Int J Biol Med Res. 2024; 15(2): 7791-7798

Contents lists available at BioMedSciDirect Publications

International Journal of Biological & Medical Research

Journal homepage: www.biomedscidirect.com

Review article

Prospect and Challenges of Xenotransplantation: A Review

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ARTICLEINFO

Keywords:
Xenotransplantation
Xenograft Rejection
Biomarkers for Xenograft

ABSTRACT

Background and Aims - The shortage of organs for transplantation has been a critical issue, leaving many patients with life-threatening conditions on waiting lists with little hope. Xenotransplantation have the potential to provide hope for patients with organ failure, offering them a chance at extended and improved quality of life. However, no single study satisfactorily evaluated the health outcome of xenotransplantation strategies. There is the lack of gold standard limit evaluation for xenotransplantation. This review aimed to address the shortage of organs for transplantation by utilizing tissue organs from animals in accordance with the Preferred Reporting Items for Systematic Reviews and MetaAnalyses extension for Scoping Review (PRISMAScR) guideline. Published english-language studies in electronic data bases included PubMed, Scopus, and Google Scholar were retrieved using specific search themes such as organ transplantation OR xenotransplantation OR xenograft AND immune responses. In this study, xenotransplantation provided hope for patients with organ failure, offering them a chance to live with improved quality of life. Despite the numerous advantages that could accrue to humans when xenografting becomes a clinical success, there are a lot of risks that are associated with xenotransplantation. The high level of immunosuppressive drugs needed to overcome immune rejection may be counterproductive, leaving the patient susceptible to other infections. Modifying xenotransplantation to suit individual patient's needs through genetic modification could reduce the risk of graft rejection. In addition, research in xenotransplantation in fields like immunology, genetics, and biotechnology could lead to a profound understanding of the human immune system.

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Introduction

Xenotransplantation is a medical procedure that involves the transplantation of tissue organs from a donor species into the recipient of a different species [1]. According to the World Health Organization (WHO), 17 people die each day waiting for an organ transplant. In 2022, only 42,000 transplants were performed, 25,499 kidney transplants, 9,528 liver transplants, 4,111 heart transplants, 2,692 lung transplants, other 950 transplants were performed in 2023 [2]. The primary aim of xenotransplantation is to address the shortage of organs for transplantation by utilizing tissue organs from animals, typically pigs which are considered suitable donors due to their physiological and anatomical similarities to humans. Early attempts at xenotransplantation go far back to the 17th century. However, xenotransplantation such as the transplantation of animal kidneys into man were often unsuccessful due to a lack of understanding of immunology [3]. 1963. Dr. Keith Reemtsma performed a series of kidney transplants from chimpanzees to humans. However, the patients survived for only a short period. In 1984, Dr. David Cooper successfully transplanted a pig heart into a baboon, demonstrating the potential of using pigs as organ donors [4].

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In the 20th century, the concept of tissue rejection became better understood. The first significant xenotransplantation procedure occurred in Advances in genetics became prominent in the early 2000s, genetic engineering techniques allowed for the modification of genetically modified pigs with organs less likely to be rejected by the human immune system. The genetic discrepancy between pigs and humans has resulted in obstacles for xenotransplantation including immunological rejection and risk of xenozoonosis. Thanks to genetically modified pigs and immunosuppressive therapy, survival time results for xenografts have improved considerably in preclinical xenotransplantation models. However, xenotransplantation remains an active ongoing research with a focus on refining genetic modifications, developing immunosuppressive regimens, and addressing safety and ethical concerns.

Main Body

The present study was carried out in accordance with the Preferred Reporting Items for Systematic Reviews and MetaAnalyses extension for Scoping Review (PRISMAScR) guidelines. English-language studies published in electronic data bases included PubMed, Scopus, and Google Scholar were retrieved using specific search themes such as organ transplantation OR xenotransplantation OR xenograft AND immune responses.. A total 112 articles were found. After exclusion based on different criteria, we screen out duplicates, and included 20 articles for this review as per the PRISMA guidelines.

Significance of xenotransplantation

Xenografting is helpful in the treatment of diseases. People with serious kidney, liver, or heart disease, diabetes, or Parkinson's disease which have defied all known treatments could be treated through xenotransplantation.

People needing bone marrow transplants could also benefit from xenotransplantation. Cellular xenotransplants for instance could treat people suffering from diabetes, Parkinson's disease, or other diseases. The treatment involves replacing specific cells or tissues which do not work properly as a result of the disease, for diabetes these cells are the islet cells of the pancreas; for Parkinson's disease, they would be brain cells. These cells are difficult to obtain from human donors. People with liver failure could be treated with an extra-corporeal (outside the body) xenotransplant using a healthy pig liver. In this process, the patient's blood circulation is made to pass through a pig liver that is kept outside the patient's body. Sometimes this is meant to be temporary until a suitable human donor is sought, but sometimes this is all that is needed to allow the person's liver to recover and start working again [5].

