Donor pigs for clinical islet xenotransplantation: Review and future directions

Matsumoto, Shinichi ; Asari, Sadaki ; Nanno, Yoshihide ; Nakamura, Hiroyuki ; Okawa, Taisuke ; Fukumoto, Takumi

(Citation)
Cell Transplantation,34:09636897251332532

(Issue Date)
2025-04

(Resource Type)
journal article

(Version)
Version of Record

Cell Transplantation
Volume 34: 1–11

DOI: 10.1177/09636897251332532
journals.sagepub.com/home/cll
Sage

Donor pigs for clinical islet xenotransplantation: Review and future directions

Shinichi Matsumoto[1,2], Sadaki Asari[1], Yoshihide Nanno[1], Hiroyuki Nakamura[1], Taisuke Okawa[1], and Takumi Fukumoto[1,2]

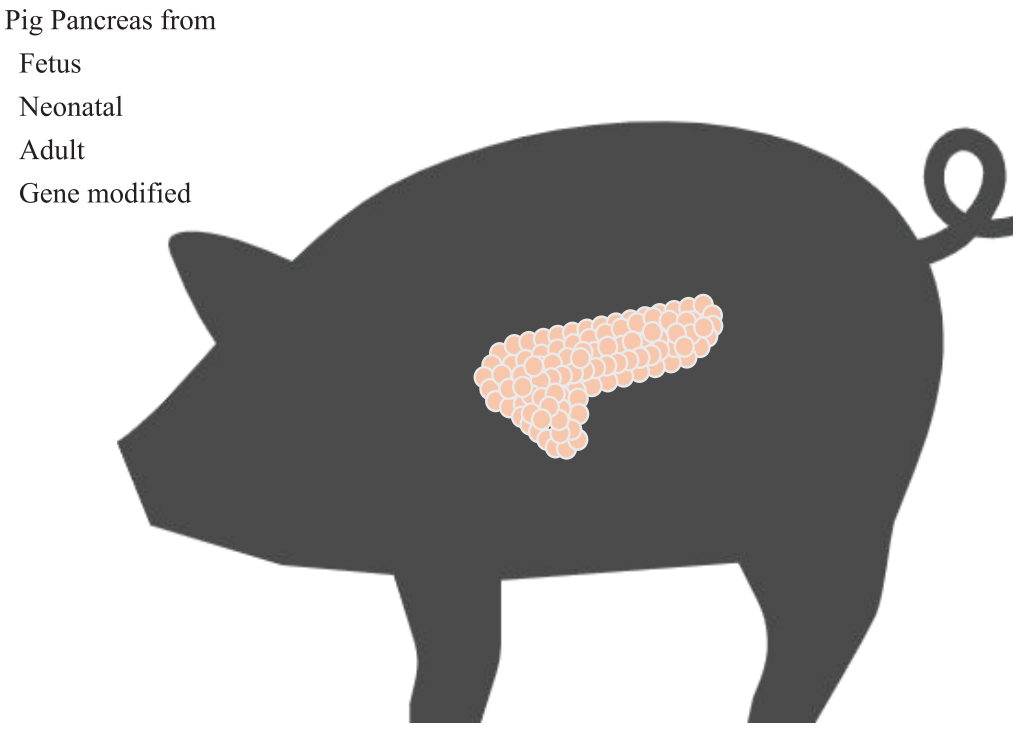
Abstract

Allogeneic islet transplantation becomes a viable option for patients with unstable type 1 diabetes. However, considering the huge number of patients with type 1 diabetes, human donor shortage is a serious issue. To overcome the donor shortage issue, xenotransplantation is an attractive option. In fact, clinical islet xenotransplantation has been conducted since 1990s. The first clinical trial was performed using fetal pigs and demonstrated the porcine pancreatic tissue could survive in human body with immunosuppressive strategies. To scale up the islet production, Canadian group established a method for islet isolation from neonatal pigs. Their method has been used for clinical islet xenotransplantation in New Zealand, Russian, Mexico, Argentina, and China. Recently Korean group published a clinical protocol for islet xenotransplantation using adult pigs. For the next generation of islet xenotransplantation, gene-modified pigs were created. Especially "superislets" created by Belgian group demonstrated promising preclinical outcomes. With advanced donor pigs, islet xenotransplantation might become a suitable treatment for the majority of type 1 diabetic patients.

Keywords

islets, islet transplantation, xenotransplantation, clinical trials

Graphical Abstract



Introduction

Allogeneic islet transplantation becomes a viable treatment for type 1 diabetic patients who experience severe hypoglycemia despite intensive diabetes management and education[1]. However, considering a limited number of pancreatic donors, xenogeneic islet transplantation is an attractive option[1]. In fact, the first islet xenotransplantation was performed in 1990[2].

The ideal donor pig for islet xenotransplantation is a fundamental question. In this review article, we assess the clinically used donor pigs and discuss future possibilities of a next generation of donor pigs.

Fetal porcine pancreas for islet xenotransplantation

Swedish group

The first clinical islet xenotransplantation was conducted by Swedish group using fetal pancreata. Fetuses ranging in age from 51 to 77 days were used[3]. The crown-rump length of the fetuses ranged from 10 to 20 cm, and the body weight from 40 to 260 g. The fetuses were immediately collected from the uterus and placed on ice during transportation to the laboratory. Removed pancreata were put on chilled Hanks' balanced salt solution (HBSS) supplemented with benzylpenicillin, and streptomycin within 1 h after killing the saw.

Their islet isolation method was as follows. The pancreata were minced and transferred to a glass vial containing collagenase. Then digestion was conducted by vigorous shaking in a 37°C water bath. After visual confirmation of disintegration, digestion was arrested by adding HBSS. Then digested pancreata were washed with HBSS and resuspended in RPMI 1640 culture medium with fetal calf serum or heat-inactivated human serum. The culture dishes were maintained at 37°C in a gas phases of 5% CO_2 in humidified air. The culture medium was changed every 2 days until the sixth or seventh day. This method provided average 10,000 islet like cell cluster per a pancreas[4]. They mentioned optimal age of the piglets, and they preferred as old as possible due to larger glands with more differentiated endocrine properties. However, with their method, late gestational (2–3 days before term) or newborn piglets were less effective. The group tested the effect of nicotinamide and sodium butyrate during culture. They found the optimal maturation of porcine fetal pancreatic cells was obtained in serum-free medium supplemented with nicotinamide. Sodium butyrate was a potent stimulus for beta cell differentiation but leaded to increase apoptotic cell death[5]. They transplanted the fetal porcine pancreatic cells into type 1 diabetic patients who had already received kidney transplantation. They successfully demonstrated positive porcine C-peptides for 200–400 days, however no apparent clinical benefits were shown.

