

Human islet isolation: semi-automated and manual methods

MICHELLE PAGET, HILARY MURRAY, CLIFFORD J BAILEY, RICHARD DOWNING

Abstract

Large yields of high-viability human islets are necessary to service the expanding programmes of islet transplantation worldwide; similarly, there is an increasing demand from diabetes researchers for a reliable and cost-effective supply of human islets. The two main isolation methods are 'semi-automated' and 'manual'. Both methods rely on prompt and careful removal and transfer of the donor pancreas to allow isolation to commence, preferably within eight hours. Each method involves exocrine digestion with high-activity collagenase (Liberase).

The semi-automated method is standardised, generally provides higher islet yields and is used for clinical transplant purposes, although it is not suitable for all donor pancreata. The manual method is less expensive and more adaptable and enables islets to be isolated for research from most donor pancreata.

Diabetes Vasc Dis Res 2007;4:7–12

Key words: human islets of Langerhans, human islet isolation, islet cell transplantation.

Introduction

Large-scale isolation of human islets is required to service expanding transplantation programmes.^{1,2} Early proof of concept studies in animals^{3,4} encouraged human islet transplantation, but progress was slow since methodological problems hampered islet isolation^{5,6} and clinical studies were limited and tentative.^{7–9}

In the year 2000 a modified islet isolation and immunosuppression regimen improved implant survival¹⁰ and several new transplant programmes emerged.^{1,2} More than 350 patients worldwide received islet transplants between 1999

and 2005.¹¹ Some 70–80% of graft recipients achieved independence from insulin injections during the first year. However, this was reduced to 10% by five years after transplant.¹²

Successful transplants typically require 6,000–9,000 islets of 150 μm diameter (or equivalent beta-cell mass) per kg bodyweight of the recipient,⁹ that is, between 0.5 and 1.0 million islets are needed per transplant recipient. A normal human pancreas may have more than one million islets, but even the most successful isolations seldom yield more than 400,000 islets, often considerably fewer.¹⁰ Therefore, transplant recipients typically require islets from 2–4 donors; not surprisingly, attention has focused on methods to improve yields and viability of islets isolated from human cadaveric donors.^{13–15} This review provides an evaluation of current islet isolation procedures, comparing the relative merits of manual and semi-automated methods.

Development of current procedures

Isolation of individual islets was undertaken initially for research purposes. Early studies used small pieces of minced rodent pancreas. These were superseded by microdissection procedures, sometimes assisted by prior pancreatic duct ligation or pilocarpine administration to reduce the volume of exocrine tissue.^{16–18} Moskalewski was the first to use the enzyme complex collagenase in 1965 to isolate viable islets from chopped guinea pig pancreata.¹⁹ Lacy and Kostianovsky improved this technique in 1967 by intraductal distension of the pancreas prior to digestion, and by employing sucrose gradients for centrifugal separation of the islets from the pancreatic digest.²⁰ Sorenson achieved more efficient separation in 1968 using Ficoll density gradients.²¹ In 1979 Downing compared venous versus ductal distension of the canine pancreas to improve islet yield²² and in 1981 Horaguchi and Merrell²³ retrogradely perfused collagenase at 37°C through the canine pancreatic duct. Ficoll density gradients further improved the yield of canine islets.²⁴

In 1984 Gray and colleagues at Oxford successfully adapted the canine methodology to human pancreata²⁵ and in 1988 Ricordi and colleagues initiated development of a semi-automated method in which the gland was placed into a sealed chamber during the digestion phase and agitated constantly to enhance dispersion of the pancreas.²⁶ The semi-automated islet isolation method was advanced by a purification step involving modification of the COBE 2991 cell processor to provide a linear continuous Biocoll density gradient for optimal separation of islets from the pancreatic digest.^{27,28} Purification of the collagenase enzyme blends (Liberase, Roche) with low endotoxin levels resulted in short-

The Islet Research Laboratory, Worcestershire Clinical Research Unit, Worcestershire Acute Hospitals NHS Trust, Newtown Road, Worcester, WR5 1HN, UK.

