



Islet Isolation From Human Pancreas With Extended Cold Ischemia Time

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ABSTRACT

The general consensus among transplant centers is that a cold ischemia time (CIT) beyond 8 hours results in reduced yields and quality of human islets. We sought to optimize the isolation process and enzymes for pancreata with extended CIT. We processed 16 extended CIT pancreata (13.2 ± 0.7 hours). Donors averaged 50.8 ± 2.6 (standard error of the mean) years old with a body mass index of 28.6 ± 1.5 . Glands were shipped in cold organ preservation solution without oxygenated perfluorocarbon. Isolations were performed under a protocol optimized for digestion with the new cGMP collagenase from Roche. Purification used continuous Eurocoll/University of Wisconsin gradients. Islets were cultured in two types of Prodo cGMP islet culture media and/or in Miami 1A media. Glucose-stimulated insulin secretion assays were performed after 8 to 16 days of culture. Prepurification yield averaged 415 ± 41 KIEQ postpurification, 359 ± 29 KIEQ (purification loss 13.5%); and postculture 317 ± 27 KIEQ (culture loss 11.7%). Our process liberated an average of 4278 IEQ/g of pancreas (97 ± 5 g). Most islets were recovered in the purest fraction (purity $79.7\% \pm 1.9\%$). Culture loss in our enhanced culture media was 11.7%. After 2 to 3 days in culture, viability was $92\% \pm 1\%$. Islets exhibited compactness and dithizone staining. Glucose-stimulated insulin secretion assays performed after 3 to 23 days in our PIM(R) media resulted in a stimulation index of 6.8 ± 1.7 (G50 to G350). We concluded that our human islet isolation process permitted the recovery of large numbers of high-quality human islets from extended CIT pancreata and that our cGMP islet culture media was superior to the current standard CMRL-based media.

ROCHE LIBERASE HI was the standard collagenase/thermolysin mixture for human islet isolation that was used by the great majority of clinical islet transplant centers until it was disallowed by the FDA in 2007. The concern was that these enzyme preparations were manufactured from bacterial cultures that were grown in broths that contained bovine brain extract, raising the possibility that they might contain prions that could cause transmissible spongiform encephalitis.¹ In response, Roche developed Roche cGMP Liberase MTF C/T, which is manufactured completely free of components from mammalian or avian tissue sources. We evaluated this new product as it was introduced in late 2008. Since then we have standardized and optimized our human islet isolation process for this new collagenase.

Currently, Prodo's human pancreases are from out-of-state donors with research consent. As a result, the cold ischemia time (CIT) for many glands is much longer compared with organs from local donations. Thus, Prodo will accept a donor pancreas that arrives within 18 hours of

aortic cross clamping, keeping total CIT to below 19 hours. We have described herein the results of 16 isolations from pancreases with extended CIT as well as culture media comparisons between our proprietary media PIM(S) and PIM(R) with commercially available Miami 1A (Mediatech).

METHODS

Technical Information

Donor pancreases were obtained with research consent from out-of-state Organ Procurement Organizations (OPOs). Islet isolation was performed under a Research Institutional Review Board

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Table 1. Demographic Data for All Pancreas Donors

Isolation ID	Donor Age (y)	BMI	Gender	Race	Cause of Death
HP-08316-01	63	39.3	F	White	Stroke
HP-08323-01	41	30.1	M	White	Head trauma
HP-08330-01	37	30.8	F	Hispanic	Stroke
HP-08339-01	66	29.4	F	White	Anoxia
HP-08343-01	57	27.5	M	White	Head trauma
HP-08355-01	34	22.5	F	White	Anoxia
HP-09006-01	58	43.3	M	Hispanic	Stroke
HP-09009-01	42	30.7	F	White	Stroke
HP-09016-01	64	29.8	F	White	Stroke
HP-09041-01	50	30.8	F	White	Stroke
HP-09061-01	51	25	F	White	Stroke
HP-09071-01	41	21.5	F	Asian	Stroke
HP-09080-01	38	27.1	M	White	Anoxia
HP-09099-01	60	28	M	White	Stroke
HP-09128-01	57	22.2	M	White	Head trauma
HP-09134-01	54	20	F	White	Stroke
Average	50.8	28.6			
SEM	2.6	1.5			

