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Chapter

Pig Islet Transplant

Masayuki Shimoda

Abstract

Islet transplantation is an effective treatment for insulin-dependent diabetes, but the shortage of donors is a problem. To overcome this, porcine islets have been widely studied as an alternative source. This chapter focuses on recent advances in porcine islet transplantation, placing particular emphasis on new transgenic pig models, islet encapsulation, and biological safety. Genetic modifications aimed at reducing the immunogenicity of islet cells to prolong graft survival or improve insulin secretory function have been reported. Microencapsulation and macroencapsulation of porcine islets may be able to control rejection with little or no immunosuppression. Also, the risk of porcine endogenous retrovirus infection is considered low because several clinical and preclinical studies have found no such evidence. Appropriate pathogen screening, animal selection, and microbiological and quality control measures should improve the safety and efficacy of porcine islet transplantation in future clinical trials.

Keywords: xenotransplantation, islet transplantation, porcine islet

1. Introduction

The islet transplantation protocol used for patients with type 1 diabetes, published by a team of researchers at the University of Alberta in 2000, was called the Edmonton Protocol and became the starting point for clinical islet transplantation [1]. The characteristics of the Edmonton Protocol were that multiple transplants were performed using multiple donors to transplant sufficient amounts of islets, no steroids were used for immunosuppression, and transplants were performed as soon as possible after islet isolation.

Clinical results were reported 5 years after the Edmonton Protocol was announced [2], and several problems were identified. For example, the insulin-free status is not sustained for a long time, the probability of being able to obtain islets of sufficient quality and quantity for transplantation even with islet isolation is about 50%, and there were many side effects, mainly from immunosuppressants.

Islet transplantation has been found to stabilize blood glucose levels and could prevent severe hypoglycemia, defined as hypoglycemia requiring another person's assistance. Because severe hypoglycemia can be life-threatening for patients with type 1 diabetes, islet transplantation will likely be positioned as a measure for preventing severe hypoglycemia. Indeed, allogeneic islet transplantation is an established treatment for severe hypoglycemia in Canada and other European countries. In addition, in 2016, a phase 3 clinical trial of allogeneic islet transplantation for type 1 diabetes patients with a history of severe hypoglycemia found that islet transplantation has a preventive effect for severe hypoglycemia [3]. Therefore, allogeneic

islet transplantation has also come to be recognized as standard treatment for severe hypoglycemia in the United States. Data on allogeneic islet transplantation are registered in the Collaborative Islet Transplant Registry (CITR). According to CITR data, the C-peptide positivity rate after islet transplantation alone was 80% after 1 year and 61% after 3 years, but the severe hypoglycemia prevention rate was 94 and 88%, respectively. This indicates that even if the concentration of C-peptide is below the lower limit of detection for a positive result (0.3 ng/ml), it would be effective in stabilizing blood glucose levels and preventing hypoglycemia. According to the data from the International Pancreas Transplant Registry, the pancreatic graft survival rate in simultaneous kidney and pancreas transplantation was 89% at 1 year and 82% at 3 years after transplantation. In other words, islet transplantation outperforms simultaneous pancreas and kidney transplantation in terms of rates of preventing severe hypoglycemia. The current status and direction of beta cell replacement therapy were discussed at a consensus meeting of the beta cell replacement therapy opinion leaders held at Oxford University in 2014 [4]. According to the consensus report, there are 15–20 million patients with type 1 diabetes worldwide, they are mostly at >20 years after onset of type 1 diabetes, 1 in 6 patients develop hypoglycemia unawareness, and ~10% of deaths in type 1 diabetes patients are due to hypoglycemia. It was announced that β cell replacement therapy was optimal for hypoglycemia in patients with hard-to-control type 1 diabetes. However, only 0.1% of patients with type 1 diabetes could receive beta cell replacement therapy due to a shortage of donors. In Japan, cardiac arrest donor islet transplantation [5] and living donor islet transplantation [6] have been carried out, but in order to fundamentally solve the donor shortage, β cell replacement therapy not relying on human organ donors is considered essential. Under these circumstances, pig organs are attracting attention as an alternative to organs from human donors.

