft organ donation due to their organ size, physiological metabolism, and immune system closely resembling those of humans [8]. And more, pigs have a lower risk of zoonosis than non-human primates and they can be reared under specific pathogen free (SPF) housing conditions further reducing risk of infections [9]

Additionally, pigs have a shorter gestation period, lasting around four months, compared to the other big animal models. Lastly, pigs are a litter-bearing species, typically giving birth to 10–15 piglets per gestation cycle. This capacity for rapid herd expansion makes pigs an attractive choice for transgenic animals and xenotransplantation [10].

These genetic modifications have been studied for years, there has been done knockout of many proteins dangerous to the human body (Figure 1). At the present, there are a lot of non-clinical studies in progress, and some clinical trials in brain-dead humans, with some promissory results [13][14]

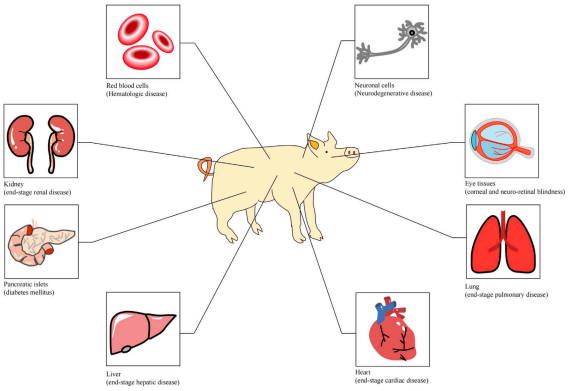

Fig. 1 - Tissues or organs that can be used for xenotransplantation (4).

However, before introducing pig cells into the human body, it is essential to modify these cells to ensure compatibility with the human system, these modifications serve to enhance the viability of pig organs within a human host. The genetic modification primarily targets the cell's DNA, the process of altering a specific part of the genome is achieved through the utilization of techniques, as zinc finger nucleases (ZFN), transcription activator-like effector (TALE) nucleases and modifications of the CRISPR/Cas-9 (Clustered Regularly Interspaced Short Palindromic Repeats) in conjunction with the associated protein Cas-9 [11]. CRISPR is the most recent technology, and it's widely used because this protein can precisely cut these sections, enabling the deactivation of existing genes or the introduction of new ones. [12]

3. Challenges

While genetic modifications contribute to the acceptance of xenogeneic organs, there remain issues related to rejection that must be carefully considered. Due to genetic disparities between the organ donor and recipient, the transplantation of xenogeneic organs triggers an immune response in the recipient, often resulting in the complete failure of the transplanted organ. Depending on the mechanism and timing of xenotransplant rejection, the following classifications have been adopted: hyperacute rejection (HAR), delayed xenograft rejection (DXR), acute cellular rejection (ACR), and chronic rejection (CR) [11].

The hyperacute rejection occurs within a few minutes following organ transplantation and invariably results in organ failure. The central role in hyperacute rejection is attributed to the damage to the endothelium and the loss of its biological functions. Pathological alterations occur within the capillaries and arterioles, leading to intravascular coagulation and substantial infiltration, predominantly consisting of neutrophils, ultimately culminating in the necrosis of the transplanted organ [11]. The primary cause of hyperacute rejection in xenografts is the existence of naturally occurring, preexisting antibodies in human plasma that specifically target the Gala(1,3)-Gal antigen found on the surface of porcine endothelial cells. In this case, porcine organs need to undergo genetic modification, specifically a knockout of the alpha-1,3-galactosyltransferase gene, before being transplanted into humans [15].

The difference between DXR and HAR, is the time it takes to take place and what types of molecules are involved in the rejection. The delayed xenograft rejection, as the name explain, takes hours or several days to take place in the body. The molecule that evolves this condition is the immunoglobulins (IgG) in the arterial lumen, it's observed a higher expression of proinflammatory genes, more chemokines and blood platelet activation [16]. This is the most complex type of rejection because it involves numerous pathways that regulate the activation of these genes. Some trials involve knocking out genes or inserting new genes into pig cells to alleviate this type of rejection. [17,18].