BMI, body mass index; SEM, standard error of the mean.

from the Western Institutional Review Board (Olympia, Wash, USA), The organs were shipped to Prodo Laboratories by same-day courier service on ice in Viaspan or HTK custodial organ preservation solution. Two-layer pancreas preservation was not used since in our hands we have not demonstrated advantages for this method over regular shipping.² The organs were trimmed, decontaminated, and cut into two pieces. Both halves were then cannulated and distended at room temperature with HBSS containing cGMP Liberase MTF, thermolysin, and DNase I (all three from Roche Diagnostics, Indianapolis, Ind, USA). The tissue was then transferred to a chamber incorporated in a digestion circuit with a heating coil. Digestion was performed at 37°C in a recirculation mode for up to approximately 10 minutes. Once sufficient

islets were liberated, the tissue digest was collected in wash media containing 10% horse serum. After several washes and incubation in Viaspan, the islets were purified using a modification of the UIC-UB procedure³ on a COBE 2991 cell processor. The islets were then cultured in our PIM(R) media. A half media change was performed after 12 to 18 hours, and by 24 to 48 hours, the islets were ready for use or for shipping to other investigators.

The islets for comparisons were introduced into respective media immediately following the isolation for 3 to 23 (average = 8) days culture. Periodically islet samples were subjected to a glucose-stimulated insulin secretion (GSIS) assay. Before starting the assay, triplicate islet samples were preconditioned by overnight culture in media with 3 mmol/L glucose, followed by two 1-hour incubations in fresh 3 mmol/L media. For the actual assay, the islets were incubated for 1 hour each in 3, 12, 20, or 20 mmol/L + 1 mmol/L IBMX, and 3 mmol/L glucose media. At the end of each incubation, the supernates were collected and frozen for insulin analysis. For DNA quantitation, islets collected at the end of the GSIS assay were lysed using 1× TE buffer with protease inhibitors and 0.1% Triton X-100. Secreted insulin was determined using ALPCO insulin enzyme-linked immunosorbent assay kits (Salem, NH, USA). DNA was quantified using the picogreen method (Invitrogen, Carlsbad, Calif, USA).

Statistical Techniques

Statistical significance was determined using two-tailed Student's *t* tests.

RESULTS

Islet isolations were performed on 16 human pancreases with extended cold ischemia. The donor demographic data are presented in Table 1. Pancreas donors were 50.8 ± 2.6 (standard error of the mean) years old, with a body mass index of 28.6 ± 1.5 . The average CIT was 13.2 ± 0.6 hours (Table 2). We performed the collagenase infusion/pancreas distention at room temperature and the digestion at 37 °C

Table 2. Data for All Islet Isolations

Isolation ID	Cold Ischemia (h)	Pancreas Total Weight (g)	Digested Weight (g)	Percent Digested (%)	Distention Time (min)	Switch Time (min)
HP-08316-01	12.25	123	97.5	79.3	15	3
HP-08323-01	13.25	139.6	129.6	92.8	16.5	8
HP-08330-01	17	89.5	80.4	89.8	15	5.5
HP-08339-01	15	115	96.4	83.8	15	7
HP-08343-01	14.25	122.9	101.9	82.9	16	6.5
HP-08355-01	10	72.8	60.9	83.7	16	8
HP-09006-01	9.5	126.8	110.2	86.9	18	5.5
HP-09009-01	14.75	109.7	99.7	90.9	12.5	8
HP-09016-01	15	116	90	77.6	15.5	10.25
HP-09041-01	19	93	85	91.4	14.5	5
HP-09061-01	12	158.8	140.5	88.5	15.5	8
HP-09071-01	10.25	84.7	74.8	88.3	13	9
HP-09080-01	10	139	110.1	79.2	15.75	7
HP-09099-01	12.5	129.8	102.8	79.2	14	6.75
HP-09128-01	13	107.3	90.3	84.2	14	7
HP-09134-01	14	96.4	81.5	84.5	18	8.5
Average	13.2	114.0	97.0	85.2	15.3	7.1
SEM	0.6	5.2	4.6	1.1	0.3	0.4

SEM, standard error of the mean.