New Zealand group

New Zealand group used fetal pancreata for islet isolation using the Korsgren islet isolation method[6]. They used term-gestation piglets delivered by hysterotomy from healthy farm stock[7]. Pigs in New Zealand are remarkably free of many porcine endemic viruses as a result of isolationist agricultural policies. A standard collagenase digestion of the minced pancreata according to Korsgren protocol was carried out. The digested pancreatic tissue was cultured in RPMI-1640 medium containing 10mM nicotinamide[7]. They encapsulated the isolated islets then transplanted the encapsulated islets into two type 1 diabetic patients without immunosuppressive drugs[7]. They successfully demonstrated positive porcine C-peptides. In addition, reduction of insulin dose and HbA1c were shown.

Neonatal porcine pancreas for islet xenotransplantation

Canadian group

Korbutt and his colleague demonstrated a large-scale isolation method using neonatal porcine pancreas[8]. They used 1-day-old to 3-day-old Landrace Yorkshire neonatal pigs (1.5–2.0 kg body weight) of either sex. Their islet isolation method was as follows. Procured pancreata were cut into fragment of 1–2 mm^3, then digested with a collagenase. The digested pancreatic tissue was filtered through a nylon screen (500 μm) then cultured in Ham's F10 tissue culture medium (10 mmol/l glucose, 50 mmol/l IBMX, 0.5% BSA, 2 mmol/l L-glutamine,10 mmol/l nicotinamide, 100 U/ml penicillin, and 100m g/ml streptomycin) for 9 days. This group collaborated with the Emory University group and transplanted the isolated neonatal porcine islets into portal vein of pancreatectomized diabetic rhesus macaques[9]. They used short-term administration of an antibody to interlukin-2 receptor

[1]Hepatobiliary Pancreatic Surgery, Kobe University Graduate School of Medicine, Kobe, Japan
[2]Medical Porcine Development Organization, Kobe, Japan

Received: February 2, 2025; revised: March 12, 2025; accepted: March 17, 2025

Corresponding Author:
Shinichi Matsumoto, Hepatobiliary Pancreatic Surgery, Kobe University Graduate School of Medicine, Kusunoki-cho 7-5-2, Chuo-ku, Kobe 650-0017, Japan.
Email: shinichi.matsumoto@medicalporcine.com

Table 1. Testing schedule for the NZ donor pig herd[12].

Microorganism	Frequency of testing	Method
Bacteria		
Leptospira tarrasovi	Quarterly	MAT
Leptospira hardjo	Quarterly	MAT
Leptospira Pomona	Quarterly	MAT
Mycoplasma hyopneumoniae	Annually	PCR
Campylobacter	Annually	Culture
Isospora	Annually	Culture
Cryptosporidium	Annually	Culture
Yersinia	Annually	Culture
E coli K88	Annually	Culture
Salmonella	Annually	Culture
Viruses		
PCV2	Quarterly	ELISA & rt-PCR
PCV1	Annually	PCR
PLHV2	Quarterly	rt-PCR
PCMV	Quarterly	rt-PCR
Rota V A-C	Annually	RT-PCR
Reo V	Annually	RT-PCR
PTV	Annually	rtRT-PCR
PEVB	Annually	RT-PCR
PHEV	Annually	RT-PCR
HEV	Quarterly	ELISA & RT-PCR
BVD	Quarterly	ELISA
AujD	Annually	ELISA
PPV	Quarterly	ELISA & rt-PCR
PRRSV	Annually	ELISA & PCR
EMCV	Annually	VNT & rtRT-PCR
Protozoa		
Toxoplasma	Quarterly	LAT

PCV2: Porcine Circovirus Type 2, PCV1: Porcine Circovirus Type 1, PLHV2: Porcine Lymphotropic Herpesvirus Type 2, PCMV: Porcine Cytomegalovirus, Rota V A-C: Rotavirus A, Rotavirus B and Rotavirus C, Reo V: Reovirus (all types), PTV: Porcine Teschovirus, PEVB: Porcine Hemagglutinating Encephalomyelitis Virus, HEV: Hepatitis E Virus, BVD: Bovine Virus Diarrhea, AujD: Aujeszky's Disease, PPV: Porcine Parvovirus, PRRSV: Porcine Reproductive and Respiratory Syndrome Virus, EMCV: Encephalomyocarditis Virus. MAT: microscopic agglutination test, ELISA: enzyme-linked immunosorbent assay, LAT: latex agglutination test, PCR: conventional nested PCR, RT-PCR: reverse transcriptase PCR, rt-PCR: real-time PCR, rtRT-PCR: real-time reverse transcriptase PCR, VNT: virus neutralization test.

and antibody to CD154, with maintenance immunosuppression using sirolimus and belatacept.

Six of the seven macaques receiving neonatal porcine islets with the costimulation blockade regimen achieved a sustained period of insulin independence for the length of survival of the recipient.

New Zealand group

In 2009, The International Xenotransplantation Association published consensus statement for source pig on clinical trials of porcine islet products in type 1 diabetes[10]. In the statement, donor pigs need to fulfill the designated pathogen-free (DPF) status. Related to the DPF, Food and Drug Administration (FDA) describes DPF as follows. This term is used to describe animals, animal herds, or animal facilities that have been rigorously documented to be free of specified infectious agents and that are maintained using well-defined routines of testing for designated pathogens and utilizing rigorous Standard Operating Procedures (SOPs) and practices of herd husbandry and veterinary care to assure the absence of the designated pathogens[11].

Table 2. Multilevel screening system for sows, donor pigs and islet cells[12].

