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# Biosafety Barrier to Xenotransplantation

*Wei Wang, Qi Liang, Wei Nie, Juan Zhang and Cheng Chen*

## Abstract

Biosafety barrier is most important for xenotransplantation clinical trial. Source animals used in xenotransplantation should be bred in a closed herd and raised in a well-controlled, pathogen-free environment with high standards of animal welfare. To ensure the source animals' freedom from known pathogens under adequate biosecurity and surveillance, extensive tests must be done. Biosafety of DPF source pig should be proved by animal model before clinical trial. In addition, inclusion criteria for transplant recipients and clinical safe transplantation protocol should be established. Comprehensive anti-immune rejection treatment based on immune tolerance program can significantly prolong the xenograft survival and reduce the adverse impact on the immune system, which is suitable for clinical application. According to the clinical follow-up plan of the xenograft recipients, the patients should come back to the hospital for a check at regular intervals after the transplantation. The database of clinical trials for xenotransplantation should be established, including specimens, paper documents, and electronic documents. The information and samples of xenotransplantation donors and recipients should be preserved for long time.

**Keywords:** biosafety barrier, donor animal, xenotransplantation, clinical trial

## 1. Introduction

The demand for a new source of organs and cells for clinical transplantation has been exacerbated for decades. And xenotransplantation (e.g., from pigs to human) could resolve this issue.

In 2008, the WHO and International Xenotransplantation Association (IXA) released a consensus statement on xenotransplantation from pig to human for clinical trials. In this statement, it proposed the criterion for biosafety of source animals in clinical trials. The source animals should be bred in a closed herd for the purpose and kept under a well-controlled and pathogen-free environment with complete animal welfare. Even source animals are housed in appropriate biosecurity and under surveillance, extensive detection must be done to ensure freedom from known pathogens and infectious disease.

Therefore, this chapter will draw attention to the significant biosafety barriers need to be overcome before xenotransplantation from pig to human can become a clinical therapy.

## **2. DPF source pig**

The term “DPF” (Designated Pathogen Free) is used to describe animals, animal herds, or animal facilities that have been rigorously documented to be free of specified infectious agents and that are maintained using well-defined routines of testing for designated pathogens and utilizing rigorous SOPs (Standard operating procedures) and practices of herd husbandry and veterinary care to assure the absence of the designated pathogens [1]. So far, there is no normative document specifying the pathogens specified in DPF pig. DPF standards are dynamic and need to be updated over time according to the geographical environment of the animal population and new pathogens emerging. Generally speaking, there are two types of pathogens that need to be excluded from DPF pig: (1) Pathogens that affect animal health; (2) Pathogens that can cause cross-species transmission.

Experts in this field met to agree on the most comprehensive list of bacteria, fungi, parasites and viruses that should not be present in DPF pig [2]. Endogenous viruses are not listed. PERV (Porcine endogenous retrovirus) is the only one endogenous virus we known in pigs [3]. PERV has three subgroups including PERV-A, PERV-B and PERV-C. In general, PERV-A and PERV-B can infect both pig and human cells, but PERV-C can only infect pig cells. It is noteworthy that PERV-A/C recombine were be found in vitro co-culture system using cells from miniature swine, which means PERV-C can also infect human cells in some condition [4]. To monitor the status of DPF pigs, the pigs’ samples including blood, serum, tissues and feces must be tested regularly.

The DPF pigs must be raised in biosecure barrier environment. Biosecure barrier facility includes many aspects.

