Human Islet Viability and Function Is Maintained During High-density Shipment in Silicone Rubber Membrane Vessels

J.P. