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RESEARCH ARTICLE

Trisected pancreas model for testing tissue dissociation enzyme combinations: a novel methodology for improving human islet yield for clinical islet transplantation

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Abstract

Purpose Human islet isolation requires a defined collagenase-protease enzyme combination for obtaining a successful islet yield. While different islet laboratories use different enzyme combinations, a systematic methodology to identify optimal enzyme combinations and their concentrations within a single donor pancreas has not been tested. In this study, we designed a trisected pancreas model to test efficacy of three clinical grade enzyme blends (VitaCyte, Roche, SERVA) within a single pancreas.

Methods Islet isolations were performed using brain-dead donor pancreases ($n = 15$) applying the enzyme-related design of experiments (DOEs) and the trisected model approach. After trimming, split each pancreas into three individual lobes (head, body, tail). As per the DOEs, the lobes were altered between different experiments, to minimize anatomical bias. Islets isolated from each lobe (27 lobes totally) were subjected to functional assessments. Insulin staining and islet area fraction were determined for tissue sections obtained from each lobe.

Results Utilizing the trisected model, we identified that the collagenase dose from three different vendors did not affect the pancreas digestion and islet yield, but islet morphology after isolation with the neutral protease and BP-protease was better than thermolysin. In addition, the head lobe yielded a lower islet mass and higher tissue volume compared to other two lobes, irrespective of enzyme combination used.

Conclusions This study demonstrates that the trisected model is a promising methodology in assessing donor and isolation associated parameters. Based on this study, we conclude that the donor characteristics and an optimal enzyme dose play a critical role in achieving higher islet yields.

Keywords Islet isolation · Islet transplantation · Trisected pancreas model · Collagenase · Thermolysin · Neutral protease · BP-protease

Abbreviations

TDE	Tissue dissociation enzyme
DOE	Design of Experiment
WU	Wunsch Units
GSIR	Glucose stimulated insulin release
NP	Neutral protease
MTF	Mammalian Tissue Free
CDA	Collagen degradation assay
Wunsch	Measurement of C2 activity

Introduction

Human islets are the proven source of cellular therapeutics for treating pancreatic anomalies such as type 1 diabetes and surgery-induced diabetes [1, 2]. However, successful transplantation requires the recovery of a sufficient number of functional islets from cadaveric or from chronic pancreatitis pancreata [3, 4]. According to the Collaborative Islet Transplant Registry (CITR), only 50% of the islet preparations are successfully transplanted [5]. Two critical factors that majorly influence the islet yield are the donor characteristics and

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the type of tissue-dissociation enzyme (TDE) blend utilized. Variability within donor pancreases is unavoidable however, isolation enzymes can be optimized to obtain a higher number of viable islets for successful clinical transplantation.

TDE blends are critical in determining the yield and quality of isolated pancreatic islets and affect clinical outcomes. The current well-known TDE blends manufactured for isolating islets for clinical transplantation are collagenase NB1/Neutral protease (NP) (SERVA, Heidelberg, Germany), mammalian tissue-free collagenase/thermolysin (Roche, Indianapolis, USA), and CIzyme collagenase HA/BP-Protease (VitaCyte, Indianapolis, USA) [6–8]. All these purified TDE blends are supplied in individual vials of collagenase and protease to minimize the lot-to-lot variation. However, utilizing these currently available clinical TDE blends, only 50% of the isolations have generated a sufficient number of islets for a single donor transplant [5]. It is still challenging for isolation laboratories to identify an ideal TDE blend for obtaining successful islet yields for transplantation. While several studies have compared the efficacy of TDE blends, the results regarding differences in islet yield and viability are still conflicting between centers [6, 9, 10]. Moreover, all digestion enzymes have never been directly compared with respect to their islet isolation outcomes from different lobes originating from the same donor pancreas [11].

Keeping these factor into consideration, in this study we have developed a miniature islet isolation protocol using a trisected pancreatic model, while retaining the entire clinical islet isolation standard protocol. Using this approach, we tested the collagenase and protease enzyme blends obtained from three different vendors. Interestingly, we identified that different collagenases did not affect pancreas digestion but NP and BP proteases were better than thermolysin. Overall, we successfully developed miniature isolation method using trisected pancreas lobe.