Xenografts give the surgeon enough time to eliminate potential pathogens. In allografting (human-to-human transplantation) organs that are usually transplanted from a brain-dead patient are given little or no time for examination to ascertain the health state of the organ, due to the urgency involved. In xenotransplantation, a donor pig is raised under controlled conditions and specifically intended for use as an organ donor. In this case, the donor pig can be extensively analyzed to eliminate all pathogens. In xenotransplantation, animal donors could be genetically modified to be resistant to many human pathogens specific to human tissues, such as HIV, hepatitis, and human cytomegalovirus. The introduction of xenotransplantation would eliminate the 'black market' in human donor organs. Due to the scarcity of human donor organs and the large number of patients on the waiting list for organ transplantation, it is believed that human organs could be procured illegally. Xenografting could save hundreds of thousands of livers. This is because, patients who otherwise would not have been eligible for transplantation because of a shortage of human organs, would receive organs and tissues through xenotransplantation. Xenotransplantation therefore could eliminate poor quality of life situations for patients, such as kidney dialysis [6].

Figure 1 Uses of xenotransplant

Despite the numerous advantages that could accrue to humans if xenografting becomes a clinical success, there are a lot of risks that are associated with xenotransplantation. These risks include: The risks of the introduction of xenosis Xenosis is the infection of humans by agents like bacteria, viruses, and fungi. The possibility of transmission of infectious agents raises questions regarding the safety of using xenotransplantation in individuals, but it could also potentially place the general public at risk. Like humans, animals may also be infected with microorganisms that could be species-specific (that is, it is not transmittable to other species). For instance, the transmissible virus of pigs causes diarrhea in pigs but does not cause any sickness in people. However, other kinds of micro-organisms are not species-specific, which means some of them can infect animals and also cause disease in humans. An example of this is influenza. The flu first infected birds and pigs and though it did not make these animals sick, when it passed to humans, it made them sick. The word xenozoonosis, therefore, refers to zoonotic diseases that may pass to humans through xenotransplant [7]. Most mammals are known to have a kind of virus embedded in their DNA known as "endogenous retroviruses." These viruses are passed from one generation to the next without causing havoc in the host species. All pigs are believed to carry such viruses called PERVs (Pig or Porcine Endogenous Retroviruses). These are normally inactive and thus do not cause disease to the pigs. The concern among scientists is that PERV may become active and infect the human cells.

The high level of immunosuppressive drugs needed to overcome immune rejection may be counterproductive. This may leave the patient susceptible to other infections. The immune system fights foreign agents that invade the body like bacteria, fungi, and viruses. Thus, suppression of the immune system would leave room for easy invasion of the body by these micro-organisms.

Xenograft Rejection

Natural killer (NK) cells play a pivotal role in the immune response to xenografts, which are transplants between different species, most commonly between animals and humans. Xenograft rejection occurs when the recipient's immune system recognizes the graft as foreign and mounts an immune response to eliminate it. NK cells are crucial components of this immune response and are involved in various aspects of xenograft rejection. NK cells are part of the innate immune system, which serves as the first line of defense against invading pathogens, including xenografts [8]. Unlike adaptive immune cells, such as T cells, NK cells do not require prior sensitization to recognize and respond to foreign entities. This innate recognition makes NK cells particularly important in the early stages of xenograft rejection.

NK cells can detect these differences and identify the graft as foreign based on their unique cell surface markers. Upon recognizing the xenograft as foreign, NK cells can release cytotoxic molecules, such as perforin and granzymes, to induce apoptosis in the graft's cells. This direct killing of graft cells is a key mechanism of xenograft rejection. NK cells are potent producers of cytokines, such as interferon-gamma (IFN-γ) [9]. These cytokines could further activate other immune cells, including macrophages and dendritic cells, to enhance the immune response against the xenograft. IFN-γ, in particular, can up-regulate the expression of MHC class I molecules on graft cells, making them more susceptible to immune recognition and attack [9]. In the presence of antibodies against the graft, NK cells can engage in antibody-dependent cell-mediated cytotoxicity (ADCC). They can bind to the Fc portion of antibodies attached to the graft's cells and subsequently induce cell lysis (Cooper, 2023). This mechanism is particularly relevant when there is pre-existing or de novo antibody production against xenograft antigens. NK cells can also influence the adaptive immune response to xenografts. By eliminating graft cells or releasing regulatory cytokines, NK cells can indirectly modulate the activation and function of alloreactive T cells, which play a crucial role in xenograft rejection [10].

NK cells may be involved in the chronic rejection of xenografts by contributing to ongoing inflammation and tissue damage. Their prolonged activation can lead to fibrosis and graft dysfunction over time. Understanding the role of NK cells in xenograft rejection is crucial for developing strategies to mitigate their impact. Various approaches, including the use of immunosuppressive drugs, genetic modifications of the graft to reduce NK cell recognition and the development of tolerance-inducing protocols, are being explored to improve the success of xenotransplants and reduce NK cell-mediated rejection.