Sows (prefollowing and postfollowing)	Donor pigs	Islet cells
Leptospira	HEV	Toxoplasma PCR
PPV	Toxoplasma serology	PCV2
Mycoplasma	Postmortem autopsy	PLHV
Toxoplasma	PCV2	PCMV
HEV	PLHV2	HEV
PLHV	PCMV	Mycoplasma
PCV2		Sterility (bacteriology and mycoplasma)
PCMV		Endotoxin

PCV2: Porcine Circovirus Type 2, PLHV2: Porcine Lymphotropic Herpesvirus Type 2. PCMV: Porcine Cytomegalovirus,, HEV: Hepatitis E Virus, PPV: Porcine Parvovirus.

To achieve DPF status, New Zealand group established the multilevel testing system consisting of testing donor herd, donor sows. They created a DPF facility, and the donor pig herd was maintained in the facility and tested regularly using a combination of culturing, serological, and polymerase chain reaction (PCR) method. Ten bacterial species, 15 viruses, and one protozoa were routinely tested[12] (Table 1). They also screen sows at both prefarrowing and postfarrowing, donor piglets, and islet cells as shown in Table 2.

They modified the Korbutt isolation method for the large scale neonatal porcine islet isolation[13]. They used 7-day-old to 17-day-old neonatal piglets. Digested pancreata were filtered through a 300 μm stainless-steel screen. Then the filtrated tissue was cultured in 500 mL disposable spinner flask at a concentration of 40 mL per gram of pancreatic tissue in RPMI-CAN serum-free medium (RPMI-1640, 0.3g/l ciprofloxacin, 1% human serum albumin, 0.12% wt/vol nicotinamide). The flasks were stirred on a four-position slow speed magnetic stirrer at 80–90 rpm in a humidified 5% CO_2 incubator at 37°C. Isolated islets were encapsulated using alginate and poly L ornithine (PLO) to avoid immunological rejection. The encapsulated neonatal porcine islets were transplanted into 16 type 1 diabetic patients in New Zealand and 22 type 1 diabetic patients in

Table 3. Designated Pathogen Free (DPF) listing of excluded pathogens at Spring Point Project.

Bacteria
- *Actinobacillus pleuropneumonia*
- *Actinobacillus suis*
- *Bacillus anthracis*
- *Bordetella bronchiseptica*
- *Brucella* sp.
- *Campylobacter* sp.
- *Chlamydia* sp.
- *Erysipelothrix* sp.
- *Haemophilus parasuis*
- *Lawsonia intracellularis*
- *Leptospira* sp.
- *Mycoplasma hyopneumoniae*
- *Mycoplasma hyorhinis*
- *Mycoplasma hyosynoviae*
- *Mycobacterium tuberculosis*
- *Mycobacterium bovis*
- *Mycobacterium avium*
- *Pasteurella multocida*
- *Pasteurella haemolytica*
- *Salmonella* sp.
- *Brachyspira* sp.
- *Staphylococcus hyicus*
- *Streptococcus suis*
- *Yersinia* sp.

Fungi:
Systemic mycoses including
o *Blastomyces* sp.,
o *Cryptococcus* sp.,
o *Histoplasma* sp.

Parasites:
- Pathogenic Protozoa including
o *Cryptosporidium parvum*,
o *Giardia* sp. *and*
o *Toxoplasma* sp.
- Helminths
- *Trichinella spiralis*
- Blood parasites

Arthropods:
- All pathogenic arthropods (eg, lice and mites)

Viruses:
- Adenovirus (Porcine)
- Bovine Viral Diarrhea Virus
- Porcine Circovirus types 1 and 2
- Encephalitis, Eastern and Western Equine
- Encephalomyocarditis Virus
- Enterovirus
- Hemagglutinating Encephalomyelitis Virus
- Hepatitis E
- Infectious Bovine Rhinotracheitis Virus
- Swine Influenza Virus
- Porcine cytomegalovirus
- Porcine Parvovirus
- Porcine Reproductive and Respiratory Syndrome Virus
- Parainfluenza 3 Virus
- Pseudorabies Virus
- Porcine Respiratory Coronavirus
- Rotavirus
- Transmissible Gastroenteritis Virus
- Vesicular Stomatitis Virus (NJ & Indiana)
- West Nile Fever Virus
- Porcine Lymphotropic Herpes virus 1 and 2

Argentina[14]. Importantly, there were no xenogeneic infection in all patients[14].

In New Zealand clinical trial, majority of patients improved the number of unaware hypoglycemia[15]. In Argentine trial, majority of patients improved HbA1c, reduced insulin requirements and reduced the number of unaware hypoglycemia[16]. Especially in Argentina trial, majority of patients were positive attitude even 10 years after receiving encapsulated neonatal porcine islet transplantation[17].

Chinese group

Between 2013 and 2017, Dr Wei Wang and his team transplanted newborn pig islets into 10 patients with type 1 diabetes[18] (ClinicalTrials.gov ID NCT03162237). The pigs were from the first Asian Designated Pathogen Free Donor Facility established by the Third Xiangya Hospital and Human XENO Biological Technology Co,. Ltd.

The neonatal pig islets (10,000 IEQ/kg) and patients' autologous T regulatory cells (2×10^6/kg) were transplanted. They used tacrolimus, mycophenolate mofetil, belatacept as immunosuppression. The condition of these patients improved substantially[18].

Spring Point Project

Spring Point Project (Minneapolis, MN, USA) created a Source Animal Facility (SAF) for generating DPF pigs for clinical xenotransplantation[19].

To enable DPF pigs should be reared within biosecure barrier facilities, so-called Source Animal Facilities (SAFs), isolated from potential pathogen sources via filtered air, filtered and disinfected water, sterilized fee, show-in and show-out access for staff, and hygienic measures including through screening of employees for potentially transmissible pathogens prior to hiring.

Origin of animals for SAF was populated with caesarian derived colostrum deprived (CDCD) piglets derived from vaccinated specific pathogen free (SPF) sows. The founder animals were selected based on the criteria that high islet of Langerhans yield from adult donors[20].

