Chapter 5

Xenotransplantation for Islets from Clinical Side

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

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.69233

Abstract

Islet transplantation can eliminate severe hypoglycemia symptoms caused by conventional treatment, and has the advantages of less trauma and complications, which is considered as the most promising treatment for type 1 diabetes mellitus (T1DM). Regulatory guidance is needed for a standard pig source. In section 1, the regulation of medical grade designed pathogen free (DPF) donor pig for clinical xenotransplantation consists of five parts: genetic quality control, microbiological surveillance, formula feeds, specification of pathological diagnosis, and requirements of environment and housing facilities. In section 2, we present the current approach and progress in pig donor selecting, pancreatic digestion, isolation and preparation of porcine islet grafts, identification and quality assessment of final islet product in clinical trials. The liver is currently the most preferred site for islet transplantation, even though it is far from ideal. A large number of alternative sites have been used for islet transplantation in experimental animal models to provide improved engraftment and long-term survival. In Section 3, we introduce some commonly used sites in xenotransplantation. The benefits and drawbacks of each parameter above are discussed in an attempt to decide which is the most suitable for clinical use and to direct future research.

Keywords: cell and tissue xenotransplantation, clinical study, designated pathogen free status, islet xenotransplantation, regulatory guidance of source pigs, islets preparation, release criteria, sites for islet transplantation

1. Regulation of medical grade designed pathogen free (DPF) donor pig for clinical xenotransplantation

Definition of PDF donor pig: the donor pig should be artificially bred and cultivated, genetic background or origin clear. The weight should not be more than 50 kg when 12-month old; WHO designated animal donor as pathogen free; pathogen infection must be prevented by biological safety barrier, antibiotics or vaccines free, for medical xenotransplantation, scientific research, teaching, production, verification, as well as other scientific experiment [1].

Part 1: Genetic quality control

In this section, the genetic classification, breeding methods, and the genetic quality criteria of inbred herd and closed herd have been discussed, which are suitable for the genetic quality control of DPF medical donor pigs [2].

1. Genetic classification and nomenclature

1.1. Genetic classification: According to different genetic characteristics, DPF pigs are divided into inbred herd and closed herd.

1.2. Nomenclature

1.2.1. Nomenclature of DPF pigs
Herd, generally named after the capital English letters, can also use capital English letters and Arabic numerals; named symbols should be short as far as possible, such as XENO.

1.2.2. Generation
The generation of herd use F in capital English letters. For example, an inbred herd for 30 generations is written as F30.

2. Breeding, including the principle of breeding, introduction, and methods

2.1. Breeding of inbred herd

2.1.1. Principle of propagation
Keep the inbred herd miniature pigs alleles homozygosity.

2.1.2. Introduction
Breeding pigs of inbred herd medical grade xenotransplantation DPF donor pigs should be from foundation herd or pedigree expansion herd, the basis of genetic background is clear, complete data (including the name of the herd, inbreeding generation, genetic characteristics, and main biological characteristics).

2.1.3. Methods
Inbred herd can be divided into foundation herd, pedigree expansion herd, and production herd. When the production supply of the inbred herd miniature pig is not very large, generally no consanguinity expanding herd, just set foundation herd and production herd.

2.2. Breeding of closed herd

2.2.1. Principle of breeding
Keep the genetic heterogeneity and genetic polymorphism of closed herd miniature pig, avoid generation growth too fast.

2.2.2. Introduction

Breeding pigs of inbred herd medical grade should be defined genetic background or source is clear, complete data (Including the species-group name, origin, genetic characteristics and main biological characteristics, etc.)

According to the way of breeding, ensuring the inbreeding generation growth in each generation under the premise of not greater than 1%, determines the minimal introduction scale. If using cycle copulation, introduction amount shall not be less than 13 pairs of unrelated pigs (within three generations, no common ancestor); if using random mating, introduction amount shall not be less than 25 pairs of unrelated pigs.

2.2.3. Methods

Keep a closed herd of medical grade xenotransplantation DPF donor pigs genetic stability, and try to avoid inbreeding.

3. The genetic quality monitoring of DPF medical donor pig

3.1. Inbred herd

3.1.1. Detection method

Microsatellite DNA markers detection method is generally used.

3.1.2. Sampling

In foundation herd, all animal breeding parents should be tested. In production herd, a random sample from each inbred herd, half male and half female, should be tested.

3.1.3. Result determination

All the alleles of microsatellite DNA markers in sample should conform to the characteristic of the herd, no new alleles appear as qualified medical grade xenotransplantation DPF donor pigs inbred herd, otherwise the sentence to unqualified.

3.1.4. Detection frequency

Genetic quality of medical grade xenotransplantation DPF donor pigs production herd should be tested at least once a year.

3.2. Closed herd

3.2.1. Detection method

Detection method is same as that of inbred herd.

3.2.2. Sampling

A random sample from each inbred herd, half males and half females, should be tested.

3.2.3. Result determination

The evaluation method of the genetic variation of the group is an average heterozygosity index or group balance state.

When the average heterozygosity lies between 0.5 and 0.7, and no obvious difference was found between observed heterozygosity and expected heterozygosity by chi-square test, the medical grade xenotransplantation DPF donor pigs can be qualified for closed herd. Or determine by whether the group is in a balance state, if there is no balanced, indicate that the gene frequency and genotype frequency of population changed, the closed herd of medical grade xenotransplantation DPF donor pigs group is not qualified.

3.2.4. Detection frequency

Genetic quality of closed herd medical grade xenotransplantation DPF donor pigs production herd should be tested at least once a year.

Part 2: Microbiological surveillance

In this section, microbiology classification of pathogens, surveillance standard, procedures, methods, rules, results determination, conclusions, sample preservation for xenotransplantation medical grade DPF donor pigs microbiology surveillance [1, 3–5] have been discussed.

Type	Number	Testing items
Bacterium	1	*Brucella* spp.
	2	*Leptospira* spp.
	3	*Serpulina hyodysenteriae*
	4	*Mycobacterium bovis*
	5	*Mycobacterium tuberculosis*
	6	*Mycobacterium avium-intracellulare complex*
	7	*Mycoplasma hyopneumoniae*
	8	*Salmonella typhi*
	9	*Shigella*
	10	*Bordetella bronchiseptica*
	11	*Pasteurella multocida*
	12	*Actinobacillus pleuropneumoniae*
	13	*Streptococcus suis* type 2
Fungi	14	*Pathogenic dermal fungi*
	15	*Cryptococcus neoformans*
	16	*Histoplasma capsulatum*
Parasites	17	*Ectozoa*
	18	*Ascaris suum*
	19	*Echinococcus* sp.
	20	*Isospora* sp.
	21	*Strongyloides ransomi*
	22	*Toxoplasma gondii*
	23	*Trichinella spiralis*
	24	*Neospora*
	25	*Fasciolopsis buski*

Type	Number	Testing items
Viruses	26	Adenovirus (porcine)
	27	Encephalomyocarditis virus
	28	Porcine influenza virus
	29	Human influenza viruses
	30	Porcine cytomegalovirus
	31	Porcine gammaherpesvirus
	32	Porcine reproductive and respiratory syndrome virus
	33	Porcine parvovirus
	34	Rotavirus
	35	Pseudorabies virus
	36	Rabies virus
	37	Foot and mouth disease virus
	38	Classical swine fever virus
	39	Japanese encephalitis virus
	40	Porcine circovirus type 2
	41	Porcine transmissible gastroenteritis virus
	42	Swine vesicular disease virus

Table 1. Microbiological testing items [3].

