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Pancreatic Ductal Perfusion at Organ Procurement Enhances Islet Yield in Human Islet Isolation

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Abstract

Objective—Pancreas preservation is a major factor influencing the results of islet cell transplantation. This study evaluated the effects of two different solutions for pancreatic ductal perfusion (PDP) at organ procurement.

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Conflict of Interest Disclosure: The authors declare no conflict of interest.

List of Supplemental Digital Content

Table S1. Composition of the solutions for PDP

Table S2. Inclusion and exclusion criteria for donor selection

Figure S1. Power analysis of the anticipated difference in the primary endpoint

Figure S2. Pairwise comparison for significant differences in islet isolation outcomes

Methods—Eighteen human pancreases were assigned to three groups: non-PDP (control), PDP with ET-Kyoto solution, and PDP with cold storage/purification stock solution. Pancreatic islets were isolated according to the modified Ricordi method.

Results—No significant differences in donor characteristics, including cold ischemia time, were observed between the three groups. All islet isolations in the PDP groups had >400,000 IEQ in total islet yield post-purification, a significant increase when compared with the control ($P = 0.04$ and <0.01). The islet quality assessments—including an *in vivo* diabetic nude mice assay and the response of high-mobility group box protein 1 to cytokine stimulation—also showed no significant differences. The proportion of TUNEL-positive cells showing apoptosis in islets in the PDP groups was significantly lower than in the control group ($P < 0.05$).

Conclusion—Both ET-Kyoto solution and cold storage/purification stock solution are suitable for PDP and consistently resulted in isolation success. Further studies with a larger number of pancreas donors should be done to compare the effects of the PDP solutions.

Keywords

organ preservation; pancreatic islet isolation; islet potency assay; islet transplantation; apoptosis

INTRODUCTION

Pancreatic islet cell transplantation is a promising treatment option, with allogeneic transplants used for patients with brittle type 1 diabetes and autologous transplants following total pancreatectomy used for patients with refractory chronic pancreatitis.^{1,2} In 2000, the Edmonton protocol opened a new era of allogeneic islet transplantation, achieving insulin independence in all islet recipients; however, there are still major barriers to its wide use, such as the need for multiple infusions with multiple donors, failed islet isolation, difficulty in maintaining long-term graft function, and the use of strong immunosuppression.³

A major element in improving islet isolation outcomes and, in turn, clinical results is pancreas preservation.⁴ To that end, pancreatic ductal perfusion (PDP) at organ procurement was originally examined and shown to be effective in rodent models, where it was called *prestorage ductal flush* and *intraductal distension* and involved use of collagenase-containing Hanks' solution or University of Wisconsin solution (UWS).^{5,6} These techniques allowed sufficient distribution of the collagenase solution in the entire pancreas, preserving pancreatic ducts and inhibiting cold ischemia injury in ductal epithelium.⁷ Sawada et al showed that ductal perfusion using UWS *without* collagenase could significantly improve islet yield and quality.⁷ UWS can prevent hypothermia-induced cell swelling during cold ischemia time.⁸ Contradictory effects have also been reported: that the UWS inhibited collagenase activity and has high viscosity, possibly resulting in poor isolation outcomes.^{9,10} Another disadvantage in the use of UWS for PDP is the β cell exhaustion caused by its high potassium level.¹¹ Thus, extracellular fluid-like solution with a low potassium level should be considered for PDP.

ET-Kyoto solution (ETKS, Otsuka Pharmaceutical Factory Inc, Naruto, Japan) was originally developed as an organ preservation solution for lung transplantation and has an

extracellular fluid-like electrolyte composition with sodium and potassium levels of approximately 100 and 44 mmol/L, respectively (Supplemental Digital Content Table S1).^{12, 13} The potassium concentration in ETKS was designed at lower level than UWS, but higher than extracellular fluid, since 40 mmol/L of potassium had benefit in keeping vascular resistance lower in preclinical lung transplantation model when compared to specially prepared ETKS with much lower potassium level (20 mmol/L) and Euro-Collins solution with higher potassium (115 mmol/L).^{14, 15} ETKS includes unique ingredients of trehalose and gluconate, which help stabilize the cell membrane and prevent cell swelling.^{16, 17} ETKS showed less inhibition of collagenase activity than UWS but had comparable benefits in islet isolation.¹⁸ Recently, PDP with ETKS coupled with the two-layer method was shown to contribute to highly successful islet isolation.^{19, 20}