Acute Humoral Xenografts Rejection

Acute humoral xenograft rejection, as the name implies, is primarily mediated by humoral (antibody-based) immune responses. The vital immune cells and processes involved in acute humoral xenograft rejection include:

1. B Cells: B lymphocytes are a type of white blood cell that plays a central role in antibody production. In acute humoral rejection, B cells are activated and differentiate into plasma cells, which are responsible for

producing antibodies [10].

2. Antibody Production: Antibodies, or immunoglobulins, are proteins produced by plasma cells in response to the presence of foreign antigens on the surface of the transplanted xenograft. These antibodies, which can be IgM or IgG antibodies, specifically recognize and bind to these antigens.

3. Antibody Binding: The produced antibodies bind to the antigens present on the xenograft's endothelial cells and other structures. This antibody binding is a critical step that marks the graft as foreign to the recipient's immune system.

4. Complement Activation: The binding of antibodies to the xenograft's antigens can trigger the activation of the complement system. Complement proteins, when activated, can form membrane attack complexes (MACs) on the endothelial cells of the graft's blood vessels [11].

Chronic Xenograft Rejection

Chronic xenograft rejection, also known as chronic rejection in xenotransplantation, refers to the immune-mediated rejection of a xenograft, which is the transplantation of organs or tissues from one species to another [12]. Chronic rejection is a long-term process that occurs over an extended period after the xenotransplant. It is characterized by a gradual deterioration of the transplanted organ's function due to various immune and non-immune factors [12]. The exact mechanisms of chronic xenograft rejection are not fully understood, but they are thought to involve a combination of immune responses and factors like vascular changes, fibrosis, and tissue damage. The immune response in chronic xenograft rejection can involve the recipient's immune system recognizing and attacking the foreign animal tissue [15]. This immune response can lead to inflammation and tissue damage over time, ultimately causing the loss of function of the transplanted organ. Strategies to prevent chronic xenograft rejection may include immunosuppressive medications, genetic engineering of donor animals to make their tissues less immunogenic, and other approaches to improve the compatibility of xenografts with the human recipient's immune system.

Mechanism of Chronic Xenograft Rejection

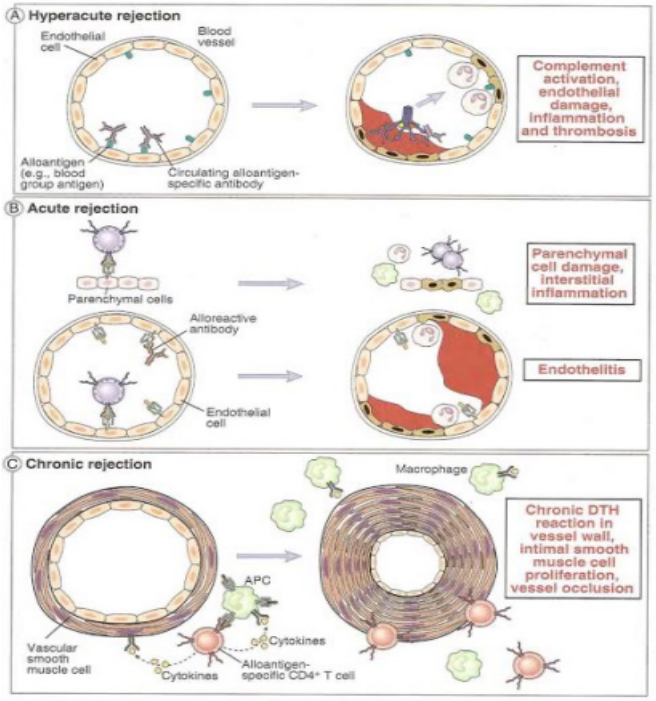
Chronic xenograft rejection is a complex and multifactorial process that involves various mechanisms, both immune and non-immune, leading to the gradual deterioration of a transplanted animal organ or tissue in a human recipient [14]. While the exact mechanisms are not fully understood, several key factors play a role in this process:

The recipient's immune system, particularly T lymphocytes, recognizes the animal organ as foreign. Over time, T cells mount an immune response against the xenograft, leading to inflammation and tissue damage. B cells can produce antibodies against animal-specific antigens (e.g., alpha-gal epitopes) present on the xenograft's surface [15]. These antibodies can lead to chronic vascular and tissue damage. Complement proteins, activated by the binding of antibodies to the xenograft, can contribute to tissue injury and inflammation.

Xenografts often suffer from endothelial injury and dysfunction. This can result in increased blood clot formation, platelet activation, and vascular constriction [13]. chronic endothelial activation and inflammation can lead to the narrowing of blood vessels, reducing blood flow to the transplanted organ. Chronic inflammation within the xenograft can trigger the deposition of collagen and other extracellular matrix components, leading to fibrosis which stiffens the tissue, impairs its function, and ultimately contributes to organ failure [16].