1. Microbiology classification: conventional (CV) minipig, clean (CL) minipig, specific pathogen free (SPF) minipig
2. Microbiological surveillance standard

2.1. Clinical observation
A visual inspection without abnormality has been done.

2.2. Microbiological testing items
Details see **Table 1**.

2.3. Microbiological testing procedures
Numbering—visual inspection—Blood sampling—Testing—Result determination.

2.4. Sample testing frequency
Testing is done at least once in every 6 months.

2.5. Sampling standard
Choose the pig which is more than 6-month-old for detection. random sampling.

3. Results determination
Results determination is according to the various microbial detection items. For antibody testing item, serum antibody negative is qualified. For antigen and nucleic acid testing item, no positive is qualified.

4. Preservation standard for microbiology testing sample
   Sample data, sample source, animal numbering, sample type and number, save by medical pathology data file management standard. Save time for 1 year.

Part 3: Formula feeds

Raw material for feed, nutrition, feed production, sanitary standard for medical grade xenotransplantation DPF donor pigs quality control of feed [6].

1. Raw material for feed
1.1. Principle of selection
   Raw material should be certified as green food, or from the product of standard green food production base. Or from self-production green food which is qualified by government. According to the mode of green food production standard.
1.2. Nutrition
   Details see **Table 2**.
1.3. Product requirements
1.3.1. Sense
   The color should be homogeneous, without mildew, caking and odor.
1.3.2. Water
   Compound feed 13.5% or less.
1.3.3. Mixing homogeneity
   Compound feed, concentrated feed mixing uniformity coefficient of variation (CV) of 7% or less; Additive premixed feed coefficient of variation (CV) of 5% or less.
1.4. Sanitary requirement
   Shall conform to the World Health Organization (World Health Organization, WHO) for medical donor animal food hygiene requirements, within the sterilization period of validity.

Item	Standard sustaining feed	Growth and reproduction feed
Water, % ≤	11.0	11.0
Crude protein, % ≥	12.0	14.0
Crude fat, % ≥	2.5	3.0
Crude fiber, % ≤	7.5	7.0
Crude ash, % ≤	8.0	7.5
Calcium, %	0.65–1.0	0.75–1.0
Phosphorus, %	0.55–0.7	0.58–0.8
Available phosphate, %	0.32–0.4	0.32–0.4

*Modified from Laboratory animal wuzhishan pig, Feed nutrients requirements. (http://down.foodmate.net/standard/sort/15/42411.html. In Chinese).

Table 2. Feed nutrition ingredient detection index*.

Part 4: Specification of pathological diagnosis

In this section, the contents and methods of pathological examination for medical grade xenotransplantation DPF donor pigs, including examination rules, procedures, clinical pathology, anatomy and results determination and conclusion have been discussed [2, 7, 8].

1. The frequency of examination, sampling requirements, sampling methods, and the number of samples

1.1. Examination frequency
Clinical pathology inspection check should be done at least once in every 6 months; anatomical pathology testing check should be done at least once in every 2 years.

1.2. Sampling requirements

1.2.1. Method
Select 6 months above medical grade xenotransplantation DPF donor pigs for detecting random sampling.

2. Clinical and pathological examination

2.1. Visual examination
Mental state, coat, skin, natural orifice, nutritional status, motions, eating, breathing, etc.

2.2. Hematology detection
RBC: Red Blood Cell, HCT: Hematocrit, MCV: Mean Corpuscular Volume, HGB: Hemoglobin, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, RDW: Red Cell Distribution Width, PLT: Platelets, MPV: Mean Platelet Volume, WBC: White Blood Cell.

2.3. Biochemistry detection
ALT: Alanine Aminotransferase, AST: Aspartate Amino Transferase, Scr:Serum Creatinine, BUN: Blood Urea Nitrogen, TP: Total Protein, ALB: Albumin, GLU: Glucose, T-BIL: Total Bilrubin, TG: Triglyceride, T-CHO: Total cholesterol.

2.4. Autopsy and pathological examination

2.4.1. Surface: the developmental status, nutritional status, mental state, sense organs, respiratory system, coat, skin, and testicles.

2.4.2. Subcutaneous: fat, mammary gland, lymph nodes (lymph nodes under the jaw, neck shallow lymph node, axillary lymph nodes, popliteal lymph nodes), and epididymis.

2.4.3. Head and neck: oral, nasal, brain, cerebellum, brainstem, pituitary gland, tonsil, thyroid, parathyroid gland.

2.4.4. Chest: pleural fluid, the thymus, the lungs and the lung, pericardium, pericardial fluid lymph node and the heart, the aorta, trachea, and bronchi.

2.4.5. Abdominal cavity: peritoneal fluid, spleen and lymph nodes, liver, gallbladder, liver door parts of the blood vessels, bile duct and lymph nodes, pancreas, kidney, adrenal, stomach, intestines, duodenum, jejunum, ileum, caecum, colon, rectum) and mesenteric lymph nodes.

2.4.6. Pelvic: bladder, ureter, prostate, seminal vesicle, ovaries, fallopian tubes, uterus, vagina.

2.5. Histopathological examination
Heart, lung, liver, spleen, kidney, stomach, jejunum, mesenteric lymph nodes, ovary/testis, and gross anatomy examination revealed abnormal organs and tissues.

3. Results determination
   Clinical pathology examination result is divided into four levels: did not see abnormality, minor abnormality, mild abnormality, and obvious abnormality. The pathological diseases are divided into congenital and infectious diseases, no matter what level, noninfectious disease excluded disease individuals, the whole group is ruled out when infectious disease occurs.

3.1. No abnormality: animal appearance without abnormality, at the same time, the blood test indices in the normal reference value range.

3.2. Minor abnormality: animals have no obvious abnormal appearance, but blood tests index one or two higher or lower, the biggest variation is not more than 20% of the normal reference value range threshold.

3.3. Mild abnormality

3.3.1. No obvious abnormal animal appearance, but more than two blood tests index higher or lower, the biggest variation is 20–50% of the normal reference value range threshold.

3.3.2. Mildly abnormal appearance, at the same time more than two blood tests index higher or lower, the biggest variation is 20–50% of the normal reference value range threshold.