Materials and methods

Donor pancreas

Human brain-dead donor pancreases ($n = 15$) were obtained through Kentucky organ donor affiliates (KODA) after informed consent was obtained as part of multiorgan procurement. The pancreas was rejected for transplantation; the donor consent stating that the pancreas was donated for research. The procured pancreases were shipped in cold University of Wisconsin solution (UW) or histidine tryptophane ketoglutarate (HTK) solution from the donor center to the islet isolation laboratory.

Islet isolation enzymes

The current clinical islet isolation TDE blends from three different enzyme suppliers (SERVA, Roche, VitaCyte) were evaluated in utilizing 9 human donor pancreases, which is 27 individual human pancreatic lobes to identify the optimal TDE blends for successful isolations. Enzyme characteristics for three different TDEs are summarized in Table 1.

Miniature islet isolation method using trisected pancreas (small lobe)

We developed a miniature islet isolation method using trisected pancreas ($n = 3$). On arrival, the pancreas was trimmed, and split equally using sterile stainless-steel measuring scale into 3 individual lobes (head, body, and tail) and then individually cannulated using the appropriate angiocatheter. The head and tail lobe were singly cannulated, while the body lobe was cannulated on both sides. During the development of miniature islet isolation techniques, in the pilot study we used "VitaCyte HA Collagenase and VitaCyte BP-Protease" as we reported earlier [Transplant Direct. 2015 Dec 23;2(1):e54].

The clinical enzyme combinations tested were altered between the lobes for every individual experiment to minimize the anatomical bias. Hence, the overall pooled data for one enzyme combination was obtained from the head, body and tail lobes from three independent pancreases. Thus the statistical significance evaluated for each enzyme combination was based on the data obtained from three different lobes, and not just a single pancreas.

Appropriate enzyme combinations from three different enzyme vendors [SERVA (collagenase 20WU/g and NP 2.0 DMC U/g), Roche (collagenase 20WU/g and Thermolysin 1000 U/g), and VitaCyte (collagenase 20WU/g and BP 23400 FITC-BSA U/g)] were evaluated for each lobe based on the DOE. In addition, the effect of three different proteases were also evaluated. Human islet isolations were performed using the standard procedure as previously described [12]. Each lobe was picked for ductal perfusion of the enzyme based on the DOE. For each lobe, the enzyme distention volume was 3.5 ml/g of pancreatic tissue. After performing enzyme distention for 12 min, each lobe was chopped into smaller pieces and digested using a modified Ricordi's semi-automated method using a 250 ml mini Ricordi chamber. The switch from digestion phase to dilution phase occurred when most of the islets were free from exocrine tissue. Digested tissue was then purified by continuous iodixanol (OptiPrep™, Axis-Shield, Oslo, Norway). The islet yield and purity were assessed by counting duplicate aliquots stained with diphenylthiocarbazone (# D5130, Sigma, St. Louis, MO). The purified islets were cultured in CMRL-1066 supplemented medium (#99–603-CV, Corning/Mediatech Inc., Manassas, VA) containing 0.5% human

Table 1 Enzyme characteristics of the currently available clinical grade tissue dissociation enzyme blends

Enzymes		Source	Specific Activity (U/mg)			
			Wunsch	CDA	C1/C2 Ratio	NPA
VitaCyte	VitaCyte CIzyme™Collagenase HA,VitaCyte, Indianapolis, IN	*Clostridium histolyticum*	4.32 ± 0.02	57,431 ± 2167	60/40	< assay limit
	BP-Protease [CIzyme™ BP Protease] VitaCyte, Indianapolis, IN	*Paenibacillus polymyxa*	NA	< assay limit	NA	119,124 ± 702 (FITC-BSA)
SERVA	SERVA Collagenase NB-1, SERVA Electrophoresis GmbH, Heidelberg, Germany	*Clostridium histolyticum*	5.2 ± 1.3	NA	NA	0.05
	Neutral Protease NP, SERVA Electrophoresis GmbH, Heidelberg, Germany	*Clostridium histolyticum*	NA	NA	NA	1.5 ± 0.6 (DMC)
Roche	Roche MTF Collagenase, Roche Applied Science, Indianapolis, IN	*Clostridium histolyticum*	6.0 ± 1.7	NA	60/40	No data
	Roche Thermolysin, Roche Applied Science, Indianapolis, IN	*Bacillus*	NA	NA	NA	NA

Wunsch-measurement of C2 collagenase activity; *CDA* Collagen degradation assay used to measure C1 collagenase activity, *NPA* Neutral protease activity

serum albumin (#1332253, Baxalta, McKesson plasma & biologics, TN) at 37 °C with 5% CO2 before being subjected to quality control assessment. This islet isolation procedure was independently repeated for the other two lobes with their appropriate enzyme combinations based on the DOE.