### **2.1 Facility environment and building**

1. The proposed DPF facility will be sited at a property to be confirmed.
2. The building will be on rural land where there are no other pig farms within a radius of 10 km. The grounds of the facility will be protected and planted with trees, and the grass mowed regularly. There are to be no other animals or livestock within the area boundary.
3. The building is to be fully protected by a secure fence and electric gate entry. The main entrance door is also to be a security door with key access and protected by security alarms 24 hours a day.
4. The facility is designed with two separate areas, outside the barrier (external) and within the barrier (termed “inside the barrier”).
5. The external area houses a delivery bay, storerooms for feed and bedding, staff lunchroom facilities, office, laundry area, external change rooms, and rooms to supply goods through the barrier.
6. Inside the barrier, the building is to have a HEPA (High efficiency particulate air) -filtered air supply and it will only contain goods that are sterile, staff who have showered and are wearing sterile clothes, and the pigs themselves which will be free of all specified diseases. There are to be two rooms holding the pig pens, internal gown-up areas, office, treatment room, reception room, and feed and bedding storerooms.

7. There will be two rooms of animal pens, a further unit for the sow farrowing, and a quarantine area, all with an air lock entry. The air pressure in all animal areas will be positive to the corridors (monitored by magnehelic gauges). Rooms will have controlled fluorescent lighting, temperature and humidity, and 15 to 20 air changes per hour of HEPA -filtered air.
8. Animal pens will have gates of the metal farm type, allowing pigs to see out and receive physical contact from other pigs and staff, with aisles between pens and a drain running in front of each row of pens. There will be windows in the walls between each pen to allow pigs to see each other.
9. Each pen will have a valve supplying filtered drinking water and individual stainless steel bowls for feed.
10. Music will be piped into the units by speakers set into the ceiling and serviced from the mezzanine level. Music will be controlled from the main office.

## **2.2 Facility operation**

1. The DPF facility will operate as a full sterile barrier facility. Therefore, all goods entering the facility must be sterile and all staff should go through a full shower procedure and gown-up in sterile suits, boots, hats and gloves. All original breeding stock in the facility will be cesarean-derived, colostrum-deprived, and hand-reared.
2. To enter the facility, staff must shower and don a complete clothing and footwear and wear gloves.
3. All activities that take place will be fully documented in the SOP Manual, including inwards receipt of goods through the facility barrier using such methods as an autoclave, dunk tank, and UV pass-through hatch.
4. There will be SOP-documented regular health screening of pigs and staff.
5. A comprehensive pest control system will be used inside and outside the building and managed by a contracted pest control company. Records of all inspections will be documented.
6. Pig care and welfare are a top priority, as described above for the Invercargill facility.

## **2.3 Health monitoring of DPF pigs**

All pigs are uniquely identified and individual records should be maintained, including animal breeding and genetic records.

1. Regular veterinary attendance at the pig facilities ensures that the staff is trained in disease recognition and that the veterinarian is called immediately in the event of signs and symptoms of disease in any animal. The veterinarian should report any such incident in writing.
2. The donor herd should continue to test the porcine pathogens and parasites.

3. All donor piglets should be necropsied by a veterinarian within 6 hours of cell harvesting. Any pathological changes must be noted and appropriate specimens taken. The veterinarians' report should be documented.
4. Donor piglet tissue retention samples collected include brain, heart, kidney, liver, lung, pancreas, and spleen. Duplicate samples are stored at 80°C in two separate locations.
5. Duplicate donor piglet serum retention samples are also stored in two separate locations.
6. In addition, duplicate final product retention samples are stored at 80°C in two separate locations.
7. A positive result in any of the infection monitoring tests described in this section, will lead to the donor animal and the batch of isolated islets being discarded.

The pigs are conveyed to the DPF breeding center. They must be disinfected in buffer rooms before entering inspection and quarantine where they are isolated for a month. After isolation the pigs give cesarean birth to the first generation of purified pigs. Compared to vaginal births Cesarean section can eliminate or reduce the risk of infecting with pathogens from sow's vagina. These newborns are fed in isolation under aseptic conditions and grow into adulthood. They are then impregnated and naturally deliver the second generation. After being tested for specified pathogens this second generation enters into a DPF area. The first generation of pigs should not be used as source pigs but the pigs in a second or higher generation can be used as DPF source pigs [1, 5].

### **3. Other biosafety issues for xenotransplantation clinical trials**

Donor pigs are the basis for ensuring the biosafety of xenograft clinical trials. Other biosafety issues are also worthy of attention, including immunosuppression protocols, clinical treatment protocols, sample/data retention programs, and case-tracking programs.