Michelle Paget, Research Associate

Hilary Murray, Research Scientist

Richard Downing, Consultant Vascular Surgeon

Diabetes Research, Life and Health Sciences, Aston University, Birmingham, B4 7ET, UK.

Clifford J Bailey, Head of Diabetes Research

Correspondence to: Dr Michelle B Paget

The Islet Research Laboratory, Worcestershire Clinical Research Unit, Worcestershire Acute Hospitals NHS Trust, Newtown Road, Worcester, WR5 1HN, UK.

Tel: +44 (0)1905 760527; Fax: +44 (0)1905 760262

E-mail: Michelle.Paget@worsacute.wmids.nhs.uk

er digestion times and less fragmentation of islets during the digestion and separation process.²⁹

Variables affecting islet yield and viability

The origin and condition of a human pancreas, as well as the method of islet isolation, can affect substantially the yield and viability of human islets for either transplant or research purposes.

Donor and pancreas variables

Pancreata from organ donors who are overweight (with a body mass index [BMI] 25–30 kg/m²) generally give significantly higher islet yields than lean donors (with a BMI < 25 kg/m²).^{30,31} Also, higher islet yields are typically achieved when a thicker capsule surrounds the islets (often observed in older donors),³² while pancreata from obese donors (BMI > 30 kg/m²) may give high islet yields consistent with islet hypertrophy and hyperplasia.³³ Donors aged 50 years or more frequently give good islet yields, but they are often associated with compromised glucose responsiveness, and high donor serum glucose levels prior to organ retrieval reduce both islet yield and viability.^{30,31} Pancreata from brain-dead organ donors may produce lower yields of less viable islets due to donor haemodynamic instability, high doses of inotropic support and the direct toxic effects of circulating brain-derived peptides.³⁴

Donor hospitalisation of less than four days prior to organ donation is preferable as islet yield and viability are generally higher than those from donors who have been hospitalised for longer periods.³⁵

Retrieval, preservation and isolation

The cooperation and experience of the organ retrieval team are vital. Packing the abdominal cavity with ice decreases the damage caused by warm ischaemia time, while rapid atraumatic removal of the organ with the capsule intact increases the likelihood of high yields of viable islets.³⁶ Rapid transport of the pancreas to an isolation centre is vital since cold ischaemia times in excess of eight hours impact negatively on islet yield and viability. However, the two-layer method of organ transport using University of Wisconsin solution (UW) and oxygenated perfluorocarbon reduces cold ischaemic damage, allowing successful isolation of islets from pancreata with up to 12 hours' cold ischaemia.^{36–38} Perfluorocarbons (PFC) are hydrocarbons in which fluorine replaces all or most of the hydrogen atoms, resulting in a high capacity for dissolving oxygen while maintaining a low oxygen-binding constant; this allows them to release oxygen more effectively than haemoglobin into surrounding tissue.³⁹ In the two-layer method, PFC protect pancreata against cold ischaemia damage by increasing the expression of inhibitors of apoptosis while down-regulating expression of pro-apoptotic genes and caspases.⁴⁰

A recent study has shown significant variation in efficacy between and within batches or lots of the collagenase blend, Liberase.⁴¹ Additionally, differences in the composition of collagenase isoforms within the blend may affect the stability and potency of the enzyme.⁴¹ The experience of the islet isolation team can also affect islet yield and viability: even

Figure 1. An image of a human pancreas that has been distended with Liberase by ductal cannulation. This image is reproduced with kind permission of Professor Camillo Ricordi of the Diabetes Research Institute at the University of Miami



high-grade preparations often yield only 20–50% recovery of available islets from a donated pancreas.⁴²