Xenografts can trigger innate immune responses, such as the activation of natural killer (NK) cells and macrophages, which can cause tissue damage and further inflammation. The xenograft may release danger signals (damage-associated molecular patterns or DAMPs), which activate the innate immune system[15] Some animal organs may not function optimally in a human host due to differences in anatomy, size, or physiology. The transplantation process itself can cause tissue damage, leading to inflammation and oxidative stress [2]. Xenografts from animals may age differently than human organs, affecting long-term function.

Figure 1. (a) Direct and indirect recognition of Allo antigens. (A) Direct alloantigen recognition occurs when T cells bind directly to intact allogeneic MHC molecules in professional APCs in a graft, as illustrated.



(B) Indirect alloantigen recognition occurs when allogeneic MHC molecules from graft cells are taken up and processed by recipient APCs, and peptide fragments of the allogenic MHC molecules are presented by recipient (self) MHC molecules. Recipient APCs may also process and present graft proteins other than allogeneic MHC molecules. (b) Recognition of allogeneic MCH molecules by T lymphocytes. Recognition of allogeneic MHC molecules may be thought of as a cross-reaction in which a T cell-specific for a self MHC molecule-foreign peptide complex (A) also recognizes an allogeneic MHC molecule whose structure resembles that of a self MHC molecule- foreign peptide complex (B and C). Peptides derived from the graft (labeled "donor peptides") may not contribute to allorecognition (B), or they may form part of the complex that the T cell sees (C). The type of T cell recognition depicted in (B) and (C) is called direct allorecognition. Reproduced with permission from Abbas and Lichtman.

T-Cell Mediated Rejection

T cells play a crucial role in mediating xenograft rejection, which is primarily a cell-mediated immune response. T cells, specifically CD4+ and CD8+ T cells, are responsible for recognizing and responding to foreign antigens on the xenograft. These antigens are presented by antigen-presenting cells (APCs) like dendritic cells. CD4+ T cells recognize peptide antigens presented on MHC class II molecules, while CD8+ T cells recognize antigens presented on MHC class I molecules. Upon recognition of xenogeneic antigens, T cells become activated. This activation leads to their proliferation and differentiation into effector T cells. CD8+ T cells, also known as cytotoxic T lymphocytes (CTLs), are

crucial for the direct destruction of xenogeneic cells. They release cytotoxic molecules like perforin and granzymes to induce apoptosis in the graft cells expressing foreign antigens. This process helps eliminate the xenograft. CD4+ T cells, often referred to as helper T cells, play a supportive role by releasing cytokines that further activate the immune response. They help coordinate the immune reaction by interacting with B cells, macrophages, and other immune cells. Some T cells that are activated during the initial immune response become memory T cells. These cells "remember" the xenogeneic antigens and remain in the recipient's body, allowing for a more rapid and robust response if a subsequent xenograft is introduced. The T cell-mediated xenograft rejection process involves the release of pro-inflammatory cytokines, recruitment of additional immune cells, and inflammation at the graft site. The ultimate goal is to eliminate the xenogeneic tissue to prevent harm to the recipient.

Strategies To Mitigate Immunological Responses Genetic engineering of donor animals. The use of pigs with one or more immune-related genes modified has made a significant advance in solid organ transplantation from pigs to other large animals and has resulted in remarkably long survival times for both recipients and grafts [17]. The use of genetically modified pigs has greatly addressed the immune barriers to xenotransplantation. Hyperacute rejection and acute cellular rejection have been nearly overcome [18], and results from these studies have greatly contributed to research on acute vascular rejection and chronic rejection. Genetically modified pigs can be created by deleting several pig genes related to the synthesis of various pig-specific antigens or by inserting human complement- and coagulation-regulatory transgenes. One approach is to genetically modify donor animals to knock out or replace certain genes that are responsible for the expression of antigens recognized by the recipient's immune system. For example, by inactivating or replacing genes associated with the production of galactose-alpha-1,3-galactose (Gal), which is a major target for xenograft rejection, it's possible to reduce the risk of hyperacute rejection [13]. Genetic engineering can be used to introduce human genes into the donor animals. This includes the expression of human complement regulatory proteins (e.g., complement regulatory protein (CD46), complement decay accelerating factor (CD55), CD59 also known as MAC inhibitory protein) to protect the transplanted organ from complement-mediated damage [19]. Additionally, the expression of human coagulation factors can help address coagulation-related issues in xenografts.

Recent advances in genome editing techniques, such as CRISPR-Cas9, offer precise control over gene modifications [20]. This technology can be employed to make targeted changes in the donor animal's genome, ensuring that the transplanted organ is less likely to be rejected by the recipient. Genetic modifications can also be used to induce immunomodulatory effects in the donor animals. For example, the expression of immune checkpoint inhibitors or immunosuppressive molecules can dampen the recipient's immune response to the xenograft. Genetic modifications may aim to promote immune tolerance in the recipient. This can be achieved through the expression of regulatory T cell (Treg)--promoting factors in the donor animal [21].

