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Evaluation of Islet Purification Methods for Making a Continuous Density Gradient and Loading Tissue

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Abstract

Islet purification is one of the most important steps of islet isolation for pancreatic islet transplantation. We previously reported that a purification method using large plastic bottles effectively achieved a high yield of islets from porcine pancreas. In this study, we evaluated the methods for making a continuous density gradient and loading tissue. One method involved loading digested tissue on top of a continuous density gradient (top loading). The other method involved mixing digested tissue with low-density solution and then making a continuous gradient (mixed loading). There were no significant differences between the 2 purification methods in terms of the islet yield, rate of viability or purity, score, or in the stimulation index after purification. Furthermore, there were no marked differences in the attainability or suitability of posttransplantation normoglycemia. Our study shows the equivalency of these 2 methods of islet purification.

Keywords

islet transplantation, islet isolation, islet purification, bottle purification, continuous density gradient

Introduction

Islet transplantation is a treatment for type 1 diabetes mellitus[1–5]. The islet isolation process consists of pancreas distension, pancreas digestion, and islet purification. The purification step is one of the most important and difficult procedures in islet isolation. The most common method of islet purification is density gradient centrifugation based on the different densities between islets and acinar tissue[1,4,6,7]. We previously reported that purification using large plastic bottles substantially improved the efficacy compared with standard purification using a COBE 2991 cell processor[8]. The high shear force involved in the standard purification method causes mechanical damage to the islets[9] but can be minimized or eliminated using the bottle purification method.

In this study, we evaluated 2 methods for making a continuous density gradient and loading tissue. One method involved loading digested tissue on top of a continuous density gradient made by changing the volumetric ratio of iodixanol and University of Wisconsin (UW) solution (top loading [TL]), which was more efficient than the method of "bottom loading"[8]. Another method involved mixing digested tissue with a low-density solution and then making a continuous gradient (mixed loading [ML]). We compared the effects of the purification methods on the outcomes of islet purification and transplantation.

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Materials and Methods

Porcine Pancreas Procurement and Pancreas Digestion

Pancreata from 3-y-old pigs (female, $N = 6$) were obtained from a local slaughterhouse. The operation was started about 10 min after the cessation of the heartbeat. All pancreata were procured using a standardized technique to minimize the warm ischemic time (WIT). After removing the pancreas, we immediately inserted a cannula into the main pancreatic duct. The pancreas was weighed, and 1 mL/g pancreas weight of modified Kyoto (MK) solution (Otsuka Pharmaceutical Factory Inc., Naruto, Japan) was infused through the intraductal cannula[10]. The pancreata were placed into the MK solution container at 4 °C for about 18 h until the islet isolation procedure[11,12]. The "operation time" was defined as the time from the start of the operation until the removal of the pancreas. The WIT was defined as the time from the cessation of the heartbeat until the placement of the pancreas into the preservation solution. The cold ischemic time was defined as the time from the placement of the pancreas into the preservation solution until the start of islet isolation.

Islet isolation was conducted as previously described[5], according to the standard Ricordi technique[13] with the modifications that were later introduced in the Edmonton protocol[1,4,14,15]. After the decontamination of the pancreas, the ducts were perfused in a controlled fashion with a cold enzyme blend of Liberase MTF (1.0 mg/mL) with thermolysin (0.075 mg/mL; Roche Diagnostics Corporation, Indianapolis, IN, USA). The distended pancreas was then cut into 7 to 9 pieces, placed in a Ricordi chamber, and gently shaken. While the pancreas was being digested by recirculating the enzyme solution through the Ricordi chamber at 37 °C, we monitored the extent of digestion with dithizone staining (2 mg/mL final concentration; Sigma Chemical Co., St. Louis, MO, USA) by taking small samples from the system. Once digestion was confirmed to be complete, the dilution solution (Center for Promotion of Education and Science, Hiroshima, Japan) was introduced into the system. The system was then cooled to stop further digestive activity. The digested tissue was collected in flasks containing 5% fetal bovine serum (FBS) (GIBCO-Invitrogen, Carlsbad, CA, USA). The phase I period was defined as the time from the placement of the pancreas in the Ricordi chamber until the start of digested pancreas collection. The phase II period was defined as the time between the start and end of collection. After the digestion, the tissue was collected and washed with fresh medium to remove the enzyme. The digested tissue was incubated in UW solution (ViaSpan, DuPont Pharmaceuticals, Wilmington, DE, USA) for 30 min before purification[16].

