Roche

Application note

Islet cell isolation from human pancreas with extended cold ischemia time (CIT)

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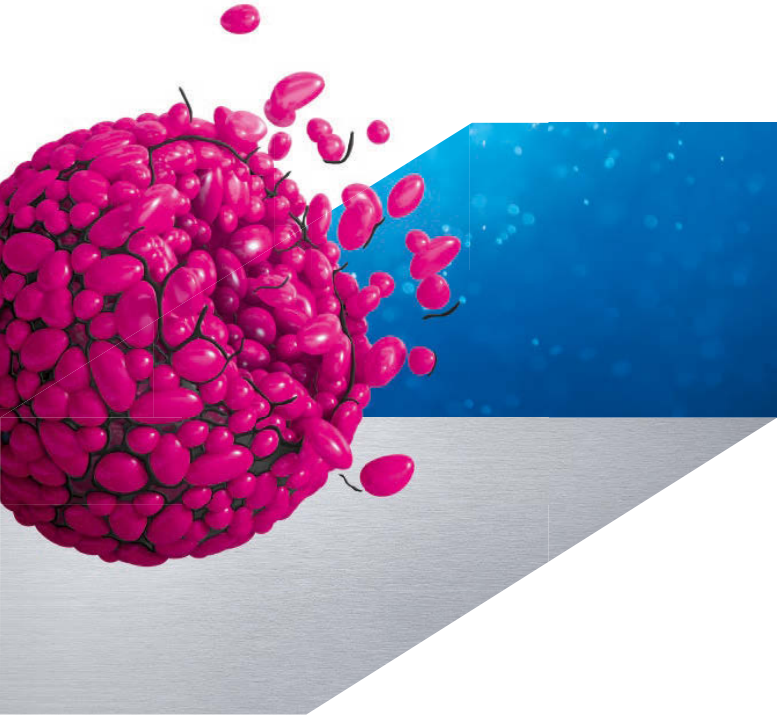

Introduction

Until early 2007 Roche Liberase HI had been the standard commercial enzyme preparation for islet cell isolation. At that time, the US FDA issued guidance discouraging the use of enzyme preparations, like Liberase HI, that are manufactured using material derived from bovine brain; which could harbor the infectious agent for transmissible spongiform encephalitis (TSE), for use in clinical islet transplantation. In an effort to better serve these customers, Roche developed a new formulation, Liberase MTF GMP Grade, which is manufactured completely free of components from mammalian or avian tissue sources. We evaluated this product as it was introduced in late 2008, and have since standardized our human islet isolation process on this enzyme preparation and successfully optimized our procedures for its use.

As a result of our lab's human pancreases coming predominantly from out-of-state donors with research consent, the cold ischemia time (CIT) for many glands is much longer as compared to those from local donations. Thus, Prodo Labs accepts donor pancreas that arrive within 18 hrs of aortal cross clamp; keeping total CIT below 19 hrs. In this paper we describe the results from 16 consecutive isolations from pancreases with such extended CIT.

Blended proteolytic enzyme for tissue dissociation.
For use in quality control/manufacturing process only.

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Materials and methods

Donor pancreases with research consent were obtained from out-of-state Organ Procurement Organizations (OPO's). The organs were shipped to Prodo Laboratories by same-day courier service on ice with Viaspan or HTK organ preservation solution. The organs were trimmed, decontaminated, and cut into two pieces. Both halves were then canulated and repeatedly distended at room temperature with 350 mL HBSS containing 0.5 grams of Collagenase I/II MTF blend together with 15 mg of Thermolysin MTF out of Liberase MTF C/T GMP Grade kit, and 5 mg of DNAse I (Roche Diagnostics), as well as 10 mM Hepes and extra calcium. Distention time was variable and was adjusted to the requirements of each specific pancreas, in effect constituting a pre-digestion phase. The tissue was then transferred to a Ricordi chamber incorporated in a digestion circuit with heating coil. Digestion was performed at 37 °C. Due to the pre-digestion phase, the actual digestion times were relatively brief (between 3 and 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 procedure on a COBE 2991 cell processor. The islets were then cultured in PIM(R) media. A half media change was performed after 12 to 18 hrs and the islets were ready for use, or for shipping to other investigators, by 24 to 48 hrs.

