Chapter 21
DCD for Islet Transplantation

Takashi Kenmochi, Takehide Asano, Naotake Akutsu, and Taihei Ito

21.1 Introduction

Pancreatic islet transplantation has the potential to become the most physiologically advantageous and minimally invasive procedure for the treatment of type 1 diabetes mellitus. Since the first clinical islet transplantation was performed at the University of Minnesota in 1974 [1], the results have been far from ideal for more than two decades in spite of an improvement of islet isolation technique by Ricordi et al. [2–4]. The introduction of the Edmonton protocol, with a highly improved rate of insulin independency, encouraged us to promote clinical islet transplantation [5, 6]. In Japan, we organized the Working Group (The Japanese Islet Transplant Registry) in 1997 under the Japanese Society for Pancreas and Islet Transplantation for the purpose of starting clinical islet transplantation. The first issue of the Working Group was to construct a system of clinical islet transplantation in Japan including the registration of the recipients, procurement of the pancreas for islet isolation and transplantation of the isolated islets. In Japan, afterwards, various problems facing to a start of clinical islet transplantation have been discussed and we completed the guideline for clinical islet transplantation in Japan. The Japanese Organ Transplant Law was enforced in 1997 and organ transplantations using brain dead (DBD) donors were finally started. Since the islet transplantation was not included in the Japanese Organ Transplant Law because it was categorized as tissue transplantation, we were able to use the pancreas only from DCD donors for islet transplantation. The first islet isolation from the human pancreas was performed in 2003.9 and the
first islet transplantation was performed in 2004.4 [7–9]. Sixty-five islet isolations and 34 islet transplantations were performed in our country from 2003.9.12 to 2007.3.11 [10].

In this chapter, we describe the current status of clinical islet transplantation using DCD donors in Japan.

21.2 Donor Criteria

Islet transplantation is categorized as tissue transplantation in Japan. Therefore, donor criteria for islet transplantation were based on the guideline issued by Japanese Society of Tissue Transplantation (JSTT) [11], which is shown in Table 21.1. According to the guideline, when a specific disease or state is observed in a donor, retrieval or transplant of tissue from the donor must not be permitted. It is necessary to conduct inspection and palpation of the donor, as detailed as possible. Interview of the donor’s next of kin and studying the donor’s medical records are also required. The results of a pathological autopsy, if performed, must be reviewed. For all tests performed, conduct the most current methodology. Criteria and methods of tests or examinations must be always updated at a tissue bank (Islet isolation center), in accordance with technical and scientific advancements and new findings in infectious diseases. (It is desirable to involve experts from related

Table 21.1 Exclusion criteria of tissue donors in general (Guideline on the safety, storage, and application of human tissue in medical practice. IV. Criteria for donor screening. Japanese Society of Tissue Transplantation (JSTT)) [11]

- Death from unknown causes
- Documented cases of sepsis or other systemic infectious diseases
- CJD (vCJD) or its suspect of infection
- Malignancy (as for primary brain tumors or solid tumors treated more than 5 years ago, and diagnosed to be cured, judgement may be given by the physicians to procure the tissue)
- Hematologic malignancies, such as leukemia or malignant lymphomas
- Severe metabolic or endocrine disorders. Autoimmune disorders such as hematological diseases or collagen disease
- Syphilis positive
- Hepatitis (HBV positive, HCV positive)
- AIDS (HIV positive)
- Adult T-cell leukemia (ATL), HTLV-1 positive
- Parovirus B19 positive
- West Nile virus positive
- SARS
- Rabies
- Other patient/donor who corresponds to exclusion criteria set for each tissue

Patients or donors must be negative in interviews and tests (serodiagnosis or NAT) for all these infections and diseases listed above. Where necessary, a patient or donor is to be tested for negative infection of cytomegalovirus and EB virus
In accordance with the principle presented in the above notification of the Pharmaceutical and Medical Safety Bureau of MHLW, retrieval of tissue is prohibited when a patient or donor corresponds to any of the following items in Table 21.1.

In addition, the Japanese Islet Transplant Registry determined following four items for donor criteria of islet transplantation: (1) age ≤ 70 years, (2) warm ischemic time ≤ 30 min, (3) exclusion criteria of the JSTT guideline (Table 21.1), and (4) recommendation of UW solution or two-layer method (TLM) for preservation.