Standard human islet isolation method (whole pancreas)

Standard human islet isolations ($n = 3$) were performed as previously reported [13]. On arrival, the pancreas was trimmed, cannulated and distended with digestive enzymes. The Vitacyte HA collagenase (20 WU/g) were mixed with VitaCyte BP Protease (23400 FITC-BSA U/g pancreas) at the time of islet isolation ($n = 3$). Enzymes were diluted in 350 mL of Hank's Balanced Salt Solution (HBSS) for each pancreas. After ductal perfusion of the enzymes, the pancreas was digested in a 600 ml Ricordi chamber using a modification of Ricordi's semi-automated method [14]. The digested tissue was then purified by continuous iodixanol (#D1556, Sigma-Aldrich, Sigma) density gradient with a COBE-2991 cell processor (Terumo BCT, Lakewood, CO). The islet yield and purity were assessed by counting duplicate aliquots stained with diphenylthiocarbazone. The purified islets were cultured overnight in CMRL-1066 supplemented medium (# 99–603-CV, Corning/Mediatech Inc., Manassas, VA) containing 0.5% human serum albumin (#1332253, Baxalta, McKesson plasma & biologics, TN) at 37 °C with 5% CO2 before being subjected to quality control assessment.

Islet quality assessment

Isolated islets from three different enzyme products (SERVA, Roche, and VitaCyte) were assessed for viability using the fluorescein diacetate/propidium iodide assay [15]. A glucose-stimulated insulin release (GSIR) test was used to assess the functional islet quality, and subsequently, the stimulation index was calculated. Briefly, after overnight culture, islet insulin secretion was assessed by in vitro static glucose challenge using low (2.8 mM/L) and high concentrations (28 mM/L) of glucose. Krebs Ringer's bicarbonate was used as base media. Under static conditions, hand-picked human islets (150–200-μm size islets) were placed in trans-well plates and pre-incubated in Krebs medium containing 2.8 mM/L glucose for 30 min at 37 °C. After 30 min of pre-incubation, the islets were switched into KREBS medium containing 2.8 mM/L glucose for 1 h, followed by a high glucose stimulus consisting of 28 mM/L for 1 h. Insulin concentration in supernatant was assessed using the Mercodia (#10–113-10, Mercodia) insulin ELISA kit. The stimulation index was calculated as a ratio of the insulin value obtained after high glucose stimulation over the insulin value obtained after low glucose stimulation [16].

Insulin staining and islet area fraction determination We assessed islet area fraction to determine the percentage of islets in different lobes. Human brain-dead donor pancreatic tissue samples from the head, body, and tail lobes ($n = 9$) were fixed in 10% formalin, paraffin embedded, and each sample was sectioned at 7 μm. Two samples from each lobe were incubated overnight with rabbit anti-insulin polyclonal antibodies at a 1:1000 dilution (#20056, Immunostar, Hudson, WI), after deparaffinization, rehydration in serial ethanol washes, heat retrieval in citrate, and blocking with 5% normal goat serum. Islets were visualized after staining with goat anti-rabbit at 1:500 dilution. After staining, the digital bright field images were acquired on a Nikon E800 photomicroscope equipped with a Nikon DXM1200 digital camera using a 1×

Table 2 Comparing islet isolation characteristics between the miniature (250 ml Ricordi Chamber) and standard islet (600 ml Ricordi Chamber) isolation utilzing "Vitacyte collageans"