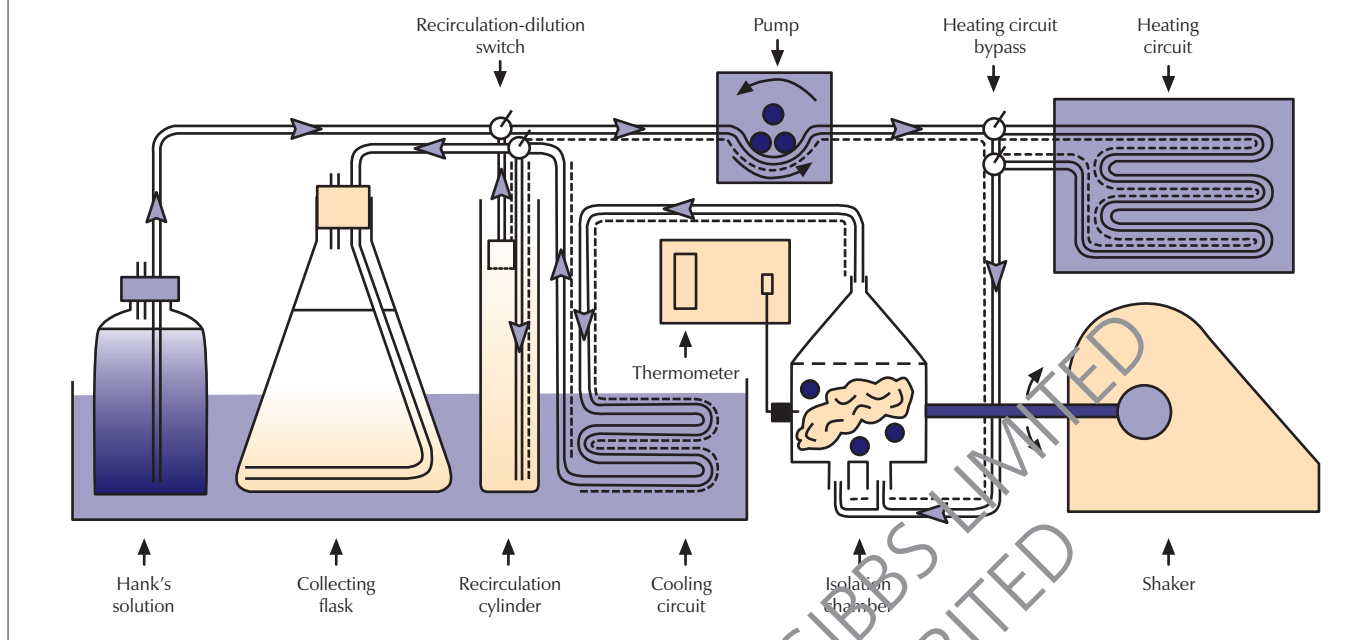
Procedures for semi-automated and manual methods of islet isolation

The initial procedure is the same for both semi-automated and manual methods. The pancreas is assessed visually for colour, shape, approximate size, obvious signs of damage and amount of fat and other attached tissue, with a view to considering minor adjustments to the later procedure (e.g. earlier sampling from a small, fatty, non-fibrous pancreas). The organ is prepared for enzyme perfusion by removing excess fat and trimming off any remaining duodenum and spleen. Great care is taken to avoid damage to the pancreatic capsule in order to maximise efficacy of enzyme infusion and organ distension. The main differences between the two isolation methods commence at this stage.

Semi-automated method

The main pancreatic duct is accessed via a central incision into the pancreas and two cannulae are inserted and sewn into place, one to infuse solutions toward the proximal, and the other toward the distal end of the organ. Infusion of cold (~4°C) Liberase solution (figure 1) via the two cannulae is achieved using a peristaltic pump to deliver the enzyme solution at a maximum pressure of 180 mmHg. The organ is placed into a sterile, sealed chamber (figure 2) with metal spheres and warmed to 37°C. Some groups process the intact organ while others cut it into 6–8 pieces. Constant agi-

Figure 2. A diagram showing the semi-automated human islet isolation procedure. This image is reproduced with kind permission of Professor Camillo Ricordi of the Diabetes Research Institute at the University of Miami



tation aids digestion, while a mesh is employed to allow tissue particles of $< 500 \mu\text{m}$ to pass through to the next phase where the enzyme activity is terminated by cooling and addition of protein (human serum albumin).

Liberase activity is stopped when a sample of the digest reveals islets that are cleaved from the exocrine tissue (figure 3).