The CDCD piglets were housed for the first 3 weeks in the quarantine nursery in isolated nursery carts outfitted with tenderfoot flooring. After 3 weeks, the animals were moved into animal holding rooms outfitted with stainless steel penning affixed to epoxy sealed concrete flooring and walls. The Designated Pathogen Free (DPF) listing of excluded pathogens was shown in Table 3.

Medical porcine development organization. In Japan, currently no DPF pig for xenotransplantation. To accelerate xenotransplantation the "Medical Porcine Development Organization" was established in Japan on December 1, 2023, at the Kobe

Table 4. Immunosuppression protocol for porcine islet xenotransplantation into diabetic nonhuman primates by Minnesota group.

	N	Induction immunosuppression	Maintenance immunosuppression
A	3	Basiliximab	FTY720, Everolimus
B	4	Basiliximab	FTY720, Everolimus, ABI 793 (CD154-specific human monoclonal antibody)
C	5	Basiliximab	FTY720, Everolimus, ABI793, Leflunomide

Table 5. Immunosuppression protocol by Seoul group.

	Anti-IBMIR	Induction Immune Suppression	Treg transfer after induction	Maintenance Immune Suppression
1	CVF, Adalimumab	ATG	1.9×10^6 on day 6	Sirolimus Anti-CD154 mAb (5C8)
2	CVF, Adalimumab	ATG	2.6×10^7 on day 6 2.7×10^7 on day 16	Sirolimus Anti-CD154 mAb (5C8)
3	CVF, Adalimumab	ATG	6.2×10^7 on day 6 8.8×10^7 on day 20	Sirolimus Anti-CD154 mAb (5C8)
4	CVF, Adalimumab	ATG	None	Sirolimus Anti-CD154 mAb (5C8)
5	CVF, Adalimumab	ATG	None	Sirolimus Anti-CD154 mAb (5C8)

University Graduate School of Medicine in the department of hepatobiliary pancreatic surgery[1]. This organization aims to create new medical care protocols and contribute to the health and welfare of the population by establishing and improving the technology to produce pigs than can be used as medical raw materials.

Adult porcine pancreas for islet xenotransplantation into nonhuman primates

Adult porcine pancreata were not used clinically. However, these pigs were used for preclinical nonhuman primate studies.

Minnesota group

In 2016, Hering et al.[21] published long-term insulin free in diabetic nonhuman primate after intraportal adult porcine islet transplantation. They used genetically unmodified wild-type adult pigs as pancreas donors. They transplanted isolated islets 25,000 IEQ/kg into streptozotocin induced diabetic nonhuman primates. They used three types of immunosuppression protocols (Table 4).

At 100 days after transplant islet xenograft survival rates were 0% in group A, 50% in group 5, and 100% in group C. However, eight out of nine recipients showed thromboembolic lesions in group B and C, probably related to ABI793 therapy. Therefore, this protocol was not proceeded in clinical trials.

Seoul group

Seoul National University group used adult miniature pig for islet xenotransplantation. They used Seoul National University miniature pig (SNU miniature pig) for their study. At first, the Seoul group conducted 68 porcine islet isolation to identify important parameters for successful islet isolation. The pigs with a mean age 25 months, median age of 24.3 months and a mean weight of 88 kg were used. The number of male and female pigs was the same. The male pigs were bred without castration. The complete pregnancy related history was recorded for the female pigs. The general condition of all pigs was carefully examined and noted. After transfer to the operative unit a week before operation, the pigs' adaptation to their new environment was monitored by observing their feeding by a veterinarian.

They divided the isolation outcomes based on islet yield, then compared the outcomes between high yield group and low yield group. The total islet yield in high yield group was 304,962 $\pm$ 25,014 IEQ and in low yield group was 142,672 $\pm$ 12,493 IEQ. They identified good or moderate pancreatic distention with collagenase, older age (>2yr), and male gender are significant parameters to obtain high total islet yield[22].

The group demonstrated long-term control of diabetes in immunosuppressed nonhuman primate by the transplantation of adult porcine islets[23]. The immunosuppression protocol is shown in Table 5.

In addition to immunosuppression, they used cobra venom factor and anti-TNF-alpha monoclonal antibody to reduce instant blood mediate reaction (IBMIR). For induction

Table 6. Anti-CD40 mAb based immunosuppression protocol by Seoul group.

	Anti-IBMIR	Induction immune suppression	Maintenance immune suppression
1	CVF, Adalimumab	ATG, Anti-CD40mAb	Sirolimus
2	CVF, Adalimumab	ATG, Anti-CD40mAb	Sirolimus
3	CVF, Adalimumab	ATG, Anti-CD40mAb	Sirolimus, Belatacept
4	CVF, Adalimumab	ATG, Anti-CD40mAb	Sirolimus, Belatacept
5	CVF, Adalimumab	ATG, Anti-CD40mAb	Sirolimus, Tacrolimus
6	CVF, Adalimumab	ATG, Anti-CD40mAb	Sirolimus, Tacrolimus
7	CVF, Adalimumab	ATG, Anti-CD40mAb	Sirolimus, Tacrolimus
8	CVF, Adalimumab	ATG, Anti-CD40mAb	Sirolimus, Tacrolimus
9	CVF, Adalimumab	ATG, Anti-CD40mAb	Sirolimus, Tacrolimus

Table 7. Clinically available immunosuppression protocol by Seoul group.

Anti-IBMIR	Induction Immune Suppression	Maintenance Immune Suppression
Adalimumab	ATG, Anakinra, Tocilizumab, IVIg, Tacrolimus	Abatacept, Belimumab, Tofacitinib, Sirolimus or methyl prednisolone or MMF,

immunosuppression, they used anti-thymoglobulin, for maintenance immunosuppression they used sirolimus. Three out of five cases, they used own Treg as shown in Table 5.

They transplanted 81,000 IEQ/kg in the first case and 100,000 IEQ/kg in all the other cases. The diabetic nonhuman primates could maintain an insulin free status more than 603 days in the first case, 167 days in the second case, 512 days in the third case, 303 days in the fourth case, and 180 days in the fifth case. This protocol used anti-CD154 mAb which is not clinical available due to the side effects. Therefore, this protocol was not proceeded in clinical trials.