2. Pancreatic islet transplantation using porcine islets

To realize successful porcine islet transplantation, exploratory clinical research began several decades ago. **Table 1** shows an overview of the history of porcine islet transplantation. In the 1990s in Sweden, Groth et al. transplanted islet cells from fetal pigs into type 1 diabetic patients on immunosuppressants after kidney transplantation [7]. Porcine C-peptide was positive for several months after transplantation, which indicated that porcine islets were successfully engrafted in the human body. Yet, no clinical effect such as a decrease in the amount of insulin injection was observed. In other works, Valdes et al. implanted an angioplasty device with newborn pig islets and Sertoli cells subcutaneously into type 1 diabetes patients [8]. Eleven patients received additional transplantation 6–9 months after the initial transplantation, and four received additional transplantation in the third year. Two patients achieved insulin-free status for several months after transplantation. In New Zealand, Elliott et al. transplanted newborn pig islets encapsulated in hydrogel microcapsules into the peritoneal cavity of type 1 diabetic patients. Because the islets were embedded in the immunoisolation capsule, no immunosuppressant was used. Insulin and glucagon staining of encapsulated pig islets, which were removed after 9.5 years of transplantation, showed that the encapsulated pig islets could be engrafted for a long time [9].

Thus, xenogeneic islet transplantation for type 1 diabetes patients using porcine islets has been performed in several clinical trials overseas. The risk of infection due to xenotransplantation was a concern.

Year	Events	Ref.
1994	Groth et al. reported that fetal pig islet transplantation to diabetic patients	[7]
1997	Patience et al. reported that PERV could infect human cells	[11]
2005	Valdes-Gonzales et al. reported a 4-year course after transplantation of neonatal pig islets and Sertoli cells	[8]
2006	Dufrane et al. showed that encapsulated adult porcine islets survived in the cynomolgus monkey body for more than 6 months	[12]
2006	Hering et al. achieved long-term insulin-free status in diabetic monkeys by transplantation of wild-type adult porcine islets	[13]
2006	Cardona et al. achieved long-term insulin independence in diabetic monkeys by transplantation of neonatal porcine islets	[14]
2007	Elliott et al. reported that about 9.5 years after transplantation, encapsulated porcine islets were recovered and insulin staining was positive	[9]
2013	Wang et al. commenced neonatal porcine islet transplantation with Tregs at Central South University, China	—
2014	Matsumoto et al. reported porcine islet transplantation under New Zealand regulations	[23]
2015	Yang et al. announced that they used CRISPR/Cas9 to inactivate all PERVs	[31]
2016	Matsumoto et al. reported clinical efficacy with encapsulated pig islet transplantation	[24]
2017	Yamaguchi et al. succeeded in creating a mouse pancreas in a rat using blastocyst complementation	[32]

Table 1.
Chronological overview of clinical and preclinical islet xenotransplantation.

3. Designated pathogen-free status and porcine endogenous retrovirus

Pigs for clinical use must have a designated pathogen-free (DPF) status, which means they are free of pathogens that can infect humans and pigs [10]. DPF status is achieved by delivering a piglet by cesarean section from a sow that has been confirmed to be free of transplacental pathogens, and after cleaning and decontamination, the piglet is placed in a biosecure barrier facility.

These facilities are defined at several levels. First, it is necessary that the facility itself be sited away from the pig farming facility. The breeding building must be completely isolated from the outside environment with an air filter, water decontamination system, radiation sterilization, and autoclave for all incoming materials. Piglets are fed with pasteurized milk, not breast milk, and enteric bacteria are provided separately. For waste disposal, especially liquid waste, special consideration is necessary to avoid backflow. Staff must pass through antiseptic showers both when entering and exiting the facility and must change into special sterilized clothes. Routine health checks of personnel are also conducted. In general, all procedures must follow standard operative procedures. It is also important to incorporate current good manufacturing practices in accordance with regulatory guidelines.