21.3 Procurement and Preservation of the Pancreas

In Japan, the pancreas from DCD donors are used for islet isolation since the pancreas from DBD donors are usually used for pancreas or pancreas/kidney transplantation according to the Japan Organ Transplant Law. The withdrawal of a respirator is rarely performed even though the donor is diagnosed to be brain dead (Maastricht category 4 [12]). Moreover, the donors usually are not examined to diagnose brain death, and thus, a cannulation into an abdominal aorta with a double-balloon catheter via a femoral artery and a systemic heparinization are not permitted before a cardiac arrest (modified Maastricht category 5 [13]). To shorten warm ischemic time and maintain a viability of the pancreas for islet isolation, we use in situ machine washout (ISMW) technique [14, 15], which was originally developed for the procurement of the kidneys from DCD donors. The efficacy of ISMW on clinical kidney transplantation from DCD donors is described in detail in another chapter. For the procurement of the pancreas from DCD donors, a double-balloon catheter position needs to be changed to perfuse celiac artery and superior mesenteric artery as well as bilateral renal arteries. We usually place the distal balloon in the aorta which is located above the celiac axis and only distal balloon is inflated. X-ray and ultrasound sonography are used for the confirmation of the collect location of the balloon. The University of Wisconsin (UW) solution is most frequently used as the perfusate of ISMW for the procurement of pancreas.

TLM, which was developed by Kuroda Y et al., was frequently used for the preservation of the pancreas before islet isolation. TLM was considered to be advantageous for the preservation of the pancreas for islet isolation, which resulted in higher yield and purity [16–22]. However, Caballero-Corbalán J et al. reported that a TLM had no beneficial effect as compared with the UW solution on human islet isolation and transplantation [23]. Currently, the use of TLM depends on the consideration of each islet isolation and transplantation center.

Procurement of the pancreas from DCD donors is different from that of the DBD donors in Japan. Since pancreatic islet transplantation is categorized as tissue transplantation, the pancreas must be procured after en bloc procurement of bilateral kidneys. Pancreas is procured with or without duodenum from the DCD donor. In back table, pancreas was isolated from the surrounding tissue including the
duodenum, spleen, blood vessels, and lymph nodes. When TLM is used for preservation of the pancreatic graft, the graft is moved into TLM container in which oxygenation was already done with 30 min oxygen bubbling. Some institutions perform the ductal injection with UW solution or other solutions into the main pancreatic duct [24–27].

21.4 Quality Control of Islet Transplantation

Quality control (QC) is important for the safe performance of islet transplantation. QC includes infection control and viability assay of the isolated islets. QC committee was placed in the Japanese Islet Transplant Registry, performing QC of all procedures in clinical islet transplantation in Japan [28]. As shown in Table 21.2, infection control is the major issue in QC. In the procurement of the pancreas from DCD donors, donor criteria must be strictly obeyed and past history of the infectious diseases should be carefully checked. Before islet isolation, the checking of the bacterial contamination of the preservation solution must be performed. Islet isolation is permitted to proceed in the authorized islet isolation centers which were acknowledged by the Japanese Society for Pancreas and Islet Transplantation. These centers have GMP-level-regulated cell processing rooms (center) and provide

| Table 21.2 Quality control of pancreatic islets of islet transplantation (Committee of Quality Control, The Japanese Islet Transplant Registry, 2006.9) [28] |
|---|
| **Quality control of pancreatic islets** |
| 1. Infection control  
  1. Donor screening and assessment of the infection  
  2. Checking of contamination during islet isolation and culture procedure  
  3. Checking of endotoxin levels of the media before transplantation |
| 2. Morphological and functional assay of the isolated islets (viability assay)  
  1. Microscopic and electron microscopic examination  
  2. Trypan blue staining  
  3. Static incubation and/or perifusion studies |
| **Algorism of quality control** |
| Procurement team  
Procurement of the pancreas: checking of the active infection and past history of the infectious diseases |
| Islet isolation center  
1. GMP level regulated cell processing room must be used (all procedures must be performed in sterile field)  
2. Islet isolation: checking of the contamination (preservation solution, isolation media, culture media, etc.), static incubation  
3. Cryopreservation of the islets: checking of the contamination (samples of cryopreserved islets), static incubation of samples of cryopreserved islets |
| Islet transplantation center  
1. Final checking of the contamination and endotoxin level of the islet suspended media |
the sterile field on all procedure of islet isolation and islet culture before transplantation. After islet isolation and culture, the media were sampled and checked for bacterial contaminations.