Hyperacute rejection and acute vascular rejection primarily involve the humoral immune system, whereas acute cellular rejection is predominantly associated with cellular immunity. The key cells involved in acute cellular rejection include NK cells and T lymphocytes [8]. Currently, the T-cell response is primarily suppressed through multigenic modifications aimed at decreasing acute cellular rejection. Pigs with the multigenic GTKO/hCRPs modification were initially created to address primary humoral immunity. However, the loss of the Gal epitope and the expression of hCRPs on porcine cell surfaces can also act to attenuate T-cell proliferation and certain cytokine-mediated responses, thereby partially inhibiting acute cellular rejection [19]

Chronic rejection manifests clinically as a gradual deterioration of the transplanted organ or tissue, occurring months or even years after transplantation. It is believed to arise from the cumulative impact of several detrimental factors. Currently, there is limited knowledge regarding chronic xenotransplant rejection [11]

Another challenging issue is the greater amount of immunosuppression that a patient with a xenotransplant must undergo compared to a patient who has undergone allotransplantation. Even in

allotransplantation, immunosuppression has profound side effects, including heightened susceptibility to infection, sepsis, malignancy, and cardiovascular disease [6]

4. Ethical issues

There are ethical concerns that need to be addressed regarding this matter, but it's important to always consider the potential benefits this technology may offer. In some cultures, porcine-based products are avoided, and there are individuals who refrain from consuming or avoid animal products due to religious or personal beliefs. Furthermore, pigs raised for use in xenografts endure a lifetime in confinement under highly sterilized conditions, which differs from their natural environment and raises concerns about the welfare of these animals [20].

Furthermore, even with aseptic measures in place, there will always be a risk of zoonotic disease transmission, especially when exposed to a patient who is already on immunosuppressive medications [21]. Porcine endogenous retroviruses (PERV) continue to pose a challenge, given their intrinsic presence within the porcine genome [11]. These endogenous retroviruses typically remain dormant, showing no signs of disease activity within the host. Nevertheless, they hold the potential to become active and infectious under specific circumstances [22]. In the context of xenotransplantation, the presence of an endogenous virus within the graft raises concerns, as it could potentially become activated and pose a pathogenic risk to the recipient [23].

Moreover, the absence of clinical data presents a challenge in obtaining informed consent. While uncertainties are inherent in any clinical trial, the consent process in xenotransplantation must account for unique uncertainties, including the potential existence of previously unidentified pig viruses capable of causing illness in humans. It becomes essential to furnish patients and their close family with adequate information, allowing them to comprehend and contemplate unforeseen potential consequences stemming from 'unknown unknown' risks [6].

5. Conclusion

Xenotransplantation represents a multifaceted undertaking, requiring the utilization of various research methods across a wide spectrum of disciplines. This field encompasses diverse areas of expertise, ranging from molecular biology, where researchers develop appropriate gene constructs and study the characteristics of transgenic animals, to animal breeding and experimental embryology, which involve the introduction of gene constructs. Furthermore, it extends to pig farming, which is essential for the breeding of pigs, as well as immunology, where the focus is on ensuring compatibility between donors and recipients. Additionally, virology plays a crucial role in this context, specifically in the detection of endogenous retroviruses. Lastly, transplant surgery is an integral part of the process, completing the interdisciplinary nature of xenotransplantation research.

The which involves the transplantation of organs cross-species, holds the potential to address the shortage of organs and potentially alleviate or eliminate waiting lists. Furthermore, it could broaden the pool of eligible patients for organ transplantation.

6. References

[1] Bihon Asfaw A, Assefa A. Animal transgenesis technology: A review. Http://WwwEditorialmanagerCom/Cogentagri 2019;5. https://doi.org/10.1080/23311932.2019.1686802.

[2] Shakweer WME, Krivoruchko AY, Dessouki SM, Khattab AA. A review of transgenic animal techniques and their applications. Journal of Genetic Engineering and Biotechnology 2023;21:1–14. https://doi.org/10.1186/S43141-023-00502-Z/TABLES/2.

[3] Perleberg C, Kind A, Schnieke A. Genetically engineered pigs as models for human disease. DMM Disease Models and Mechanisms 2018;11. https://doi.org/10.1242/DMM.030783/2587.

[4] Campbell KHS. A background to nuclear transfer and its applications in agriculture and human therapeutic medicine*. J Anat 2002;200:267–75. https://doi.org/10.1046/J.1469-7580.2002.00035.X.

[5] Aschheim K, DeFrancesco L. Xenotransplantation: how close are we? Nature Biotechnology 2023 41:4 2023;41:452–60. https://doi.org/10.1038/s41587-023-01730-x.