Nevertheless, in coculture of PK-15 pig kidney cell line (PK15 cells), and human fetal kidney cells 293 (HEK293 cells), infection of HEK293 cells by porcine endogenous retrovirus (PERV) naturally released from PK15 cells has been reported [11]. The problem of PERV infection via porcine xenotransplantation has emerged, and because PERV-A and PERV-B are integrated into all porcine genes, they are extremely difficult to eliminate. Thus, with regard to PERV, instead of exclusion, denial of infectivity and monitoring of transplanted patients and their close relatives are recommended.

4. Pig islet transplantation experiment using nonhuman primates

Dufrane et al. demonstrated that mature pig islets embedded in alginate capsules and transplanted into cynomolgus monkeys without immunosuppressants survived up to 6 months after transplantation [12]. Hering et al. at the University of Minnesota reported that wild-type (unmodified) adult porcine islets transplanted into the portal vein of rhesus monkeys with streptozotocin-induced diabetes mellitus achieved long-term insulin independence [13]. Also, Cardona et al. from the University of Alberta reported that wild-type newborn porcine islets transplanted into the portal vein of monkeys with pancreatectomy-induced diabetes resulted in long-term insulin-free status [14]. Recently, Park et al. reported more advances with modification of immunosuppressants [15]. These reports have brought great hope for islet transplantation using porcine islets. However, the importance of prevention of infections including PERV has been recognized.

5. Guidelines

While xenotransplantation holds great promise for overcoming donor shortages, the global problem of xenogeneic infection must be considered. Therefore, in 2008 the World Health Organization (WHO) held a conference on xenotransplantation in Changsha, China, and presented the main points as the First WHO Global Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials [16]. This statement, referred to as the Changsha Communique, is the basis for xenotransplantation worldwide. The content summary is shown in **Table 2**.

Based on the Changsha Communique, in 2009 the International Xenotransplantation Association (IXA) announced a consensus statement of conditions for the initiation of clinical trials of porcine islet products for type 1 diabetes [17]. This consensus statement consists of seven chapters and addresses the requirements of the Changsha Communique. Because remarkable progress has been made in research in this field, the statement should be updated.

Since the consensus statement for the initiation of xenogeneic islet transplantation in IXA was announced in 2009, clinical findings of xenotransplantation including clinical xenogeneic islet transplantation in New Zealand have been accumulated, and the consensus statement was updated in 2016 [18]. The contents of the chapters are:

Chapter 1. Key ethical requirements and progress toward the definition of an international regulatory framework: ethical requirements and progress toward establishing an international regulatory framework.

Chapter 2. Source pigs: pig requirements for donor sources.

Chapter 3. Pig islet product manufacturing and release testing: manufacturing, quality control, and release testing.

Chapter 4. Pre-clinical efficacy and complication data required to justify a clinical trial: appropriate pre-clinical trial.

Chapter 5. Strategies to prevent transmission of porcine endogenous retroviruses: concept and prevention strategy for PERV.

Chapter 6. Patient selection for pilot clinical trials of islet xenotransplantation: appropriate patient selection.

Chapter 7. Informed consent and xenotransplantation clinical trials: ideal informed consent procedure.

In particular, because PERV infection and cross-species infection did not occur at all, these infections were regarded as a “theoretical risk” and were considered