Since anti-CD154 mAb is not clinically available, the Seoul group assessed anti-CD40 antibody-based immunosuppression despite anti-CD154 mAb, in the pig to nonhuman primate islet xenotransplantation model[24]. They conducted nine cases, and their immunosuppression protocol was described in Table 6.

The streptozotocin induced diabetic nonhuman primates received approximately 100,000 IEQ/kg islets in their portal veins. The nonhuman primates could maintain insulin free periods for 4 and 5 days in the first and second cases. The insulin free periods were prolonged for 25 and 8 days in the third and fourth cases. The insulin free periods were more than 60, 266, 10, 14, and 3 days in the fifth, sixth, seventh, eighth, and ninth cases, respectively. They concluded that anti-CD40 mAb combined with tacrolimus was effective in prolonging porcine islet graft survival, but anti-CD40 mAb was not as effective as anti-CD154 mAb in terms of preventing early islet loss.

The Seoul group created a new immunosuppression protocol using clinically available immunosuppression[25]. The protocol is shown in Table 7.

They transplanted 55,600 to 102,000 IEQ/kg porcine islets into streptozotocin induced diabetic nonhuman primates (n = 7). The durations of normoglycemia with less than half doses of exogenous insulin were more than 200, 45, 194, 80, 210, 93, and more than 222 days. They concluded that the study could contribute greatly to the initiation of islet xenotransplantation clinical trials.

The Seoul group published "Clinical Trial Protocol for Porcine Islet Xenotransplantation in South Korea" in 2024[26]. A clinical trial, titled "A single-center, open-label, sponsor-initiated clinical trial to assess the safety and efficacy of porcine islet transplantation on diabetes patients" has received approval by the Korean Ministry of Food and Drug Safety.

The donor pig is the DPF grade Seoul National university (SNU) miniature pigs aged more than 2 years. Preceding transplantation, porcine islets sourced from DPF pig will undergo isolation, commencing 6 days prior to the procedure, continuing until the total islet count (10,000 islet equivalent /kg of the patient body weight) is achieved.

They explained that the review process by Korean Ministry were categorized into the source of pigs and the definition of DPF status, islet product release criteria, concerns about zoonosis, the use of immunosuppressants (Table 8), patient selection and inclusion/exclusion criteria, and the sample archive. Thus, islet xenotransplantation using adult pigs are ready for the clinical trial.

Dresden group

In 2017, Dresden Group demonstrated favorable outcome of experimental islet xenotransplantation without immunosuppression using a unique device in a nonhuman primate model of diabetes[27]. The donor pigs were female retired breeder

Table 8. Clinical protocol for porcine islet xenotransplantation by Seoul group.

	Cytokine blockers	Induction Immune Suppression	Maintenance Immune Suppression
1	Adalimumab, Anakinra,	ATG	Sirolimus, Tacrolimus
2	Adalimumab, Anakinra	ATG, Belimumab	Sirolimus, Tacrolimus followed by Tofacitinib

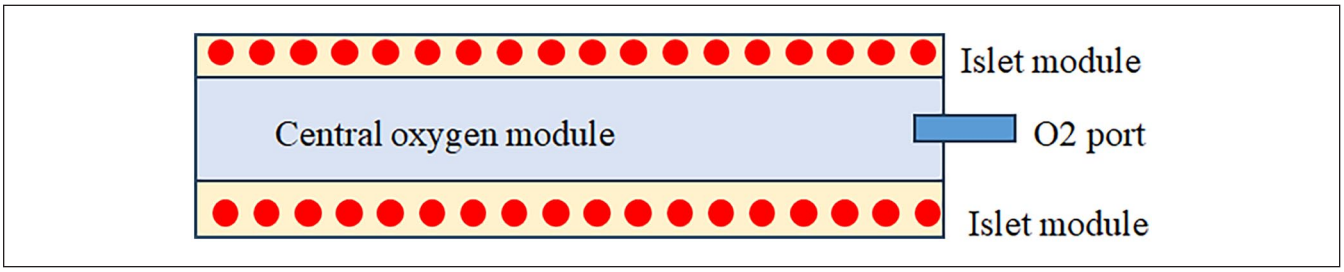


Figure 1. The chamber system for islet macroencapsulation and encapsulated porcine islets described by Dresden team. The system is built from two islet modules containing the islets immobilized in alginate, and central oxygen module. Oxygen-enriched gas mixture (95% oxygen) is directly injected into central oxygen module, and oxygen is delivered into islet modules. This system allows islet survival due to avoiding the ischemic issue.

Goettingen minipigs (Ellegard) with an average of 3–4 years and average pregnancies of three to four were used[27]. Before organ retrieval, the animals were housed under standard conditions (19°C–23°C; 40%–70% relative humidity; 12:12 h day: night cycle) and fed twice daily with special minipig pelleted food (Ellegard). Water was provided ad libitum.

Before this nonhuman study, they conducted 21 porcine islet isolations[28]. They used four young minipigs and 17 retired breeder minipigs and found that the usage of retired breeder animals resulted in high quality and quantity of islets. Therefore, for the nonhuman primate study, female retired breeder minipigs with an average age of 3–4 years and average pregnancies of three to four times were used. They isolated islets using Ricordi method and purified islets with discontinuous gradient centrifugation. Isolated islets were immobilized in alginate and put into the unique device (Fig. 1). They demonstrated that a diabetic nonhuman primate maintained excellent blood glucose levels more than 6 months with positive porcine C-peptide.