The cold storage/purification stock solution (CSPS) (Mediatech, Inc, Manassas, VA, USA) has a sodium-potassium composition similar to that of extracellular fluid (Supplemental Digital Content Table S1) and contains histidine, allowing a robust buffering capacity.²¹ Histidine-lactobionate-based preservation solution has been reported to improve the viability of purified islets up to 48 hours.²²

Both ETKS and CSPS have an electrolyte composition of higher sodium and lower potassium levels compared to UWS, which should be beneficial for PDP, while each solution has its own unique ingredients to improve pancreas or islet preservation. No reports have directly compared ETKS and CSPS solution for PDP. Hence, we designed a prospective study to investigate the impact of the two PDP solutions on islet isolation outcomes, comparing with control group without ductal perfusion at organ procurement. Total islet yield after purification and other islet quality parameters were used for the primary and secondary endpoints. We also evaluated apoptosis in islets immediately after cold ischemia time as an ancillary study to elucidate the influence of PDP solutions on islets before the isolation.

MATERIALS AND METHODS

Study Design and Donor Criteria

This study was designed as a prospective trial, and the donor criteria listed in Supplemental Digital Content Table S2 were defined prior to the study initiation, according to the international trial of the Edmonton protocol.²³ The recovered pancreas was assigned to one of three groups—no PDP (control), PDP with ETKS, or PDP with CSPS—with the goal of avoiding significant differences in donor characteristics among the groups, particularly for age and body mass index.

A power analysis was completed to determine the appropriate number of donors for this study (Supplemental Digital Content Figure S1). Based on our previous observation,¹⁹ we expected a difference between the control group and the PDP groups of $2.5 \pm 1.4 \times 10^5$ IEQ (average $\pm$ SD) for the primary endpoint. The analysis revealed that a minimum of 6 donors per group should be evaluated to detect the anticipated difference with a statistical power of $\beta = 0.80$ and $\alpha = 0.05$.

The primary endpoint was total islet yield after purification, represented by a standard number of islet equivalents (IEQ).²⁴ The secondary endpoints included islet yield per pancreas weight postdigestion and postpurification (IEQ/g) and islet quality evaluated by viability, stimulation index of the glucose-stimulated insulin release assay, and in vivo nude mice assay. As an ancillary study to explain the effect of PDP, we evaluated apoptosis by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay in the pancreas immediately after cold preservation and measured high-mobility group box protein 1 (HMGB1) level with 10 islet cultures in each group in order to assess islet damage after the isolation.^{25,26,23}

Human Pancreas Procurement

Research-grade human pancreases from brain-dead donors were provided through the local organ procurement organizations (Southwest Transplant Alliance, Dallas, TX, and LifeGift, Houston, TX). Pancreases were removed en bloc with a standard procedure after vascular perfusion with UWS or histidine-tryptophan-ketoglutarate (HTK) solution. Immediately after the pancreas procurement, PDP was performed, wherein an 18 or 20 G cannula was inserted into the main pancreatic duct from the pancreatic head after removing the spleen and duodenum.¹⁹ Approximately 1 mL/g of either ETKS or CSPS was then injected intraductally.²⁷ After completion of PDP, the pancreas was preserved with the two-layer method using the corresponding preservation solutions of ETKS or CSPS and perfluorocarbon.^{28,29} No PDP was performed for the control group, but the recovered pancreas was preserved with the two-layer method with CSPS and perfluorocarbon ($n = 6$).

TUNEL Assay with Pancreas Tissue Before Islet Isolation

The TUNEL assay was performed with an ApopTag fluorescein in situ apoptosis detection kit (Millipore, Billerica, MA, USA).³⁰ Pancreas tissue was taken from the pancreas body and prepared for paraffin sectioning. Digoxigenin-labeled nucleotides were added to DNA fragments by terminal deoxynucleotidyl transferase, and then fluorescein-labeled antidigoxigenin antibodies were bound to the digoxigenin. Apoptotic cells—those that appeared fluorescently green in the islet area stained by anti-insulin antibody (Sigma-Aldrich, St. Louis, MO, USA)—were manually counted. Similarly, manual counts were made of nuclei stained by the blue signal of 4', 6-diamidino-2-phenylindole, dihydrochloride (DAPI) (Sigma-Aldrich, St. Louis, MO, USA). The proportion of TUNEL-positive cells in an islet or acinar cell area was evaluated.

