

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



## Chapter

# Porcine Islet Cell Xenotransplantation

*Rajeswar Chinnuswami, Abid Hussain,  
Gopalakrishnan Loganathan, Siddharth Narayanan,  
Gene D. Porter and Appakalai N. Balamurugan*

## Abstract

This article reviews the rationale, sources and preparation of pig islets for xenotransplantation. Pancreatic islet cell transplantation is an attractive alternative and an effective treatment option for type 1 diabetes, however, donor pancreas shortages prevent islet transplantation from being a widespread solution as the supply cannot possibly equal the demand. Porcine islet xenotransplantation has the potential to address these shortages, and recent preclinical and clinical trials show promising scientific support. Pig islets provide a readily available source for islet transplantation, with the recent trials in non-human primates (NHPs) demonstrating their potential to reverse diabetes. The risk of zoonosis can be reduced by designated pathogen-free breeding of the donor pigs, but porcine endogenous retroviruses (PERVs) which are integrated into the genome of all pigs, are especially difficult to eliminate. However, clinical trials have demonstrated an absence of PERV transmission with a significant reduction in the number of severe hypoglycemic episodes and up to 30% reduction in exogenous insulin doses. A number of methods are currently being tested to overcome the xenograft immune rejection. Some of these methods include the production of various transgenic pigs to better xenotransplantation efficiency and the encapsulation of islets to isolate them from the host immune system. Furthermore, ongoing research is also shedding light on factors such as the age and breed of the donor pig to determine the optimal islet quantity and function.

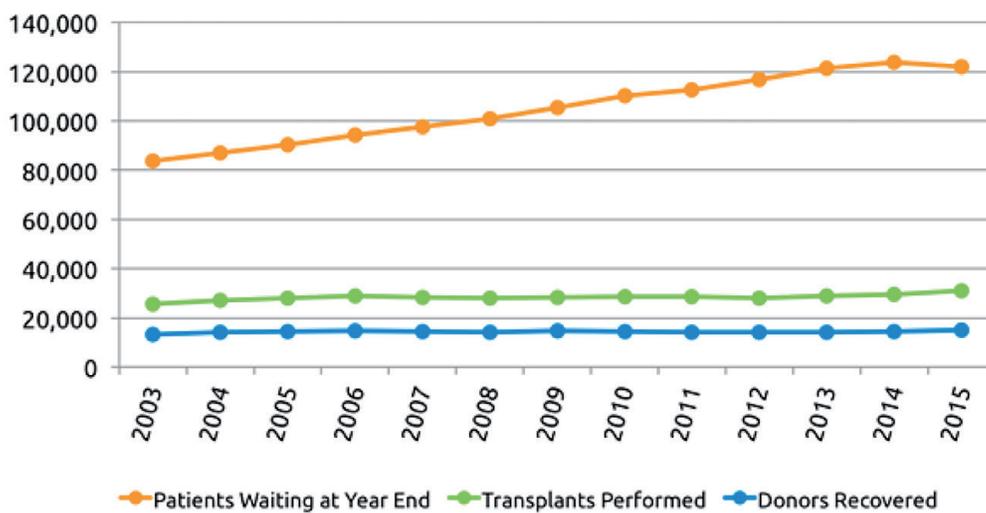
**Keywords:** type 1 diabetes, xenotransplant, porcine islets, encapsulation, transgenic

### Keypoints

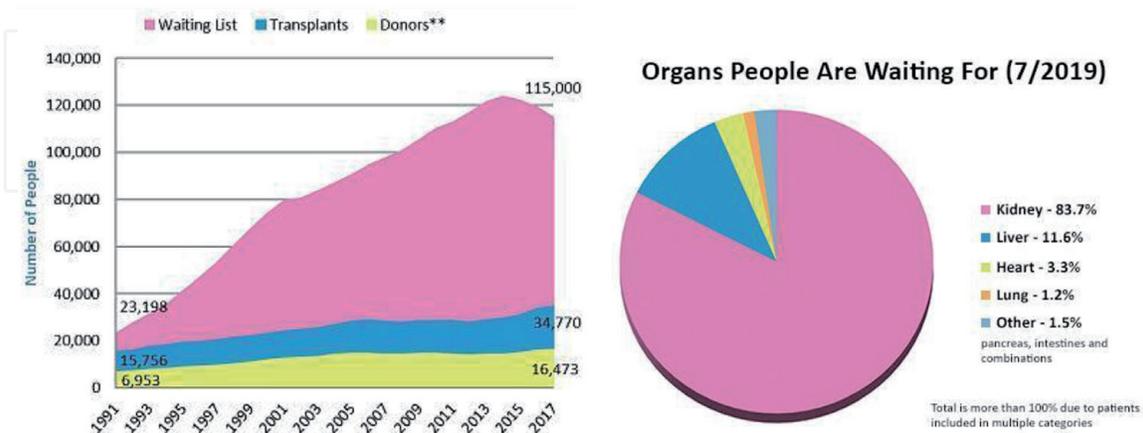
- Preclinical studies show improvements in pig islet survival after transplantation.
- Clinical pig islet xenotransplantation studies prove no transmission of PERV.
- Pig islets can be successfully transplanted using encapsulation technology.

## 1. Introduction to islet xeno-transplantation

Exogenous insulin is the most common treatment option for type I diabetes (insulin-dependent diabetes mellitus), a chronic metabolic disorder caused by the failure of the beta cells of pancreatic islets most often due to T-cell mediated auto immune reaction which result in hyperglycemia [1]. While the standard insulin therapy treats patients with diabetes, however, it does not cure the disease, nor does it prevent the development of the secondary complications leading to end stage organ failures along with its morbidity and mortality [2]. Technical advancements in the production of exogenous insulin, better glucose monitoring system and optimal insulin therapy can reduce HbA1C but still has not addressed the issues of increasing hypoglycemic episodes in patients. Achievement of normoglycemia and exogenous insulin independence is the goal of diabetes treatment. The International Diabetes Federation (IDF) estimated the number of adults suffering from DM in 2017 to be 425 million: this number is expected to increase to 629 million patients in 2040 [3]. Whole pancreas and pancreatic islet transplantation



### Recent Trend:



As of July 2019, there are more than 113,000 candidates for transplant on the U.S. national waiting list. Two out of every three people on the waiting list are over the age of 50. Almost 2,000 children under 18 are on the waiting list. Over 67,000 people (59 percent) on the list are ethnic minorities.

**Figure 1.**

Trends in the number of organ donors (blue), organ transplants (green), and patients on the waiting list (Orange) in the US, 2003–2015. In 2003, there were 13,285 donors, 25,473 organ transplants, and 83,731 patients on the waiting list. By 2015, there were 15,068 donors, 30,975 organ transplants and 122,071 patients on the waiting list. Source: <http://optn.transplant.hrsa.gov>.

are effective treatment options for diabetes by which insulin independence in T1D patients can be achieved [4]. Unfortunately, both whole organ and cellular transplantation face challenges due to a wide gap between the ever-increasing transplant waiting list and the supply of donor organs [5]. Data from the Organ Procurement and Transplant Network (OPTN) from 2003 to 2015, indicates a 145% increase in the wait list for all organs, while donor availability increased by only 113% (**Figure 1**) [6]. Similarly, the total number of pancreases available is insufficient to match the need for pancreatic islet allo-transplantation [7–9].

Due to this shortage, xenotransplantation using porcine islets has emerged as a potential alternative source for beta cell replacement. Porcine islets have structural and physiological similarities to human islets. Porcine insulin (differs from human insulin by only one amino acid) is used to treat diabetes in clinical practice [10, 11]. Intact functional islets have been successfully isolated from the pig pancreas [12], and these islets have shown the ability to reverse diabetes when transplanted into NHPs [13]. This review article will present the evolution, current practices, challenges and perspectives for pig islet xenotransplantation.

## **2. History of islet xeno-transplantation**

Xenotransplantation has been attempted for the past 300 years or so and blood xenotransfusion was tried as early as the seventeenth century by Jean Baptiste Denis [14]. This was later followed by corneal transplantations from pigs to humans and kidney transplantations in NHP [15, 16]. The first pancreatic xenotransplantation was performed by Watson et al., implanted three ovine fragments into the subcutaneous plane of a diabetic patient. Though clinically significant blood glucose reduction was not demonstrated, the blood sugar level did decrease [17]. This pioneering work was followed by many experimental xenotransplantations, but results were mostly inconclusive [18–21]. Shumakov et al. reported 53 fetal porcine xenotransplants and 18 fetal bovine xenotransplants in diabetic patients [22]. A century later, Groth et al. performed clinical xenotransplantation trial using fetal porcine islet cell-like clusters (ICCs) and provided preliminary data regarding the function and survival of grafts. After porcine islets were transplanted into 10 insulin-dependent diabetic kidney-transplant patients, detectable levels of porcine C-peptide were identified in the urine for up to 400 days and in one case, renal graft biopsy showed insulin and glucagon positive cells after staining [23]. Several xenotransplantation studies have also been performed in NHPs [20], and have succeeded in reversing diabetes [24–27] and in reducing daily insulin dosage requirement [28]. Transplanted porcine islet grafts were also shown to survive and function in NHPs for longer than 6 months with immunosuppression [25, 27, 29]. The longest survival rate is now over 603 days according to Shin et al., [30]. Studies have also shown that microencapsulation of the transplanted islets and immune-isolation lead to better survival rate without the need for aggressive immunosuppressive therapy [26].