Gene modified pigs: next generation of donor pigs

Australian group

Australian group used gene modified neonatal pig islets for the treatment of diabetic baboons[29]. They used alpha 1,3-galactosyltransferase knock out (GTKO) pigs and CD55-CD59-HT triple-transgenic pigs. CD55-CD59-HT triple transgenic pigs express human CD55, CD 59 and alpha 1,2-fucosyltransferase. These two kinds of pigs were mated to produce GTKO/CD55-CD59-HT piglets which are used as donors. 1-day-old to 3-day-old newborn piglets of either sex obtained from a registered pig supply unit were used[30]. They used the modified Korbutt method for islet isolation[30]. The digested tissue was washed thrice in ice-cold HBSS, filtered through a 500 μM sieve and plated into Petri dishes with culture media. Culture media consists of Ham's F10 medium, 10mM glucose, 50 mM isobutylmethylxanthine, 10% pooled autologous pig sera, 2mM L-glutamine, 10 mM nicotinamide, 100 U/ml penicillin and 100 microg/ml streptomycin, 0.236g/l $CaCl_2$, 80 mM HEPES, and 21.3% $NaHCO_3$. The cells were cultured for 6 days at 37°C in 5% CO_2. They transplanted approximately 10,000 to 57,000 IEQ/kg into five diabetic baboons via an inferior mesenteric vein. One baboon received islets twice, therefore total 6 transplantations were performed. The immunosuppression consists of anti-CD2 mAb diliximab, anti-CD154 mAb 5C8, Belatacept, and Tacrolimus. All diabetic baboon achieved insulin independent at average 87 ± 43 days posttransplant and remained for 397 ± 174 days. Maximum graft survival was 675 days. Interestingly, liver biopsies showed functional islets with no evidence of the inflammatory blood mediated inflammatory reaction (IBMIR) and minimal leukocyte infiltration. They concluded that the genetic modification of the donor pig enables attenuation of early islet xenograft injury, and in conjunction with judicious immunosuppression provides excellent long-term function and graft survival in the diabetic baboon model.

Pittsburg group

Pittsburg group used gene modified pig for islet xenotransplantation[31]. They compared islets from two wild type pigs, two female GTKO pigs, and seven female human complement regulatory protein (hCD46) transgenic pig. The wild

Table 9. Outcomes of preclinical study for gene modified porcine islet xenotransplantation into nonhuman primate by Pittsburg group.

	Dose IEQ/kg	Insulin free	Duration of insulin free (days)
Wild type	100,000	Yes	5
Wild type	100,000	Yes	36
GTKO	85,000	No	NA
GTKO	100,000	Yes	17
Gene modified	90,000	Yes	87
Gene modified	95,000 + 100,000	No	NA
Gene modified	94,000	Yes	92
Gene modified	100,000 + 100,000	Yes	91
Gene modified	100,000	Yes	396

Table 10. Multi-gene modified pig and transplant outcomes by Pittsburg group.

	Modified genes	Islet Yield (IEQ)	Tx Islets (IEQ/kg)	Long-term islet function
3GE	GTKO/CD46/CD39	325,420	100,000	No
		300,750		
4GE	GTKO/CD46/TFPI/CTL4-Ig	338,083	100,000	No
		399,083	100,000	Yes
5GE	GTKO/CD46/TFPI/CTL4-Ig/CD39	331,846	75,000	Yes
		149,606	50,000	No

type pigs were female outbred large white pigs. The body weight of all pigs were more than 180 kg and ages were between 7 months and 2 years. Islets were isolated using Ricordi method followed by purification using discontinuous gradient centrifugation[32].

A total of 85,000 to 100,000 IEQ/kg islets were transplanted into nine streptozotocin induced diabetic nonhuman primates (Table 9). In all diabetic nonhuman primate, ATG was administered for induction immunosuppression and mycophenolate mofetil (MMF), anti-CD154 were used for maintenance immunosuppression. Aspirin was also used to minimize coagulation related islet damages. At the time of transplantation, low molecular weight dextran sulfate, prostacyclin, and methyl prednisolone were administered to prevent IBMIR[33].

Majority of transplanted nonhuman primates achieved insulin free status (Table 9). However, nonhuman primates which received islets from the gene modified porcine showed longer insulin free durations. The authors mentioned no complement activation were observed in nonhuman primates which received islets from the gene modified porcine.

The Pittsburgh group further tested gene modified pigs for islet transplantation into streptozotocin induced diabetic nonhuman primates (cynomolgus monkeys)[34]. In addition to GTKO/CD46, they further modified with human CD39, human tissue factor pathway inhibitor (hTFPI), and porcine CTLA4-Ig. Human CD 39 was shown to decrease platelet activation. hTFPI was aimed at inhibition of IBMIR. The porcine CTLA4-Ig was used to inhibit the cellular immune response.

Three groups were created for this study and six female pigs aged 16 to 36 months, weighing 159–181 kg were sources of islets (Table 10). Islets were transplanted into streptozotocin induced five diabetic nonhuman primates. One nonhuman primate received isolated islets from two 3GE pigs. The other four nonhuman primates received islets from one 4GE or 5GE pigs respectively. All nonhuman primate received prostacyclin, methylprednisolone, dextran sulfate, and aspirin for anticoagulant and/or anti-inflammatory effects; ATG for induction, and MMF and anti-CD154mAb for maintenance immunosuppression.

To monitor early islet destruction after transplantation, porcine C-peptide levels were measured at 1, 2 and 24 h. Porcine C-peptide levels after 1-h transplantation were significantly lower in all nonhuman primate with 3-4-5 GE islets compared with previous nonhuman primates with no or one GE pig islets. This demonstrated islets from 3-4-5 GE pigs caused less immediate islet destruction which could reduce the IBMIR.

However, only two nonhuman primates demonstrated long-term islet function. They concluded that despite encouraging effect on early isle loss, GE islet grafts did not demonstrate consistency regarding a long-term success.

Belgian group "superislets"

Belgian group demonstrated that beta cell specific gene modified pig islets using adenoviral vectors to transduce modified GLP-1 and activated type 2 muscarinic receptor (M3R) enhanced insulin secretary ability[35]. They successfully transduced GLP-1 and M3R into both neonatal and

adult porcine islets. Adult pigs were Belgian Landrace pigs more than 200 kg body weight. Insulin secretary abilities were 12.5-fold increase in neonatal islets and 6.1-fold increase in adult porcine islets.

More recently they created transgenic pig expressing the GLP-1M3R cassette under the porcine insulin promoter (InsGLP-1M3R)[36]. They used genetically modified both adult and neonatal porcine for islet isolation. The body weights of adult pigs were more than 200kg. Islets were isolated using collagenase digestion and discontinuous gradient purification for the adult pigs and Korbutt method for the neonatal pigs. They described islet yield from one pig was between 25,000 to 40,000 IEQ from neonatal pigs and 50,000 to 80,000 IEQ from adult pigs. However, they also mentioned islet isolation is reproducible/easy in neonatal and variable/difficult in adult pigs.