Islet Isolation Characteristics	Miniature Islet Isolation ($n = 3$)	Standard Islet Isolation ($n = 3$)	
Pancreas	Trisected lobe (small lobe)	Whole Pancreas	*P* value
Trimmed Pancreas Weight (gram)	34 ± 11	91.3 ± 8.0	0.0019
Collagenase (Wunsch U/g)	20	20	NA
BP Protease (FITC-BSA U/g)	23,400	23,400	NA
Enzyme Distention Volume (ml) per gram pancreas	3.5	3.5	NA
Ricordi Chamber Size (ml)	250	600	NA
Digestion time (min)	21 ± 3	22.3 ± 4.2	0.685
Islet Morphology Score*	7.5 ± 0.6	8.0 ± 0.5	0.329
Undigested Pancreas Weight (%)	11.7 ± 6	16.4 ± 5.1	0.359
Digest Pellet Volume / gram pancreas	0.41 ± 0.15	0.49 ± 0.26	0.672
Digest Count IEQ/g pancreas	3430 ± 1572	3504 ± 2084	0.963
Post Purification Islet Recovery (%)	83.2 ± 5.3	81 ± 6.5	0.673
Viability (by FDA/PI)	90 ± 2.2	89 ± 4.2	0.733

* Islet Morphology score (1–10; 1 lowest and 10 is highest)

objective lens for IAF application. Captured images were then imported to image-pro plus for morphometric evaluation. The total tissue area was determined by tracing the perimeter of the tissue and excluding any voids within the tissue. A threshold was determined for cells staining positive for insulin. This threshold was used to count the number of positive pixels within the outlined region. The total number of islets and the surface area occupied by each were measured. In comparison to the total tissue area, the islet area fraction (IAF) was determined [17]

Statistical data analyses

The different enzyme combinations were evaluated in this study according to the DOE. For enzyme combinations in the DOE, variables were tested for statistical significance by least-squares analysis of variance for a 3 × 3 factorial design using JMP 8 software [18] (SAS Institute Inc., Cary, NC). Each enzyme combination was tested in triplicates for each lobe of the pancreas. All results were expressed in arithmetic mean ± standard deviation. Other than assessing effects of various enzyme vendor products on same pancreas, the overall regional heterogeneity of islets between head, body and tail, results were compared. Statistical comparison was performed using the non-parametric Kruskal-Wallis test followed by the post-hoc Dunn method for multiple comparisons and the Friedman test followed by the Dunn correction, respectively. Statistical significance for the analysis was set at $P < 0.05$.

Results

Effectiveness of miniature islet isolation method using trisected pancreas comparable to standard islet isolation

In our preliminary studies, we first compared the miniature islet isolation characteristics (digestion time, islet score, digest count (IEQ/g), undigested tissue percentage, pellet size per

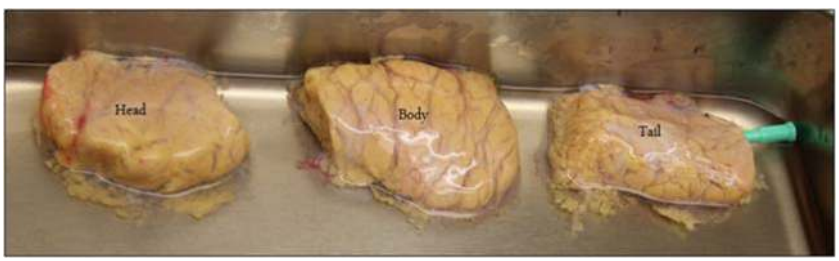

Fig. 1 The human trisected pancreatic lobe model: A figure demonstrating the three lobes (head, body and tail) from a single donor pancreas (trisected model) for evaluating the clinical grade TDE blends from three different enzyme vendors (Vitacyte/Roche/SERVA)

Table 3 Trisected pancreatic islet isolation outcome from three different enzyme providers Vitacyte/Roche/SERVA