1	Xenotransplantation can be used to treat serious diseases such as diabetes, heart disease, and kidney disease. Also, patients who cannot currently receive transplants may be able to receive transplants
2	Medical animals can provide high-quality cells, tissues, and organs. Genetically modified animals may further improve outcomes. Medical animals are limited to closed colonies. Breeding should be done at a well-controlled pathogen-free facility, with high standards for animal welfare. Medical animals are verified by testing for the absence of known pathogens and, moreover, must be kept free of infectious diseases by continuous observation
3	Xenotransplantation is a complex procedure with risk of rejection, poor graft function, and known or unknown infections. There is a risk of developing serious or new infections, and patients, relatives, or other humans and animals may be infected
4	Because of the risk to the community at large, clinical trials of xenotransplantation should be conducted under strict regulation. Xenotransplantation should not be performed in the absence of national regulations. These regulations should have legal basis and be able to prohibit nonregulatory transplants. Furthermore, this regulatory framework should ensure transparency to the general public and should include both science and ethics
5	Given the risk to the community, the benefit to the patient should be high. In particular, preclinical studies should be conducted using animal experiments with predictable effects to demonstrate the safety and efficacy recommended by the international scientific community. Proposed clinical trials should be assessed by the relevant regulatory authorities to minimize risk
6	Personnel responsible for clinical trials should explain the inclusion criteria in order to justify the clinical trial. Patient selection must be done at the patient's own discretion based on informed consent. Patients and relatives must be effectively educated to ensure compliance and minimize risks to themselves and to society
7	Participation in xenotransplantation usually takes a long time. Samples from donor animals, patients before and after surgery, and all records should be kept. Patients who have had transplants need lifetime follow-up, and close relatives may need similar follow-up. The results of clinical trials should be analyzed rigorously. Patients who have undergone xenotransplantation should be registered in an appropriate database, which should also be able to track donor animals. At the same time, the patient's privacy has to be protected. All records, data, and samples must be prepared for submission to regulatory authorities for a designated period
8	The health-care team must have adequate experience and an understanding of the risks to the patient, the health-care team itself, and the community. Because of the risk of transmission to the community, a system of vigilance and surveillance should be established to ensure that any infection associated with the xenotransplant will be identified and addressed immediately
9	There is a need to establish a system for worldwide information exchange, prevention of unregulated xenotransplantation, vigilance and monitoring of xenotransplantation, and response in case of suspected infection
10	Considering the benefits of successful xenotransplantation, from the early stages, the treatment should be considered widely accepted as the treatment is completed, and the public sector is recommended to support

Table 2.
Summary of the contents of the Changsha communique.

unlikely under the adequate control of suitable donors and recipients. In addition, clinical data have been accumulated, infection diagnostic techniques have progressed, clinical protocols have been improved, the risk of PERV-related infection is better understood, DPF facilities and dietary restriction methods have advanced, and the role of sample archives has been clarified. As a result of these efforts, cost-effective generation of donor pigs will be possible, and it is expected that porcine islets will be delivered to many patients who truly need this treatment modality.

Some countries have responded to this consensus statement. In Japan, the Ministry of Health, Labour and Welfare also revised the "guidelines on public health infection problems associated with the implementation of xenotransplantation" in 2016.

Recently, in response to the resumption of clinical xenotransplantation, the Third WHO Global Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials was held in 2018, and the contents were announced as the 2018 Changsha Communique [19]. The points of the revision are:

1. Prohibition of clinical trials in countries without national regulations and subsequent prohibition of medical tourism to such countries

2. Emphasis on reproducible preclinical data

3. Development of quality control measures and standards for genetically modified pigs

4. Deletion of sample retention period requirements

The Communique emphasizes safety while taking into consideration the actual situation of clinical xenotransplantation, social conditions, and technological advances.

6. Encapsulation of islets

Although islet transplantation has proved to be successful for patients with type 1 diabetes, one of the limitations is the requirement for lifelong immunosuppression. An encapsulation strategy that can prevent rejection of xenogeneic islets can potentially overcome this challenge (**Figure 1**). Such capsules have fine holes that allow the passage of oxygen, glucose, and insulin but not immune cells. Blocking immune cells allows islet transplantation without the need for immunosuppressants. The capsules have been studied in various materials and sizes. There are three main sizes: macro, micro, and nano [20, 21]. The macro-capsule is used to seal islets in centimeter-order devices, which are easy to handle and can be removed and replaced. However, the problem is that substance permeability is low, and foreign body reactions are likely to occur, and the survival rate of internal cells is low. The microcapsule is several hundreds of micrometers to millimeters order in size, is