Immunosuppressive Medications

Immunosuppressive medications play a crucial role in preventing xenograft rejection, which is the immune response mounted by the recipient's body against transplanted organs or tissues from a different species [22]. In the context of xenotransplantation, where organs or tissues from animals are used for transplantation into humans, the immune response can be particularly strong due to the foreign nature of the graft. Immunosuppressive drugs are essential to manage this immune response and improve the chances of a successful xenograft. Immunosuppressive drugs, such as calcineurin inhibitors (cyclosporine and tacrolimus), work by inhibiting the activation of T-cells, which are key players in the immune response [23]. By preventing T-cell activation, these drugs reduce the recipient's immune system's ability to recognize and attack the xenograft as foreign [24].

Immunosuppressive medications also target other immune cells and molecules involved in the inflammatory response, including cytokines and antibodies. This dampening of inflammation helps to minimize the immune attack on the transplanted xenograft. Xenotransplants can trigger the production of antibodies directed against antigens from the donor animal, which can lead to graft rejection. Immunosuppressive drugs like mycophenolate mofetil can inhibit the production of antibodies, reducing the risk of hyperacute or acute antibody-mediated rejection. Xenotransplants often require more intense and prolonged immunosuppressive therapy compared to allografts (transplants between individuals of the same species). Maintenance of immunosuppression is necessary to prevent chronic rejection, which can occur over time due to ongoing immune responses. To achieve the best results in xenotransplantation, a combination of immunosuppressive drugs is often used. This approach allows for lower doses of individual drugs, reducing the risk of side effects while providing comprehensive immune suppression.

Immunosuppressive therapy in xenotransplantation requires careful monitoring of the recipient's immune status and graft function. The dosage and combination of drugs may need to be adjusted based on the individual's response to treatment.

It's important to balance the benefits of immunosuppression with potential side effects, including an increased risk of infection, cancer, and metabolic disorders [25]. Clinicians must carefully manage these risks to ensure the overall well-being of the transplant recipient. While immunosuppressive medications are essential for preventing xenograft rejection, there are challenges and considerations. The long-term use of these drugs carries risks, and there's a need for ongoing research to develop more targeted and specific immunosuppression strategies, minimizing side effects and improving the safety and efficacy of xenotransplantation.Side Effects of Immunosuppressive Drugs.

Some of the risks of immunosuppressive medications are:

Immunosuppressive drugs weaken the recipient's immune system, making them more susceptible to infections. This includes bacterial, viral, and fungal infections. Opportunistic pathogens that may not normally cause illness in healthy individuals can become serious threats to transplant recipients.

Long-term use of immunosuppressive medications can increase the risk of developing certain cancers, particularly skin cancers, lymphomas, and other malignancies [26]. The immune system plays a crucial role in identifying and destroying cells that have become cancerous, and suppressing the immune response can allow these cells to proliferate. Some immunosuppressive drugs, like corticosteroids [12], can lead to metabolic disorders such as diabetes and high blood pressure. These conditions can have a significant impact on the overall health of the recipient.

Certain immunosuppressive medications may contribute to cardiovascular complications, including hypertension and elevated cholesterol levels, which can increase the risk of heart disease (Thurman et al. 2020). Many immunosuppressive drugs are processed by the kidneys and can potentially lead to kidney dysfunction or damage over time, a condition known as nephrotoxicity.

Some recipients of immunosuppressive medications may experience neurological side effects, including mood changes, depression, and cognitive impairment. Immunosuppressive drugs can affect the bone marrow's ability to produce blood cells, potentially leading to anemia, low platelet counts, or low white blood cell counts.

Medications like corticosteroids may cause gastrointestinal problems, including gastritis, ulcers, or gastrointestinal bleeding [15]. In addition to the above, there are specific risks associated with the particular type of immunosuppressive medications used in xenotransplantation, such as interactions with other drugs, side effects related to their mechanisms of action, and individual patient variability in drug response.

Biomarkers For Xenograft Rejection

Table 1. Biomarkers for xenograft rejection

Biomarker classification	Biomarker	Sample type	Application
Intra-graft biomarkers	C4d	Pig-to-human kidney	Marker of inflammation
	CD68	Pig-mouse models	Identify macrophage infiltration
	CD3	Pig-mouse models	Identify T-cell infiltration
	NK1.1 and DX5	Peritoneal mouse cells	Identify NK cell infiltration
	TLR2 mRNA and protein (↑)	Porcine iliac artery endothelial cells	Marker of immune rejection
	CCL2 and CXCL8 (↑)	Porcine cells	Marker of immune rejection
Serum biomarkers	Non-α-Gal IgM and IgG antibodies (↑)	Pig-to-human kidney	Marker of immune rejection
	cDNA	Pig-mouse models	Increased levels precede immune
	cfDNA	Pig-to-baboon hearts	rejection
	ssc-miR-199b	Liver, heart, and lung	Predicts transplant prognosis
	miR-146a (↓)	Mouse-to-rat cardiac models	Marker of immune rejection,
	miR-155 (↑)	Mouse-to-rat cardiac models	the potential target of immunotherapy
	C3 (↑)	Pig-to-non-human cornea	Increased before tissue rejection

Keywords: NK: natural killer; TLR2: toll-like receptor-2; cpsDNA: circulating pig-specific DNA; cfDNA: cell-free DNA; mRNA: messenger ribonucleic acid; CCL2: C–C motif chemokine ligand-2; CXCL8: C-X-C motif chemokine ligand-8; DNA: deoxyribonucleic acid.