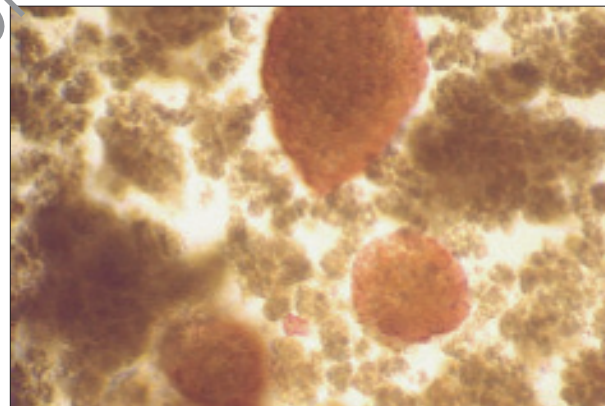
Once digestion is complete, the tissue pellet is recovered and suspended in UW solution. This increases the density of the exocrine tissue, resulting in a more distinct difference in densities between the endocrine and exocrine fractions of the digest, and thereby improving the efficacy of density gradient separation.

The tissue pellet is then loaded onto a continuous Biocoll (ficoll) density gradient on a COBE 2991 cell processor, and centrifugation at 1,800 rpm for 10 minutes results in islets forming bands upon reaching their specific density. The COBE is cooled to 4°C during this procedure to prevent further enzyme activity from damaging structural integrity of the islets and to protect the cells from gradient toxicity. Upon completion of density gradient separation, the tissue is collected in aliquots with varying islet:exocrine ratios. The islets are contained in the least dense component of the gradient: hence the initial aliquots retrieved from the COBE contain highly purified islets and as gradient density increases the presence of exocrine tissue is observed. Aliquots with high purity and good yields of islets are combined to provide the final islet preparation.

For research purposes the islets are maintained in tissue culture, with a culture medium such as medium 199 or CMRL, in a humidified incubator at 30°C with 5% CO_2 .

If the islets are intended for transplant, the currently preferred procedure is to maintain them in media such as Miami #1 with 0.5% human serum albumin and to effect

Figure 3. Human pancreatic digest prior to density gradient separation of the islets from the exocrine tissue – image from the Islet Research Laboratory, Worcester, UK. The islets of Langerhans are stained red with dithizone



transplant as soon as possible after isolation. However, some studies have suggested a period of culture during which the immunogenicity of the islets may be reduced, potentially enabling the use of less severe immunosuppression.^{43,44}

Manual method

Liberase is injected into the pancreatic duct through a syringe with a pressure of up to 300 mmHg. The distended pancreas is placed into a sterile container, warmed to 37°C and agitated gently to aid digestion. Frequent samples of the digest are taken to monitor the digestion process by noting the presence of free islets. Dithizone is used to stain the islets to distinguish them clearly from exocrine tissue. Upon com-

pletion of digestion, enzyme activity is terminated by cooling and the addition of bovine serum albumin (BSA).

The digest is then added to cold UW solution to increase the difference in densities between the endocrine and exocrine fractions. More efficient islet isolation is obtained using centrifugation on a discontinuous density gradient prepared with Hanks' balanced salt solution and Histopaque (polysucrose and sodium diatrizoate). After centrifugation, the interface containing islets is removed and prepared for culture⁴⁵ (figure 4) in either conventional static culture or a rotational cell culture system⁴⁶ in suitable media and maintained in a humidified incubator at 30°C with 5% CO₂.