## **3. Pig islets as alternative source**

The success of porcine insulin and its role in the treatment of T1D has been well established since its discovery in the 1920s [11, 12, 25]. The structural and physiological similarities between human and pig organs, along with its unlimited supply, have made them an excellent translational research model [25]. Insulin extracted from pig islets has been used for the treatment of diabetes for decades [10, 11, 20, 33]. Because porcine islets produce insulin patterns similar to those found in

humans, and because they are readily available [20], studies strongly suggest that islets obtained from pigs could be a promising substitute for human islets in the treatment of T1D. Recent studies on genetically engineered pigs suggest that these pigs are more suitable for xenotransplantation. For example, alpha 1,3-galactosyl-transferase gene knockout (GTKO) pigs, have decreased the incidence of immune-rejection and improved compatibility between the donor and recipient [31–36].

The major advantages for using pigs as an islet source for xenotransplantation are as follows:

1. Ethically acceptable source.
2. The pig pancreas has structural and physiological similarities to the human organ.
3. Unlimited availability.
4. Easy to breed and produce large litters.
5. Rapid growth into adult organs (6 months).
6. Significantly low cost of maintenance.
7. Elective and emergent availability of the organs.
8. Low risk of zoonosis.
9. Facilities available to breed pigs under 'clean' conditions.
10. Obviates 'cultural barriers' to human organ transplant (e.g. Japan); illegal organ trafficking; deleterious effects on organs in brain dead patients; living donor organ donation.
11. Advanced and safe immunosuppression protocols.
12. Cloning and genetic modification of cells to reduce immune destruction.
13. Islet encapsulation to combat immune challenge.

Modified from Eksler et al. [5]; Cooper et al. [37, 38]; Cheng et al. [20].

#### **4. Selection of pig and sources of pig islets**

Islet quantity and quality varies with the breed of pigs. Readily available market pigs have shown to yield lower when compared to the well-studied breeds of pigs like Landrace pigs, Chicago Medical School (CMS) miniature pigs and Chinese Wuzhishan (WZS) miniature pigs [23, 25, 27]. Two major factors which have been studied in relation to the source of pig islets for xenotransplantation are the breed and age of the donors. Some well-studied breeds are the Landrace pigs, Chicago Medical School (CMS) miniature pigs, and the Chinese Wuzhishan (WZS) miniature pigs. Market weight pigs are easily available, but studies have shown lower yields than for other breeds [39]. Landrace pigs have been shown to yield large sized (>250  $\mu\text{m}$ ) islets with a high islet volume density [39, 40]. Adult Chicago Medical School (CMS) miniature pigs

are bred under specific pathogen-free (SPF) conditions, and contain large-sized islets. The yield is greater than market or other miniature pigs ( $9589 \pm 2838$  IEQ/g), making CMS pigs one of the best sources for obtaining islets [39, 41–45]. Another miniature pig, the Chinese Wuzhishan (WZS) pig has also shown an islet yield greater than that of market pigs [39, 46]. Though no consensus has been arrived at the optimal breed for the preclinical/clinical studies, these breeds has been well documented to yield better islets than others. Higher expression of extracellular matrix (ECM) protein in islet capsules makes isolation easier and German Landrace pigs have higher ECM [24].

Additionally, age [43, 44, 47–49] and size of the donor pigs [36, 50–52] are major factors that affect islet isolation outcomes. Some studies have also suggested that gender may play a role in the final islet yield [39, 53, 54]. Pig islets can be obtained at four distinct life-stages: embryonic, fetal, neonatal and adult [55], and **Table 1** summarizes the significance, advantages, and disadvantages of pig islets from

Islet source	Significance	Advantages	Disadvantages
Embryonic	In the dorsal pancreatic primordial, strands of insulin positive cells are seen as early as week 4 [43]. From week 13, cells exhibiting intense immunoreactivity for insulin are distributed throughout the pancreas [43, 57].	Embryonic pancreatic tissue exhibit predominantly insulin-positive beta cells without evidence of alpha cells [43, 58]. Use of embryonic primordial pancreas is better than pluripotent stem cells as they do not need steering toward pancreatic differentiation and have lower risk of teratoma [59]. Following transplantation, the exocrine tissue does not proliferate. Hence, there is decreased immune response and inflammatory complications. Pancreatic primordia obtained on day 28 successfully reversed diabetes in rhesus monkey when compared to that obtained on day 35, which underwent rejection [43, 60, 61].	Immaturity takes 8–12 weeks (~6 months) for maturation <i>in vivo</i> [43]. Poor insulin response post-transplantation due to immaturity [39, 62–64]. Higher expression of alpha-1,3 galactose (Gal) when compared to adult—more susceptible for humoral rejection. Low yield—only a small number of islets can be isolated, requiring large number of pigs which limits large scale clinical application, with ethical issues.
Fetal	Porcine islets are isolated from fetuses of 60–69 days gestational age [36, 65]. Islets lack a definite shape and capsule and are organized in clusters (ICCs) [36]. These cellular clusters are composed of <40% endocrine cells (6–8% beta cells) with the majority being the cytokeratin-positive epithelial cells [65]. Their ability to proliferate makes them a potential source of islet cells [27, 36, 66–68].	Isolation process is very simple, involving digestion of the pancreatic tissue to free the islet clusters [65, 69]. No gradient purification necessary. Easily scalable to provide clinical product. Isolation not dependent on the enzyme collagenase, (activity is variable between enzyme lots). The use of alpha 1,3-galactosyltransferase GTKO strains has demonstrated better transplant outcomes than wild-type strains [43, 70].	Cellular culture is required for 5–9 days to form cellular aggregates. Maturation of islets is delayed Demands higher number of pigs to provide sufficient islets due to lower yield [27, 36, 71]. Because of their clustered appearance, it is difficult to separate islets from the surrounding exocrine and other non-islet cells [36].

Islet source	Significance	Advantages	Disadvantages
Neonatal	<p>The neonatal period is up to 30 days after birth. NPIs are usually obtained from the pancreas within the first week of life [43].</p> <p>NPIs comprise ~35% of endocrine cells and ~57% of epithelial cells—islet precursor cells [39, 72, 73].</p> <p>Correct hyperglycaemia in diabetic animal models as the precursor cells also differentiate and proliferate into beta cells [27, 36, 39, 74, 75].</p> <p>The cellular aggregates are composed of &lt;40% endocrine cells (20–25% beta cells) with majority being cytokeratin-positive epithelial cells [65].</p> <p>About 10–13 days after birth, the ICCs begin to resemble adult islets [43, 57].</p>	<p>Isolation process is very simple—the process involves digesting pancreatic tissue simply to free islet clusters [65, 72].</p> <p>No gradient purification.</p> <p>Easily scalable to provide clinical product.</p> <p>Isolation not dependent on the enzyme collagenase (activity is variable between lots).</p> <p>Isolation process is less expensive than for adult islets.</p> <p>Maintenance of neonates is easy and inexpensive as they are maintained only for few days postpartum.</p> <p>Exhibit strong resistance to inflammatory and hypoxia-induced injury.</p> <p>Lower T-cell reactivity than adult pigs [39, 76, 77].</p> <p>Potential alternative to adult pig islets as xenografts.</p>	<p>Maturation is delayed when compared to adult islets but is faster than for fetal ICCs.</p> <p>Cellular culture is required for 5–9 days to form cellular aggregates.</p> <p>Lower yield—limits clinical usage. Only 50,000 aggregates can be obtained from a single pancreas when compared to adult.</p>
Adult	<p>Adult pig islets (APIs) are the major source of islet cells for xenotransplantation [39, 78–80].</p> <p>APIs are well differentiated with distinct and intact capsule and vasculature with very few insulin positive cells outside these islets [43, 57].</p> <p>Antigenicity is from N-linked sugars and not from Gal Ag [39, 43, 81–83].</p> <p>The expression of Gal Ag decreases and becomes negligible as the pig reaches adulthood [43, 81–86].</p> <p>&gt;2 yrs. is the optimal age [36, 39, 50, 54, 87].</p> <p>Adult islets are predominantly islet endocrine cells.</p>	<p>Morphologically distinct—can be extracted and purified as a single unit [36].</p> <p>Mature cells—response to hyperglycemia is immediate following transplantation without latency [36, 39, 43, 87–90].</p> <p>Insulin independence in diabetic NHPs is achieved when <math>\geq 10,000</math> IEQs are transplanted. (islets pooled from 2 to 4 adult pigs) [39, 80].</p> <p>Do not require culturing of the isolated islets [65].</p> <p>Islet yield is greater than for fetal and neonatal pigs [43, 78, 91].</p>	<p>Isolation is technically challenging, complex and expensive [36, 43, 65, 79, 92–94].</p> <p>More fragile islets [65].</p> <p>Difficult to scale-up [65].</p> <p>Highly dependent on the enzyme lot and activity [65].</p> <p>Requires gradient purification [65].</p> <p>Very high cost of maintenance and breeding in a clean isolated environment [36, 43, 47].</p> <p>Bigger size of the animal is associated with surgical complications during organ procurement [36, 50].</p>

**Table 1.**  
Different sources of pig islets; significance, advantages, and disadvantages.

different donor life-stages. Adult pigs have been preferred for their higher yield of mature islet cells that have the potential to secrete insulin soon after transplantation. However, the higher costs, fragility of the islets and the difficulty in isolation are the disadvantages. Neonatal and fetal islets are easy and inexpensive to isolate but the main disadvantage is the significant delay in functioning after transplantation due to their immaturity and their high expression of Galactose- $\alpha$ -1,3-galactose ( $\alpha$ Gal), the major antigenic target for primate anti-pig antibodies [56].