Table 3. Quantification of Islet Number, Purity, and Size, Before and After Purification, as well as After Culture

Isolation ID	Tissue (mL)	Pre IEQ	Post IEQ	% Loss After Purification	Postculture IEQ	% Loss After Culture	Main Prep Purity	Main Prep Index
HP-08316-01	35	592,853	542,386	8.5	477,516	12.0	80	2.3
HP-08323-01	37	215,762	189,975	12.0	158,945	16.3	70	0.9
HP-08330-01	30	604,693	429,670	28.9	398,676	7.2	90	1.1
HP-08339-01	33	373,778	380,306	-1.7	337,465	11.3	90	0.8
HP-08343-01	26	204,218	234,606	-14.9	267,427	-14.0	85	0.5
HP-08355-01	38	563,110	212,188	62.3	345,475	-62.8	90	1.6
HP-09006-01	43	793,817	586,089	26.2	549,136	6.3	77	1.3
HP-09009-01	45	432,400	498,594	-15.3	254,104	49.0	85	3.1
HP-09016-01	20	348,508	336,101	3.6	240,561	28.4	85	0.9
HP-09041-01	35	290,350	233,033	19.7	291,795	-25.2	75	0.4
HP-09061-01	35	345,597	330,645	4.3	257,631	22.1	73	2.5
HP-09071-01	37	300,902	267,090	11.2	172,927	35.3	80	2.4
HP-09080-01	38	539,109	433,161	19.7	375,969	13.2	75	0.8
HP-09099-01	35	329,667	370,000	-12.2	407,680	-10.2	80	0.5
HP-09128-01	30	262,764	328,900	-25.2	217,000	34.0	70	0.6
HP-09134-01	24	437,340	366,276	16.2	322,630	11.9	70	0.3
Average	33.8	414,679	358,689	13.5	317,184	11.7	79.7	1.2
SEM	1.6	40,995	29,436	5.3	26,727	6.7	1.8	0.2

IEQ, islet equivalents; SEM, standard error of the mean.

for an average of 7.1 minutes (“Switch time” in Table 2). The collection phase was constant for each process (approximately 21 minutes).

Our process yielded an average of 415 ± 41 KIEQ islets prepurification (Table 3), namely, 4278 IEQ per gram digested pancreas. After COBE gradient purification, we averaged 359 ± 29 KIEQ. Thus, 13.5% of the islets were not recovered from the gradient. After 2- to 3-day culture in PIM(R), we recovered 317 ± 27 KIEQ (average culture loss 11.7%; see Table 3 and Fig 1). PIM(R) culture resulted in rapid rounding of the islets into a compact state (Fig 2A,B) with retention of DTZ staining (Fig 2C).

We determined the functional characteristics of the isolated islets after culture in our proprietary PIM(S) and PIM(R) media, as compared to the commercially available Miami 1A (Mediatech, Herndon, Va, USA). All three media were supplemented with human AB serum to an end

concentration of 5%. We performed GSIS assays on islets cultured for 3 to 23 days in each of the three media (Fig 3). The PIM(R) media was clearly superior to Miami 1A media, resulting in the highest absolute as well as fold-over-basal GSIS insulin secretion (G50 to G350 stimulation index of 6.8 ± 1.7). The GSIS results between PIM(R) and Miami 1A at 12 mmol/L glucose, 20 mmol/L glucose, and 20 mmol/L glucose + 1 mmol/L IBMX were all statistically significant ($P = .02, .02, \text{ and } .01$, respectively).