3.4. Obvious anomaly

3.4.1. No obvious abnormal animal appearance, but more than two blood tests index higher or lower, the biggest variation is more than 50% of the normal reference value range threshold.

3.4.2. Animal mildly abnormal appearance, at the same time more than two blood tests index higher or lower, the biggest variation is more than 50% of the normal reference value range threshold.

3.4.3. Animal appearance is apparently abnormal

Part 5: Requirements of environment and housing facilities

In this section, the requirements of facilities, environmental conditions and drinking water, cushion, cage and transport standard for medical grade xenotransplantation DPF donor pigs [2, 8–11] have been discussed.

1. Construction

1.1. Building site

1.1.1. Chooses a good air quality and natural environment

1.1.2. Should be far away from the urban residential area and places have serious air pollution, vibration or noise of railway, docks, airports, roads, factories, storage, storage area, slaughtered live herd and poultry farms, factories, etc.

1.1.3. Facilities should be reliable to avoid other animal feeding cross infection

1.2. Sanitary requirements

1.2.1. External environment should be clean and tidy, easy to clean, and disinfectant. Drainage should be unblocked, without waste and sewage accumulation.

1.2.2. Set entrance for people, animals, objects, vehicles is dedicated, special disinfection facilities and equipment.

1.2.3. There should be measures to prevent exotic animals in and the experimental animals out.
1.2.4. Structure of the barrier should be solid, nontoxic, and without any radioactive material.
1.3. Facilities requirements
1.3.1. Doors and windows of the building should have good sealing; the observation window should be set up in the feeding door, set appropriate buffer room door interlock device.
1.3.2. Air-tight door of barrier environment facilities should be open in the direction of the higher air pressure, and can be automatically shut down.
1.3.3. The stairs width should not be less than 1.2 m; the corridor width generally should not be less than 1.5 m. The slot width should not be less than 1.0 m. The door width should meet the requirements for equipment to be in and out, width of which should not be less than 0.8 m.
1.3.4. Barrier environment facilities should be according to the need to maintain the correct pressure direction.
1.3.5. Breeding should be a reasonable organization between the location of the outlet and the inlet air flow, avoid blind angle and short circuit.
1.3.6. The pipe orifice of clean areas toward nonclean areas should be sealed. Drains, tank, pipe slope should guarantee the smooth drainage with no dirt accumulation. Drainage pipe diameter should not less than DN150.
1.3.7. There should be an established environmental monitoring system and the level of other facilities according to the need to set up the environment monitoring system.
1.4. Construction requirements
1.4.1. Passageway of goods should set up a ramp or unloading platform. The ramp slope should not be more than 1/10.
1.4.2. Rooms with the drain, drainage slope should not be less than 1%; the ground should be tested for waterproof processing.
1.4.3. Animal feeding room and lab should be set separately.
1.4.4. The autoclave sterilization equipment should be set between cleaning and disinfection room and clean storage room.
1.4.5. Production area (lab area); the height should not be less than 4.2 m.
1.4.6. The surface of Windows and doors, walls, ceiling, floor (ground) surface in clean area should be smooth, the structure and construction cracks reliably airtight measures should be adopted, metope and ground intersect position should have a radius of not less than 30 mm arc processing.
1.5. Water supply and drainage requirements
1.5.1. Water supply
1.5.1.1. The area of water purification should meet the requirements of sterilization.
1.5.1.2. Water supply system in production area (lab area) should be well equipped with the technology layer.
1.5.1.3. Purification pipeline crossing the wall, reliably sealing measures should be taken.
1.5.1.4. Water supply pipe and pipe fittings of purification area should be of corrosion resistance and provided with convenient and reliable connections.

1.5.2. Drainage
1.5.2.1. The septic tank must be individually set up for the drainage of production and lab area.
1.5.2.2. Drainage in barrier environment facilities should be set apart from subsistence drainage.
1.5.2.3. Purification area should not be through the drainage riser.
1.5.2.4. Pipes should be rust and corrosion free.
1.5.2.5. The drain floor of purification area should be airtight.
2. Layout
2.1. Overall layout
2.1.1. According to DPF medical donor pig's physiological needs and behavior characteristics, design and built their living facilities, and to strictly control the in and out of the personnel, goods, animals and the air.
2.1.2. Production area includes quarantine inspection room, the buffer room, shower rooms, corridors, clean storeroom, post disinfection room and board, mating, pregnancy, childbirth, breastfeeding, piglets, breeding pig house, etc.
2.1.3. Lab area includes buffer room, animals bath room, clean storeroom, post disinfection room, corridors, quarantine inspection room, preparation room, operating room, postoperative observation room, breeding room, etc.
2.2. Requirements for the main locale
2.2.1. Breeding room setup requirements
2.2.1.1. Water system should prevent reflux and alien species.
2.2.1.2. Breeding room should be equipped with appropriate feeding equipment and capture tools. Equipment and tools shall ensure to be firm and will not harm the animal.
2.2.2. Operating room setup requirements
2.2.2.1. Should set up comprehensive laboratory, equipped with necessary equipment and according to the demand.
2.2.2.2. Should set up isolation room to independently observe injured and suspected diseased animals.
2.2.2.3. Should set up quarantine room for new animals.
2.2.2.4. According to the need to set up postoperative observation room.
2.2.3. The auxiliary area setup requirements
2.2.3.1. Environmental controls should be strict in the feed storeroom, preventing pathogenic microorganism pollution, parasites pollution, and alien species.
2.2.3.2. Should set up storeroom with storage cages and instruments.
2.2.3.3. Disinfection room space should be accessible for cleaning process. Before and after cleaning equipment should be placed separately. The walls and floor waterproof treatment should be done.
2.2.3.4. Specialized room and (or) equipment should be set up for animal bodies and waste storage
2.2.3.5. Should set up the observation corridor, or observation area, or set up a video surveillance system, used for observing animals.
3. Feeding conditions
3.1. Fence

Item		Index
Temperature, °C		20–26
Daily temperature difference, °C, ≤		4
Relative humidity, %		40–80
Pressure gradient in the same area, Pa, ≥		10
Air velocity, m/second, ≤		0.2
ventilation rate, /hour, ≥		15
Air cleanliness, level		7
Mean concentration of settled bacteria/0.5 hours/Φ 90 mm plating, ≤		3
Detection rate of designed pathogen, %, ≤		0
Ammonia concentration, mg/m^3, ≤		14
Noise, dB(A), ≤		60
Illuminance,lx	Working, ≥	200
	Animal	100–200
Photoperiod, hour		12–14/12–10

*Modified from Laboratory animal wuzhishan pig, Feed nutrients requirements. (http://down.foodmate.net/standard/sort/15/42411.html. In Chinese.)

Table 3. Environment factor index*.

3.1.1. Choose a material that is nontoxic, washable, high temperature resistant, and easy to sterilize.

3.1.2. Bar size should meet the miniature pig lying down, feed intake and defecation, or use the fence to establish different function areas. Fences should be strong.