Islet Purification

Islets were purified with a continuous density gradient of iodixanol-UW solution (Optiprep®, Sigma-Aldrich, St. Louis, MO, USA) as previously reported[6,17–19]. We combined iodixanol with UW solution (IU solution) to generate a new purification solution. Low-density (1.075 g/cm^3) and high-density (1.085 g/cm^3) solutions were produced by changing the volumetric ratio of iodixanol and UW solution as reported previously[6]. Before purification by IU solution, we calculated the density of the digested tissue. During this step, digested tissue (0.2 mL; after incubation in UW solution and prior to purification) was added to six 5 mL test tubes (Corning Japan, Tokyo, Japan) of different densities (1.085, 1.090, 1.095, 1.100, 1.105, and 1.110 g/cm^3), and these tubes were centrifuged at 235× g (1,000 rpm) for 5 min. The density at which most of the digested tissue floated was defined as the density of the digested tissue. According to the outcome of the density determination step, we determined the necessary density of the high-density IU solution and added an appropriate amount of iodixanol to the high-density IU solution (Table 1).

Islet purification was performed by digested tissue (≤20 mL of tissue/run) with continuous gradients using low-density and density-adjusted high-density solutions in bottles (size 500 mL; NALGENE, Rochester, NY, USA). The gradient was produced with a gradient marker (Biorep Technologies, Miami Lakes, FL, USA) and candy cane–shaped stainless steel pipes (length 30 cm; UMIHIRA, Kyoto, Japan). One method involved making a continuous gradient and then loading digested tissue in UW solution (50 mL) on top of the continuous density gradient (TL). Another method involved mixing digested tissue in UW solution (50 mL) with low-density solution (160 mL) and then making a continuous gradient (ML). The density of the low-density solution after mixture with digested tissue was 1.0645. The theoretical density on each continuous gradient is shown in Fig. 1A. The volumes of low-density and high-density solutions are shown in Table 1. The bottles were centrifuged at 235 g (1,000 rpm) for 5 min at 4 °C. After centrifugation, approximately 9 fractions (50 mL each) were collected and examined for purity.

Islet Evaluation

The crude number of islets in each diameter class was determined by counting the islets after dithizone staining (2 mg/mL final concentration) using an optical graticule (Olympus, Tokyo, Japan). The crude number of islets was then converted to the standard number of islet equivalents (IEs; diameter standardized to 150 μm)[20]. The gross morphology was qualitatively assessed by 2 independent investigators scoring the islets for shape (flat vs. spherical), border (irregular vs. well-rounded), integrity (fragmented vs. solid/compact), uniformity of staining (not uniform vs. perfectly uniform), and diameter (least desirable: all cells <100 μm/most desirable: more than 10% of cells >200 μm)[16]. Islet recovery was defined as the percentage of IEs recovered after purification divided by the IEs before purification. The islet viability after purification was assessed using double fluorescein diacetate/propidium iodide (fluorescein

Table 1. Characteristics of Each High-density Purification Solution, Volumetric Ratio of Iodixanol and High-density IU Solution, and the Contents of the TL and ML Bottles.

High-density solution for TL						
High-density solution[a]	150	150	150	150	150	150
Iodixanol[b]	0	3.3	6.7	10.2	14.0	17.9
Total volume (mL)	150	153.3	156.7	162.2	164.0	167.9
High-density solution for ML						
Density (g/cm^3)	1.085	1.090	1.095	1.100	1.105	1.110
High-density solution[a]	200	200	200	200	200	200
Iodixanol[b]	0	4.4	8.9	13.6	18.6	23.8
Total volume (mL)	200.0	204.4	208.9	213.6	218.6	223.8

Contents of TL and ML bottles	TL	ML
Cap solution	50	0
UW solution with digested tissue	50	50[c]
Low-density IU solution	160	160[c]
High-density IU solution	150–167.9	200–223.8
Total volume (mL)	410–427.9	410–433.8

Abbreviation: IU = iodixanol with University of Wisconsin solution; TL = top loading; ML = mixed loading; UW = University of Wisconsin.
[a]The density of the high-density IU solution is 1.085 g/cm^3.
[b]The density of the iodixanol solution is 1.320 g/cm^3.
[c]The digested tissue and low-density solution were mixed before making a continuous gradient.