Viability assays of the isolated islets include the results of the isolation such as yield and purity of the islets, volume of the pellet of islets, morphological examination using microscope with dithizone staining, and electron microscope and functional assays.

The Japanese Islet Transplant Registry determined static incubation as the standard method of functional assay of the isolated islets. The technique of the static incubation is shown as follows. Briefly, five aliquots of ten islets were placed into 12-well Transwell microplates with 1 mL RPMI 1640 containing 3.3 mmol/L d-glucose and 0.1 % BSA as the basal medium. After 60 min, the culture Transwells were transferred into new 12-well microplates with RPMI 1640 containing 20 mmol/L d-glucose and 0.1 % BSA (glucose stimulation). After 60 min, the culture Transwells were transferred to new 12-well microplates to add basal medium again for an additional 60 min culture. Each medium was centrifuged and immediately frozen for a later assay of the insulin concentration by ELISA. The stimulation index was calculated by comparing the insulin content in the glucose stimulation medium with the second basal medium.

In addition to the static incubation, we perform the perifusion study to assess the ability of insulin release from the islets. Perfusion study was performed using following technique. Using our original perifusion system, the 50 islets were perifused in a low-glucose medium (Krebs Ringer solution with 3.3 mM d-glucose) for 80 min and then perifused in a high-glucose medium (Krebs Ringer solution with 16.7 mM d-glucose) for 30 min, followed by 60 min perfusion in a low-glucose medium. Stimulation indices were calculated from the ratio of insulin released into the low-glucose medium and the high-glucose medium.

Whether the isolated islets are alive or dead is the first viability assay. Chromogenic dyes such as neutral red or trypan blue are able to visualize live or dead cells. The islet, however, consists of a number of cells; therefore, chromogenic dyes cannot evaluate the islet itself, while fluorescein diacetate (FDA) is used to quantify the proportion of cells that are intact or that are damaged, which can evaluate the islet more precisely [29]. Live islet is not necessarily able to respond appropriately to glucose stimulation; therefore, in vitro biochemical techniques have predominantly been used to assess islet viability. Insulin release from islets after stimulation with high glucose concentration has commonly been used in static incubation or perifusion study [30–33]. Glucose utilization [32], protein synthesis [34–36], and oxygen utilization [37] have also been used to assess islet function.

In addition to in vitro studies, syngeneic or autologous islet transplantation is preferable to assess islet viability, which simulates the outcome of islet transplantation. Nude (athymic) mice, which were induced with diabetes by intravenous injection with streptozotocin, are commonly used for in vivo assay of human islets [38, 39].

According to the outcome of islet isolation, the Japanese Islet Transplant Registry determined the criteria for fresh islet isolation. When the outcome of the isolation fulfill the criteria ((1) yield ≥5,000 IEQ/kg (recipient body weight); (2) purity
Table 21.3 The rules for recipient selection from the recipient pool in Japan [28]

1. Recipients must be registered to the islet isolation center
2. ABO blood type
3. Second or third transplantation
4. Waiting days

Table 21.4 Indications for the recipient of islet transplantation in Japan [28]

Indications
1. Insulin dependent diabetes mellitus (serum C-peptide level: <0.1 ng/mL)
2. Unstable blood glucose levels even under the treatment by a diabetologist
3. Age: <75 years old (preferable)
4. Sufficient informed consent obtained from the patient, family, and family doctor

Contraindications
1. Severe heart disease or severe liver disease
2. Alcoholism
3. Active infectious disease
4. Malignancy
5. Severe obesity
6. Untreated retinopathy
7. Others

≥30 %; (3) final cell pellet ≤10 ml; (4) viability ≥70 %; and (5) endotoxin ≤5 EU/kg (recipient body weight)), the recipient is selected from the recipient pool by the Japanese Islet Transplant Registry according to the rules for recipient selection (Table 21.3) [28]. The patients were registered to each transplantation center according to the indications of the recipient for islet transplantation in Japan (Table 21.4) [28].