## 5. Pig islet isolation

Adult pig islet preparation is very similar to human islet isolation methods [55] but the digestion process is a lot more gentler as the porcine islets are extremely fragile. Methods of islet preparation may vary depending on the life-stage of the donor pancreas. Fetal pig ICCs and neonatal pig islets (NPIs) are immature cells and can be easily isolated by enzymatic digestion [55] but must subsequently be cultured prior to transplantation to promote re-aggregation of islet clusters and to help eliminate exocrine cells [55]. The digestion procedure for the adult pig pancreas is significantly different over the fetal or neonatal pancreas. Many factors, such as the type of donor pigs, blood exsanguination, warm ischemia time, cold ischemia time, enzyme lot and activity, perfusate, and the isolation-purification process significantly affect the final islet yield, function and viability [39, 54, 55, 79, 95–97].

### 5.1 *In vitro* and *in vivo* assessment of pig islet function

*In vitro* studies investigating the insulin response of islets from donor pigs of different ages have shown that the insulin response from adult pig islets is more pronounced and sustained, and that they have a higher stimulation index over young pigs [36]. Islets from different age of donor pigs have also been compared *in vivo*. Two groups of diabetic nude mice populations were implanted with either young and young adult porcine islets or adult islets. One out of 11 recipients of young and young-adult islets achieved normoglycemia, whereas 32 out of 39 transplanted with adult islets became normal, the blood glucose reaching normal range within 4 weeks post-transplantation. Graft function was confirmed as the cause for normoglycemia, as all 32 mice reverted back to hyperglycemia after islet graft removal [36]. Many studies using NHP models have demonstrated the benefits of the pig islets as xenotransplants, with a potential cure for diabetes [25, 39, 98–101]. These studies have shown diabetes reversal with prolonged graft survival in diabetic NHPs.

### 5.2 Hurdles for xenotransplantation

Prevention of the transmission of porcine endogenous retrovirus (PERV) and immunological reactions have been the major hurdles for xenotransplantation in preclinical and clinical trials. Though the risks of zoonosis have been downplayed significantly with the introduction of genetically modified pigs, immunological responses like instant blood mediated inflammatory response (IBMR) dictate the success of the graft survival. One of the most important risk to overcome during xenotransplantation is the prevention of zoonosis [102]. Porcine endogenous retroviruses (PERVs) are of special concern as they are found integrated with porcine genomes and are difficult to eliminate [102]. The degree of risk of PERV being able to infect the human host is unknown, but evidence has shown that PERV can infect human cells when co-cultured with human EK-293 cells [55, 103]. Cross-species transmission has also been documented in pig to SCID mice xenotransplantation [55, 104]. However, no evidence of transmission has been documented in T1D patients who received porcine islet transplants, even after prolonged follow-up [55, 105].

Apart from PERV, other pathogenic organisms including the herpes virus, lymphotropic herpes virus, and cytomegalovirus can also be transmitted. [55]. Methods of combatting these pathogens include careful assessment and screening protocols, designated pathogen-free (DPF) breeding and housing of PERV gene knockout pigs, all of which can help minimize the risk of zoonotic infections [29]. DPF herds

Immune related islet injury	Genetic modifications	References
Ischemia/reperfusion injury and inflammatory cytokine related injury	Expression of human heme oxygenase-1 GTKO pigs/hCRP pigs	[55, 109] [110–112]
Humoral rejection	GTKO CD46 (membrane cofactor protein) CD59 (MAC-inhibitory protein) CD55 (decay accelerating factor)	[55, 113–115] [116] [117]
IBMIR and coagulation dysfunction	TF knockout and overexpression of human antithrombotic genes (CD39/thrombomodulin) ENTPD1 expression Mesenchymal stem cell (MSC) co-transplantation	[55, 118] [39, 119] [110, 120]
Cellular rejection	CTLA4Ig gene expression GTKO pigs/hCRP pigs MSC co-transplantation—downregulate T-cell response (immunomodulator)	[55, 121] [110–112, 122] [39, 120, 123] [117]

**Table 2.**  
*Genetic modifications in pigs to overcome immunological rejection.*

must be free from a comprehensive and list of specified microorganisms [29, 106] and meticulous documentation and standard operating procedures (SOPs) must be implemented to maintain this status [29] including feed restrictions [29].

### 5.3 Immunological response

Pig islet cells express different surface proteins that play a major role in the immunological rejection seen following transplantation [102, 107]. Immunological responses are much more complex than seen in allo-transplantation [102]. Immune mediated inflammatory response have been brought down by significantly by genetic modifications as summarized in **Table 2**. Hyper acute rejection (HAR), Instant blood mediated inflammatory response (IBMIR), and cellular rejection are the types of responses seen in graft rejection of which IBMIR is the most crucial. Portal vein site provides good revascularization and drainage for islet transplantation but due to the severe complications like bleeding, thrombosis, and hepatic steatosis, it is no longer an optimal site [108]. Immunological issues observed during xenotransplantation are similar to those seen in allo-transplantation but are much more complex [102]. Pig islets express different types of surface proteins, and these play a critical role in the immunologic rejection seen following transplantation [107]. Multiple genetic modifications in pigs have been proposed to significantly reduce immune mediated inflammatory response, and these are summarized in **Table 2**.

There are four known major routes for islet cell loss following transplantation and these are summarized in the following sections.

#### 5.3.1 Hyper acute rejection (HAR)

HAR occurs due to the presence of pre-existing host antibodies to surface proteins on the porcine islets. These surface proteins can be broadly categorized into Gal and non-Gal proteins [34, 38, 110]. The Gal epitope is absent in humans, apes and old-world monkeys but many bacteria, NHP and new world monkeys express

the Gal epitope abundantly. In pigs, the expression of Gal antigens decreases as they grow into adults [84, 102, 110, 124, 125].

As the human body is continuously exposed to micro-organisms (including bacteria), it develops immunity to the Gal antigen and has pre-formed, circulating anti-Gal antibodies [107, 126], which make up around 1% of the circulating antibodies [102, 124]. Once the pigs islets are transplanted, these pre-formed antibodies kill the islet cells rapidly by complement mediated destruction [107, 124] resulting in substantial islet loss [102, 107, 127].

Antibodies are also produced for other surface epitopes (non-Gal Ag) such as N-glycolylneuraminic acid (NeuGc) also known as Hanganutzu-Deicher and beta 1,4 N-acetylgalactosaminyltransferase (B4GALNT2) [107, 128–130] which are also involved in complement mediated destruction of xenografts [107].

There are two known strategies for prevention of HAR. Knockout of genes responsible for adding the Gal epitope and other epitopes such as Neu5Gc to the cell surface can prevent their expression [34, 102]. Secondly, expression of complement regulatory proteins such as hCD46, hCD55 and hCD59 can be induced on the surface of the islet cells [102, 131]. Double knockout pigs (deficient in alpha-gal (GTKO) and Neu5Gc) have been produced, which has significantly reduced the incidence of humoral rejection [102, 132]. The Gal antigen is highly expressed in fetal and neonatal pig pancreas, but its expression decreases as the pigs reach adulthood. The use of GTKO pigs is more validated when using fetal or neonatal pancreas [85, 116], but is not as essential when using adult pigs [116]. However, increasing titres of anti-Gal IgG antibody have been noted when immunosuppression is stopped after adult pig islet transplant [30, 116], so GTKO pigs may prove beneficial even for islets isolated from adult pigs.

### 5.3.2 Instant blood mediated inflammatory reaction (IBMIR)

Following the intra-portal infusion of the pig islets, the elevated expression of tissue factor by the islets initiates IBMIR [39]. The IBMIR contributes to significant islet loss in the early post-transplant phase through a series of events involving simultaneous complement activation (alternative pathway) [81, 86], activation of intrinsic and extrinsic coagulation pathways, and platelet activation (platelet aggregates around the islets P6) followed by neutrophil and monocyte infiltration [110, 116, 133, 134]. IBMIR can result in 60–80% of islet loss in the immediate post-transplant period [39, 55, 110, 118, 135], but studies in NHPs have shown that if a sufficient number of islet cells survive, they can establish normoglycemia for several months [110]. Genetically modified pigs have been produced [110, 136] to combat IBMIR by decreasing the load of xenoantigens but it failed to provide long-term protection against host response [137]. Experimental studies involving control of complement activation by cobra venom factor, and platelet aggregation and coagulation by anti-platelet agents and low molecular weight heparins are not proven clinically safe, [138, 139]. Peritoneal cavity and omentum offer alternative sites for transplantation of encapsulated islets [140].