Enzyme Combination	Cizyme + BP Protease	MTF + Thermolysin	NB1 + NP	
Pancreas Lobe	(Head, Body, Tail) Mean	(Head, Body, Tail) Mean	(Head, Body, Tail) Mean	*P* Value
Donor Age (year)	40 ± 19	40 ± 19	40 ± 19	0.99
Height (cm)	170 ± 11	170 ± 11	170 ± 11	0.99
Weight (Kg)	77 ± 20	77 ± 20	77 ± 20	0.99
Gender (M/F)	33%M; 66%F	33%M; 66%F	33%M; 66%F	NA
Body Mass Index (kg/m2)	26 ± 6	26 ± 6	26 ± 6	0.99
Trimmed Pancreas Weight (gram)	28 ± 15	28 ± 10	28 ± 14	0.96
Collagenase (Wunsch U/gram)	20	20	20	NA
Neutral Protease/Thermolysin/BP Protease	23400	1000	2	NA
Digestion time (min)	18 ± 3	15 ± 4	18 ± 3	0.57
Islet Morphology Score	8.0 ± 0.5	7.0 ± 0.5	8.5 ± 0.5	0.22
Phase 2 Time (min)	29 ± 1	27 ± 3	30 ± 4	0.38
Undigested Pancreas Weight (gram)	4 ± 1	2 ± 1	7 ± 7	0.32
Digest Pellet Volume (mL)	9 ± 6	13 ± 3	11 ± 3	0.37
Digest Count IEQ	82471 ± 31887	81559 ± 28544	80569 ± 28222	0.96
Digest Count IEQ/g pancreas	3109 ± 1559	3089 ± 1661	3427 ± 1892	0.84
Post Purification Islet Recovery (%)	82 ± 6	85 ± 2	79 ± 5	0.32
Viability (by FDA/PI)	91 ± 2	93 ± 2	90 ± 2	0.32
GSIR (Glucose Stimulated Insulin Release)	2.0 ± 0.8	2.5 ± 1.0	2.3 ± 0.5	0.91

gram tissue, post purification islet recovery percentage and islet viability) with standard islet isolation procedure. The results from these experiments are summarized in Table 2. When the islet isolation characteristics were compared between the two procedures, there was no statistical significance between islet isolation characteristics, except for the pancreatic lobe size as shown in Table 2.

Determining the effects of three different enzymes (VitaCyte/Roche/ SERVA)

After standardizing the miniature islet isolation method, it was applied on a DOE to test the current clinical grade enzyme products from three different enzyme vendors (VitaCyte, Roche, and SERVA), utilizing trisected pancreatic lobe model (Fig. 1). The standard doses of collagenase and protease from each vendor was utilized and the data is summarized in Table 3. Each enzyme combination was tested on the head, body and tail lobes from three different pancreases. The arithmetic mean of these three values obtained after quantifying isolation outcomes are listed within the 3 columns (Vitacyte/Roche/SERVA) of Table 3.

We observed that the digestion profile across the enzyme groups were similar. Since the donor characteristics were the same for all groups, many isolation characteristics (for example, pancreas weight, BMI, digest count) were not statistically significant (Table 3). The digest IEQ/g pancreas obtained from SERVA (3427 ± 1892), VitaCyte (3109 ± 1559), and Roche (3089 ± 1661) were not statistically significant either. However, utilizing Roche enzymes resulted in greater number of medium to small size islets when compared to SERVA or VitaCyte enzyme groups. The overall data indicate that utilizing the enzymes from three vendors resulted in similar isolation outcomes but there was a difference in islet size for thermolysin combination when compared to the collagenase groups with neutral protease and BP-protease.

Quality of islets after isolation were measured by FDA/PI viability assessment and glucose-stimulated insulin release (GSIR). The viability and stimulation index calculated from GSIR were 90 ± 2.0% and 2.3 ± 0.5 in the NB1 + NP group, 93 ± 2% and 2.5 ± 1.0 in the MTF + Thermolysin group; and 91 ± 2% and 2 ± 0.8 in the Cizyme+BP Protease group, respectively (Table 3).

Overall, the results so far indicate that there was no significant difference in islet yield and function across the enzyme