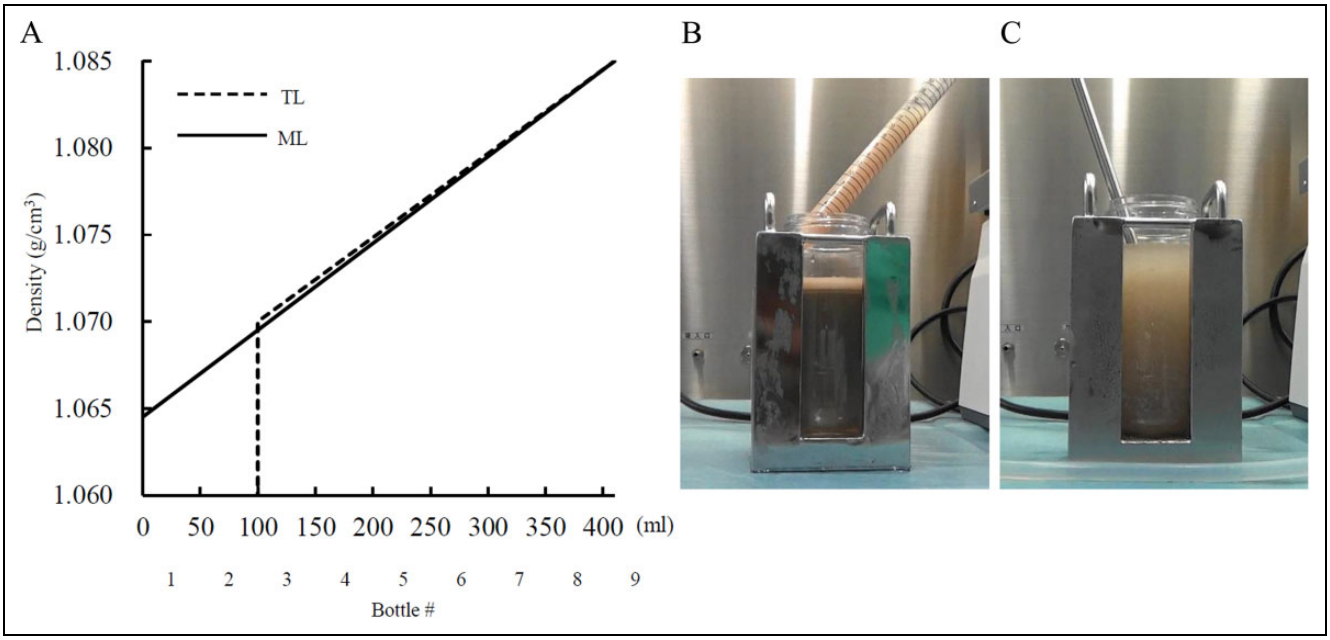

Fig. 1. Density of top loading (TL) and mixed loading (ML) gradients and loading of digested tissue. (A) Theoretical density of the TL and ML gradients. Since the densities of fractions 1 and 2 on the TL gradient were constant (fraction 1: density of cap solution, 1.007 g/cm^3; fraction 2: density of University of Wisconsin solution, 1.047 g/cm^3), the data for these fractions are not shown. (B) A picture of the TL gradient. (C) A picture of the ML gradient.

diacetate/propidium iodide, Sigma-Aldrich) staining to simultaneously visualize the living and dead islet cells[1,4,20]. Fifty islets were inspected, and their individual viability was determined visually. The average viability was then calculated[5].

The islet function was assessed by monitoring the insulin secretory response of the purified islets during glucose stimulation using a procedure described by Shapiro et al[1,4]. Briefly, 1,200 IEs were incubated with either 2.8 mM or

25 mM glucose (Sigma-Aldrich) in Roswell Park Memorial Institute (RPMI) 1640 (Sigma-Aldrich) for 2 h at 37 °C and 5% CO_2. The supernatants were collected, and the insulin levels were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (ALPCO Insulin ELISA kit; ALPCO Diagnostics, Windham, NH, USA). The stimulation index was calculated by determining the ratio of insulin released from the islets in high glucose to the insulin released in low glucose. The data were expressed as the mean ± standard error (*SE*) of the mean.