### 5.3.3 Cellular rejection

Cellular rejection, a CD4<sup>+</sup> T-cell-dependent process [55, 141–143], plays a major role in islet destruction [39, 118, 144, 145]. Acute cellular rejection occurs within 24 h to 20 days post-transplant, and is characterized by a massive infiltration of macrophages and T-cells (CD4<sup>+</sup> and CD8<sup>+</sup> cells). Two signaling pathways required for the full activation of T cells are the T cell receptor signaling, and the co-stimulatory signaling [55, 146]. Since T cell activation requires double signaling

involving TCRs and co-stimulatory molecules [39], blockade of co-stimulatory cell surface molecules such as CD870/86- CD28 and/or CD40L (CD154)- CD40 have significantly improved graft survival, even without immunosuppression [39, 147–149]. The addition of targeted immunosuppression to multi-molecular blockade may further increase effectiveness, and provide an even more promising option to prevent cellular destruction of the transplanted islets [39].

#### *5.3.4 Islet cell revascularization*

Islet revascularization is critical for the survival of transplanted pig islets. Islet grafts are cut off from their native vascular supply and after transplantation, are solely dependent on diffusion for nutrient supply, until functional revascularization is established with the host vasculature. This process takes place within 10–14 days post-transplantation [41, 49, 141].

## **6. Islet encapsulation approaches**

Islet encapsulation provides the means for islet cell survival in the absence of immunosuppressive drugs. The principle of encapsulation is that transplanted cells are contained within an artificial compartment separated from the immune system by a semipermeable membrane. The capsule should protect the cells from potential damage caused by antibodies, complement proteins, and immune cells. Therefore, the capsule is often referred to as an “immunoisolation device.” As well as the protective mechanism provided by the capsules, islet cells within the capsules can also release insulin to control blood glucose levels, since this membrane enables small molecules to diffuse in (glucose, oxygen, and nutrients) and out (metabolic wastes) [39, 150–152]. Thus, the encapsulation system is also regarded as a “bio-artificial pancreas.” The immunoisolation device or bioartificial pancreas can be commonly separated into two categories, intravascular and extravascular devices. The latter can further be divided into macroencapsulation and microencapsulation devices. Intravascular and extravascular classifications are based on whether or not it is connected directly to the blood circulation.

The macroencapsulation and microencapsulation classifications depend on whether it contains one or more islets in the device [153, 154]. Alginate is the most commonly used capsule material for microencapsulation, but other materials such polyethylene glycol have also been tested [153].

Although the capsule is selectively permeable, islets can be damaged due to hypoxia or inadequate nutrients, and slow glucose and insulin diffusion can delay insulin response to changing glucose levels [155]. Despite the protection offered from direct immune attack, islets can still be damaged by immune responses. Inflammatory cytokines, produced against the capsules can enter the capsule and damage islets. The encapsulated islets themselves may release such cytokines and cause self-damage [156]. Approaches investigated to overcome these problems include testing different sites of implantation, creating biocompatible capsules, and optimizing the capsule size. The use of genetically engineered pig islets within capsules to promote graft survival and function have also been studied [156]. Several clinical trials of encapsulated pig islets to improve long-term survival outcomes of xenografts are currently being conducted around the world [117, 157]. A phase I/IIa clinical study in Moscow has tested the clinical applicability of a commercially available encapsulated pig islet product called Diabecell [39, 158, 159]. Additional phase I/IIa clinical trials are ongoing in New Zealand and Argentina. These trials have demonstrated an absence of PERV transmission, a significant

reduction in the number of severe hypoglycaemic episodes and up to 30% reduction in exogenous insulin doses [29, 160]. A 10 year follow up of another study involving xenotransplantation of encapsulated porcine islets into the peritoneum of a T1D patient has shown long-term islet survival and function, with no evidence of PERV infection [39, 150].

## 7. Regulatory aspects

Any new therapeutic substance or procedure, safety and efficacy of the drug substance have been inveterate before starting government approved clinical trials. In line with guidance in consensus statements from the International Xenotransplantation Association and the WHO on xenotransplantation, geographical location will impact choice of the microbiological mitigation strategy. Risk management at the source would include the definition of pathogens circulating in the countries of origin [161], establishment of reliable detection, and screening methods and assessment of risk from animal feed. Given the source animals to be utilized will be from specific pathogen-free/designated pathogen-free or high hygienic herds from a single location, the pathogen risk compared with standard slaughter herd animals is significantly reduced. Further testing during the manufacturing process, that is, islet isolation and encapsulation will provide tissue specific data that should further confirm safety of the final product. Moreover, alginate encapsulation allows keeping the islets in culture for longer periods thus giving enough time to perform viral screening on islet products before transplantation. Other release quality controls related to islet morphology, viability, purity, quantity, and potency should also be established in order to guarantee that only well characterized and functional islet preparations are used in patients. The use of genetically modified donor pigs to reduce islet cells immunogenicity and improve their secretory function stipulates that these genetic modifications should be well characterized. Integration of transgene expression cassettes should be in well-defined genomic locations, preferably in the form of a single-targeted integration that would ensure stable expression of the transgene across herds without affecting other cell functions or rendering them tumorigenic. In this context, it should be noted that encapsulation limits the risk of tumor cells spreading since it confines the cells and eliminates the need for immunosuppression meaning that in case the integrity of the encapsulation device would be compromised, xenogeneic pig cells would most probably be rejected by the host immune system. The use of nonhuman primates in research is subjected to very strict ethical and regulatory considerations but the pig-to-primate model is still considered as a gold standard for pig islet xenotransplantation, so that safety and efficacy data obtained using this model are required before initiating clinical studies [162].

## 8. Conclusion

Porcine islets represent an excellent alternative source to replace human islets in diabetic patients. Pig islets can be obtained from different life-stages (embryos to adults) and has several other advantages making it an indispensable resource for xenotransplantation. Active research have resulted in standardization of protocols, thereby bettering isolation outcomes. In addition, incorporation of multiple strategies such as generating transgenic pigs together with developing cellular and molecular therapies to sustain long-term xenograft survival have brought porcine islets closer to clinical applications. Despite the risk of zoonosis and other factors which

contribute to islet loss post-transplantation, tremendous progress has been made within the field such as developing encapsulated islets to combat host immunity and utilizing host stem cells to aide islet revascularization. Pig islet xenotransplantation currently acts as a bridge between allo-transplantation and stem-cell therapies. With all the tremendous progress made within the field, ongoing research focuses on a better understanding of various factors such as donor characteristics, isolation procedures, microbiological safety, and immunological tolerance to improve pig islet yield, function and transplantation outcomes. Furthering this understanding will require multiple clinical trials directed toward establishing porcine islets as a safe, effective and robust alternative for treating patients with T1D.

### **Acknowledgements**

The authors sincerely thank Kentucky Organ Donor Affiliates (KODA) for the supply of human pancreases for our research programs.

### **Conflicts of interest**

None.

### **Financial support and sponsorship**

The authors thank the Jewish Heritage Fund for Excellence for providing generous support to our program.

### **Author details**

Rajeswar Chinnuswami, Abid Hussain, Gopalakrishnan Loganathan, Siddharth Narayanan, Gene D. Porter and Appakalai N. Balamurugan\*  
Clinical Islet Cell Laboratory, Department of Surgery, Cardiovascular Innovation Institute, University of Louisville, Louisville, KY, United States