InsGLP-1M3R islets isolated from neonatal and adult pigs secrete up to 15-fold more insulin in response to glucose stimulation compared with wild-type islets. InsGLP-1M3R islets also proved to be more efficient in treating diabetes in a rodent model as shown by a significantly higher percentage of normoglycemic recipients and higher porcine C-peptide levels. Due to high insulin secretary ability, they named InsGLP-1M3R islets as "superislets."

Conclusion

Fetal pigs were used for the first clinical trial of islet xenotransplantation by Swedish group followed by New Zealand group. To scale up islet isolation, Korbutt and his colleagues used neonatal pigs and developed "Korbutt neonatal islet isolation method" which is a breakthrough development. In fact, recent clinical trials in New Zealand, Argentina, Mexico, and China were all used neonatal pigs with the Korbutt method. Due to reproducibility and easiness of islet isolation, neonatal pigs have been used for the clinical trials. However, Seoul group developed an excellent protocol using adult pigs and ready for clinical trials.

For the next generation of donor pig, gene modification seems attractive approach. Islets from gene modified pigs can cause less IBMIR and less immunogenic reaction which should contribute clinical outcomes. Eventually, "superislets" should improve the efficacy of islet transplantation which might more effective than pancreas transplantation.

With advanced donor pigs, islet xenotransplantation might become a suitable treatment for the majority of type 1 diabetic patients.

Acknowledgments

No acknowledgment.

ORCID iD

Shinichi Matsumoto https://orcid.org/0000-0001-9959-7756

Ethical Considerations

This is a review article, and all information was gathered from published literatures. Therefore, no ethical assessment was required.

Informed Consent/ Patient Consent

This study did not conduct any clinical trials, therefore, no informed consent existed.

Author Contributions

SM conceptualized and designed the review, conducted the literature search and analysis, interpreted the findings, and prepared the manuscript. All other authors conceptualized and designed the review, conducted interpreted the findings and reviewed the manuscript. All authors approved the final manuscript.

Trial Registration Number/Date

This study did not conduct any clinical trials, therefore, no registration number existed.

Grant Number

There is no grant number.

Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was funded by Japan IDDM network, type 1 diabetes research fund

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data Availability Statement

All information was gathered from published literatures. Therefore, all data are available.

References

1. Matsumoto S, Matsumoto K. Clinical islet xenotransplantation: development of isolation protocol, anti-rejection strategies and clinical outcomes. *Cells*. 2024;13:828.
2. Groth CG, Korsgren O, Tibell A, Tollemar J, Möller E, Bolinder J, Ostman J, Reinholt FP, Hellerström C, Andersson A. Transplantation of porcine fetal pancreatic to diabetic patients. *Lancet*. 1994;344:1402–404.
3. Korsgren O, Sandler S, Landstrom AS, Jansson L, Andersson A. Large-scale production of fetal porcine pancreatic islet like cell clusters. *Transplantation*. 1988;45:509–14.
4. Groth CG, Tibell A, Wennberg L, Bennet W, Lundgren T, Rydgard KJ, Lundin S, Lindeborg E, Korsgren O. Clinical