#### **3.1 Immunosuppression and tolerance-inducing strategies for xenotransplantation**

The principal challenges that must be faced to make xenotransplantation a clinical reality, which include determining a repeatable strategy for efficient preparation of xenogeneic tissues and organs and tracing the potential transmission of porcine pathogens to human. In addition, it is necessary to overcome the rejection barrier with clinically practicable immunosuppression and tolerance induction strategies. The application of xenotransplantation faces insurmountable immunological barriers, including: (1) hyperacute rejection (complement activation mediated by antibody) which is triggered by natural xenoreactive antibodies against Gal (1,3) and non-Gal antigens, (2) acute rejection of humoral xenograft which is mediated by antibodies that are dependent on T cells, (3) acute cellular xenograft rejection due to T cell mediated cellular responses.

##### *3.1.1 Immunosuppression protocols for xenotransplantation*

Continuous administration of multiple immunosuppressive drugs has been required and attempts to minimize immunosuppression. 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. One or more rounds of immuno-adsorption or plasmapheresis are essential to remove antibodies from the recipient's circulation. These regimens are often associated with serious side effects such as pancytopenia and sepsis.

The xenogeneic T cell response is supposed to be similar to that of typical allogenic responses, even larger. Consider this challenging barrier, most successful immunosuppressive therapy include a T cell depletion method like mono- or polyclonal anti-T cell antibodies, chemotherapeutic agents like cyclophosphamide, or whole body or thymic radiation therapy [6]. And anti-thymocyte globulin (ATG) is still the most commonly utilized option.

The engagement of TCR (T cell receptor) with foreign antigen without co-stimulatory signal will lead to T cells unresponsive to the antigen (known as T-cell anergy), thereby suppressing antigen induced response. The possible mechanism was that the CTLA4Ig fusion protein blocked CD28/B7 co-stimulatory signaling of the primary pathway, which eventually induced differentiation bias of T helper cells (Th cells [7]). Anti-CD154 antibodies, known to be effective in blocking indirect pathway of allorecognition [6], is also a critical component of effective immunosuppressive strategies in preventing cellular rejection in pig-to-NHPs (Non-human primates) xenotransplantation [8] yet its clinical application is restricted due to high risk of thromboembolic complications [9]. However, in pig-to-NHPs models, immune tolerance achievement approached by utilizing co-stimulatory blocking agents and other immunosuppressants in long-term treatments.

The transgenic pigs expressing graft-protecting factors has been shown to require a less toxic immunosuppressive protocol [10] which gives another path to explore. Using advanced gene editing technologies, xenotransplantation from multitransgenic alpha-1,3-galactosyltransferase knockout pigs (GTKO pigs) has demonstrated marked prolongation of xenograft survival. In addition, the incidence of hyperacute rejection was further reduced with organs from the GTKO pigs expressing one or more human complement-regulatory proteins (GTKO/hCRPs pigs), such as CD46, CD55, or CD59.

### *3.1.2 Tolerance-inducing strategies across xenogeneic immunological barriers*

A better but much more complex approach is to try to achieve immunological tolerance to the xenograft. Three successful tolerance induction approaches have been explored in large animal models: the use of mixed hematopoietic chimerism [11, 12], T regulatory cells [13, 14] and thymic transplantation [15]. It has been demonstrated that tolerance is possible in humans by successful clinical application of the mixed chimerism approach to renal transplantation [16] and by the T regulatory cell approach to liver allografts [17]. Despite the greater immunologic differences between species than within species, both mixed chimerism and thymic transplantation approaches have been shown to be capable of tolerizing human T cells to porcine xenografts in humanized mouse models [18]. Moreover, treatment with in vitro expanded regulatory T cells (Treg) prevents porcine xenograft rejection in humanized NOD-SCID IL-2 receptor gamma null (NSG) mice by the suppression of the T cell-mediated graft destruction, which suggesting the feasibility of pig-to-primate xenograft tolerance.

