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Review

Current progress in xenotransplantation and organ bioengineering

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HIGHLIGHTS

- Xenotransplantation and bioengineering could solve the donor organ shortage crisis.
- Bioengineered organs and xenografts are challenged by immunobiological barriers.
- Further work is needed to generate transplantable complex organs or xenografts.

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ABSTRACT

Organ transplantation represents a unique method of treatment to cure people with end-stage organ failure. Since the first successful organ transplant in 1954, the field of transplantation has made great strides forward. However, despite the ability to transform and save lives, transplant surgery is still faced with a fundamental problem the number of people requiring organ transplants is simply higher than the number of organs available. To put this in stark perspective, because of this critical organ shortage 18 people every day in the United States alone die on a transplant waiting list (U.S. Department of Health & Human Services, http://organdonor.gov/about/data.html). To address this problem, attempts have been made to increase the organ supply through xenotransplantation and more recently, bioengineering. Here we trace the development of both fields, discuss their current status and highlight limitations going forward. Ultimately, lessons learned in each field may prove widely applicable and lead to the successful development of xenografts, bioengineered constructs, and bioengineered xeno-organs, thereby increasing the supply of organs for transplantation.

1. Introduction

Regenerative medicine and xenotransplantation share the same goal – that of replacing diseased organs with newly functioning ones. Both of these fields have the potential to mitigate the ever-growing demand for transplantable organs and reduce waiting list mortality by creating a new, inexhaustible supply of organs.

2. History of clinical xenotransplantation

Clinical animal-to-human solid organ transplants have been performed on a number of occasions in critical situations (Table 1). In 1964, James D. Hardy attempted a chimpanzee to human heart transplant [1]. Subsequently Christiaan Barnard's team performed heart xenotransplants using baboon and chimpanzee donors [2]. In these early cases patients survived hours to days, with death attributed to the small size of primate hearts being unable to support the circulation of adult humans. Other cases highlighted the immunological barriers that had to be overcome for xeno-transplants to work. Notably, a team lead by Leonard L. Bailey in California performed a heart transplant on Baby Fae, in which size was not an issue but the graft failed at 20 days due to humoral rejection [3]. Despite the poor outcomes in initial xenotransplants,

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Table 1
Milestones in clinical xenotransplantation.

Year	Donor	Patient survival	Surgeon	Refs.
1963/64 (13×)	Chimpanzee kidneys	Up to 9 months	Keith Reemtsma	[4]
1963/64 (6×)	Baboon kidneys	19 to 98 days	Thomas Starzl	[5]
1964	Chimpanzee heart	2 h	James Hardy	[1]
1977 (2×)	Baboon and chimpanzee hearts	6 h and 4 days	Christiaan Barnard	[2]
1984	Baboon heart	20 days	Leonard Bailey	[3]
1992	Baboon liver	70 days	Thomas Starzl	[6,7]

a few cases provided optimism that this form of organ replacement could work. In a series of chimpanzee-to-human kidney transplants performed by Keith Reemtsma, a patient was reported to have survived for nine months [4]. Thomas E. Starzl's group reported the survival 19–98 days in 6 cases of baboon-to-human transplantation [5]. In 1992, Starzl and colleagues reported 70 day survival in baboon-to-human liver xenotransplants. [6,7]

3. Optimal donor species for xenotransplantation

While non-human primates are phylogenetically the most similar to humans, the use of these animals as xenotransplant donors has several drawbacks. The most pertinent of these include their small sizes, infection risks, long gestation and growth periods and ethical concerns. For these reasons, swine are currently considered the most likely xenotransplant donors. Swine are easy to breed, can be produced in germ-free conditions, their organs reach a size that can provide life-supporting functions to human recipients and the ethical barriers precluding nonhuman primate research are present to a lesser degree. Furthermore, swine can be genetically manipulated to permit immune challenges to be overcome. However, there are still major obstacles before swine can be successfully used as a source of organs. First, the immunological responses of the recipient against the graft need to be controlled. These include hyperacute rejection (characterized by binding of naturally-occuring xenoreactive antibodies that trigger the complement cascade), acute antibody-mediated rejection (acute humoral xenograft rejection, AHXR), acute cellular rejection, and chronic rejection. Second, xenografts may have physiological limitations, such as molecular incompatibilities in the coagulation system, which preclude their use in providing functional replacement of a failing organ. Third, risk of transmissible infections such as porcine endogenous retroviruses (PERVs) affecting humans. To date there has been no case where PERVs have caused an infection in humans that were exposed to porcine tissues [8]. However, pig cells can transmit PERV to human cells *in vitro* [9], which makes it mandatory that all potential recipients of porcine organs and cells, as well as their families be monitored closely. The risk of contracting West Nile virus, rabies and HIV from swine organs is considered vanishingly low [10].