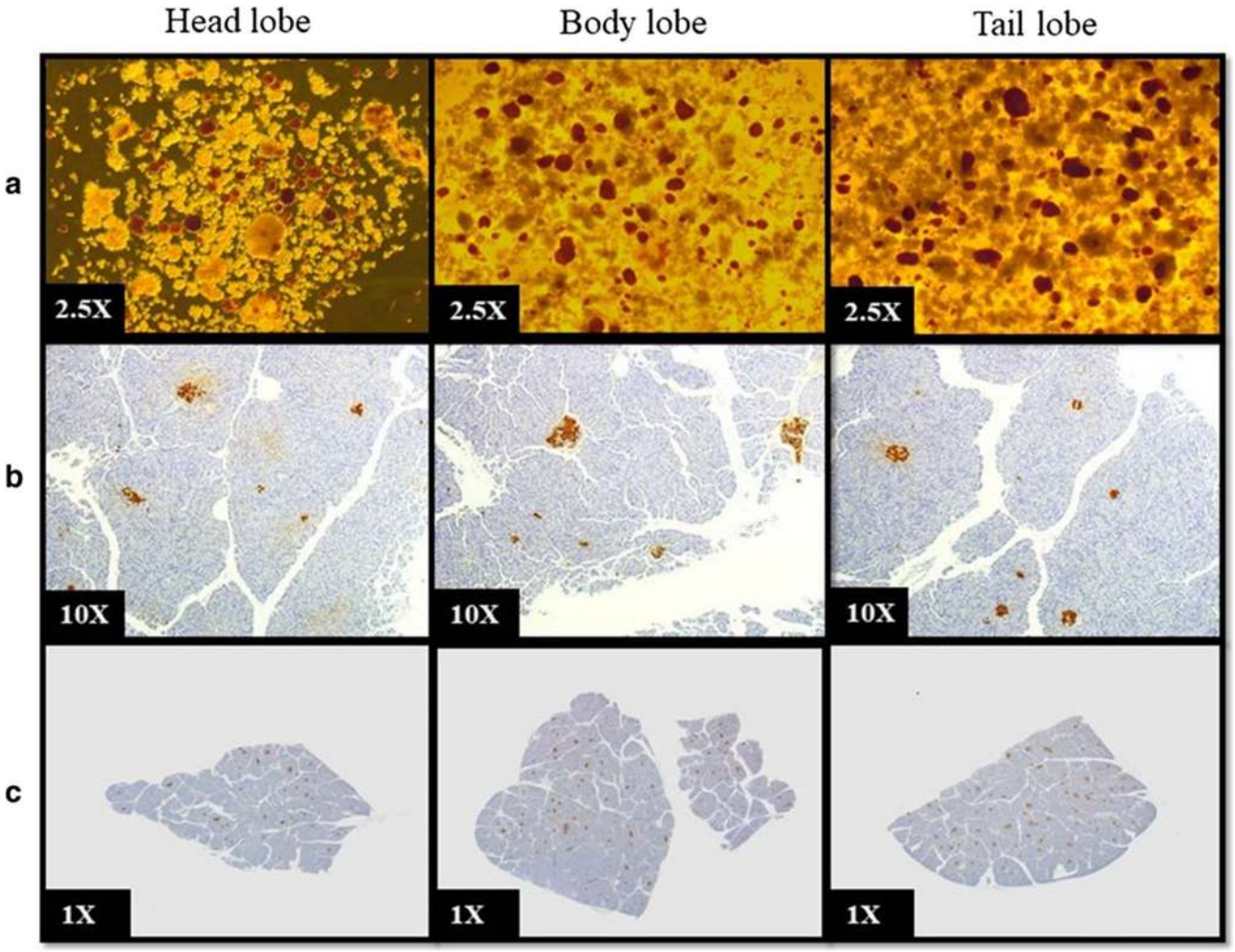

Fig. 2 Islet dithizone and Insulin immunohistochemistry staining images: **a** Pre-purified islet preparations from head, body, and tail lobes were stained with dithizone (red color). Images were taken at 2.5× magnification using inverted microscope. **b** Multiple sections were taken from head, body and tail lobe of the human pancreas and insulin stainings were pefromed with immunohistochemistry method. A representative image from each lobe was taken at 10× magnification. **c** A similar immunohistochemistry images of insulin as (**b**) for head, body, and tail were taken at a lower magnification (1×) for islet area fraction application

Table 4 Regional heterogeneity of islet number in the human pancreas (Head, Body, Tail)