[6] Leon-Villapalos J, Eldardiri M, Dziewulski P. The use of human deceased donor skin allograft in burn care. Cell Tissue Bank 2010;11:99–104. https://doi.org/10.1007/S10561-009-9152-1/METRICS.

[7] Xi J, Zheng W, Chen M, Zou Q, Tang C, Zhou X. Genetically engineered pigs for xenotransplantation: Hopes and challenges. Front Cell Dev Biol 2023;10:1093534. https://doi.org/10.3389/FCELL.2022.1093534/BIBTEX.

[8] Fischer K, Schnieke A. Xenotransplantation becoming reality. Transgenic Res 2022;31:391–8. https://doi.org/10.1007/S11248-022-00306-W.

[9] Hay AN, Farrell K, Leeth CM, Lee K. Use of genome editing techniques to produce transgenic farm animals. Adv Exp Med Biol 2022;1354:279. https://doi.org/10.1007/978-3-030-85686-1_14.

[10] Hryhorowicz M, Zeyland J, Słomski R, Lipiński D. Genetically Modified Pigs as Organ Donors for Xenotransplantation. Mol Biotechnol

2017;59:435. https://doi.org/10.1007/S12033-017-0024-9.

[11] Mengstie MA, Wondimu BZ. Mechanism and Applications of CRISPR/Cas-9-Mediated Genome Editing. Biologics 2021;15:353–61. https://doi.org/10.2147/BTT.S326422.

[12] Porrett PM, Orandi BJ, Kumar V, Houp J, Anderson D, Cozette Killian A, et al. First clinical-grade porcine kidney xenotransplant using a human decedent model. Am J Transplant 2022;22:1037–53. https://doi.org/10.1111/ajt.16930.

[13] Ryan Chaban, David K. C. Cooper. The First Clinical Pig Heart Transplant and the Future of Cardiac Xenotransplantation. 2022.

[14] Montgomery RA, Stern JM, Lonze BE, Tatapudi VS, Mangiola M, Wu M, et al. Results of Two Cases of Pig-to-Human Kidney Xenotransplantation. N Engl J Med 2022;386:1889–98. https://doi.org/10.1056/nejmoa2120238.

[15] Zhou Q, Li T, Wang K, Zhang Q, Geng Z, Deng S, et al. Current status of xenotransplantation research and the strategies for preventing xenograft rejection. Front Immunol 2022;13. https://doi.org/10.3389/FIMMU.2022.928173.

[16] Iwase H, Ekser B, Hara H, Phelps C, Ayares D, Cooper DKC, et al. Regulation of human platelet aggregation by genetically modified pig endothelial cells and thrombin inhibition. Xenotransplantation 2014;21:72–83. https://doi.org/10.1111/XEN.12073.

[17] Petersen B, Ramackers W, Lucas-Hahn A, Lemme E, Hassel P, Queißer AL, et al. Transgenic expression of human heme oxygenase-1 in pigs confers resistance against xenograft rejection during ex vivo perfusion of porcine kidneys. Xenotransplantation 2011;18:355–68. https://doi.org/10.1111/J.1399-3089.2011.00674.X.

[18] Xu XC, Goodman J, Sasaki H, Lowell J, Mohanakumar T. Activation of natural killer cells and macrophages by porcine endothelial cells augments specific T-cell xenoresponse. Am J Transplant 2002;2:314–22. https://doi.org/10.1034/J.1600-6143.2002.20405.X.

[19] Loike JD, Kadish A. Ethical rejections of xenotransplantation? The potential and challenges of using human-pig chimeras to create organs for transplantation. EMBO Rep 2018;19. https://doi.org/10.15252/EMBR.201846337.

[20] Carrier AN, Verma A, Mohiuddin M, Pascual M, Muller YD, Longchamp A, et al. Xenotransplantation: A New Era. Front Immunol 2022;13. https://doi.org/10.3389/fimmu.2022.900594.

[21] Blusch JH, Patience C, Martin U. Pig endogenous retroviruses and xenotransplantation. Xenotransplantation 2002;9:242–51. https://doi.org/10.1034/J.1399-3089.2002.01110.X.

[22] Entwistle JW, Sade RM, Drake DH. Clinical xenotransplantation seems close: Ethical issues persist. Artif Organs 2022;46:987–94. https://doi.org/10.1111/AOR.14255.

https://www.fda.gov/vaccines-blood-biologics/xenotransplantation