Pancreatic Islet Isolation

Islet processing was performed based on the modified Ricordi method in accordance with current good manufacturing practice at Baylor Research Institute's Islet Cell Laboratory (Dallas, TX) as previously described.^{31,32} Briefly, after decontaminating the pancreas surface with 1% povidone-iodine and 1 g of cephalosporin, the chilled collagenase enzyme solution was perfused into the pancreatic duct for 10 minutes. Then, the distended pancreas was cut into approximately 10 pieces. The pancreas pieces were put in the Ricordi chamber and digested by circulating the collagenase solution at 37°C. After dilution and recombination of digested tissue, the islets were purified with continuous density gradient

using iodixanol followed by adjustment of islet density.³² The proportion of undigested tissue weight was calculated by undigested tissue weight dividing by trimmed pancreas weight. The recovery rate in final preparation was calculated by the total islet yield (IEQ) after purification divided by that after digestion.

In Vitro Islet Evaluations

Viability was evaluated with fluorescein diacetate (10 μ M)/propidium iodide (15 μ M) staining.³³ The average viability in 50 islets was calculated. Islet yield was assessed using dithizone staining (Sigma Chemical Co., St. Louis, MO, USA) (2 mg/mL) and converted to a standard number of IEQ (diameter standardizing to 150 μ m).²⁴

The glucose-stimulated insulin release assay was performed as follows. After overnight culture, triplicates of 150 IEQ islets were incubated with low (1.67 mM) and high (16.7 mM) concentrations of glucose in functionality/viability medium CMRL1066 (Mediatech Inc, Manassas, VA, USA) for 1 h at 37°C. Insulin concentrations were measured with an enzyme-linked immunosorbent assay (ELISA) kit (ALPCO Diagnostics, Salem, NH, USA) and a spectrophotometer (BioTek Instruments, Inc, Winooski, VT, USA). The stimulation index was calculated by dividing the insulin concentration in the high-glucose solution by that in the low-glucose solution based on three independent measurements.

Islet Culture with Cytokines and HMGB1 Measurement

Isolated human islets were cultured in CMRL1066 (Mediatech, Inc, Manassas, VA, USA) at 37°C in 95% air and 5% CO₂ for 24 hours after islet isolation. Islets were washed twice with culture medium after initial culture and then cultured again under the same conditions without adding cytokines for 48 hours (nonstimulation group). In the stimulation group, islets were cultured with a cytokine cocktail consisting of 20 ng/mL of recombinant human interferon- γ , tumor necrosis factor- α , and interleukin-1 β (Sigma-Aldrich Co., St. Louis, MO, USA) for 48 hours after medium exchange.^{25, 26} The amount of HMGB1 in the medium after culturing for 48 hours was measured with the HMGB1 ELISA kit (IBL International GmbH, Hamburg, Germany). The amounts of HMGB1 in media were normalized to the total DNA of cultured islets (dsDNA Assay Kit, Molecular Probes, Inc, Eugene, OR, USA).

In Vivo Assessment

Nude male mice (Harlan, Houston, TX, USA) were used as the recipients. A single dose (180 mg/dL) of streptozotocin (Sigma-Aldrich Co., St. Louis, MO, USA) was administered intravenously on day -2, and hyperglycemia >300 mg/dL was confirmed twice in each mouse before transplantation. An islet mass of 2,500 IEQ as curable dose was injected into the kidney capsule.³⁴ Nonfasting blood glucose levels were measured using Accu-Chek Aviva (Roche Diagnostics, Indianapolis, IN, USA) three times a week in all the recipients for 30 days after islet transplantation. Normoglycemia was defined as two consecutive blood glucose levels reading <200 mg/dL.^{25, 26} This study was approved by the Institutional Animal Care and Use Committee at Baylor Research Institute (Dallas, TX).

Statistical Assessments

Statistical evaluations were performed with GraphPad Prism version 6.03 (GraphPad Software, Inc., San Diego, CA, USA). Numerical and categorical values between three groups were evaluated with the Kruskal-Wallis test followed by post-hoc Dunn's method for independent samples and the Pearson Chi-square test, respectively. Numerical and categorical data were expressed as median (range) and number (percentage), respectively. Survival curves of curative rate were evaluated with the Mantel-Cox log-rank test. Results were considered statistically significant when a two-sided *P* value was <0.05 .

RESULTS

Donor Characteristics

Eighteen human islet isolations, six in each study group, were performed for this study between October 2007 and May 2013. There were no significant differences in donor characteristics among the three groups (Table 1), and none of the donors had any warm ischemia time.