In Vivo Assessment

Six-wk-old nude mice (male; Charles River Laboratories Japan, Inc., Kanagawa, Japan; $N = 24$) were rendered diabetic by a single intraperitoneal injection of 220 mg/kg of streptozotocin (STZ; Sigma-Aldrich). Hyperglycemia was defined as when a glucose level of >350 mg/dL was detected twice (consecutively) after the administration of STZ. The 2,000 IE porcine islets obtained from each group were transplanted into the renal subcapsular space of the left kidney of a diabetic nude mouse as described previously[21–23]. During the 30-d posttransplantation period, the nonfasting blood glucose levels were monitored 3 times per week. Normoglycemia was defined when 2 consecutive blood glucose–level measurements were <200 mg/dL. No statistically significant differences were observed in either the pretransplantation blood glucose levels or the pretransplantation body weight among the 2 groups of mice. Glucose was measured using an ACCU-CHEK® Compact Plus kit (Roche Diagnostics K.K., Tokyo, Japan) in accordance with the manufacturer's instructions. All of the mouse studies were approved by the Institutional Animal Care and Use Committee of University of the Ryukyus.

Statistical Analyses

The data are represented as means ± *SE*. The differences between 2 groups were analyzed using Student's *t*-test or the Kaplan–Meier log-rank test. *P* values of <0.05 were considered to indicate statistical significance.

Results

Characteristics of the Isolated Porcine Islets

Porcine islet isolation was performed using TL or ML as shown in Fig. 1B and C, respectively. The characteristics of the porcine pancreas and islets before purification are shown in Table 2. There were no significant differences in the islet yield after purification (TL group: 190,705 ± 59,841 IE; 1,932 ± 271 IE/g; BP-B group: 192,077 ± 66,414 IE; and 1,947 ± 717 IE/g; Fig. 2B) or in the post-purification recovery rate, viability, purity, or score (Table 3). Thus, the 2 solutions achieved a similar level of efficiency in islet purification.

Table 2. The Characteristics of the Tissue and Procedures before Purification.

	$N = 6$
Pancreas size (g)	101.1 ± 7.1
Operation time (min)	6.5 ± 1.1
Warm ischemic time (min)	28.5 ± 1.3
Cold ischemic time (min)	1,103.3 ± 13.9
Phase I period (min)	11.3 ± 0.7
Phase II period (min)	35.7 ± 1.3
Undigested tissue (g)	10.6 ± 2.0
Islet yield before purification (IE)	466,648 ± 106,463
Islet yield before purification (IE/g)	4,686 ± 1,141

Note: The data are expressed as the means ± standard error. IE = islet equivalent.

In Vitro Assessment

To assess the islet quality in each group in vitro, the stimulation index of the isolated islets was measured. There were no significant differences between the 2 solutions in the stimulation index (TL group: 2.24 ± 0.06, $N = 6$; ML group: 2.32 ± 0.15, $N = 6$; Fig. 3). These data suggest that the islets in the 2 groups were of similar quality in vitro.

In Vivo Assessment

To assess the islet graft function of each group in vivo, 2,000 IEs from each group were transplanted below the kidney capsule of STZ-induced diabetic nude mice. The blood glucose levels of 9 of the 12 mice (75.0%) from the TL group and 10 of the 12 mice (83.3%) from the ML group decreased gradually after islet transplantation until they reached normoglycemia. The blood glucose levels remained stable thereafter (Fig. 4) and returned to the pretransplantation levels after the removal of the islet-bearing kidneys (30 d posttransplantation). Posttransplantation normoglycemia was similarly attainable in the 2 groups. These data suggest that the islets of the 2 groups were of similar quality.

Discussion

Islet purification using a COBE 2991 cell processor is the gold standard method for clinical islet isolation. However, the high shear force associated with the method causes mechanical damage to the islets[9]. We previously reported that shear stress was substantially reduced by bottle purification, and the size of the islets purified by the bottle method was significantly larger than that of the islets purified by COBE purification[8]. We also reported that the TL method has several advantages compared with bottom loading, such an increased islet recovery rate[8]. One issue associated with bottle purification using the TL method was the occasional clumping of a pellet of digested tissue on the wall of the bottle. To overcome the issues associated with bottle purification, this study was performed.