21.5 Outcome of Clinical Islet Transplantation from DCD Donors in Japan

Sixty-five islet isolations were performed at five authorized centers of islet isolation and transplantation from December 2003 to March 2007. Only one isolation (the first isolation in Japan) was performed using the pancreas from DBD donor and the other 64 isolations were performed using DCD donors. Thirty-four successful isolations (53.1 % of 64 isolations from DCD donors) fulfilled the fresh islet transplantation criteria and were transplanted to the 18 patients. The analysis of the factors influencing the results of the islet isolation demonstrated that the donor age and a warm ischemic time did not affect the results. In contrast, duration of low blood pressure of the donor, cold ischemic time, and usage of Kyoto solution for preservation was significantly influenced factors [40]. Multivariate analysis selected usage of Kyoto solution as most important.
All 18 recipients were type 1 diabetic patients with the history of diabetes from 6 to 37 years. The age of the recipients ranged from 16 to 61 years (36.2 ± 10.9 years) and the gender was 5 males/13 females. Four patients underwent two sequential islet transplantations and six patients underwent three sequential islet transplantations. Out of four patients who underwent two islet transplantations, one patient showed insulin independency, and two out of six patients who underwent three islet transplantations achieved insulin independency. However, all of these patients returned to insulin dependency from 2 weeks to 6 months after achieving insulin independency. The islet transplantation technique was performed according to the Edmonton Protocol. The isolated islets were transplanted into the portal vein using an angiocatheter via an ultrasonography-guided puncture. A steroid-free immunosuppressive regimen was used with sirolimus, tacrolimus, and anti-CD25 antibody according to the Edmonton Protocol5. As is shown in Fig. 21.1, the serum C-peptide levels were 0.5 ± 0.4 ng/mL after the first, 0.4 ± 0.2 ng/mL after the second, and 0.8 ± 0.4 ng/mL after the third islet transplantation. The positive insulin secretion from the islets, as indicated by the serum C-peptide levels, resulted in the decreased insulin requirement: 39.7 ± 18.0 U/day before, 24.2 ± 110 U/day after the first, 21.4 ± 11.5 U/day after the second, and 21.0 ± 7.7 U/day after the third islet transplantation. In addition, the hemoglobin A1C levels (JDS) decreased from 8.8 ± 1.8 % before transplantation to 7.5 ± 1.4 % after the first, 6.5 ± 1.4 % after the second, and
6.2 ± 1.2 % after the third islet transplantation. All patients obtained stabilized blood glucose levels which resulted in the disappearance of hypoglycemic unawareness. Overall graft survival defined as C-peptide level more than or equal to 0.3 ng/mL was 76.5 %, 47.1 %, and 33.6 % at 1, 2, and 3 years, respectively, whereas corresponding graft survival after multiple transplantations was 100 %, 80.0 %, and 57.1 %, respectively [40]. Almost all patients showed, however, negative C-peptide level at 5 years after the first islet transplantation.

21.6 Outcome of Clinical Islet Transplantation from DCD Donors in Chiba East National Hospital

From September 2003 to April 2007, 23 islet isolations were performed from the pancreas of non-heart-beating donors in our institution (GMP grade Bio-clean Cell Processing Center (CPC), Clinical Research Center, Chiba-East National Hospital). The age of the donors ranged from 10 to 69 years (37.5 ± 18.0 years), and the gender was 13 males/10 females. The causes of death were cerebrovascular disorder (ten cases), hypoxic encephalopathy (seven cases), trauma (five cases), and one brain tumor. After cardiac arrest, the pancreas were procured using our ISMW technique. In 9 of the 23 donors, permission was not given to insert a catheter into the aorta for systemic heparinization before a cardiac arrest, which resulted in a prolonged warm ischemic period. The pancreas were preserved using a TLM or a simple cold storage in UW solution and were transported to our CPC.

The islet isolation was performed according to the Edmonton Protocol with some modifications [41–48]. Briefly, the pancreas was distended with a cold Liberase solution (Liberase HI, Roche Diagnostics™, IN) by a ductal injection. Thereafter, the distended pancreas were cut into several pieces and put into a Ricordi chamber and digested using a closed automated system at 37 °C. The shaking of the Ricordi chamber was performed either by hand or by a shaking apparatus. The pancreatic digests were collected in a flask on ice and were purified on a Euro-Ficoll discontinuous solution using a COBE 2991 cell processor. When the results of the isolation fulfilled the criteria for fresh islet transplantation in Japan, the islets were immediately transplanted to the recipient.