Primary Outcome: Total Islet Yield

The total islet yield postpurification was $304 [168\text{--}554] \times 10^3$ IEQ in the control group, $674 [481\text{--}975] \times 10^3$ IEQ in the ETKS group, and $742 [624\text{--}1,057] \times 10^3$ IEQ in the CSPS group (Fig. 1). Statistically significant differences were found between the control and both the ETKS and CSPS groups ($P = 0.04$ and <0.01), but not between the ETKS and CSPS groups ($P = 1.0$). All islet isolations in the PDP groups had $>400,000$ IEQ.

Secondary Outcomes for Islet Isolation

Significant differences between the control and PDP groups were found for the secondary outcomes for the isolated islet mass: total islet yield postdigestion, islet yield per trimmed pancreas weight postdigestion, and islet yield per trimmed pancreas weight postpurification (Fig. 2). No significant differences, however, were detected between the ETKS and CSPS groups.

The trimmed pancreas weight was not significantly different between the three groups (Table 2), but significant differences were seen in digestion time, undigested tissue weight, and the proportion of undigested tissue ($P = 0.03$, 0.01 , and 0.006 , respectively).

Islet Quality Assessment

No significant differences were observed in the islet purity assessment and stimulation index between the three groups, but there was a marginally significant difference in viability between the control and CSPS groups (Table 2). As shown in Figure 3, there was no significant difference in the groups' curative rate based on an *in vivo* nude mice assay using human islet preparations (P with the Mantel-Cox log-rank test = 0.57). Furthermore, HMGB1 levels on the static and cytokine-stimulated conditions and the fold changes did not show any statistically significant differences between the three groups (Fig. 4). The proportion of TUNEL-positive cells in an islet was $<10\%$ in both PDP groups, but significant differences were seen between the control and the ETKS and CSPS groups ($P <$

0.05 and <0.01, respectively) (Fig. 5). Similar results were observed in acinar cell area; the proportions of TUNEL-positive cells in PDP groups were significantly lower than those in control ($P < 0.05$, Fig 5B).

DISCUSSION

Preservation of the pancreatic duct is essential in human islet cell transplantation since wide distribution of collagenase solution in the pancreatic duct is a critical procedure in pancreas digestion.³⁵ We examined two different solutions of ETKS and CSPS with higher sodium and lower potassium levels, compared to UWS, for PDP and found no significant differences in islet yield or islet quality between the two groups, but a significant increase in islet yield compared to the control with no intra-pancreatic duct preservation solution delivery. The final islet preparations in all isolations in the PDP groups had $>4.0 \times 10^5$ IEQ, which is a higher yield than the isolation success defined in previous reports.^{36,37} Thus, both ETKS and CSPS can be used for PDP in pancreas preservation and are likely to result in isolation success. Islet investigators can implement PDP using either ETKS or CSPS, based on what is more accessible to them; CSPS has been used for islet purification in a multicenter trial on allogeneic islet transplantation in the USA and Europe, and ETKS has been applied in Japan.^{18,38,39} In the USA, ETKS is not commercially available, whereas CSPS is extensively used in major islet isolation centers. Hence, based on results from this study, CSPS can be a solution of choice for PDP.

No statistically significant differences between the ETKS and CSPS groups were found in the variables related to islet isolation and ancillary studies as well. The stimulated HMGB1 levels, the fold change and proportion of TUNEL positive cells were slightly lower in CSPS than ETKS group, supporting feasibility of PDP with CSPS. The differences between the two solutions include higher level of potassium, containing gluconate and trehalose and higher osmolality in ETKS *versus* containing lactobionate, raffinose and histidine in CSPS (Supplemental Digital Content Table S1). Since the two solutions have been developed and investigated with different background, –ETKS was originally designed for lung preservation and CSPS was for islet preservation after pancreas digestion–, it is very difficult to mention the effect of individual component in PDP and islet isolation outcomes.

The effect of PDP in the outcome of pancreatic islet isolation is still controversial. Nakanishi et al showed that PDP was effective in a rodent model of islet isolation but observed no additive effect when vascular perfusion was simultaneously performed.⁴⁰ In present study, we obtained islet yields with PDP similar to those of our previous report, showing significantly higher islet mass of approximately 6.0×10^5 IEQ when compared with control.¹⁹ We believe that additional benefits of PDP are possible in human islet isolation since vascular perfusion with UWS or HTK solution was performed in both control and PDP groups and we consistently obtained higher islet yield with PDP in this study. Previously, it was shown that PDP significantly improved the viability of pancreatic duct cells as well as acinar and islet cells, using morphological evaluation with Trypan blue and TUNEL assay, along with increased islet yield.^{7,18} PDP was able to inhibit trypsin activity during pancreas digestion.¹⁸ In addition, we reported improved distribution of collagenase enzyme in human pancreas preserved with PDP, where the collagenase was successfully detected into islet