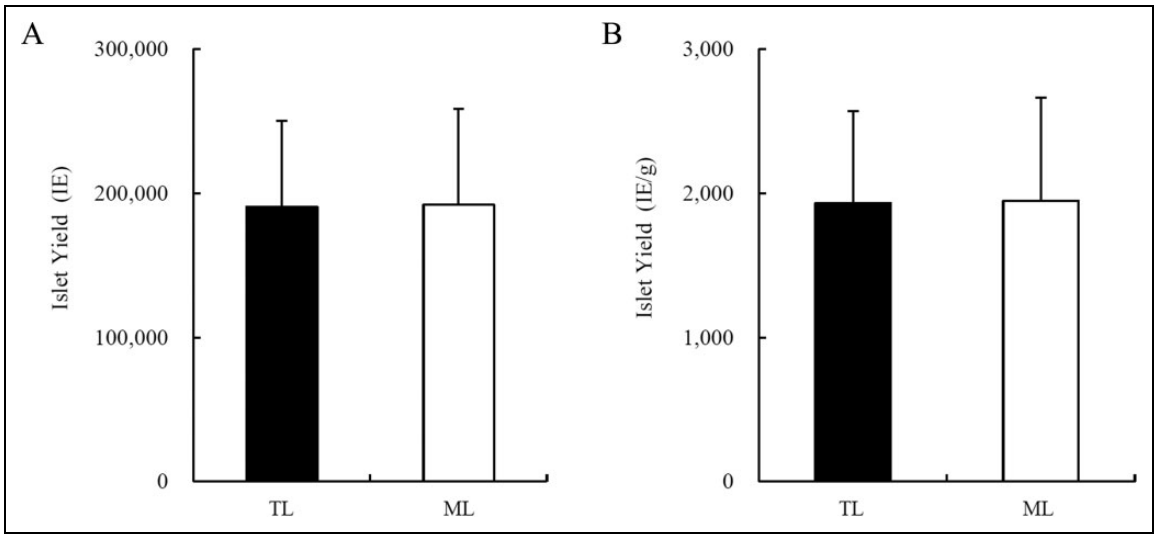

Fig. 2. The islet yields. (A) The islet yield after purification. (B) The islet yield per pancreas weight after purification. The data are expressed as the mean ± standard error. TL = top loading ($N = 6$); ML = mixed loading ($N = 6$); IE/g = islet equivalent per pancreas weight.

Table 3. The Islet Characteristics after Purification.

	TL ($N = 6$)	ML ($N = 6$)
Postpurification recovery (%)[a]	77.4 ± 9.3	76.6 ± 9.9
Viability (%)	96.1 ± 0.3	96.6 ± 0.7
Purity (%)	53.3 ± 3.7	60.0 ± 7.4
Score	9.3 ± 0.3	9.1 ± 0.3

Note: The data are expressed as the means ± standard error. IE = islet equivalent; TL = top loading; ML = mixed loading.
[a]Postpurification recovery (%) = IE after purification/(IE before purification/2) × 100.

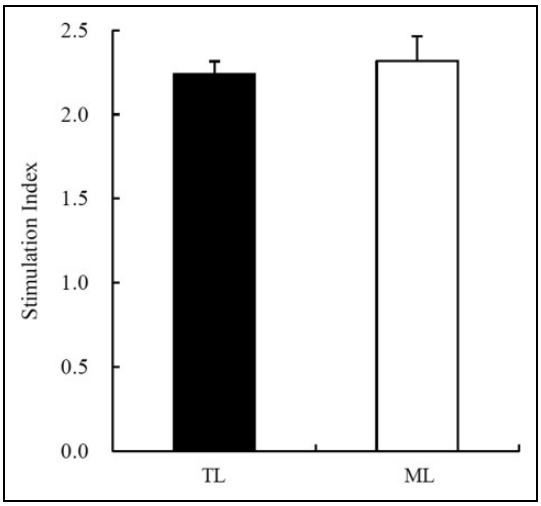

Fig. 3. The stimulation index of the isolated islets. The stimulation index was calculated by determining the ratio of insulin released from islets in high-glucose media to that released in low-glucose media. The data are expressed as the mean ± standard error ($N = 6$, each).