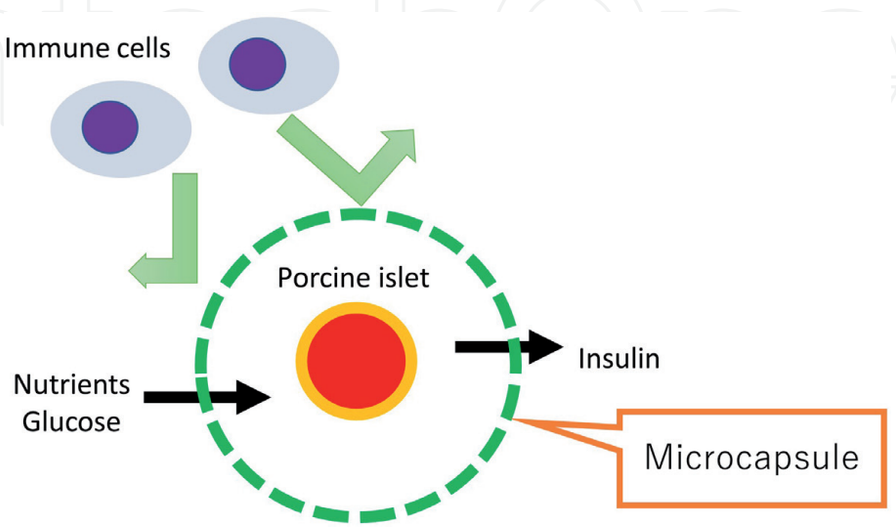

Figure 1.
Schematic representation of an encapsulated porcine islet. Pancreatic islets isolated from DPF pig are encapsulated with an immunoisolation hydrogel. The capsule has fine holes that allow passage of oxygen, glucose, and insulin but not immune cells.

made mainly of hydrogel, and contains one to several islets. It is compatible in terms of substance permeability and immune isolation ability. However, it is too large for endovascular transplantation and recovery after transplantation is difficult. The nano-capsule has a thin-layered surface coating enclosing pancreatic islets comprising a variety of polymers and therapeutic agents. The permeability is high but stability is an issue. In addition, a surface modification with immune-privileged cells is another concept of encapsulation. Each of these encapsulation techniques has advantages and disadvantages, but the technique is very promising.

7. Micro-encapsulated neonatal porcine islet transplantation

In 1980, Lim and Sun applied microcapsules in diabetes treatment, showing prolonged islet graft survival using alginate-poly-L-lysine-polyethyleneimine microcapsules [22]. Since then, this promising technology has been considerably improved.

In 2014, clinical results were reported in which neonatal porcine islets isolated from DPF pigs encapsulated with alginate and poly-(L)-ornithine were transplanted in 14 patients with type 1 diabetes [23]. The patients were divided into four groups according to transplantation dose, and 5000, 10,000, 15,000, and 20,000 IEQ/kg of encapsulated islets were transplanted intraperitoneally, respectively, according to body weight. No immunosuppressant was used. After transplantation, in the low-dose groups of 5000 and 10,000 IEQ/kg, the frequency of occurrence of hypoglycemia unawareness was halved compared to that before transplantation.

In 2016, the same group reported results of a clinical trial in which 5000 and 10,000 IEQ/kg of encapsulated neonatal porcine islets were transplanted twice at intervals of 3 months [24]. After transplantation, HbA1c decreased significantly in all patients, and the frequency of occurrence of hypoglycemia unawareness was significantly reduced in the group that received a transplant of 10,000 IEQ/kg twice. Moreover, the group that received a transplant of 10,000 IEQ/kg maintained an average HbA1c of ≤7% over 2 years after transplantation and showed a long-term effect. Clinical effects have been shown in islet xenotransplantation.

8. Porcine islet transplantation combined with regulatory T cell (Treg)

A clinical trial of transplantation of neonatal porcine islets and autologous Tregs in type 1 diabetes patients is underway and is being conducted by Wang et al., Central South University, China (ClinicalTrials.gov Identifier NCT03162237). The transplanted dose is 10,000 IEQ/kg of islets, 2×10^6/kg of Tregs, and the immunosuppressants are tacrolimus, mycophenolate mofetil, and belatacept. The primary end point is stable blood glucose level and prevention of ketoacidosis and hypoglycemia and a 30% reduction in required insulin. The authors reported that the condition of these patients improved substantially (http://en.xy3yy.com/document/show_12/184.html). These results are encouraging and add value to this field of research.