DISCUSSION

To optimize the quality of islets for clinical transplantation, centers typically concentrate their isolation efforts on donor pancreases with CITs that are as short as possible and generally less than 8 hours.^{4–8} Since Prodo Laboratories receives the majority of its human donor pancreases from

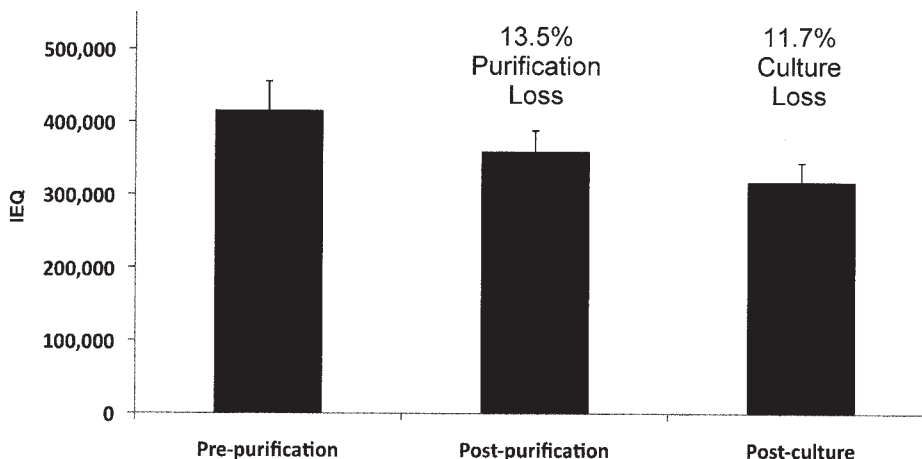


Fig 1. Quantification of number of islet equivalents before and after purification and after 2-day culture (average \pm standard error of the mean over all isolations). IEQ.

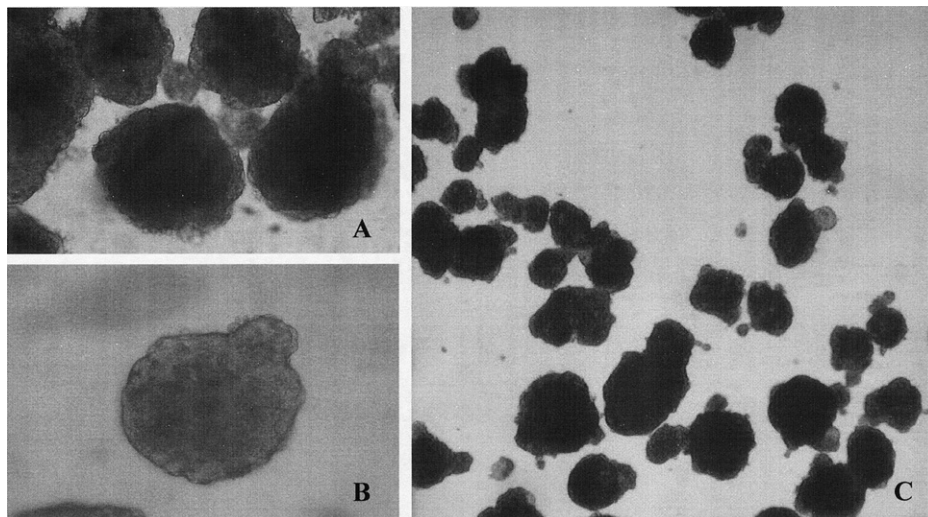


Fig 2. Recovery of islets during culture in PIM(R) media. The islets regained their rounded and smooth morphology within 12 to 24 hours of culture after isolation (A, B) and exhibited intense dithi-zone staining capacity (C).

out-of-state OPOs, the CIT incurred by the glands typically is much longer, averaging 13.2 hours.

The Roche cGMP Liberase MTF C/T is a new addition to the arsenal of enzymes available for human islet isolation. The main advantage of this product over other enzymes is that no materials of mammalian origin were used in its production, thereby eliminating the risk of prion contaminants.¹ As our data showed, we adapted our islet isolation procedures to accommodate glands with extended CIT. Our isolation procedure, together with the use of Roche cGMP Liberase MTF C/T collagenase and thermolysin, achieved isolation of large numbers of high-quality human islets from pancreases with extended CIT of up to 19 hours.