Our technique of islet transplantation is the same procedure as in the Edmonton Protocol. The immunosuppression protocol was achieved by a triple therapy using sirolimus, tacrolimus, and anti-CD25 antibody. Four patients underwent six islet transplantations. All patients were type 1 diabetic patients (one male, three females) complaining of frequent hypoglycemic unawareness and showing an undetectable serum C-peptide levels (<0.03 ng/mL). The patient ages ranged from 16 to 33 years old. Two patients underwent two sequential islet transplantations.

The islet yield was 400–491,040 IEQ (mean 148,511) and the final purity was 1–70% (mean, 35.3). The stimulation index of static incubation was 1.38–11.69. Six isolations were used for transplantation because they fulfilled the Japanese criteria (success rate: 26.1 %). All patients, including two cases of single transplantation,
showed a positive serum C-peptide level (0.4–0.8 ng/mL) immediately after transplantation. Although insulin independency was not achieved, all patients achieved stabilization of blood glucose levels, reduced requirement of insulin, and the disappearance of hypoglycemic unawareness. The hemoglobin A1C levels (JDS) were significantly decreased from 9.4 ± 3.1 % to 6.4 ± 0.6 % at 4 months after transplantation. Although stomatitis and diarrhea, which were considered as the side effects of sirolimus, were observed in two patients, severe complications did not occur. In patient #1, the serum C-peptide levels decreased gradually after islet transplantation (Fig. 21.2). Blood glucose levels were, however, again stabilized after the second islet transplantation (Fig. 21.3). However, all four patients showed negative C-peptide levels at 5 years after the first islet transplantation.

21.7 Conclusions

Islet transplantation employing DCD can ameliorate severe hypoglycemic episodes, significantly improve HbA1c levels, sustain significant levels of C-peptide, and achieve insulin independence after multiple transplantations. Thus, DCD can be an important resource for islet transplantation if used under strict releasing criteria and...
in multiple transplantations, particularly in countries where heart-beating donors are not readily available.

However, the outcome of clinical islet transplantation using Edmonton protocol is not satisfactory results. In particular, long-term islet survival has not been achieved, resulting in poor achievement of insulin independency. Based on these results, the Japanese Islet Transplant Registry currently introduced a newly designed clinical islet transplantation protocol including an immunosuppressive protocol using anti-thymus globulin and anti TNF α, and the use of DBD donors.