3.2. Manger

3.2.1. Choose a material that is nontoxic, washable, high temperature resistant, and easy to sterilize.

3.2.2. The size of the manger should allow all the animals to eat at the same time.

4. Environment

Details see **Table 3**.

5. Waste disposal

5.1. Sewage

Primary sewage treatment equipment or anaerobic tank should be equipped.

5.2. General waste

Waste packing should be gathered for disposal. Disposable coverall, masks, hats, gloves, and experiment of waste should be treated harmless. Injection needles, razor blades sharp items should be collected in toolbox processed by corresponding qualifications organization.

5.3. Infectious waste

Infectious waste must be high pressure sterilized before processing.

5.4. Animal waste
Animal bodies and tissues should be loaded in special bags stored in the refrigerator or freezer, gathered for harmless disposal.

6. Transport

6.1. Transport cages

6.1.1. Should be strong and can prevent animal damage and escape, have feces and urine collection device, and in accordance with the requirements of the animal health and welfare. Suitable for carrying, is conducive to protect animals, and handling personnel safety.

6.1.2. In accordance with the corresponding microbial control environment, easy to clean, and disinfectant.

6.1.3. With peripheral filter membrane, internal solid cages of biological security isolation function.

6.2. Transportation

6.2.1. The transportation shall be equipped with air conditioning and other equipment and able to keep the environment temperature stable.

6.2.2. It should be able to ensure that there is enough fresh air and the shipping space of the cages, meets the needs of the animals' health, safety, and comfort.

6.2.3. The transportation can be disinfected.

6.2.4. Long-distance transportation (more than 6 hours) should provide drinking water, feed whenever necessary.

Conclusion

In conclusion, after strict genetic quality control, close monitoring of pig breeding condition and process, and extensive microbiological screening, we have selected a DPF herd as donors for cell xenotransplantation. This herd is free from all tested conventional and xenotransplantation related pathogens. It can not only minimize microbial negative impact, but also be likely to reduce swine pathogen infection risk, which will promote the development of clinical xenotransplantation from pig donor sources.

Isolation, purification, and quality control of islet in clinical porcine islet xenotransplantation

Islet cells are mainly divided into three types according to different hormone secretion, namely glucagon secretion α-cells, insulin secretion β-cells, and somatostatin secretion δ-cells. β-cells can regulate insulin release by sensing the change of glucose level in order to maintain euglycemia. Islet transplantation can eliminate severe hypoglycemia symptoms caused by conventional treatment and has the advantages of less trauma and complications, which is considered as the most promising treatment for type 1 diabetes mellitus (T1DM). In recent years, with the maturing of islet cell transplantation technology and the development of the clinical application, pancreatic islet transplantation has gradually showed satisfactory and

prospective approach in the treatment of T1DM. Nonetheless, the donors' shortage still precluded the development and progression of clinical islet transplantation. Xenotransplantation offers an effective and feasible solution for this limitation. Among many potential candidates, pig is considered as the most ideal donor for future clinical applications. Although a number of encouraging findings have been reported in preclinical trails, the clinically pervasive application of pig islet xenotransplantation still faces the challenges such as inadequate supply of islet cells with high-quality. This section will discuss the current approach and progress in pig donor selecting, isolation, and preparation of porcine islet grafts, quality control, and release criteria of final product in clinical trials.

1. Selection of islet from different sources of donor pig

1.1. Comparison of characteristics of newborn pigs, young pigs, and adult pigs

Islets may be harvested from newborn (neonatal), young, or adult pigs for transplantation into human in clinical trials. For human recipients, pig donor sources not only comply with the regulation of medical grade DPF donor pig for clinical xenotransplantation, but also must conform to human islets in terms of structure and function to regulate the high blood glucose level found in T1DM patients. Despite several years of investigation, no real consensus has been established with regard to the best pig strain to provide enough viable and functional isolated porcine islets for xenotransplantation [12].

Newborn or neonatal (aged 1–5 days) islet-like cell clusters, (NICCs) have several advantages as the preferred source of β-cells for xenotransplantation. Compared to young or adult pig islets, they can be procured and are easy to isolate by enzymatic digestion [13–15] and there is less batch-to-batch variation between isolations. The main problem with NICCs is the need to culture for maturation to achieve functionality, which is as a potential source of cells because they have an inherent ability to proliferate and differentiate *in vivo* [16]. The NICCs are composed of fully differentiated endocrine cells (35%) and endocrine precursor cells (57%) [17].

Reducing warm ischemia time in the surgical procedure is indispensable for processing of pancreases from young or juvenile (12–15 weeks) pigs. However, culturing between weeks 5 and 24 is required for further maturing of the islets acquired from young pigs, which is unpractical for clinical transplantation owing to isolation difficulties and immature capability [17, 18].

Isolation and purification of islets acquired from adult pigs (>2 years) is still demanding to conduct and repeat, although there were many experiences in this field previously. The mature islets from adult pigs are physiologically similar to humans, which can secrete insulin in response to a glucose challenge. However, adult pig islets require mature pigs of more than 2 years of age [19], furthermore, the logistical considerations of keeping and handling large numbers of adult donor pigs in specific pathogen free housing for this period is impractical, which are not present with neonatal or juvenile pigs.

Dufrane D's group reported a protocol providing a greater than 90% chance to obtain a sufficient islet yield for adult pig islet xenotransplantation into no human primates [20].

Furthermore, they demonstrated that the β-cell percentage within islets is significantly affected by pig age (87.0 ± 3.3% versus 82.1 ± 3.6% for young and adult pig donors, respectively) [21]. This was directly correlated with a significant difference in non–β-cell composition between islets from young and adult pig donors (11.8 ± 3.3% versus 16.2 ± 3.4%, respectively).

1.2. Comparison of different methods for isolation and purification of islets of newborn pigs, young pigs, and adult pigs

1.2.1. NICC isolation

Once removed, the pancreata of neonatal pigs were finely chopped and digested with collagenase Type V, 1 mg/ml (Sigma-Aldrich) at 37°C. The digested tissue was washed in Hank's balanced salt solution (HBSS) (Gibco) and filtered through a 500-μm sieve. Tissue was plated into dishes (No tissue culture treated) with Hams F-10 medium (Gibco) containing 10 mM glucose, 50 mM isobutylmethylxanthine, 10% porcine serum, 2 mM L-glutamine, 10 mM nicotinamide, 100 U/ml penicillin, and 100 μg/ml streptomycin, CaCl2 0.236 g/l, hepes 80 mM, NaHCO3 21.3%(Sigma-Aldrich), with full media changes every other day. The cells were cultured at 37°C and 5% CO_2.

NICCs were isolated and cultured for up to 27 days postisolation. Number of islet equivalents (IEQ), viability, and function were analyzed each week to determine whether time in culture was important for NICC function *in vivo*. It is reported that culture of NICC for 12 days provided the best outcome of viability and function *in vivo* posttransplantation, which was revealed by better reversal of diabetes, and lower levels of TF expression and higher expression of insulin, glucagon, and Bcl-2 with acceptable cell loss in terms of time and expense [22].