Comparison of semi-automated and manual methods

Cost

The manual method of islet isolation is less expensive to establish than the semi-automated method as it does not require construction or purchase of digestion chambers, gradient makers, peristaltic pumps or a COBE cell processor. The use of consumables such as density gradients, serum and physiological salt solutions is also reduced when the manual method is employed. The two methods use the same amount of Liberase, but the semi-automated method requires more laboratory staff than the manual method and as such adds further to the costing, making the manual method generally a more cost-efficient procedure for isolating islets. The average cost of a single isolation is presently about £3,000 by the manual method, while the cost for the semi-automated method varies between £5,000 and £14,000 (personal communication, Dr S Hughes, Oxford).⁴⁷

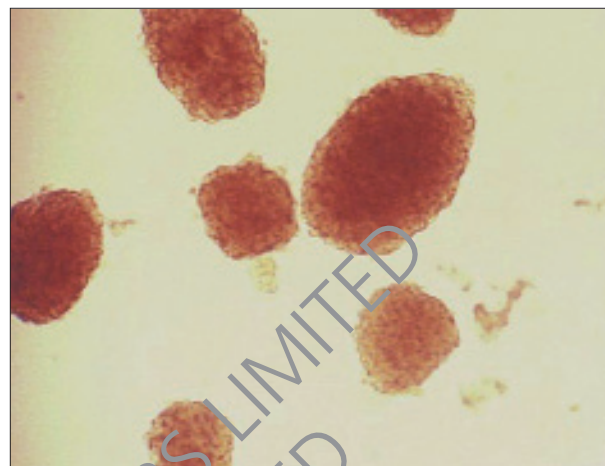
Yield

Neither the manual nor the semi-automated method extracts all of the approximately one million islets from a pancreas, but the semi-automated method generally produces higher yields than the manual method. With so many factors affecting the isolation process, it is not surprising that yields vary from as low as ~100,000 IEQ (islet equivalents, ~150 µm) to more than 400,000 IEQ per pancreas with the semi-automated method.^{30,32,36,38} The yields from the manual method also vary considerably, from ~80,000 IEQ to 300,000 IEQ, depending on the quality of the pancreas.

Leading centres using the semi-automated method report successful isolation of clinical grade islets from 25–75% of pancreata received,³¹ while the manual method employed within our laboratory results in the isolation of viable islets from at least 90% of pancreata received. Thus, the semi-automated method gives a higher yield per pancreas, but is suitable for fewer pancreata than the manual method.

The question of how pure islet preparations should be for transplant has produced two schools of thought. According to the first school, the beta-cell preparation should be as pure as possible to replace mostly endocrine tissue; according to the second, because communication between the endocrine and exocrine fractions may be important to islet function, viability and survival, the presence of exocrine tissue could be helpful. Attempting to achieve very high purity

Figure 4. Human islets isolated at the Islet Research Laboratory in Worcester, UK using the manual method of islet isolation. The islets have been separated from the exocrine tissue by density gradient centrifugation and are stained red with dithizone



may lead to an overall loss of beta-cell yield as there will be some overlap in the density ranges of the endocrine and exocrine fractions during density gradient centrifugation.

Islet preparations acquired using the semi-automated method of isolation are used for transplant when a purity of >30% has been achieved.⁴⁸ The manual method generally results in islet preparations with a range of purity of 60–85%. Recent evidence suggests that the presence of some exocrine tissue in the transplant material may improve graft performance, possibly by providing a source of beta-cell progenitors.⁴⁹

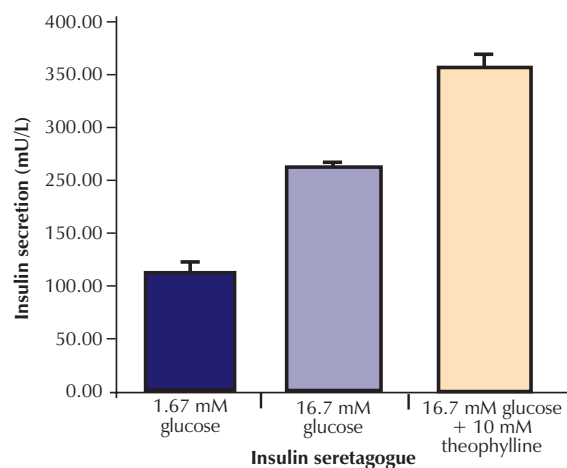
Application

The semi-automated method of islet isolation has been standardised and conforms to the strict regulations governing the quality of tissue suitable for clinical transplantation. Hence, this method is preferred for centres involved in an islet transplant programme. (Manual methods would require separate validation.) Although the semi-automated method tends to result in higher islet yields per isolation when it is successful, the purity of the islet preparations obtained in this way is not necessarily superior to those obtained by the manual method. Due to the lower cost and staff requirements of the latter, the technique lends itself well to the purpose of acquiring good yields of high-viability islets for research purposes. When semi-automated isolation gives an insufficient number of islets for transplantation, or when the islets are deemed unsuitable for reasons other than viability, these islets may be used for research.