9. Gene editing and blastocyst complementation

One of the advantages of xenotransplantation is the possibility of genetic modification in the donor. Advances in gene editing, such as the CRISPR/Cas9 system, have facilitated editing of specific genes.

Recent advances in genetic engineering and gene editing of donor pigs may overcome the challenge of islet rejection and improve their engraftment and ability to secrete insulin. The required set of genetic modifications will depend on the source of islets (fetal, neonatal, and adult), mode of delivery (encapsulated, free), and the transplantation site. Genetic modification of pigs has been developed mainly via deletion of one or more of the major porcine antigens such as GGTA1, CMAH, and β4GalNT2, and/or insertion of human complement (such as hCD46, hCD55, and hCD59) which suppress the coagulation reaction [25, 26], and/or knockout or insertion of other genes. Simultaneous knockout of two or three major pig antigens has been achieved, and consequently the binding of human antibodies to these cells is significantly reduced. Other genes include the expression of proteins that inhibit co-stimulation of T cells such as hCTLA4Ig [27]. The combinations of multiple gene editing were promising [28, 29]. Currently, the modifications being carried out in pigs span over 24 genes including coagulation regulatory genes, immune cell regulatory genes, and anti-inflammatory genes [30]. Simultaneous modification of more than five genes has been performed in some pigs [30]. These genetically modified pigs will contribute to the improvement of transplantation outcome.

The technology has also been applied to elimination of PERV, and Yang et al. of Harvard University reported inactivation of all PERV genomes using the CRISPR/Cas9 system [31]. They launched a venture company called eGenesis, aiming to create a human friendly medical pig with the added advantage of PERV inactivation. Thus, it is considered that a medical pig suitable for islet transplantation will be created by gene editing technology.

Yamaguchi et al. of the University of Tokyo complemented mouse-induced pluripotent stem cells (iPS), cells with blastocysts of pancreatic-deficient rats, and succeeded in inducing the rats to develop mouse pancreas [32]. The pancreas derived from mouse iPS, which was produced by this blastocyst complementation method, was the size of the rat pancreas and had a sufficient number of pancreatic islets that could be isolated for transplantation to the mouse. These islets were transplanted with small amounts of an immunosuppressant drug to diabetic mice syngeneic with the iPS cells to normalize blood glucose levels. In addition, this research group also succeeded in inducing apancreatic pigs to produce different pig-derived pancreases by blastocyst complementation [33]. In the future, it may be possible to use a human iPS cell line to generate a medical pig for a human pancreas by blastocyst complementation. If the patient's own iPS-derived pancreas can be obtained from a pig, it is essentially an autologous transplantation, and it thus becomes possible to perform islet transplantation without the need for immunosuppressants.

10. Summary

Allogeneic islet transplantation is being established as a standard treatment for hypoglycemia unawareness and severe hypoglycemia, but a shortage of human donors has become a problem. Islet xenotransplantation using DPF pigs is considered as a promising fundamental solution to the donor shortage. However, cross-species infection, especially PERV infection, poses risks to the community, and discussions among key opinion leaders have been implemented by the WHO. As a result, the IXA Consensus Statement was published in 2016, envisioning a future where cost-effective delivery of islet transplants to diabetic patients is facilitated by medical pigs. With the risk of infection always kept in mind, cases of clinical islet xenotransplantation have been accumulated, and steady progress has been made toward a feasible, safe, and effective treatment for diabetic patients. In addition, the development of donor pigs optimal for transplantation using the

recently publicized CRISPR/Cas9 technology and blastocyst complementation that could enable the creation of an individual's pancreas in pigs could provide for safer and more effective islet xenotransplantation. Proper pathogen screening, animal selection, microbiological control, and long-term monitoring of recipients will be required for clinical application of porcine islet transplantation.

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