1.2.2. Islet isolation from young pigs

For young pigs, briefly, the pancreas was harvested using rapid surgical procurement (<5 min) and placed in organ preservation solution. Cold ischemia time was limited to less than 30 min. The pancreas was then washed in cold (4°C) HBSS supplemented with hepes and trimmed of surrounding adipose and lymphatic tissue in a sterile environment. The pancreatic tissue was then minced into 2–3 mm^3 pieces and digested at 37°C using sigma type V collagenase (2.5 mg/mL in HBSS). The mean digestion time was around 16 min. The islet tissue clusters (50–500 μm) isolated using the method above were allowed to mature into complete islets during culture *in vitro* at 37°C, 5% CO_2 at first in recovery maturation media (Optatio LLC) supplemented with 10% porcine serum, 417 mM dornase α, 215 mM aprotinin, 0.5 mM pefabloc, and then in a novel maturation media (Optatio LLC) 48 hours later, supplemented with 10% porcine serum [23, 24].

1.2.3. Islet isolation from adult pigs

The factors as follows influence the islet yield of pancreas from adult pigs: (1) pancreas acquisition (exsanguination and warm ischemia time), (2) the ingredient of cold storage solutions, (3) the various methods of pancreas digestion and purification, and (4) the endotoxin content and enzymatic activity. Researchers concluded some variables through a variety of investigation, which could enhance the yield of islets, for example, the application of blood exsanguination before pancreas procurement, a warm ischemia time within 10 min, the concentration of <30 EU of endotoxin in Liberase batches, etc. To the contrary, the isolation technique (dynamic versus static) and the storage

method (short-term versus long-term) and solution (UW versus modified UW) did not obviously affect the islet yield. Additionally, there was a positive relationship between isolated islet number and the number of islets/cm^2 or with the percentage of large islets shown by the pancreas biopsies. Pig pancreases containing more than 82 islets/cm^2 and more than 42% of large islets (>100 μm); thus, enabled more than 120,000 islet equivalents to be harvested in 90% of the cases [20].

2. Methods for islet quality control

To ensure the safety and efficiency of islet transplantation, all kinds of measurements have been processed. The quality parameters of islet products involved sterility, purity, viability and activity, cell population, and functionality. It has been suggested that transplantation of poor quality islet product would cause the inconsistencies of the ability of islet transplants to reverse diabetes [25, 26], so islet quality control is critical to both determining the suitability of islets for transplantation as well as to maintain a long-term functional graft in recipients posttransplantation.

2.1. Biological safety

As the main aim of clinical islet isolation is transplantation into a recipient, biological safety of the final product is an essential criterion for product release. This is particularly significant as recipients are immunosuppressed and thus are at an increased risk that infectious pathogens enter the recipient as part of the transplant product and cause infectious disease [27].

To test for the microbial sterility, sample aliquots are taken from the culture medium post-purification and post-culture respectively. Two aliquots from each time point are inoculated aseptically into bactec™ culture vials (Becton Dickinson) specific for aerobic (tryptic soy broth) and anaerobic (soybean-casein digest broth) bacteria culture for 72 hours [28, 29]. Furthermore, samples are also cultured for fungi, mycoplasma, and mycobacteria. At last, the final product is tested for a large series of viruses, consisting of more than 28 viruses.

Endotoxin contaminants are known to lead to islet cell damage and early graft loss. Additionally, microbial contamination is likely to occur at various stages throughout the islet isolation, purification, and culture procedure. The reagents and supplies are possible sources of endotoxins in islet preparations [30, 31], but the most likely source of contaminations is the donor duodenum during pancreas surgical retrieval, as observed from testing of the solution in which the sample of retrieved pancreas is preserved [32].

It is very crucial to determine the sterility of islet preparations for transplantation, and several measures are in place to reduce risk of contamination after isolation and culture. Antibiotics are usually supplied to culture media, and aliquots are taken for Gram staining, endotoxin content measurement, and microbiological culture both after isolation and pretransplant after culturing. In terms of islets release, a negative Gram stain is required, as well as endotoxin content <5 endotoxin units (EU)/kg recipient weight [33]. Several studies have demonstrated that no clinical infection was observed in recipients and long-term graft survival remained unaffected by using these criteria [32].

2.2. Biological characteristics

2.2.1. Quantity (islet equivalent determination, islet count standard)

Islet count was determined by the number of islet equivalents (IEQ = conversion of actual number of islets into number of islets with a diameter of 150 μm) [34]. The final purity of islet products after purification was calculated as the ratio between islets (stained in red by dithizone) and exocrine tissue (unstained by dithizone) on an inverted phase contrast microscope with a calibrated grid in the eyepiece.

2.2.2. Activity

Dithizone staining was also used to determine percentage of purity over the maturation period. Islet viability was analyzed using Newport Green (NPG) (Invitrogen) and propidium iodide, imaged using fluorescence microscopy, and quantified with a microplate reader.

2.2.3. β cell purity

2.2.3.1. Flow cytometry

Newport Green (NPG PDX acetoxymethyl ether) binds to zinc present in β-cells. Apoptosis was measured by tetramethylrhodamine ethyl ester perchlorate (TMRE) selectively binding to mitochondrial membranes. 7-aminoactinomycin D (7-AAD) binds to DNA in dead cells as their membrane permeability is altered. NICCs were dispersed by accutase (Sigma-Aldrich) and then stained, respectively, with NPG, TMRE, and 7-AAD to determine the proportion of β-cells, viable cells, and dead cells. To obtain a numeric product, the β-cell viability index was calculated according to the following formula ((%βcells × %βcells viability)/10,000) [35].

2.2.3.2. DNA content

Two samples of 200 IEQs of islets were obtained and stored at 20°C after washing with citrate buffer. Samples were sonicated and used to measure the DNA content using the Quant-iT Pico Green dsDNA Assay Kit (Invitrogen). Fluorescent excitations ~480 nm, emission ~520 nm was used to read samples by staining with Pico Green fluorescent reagent.

2.2.3.3. Insulin/DNA ratio

Cell suspensions of each sample were homogenized by ultrasonication on ice prior to detection of DNA content using a Quanti-iT Pico Green dsDNA Assay Kit (Invitrogen) and insulin content with an porcine insulin RIA kit (Biosource), respectively. Insulin capacity was obtained by the ratio of the insulin content to the DNA content in microgram in 1000 NICC IEQ [25].