4. Overcoming the immune response to pig xenotransplants by genetic engineering

Unlike the rejection of allografts, which is mainly governed by T cells, the immune response to porcine xenografts primarily occurs hyperacutely, mediated by pre-formed, natural antibodies to the Galactosyl-alpha(1,3)galactose (Gal) epiotope that is highly expressed on porcine endothelium. Knocking out the gene for α1,3-galactosyltransferase prevents the expression of the Gal epitope on porcine tissues. This helped overcome hyperacute rejection in pig-to-primate studies [11]. Despite this, non-Gal antibodies may still activate the porcine endothelium, leading to microvascular thrombosis and graft loss. Byrne and colleagues identified many non-Gal antigens on porcine endothelium which are members of the heat shock protein family [12]. If the next generation of genetically modified pigs can address these non-Gal antigens, another significant step towards controlling the humoral response to xenotransplants will be made.

Aside from the humoral response, efforts have also been made to control the innate immune response to xenotransplantation by engineering pigs that: 1) express human CD47, a marker for "self", to prevent organ damage caused by macrophages [13,14]; 2) express the inhibitory receptor HLA-E to control the NK cell response [15]; or 3) express complement regulators, such as human decay accelerating factor (hDAF) [16] or membrane cofactor protein (hCD46). However, more important for graft survival was the supraphysiologic expression of these complement regulators rather than the fact that they were human [17].

Thrombotic microangiopathy caused by immunologic mechanisms is compounded in the xenotransplant setting due the molecular incompatibilities in the coagulation cascade between pig and primate. To overcome this, efforts are underway to allow for the expression of human anticoagulants on pig endothelium. These include expression of human CD39 to inhibit platelets, human thrombomodulin to allow activation of the human anticoagulant protein C and tissue factor pathway inhibitor to prevent the initiation of the extrinsic pathway of coagulation [18].

5. Immunosuppression protocols for xenotransplantation

Immunosuppression in preclinical models of xenotransplantation usually consists of B-cell and plasma cell therapeutics like Rituximab and Bortezomib in addition to the standard triple drug immunosuppression [19]. For example, peritransplant B-cell depletion using 4 weekly doses of anti-CD20 antibody, along with ATG, anti-CD154 and MMF-based immunosuppressive regimen resulted in prolonged survival of Gal-knock out (Gal-KO)/human CD46 transgenic pig cardiac xenografts (up to 236 days) [20]. One or more rounds of immuno-adsorption or plasmapheresis are necessary to remove antibodies from the recipient's circulation. These regimens are often associated with severe side effects like pancytopenia and sepsis.

Considering that systemic immunosuppression needs to be higher in xenotransplantation than in allotransplantation, another strategy to counteract this effect is to express immunosupppressive molecules like CTLA-4Ig on the pig endothelium [21]. However, the step-wise approach of gene knock-in or gene knock-out is limited in scope; kidneys from pigs who were Gal-KO and transgenic for human CD55 (hCD55), hCD59, hCD39, and fucosyl-transferase (hHT) showed limited improved survival in baboons [22].

An alternative but much more complex approach is to try to achieve immunological tolerance to the xenograft. In clinical allotransplantation, the tolerance approach has already proven to be successful by achieving transient mixed hematopoietic chimerism by donor bone marrow co-transplantation with kidney allografts. A number of patients at Massachusetts General Hospital have been living immunosuppression-free for several years now after receiving their kidney along with bone marrow of the donor [23,24]. Apart from generating bone marrow-based chimerism, another tolerance approach would be to transplant xenogeneic thymus to promote recipient thymopoiesis, which could induce T cell tolerance to solid organ xenografts. Indeed, based on this approach, Yamada and colleagues have reported over 80 day survival of a life-supporting kidney transplant in a pig-to-primate model [25,26].