surface area without islet damage.⁴¹ Therefore, PDP can contribute to maintaining higher viability of duct cells as well as acinar and islet cells, but also keeping pancreas micro-structure intact, resulting in significantly higher islet yield. Clinically applicable test(s) such as biomarkers in pancreatic duct fluid or biopsy using very tiny tissue before islet isolation would provide more reliable information on the effect of PDP and this is an important area for future study. Of note, pancreas preservation in all three groups was performed with two layer method in the present study, thus, all groups should have benefits of two layer method in islet isolation outcomes as well as prevention of cell swelling and apoptosis during pancreas preservation.^{42, 43}

Several limitations should be noted in this study. The minimum number of pancreas donors was calculated by power analysis using our previous observation.¹⁹ A larger number of donors ($n = 52$ per group for statistical $\beta = 0.8$, $\alpha = 0.05$ and two-tailed test), however, would be required if the anticipated difference in total islet yield postpurification between the two PDP groups is $1.0 \pm 1.8 \times 10^5$ IEQ, as shown here. Thus, the power in this study was not sufficient to detect smaller differences, and further studies with larger cohorts are needed. Also, randomization with categorized donor information should be planned to obtain robust results. This study did not aim to investigate islet isolation using pancreases with a prolonged cold ischemia time. Specific clinical biomarker to indicate viability of pancreatic duct cells should be developed and validated in large study, which would provide link between PDP and islet isolation outcomes since it was demonstrated that HMGB1 was specifically expressed in pancreatic islets, not duct cells.³⁰

In conclusion, no significant differences in islet isolation outcomes were observed between the extracellular fluid-like PDP solutions, while we consistently achieved isolation success when PDP was implemented. Both ETKS and CSPS can be safely used for PDP. Further study with a larger number of pancreas donors and well-planned randomization should be undertaken to compare the effect of PDP solutions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Effect of PDP using ETKS or CSPA compared with a control group for the study's primary outcome: islet yield after purification. Dot plots with median (bold bars) and interquartile range (gray bars) are shown. Significant differences were observed between the control and ETKS and CSPA groups (* $P < 0.05$ and ** $P < 0.01$). The dotted line shows 400,000 IEQ; both ETKS and CSPA groups resulted in >400,000 IEQ of final islet yield. PDP indicates pancreatic ductal preservation; ETKS, ET-Kyoto solution; CSPA, cold storage/purification stock solution.

FIGURE 2.

Effect of PDP using ETKS or CSPS compared with a control group for the study's secondary outcomes: **(A)** total islet yield postdigestion, **(B)** islet yield per trimmed pancreas weight postdigestion, and **(C)** islet yield per trimmed pancreas weight postpurification. Dot plots with median (bold bars) and interquartile range (gray bars) are shown. PDP indicates pancreatic ductal preservation; ETKS, ET-Kyoto solution; CSPS, cold storage/purification stock solution. * $P < 0.05$ and ** $P < 0.01$.

FIGURE 3.

Effect of PDP in an *in vivo* nude mice assay. The curative rates for the control group (solid line, $n = 6$), ETKS PDP group (long dotted line, $n = 16$), and CSPS PDP group (short dotted line, $n = 17$) are shown. No significant difference in the curative rate was observed in the three groups ($P = 0.57$). PDP indicates pancreatic ductal preservation; ETKS, ET-Kyoto solution; CSPS, cold storage/purification stock solution.

FIGURE 4.

Effect of PDP in an HMGB1 assay assessing islet damage after isolation. No significant differences were seen in HMGB1 levels between the control group, ETKS PDP group, and CSPA PDP group in (A) a static culture (no stimulation) ($P = 0.31$); (B) a culture with cytokine stimulation ($P = 0.15$); or (C) fold change ($P = 0.32$). The bar, box, and bold line indicate range, interquartile range, and median, respectively. PDP indicates pancreatic ductal preservation; HMGB1, high-mobility group box protein 1; ETKS, ET-Kyoto solution; CSPA, cold storage/purification stock solution.

FIGURE 5.