\*Address all correspondence to: bala.appakalai@louisville.edu

### **IntechOpen**

© 2020 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

## References

- [1] Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. *Lancet*. 2014;**383**(9911):69-82
- [2] Sena CM, Bento CF, Pereira P, Seica R. Diabetes mellitus: New challenges and innovative therapies. *The EPMA Journal*. 2010;**1**(1):138-163
- [3] Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, et al. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: Systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. *Lancet*. 2011;**378**(9785):31-40
- [4] Kitzmann JP, Karatzas T, Mueller KR, Avgoustiniatos ES, Gruessner AC, Balamurugan AN, et al. Islet preparation purity is overestimated, and less pure fractions have lower post-culture viability before clinical allotransplantation. *Transplantation Proceedings*. 2014;**46**(6):1953-1955
- [5] Ekser B, Cooper DK, Tector AJ. The need for xenotransplantation as a source of organs and cells for clinical transplantation. *International Journal of Surgery*. 2015;**23**(Pt B):199-204
- [6] Organ Procurement and Transplantation Network (OPTN). 2017. Available at: <http://optn.transplant.hrsa.gov>
- [7] Rood PP, Bottino R, Balamurugan AN, Fan Y, Cooper DK, Trucco M. Facilitating physiologic self-regeneration: A step beyond islet cell replacement. *Pharmaceutical Research*. 2006;**23**(2):227-242
- [8] Robertson RP. Islet transplantation as a treatment for diabetes—A work in progress. *New England Journal of Medicine*. 2004;**350**(7):694-705
- [9] Berney T, Buhler LH, Morel P. Pancreas allocation in the era of islet transplantation. *Transplant International*. 2005;**18**(7):763-767
- [10] Home PD, Alberti KG. The new insulins. Their characteristics and clinical indications. *Drugs*. 1982;**24**(5):401-413
- [11] Sonnenberg GE, Berger M. Human insulin: Much ado about one amino acid? *Diabetologia*. 1983;**25**(6):457-459
- [12] Ricordi C, Socci C, Davalli AM, Staudacher C, Baro P, Vertova A, et al. Isolation of the elusive pig islet. *Surgery*. 1990;**107**(6):688-694
- [13] van der Windt DJ, Bottino R, Kumar G, Wijkstrom M, Hara H, Ezzelarab M, et al. Clinical islet xenotransplantation: How close are we? *Diabetes*. 2012;**61**(12):3046-3055
- [14] Cooper DKC, Ekser B, Tector AJ. A brief history of clinical xenotransplantation. *International Journal of Surgery*. 2015;**23**(Pt B):205-210
- [15] Hara H, Cooper DK. The immunology of corneal xenotransplantation: A review of the literature. *Xenotransplantation*. 2010;**17**(5):338-349
- [16] Cooper DKC. Early clinical xenotransplantation experiences—An interview with Thomas E. Starzl, MD, PhD. *Xenotransplantation*. 2017;**24**(2)
- [17] Williams PW. Notes on diabetes treated with grafts of sheeps' pancreas. *BMJ*. 1894;**19**:1303-1304
- [18] Morris J. Pioneer attempts to cure diabetes by pancreatic transplantation. *The Medical journal of Australia*. 1988;**149**(11-12):634
- [19] Pybus F. Notes on suprarenal and pancreatic grafting. *The Lancet*. 1924;**204**(5272):550-551

- [20] Cheng M. Islet xeno/transplantation and the risk of contagion: Local responses from Canada and Australia to an emerging global technoscience. *Life Sciences, Society and Policy*. 2015;**11**:12
- [21] Hitchcock CR, Kiser JC, Telander RL, Seljeskog EL. Baboon renal grafts. *Journal of the American Medical Association*. 1964;**189**(12):934-937
- [22] Shumakov VI, Bljumkin VN, Ignatenko SN, Skaletsky NN, Slovesnova TA, Babikova RA. The principal results of pancreatic islet cell culture transplantation in diabetes mellitus patients. *Transplantation Proceedings*. 1987;**19**(1 Pt 3):2372
- [23] Groth C, Tibell A, Tollemar J, Bolinder J, Östman J, Möller E, et al. Transplantation of porcine fetal pancreas to diabetic patients. *The Lancet*. 1994;**344**(8934):1402-1404
- [24] Hering BJ, Walawalkar N. Pig-to-nonhuman primate islet xenotransplantation. *Transplant Immunology*. 2009;**21**(2):81-86
- [25] Hering BJ, Wijkstrom M, Graham ML, Hårdstedt M, Aasheim TC, Jie T, et al. Prolonged diabetes reversal after intraportal xenotransplantation of wild-type porcine islets in immunosuppressed nonhuman primates. *Nature Medicine*. 2006;**12**(3):301-303
- [26] Dufrane D, Goebbels R-M, Saliez A, Guiot Y, Gianello P. Six-month survival of microencapsulated pig islets and alginate biocompatibility in primates: Proof of concept. *Transplantation*. 2006;**81**(9):1345-1353
- [27] Cardona K, Korbitt GS, Milas Z, Lyon J, Cano J, Jiang W, et al. Long-term survival of neonatal porcine islets in nonhuman primates by targeting costimulation pathways. *Nature Medicine*. 2006;**12**(3):304-306
- [28] Torrie B. More trials of pig cells to help treat type 1 diabetics. *The Dominion Post*. 2012
- [29] Ellis CE, Korbitt GS. Justifying clinical trials for porcine islet xenotransplantation. *Xenotransplantation*. 2015;**22**(5):336-344
- [30] Shin JS, Kim JM, Kim JS, Min BH, Kim YH, Kim HJ, et al. Long-term control of diabetes in immunosuppressed nonhuman primates (NHP) by the transplantation of adult porcine islets. *American Journal of Transplantation*. 2015;**15**(11):2837-2850
- [31] Cooper DK, Koren E, Oriol R. Genetically engineered pigs. *Lancet*. 1993;**342**(8872):682-683
- [32] Koike C, Friday RP, Nakashima I, Luppi P, Fung JJ, Rao AS, et al. Isolation of the regulatory regions and genomic organization of the porcine alpha1,3-galactosyltransferase gene. *Transplantation*. 2000;**70**(9):1275-1283
- [33] Koike C, Fung JJ, Geller DA, Kannagi R, Libert T, Luppi P, et al. Molecular basis of evolutionary loss of the alpha 1,3-galactosyltransferase gene in higher primates. *The Journal of Biological Chemistry*. 2002;**277**(12):10114-10120
- [34] Phelps CJ, Koike C, Vaught TD, Boone J, Wells KD, Chen SH, et al. Production of alpha 1,3-galactosyltransferase-deficient pigs. *Science*. 2003;**299**(5605):411-414
- [35] Kolber-Simonds D, Lai L, Watt SR, Denaro M, Arn S, Augenstein ML, et al. Production of alpha-1,3-galactosyltransferase null pigs by means of nuclear transfer with fibroblasts bearing loss of heterozygosity mutations. *Proceedings of the National Academy of Sciences of the United States of America*. 2004;**101**(19):7335-7340

- [36] Bottino R, Balamurugan AN, Smetanka C, Bertera S, He J, Rood PP, et al. Isolation outcome and functional characteristics of young and adult pig pancreatic islets for transplantation studies. *Xenotransplantation*. 2007;**14**(1):74-82
- [37] de Bock MI, Roy A, Cooper MN, Dart JA, Berthold CL, Retterath AJ, et al. Feasibility of outpatient 24-hour closed-loop insulin delivery. *Diabetes Care*. 2015;**38**(11):e186-e1e7
- [38] Cooper DK, Ayares D. The immense potential of xenotransplantation in surgery. *International Journal of Surgery*. 2011;**9**(2):122-129
- [39] Hu Q, Liu Z, Zhu H. Pig islets for islet xenotransplantation: Current status and future perspectives. *Chinese Medical Journal*. 2014;**127**(2):370-377
- [40] Kirchoff N, Hering BJ, Geiss V, Federlin K, Bretzel RG. Evidence for breed-dependent differences in porcine islets of Langerhans. *Transplantation Proceedings*. 1994;**26**(2):616-617
- [41] Kim JH, Kim HI, Lee KW, Yu JE, Kim SH, Park HS, et al. Influence of strain and age differences on the yields of porcine islet isolation: Extremely high islet yields from SPF CMS miniature pigs. *Xenotransplantation*. 2007;**14**(1):60-66
- [42] Schuurman HJ. The international xenotransplantation association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 2: Source pigs. *Xenotransplantation*. 2009;**16**(4):215-222
- [43] Nagaraju S, Bottino R, Wijkstrom M, Trucco M, Cooper DK. Islet xenotransplantation: What is the optimal age of the islet-source pig? *Xenotransplantation*. 2015;**22**(1):7-19
- [44] Jay TR, Heald KA, Carless NJ, Topham DE, Downing R. The distribution of porcine pancreatic beta-cells at ages 5, 12 and 24 weeks. *Xenotransplantation*. 1999;**6**(2):131-140
- [45] Shin J-S, Jang J-Y, Park S-K, Choi J-W, Kim S-Y, Min B-H, et al. Extremely high islet yield enables one-donor-one recipient intraportal islet transplantation with enough islet mass in pig-to-non-human primate model. *Xenotransplantation*. 2013;**20**(5):333
- [46] Jiang X, Qian T, Linn T, Cao L, Xiang G, Wang Y, et al. Islet isolation and purification from inbred Wuzhishan miniature pigs. *Xenotransplantation*. 2012;**19**(3):159-165
- [47] Jay TR, Heald KA, Downing R. Effect of donor age on porcine insulin secretion. *Transplantation Proceedings*. 1997;**29**(4):2023
- [48] Mueller KR, Balamurugan AN, Cline GW, Pongratz RL, Hooper RL, Weegman BP, et al. Differences in glucose-stimulated insulin secretion in vitro of islets from human, nonhuman primate, and porcine origin. *Xenotransplantation*. 2013;**20**(2):75-81
- [49] Socci C, Ricordi C, Davalli AM, Staudacher C, Baro P, Vertova A, et al. Selection of donors significantly improves pig islet isolation yield. *Hormone and Metabolic Research Supplement*. 1990;**25**:32-34
- [50] Dufrane D, Goebbels R, Fdilal I, Guiot Y, Gianello P. Impact of porcine islet size on cellular structure and engraftment after transplantation: Adult versus young pigs. *Pancreas*. 2005;**30**(2):138-147
- [51] Hubert T, Jany T, Marcelli-Tourvieille S, Nunes B, Gmyr V, Kerr-Conte J, et al. Acute insulin response of donors is correlated with pancreatic

islet isolation outcome in the pig. *Diabetologia*. 2005;**48**(10):2069-2073

[52] Krickhahn M, Buhler C, Meyer T, Thiede A, Ulrichs K. The morphology of islets within the porcine donor pancreas determines the isolation result: Successful isolation of pancreatic islets can now be achieved from young market pigs. *Cell Transplantation*. 2002;**11**(8):827-838