Islet isolation characteristics	Head	Body	Tail	*P* value
Trimmed Pancreas Weight (g)	29 ± 13	27.3 ± 11	28 ± 15	0.89
Digestion time (min)	18.6 ± 2.5	15.6 ± 3.2	16.3 ± 3.1	<0.001
Phase 2 Time (min)	29 ± 2.5	27.6 ± 2.0	28 ± 3.2	0.13
Undigested Pancreas Wt. (g)	5.3 ± 2.3	3.3 ± 1.5	2.1 ± 0.5	<0.0001
Digest Pellet Volume (mL)	15.3 ± 1.1	11.4 ± 2.2	12 ± 2.4	<0.0001
Digest Count IEQ	54,311 ± 23,215	79,045 ± 31,115	97,795 ± 42,317	<0.0001
Digest Count IEQ/g pancreas	1774 ± 857	3096 ± 1357	3914 ± 1992	<0.0001
Post Purification Islet Recovery (%)	80 ± 4.5	83.2 ± 2.8	81.2 ± 5.0	0.16
Viability (by FDA/PI)	91.1 ± 3	91.5 ± 1.5	90.5 ± 2.1	0.27
GSIR (Glucose Stimulated Insulin Release)	2.2 ± 0.6	2.3 ± 1.0	2.1 ± 0.7	0.64

groups, and that thermolysin may have an effect on islet size compared to the other two proteases. Particularly, more number of solid intact islets (>200 microns) were present in the two proteases than the thermolysin group (data not shown).

Heterogeneity of islet number in the human pancreas (head, body, tail)

We also examined the regional heterogeneity of islets in the human pancreas by comparing head, body, and tail lobes. The mean islet yield obtained from the tail lobe was 3914 ± 1992 IE/g and it was statistically significant ($P < 0.0001$) when compared to the body (3096 ± 1357 IE/g) and head lobes (1774 ± 857 IE/g) of the pancreas. The mean digested tissue pellet volume and undigested tissue obtained from the head lobe was 15.3 ± 1.1 and 5.3 ± 2.3 significantly higher (P = <0.0001) compared to body (11.4 ± 2.2 and 3.3 ± 1.3) and tail (12 ± 2.4 and 3.3 ± 1.3) lobes. The regional distribution of islets were further confirmed in the head, body, tail lobes of the human pancreatic tissue section by insulin immunohistochemistry followed by islet area fraction (IAF) determination as shown in Fig. 2c.

Based on the quantitative analysis on averages the tail followed by the body lobe of the pancreas contained a significantly higher number of islets which contributes to the higher percent of the total IAF when compared to the head lobe (Tail: 1.94 ± 1.1%; Body: 1.53 ± 0.74%; Head: 0.87 ± 0.47%, P = <0.0001). The higher density of islet distribution in the tail and body region was directly reflected to the islet yield as shown in Table 3. However, there was no regional difference of isolated islets in insulin secretory release to glucose in vitro (Table 4. SI: Head −2.2 ± 0.6; Body-2.3 ± 1.0; and Tail-2.1 ± 0.7 respectively).

Discussion

This study demonstrates the development and evaluation of a trisected pancreas model in assessing commercial TDE blends from three vendors (VitaCyte, Roche, and SERVA). One of the key benefits of incorporating such a study model is its ability to estimate the effectiveness of multiple combinations and doses within a single donor pancreas to overcome anatomical bias. This is important because cost-effective and robust manufacturing of islet preparation is critical for successful islet transplantation.

In the absence of Liberase-HI [19], significant progress has been made by islet isolation centers by standardizing different TDE blends from different enzyme suppliers [20]. However, isolating a sufficient number of islets for successful transplantation is achievable only 50% of the times [5]. Isolation centers still focus on identifying optimal TDE blends for obtaining a sufficient number of islets for successful transplantations. Studies have compared two or more TDE blends by utilizing various donor pancreases. [6, 9, 10]. However, taking into account that these studies analyzed different enzymes, and different isolation protocols or different donor pancreases, make it is difficult to conclude which TDE enzyme blend best suits the islet isolation procedure. Thus many laboratories select the specific TDE blend based on their empirical experience. Comparisons of different TDE blends has been performed using multiple donor pancreases and retrospectively (as shown in Table 5) [6–9, 21–23]. To overcome this challenge, we initially developed a tube method of islet isolation for testing various enzyme combinations in a single donor pancreas to overcome the donor-to-donor variability. However, to have a comparable model which mimics the current standard islet isolation protocol we introduced a trisected

Table 5 Summary of islet isolation outcome using different tissue dissociation enzyme blends from selected reports