The islets used for the media comparisons were introduced into their respective media immediately following the isolation, and cultured for an average of 8 days. Periodically islet samples were subjected to a Glucose Stimulated Insulin Secretion (GSIS) assay. Before starting the assay, triplicate islet samples were first pre-conditioned by overnight culture in media with 3 mM glucose, followed by two 1 hr incubations in fresh 3 mM media. For the actual assay, the islets were incubated for 1hr each in 3 mM, 12 mM, 20 mM, 20 mM + 1 mM IBMX, and 3 mM glucose media, respectively. At the end of each incubation the supernatant was collected and frozen for insulin analysis.

For DNA quantification, the islets were collected at the end of the GSIS assay and lysed using 1X TE buffer with Protease inhibitors and 0.1% Triton X-100. Secreted insulin, as well as insulin content was determined using ALPCO Insulin ELISA kits. DNA was quantified using the picogreen method (Invitrogen).

Isolation ID	Donor age	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**			

Table 1: Donor demographics

Isolation ID	Cold ischemia (Hrs)	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	**0.7**	**5.7**	**5.0**	**1.2**	**0.4**	**0.4**

Table 2: In process data

Results

Islet isolations were performed on 16 human pancreases with extended cold ischemia. The demographic data of the donors is presented in Table 1. Research consent had been obtained for all donors. Pancreas donors were 50.8 ± 2.6 (SEM) years old, with a BMI of 28.6 ±1.5 The average Cold Ischemia Time was 13.2 ± 0.7hrs (Table 2).

We performed the collagenase infusion/pancreas distention at room temperature. The distention phase was thus in effect a pre-digestion step. This phase was customized for every pancreas individually to try to get every pancreas to the same approximate degree of softness and tissue integrity before actual digestion started. Distention times ranged from 12.5 – 18 minutes (Table 2). The actual digestion was at 37 °C and averaged 7.1 ± 0.4 minutes (Switch time in Table 2). The dilution phase was constant for each process (average 21 min.).

Our process yielded an average of 415 ± 41 KIEQ islets pre-purification (Table 3). This amounted to an average of 4,275 Islet Equivalents (IEQ) per gram of digested pancreas. After COBE gradient purification we averaged 359 ± 29 KIEQ (average gradient loss: 9.0 ± 5.3%).

After culture for 2–3 days in PIM(R), we recovered 317 ± 27 KIEQ (average culture loss 8.4 ± 6.7%) (See Table 3 and Figure 1).

PIM(R) culture resulted in rapid rounding of the islets into a compact state with excellent retention of DTZ staining capability (Figure 2).

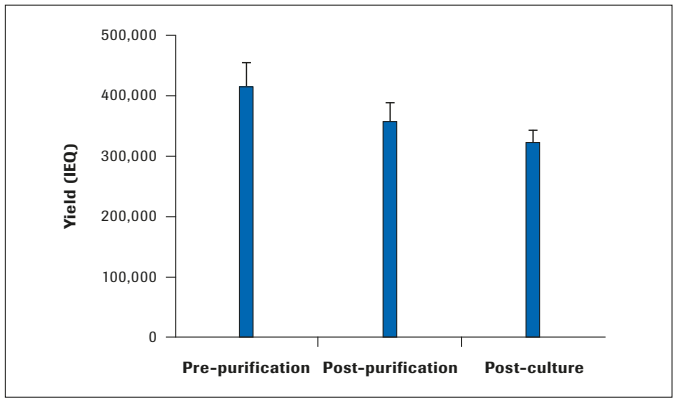

Figure 1: Isolation yield

After culture for an average of eight days in PIM(R) media, the islets had excellent Glucose Stimulated Insulin Secretion (GSIS) with an average insulin secretion of 3.5 fold over basal (3 mM versus 20 mM) and a maximum insulin secretory response of 8.5 fold over basal in the presence of IBMX (3 mM versus 20 mM + 1 mM IBMX). (See Figure 2.)

We also compared the functional and morphological characteristics of the islets after culture in our proprietary PIM(S) and PIM(R) media, as well as in the commercially available Miami 1A. All three media were supplemented with Human AB serum to an end-concentration of 5 %. (Data summarized in Figure 3.)

Islet purity was assessed by Dithizone (DTZ) staining. Purity was highest for islets that had been cultured in PIM(R). Intermediate purity was obtained in PIM(S), and lowest purity in Miami 1A.