Clinical Trials and Progress

In xenotransplantation, the choice of the donor species is crucial. Pigs are commonly used as donors due to their physiological and anatomical similarities to humans. Recipients undergo thorough medical assessments to determine their suitability for the transplant. Compatibility and potential risks, such as xenozoonoses (infectious diseases that can be transmitted from animals to humans), are assessed. Since the recipient's immune system may recognize the xenograft as foreign tissue, immunosuppressive drugs are often used to prevent rejection. The actual transplantation procedure involves removing the donor organ or tissue and implanting it into the recipient. Post-transplant, recipients are closely monitored for signs of rejection or other complications. This may involve various clinical tests and imaging. In September 2020, at the University of Alabama at Birmingham, two genetically modified porcine kidneys were transplanted into a human brain-dead decedent [27]. Months later, another similar investigational xenotransplant surgery procedure was performed at New York University, using the genetically engineered kidneys from a pig without an alpha-gal gene, which is responsible for a rapid antibody-mediated rejection of porcine organs by humans [28].

In 2022, xenograft eams independently transplanted genetically modified porcine kidneys into brain-dead patients. The porcine kidneys remained viable and functional in the recipients for 54 hours and 74 hours, respectively, with no significant signs of acute rejection. In 2022, researchers transplanted two kidneys from a pig with 10 gene edits into brain-dead patients. The transplanted porcine kidneys produced urine and showed no significant HAR in the 74-hour experiment. However, it failed to clear creatinine and suffered thrombotic microangiopathy.

In January 2022, a 57-year-old patient with terminal heart disease underwent the First successful xenotransplant of a genetically modified pig heart at the Maryland Medical Center. Although ultimately succumbing to heart failure associated with multiple factors, the patient survived for two months. The autopsy result highlighted the transmission of porcine cytomegalovirus (pCMV) as a primary factor associated with the outcome. In addition, increasing amounts of antibodies against the donor pig were detected in the patient's blood over the last month of his life, suggesting that the immunosuppressive treatment regimen used, which was minimized because of recurrent infectious complications, may not have been sufficient to prevent immune injury to the xenograft [29].

On the positive side, they demonstrated that a pig heart can sustain human life for at least one month in a patient who was already in a physiologically depleted state. On the negative side, the findings revealed the harm of conveying pCMV in the grafted organ. This case underscores the attention to detail necessary in the care and screening of donor animals, and the importance of patient selection, to allow fair evaluation of this new
technology.

Alternative Xenotransplantation Models

Alternative xenotransplantation models refer to approaches and models other than the use of pigs as organ donors for human transplantation. These alternatives aim to overcome the challenges and ethical concerns associated with xenotransplantation while providing viable solutions for organ transplantation. Here are some of the alternative xenotransplantation models and approaches:

1. Non-Human Primates (NHPs):

NHPs, such as macaques and baboons, are often used as alternative xenotransplantation models due to their genetic proximity to humans. They provide valuable insights into organ transplantation and immunological responses [30]. While NHPs can simulate the human response to some extent, they are limited in supply and raise ethical concerns due to their close relationship with humans.

2. Gene-Edited Animals:

Instead of using traditional xenotransplantation models, researchers are exploring the use of gene-edited animals, like pigs with specific genetic modifications [30]. These animals are designed to have reduced immunogenicity and better compatibility with human recipients. Gene-edited animals can serve as alternative models to study organ transplantation and rejection while addressing some ethical concerns.

3. Organ Decellularization and Recellularization:

This approach involves taking organs from animals and removing their cells, leaving behind a scaffold.Human cells are then recellularized onto this scaffold, creating a personalized organ that is less likely to be rejected. This technique, although not strictly xenotransplantation, offers an alternative by using animal organs as a scaffold.

4. Tissue Engineering and 3D Printing:

Tissue engineering and 3D printing technologies are advancing rapidly, allowing researchers to create custom-made organs using a patient's cells.

This approach reduces the need for xenotransplantation by focusing on growing human organs from scratch.

5. Stem Cell-Derived Organs:

Researchers are investigating the potential of using induced pluripotent stem cells (iPSCs) to generate transplantable organs.
iPSCs can be differentiated into various cell types, including those necessary for organ construction.

6. Xenogeneic Cell Transplantation:

Rather than whole organs, some studies are exploring the transplantation of specific cells or tissues from animals to humans. This may include islet cell transplantation from pigs to treat diabetes or other targeted applications [30].

7. In-Vitro Organ Models:

In vitro models, such as organ-on-a-chip and organoids, offer alternatives for studying organ function and disease without the need for animal or human organs.These alternative models provide researchers with various tools to study transplantation biology, immunology, and regenerative medicine while mitigating some of the challenges associated with traditional xenotransplantation. Each approach has its advantages and limitations, and the choice of model depends on the specific research goals and ethical considerations.