For xenografts, the level of immunosuppressive agents needed to fully suppress immune responses is greater than for allografts, which would likely lead to greater side effects. Thus, adoption of tolerance strategies is inevitable. Even though current immunosuppression seems to be controlling T cell responses in long-term acceptors [19, 20], it appears likely that low levels of T cell-dependent antibodies [21] and

activation of innate responses still develop [22], potentially leading to xenograft loss. Tolerance induction has the potential to avoid such persistent immune reactivity and therefore overcome the antibody-mediated response as well. Although tolerance induction in vivo has not yet been achieved in pig-to-baboon models, recent results are encouraging that this goal will be attainable through genetic engineering of porcine donors. It may be that current and future suppressive regimens that fully suppress the immune system will function sufficiently to benefit rejection of xenograft. Regardless of application, the study of tolerance continues to provide an excellent way to explore the functioning and control the immune system.

### **3.2 Data archive for xenotransplantation clinical trials**

A database of clinical trials for pig islet xenotransplantation should be established, including specimens, paper documents, and electronic documents.

The information of xenotransplantation donors, including the number of animals, test reports, will be preserved for long time. All the samples will be prepared in duplicate and one for long-period preservation in  $-80^{\circ}\text{C}$  refrigerator or liquid nitrogen tank. The information of transplant recipients and his/her spouses, such as name, hospital number, clinical data and patient records, will be recorded and maintained for long. When the patient comes to the hospital for review, the sample should be kept, including the following [23]: (1) all serum and plasma of the recipient and his/her spouse will be prepared in duplicate [24]; (2) storage of all samples at  $-80^{\circ}\text{C}$  or liquid nitrogen tank for long time; (3) preservation of samples for post-transplant cytokine detection, pathogen detection, etc.; and (4) development of standard operating procedures.

### **3.3 Postoperative follow-up**

The purpose of follow-up after xenotransplantation is to monitor the occurrence of rejection and adverse events. The goal of patient management is to improve their understanding of the disease, actively participate in and achieve partial self-management, improve compliance and achieve long-term survival and higher quality of life.

Postoperative follow-up of biosafety of clinical trials of recipients and spouses include: time-point, biosafety assays and treatment plan. (1) The patient and their spouses was reviewed 1 month before surgery, 1 month, 3 months, 6 months, 12 months, 2 years, 3 years, 4 years, and 5 years after xenotransplantation, and the sample in duplicate was kept. (2) Biosafety assays include fungal, bacterial, parasitic, viral, nucleic acid, cytokine and lymphocyte population detection. (3) If the biosafety assays are negative, the patient continues the symptomatic treatment, but if positive, then quarantine and treatment, personal protection and report to CDC (Centers for Disease Control and Prevention).

The medical record about postoperative follow-up of a xenograft recipient must contain the following information including the recipient's health status, all xenograft-related information, such as: (1) the contact information system of xenograft recipients. (2) If there is an infection related to xenotransplantation, or the pathogen from xenogeneic origin is identified, the health department of local government and the NHFPC (National health and family planning commission) shall be notified promptly. (3) The institution must have a reliable specimen and data preservation system and a complete information reporting system with the competent department. (4) The protocol must clearly address how patients are monitored for efficacy, biosafety, and period, including the draft clinical follow-up plan of xenotransplantation recipients.

## 4. Conclusions

Source donor pigs fulfilling the Designated Pathogen-Free (DPF) status have been available from a closed colony by GMP (Good Manufacturing Practice) rigorous routines, operational SOPs and rigorous data retention. Above all are very important for the operation of GMP barrier facility for biosafety of DPF source pig. A list of designated pathogens has been excluded from the DPF donor pig by long-term monitoring program of microbiological surveillance and pathological diagnosis. In addition, the consistently known DPF animals should be bred, grown and developed normally in the closed colony.

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## Conflict of interest

The authors declare no conflict of interest.

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