Key words: islet of Langerhans, isolation, iodixanol , Optiprep, pig

No Porcine Islet Loss During Density Gradient Purification in a Novel Iodixanol in University of Wisconsin Solution

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THE MARKED fragility and rapid dissociation and loss of (juvenile) porcine islets during the isolation and especially the purification process is considered a major barrier on the way to pig-to-man islet transplantation. We developed a novel simple density gradient of iodixanol (Optiprep™) in University of Wisconsin solution (UWS) which allows the purification of juvenile islets with no loss or fragmentation, and an improved purity and viability as compared to conventional ficoll-sodium-diatrizoate (Histopaque) gradients.

MATERIALS AND METHODS

Optiprep-UWS Gradient

Optiprep™ (Nycomed Pharma, Oslo, Norway) is a 60% iodixanol in water solution. We prepared a Working Optiprep Solution (WOP) by mixing equal volumes of Optiprep and a 2-times concentrated regular UWS. The bottom solution of our gradient is prepared in 50 mL tubes by mixing 10 mL of the WOP and 20 mL digest (in UWS). Density is ~ 1.10 g/mL and osmolality is ~380 mOsm. The bottom is over-layered with a 1.095 density solution of Optiprep in RPMI-1640 containing 20 mmol/L HEPES (a mixture of 9.1 mL Optiprep and 25 mL RPMI) which essentially prevents the contamination of the islet prep with single acinar cells from the bottom solution during harvesting. The gradient is topped with RPMI.

Islet Isolation

The design and methods are illustrated in Fig 1. A portion of the body of pancreases explanted from ~ 6 mo old slaughterhouse pigs (n = 8) was used for islet isolation.

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Islets were isolated following a 17 minutes warm- and 90 minutes cold ischemic time, by intraductal 2 mg/mL collagenase-P (Boehringer, Mannheim, Germany) and 4 mmol/L Pefabloc-SC (Boehringer) in UWS digestion at 37°C, and washing and sieving (400-μm mesh) in UWS on ice. Half of the digest was pelleted, and bottom-loaded in Histopaque (Sigma, St Louis, Mo) 1.119, 1.077 g/mL, UWS gradients. After centrifugation at 500*g* for 5 minutes at 4°C, purified islets were obtained from the upper two layers. The other half of the digest in UWS was — as detailed above — mixed with half the volume of WOP to prepare the bottom Optiprep-UWS, and overlayered with an 1.095 g/mL Optiprep in RPMI solution, and topped with plain RPMI. Purified islets were obtained, after centrifugation as above, from the top interface.

For morphometry multiple 25-μL aliquots of the islet suspensions were stained with dithizone, and the mean diameters of all islets ≥50 μm, were recorded in 25-μm increments for calculation of the number of islet equivalents (IEQs), and the volume-average diameter of islets. Viability was assessed both by fluorometry using acridine orange and propidium iodide, and by trypan-blue staining.

Statistical Analysis

Results are expressed as means ± SE. Differences were analyzed by analysis of variance for repeated measures, and considered not significant (NS) at $P > .05$.

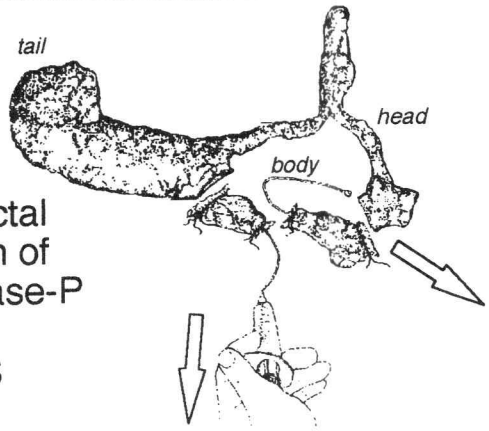
RESULTS AND DISCUSSION

In the digest, islet yield was 1007 ± 182 IEQs/g pancreas and the volume-average diameter of the islets was 89 ± 5 μm. After Optiprep purification yield was 928 ± 160 (NS vs digest), purity was 86 ± 3%, islet size was 107 ± 6% of the size before purification, and viability was 75 ± 3% (NS vs digest). After Histopaque purification, yield decreased to 58 ± 10% of the yield before purification ($P < .005$), size to 93 ± 3% ($P < .05$), purity to 61 ± 9% ($P < .05$) and viability to 54 ± 4% ($P < .001$). Thus, our novel, simple, Optiprep-UWS gradient allows the complete recovery of highly purified, viable, pig islets. Preservation of cell viability in UWS during isolation and purification appears to be the key to the successful outcome.

Legend to figure 1

Fig 1. Illustration of the design of the study, and methods of islet isolation and purification.

Slaughterhouse pig pancreas (n=8)

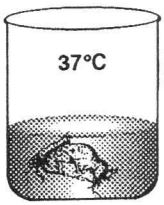


Intraductal injection of Collagenase-P in UWS

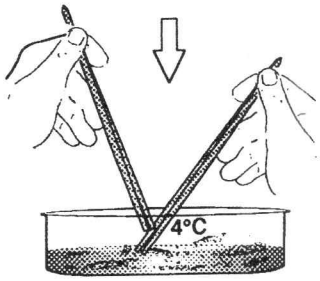
The other part of the body was likewise processed using Hanks' solution (not UWS) during isolation.

These experiments are reported next Saturday

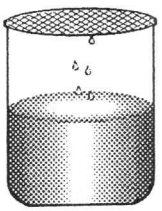
Incubation in UWS at 37°C



Dispersion in cold UWS

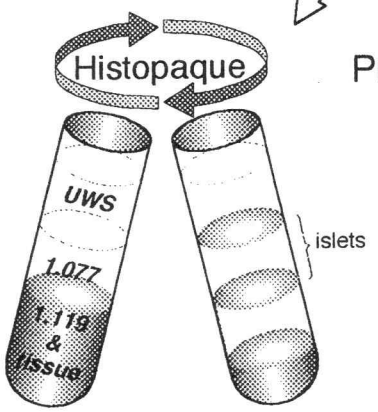


Sieving & Shaking of retained tissue



Purification

Histopaque



Optiprep