We showed that the isolation and transplant outcomes did not differ between the 2 purification methods. Although the ML method did not show any tissue clumping on the wall of the bottle, the purification efficacy was similar between the 2 groups. The reason for this lack of a substantial difference may be the same as why the TL method was superior to bottom loading. With the ML method, there was some tissue near the bottom of the bottle, which resulted in a lower efficacy than with the TL method, but this was balanced by the reduced clumping with the ML method compared with the TL method, which increased the efficacy. The efficacy of the ML method may be similar to that of the TL method.

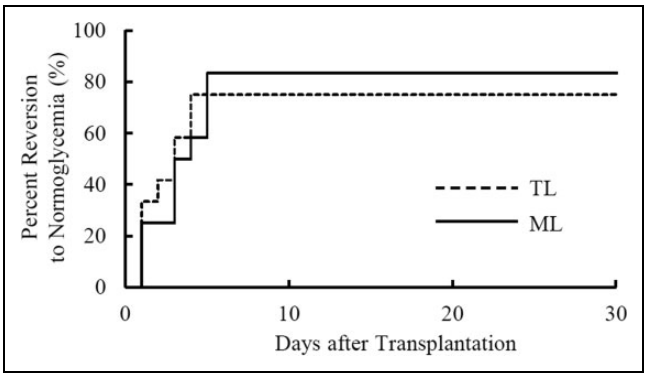

Fig. 4. Islet transplantation into diabetic nude mice. The percentage of streptozotocin-induced diabetic nude mice, in which normoglycemia was achieved after islet transplantation, is depicted. A total of 2,000 islet equivalents were transplanted below the kidney capsule in the diabetic nude mice. Normoglycemia was defined as 2 consecutive posttransplant blood glucose levels of <200 mg/dL (TL = top loading group [$N = 12$]; ML = mixed loading group [$N = 12$]).

Islets constitute approximately 2% to 5% of pancreatic tissue. By purifying the islets, the acinar tissue is removed, and the tissue volume that needs to be transplanted is greatly reduced[1,4]. Purification minimizes the risks associated with islet infusion through the portal vein (such as increased portal pressure and thrombosis). Since islet purification is one of the most important steps for islet isolation, we will

investigate more efficient methods for islet purification in a future study.

Ethical Approval

All of the mouse studies were approved by the Institutional Animal Care and Use Committee of University of the Ryukyus.

Statement of Human and Animal Rights

All of the mouse studies were approved by the review committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences and Graduate School of Medicine, University of the Ryukyus.

Statement of Informed Consent

Not Applicable.