Isolation ID	Tissue (mL)	Pre IEQ	Post IEQ	% Loss after purification	Post culture IEQ	% Loss after culture	Main prep final 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	50	0.9
HP-08330-01	30	604,693	429,670	28.9	398,676	7.2	88	1.4
HP-08339-01	33	373,778	380,306	-1.7	337,465	11.3	83	1.1
HP-08343-01	26	204,218	234,606	-14.9	267,427	-14.0	80	0.5
HP-08355-01	38	563,110	212,188	62.3	345,475	-62.8	91	1.4
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	86	3.1
HP-09016-01	20	348,508	336,101	3.6	240,561	28.4	85	1.4
HP-09041-01	35	290,350	233,033	19.7	291,795	-25.2	80	0.6
HP-09061-01	35	345,597	330,645	4.3	257,631	22.1	68	3.5
HP-09071-01	37	300,902	267,090	11.2	172,927	35.3	77	0.7
HP-09080-01	38	539,109	433,161	19.7	375,969	13.2	74	1.1
HP-09099-01	35	329,667	370,000	-12.2	407,680	-10.2	70	0.6
HP-09128-01	30	262,764	328,900	-25.2	217,000	34.0	69	0.3
HP-09134-01	24	437,340	366,276	16.2	322,630	11.9	81	0.4
Average	**33.8**	**414,679**	**358,689**	**9.0**	**317,184**	**8.4**	**77.4**	**1.3**
SEM	**1.6**	**40,995**	**29,436**	**5.3**	**26,727**	**6.7**	**2.5**	**0.2**

Table 3: Isolation

* Main Prep Index = Index of islets from the main preparation, or the fraction containing the bulk of the islets.

We also quantified compactness by grading islets that had been cultured for an average of 6.3 days on a scale of 0 (islets completely folded open) to 5 (islets completely rounded up). The results show that PIM(S) performed best, closely followed by PIM(R). The lowest compactness was observed for islets cultured in Miami 1A (Figure 3).

Similar results were obtained for fusing/chaining of the islets. On a scale of 0 (no fusing/chaining) to 5 (all islets clumped/fused together) the smallest amount of fusing/chaining was observed in PIM(S) media. Intermediate levels of fusing/chaining were observed with PIM(R) media, and about twice the amount for islets cultured in Miami 1A.

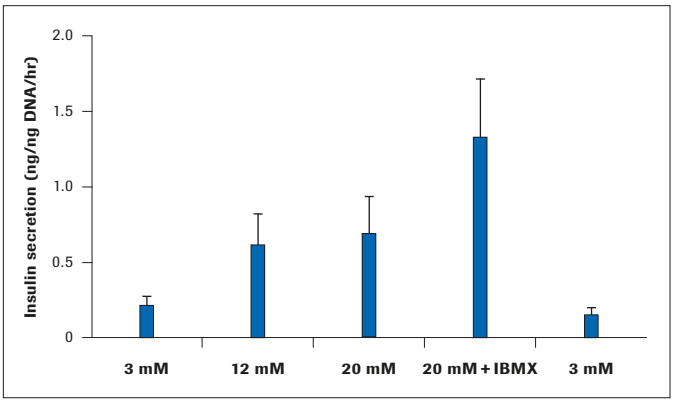

Figure 2: Glucose stimulated insulin secretion

Conclusions

- Our isolation procedure, together with the use of Roche Liberase MTF C/T GMP Grade, and DNase I, allows for the isolation of large numbers of viable human islets from pancreases with extended cold ischemia of up to 19 hrs.
- Culture of human islets in PIM(R) recovery media for up to 48 hrs, after isolation with Liberase MTF and DNase I, results in excellent and rapid islet recovery from isolation trauma. Islets feature excellent DTZ staining capability and insulin secretory function.
- Culture of islets in PIM(R) and PIM(S) media results in improved purity, compactness, insulin content and secretory capacity, as compared to culture in Miami 1A media.

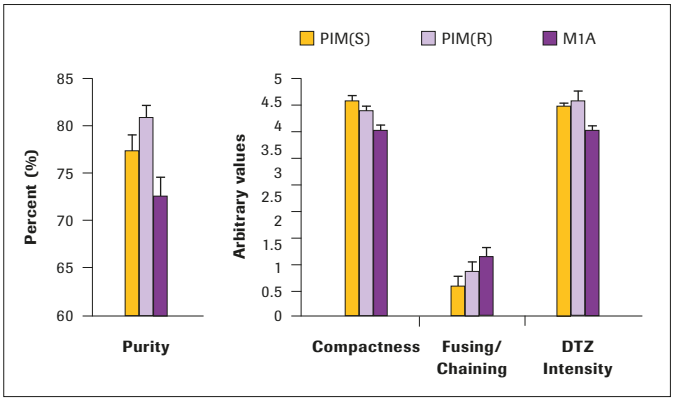

Figure 3: Post-culture functional and morphologic characteristics

Regulatory disclaimer

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