2.2.3.4. Islet function identification

In vitro insulin capacity of the islet products was determined using glucose-stimulated insulin release (GSIS). Eight hundred IEQ porcine islets were incubated in low glucose solution (2.8 mM) for 1 hour and then incubating half the cells in low glucose and half in high glucose (25 mmol/l) solution. The stimulation index was obtained as the ratio by dividing the average high glucose by the average low glucose value. Insulin levels were analyzed using a standard porcine insulin enzyme-linked immunosorbent assay (Porcine Insulin ELISA; Mercodia), and absorbance was measured using a microplate reader [36].

Alternative cell functional assays to determine the metabolic activity such as ATP content and oxygen consumption rate can also be frequently used as release

criteria. These assays utilize small number of islets from the pooled product, and can be performed quickly prior to transplantation. Furthermore, they offer a good indication of the metabolic activity and potential functional capacity of the product. Finally, it should also include the reversal of diabetes in immune-deficient mice relative to dose.

The FDA regulations place islets isolated for transplant therapy under the biological products, requiring the released preparation to demonstrate product stability and consistency between lots in addition to complying with standards of product identity, safety, purity, and potency [37, 38]. The release criteria, formally, are based on porcine islet count per recipient weight (10,000–20,000 IEQ/kg for single transplant), with purity greater than 30% (assessed using dithizone staining), viability greater than 70% (assessed using Newport Green/TMRE/7-AAD staining), endotoxin concentration <5 (EU) /kg recipient weight, and no detectable organisms in a Gram stain prior to transplantation, as well as to a glucose stimulation index (ratio of stimulated insulin secretion/basal insulin secretion) ≥1 [39, 40]. Criteria based on these are currently, in formal, applied at the institution for cell transplantation and gene therapy at the 3rd Xiangya Hospital of Central South University, China, where we require each islet preparation from neonatal pigs to reach the determined thresholds of islet number/mass, viability, purity, and sterility before the product is released for transplantation [41].

Conclusion

Based on the remarkable progress of islet cell transplantation technology in the experimental and clinical studies, the islet xenotransplantation from porcine donors expected to become one of the potential and fundamental treatments for type 1 diabetes mellitus. The effective separation and purification of functional pig islet for transplantation, has always been a hot research topic in the field of heterogeneous islet transplantation. With the continuous development of suitable sources of pig donors, modification of isolation and purification technology, the improvement of quality control system of islet products, how to establish simple, economic and standardized graft preparation, and evaluation standard as soon as possible will promote islet xenotransplantation technology make greater progress and enter the next step of clinical studies, which will benefit the patients with diabetes by the tangible therapy in the very near future. However, remaining questions and detailed problems need to be adequately addressed.

Current alternative sites for islet transplantation

A suitable transplantation site could accommodate a large volume of islets for transplantation in an ample space, which is very close to vasoganglion providing enough oxygen and nutrients in the course of revascularization. Furthermore, it should avoid the reduction of early islet from host immune and inflammatory responses, while if necessary, the site is accessible to

transplantation processes [42]. It is a priority of research to define extrahepatic engraftment sites. The purpose of ongoing studies is to find a microenvironment that could offer prompt transplantation and make the inflammation and islet cell death to a minimum at early stage. At the same time, it could realize continuous function, which is of particular interest for researchers. It has been demonstrated in experimental animal models that islet grafts transplantation with or without the strategy of bioengineering in a number of extrahepatic sites, even though translation in clinic for some is unclear [43–45]. Numerous sites have been proposed and tested, both experimentally and in some cases clinically, including the liver, kidney subcapsule, bone marrow, immune privileged sites, and peritoneum spaces. While some alternative sites may be advantageous in experimental models, their feasibility and translation into clinical settings is limited up to date. While it has been proved in clinic that the infusion of intraportal islet could abolish T1DM, there has been long a need for finding a selectable engraftment site to optimize clinical results in the long term. Experimental research has offered potential alternatives to repair normoglycemia, even though a number of methods have implied limitations in terms of technology and/or physiology.

Liver

Intrahepatic islet infusion via the portal vein accounts for all clinical islet transplants conducted worldwide. Percutaneous portal vein infusion under ultrasound and fluoroscopic guidance offers a minimally invasive procedure with the ability to regulate glycemic levels through portal insulin delivery [46]. Alternatively, in patients at risk for bleeding, the transplant is performed by cannulation of a tributary of the portal vein using open surgery (minilaparotomy) or laparoscopic approach. It is worth noting the potential procedural risks such as portal thrombosis and bleeding [47]. A significant amount of intraportal islets are lost immediately after transplantation due to instant blood-mediated inflammatory reaction (IBMIR), which negatively influences islet grafts through expression of tissue factor, resulting in platelet adherence, activation, clot formation, and lymphocyte recruitment. In addition, xenotransplantation has more severe obstructions than those of clinical islet allotransplantation because IBMIR is still a major obstacle for islet transplantation. In pig-to-NHP islet xenotransplantation, although the precise mechanisms are yet to be illuminated, simultaneous activation of complement, coagulation, and platelets occurred immediately after monocyte and neutrophil infiltration, which play a pivotal role in this very early islet destruction. In order to solve this problem, a variety of genetically engineered pigs have been developed. The generalized strategies to introduce porcine genetic modification are knocking down or knocking out certain genes for polysaccharide antigens, e.g., α-Gal, knocking in human complement regulatory proteins, coagulation and cellular immune response regulatory proteins, respectively, or combinations of the above genetic modifications. Technical speed development for genetically engineering modified pigs provides another approach to realize clinical islet xenotransplantation [48].

Peritoneum

The peritoneum has the advantages of accepting both unpurified and microencapsulated islets as tested in many experimental studies. It is unfortunate that research in the

murine mode indicated that a great number of islets are made requests for hyperglycemia reversion, while the insufficiency of parasympathetic re-innervation of the transplant is related to abnormal glucose tolerance tests [49] and morphologic alteration in islet architecture [50]. In the nonobese diabetic mouse mode, intraperitoneal syngeneic islets microencapsulated in 5% agarose hydrogel resisted to the autoimmune attack [51]. In terms of technique, a latest research suggested a minimally invasive laparoscopic process for microencapsulated islet autotransplantation in nonhuman primates [52]. As a result, the transplantation site was promptly applied in a successful clinical experiment [53] after the process of intraperitoneal alginate capsulated islets from neonatal pig was proved safe in the NHP mode [54].

Bone marrow

Bone marrow (BM) may represent an ideal microenvironment for islets, attribute to its protected and extravascular (but well vascularized) microenvironment, its broad distribution, and its easy access. Because of its broad distribution and easy access, BM has the potential to overcome not only the physiologic loss of islets, but also the technical limitations and complications encountered with the intraportal infusion [55]. A previous research reported that BM could provide an immunoprotected microenvironment that allogeneic, syngeneic, and xenogeneic islet could survive in nondiabetic rat models without immunosuppression [56]. Based on this, an ongoing pilot clinical trial at San Raffaele Scientific Institute in Milan aims to evaluate the safety and feasibility of BM as a potential site for islet auto/allotransplantation. Some research results showed that the BM is a more suitable site than the liver for the implantation of islets in murine model [57]. However, further research is required to determine whether the results can be reproduced in large animals and eventually in humans.