Declaration of Conflicting Interests

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References

1. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med. 2000;343(4):230–238.
2. Froud T, Ricordi C, Baidal DA, Hafiz MM, Ponte G, Cure P, Pileggi A, Poggioli R, Ichii H, Khan A, et al. Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience. Am J Transplant. 2005;5(8):2037–2046.
3. Hering BJ, Kandaswamy R, Ansite JD, Eckman PM, Nakano M, Sawada T, Matsumoto I, Ihm SH, Zhang HJ, Parkey J, et al. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. JAMA. 2005;293(7):830–835.
4. Shapiro AM, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, Secchi A, Brendel MD, Berney T, Brennan DC, et al. International trial of the Edmonton protocol for islet transplantation. N Engl J Med. 2006;355(13):1318–1330.
5. Noguchi H, Iwanaga Y, Okitsu T, Nagata H, Yonekawa Y, Matsumoto S. Evaluation of islet transplantation from non-heart beating donors. Am J Transplant. 2006;6(10):2476–2482.
6. Noguchi H, Ikemoto T, Naziruddin B, Jackson A, Shimoda M, Fujita Y, Chujo D, Takita M, Kobayashi N, Onaca N, et al. Iodixanol-controlled density gradient during islet purification improves recovery rate in human islet isolation. Transplantation. 2009;87(11):1629–1635.
7. Anazawa T, Matsumoto S, Yonekawa Y, Loganathan G, Wilhelm JJ, Soltani SM, Papas KK, Sutherland DE, Hering BJ, Balamurugan AN. Prediction of pancreatic tissue densities by an analytical test gradient system before purification maximizes human islet recovery for islet autotransplantation/allotransplantation. Transplantation. 2011;91(5):508–514.
8. Shintaku H, Okitsu T, Kawano S, Matsumoto S, Suzuki T, Kanno I, Kotera H. Effects of fluid dynamic stress on fracturing of cell-aggregated tissue during purification for islets of Langerhans transplantation. J Phys D Appl Phys. 2008;41(11):115507.
9. Shimoda M, Noguchi H, Fujita Y, Takita M, Ikemoto T, Chujo D, Naziruddin B, Levy MF, Kobayashi N, Grayburn PA, et al. Islet purification method using large bottles effectively achieves high islet yield from pig pancreas. Cell Transplant. 2012;21(2–3):501–508.
10. Noguchi H, Ueda M, Hayashi S, Kobayashi N, Okitsu T, Iwanaga Y, Nagata H, Nakai Y, Matsumoto S. Ductal injection of preservation solution increases islet yields in islet isolation and improves islet graft function. Cell Transplant. 2008; 17(1–2):69–81.
11. Katayama A, Noguchi H, Kuise T, Nakatsuka A, Hirota D, Kataoka HU, Kawai T, Inoue K, Imagawa N, Saitoh I, et al. Comparison of new preservation solutions, HN-1 and University of Wisconsin solution, in pancreas preservation for porcine islet isolation. Cell Med. 2013;6(1–2):3–8.
12. Noguchi H, Ueda M, Nakai Y, Iwanaga Y, Okitsu T, Nagata H, Yonekawa Y, Kobayashi N, Nakamura T, Wada H, et al. Modified two-layer preservation method (M-Kyoto/PFC) improves islet yields in islet isolation. Am J Transplant. 2006;6(3):496–504.
13. Ricordi C, Lacy PE, Finke EH, Olack BJ, Scharp DW. Automated method for isolation of human pancreatic islets. Diabetes. 1988;37(4):413–420.
14. Noguchi H, Naziruddin B, Shimoda M, Chujo D, Takita M, Sugimoto K, Itoh T, Onaca N, Levy MF, Matsumoto S. A combined continuous density/osmolality gradient for supplemental purification of human islets. Cell Med. 2012;3(1–3):33–41.
15. Ricordi C. Islet transplantation: a brave new world. Diabetes. 2003;52(7):1595–1603.
16. Kawai T, Noguchi H, Kuise T, Nakatsuka A, Katayama A, Imagawa N, Kataoka HU, Saitoh I, Noguchi Y, Watanabe M, et al. Comparison of incubation solutions prior to the purification of porcine islet cells. Cell Med. 2013;6(1–2):9–14.
17. Noguchi H, Naziruddin B, Shimoda M, Fujita Y, Chujo D, Takita M, Peng H, Sugimoto K, Itoh T, Kobayashi N, et al. Evaluation of osmolality of density gradient for human islet purification. Cell Transplant. 2012;21(2–3):493–500.
18. Miyagi-Shiohira C, Kobayashi N, Saitoh I, Watanabe M, Noguchi Y, Matsushita M, Noguchi H. Comparison of purification solutions with different osmolality for porcine islet purification. Cell Med. 2016;9(1–2):53–59.
19. Miyagi-Shiohira C, Kobayashi N, Saitoh I, Watanabe M, Noguchi Y, Matsushita M, Noguchi H. The evaluation of islet purification methods that use large bottles to create a continuous density gradient. Cell Med. 2016;9(1–2):45–51.

20. Ricordi C, Gray DW, Hering BJ, Kaufman DB, Warnock GL, Kneteman NM, Lake SP, London NJ, Socci C, Alejandro R, et al. Islet isolation assessment in man and large animals. Acta Diabetol Lat. 1990;27(3):185–195.
21. Noguchi H, Matsushita M, Okitsu T, Moriwaki A, Tomizawa K, Kang S, Li ST, Kobayashi N, Matsumoto S, Tanaka K, et al. A new cell-permeable peptide allows successful allogeneic islet transplantation in mice. Nat Med. 2004;10(3): 305–309.
22. Noguchi H, Naziruddin B, Jackson A, Shimoda M, Fujita Y, Chujo D, Takita M, Peng H, Sugimoto K, Itoh T, et al. Comparison of ulinastatin, gabexate mesilate, and nafamostat mesilate in preservation solution for islet isolation. Cell Transplant. 2012;21(2–3):509–516.
23. Noguchi H, Naziruddin B, Jackson A, Shimoda M, Ikemoto T, Fujita Y, Chujo D, Takita M, Peng H, Sugimoto K, et al. Fresh islets are more effective for islet transplantation than cultured islets. Cell Transplant. 2012;21(2–3):517–523.