[53] Jin SM, Shin JS, Kim KS, Gong CH, Park SK, Kim JS, et al. Islet isolation from adult designated pathogen-free pigs: Use of the newer bovine nervous tissue-free enzymes and a revised donor selection strategy would improve the islet graft function. *Xenotransplantation*. 2011;**18**(6):369-379

[54] Kim HI, Lee SY, Jin SM, Kim KS, Yu JE, Yeom SC, et al. Parameters for successful pig islet isolation as determined using 68 specific-pathogen-free miniature pigs. *Xenotransplantation*. 2009;**16**(1):11-18

[55] Zhu HT, Wang WL, Yu L, Wang B. Pig-islet xenotransplantation: Recent progress and current perspectives. *Frontiers in Surgery*. 2014;**1**:7

[56] Fang J, Walters A, Hara H, Long C, Yeh P, Ayares D, et al. Anti-gal antibodies in alpha1,3-galactosyltransferase gene-knockout pigs. *Xenotransplantation*. 2012;**19**(5):305-310

[57] Alumets J, Hakanson R, Sundler F. Ontogeny of endocrine cells in porcine gut and pancreas. An immunocytochemical study. *Gastroenterology*. 1983;**85**(6):1359-1372

[58] Eventov-Friedman S, Tchorsh D, Katchman H, Shezen E, Aronovich A, Hecht G, et al. Embryonic pig pancreatic tissue transplantation for the

treatment of diabetes. *PLoS Medicine*. 2006;**3**(7):e215

[59] Hammerman MR. Development of a novel xenotransplantation strategy for treatment of diabetes mellitus in rat hosts and translation to non-human primates. *Organogenesis*. 2012;**8**(2):41-48

[60] Rogers SA, Chen F, Talcott MR, Faulkner C, Thomas JM, Thevis M, et al. Long-term engraftment following transplantation of pig pancreatic primordia into non-immunosuppressed diabetic rhesus macaques. *Xenotransplantation*. 2007;**14**(6):591-602

[61] Rogers SA, Liapis H, Hammerman MR. Normalization of glucose post-transplantation of pig pancreatic anlagen into non-immunosuppressed diabetic rats depends on obtaining anlagen prior to embryonic day 35. *Transplant Immunology*. 2005;**14**(2):67-75

[62] Otonkoski T, Ustinov J, Rasilainen S, Kallio E, Korsgren O, Hayry P. Differentiation and maturation of porcine fetal islet cells in vitro and after transplantation. *Transplantation*. 1999;**68**(11):1674-1683

[63] Tan C, Tuch BE, Tu J, Brown SA. Role of NADH shuttles in glucose-induced insulin secretion from fetal beta-cells. *Diabetes*. 2002;**51**(10):2989-2996

[64] Bogdani M, Suenens K, Bock T, Pipeleers-Marichal M, In't Veld P, Pipeleers D. Growth and functional maturation of beta-cells in implants of endocrine cells purified from prenatal porcine pancreas. *Diabetes*. 2005;**54**(12):3387-3394

[65] Korbitt GS. What type of islets should be used? *Xenotransplantation*. 2008;**15**(2):81-82

- [66] Vo L, Tuch BE, Wright DC, Keogh GW, Roberts S, Simpson AM, et al. Lowering of blood glucose to nondiabetic levels in a hyperglycemic pig by allografting of fetal pig isletlike cell clusters. *Transplantation*. 2001;**71**(11):1671-1677
- [67] Korsgren O, Christofferson R, Jansson L. Angiogenesis and angioarchitecture of transplanted fetal porcine islet-like cell clusters. *Transplantation*. 1999;**68**(11):1761-1766
- [68] Luca G, Nastruzzi C, Calvitti M, Becchetti E, Baroni T, Neri LM, et al. Accelerated functional maturation of isolated neonatal porcine cell clusters: In vitro and in vivo results in NOD mice. *Cell Transplantation*. 2005;**14**(5):249-261
- [69] Korsgren O, Jansson L, Eizirik D, Andersson A. Functional and morphological differentiation of fetal porcine islet-like cell clusters after transplantation into nude mice. *Diabetologia*. 1991;**34**(6):379-386
- [70] Thompson P, Badell IR, Lowe M, Cano J, Song M, Leopardi F, et al. Islet xenotransplantation using gal-deficient neonatal donors improves engraftment and function. *American Journal of Transplantation*. 2011;**11**(12):2593-2602
- [71] Kin T, Korbitt GS, Kobayashi T, Dufour JM, Rajotte RV. Reversal of diabetes in pancreatectomized pigs after transplantation of neonatal porcine islets. *Diabetes*. 2005;**54**(4):1032-1039
- [72] Korbitt GS, Elliott JF, Ao Z, Smith DK, Warnock GL, Rajotte RV. Large scale isolation, growth, and function of porcine neonatal islet cells. *The Journal of Clinical Investigation*. 1996;**97**(9):2119-2129
- [73] Dufrane D, Gianello P. Pig islets for clinical islet xenotransplantation. *Current Opinion in Nephrology and Hypertension*. 2009;**18**(6):495-500
- [74] Trivedi N, Hollister-Lock J, Lopez-Avalos MD, O'Neil JJ, Keegan M, Bonner-Weir S, et al. Increase in beta-cell mass in transplanted porcine neonatal pancreatic cell clusters is due to proliferation of beta-cells and differentiation of duct cells. *Endocrinology*. 2001;**142**(5):2115-2122
- [75] Nielsen T, Yderstraede K, Schrøder H, Holst JJ, Brusgaard K, Beck-Nielsen H. Functional and immunohistochemical evaluation of porcine neonatal islet-like cell clusters. *Cell Transplantation*. 2003;**12**(1):13-25
- [76] Emamaullee JA, Shapiro AMJ, Rajotte RV, Korbitt G, Elliott JF. Neonatal porcine islets exhibit natural resistance to hypoxia-induced apoptosis. *Transplantation*. 2006;**82**(7):945-952
- [77] Bloch K, Assa S, Lazard D, Abramov N, Shalitin S, Weintrob N, et al. Neonatal pig islets induce a lower T-cell response than adult pig islets in IDDM patients. *Transplantation*. 1999;**67**(5):748-752
- [78] O'Neil JJ, Stegemann JP, Nicholson DT, Gagnon KA, Solomon BA, Mullon CJ. The isolation and function of porcine islets from market weight pigs. *Cell Transplantation*. 2001;**10**(3):235-246
- [79] Dufrane D, D'Hoore W, Goebbels RM, Saliez A, Guiot Y, Gianello P. Parameters favouring successful adult pig islet isolations for xenotransplantation in pig-to-primate models. *Xenotransplantation*. 2006;**13**(3):204-214
- [80] Korbitt GS. The international xenotransplantation association consensus statement on conditions for undertaking clinical trials of porcine islet

products in type 1 diabetes—Chapter 3: Pig islet product manufacturing and release testing. *Xenotransplantation*. 2009;**16**(4):223-228

[81] Komoda H, Miyagawa S, Kubo T, Kitano E, Kitamura H, Omori T, et al. A study of the xenoantigenicity of adult pig islets cells. *Xenotransplantation*. 2004;**11**(3):237-246

[82] Bennet W, Bjorkland A, Sundberg B, Davies H, Liu J, Holgersson J, et al. A comparison of fetal and adult porcine islets with regard to gal alpha (1,3) gal expression and the role of human immunoglobulins and complement in islet cell cytotoxicity. *Transplantation*. 2000;**69**(8):1711-1717

[83] Rayat GR, Rajotte RV, Hering BJ, Binette TM, Korbitt GS. In vitro and in vivo expression of Galalpha-(1,3)gal on porcine islet cells is age dependent. *The Journal of Endocrinology*. 2003;**177**(1):127-135

[84] McKenzie IF, Koulmanda M, Mandel TE, Sandrin MS. Pig islet xenografts are susceptible to "anti-pig" but not gal alpha(1,3)gal antibody plus complement in gal o/o mice. *Journal of Immunology*. 1998;**161**(10):5116-5119

[85] Diswall M, Angstrom J, Schuurman HJ, Dor FJ, Rydberg L, Breimer ME. Studies on glycolipid antigens in small intestine and pancreas from alpha1,3-galactosyltransferase knockout miniature swine. *Transplantation*. 2007;**84**(10):1348-1356

[86] Omori T, Nishida T, Komoda H, Fumimoto Y, Ito T, Sawa Y, et al. A study of the xenoantigenicity of neonatal porcine islet-like cell clusters (NPCC) and the efficiency of adenovirus-mediated DAF (CD55) expression. *Xenotransplantation*. 2006;**13**(5):455-464

[87] Yonekawa Y, Matsumoto S, Okitsu T, Arata T, Iwanaga Y, Noguchi H, et al.