References

1. Najarian JS, Sutherland DE, Matas AJ, Steffes MW, Simmons RL, Goetz FC. Human islet transplantation: a preliminary report. Transplant Proc. 1977;9:233–6.
2. International Islet Transplant Registry. Newsletter No. 8. 1999.
3. Ricordi C, Finke EH, Lacy PE. A method for the mass isolation of islets from the adult pig pancreas. Diabetes. 1986;35:649–53.
4. Ricordi C, Lacy PE, Finke EH, Olack BJ, Scharp DW. Automated method for isolation of human pancreatic islets. Diabetes. 1988;37:413–20.
5. Shapiro AM, Lakey JR, Ryan EA, Korbett GS, Toth E, Warnock GL, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med. 2000;343:230–8.
6. Ryan EA, Lakey JR, Rajotte RV, Korbett GS, Kin T, Imes S, et al. Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. Diabetes. 2001;50:710–9.
7. Matsumoto S, Okitsu T, Iwanaga Y, Noguchi H, Nagata H, Yonekawa Y, et al. Successful islet transplantation from nonheartbeating donor pancreata using modified Ricordi islet isolation method. Transplantation. 2006;82:460–5.
8. Matsumoto S, Tanaka K. Pancreatic islet cell transplantation. J Hepatobiliary Pancreat Surg. 2005;12:227–30.
9. Saito T, Ise K, Sato Y, Gotoh M, Matsumoto S, Kenmochi T, et al. The start of an islet transplantation program in Japan. Transplant Proc. 2005;37:3424–6.
10. Kenmochi T, Asano T, Maruyama M, Saigo K, Akutsu N, Iwashita C, Ohtsuki K, Ito T. Clinical islet transplantation in Japan. J Hepatobiliary Pancreat Surg. 2009;16(2):124–30.
11. Japanese Society of Tissue Transplantation (JSTT) ed. Guideline on the safety, storage, and application of human tissue in medical practice. IV. Criteria for donor screening. http://www.jstt.org/htm/guideline/Safety.pdf.
12. Kootstra G, Daemen J, Oomen A. Categories of non-heart-beating donors. Transplant Proc. 1995;27:2893–4.
13. Sanchez-Fructuoso A, et al. Renal transplantation from non-heart-beating donors: a promising alternative to enlarge the donor pool. J Am Soc Nephrol. 2000;11:350–8.
14. Arita S, Asano T, Kenmochi T, Enomoto K, Isono K. An initial wash-out solution for “in situ machine wash-out”. Transplant Proc. 1991;23:2589.
15. Asano T, Enomoto K, Ohtsuka M, Goto T, Nakagohri T, Kenmochi T, Ochiai T, Isono K. Usefulness of rapid machine cooling in the procurement of livers. Transplant Proc. 1989;21(1 Pt 2):1307–8.
16. Goto T, Tanioka Y, Sakai T, Terai S, Kamoda Y, Li S, et al. Application of the two-layer method on pancreas digestion results in improved islet yield and maintained viability of isolated islets. Transplantation. 2007;77:754–8.
17. Kin T, Mirbolooki M, Salehi P, Tsukada M, O’Gorman D, Imes S, et al. Islet isolation and transplantation outcomes of pancreas preserved with University of Wisconsin solution versus two-layer method using preoxygenated perfluorocarbon. Transplantation. 2006;82:1286–90.
18. Takahashi T, Tanioka Y, Matsuda T, Toyama H, Kakinoki K, Li S, et al. Impact of the two-layer method on the quality of isolated pancreatic islets. Hepatogastroenterology. 2006;53:179–82.
19. Tanaka T, Suzuki T, Tanioka Y, Sakai T, Kakinoki K, Goto T, et al. Possibility of islet transplantation from a nonheartbeating donor pancreas resuscitated by the two-layer method. Transplantation. 2005;80:738–42.
20. Tsujimura T, Kuroda Y, Churchill TA, Avila JG, Kin T, Shapiro AM, et al. Short-term storage of the ischemically damaged human pancreas by the two-layer method prior to islet isolation. Cell Transplant. 2004;13:67–73.
21. Tsujimura T, Kuroda Y, Kin T, Avila JG, Rajotte RV, Korbutt GS, et al. Human islet transplantation from pancreases with prolonged cold ischemia using additional preservation by the two-layer (UW solution/perfluorochemical) cold-storage method. Transplantation. 2002;74:1687–91.
22. Tanioka Y, Sutherland DE, Kuroda Y, Gilmore TR, Asaheim TC, Kronson JW, et al. Excellence of the two-layer method (University of Wisconsin solution/perfluorochemical) in pancreas preservation before islet isolation. Surgery. 1997;122:435–42.
23. Caballero-Corbalán J, Eich T, Lundgren T, Foss A, Felldin M, Källen R, et al. No beneficial effect of two-layer storage compared with UW-storage on human islet isolation and transplantation. Transplantation. 2007;84:864–9.
24. Sawada T, Matsumoto I, Nakano M, Kirchhoff N, Sutherland DE, Hering BJ. Improved islet yield and function with ductal injection of University of Wisconsin solution before pancreas preservation. Transplantation. 2003;75(12):1965–9.
25. Matsumoto S, Noguchi H, Shimoda M, Ikemoto T, Naziruddin B, Jackson A, Tamura Y, Olson G, Fujita Y, Chuo D, Takita M, Kobayashi N, Onaca N, Levy M. Seven consecutive successful clinical islet isolations with pancreatic ductal injection. Cell Transplant. 2010;19(3):291–7.