Advantages of Alternative Xenotransplantation Models:

1. Immunological Understanding: These models provide valuable insights into the immunological aspects of transplantation without the need for animals, helping researchers better understand immune responses.

2. Customization: Techniques like organ decellularization and recellularization allow for the creation of personalized organs using the patient's cells, reducing the risk of rejection.

3. Regenerative Medicine: Stem cell-derived organs and tissue engineering are at the forefront of regenerative medicine, offering potential solutions for organ replacement that are not reliant on animal donors.

4. Reduced Zoonotic Risk: Alternative models reduce the risk of zoonotic diseases, as there is no direct interaction with animal organs.

Challenges of Alternative Xenotransplantation Models:

1. Complexity: Many alternative models, such as 3D printing and tissue engineering, are still in the experimental stage and require further development before they can be used for clinical transplantation.

2. Immunological Challenges: Even in alternative models, immune responses and rejection can still be significant challenges, particularly in personalized organ engineering.

3. Regulatory Approval: Developing and gaining regulatory approval for these innovative techniques can be a time-consuming and challenging process.

4. Cost and Accessibility: Some alternative models, especially those involving cutting-edge technologies like 3D printing, can be expensive and may not be accessible to all patients.

5. Long-Term Viability: Ensuring the long-term viability and functionality of alternative models, such as 3D-printed organs, remains a substantial challenge.

6. Limited Availability: Availability and practicality may be limited for some alternatives. For instance, not all patients may have suitable cells for personalized organ generation of Preclinical Models: Unlike pig-to-non-human primate xenotransplantation, alternative models may lack well-established preclinical animal models for testing.

7. Public Acceptance: Acceptance and adoption of these innovative techniques may vary among patients, and public perception can be a significant factor.

Recent Advances in Xenotransplantation

(A) Genetic modification engineering in xenotransplantation involves the deliberate alteration of the genetic material of donor animals, typically pigs, to produce organs that are more compatible with human recipients. This sophisticated approach is aimed at overcoming the immunological barriers that have historically hindered the success of xenotransplantation.

1. Galactose-α-1, 3-Galactose (Gal) Knockout

One major hurdle in xenotransplantation is the hyperacute rejection caused by the presence of the Gal antigen. Genetic engineering has been used to knock out the gene responsible for producing this antigen in pigs, which are commonly considered donor animals.

2. Humanizing Pig Organs

To make pig organs more compatible with the human immune system, researchers have introduced human genes involved in immune regulation. This helps in reducing the likelihood of rejection and improving the overall acceptance of the transplanted organ.

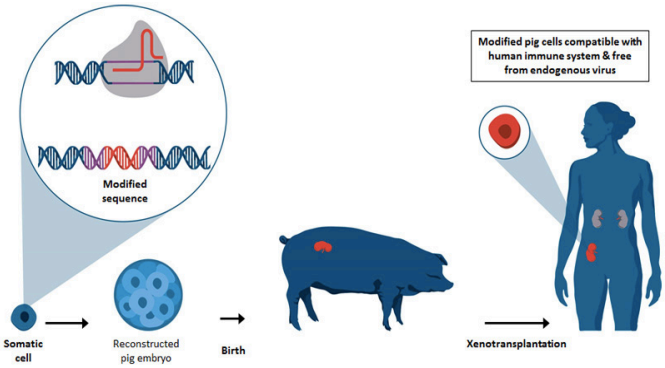
3. Transgenic Pigs with Complement Regulatory Proteins

Complement system activation is a significant contributor to organ rejection. Genetic modifications involve introducing genes that produce human complement regulatory proteins in pigs. This helps in modulating the immune response and preventing rejection.

4. Virus-Resistant Pigs

Concerns about the potential transmission of porcine viruses to humans have led to genetic modifications aimed at making pigs resistant to specific viruses. This enhances the safety of xenotransplantation by minimizing the risk of cross-species viral infections. (B) CRISPR/Cas9 Technology in XenotransplantationCRISPR/Cas9 (clustered regularly interspaced short palindromic repeats linked to Cas nuclease) essentially describes a replicate of a virus 'built' into the genetic code of bacteria within an organism so it can recognize it and come up with its immune response to protect itself against said virus [31]. This immune response comes in the form of a CRISPR/Cas- Complex made out of RNA molecules and Cas proteins, the RNA molecules serve to identify and find the virus while the Cas proteins splice it, thus rendering the attacking virus useless CRISPR/Cas9 has revolutionized genetic engineering and has shown great promise in addressing specific challenges in xenotransplantation. CRISPR/Cas9 gene editing technology has significantly accelerated the development of multi-gene-modified pigs to address the major immunological and physiological incompatibilities between pigs and humans. These gene edits include the knockout (KO) of the three porcine-specific glycan epitopes responsible for hyperacute rejection and human transgene expression targeting the coagulation and complement pathways. The first pigs modified against HAR (using the CRISPR/Cas9 system) with a triple knock-out of GGTA1, CMAH, and β4GalNT2 were obtained in 2015.