S. No	Published Studies	Isolation Variables	Isolation Enzymes		
1	Szot G et al. [7]	Post Purification IEQ/g	Roche Liberase-HI	Serva-NB1	*P* value
			4888 ± 232 (n = 9)	5862 ± 1843 (*n* = 14)	N.S.
2	Caballero-Corbalán J et al., [21]	Post Purification IEQ/g	VitaCyte-HA	Serva-NB1	
			2110 ± 242 (*n* = 18)	2047 ± 212 (n = 18)	N.S.
3	Brandhorst H et al. [22]	Post Purification IEQ/g	Roche Liberase-HI	Serva-NB1	
			4010 ± 232 (*n* = 101)	2979 ± 149 (*n* = 96)	N.S.
4	Balamurugan A.N. et al. [8]	Post Purification IEQ/g	VitaCyte-HA	Serva-NB1	
			4147 ± 1759 (n = 14)	2134 ± 1524 (*n* = 27)	0.002
5	O'Gorman et al. [9]	Post Purification IEQ/g	Serva-NB1	Roche MTF	
			3836 ± 390 (*n* = 24)	4249 ± 424 (*n* = 17)	N.S.
6	R. Misawa et al. [23]	Post Purification IEQ/g	Roche Liberase-HI	Serva-NB1	
			5931.5 ± 398.4 (*n* = 50)	5672.8 ± 516.0 (*n* = 40)	N.S.
7	Itzia Iglesias et al. [6]	Post Purification IEQ/g	Roche Liberase-HI	Serva-NB1	
			2850 ± 259 (*n* = 46)	2646 ± 252 (*n* = 20)	N.S.

pancreatic model. Utilizing this novel approach, we tested the collagenase and protease enzyme blends obtained from three different vendors. The human pancreas was split into 3 lobes - head, body, and tail and then individually cannulated using the appropriate angiocatheter. These lobes were used to assess different enzyme doses, combinations, from three different vendors. The 3 different lobes were altered on every individual experiment to minimize the difference caused by cold ischemic time, and one lobe served as a control to minimize anatomical bias.

Using this model, in this study, we observed that there was no significant difference in the islet isolation characteristics across the three different enzyme groups. While protease being an important component of the TDE enzyme blend may have harmful effects during islet isolation. Thermolysin has a more severe effect over the other two proteases, resulting with more number of smaller micron sized islets (data not shown). In support of this observation, our previous study utilizing a bisected pancreatic lobe model, showed similar results where thermolysin had a negative impact on islet integrity over enzymes blends incorporated with neutral proteases [13]. In this study there was no significant difference in the islet yields when compared between the three enzyme groups. Interestingly, the body and tail portion of the pancreas resulted with a significantly higher number of islets when compared to the head portion, irrespective of the TDE blend used during isolation. We further confirmed these observations by staining the individual pancreatic tissue sections obtained from the head, body, and tail portion of the pancreas with insulin immunostaining as shown in Fig. 2. The trisected model offers a more thorough and controlled experimental methodology by assessing all three different TDE blends within a single pancreas. The limitation of the trisected model is that, although it is reproducible and comparable with the current islet isolation protocol, it is technically challenging due to the time and resources consumed for isolation. A recent study demonstrated a slide-based digestion method to assess digestion of the human pancreas extracellular matrix (ECM) by TDE blends used during clinical islet isolation [24]. Despite being a simple method, it does not simulate the clinical islet isolation standard protocol.

The current human islet isolation technique requires a synergistic combination of collagenase and a protease, our data presents consolidated information on the different proteases on islet integrity. Our study also analyzed whether collagenase alone has any effect on the islet yield. In addition, we also observed that utilizing collagenase alone may not be detrimental to islet integrity, though we also observed that this enzyme alone may not be enough to obtain successful human islet isolation generating free islets (data not shown). While collagenase is necessary to degrade the different fibrillar and non-fibrillar collagen structures, presence of a protease in an enzyme blend is important to digest other ECM proteins to liberate islets. Future studies utilizing this novel trisected methodology is likely to help identify and fine-tune optimal enzyme blends (collagenase and protease) that results in maximizing islet yields for clinical transplantations.

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Compliance with ethical standards

Conflict of interest G.L., and A.N.B. participated in all experiments and G.L., S.V, and A.N.B, participated in article preparation. G.L., S.V., and A.N.B. participated in study design and statistical analyses of data. G.L., A.N.B. participated in data collection. S.V., and A.N.B. provided expert opinion in data analyses. All authors listed in this manuscript have no conflicts of interest to disclose.

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