Effective islet isolation method with extremely high islet yields from adult pigs. *Cell Transplantation*. 2005;**14**(10):757-762

[88] Davalli AM, Bertuzzi F, Socci C, Scaglia L, Gavazzi F, Freschi M, et al. Paradoxical release of insulin by adult pig islets in vitro. Recovery after culture in a defined tissue culture medium. *Transplantation*. 1993;**56**(1):148-154

[89] Gouin E, Rivereau AS, Duvivier V, Darquy S, Larher E, You S, et al. Perfusion analysis of insulin secretion from specific pathogen-free large-white pig islets shows satisfactory functional characteristics for xenografts in humans. *Diabetes & Metabolism*. 1998;**24**(3):208-214

[90] Holmes MA, Clayton HA, Chadwick DR, Bell PR, London NJ, James RF. Functional studies of rat, porcine, and human pancreatic islets cultured in ten commercially available media. *Transplantation*. 1995;**60**(8):854-860

[91] Ricordi C, Finke EH, Lacy PE. A method for the mass isolation of islets from the adult pig pancreas. *Diabetes*. 1986;**35**(6):649-653

[92] Dufrane D, Goebbels RM, Guiot Y, Squifflet JP, Henquin JC, Gianello P. A simple method using a polymethylpenten chamber for isolation of human pancreatic islets. *Pancreas*. 2005;**30**(3):e51-e59

[93] Brandhorst D, Brandhorst H, Hering BJ, Federlin K, Bretzel RG. Islet isolation from the pancreas of large mammals and humans: 10 years of experience. *Experimental and Clinical Endocrinology & Diabetes*. 1995;**103**(Suppl 2):3-14

[94] Toso C, Brandhorst D, Oberholzer J, Triponez F, Buhler L, Morel P. Isolation of adult porcine islets of Langerhans. *Cell Transplantation*. 2000;**9**(3):297-305

- [95] Kin T, Shapiro AM. Surgical aspects of human islet isolation. *Islets*. 2010;**2**(5):265-273
- [96] Goto M, Imura T, Inagaki A, Ogawa N, Yamaya H, Fujimori K, et al. The impact of ischemic stress on the quality of isolated pancreatic islets. *Transplantation Proceedings*. 2010;**42**(6):2040-2042
- [97] Anazawa T, Balamurugan AN, Papas KK, Avgoustiniatos ES, Ferrer J, Matsumoto S, et al. Improved method of porcine pancreas procurement with arterial flush and ductal injection enhances islet isolation outcome. *Transplantation Proceedings*. 2010;**42**(6):2032-2035
- [98] Lee JI, Shin JS, Jung WY, Lee G, Kim MS, Kim YS, et al. Porcine islet adaptation to metabolic need of monkeys in pig-to-monkey intraportal islet xenotransplantation. *Transplantation Proceedings*. 2013;**45**(5):1866-1868
- [99] Thompson P, Badell IR, Lowe M, Turner A, Cano J, Avila J, et al. Alternative immunomodulatory strategies for xenotransplantation: CD40/154 pathway-sparing regimens promote xenograft survival. *American Journal of Transplantation*. 2012;**12**(7):1765-1775
- [100] Thompson P, Cardona K, Russell M, Badell IR, Shaffer V, Korbitt G, et al. CD40-specific costimulation blockade enhances neonatal porcine islet survival in nonhuman primates. *American Journal of Transplantation*. 2011;**11**(5):947-957
- [101] van der Windt DJ, Bottino R, Casu A, Campanile N, Smetanka C, He J, et al. Long-term controlled normoglycemia in diabetic non-human primates after transplantation with hCD46 transgenic porcine islets. *American Journal of Transplantation*. 2009;**9**(12):2716-2726
- [102] Denner J. Recent progress in xenotransplantation, with emphasis on virological safety. *Annals of Transplantation*. 2016;**21**:717-727
- [103] Yu P, Zhang L, Li SF, Li YP, Cheng JQ, Lu YR, et al. Long-term effects on HEK-293 cell line after co-culture with porcine endogenous retrovirus. *Transplantation Proceedings*. 2005;**37**(1):496-499
- [104] van der Laan LJ, Lockey C, Griffeth BC, Frasier FS, Wilson CA, Onions DE, et al. Infection by porcine endogenous retrovirus after islet xenotransplantation in SCID mice. *Nature*. 2000;**407**(6800):90-94
- [105] Valdes-Gonzalez R, Dorantes LM, Bracho-Blanchet E, Rodríguez-Ventura A, DJG W. No evidence of porcine endogenous retrovirus in patients with type 1 diabetes after long-term porcine islet xenotransplantation. *Journal of Medical Virology*. 2010;**82**(2):331-334
- [106] Onions D, Cooper DK, Alexander TJ, Brown C, Claassen E, Foweraker JE, et al. An approach to the control of disease transmission in pig-to-human xenotransplantation. *Xenotransplantation*. 2000;**7**(2):143-155
- [107] Bottino R, Trucco M. Use of genetically-engineered pig donors in islet transplantation. *World Journal of Transplantation*. 2015;**5**(4):243
- [108] Bhargava R, Senior PA, Ackerman TE, Ryan EA, Paty BW, Lakey JR, et al. Prevalence of hepatic steatosis after islet transplantation and its relation to graft function. *Diabetes*. 2004;**53**(5):1311-1317
- [109] Yeom HJ, Koo OJ, Yang J, Cho B, Hwang JI, Park SJ, et al. Generation and characterization of human heme oxygenase-1 transgenic pigs. *PLoS One*. 2012;**7**(10):e46646

- [110] Ekser B, Ezzelarab M, Hara H, van der Windt DJ, Wijkstrom M, Bottino R, et al. Clinical xenotransplantation: The next medical revolution? *Lancet*. 2012;**379**(9816):672-683
- [111] 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. *American Journal of Transplantation*. 2002;**2**(4):314-322
- [112] Saethre M, Schneider MK, Lambris JD, Magotti P, Haraldsen G, Seebach JD, et al. Cytokine secretion depends on Gal $\alpha$ (1,3)gal expression in a pig-to-human whole blood model. *Journal of Immunology*. 2008;**180**(9):6346-6353
- [113] Diamond LE, Quinn CM, Martin MJ, Lawson J, Platt JL, Logan JS. A human CD46 transgenic pig model system for the study of discordant xenotransplantation. *Transplantation*. 2001;**71**(1):132-142
- [114] Liu D, Kobayashi T, Onishi A, Furusawa T, Iwamoto M, Suzuki S, et al. Relation between human decay-accelerating factor (hDAF) expression in pig cells and inhibition of human serum anti-pig cytotoxicity: Value of highly expressed hDAF for xenotransplantation. *Xenotransplantation*. 2007;**14**(1):67-73
- [115] Le Bas-Bernardet S, Tillou X, Poirier N, Dilek N, Chatelais M, Devalliere J, et al. Xenotransplantation of galactosyl-transferase knockout, CD55, CD59, CD39, and fucosyl-transferase transgenic pig kidneys into baboons. *Transplantation Proceedings*. 2011;**43**(9):3426-3430
- [116] Park CG, Bottino R, Hawthorne WJ. Current status of islet xenotransplantation. *International Journal of Surgery*. 2015;**23**(Pt B):261-266
- [117] Cooper DK, Ekser B, Ramsoondar J, Phelps C, Ayares D. The role of genetically engineered pigs in xenotransplantation research. *The Journal of Pathology*. 2016;**238**(2):288-299
- [118] Ekser B, Cooper DK. Overcoming the barriers to xenotransplantation: Prospects for the future. *Expert Review of Clinical Immunology*. 2010;**6**(2):219-230
- [119] Ma X, Ye B, Gao F, Liang Q, Dong Q, Liu Y, et al. Tissue factor knockdown in porcine islets: An effective approach to suppressing the instant blood-mediated inflammatory reaction. *Cell Transplantation*. 2012;**21**(1):61-71
- [120] Ezzelarab M, Ayares D, Cooper DK. The potential of genetically-modified pig mesenchymal stromal cells in xenotransplantation. *Xenotransplantation*. 2010;**17**(1):3-5
- [121] Klymiuk N, van Buerck L, Bahr A, Offers M, Kessler B, Wuensch A, et al. Xenografted islet cell clusters from INSLEA29Y transgenic pigs rescue diabetes and prevent immune rejection in humanized mice. *Diabetes*. 2012;**61**(6):1527-1532
- [122] Londrigan SL, Sutherland RM, Brady JL, Carrington EM, Cowan PJ, d'Apice AJ, et al. In situ protection against islet allograft rejection by CTLA4Ig transduction. *Transplantation*. 2010;**90**(9):951-957
- [123] Ezzelarab M, Ezzelarab C, Wilhite T, Kumar G, Hara H, Ayares D, et al. Genetically-modified pig mesenchymal stromal cells: Xenoantigenicity and effect on human T-cell xenoresponses. *Xenotransplantation*. 2011;**18**(3):183-195
- [124] Galili U. The alpha-gal epitope and the anti-gal antibody in xenotransplantation and in cancer