Functional integrity of isolated islets

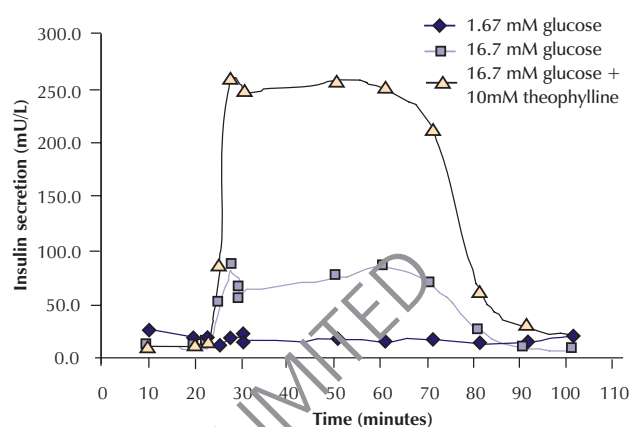
The morphological status of viable human islets obtained by either the semi-automated or manual isolation procedures, as indicated by dithizone staining (figures 3 and 4), does not take account of the highly variable functional capacity of these islets.⁴⁶ Moreover, the preservation of

Figure 5. Static incubation insulin secretion from human islets isolated at the Islet Research Laboratory in Worcester, UK by the manual method of islet isolation



Values are mean \pm SEM, n=6, 20 islets/2 ml challenge solution

Figure 6. Insulin secretion by perfusion, showing the dynamic phasic response from human islets isolated at the Islet Research Laboratory in Worcester, UK by the manual method of islet isolation



Values are insulin release from 100 human islets per chamber. At time 20 min 16.7 mM glucose and 16.7 mM glucose + 10 mM theophylline were introduced and returned to 1.67 mM at time 70 min to show a reduction to basal insulin secretion

optimal function for the period between isolation and implantation can be crucial to successful transplantation. Prior to transplant, the insulin secretory competence of islets is typically assessed during a short-term (1–2 hour) static incubation of islets in a low (sub-stimulatory) and high (stimulatory) concentration of glucose. The total cellular insulin content of a sample of the islets is also measured to help determine functional status.¹⁰ In our laboratory the insulin response to other secretagogues, such as glucose with theophylline, is routinely assessed, providing a useful indicator of functional capacity (figure 5). Furthermore, integrity of the dynamic phasic response to these secretagogues is a valuable indicator of research quality islets (figure 6). An important focus for future research will be the preservation of high-quality islets to offset partially the current use of multiple donor pancreata in an attempt to obtain sufficient islets for sustained insulin independence of the recipient.^{10–13}

Conclusion

Particular attention is now being directed to the quantity and quality of human islets in order to meet the demands of expanding transplant programmes and increased interest in diabetes research worldwide. The semi-automated and manual methods offer different advantages and disadvantages. Notably, the manual method can be used to retrieve viable islets for research from pancreata that may be considered unsuitable or unlikely to yield highly viable islets for transplant by the semi-automated method. The manual method is likely to appeal to researchers who require a cost-effective and reliable technique for acquiring sufficient numbers of high-quality human islets for their research. However, a successful isolation using the semi-automated method generally results in a larger islet yield than the manual method and this technique has been approved to produce clinical grade islets for transplantation. There is an important case to

be made for having both methods available to maximise the use of limited donor tissue.

Acknowledgements

Many thanks to Dr Steve Hughes from the Islet Transplantation Research Group, University of Oxford, for his input regarding the cost of semi-automated islet isolation in his laboratory. This work was part-funded by a grant from the Rowlands Trust.

Conflict of interest

None declared.

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