Figure 2 Shows the basic theoretical steps from the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) to an edited cell to a successful transplantation.



Here's an overview of how CRISPR/Cas9 is being utilized in the field of xenotransplantation

1. Galactose-α-1, 3-Galactose (Gal) Knockout:
CRISPR/Cas9 has been employed to precisely target and knock out the gene responsible for the expression of the Gal antigen in pig cells. This modification helps overcome the hyperacute rejection observed in xenotransplantation due to the human immune system's response to this antigen.

2. Immune System Modification
CRISPR/Cas9 allows for the precise editing of pig genes related to the immune system. By incorporating or modifying genes associated with immune tolerance in humans, researchers can reduce the likelihood of rejection when pig organs are transplanted into humans.

3. Precise Gene Editing for Compatibility
Researchers use CRISPR/Cas9 to make specific edits to pig genes associated with organ compatibility. This targeted approach allows for the customization of pig organs to be more similar to human organs, reducing the risk of rejection and improving overall transplant success rates.

4. Virus Resistance
CRISPR/Cas9 can be used to engineer pigs with resistance to specific viruses, addressing concerns about the potential transmission of porcine viruses to humans. This enhances the safety of xenotransplantation and minimizes the risk of viral infections.

5. off-Target Effects Mitigation
As with any gene-editing technology, there is a risk of off-target effects. Researchers in xenotransplantation use advanced CRISPR/Cas9 techniques and bioinformatics tools to minimize these off-target effects, ensuring the safety and precision of genetic modifications.

6. Accelerated Research and Development

CRISPR/Cas9 expedites the research and development process by providing a faster and more efficient way to make genetic modifications. This acceleration is crucial in addressing the urgent need for viable solutions to the shortage of organs for transplantation.

Ethical Considerations

With the power to make precise and extensive genetic modifications, CRISPR/Cas9 in xenotransplantation raises ethical considerations. Issues such as the potential unintended consequences of gene editing and the creation of designer organs prompt discussions about the responsible use of this technology.
Ethical And Regulatory Considerations
Establishing clear protocols for transparent reporting of research findings, including both successes and failures, to foster scientific accountability and maintain public trust. Institutional Review Boards (IRBs) rigorously evaluate xenotransplantation protocols to ensure that research adheres to ethical standards, patient safety is prioritized, and potential risks are minimized. Engaging the public through open forums, educational campaigns, and dialogue to address concerns, gather input, and incorporate diverse perspectives in decision-making processes.

Ethical evaluation of the potential benefits of xenotransplantation, such as addressing organ shortages, against the risks of unknown zoonotic infections.

(1) Animals are not objects, they are living beings. Pigs shouldn't be considered as donors because the definition of donation involves voluntarily giving something [31]. Critics argue that, as they are not fit to communicate (or even develop) their own decisions they should not be exploited in such a way. Additionally, their lives would be limited to a confined, sterile location to prevent any epigenetic changes (Olivia, 2018). An animal-friendly alternative could be to find a way to grow entire human organs ex-vivo (outside the living organism) from the patient's tissue for autotransplantation.

2) Religious aspects. Many religions such as mainstream branches of Islam forbid the slaughtering of pigs for personal use. Because there are 1.6 billion Muslims worldwide, pig/human xenotransplantations would be limited to non-muslim countries.

3) Risk of disease transmission. Concerns are indicating, that with the transplantation of animal cells, there would also be a high risk of transferring diseases or retroviruses which are harmless for pigs but potentially life-threatening to humans.

4) Risk of rejection. There is a high likelihood that the immune system of a patient would directly attack the newly implanted organs, causing the operation to fail. This would bring the patient in a situation risking his life, with the possibility of death.

Conclusion

Xenotransplantation have the potential to provide for patients with organ failure with improved quality of life. Modifying xenotransplants to suit individual patient's needs, through genetic modification could reduce the risk of graft rejection.

Summary key points and abbreviations

AHXR: acute humoral xenograft rejection
AMR: antibody-mediated rejection
CRISPR: clustered regularly interspaced short palindromic repeats
HAR: hyperacute rejection
NHPs: nonhuman primates
WBC: white blood cell
UAB: University of Alabama at Birmingham
UMB: University of Maryland, Baltimore
PERVEs: porcine endogenous retroviruses
NK: natural killer cells
ACP: Antigen-presenting cells
CTLS: cytotoxic t lymphocytes
HLAS: human leukocyte antigen
IFN: interferon gamma
ADCC: antibody-dependent cell-mediated cytotoxicity
GAL: galactose
pCMV: porcine cytomegalovirus infection
HCD46: human complement regulatory protein
IPSCS: Induced pluripotent stem cells

Acknowledgments
The authors acknowledged the Bingham University for accessing free data services.

Conflict of Interest
Nothing to declare

Funding: None

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