Effect of PDP evaluated through a TUNEL assay to count the number of apoptotic cells. The proportion of TUNEL-positive cells in the islet area was significantly lower in the PDP groups than in the control group (A). Similarly, those in non-islet area were significantly lower in PDP groups than control group (B). Representative fluorescent stains of insulin (red), nuclei (blue), and TUNEL (green) are shown for (C) the ETKS PDP group, (D) the CSPS PDP group, and (E) the control group. PDP indicates pancreatic ductal preservation; ETKS, ET-Kyoto solution; CSPS, cold storage/purification stock solution. $*P < 0.05$, $**P < 0.01$.

TABLE 1

Donor characteristics

Variables	Control (<i>n</i> = 6)	Pancreatic ductal preservation		P value
		ETKS (<i>n</i> = 6)	CSPS (<i>n</i> = 6)	
Age (year)	51 [20–68]	42 [26–54]	50 [27–51]	0.62
Gender: female (<i>n</i> , %)	3 (50)	4 (67)	3 (50)	0.80
Body weight (kg)	93.8 [63.0–115.6]	81.1 [72.5–99.8]	97.9 [72.7–125.7]	0.52
Body mass index (kg/m ²)	30.5 [20.5–44.5]	29.3 [25.0–36.6]	32.8 [24.4–38.1]	0.83
Body surface area (m ²)	1.98 [1.77–2.37]	1.93 [1.76–2.10]	2.13 [1.86–2.45]	0.20
Cause of death (<i>n</i> , %)				0.70
Cerebrovascular stroke	3 (50)	3 (50)	4 (67)	
Head trauma	3 (50)	2 (33)	1 (17)	
Other	0 (0)	1 (17)	1 (17)	
Mechanism of death (<i>n</i> , %)				0.53
Intracranial hemorrhage or stroke	3 (50)	4 (67)	4 (67)	
Trauma without abdominal injury	3 (50)	2 (33)	1 (17)	
Cardiovascular	0 (0)	0 (0)	1 (17)	
Length of hospitalization (days)	4 [3–9]	3 [2–10]	3 [1–9]	0.44
Cold ischemia time (min)	282 [153–390]	196 [159–394]	167 [82–220]	0.17

Median [range] or numbers (percentage) are shown. Numerical and categorical values were evaluated with the Kruskal-Wallis test for independent samples and the Pearson Chi-square test, respectively. ETKS indicates ET-Kyoto solution; CSPS, cold storage/purification stock solution.

TABLE 2

Islet isolation results

Variables	Control (n = 6)	Pancreatic ductal preservation		P value
		ETKS (n = 6)	CSPS (n = 6)	
<i>Pancreas digestion</i>				
Trimmed pancreas weight (g)	111 [58–150]	111 [68–142]	100 [83–125]	0.75
Digestion time (min)	19 [14–24]	14 [12–16]	14 [9–16]	0.03 *
Dilution time (min)	50 [34–58]	50 [26–78]	49 [28–55]	0.83
Undigested tissue weight (g)	29 [9–44]	5 [1–11]	9 [2–28]	0.01 *
Proportion of undigested tissue in trimmed pancreas weight (%)	24 [9–39]	6 [1–8]	9 [2–27]	0.006 *
Tissue volume postdigestion (mL)	38 [20–50]	43 [35–60]	35 [24–45]	0.22
Total islet yield postdigestion ($\times 10^3$ IEQ)	451 [308–709]	841 [638–1284]	830 [661–1215]	0.01
Islet yield per pancreas weight ($\times 10^3$ IEQ/g)	4.19 [3.32–5.50]	8.97 [4.81–12.2]	8.45 [5.87–14.7]	0.006
<i>Final preparation</i>				
Islet yield per pancreas weight ($\times 10^3$ IEQ/g)	2.90 [2.40–4.29]	6.04 [5.04–9.24]	7.61 [5.09–10.5]	0.002
Tissue volume (mL)	15 [3–27]	10 [2–12]	10 [5–21]	0.80
Recovery rate (%)	73 [52–100]	74 [56–100]	91 [70–100]	0.44
Embedded islets (%)	20 [8–90]	20 [0–33]	20 [7–51]	0.97
Average purity (%)	58 [33–74]	69 [46–75]	64 [52–78]	0.50
Viability (%)	98 [95–100]	96 [94–98]	95 [93–96]	0.05 *
Stimulation index	2.1 [1.2–6.9]	8.0 [1.0–22.0]	5.4 [1.8–13.2]	0.12

Medians [ranges] are shown. The Kruskal-Wallis test was performed for statistical evaluation. ETKS indicates ET-Kyoto solution; CSPS, cold storage/purification stock solution.

* Results on the pairwise comparison are shown in Supplemental Digital Content Figure S2.