Kidney capsule

The kidney capsule has been applied as a potential site for experimental islet transplants in murine models [58], despite its poor blood supply in a relative manner [59] and the fact that it does not supply an oxygen-rich microenvironment. Correspondingly, the surgical process in murine modes is easy, which lead to hyperglycemia reversion in several days. In addition, it has the advantages on transplant restoration post-nephrectomy by both histologic research and function test on glucose metabolism. Compared with the number of liver, islets that could reverse chemically-induced diabetes were less when transplanted under kidney capsule in a syngeneic murine model of islet transplantation, mainly because the microenvironment for intraliver engraftment is less ideal [60]. In addition, a smaller islet mass is demanded to reverse hyperglycemia in the renal subcapsular space [61, 62]. Furthermore, islet transplants under the kidney capsule allow the cotransplantion with endothelial cells [63], MSCs [64], and BM stem cells [65]. In humans, the invasive surgical procedure is used to release the islets under the kidney capsule, which is the really limited space for a high transplant mass. Additionally, the diabetic nephropathy of recipients signifies the kidney capsule is not an ideal site for islet grafts [66].

Conclusion

Although many implantation sites have been proposed, few have found their way into the clinical setting. The experts suggest that islets infusion into the liver through the portal vein has been the chief approach of option. Well characterized sites, e.g., the kidney capsule and other immunoprivileged sites, are significant experimental models but with some limitations in applicability in the clinical setting. Nevertheless, there is clinical potential for possible utilization of both the peritoneum and bone marrow sites; however, further research is required before therapeutic advances can be made here.

Acknowledgments

The authors would like to thank Bin Ye, Pengfei Rong, Xiaoqian Ma, Cejun Yang, Qiong Dong, Shengwang Zhang for their assistance with this chapter.

Author details

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

*Address all correspondence to: cjr.wangwei@vip.163.com

Institute for Cell Transplantation and Gene Therapy of the Third Xiangya Hospital, Central-South University, Changsha, China

References

[1] 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

[2] Korbutt 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):223-227

[3] Fishman JA. Infection in xenotransplantation. Journal of Cardiac Surgery. 2001;**16**(5):363-373

[4] Fishman JA, Patience C. Xenotransplantation: Infectious risk revisited. American Journal of Transplantation. 2004;**4**(9):1383-1390

[5] Fishman JA, Scobie L, Takeuchi Y. Xenotransplantation-associated infectious risk: A WHO consultation. Xenotransplantation. 2012;**19**(2):72-81

[6] Vanderpool HY. The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes–Chapter 7: Informed consent and xenotransplantation clinical trials. Xenotransplantation. 2009;**16**(4):255-262

[7] Denner J, Schuurman HJ, Patience C. The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 5: Strategies to prevent transmission of porcine endogenous retroviruses. Xenotransplantation. 2009;**16**(4):239-248

[8] Cooper DK, Casu A. 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. 2009;**16**(4):229-238

[9] Vajta G, Zhang Y, Machaty Z. Somatic cell nuclear transfer in pigs: Recent achievements and future possibilities. Reproduction Fertility and Development. 2007;**19**(2):403-423

[10] Cozzi E, 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 1: Update on national regulatory frameworks pertinent to clinical islet xenotransplantation. Xenotransplantation. 2016;**23**(1):14-24

[11] Rayat GR, 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 3: Porcine islet product manufacturing and release testing criteria. Xenotransplantation. 2016;**23**(1):38-45

[12] Prabhakaran S, Hering BJ. What strain of pig should be used? Xenotransplantation. 2008;**15**:83-88

[13] Cardona K, Korbutt GS, Milas Z, et al. Long-term survival of neonatal porcine islets in nonhuman primates by targeting costimulation pathways. Nature Medicine. 2006;**12**:304-309

[14] Rajotte RV. Isolation and assessment of islet quality. Xenotransplantation. 2008;**15**:93-97

[15] Korbutt GS, Elliott JF, Ao Z, et al. Large scale isolation, growth, and function of porcine neonatal islet cells. Journal of Clinical Investigation. 1996;**97**:2119-2147

[16] Dufrane D, Gianello P. Pig islet for xenotransplantation in human: Structural and physiological. Transplantation Reviews. 2012;**26**:183-188

[17] Jay TR, Heald KA, Carless NJ, et al. The distribution of porcine pancreatic beta-cells at ages 5, 12 and 24 weeks. Xenotransplantation. 1999;**6**:131-170

[18] Dufrane D, Goebbels RM, Fdilat I, et al. Impact of porcine islet size on cellular structure and engraftment after transplantation: Adult versus young pigs. Pancreas. 2005;**30**:138-184

[19] Mackenzie Dah D, Sollinger HW. Xenogeneic transplantation of porcine islets: An overview. Transplantation. 2003;**76**:887-891

[20] Dufrane D, D'hoore W, Goebbels RM, et al. Parameters favouring successful adult pig islet isolations for xenotransplantation in pig-to primate models. Xenotransplantation. 2006;**13**:204-217

[21] Cabrera O, Berman DM, Kenyon NS, et al. The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**:23342342

[22] Jimenez-Vera E, Davies S, Phillips P,et al. Long-term cultured neonatal islet cell clusters demonstrate better outcomes for reversal of diabetes: In vivo and molecular profiles. Xenotransplantation. 2015;**22**:114-123

[23] Krishnan R, Buder B, Alexander M, et al. Juvenile porcine islets can restore euglycemia in diabetic athymic nude mice after xenotransplantation. Transplantation. 2015;**99**:710-716

[24] Lamb M, Laugenour K, Liang O, Alexander M, Foster CE, Lakey JR. In vitro maturation of viable islets from partially digested young pig pancreas. Cell Transplantation. 2014;**23**(3):263-272

[25] Ma XQ, Ye B, Gao F, et al. Tissue factor knockdown in porcine islets: An effective approach to suppressing the instant blood-mediated inflammatory reaction. Cell Transplantation. 2012;**21**:61-71.