26. Anazawa T, Balamurugan AN, Papas KK, Avgoustiniatos ES, Ferrer J, Matsumoto S, Sutherland DE, Hering BJ. Improved method of porcine pancreas procurement with arterial flush and ductal injection enhances islet isolation outcome. Transplant Proc. 2010;42(6):2032–5.
27. Shimoda M, Itoh T, Sugimoto K, Iwashita S, Takita M, Chuo D, Sorelle JA, Naziruddin B, Levy MF, Grayburn PA, Matsumoto S. Improvement of collagenase distribution with the ductal preservation for human islet isolation. Islets. 2012;4(2):130–7.
28. Kenmochi T, Matsumoto S, Tanioka Y, Saito T, Ono J, Okamoto M, et al. Manual for clinical islet transplantation in Japan, 3rd ed. In: Fukushima H, editor. The Japanese Society for Pancreas and Islet Transplantation. Fukushima, Japan; 2006. p. 1–55.
29. Ricordi C, Gray DWR, Hering BJ, et al. Islet isolation assessment in man and large animals. Acta Diabetol Lat. 1990;27:185–95.
30. Gray DWR, McShane P, Grant A, Morris PJ. A method for isolation of islets of Langerhans from the human pancreas. Diabetes. 1984;33:1055–61.
31. Andersson A, Borg H, Groth CG, et al. Survival of isolated human islets of Langerhans maintained in tissue culture. J Clin Invest. 1976;57:1295–301.
32. Ashcroft SJH, Bassett JM, Randle PJ. Isolation of human pancreatic islets capable of releasing insulin and metabolizing glucose in vitro. Lancet. 1971;1:888–9.
33. Ballinger WF, Lacy PE. Transplantation of intact pancreatic islets in rats. Surgery. 1972; 72:175–86.
34. Sutherland DE, Matas AJ, Steffes MW, Najarian JS. Infant human pancreas. A potential source of islet tissue for transplantation. Diabetes. 1976;25(12):1123–8.
35. Davalli AM, Ricordi C, Socci C, Braghi S, Bertuzzi F, Fattor B, Di Carlo V, Pontiroli AE, Pozza G. Abnormal sensitivity to glucose of human islets cultured in a high glucose medium: partial reversibility after an additional culture in a normal glucose medium. J Clin Endocrinol Metab. 1991;72(1):202–8.
36. Kneteman NM, Rajotte RV. Isolation and cryopreservation of human pancreatic islets. Life Support Syst. 1985;3 Suppl 1:712–8.
37. Scharp DW, Lacy PE, Finke E, Olack B. Low-temperature culture of human islets isolated by the distention method and purified with Ficoll or Percoll gradients. Surgery. 1987;102(5):869–79.
38. Povlsen CO, Skakkebaek NE, Rygaard J, Jensen G. Heterotransplantation of human foetal organs to the mouse mutant nude. Nature. 1974;248(445):247–9.
39. Usadel KH, Schwedes U, Bastert G, Steinau U, Klempa I, Fassbinder W, Schöffling K. Transplantation of human fetal pancreas: experience in thymusaplastic mice and rats and in a diabetic patient. Diabetes. 1980;29 Suppl 1:74–9.
40. Saito T, Gotoh M, Satomi S, Uemoto S, Kenmochi T, Itoh T, Kuroda Y, Yasunami Y, Matsumoto S, Teraoka S, Working Members of the Japanese Pancreas and Islet Transplantation Association. Islet transplantation using donors after cardiac death: report of the Japan islet transplantation registry. Transplantation. 2010;90(7):740–7.
41. Kenmochi T, Asano T, Jingu K, Matsui Y, Maruyama M, Akutsu N, et al. Effectiveness of hydroxyethyl starch (HES) on purification of pancreatic islets. J Surg Res. 2003;111:16–22.
42. Kenmochi T, Asano T, Jingu K, Matsui Y, Maruyama M, Miyauchi H, et al. Purification of pancreatic islets using hydroxyethyl starch-Collins solution. Transplant Proc. 2001;33:670–1.
43. Kenmochi T, Miyamoto M, Une S, Nakagawa Y, Moldovan S, Navarro RA, et al. Improved quality and yield of islets isolated from human pancreata using a two-step digestion method. Pancreas. 2000;20:184–90.
44. Kenmochi T, Asano T, Jingu K, Iwashita C, Miyauchi H, Takahashi S, et al. Development of a fully automated islet digestion system. Transplant Proc. 2000;32:341–3.
45. Mullen Y, Arita S, Kenmochi T, Une S, Smith CV. A two-step digestion process and lap-1 cold preservation solution for human islet isolation. Ann Transplant. 1998;2:40–5.
46. Kenmochi T, Miyamoto M, Sasaki H, Une S, Nakagawa Y, Moldovan S, et al. Lap-1 cold preservation solution for isolation of high-quality human pancreatic islets. Pancreas. 1998; 17:367–77.
47. Iwashita C, Asano T, Kenmochi T, Jingu K, Uematsu T, Nakagohri T, et al. Combined method of mechanical chopper and automated two-step digestion technique for islet isolation from canine pancreas. Transplant Proc. 1996;28:337–8.
48. Jingu K, Asano T, Kenmochi T, Enomoto K, Uematsu T, Nakagohri T, et al. Combined method of mechanical chopper and automated digestion system for islet isolation. Transplant Proc. 1996;26:634–6.