- immunotherapy. *Immunology and Cell Biology*. 2005;**83**(6):674-686
- [125] Dai Y, Vaught TD, Boone J, Chen SH, Phelps CJ, Ball S, et al. Targeted disruption of the alpha1,3-galactosyltransferase gene in cloned pigs. *Nature Biotechnology*. 2002;**20**(3):251-255
- [126] Koike C, Uddin M, Wildman DE, Gray EA, Trucco M, Starzl TE, et al. Functionally important glycosyltransferase gain and loss during catarrhine primate emergence. *Proceedings of the National Academy of Sciences of the United States of America*. 2007;**104**(2):559-564
- [127] Kobayashi T, Cooper DK. Anti-Gal, alpha-Gal epitopes, and xenotransplantation. *Subcellular Biochemistry*. 1999;**32**:229-257
- [128] Bouhours D, Pourcel C, Bouhours JE. Simultaneous expression by porcine aorta endothelial cells of glycosphingolipids bearing the major epitope for human xenoreactive antibodies (gal alpha 1-3Gal), blood group H determinant and N-glycolylneuraminic acid. *Glycoconjugate Journal*. 1996;**13**(6):947-953
- [129] Padler-Karavani V, Varki A. Potential impact of the non-human sialic acid N-glycolylneuraminic acid on transplant rejection risk. *Xenotransplantation*. 2011;**18**(1):1-5
- [130] Byrne GW, Du Z, Stalboerger P, Kogelberg H, McGregor CG. Cloning and expression of porcine beta1,4 N-acetylgalactosaminyl transferase encoding a new xenoreactive antigen. *Xenotransplantation*. 2014;**21**(6):543-554
- [131] Petersen B, Carnwath JW, Niemann H. The perspectives for porcine-to-human xenografts. *Comparative Immunology, Microbiology and Infectious Diseases*. 2009;**32**(2):91-105
- [132] Lutz AJ, Li P, Estrada JL, Sidner RA, Chihara RK, Downey SM, et al. Double knockout pigs deficient in N-glycolylneuraminic acid and galactose alpha-1,3-galactose reduce the humoral barrier to xenotransplantation. *Xenotransplantation*. 2013;**20**(1):27-35
- [133] Goto M, Tjernberg J, Dufrane D, Elgue G, Brandhorst D, Ekdahl KN, et al. Dissecting the instant blood-mediated inflammatory reaction in islet xenotransplantation. *Xenotransplantation*. 2008;**15**(4):225-234
- [134] van der Windt DJ, Marigliano M, He J, Votyakova TV, Echeverri GJ, Ekser B, et al. Early islet damage after direct exposure of pig islets to blood: Has humoral immunity been underestimated? *Cell Transplantation*. 2012;**21**(8):1791-1802
- [135] Korsgren O, Lundgren T, Felldin M, Foss A, Isaksson B, Permert J, et al. Optimising islet engraftment is critical for successful clinical islet transplantation. *Diabetologia*. 2008;**51**(2):227-232
- [136] Moberg L, Johansson H, Lukinius A, Berne C, Foss A, Kallen R, et al. Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. *Lancet*. 2002;**360**(9350):2039-2045
- [137] Hawthorne WJ, Salvaris EJ, Phillips P, Hawkes J, Liuwantara D, Burns H, et al. Control of IBMIR in neonatal porcine islet xenotransplantation in baboons. *American Journal of Transplantation*. 2014;**14**(6):1300-1309
- [138] Vogel CW, Fritzinger DC, Hew BE, Thorne M, Bammert H. Recombinant cobra venom factor. *Molecular Immunology*. 2004;**41**(2-3):191-199

- [139] Ozmen L, Ekdahl KN, Elgue G, Larsson R, Korsgren O, Nilsson B. Inhibition of thrombin abrogates the instant blood-mediated inflammatory reaction triggered by isolated human islets: Possible application of the thrombin inhibitor melagatran in clinical islet transplantation. *Diabetes*. 2002;**51**(6):1779-1784
- [140] Mourad NI, Gianello PR. Xenoislets: Porcine pancreatic islets for the treatment of type I diabetes. *Current Opinion in Organ Transplantation*. 2017;**22**(6):529-534
- [141] Gill RG, Wolf L, Daniel D, Coulombe M. CD4+ T cells are both necessary and sufficient for islet xenograft rejection. *Transplantation Proceedings*. 1994;**26**(3):1203
- [142] Olack BJ, Jaramillo A, Benschoff ND, Kaleem Z, Swanson CJ, Lowell JA, et al. Rejection of porcine islet xenografts mediated by CD4+ T cells activated through the indirect antigen recognition pathway. *Xenotransplantation*. 2002;**9**(6):393-401
- [143] Koulmanda M, Laufer TM, Auchincloss H Jr, Smith RN. Prolonged survival of fetal pig islet xenografts in mice lacking the capacity for an indirect response. *Xenotransplantation*. 2004;**11**(6):525-530
- [144] Tonomura N, Shimizu A, Wang S, Yamada K, Tchipashvili V, Weir GC, et al. Pig islet xenograft rejection in a mouse model with an established human immune system. *Xenotransplantation*. 2008;**15**(2):129-135
- [145] Scalea J, Hanecamp I, Robson SC, Yamada K. T-cell-mediated immunological barriers to xenotransplantation. *Xenotransplantation*. 2012;**19**(1):23-30
- [146] Trikudanathan S, Sayegh MH. The evolution of the immunobiology of co-stimulatory pathways: Clinical implications. *Clinical and Experimental Rheumatology*. 2007;**25**(5 Suppl 46):S12-S21
- [147] Tian M, Lv Y, Zhai C, Zhu H, Yu L, Wang B. Alternative immunomodulatory strategies for xenotransplantation: CD80/CD86-CTLA4 pathway-modified immature dendritic cells promote xenograft survival. *PLoS One*. 2013;**8**(7):e69640
- [148] Contreras JL. Extrahepatic transplant sites for islet xenotransplantation. *Xenotransplantation*. 2008;**15**(2):99-101
- [149] Kumagai-Braesch M, Ekberg H, Wang F, Osterholm C, Ehrnfelt C, Sharma A, et al. Anti-LFA-1 improves pig islet xenograft function in diabetic mice when long-term acceptance is induced by CTLA4Ig/anti-CD40L. *Transplantation*. 2007;**83**(9):1259-1267
- [150] Elliott RB, Escobar L, Tan PL, Muzina M, Zwain S, Buchanan C. Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation. *Xenotransplantation*. 2007;**14**(2):157-161
- [151] Meyer T, Höcht B, Ulrichs K. Xenogeneic islet transplantation of microencapsulated porcine islets for therapy of type I diabetes: Long-term normoglycemia in STZ-diabetic rats without immunosuppression. *Pediatric Surgery International*. 2008;**24**(12):1375-1378
- [152] Zhu HT, Lu L, Liu XY, Yu L, Lyu Y, Wang B. Treatment of diabetes with encapsulated pig islets: An update on current developments. *Journal of Zhejiang University Science B*. 2015;**16**(5):329-343
- [153] Weir GC. Islet encapsulation: Advances and obstacles. *Diabetologia*: Clinical and Experimental Diabetes and Metabolism. 2013;**56**(7):1458-1461

- [154] Teotia RS, Kadam S, Singh AK, Verma SK, Bahulekar A, Kanetkar S, et al. Islet encapsulated implantable composite hollow fiber membrane based device: A bioartificial pancreas. *Materials Science & Engineering C*. 2017;**77**:857-866
- [155] Korsgren O. Islet encapsulation: Physiological possibilities and limitations. *Diabetes*. 2017;**66**:1748-1754
- [156] Cooper DK, Matsumoto S, Abalovich A, Itoh T, Mourad NI, Gianello PR, et al. Progress in clinical encapsulated islet xenotransplantation. *Transplantation*. 2016;**100**(11): 2301-2308
- [157] Wynyard S, Nathu D, Garkavenko O, Denner J, Elliott R. Microbiological safety of the first clinical pig islet xenotransplantation trial in New Zealand. *Xenotransplantation*. 2014;**21**(4):309-323
- [158] Tan PL. Company profile: Tissue regeneration for diabetes and neurological diseases at Living Cell Technologies. *Regenerative Medicine*. 2010;**5**(2):181-187
- [159] Elliott RB, Living Cell T. Towards xenotransplantation of pig islets in the clinic. *Current Opinion in Organ Transplantation*. 2011;**16**(2):195-200
- [160] Garkavenko O, Durbin K, Tan P, Elliott R. Islets transplantation: New Zealand experience. *Xenotransplantation*. 2011;**18**(1):60
- [161] Spizzo T, Denner J, Gazda L, Martin M, Nathu D, Scobie L, et al. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 2a: Source pigs—Preventing xenozoonoses. *Xenotransplantation*. 2016;**23**(1):25-31
- [162] Cooper DK, Bottino R, Gianello P, Graham M, Hawthorne WJ, Kirk AD, et al. First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes-- chapter 4: Pre-clinical efficacy and complication data required to justify a clinical trial. *Xenotransplantation*. 2016;**23**(1):46-52