[26] Shapiro AM, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, et al. International trial of the Edmonton protocol for islet transplantation. The New England Journal of Medicine. 2006;**355**(13):1318-1348

[27] Taylor GD, Kirkland T, Lakey J, Rajotte R, Warnock GL. Bacteremia due to transplantation of contaminated cryopreserved pancreatic islets. Cell Transplantation. 1994;**3**(1):103-108

[28] O'Connell PJ, Hawthorne WJ, Holmes-Walker DJ, et al. Clinical islet transplantation in type 1 diabetes mellitus: Results of Australia's first trial. Medical Journal of Australia. 2006;**184**(5):221-225

[29] Kin T, Rosichuk S, Shapiro AM, et al. Detection of microbial contamination during human islet isolation. Cell Transplantation. 2007;**16**(1):9-13

[30] Berney T, Molano RD, Cattan P, et al. Endotoxin-mediated delayed islet graft function is associated with increased intra-islet cytokine production and islet cell apoptosis. Transplantation. 2001;**71**(1):125-156

[31] Vargas F, Vives-Pi M, Somoza N, et al. Endotoxin contamination may be responsible for the unexplained failure of human pancreatic islet transplantation. Transplantation. 1998;**65**(5):722-728

[32] Bucher P, Mathe Z, Bosco D, et al. Microbial surveillance during human pancreatic islet isolation. Transplantation Proceedings. 2004;**36**(4):1147-1154

[33] Gala-Lopez B, Kin T, O'Gorman D, et al. Microbial contamination of clinical islet transplant preparations is associated with very low risk of infection. Diabetes Technology & Therapeutics. 2013;**15**(4):323-329

[34] Hawthorne WJ, Simond DM, Stokes R, et al. Pre-clinical model of composite foetal pig pancreas fragment/renal xenotransplantation to treat renal failure and diabetes. Xenotransplantation. 2011;**18**:390-399

[35] Ichii H, Inverardi L, Pileggi A, et al. A novel method for the assessment of cellular composition and beta-cell viability in human islet preparations. American Journal of Transplantation. 2005;**5**:1635-1645

[36] Kobayashi T, Arefanian H, Harb G, et al. Prolonged survival of microencapsulated Cell Transplant. Transplantation. 2008;**17**:1243-1256

[37] Linetsky E, Ricordi C. Regulatory challenges in manufacturing of pancreatic islets. Transplantation Proceedings. 2008;**40**(2):424-429

[38] U.S. Department of Health and Human Services FDA. Guidance for industry: Considerations for allogeneic pancreatic Islet cell products 2009 [updated 15/09/14.]

[39] Balamurugan AN, Naziruddin B, Lockridge A, et al. Islet product characteristics and factors related to successful human islet transplantation from the Collaborative Islet Transplant Registry (CITR) 1999-2010. American Journal of Transplantation. 2014;**14**(11): 2595-2606

[40] Papas KK, Suszynski TM, Colton CK. Islet assessment for transplantation. Current Opinion in Organ Transplantation. 2009;**14**(6):674-682

[41] Wang W, Mo ZH, Ye B, et al. A clinical trial of xenotransplantation of neonatal pig islets for diabetic patients. Journal of Central South University (Medical Sciences). 2011;**36**(12): 1134-1140

[42] Veriter S, Gianello P, Dufrane D. Bioengineered sites for islet cell transplantation. Current Diabetes Reports. 2013;**13**(5):745-755

[43] Cantarelli E, Piemonti L. Alternative transplantation sites for pancreatic islet grafts. Current Diabetes Reports. 2011;**11**:364-374

[44] Smink AM, Faas MM, de Vos P. Toward engineering a novel transplantation site for human pancreatic islets. Diabetes. 2013;**62**:1357-1364

[45] Brady AC, Martino MM, Pedraza E, et al. Pro-angiogenic hydrogels within macroporous scaffolds enhances islet engraftment in an extrahepatic site. Tissue Engineering Part A. 2013;**19**(23-24):2544

[46] Rajab A. Islet transplantation: Alternative sites. Current Diabetes Reports. 2010;**10**(5): 332-337

[47] Barshes NR, Lee TC, Goodpastor SE, et al. Transaminitis after pancreatic islet transplantation. Journal of the American College of Surgeons. 2005;**200**(3):353-361

[48] Park CG, Bottino R, Hawthorne WJ. Current status of islet xenotransplantation. International Journal of Surgery. 2015;**23**(Pt B):261-266

[49] Fritschy WM. The efficacy of intraperitoneal pancreatic islet isografts in the reversal of diabetes in rats. Transplantation. 1991;**52**(5):777-783

[50] Lorenz D, et al. Transplantation of isologous islets of Langerhans in diabetic rats. Acta diabetologica Latina. 1975;**12**(1):30-40

[51] Kobayashi T, et al. Indefinite islet protection from autoimmune destruction in non-obese diabetic mice by agarose microencapsulation without immunosuppression. Transplantation. 2003;**75**(5):619-625

[52] Qi M, et al. A recommended laparoscopic procedure for implantation of microcapsules in the peritoneal cavity of nonhuman primates. Journal of Surgical Research. 2011;**168**(1):e117-123

[53] Calafiore R, et al. Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes: First two cases. Diabetes Care. 2006;**29**(1):137-145

[54] Elliott RB, et al. Intraperitoneal alginate-encapsulated neonatal porcine islets in a placebo-controlled study with 16 diabetic cynomolgus primates. Transplantation Proceedings. 2005;**37**(8):3505-3512

[55] Casey JJ, Lakey JR, Ryan EA, et al. Portal venous pressure changes after sequential clinical islet transplantation. Transplantation. 2002;**74**:913-915

[56] Salazar-Banuelos A, et al. Pancreatic islet transplantation into the bone marrow of the rat. The American Journal of Surgery. 2008;**195**(5):674-678

[57] Cantarelli E, Melzi R, Mercalli A, et al. Bone marrow as an alternative site for islet transplantation. Blood. 2009;**114**(20):4566-4574

[58] Szot GL, Koudria P, Bluestone JA. Transplantation of pancreatic islets into the kidney capsule of diabetic mice. Journal of Visualized Experiments. 2007;**9**:404

[59] Carlsson PO, et al. Chronically decreased oxygen tension in rat pancreatic islets transplanted under the kidney capsule. Transplantation. 2000;**69**(5):761-766

[60] Van Suylichem PT, et al. Rat islet isograft function. Effect of graft volume and transplantation site. Transplantation. 1994;**57**(7):1010-1016

[61] Lacy PE, Ricordi C, Finke EH. Effect of transplantation site and alpha L3T4 treatment on survival of rat, hamster, and rabbit islet xenografts in mice. Transplantation. 1989;**47**(5):761-766

[62] Mellgren A, Schnell Landstrom AH, Petersson B, et al. The renal subcapsular site offers better growth conditions for transplanted mouse pancreatic islet cells than the liver or spleen. Diabetologia. 1986;**29**(9):670-672

[63] Song HJ, et al. Prolongation of islet graft survival using concomitant transplantation of islets and vascular endothelial cells in diabetic rats. Transplantation Proceedings. 2010;**42**(7):2662-2666

[64] Rackham CL, et al. Co-transplantation of mesenchymal stem cells maintains islet organisation and morphology in mice. Diabetologia. 2011;**54**(5):1127-1135

[65] Sakata N, et al. Bone marrow cell cotransplantation with islets improves their vascularization and function. Transplantation. 2010;**89**(6):686-693

[66] Jindal RM, Sidner RA, McDaniel HB, et al. Intraportal vs kidney subcapsular site for human pancreatic islet transplantation. Transplantation Proceedings. 1998;**30**(2):398-399

IntechOpen