Culture of the isolated islets in PIM(R) recovery media for up to 48 hours resulted in excellent, rapid islet recovery from the trauma of isolation. Long-term culture of islets in PIM(R) and PIM(S) media engendered increased insulin secretory capacity as compared with Miami 1A media.

REFERENCES

1. Masujin K, Matthews D, Wells GAH, et al: Prions in the peripheral nerves of bovine spongiform encephalopathy-affected cattle. *J Gen Virol* 88:1850, 2007
2. Hering BJ, Matsumoto I, Sawada T, et al: Impact of two-layer pancreas preservation on islet isolation and transplantation. *Transplantation* 74:1813, 2002

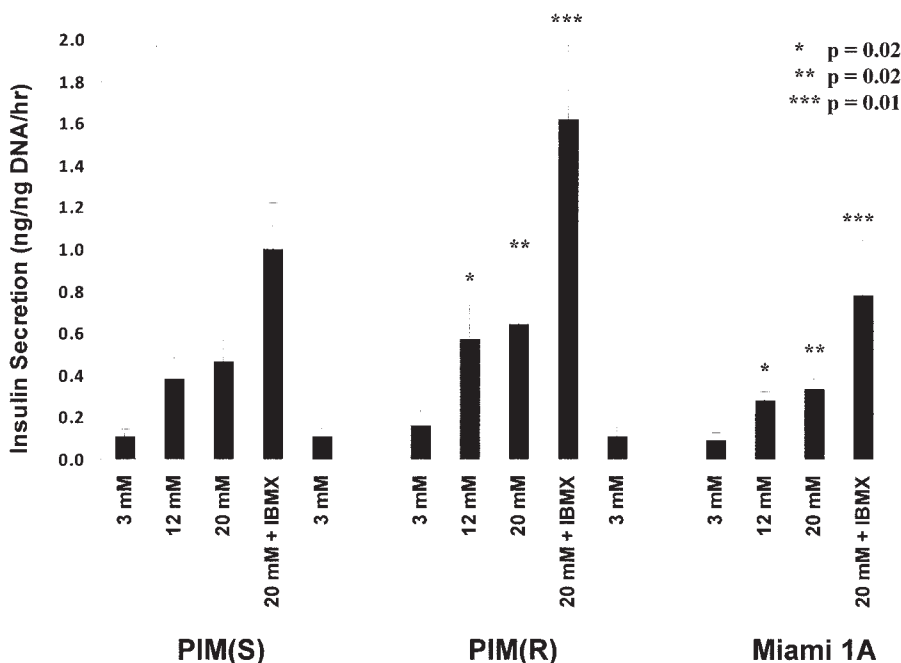


Fig 3. Comparison of insulin secretion from islets cultured in PIM(S), PIM(R), or Miami 1A media. Glucose-stimulated insulin secretion assays were performed as indicated in the text. Islets cultured in PIM(R) media exhibited statistically significant higher glucose or IBMX-stimulated insulin secretion as compared to islets that had been cultured in Miami 1A media.

3. Barbaro B, Salehi P, Wang Y, et al: Improved human pancreatic islet purification with the refined UIC-UB density gradient. *Transplantation* 84:1200, 2007
4. Pileggi A, Ribeiro MM, Hogan AR, et al: Effects of pancreas cold ischemia on islet function and quality. *Transplant Proc* 41:1808, 2009
5. Zeng Y, Torre MA, Karrison T, et al: The correlation between donor characteristics and the success of human islet isolation. *Transplantation* 57:954, 1994
6. Lakey JR, Rajotte RV, Warnock GL, et al: Human pancreas preservation prior to islet isolation. Cold ischemic tolerance. *Transplantation* 59:689, 1995
7. Hanley SC, Paraskevas S, Rosenberg L: Donor and isolation variables predicting human islet isolation success. *Transplantation* 85:950, 2008
8. Toso C, Oberholzer J, Ris F, et al: Factors affecting human islet of Langerhans isolation yields. *Transplant Proc* 34:826, 2002