aspects and perspectives in islet xenotransplantation. *J Hepatobiliary Pancreat Surg*. 2000;7:364–69.
5. 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;68:1674–83.
6. Elliott RB, Escobar L, Tan PLJ, Muzina M, Zwain S, Buchanan C. Live encapsulated porcine islets from a type 1 diabetic patients 9.5 yr after xenotransplantation. *Xenotransplantation*. 2007;14:157–61.
7. Elliott RB, Escobar L, Garkavenko O, Croxson MC, Schroeder BA, McGregor M, Ferguson G, Beckman N, Ferguson S. No evidence of infection with porcine endogenous retrovirus in recipients of encapsulated porcine islet xenografts. *Cell Transplantation*. 2000;9:893–901.
8. Korbutt GS, Elliott JF, Ao Z, Smith DK, Warnock GL, Rajotte RV. Large scale isolation, growth and function of porcine neonatal islet cells. *J Clin Invest*. 1996;97:2119–29.
9. Cardona K, Korbutt GS, Milas Z, Lyon J, Cano J, Jiang W, Bello-Laborn H, Hacquoil B, Strobert E, Gangappa S, Weber CJ, et al. Long-term survival of neonatal porcine islets in nonhuman primates by targeting costimulation pathways. *Nat Med*. 2006;12:304–306.
10. Schurrman 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;16:215–22.
11. U.S. Food and Drug Administration. *Guidance for industry: source animal, product, preclinical, and clinical issues concerning the use of xenotransplantation products in humans*. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/source-animal-product-preclinical-and-clinical-issues-concerning-use-xenotransplantation-products [accessed 2025 Mar 25].
12. Wynyard S, Natsu D, Garkavenko O, Denner J, Elliott R. Microbiological safety of the first clinical pig islet xenotransplantation in New Zealand. *Xenotransplantation*. 2014; 21:309–23.
13. Hillberg AL, Kathirgamanathan K, Lam Janice BB, Law LY, Garkavenko O, Elliott RB. Improving alginate-poly-L-ornithine-alginate capsule biocompatibility through genipin crosslinking. *J Biomed Mater Res Part B*. 2013;101:258–68.
14. Matsumoto S, Wynyard S, Giovannangelo M, Hemdev SL, Abalovich A, Carulla ME, Wechsler C. Long-term follow-up for the microbiological safety of clinical microencapsulated neonatal porcine islet transplantation. *Xenotransplantation*. 2020;27:312631.
15. Matsumoto S, Tan P, Baker J, Durbin K, Tomiya M, Azuma K, Doi M, Elliott RB. Clinical porcine islet xenotransplantation under comprehensive regulation. *Transplant Proc*. 2014;46:1992–95.
16. Matsumoto S, Abalovich A, Wechsler C, Wynyard S, Elliott R. Clinical benefit of islet xenotransplantation for the treatment of type 1 diabetes. *EBioMedicine*. 2106;12:255–62.
17. Matsumoto S, Abalovich A, Wynyard S, Carulla ME, Abalovich D. Patients' opinions 10 years after receiving encapsulated porcine islet xenotransplantation without immunosuppression. *Xenotransplantation*. 2023;30:e12798.
18. Wang Y, Lei T, Wei L, Du S, Girani L, Deng S. Xenotransplantation in China: present status. *Xenotransplantation*. 2019;26:e12490.
19. Noordergraaf J, Schucker A, Martin M, Schuurman HJ, Ordway B, Cooley K, Sheffler M, Theis K, Armstrong C, Klein L, Hansen D, et al. Pathogen elimination and prevention within a regulated, Designated Pathogen Free, close pig herd for long-term breeding and production of xenotransplantation materials. *Xenotransplantation*. 2018;25:e12428.
20. Prabhakaran S, Hering B. What strain of pig should be used? *Xenotransplantation*. 2008;23:68–79.
21. Hering BJ, Wijkstrom M, Graham ML, Hardstedt M, Asheim T, Jie T, Ansite JD, Nakano M, Cheng J, Li W, Moran K, et al. Prolonged diabetes reversal after intraportal xenotransplantation of wild-type porcine islets in immunosuppressed nonhuman primates. *Nat Med*. 2006;12:301–303.
22. Kim HI, Lee SY, Jin SM, Kim KS, Yu JE, Yeom SC, Yoon TW, Kim JH, Ha J, Park CG, Kim SJ. Parameters for successful pig islet isolation as determined using 68 specific-pathogen-free miniature pigs. *Xenotransplantation*. 2009;16:11–18.
23. Shin JS, Kim JM, Kim JS, Min BH, Kim YH, Kim HJ, Jang JY, Yoon IH, Kang HJ, Kim J, Hwang ES, et al. Long-term control of diabetes in immunosuppressed nonhuman primates (NHP) by the transplantation of adult porcine islets. *Am J Transplant*. 2015;15:283–50.
24. Shi JS, Kim JM, Min BH, Yoon IH, Kim HJ, Kim JS, Kim YH, Kang SJ, Kim J, Kang HJ, Lim DG, et al. Pre-clinical results in pig-to-non-human primate islet xenotransplantation using anti-CD40 antibody (2C10R4)-based immunosuppression. *Xenotransplantation*. 2018;25:e12356.
25. Kim JM, Hong SH, Chung H, Shin JS, Min BH, Kim HJ, Kim J, Hwang ES, Kang HJ, Ha J, Park CG. Long-term porcine islet graft survival in diabetic non-human primates treated with clinically available immunosuppressants. *Xenotransplantation*. 2021;28:e12659.
26. Kim BJ, Shin JS, Min BY, Kim JM, Park CG, Kang HJ, Hwang ES, Lee WW, Kim JS, Kim HJ, Kwon I, et al. Clinical trial protocol for porcine islet xenotransplantation in South Korea. *Diabetes Metab J*. 2024;48:1160–68.
27. Ludwig B, Ludwig S, Steffen A, Knauf Y, Zimerman B, Heinke S, Lehmann S, Schubert U, Schmid J, Bleyer M, Schonmann U, et al. Favorable outcome of experimental islet xenotransplantation without immunosuppression in a nonhuman primate model of diabetes. *Proc Natl Acad Sci USA*. 2017;114:11745–50.
28. Steffen A, Kiss T, Schmid J, Schubert U, Heinke S, Lehmann S, Bornstein S, Ludwig B, Ludwig S. Production of high-quality islets from Goettingen minipigs: choice of organ preservation solution, donor pool, and optimal cold ischemia time. *Xenotransplantation*. 2017;24:e12284.
29. Hawthrone WJ, Salvaris EJ, Chew YV, Burns H, Hawkes J, Barlow H, Hu M, Lew AM, Nottle MB, O'Connell PJ, Cowan PJ. Xenotransplantation of genetically modified neonatal pig islets cures diabetes in baboons. *Front Immunol*. 2022;13:898948.
30. Jimenez-Vera E, Davies S, Phillips P, O'Connell PJ, Hawthorne WL. Long-term cultured neonatal islet cell clusters demonstrate better outcomes for reversal of diabetes: in vivo and molecular profiles. *Xenotransplantation*. 2015;11:114–23.

31. Van der Windt DJ, Bottino R, Casu A, Campanile N, Smetanka C, He J, Murase N, Hara H, Ball S, Loveland BE, et al. Long-term controlled normoglycemia in diabetic non-human primates after transplantation with hCD46 transgenic porcine islets. *Am J Transplant*. 2009;9:2716–26.
32. Bottino R, Balamurugan AN, Smetanka C, Bertera S, He J, Rood PPM, Cooper DKC, Trucco M. Isolation outcome and functional characteristics of young and adult pig pancreatic islets for transplantation studies. *Xenotransplantation*. 2007;14:74–82.
33. Johansson H, Goto M, Siegbahn A, Elgue G, Korsgren O, Nilsson B. Low molecular weight dextran sulfate: a strong candidate drug to block IBMIR in clinical islet transplantation. *Am J Transplant*. 2006;6:305–12.
34. Bottino R, Wijkstrom M, van der Windt DJ, Hara H, Ezzelarab M, Murase N, Bertera S, He J, Phelps C, Ayares D, Cooper DK, et al. Pig-to-monkey islet xenotransplantation using multi-transgenic pigs. *Am J Transplant*. 2014;14:2275–87.
35. Mourad NI, Peroa A, Xhema D, Galli C, Gianello P. Transgenic expression of glucagon-like peptide-1 (GLP-1) and activated muscarinic receptor (M3R) significantly improves pig islet secretory function. *Cell Transplant*. 2017;26:901–11.
36. Mourad NI, Peroa A, Xhema D, Duchi R, Lagutina I, Galli C, Gianello P. Double transgenic neonatal porcine islets as an alternative source for beta cell replacement therapy. *Proc Natl Acad Sci USA*. 2024;12:e2409138121.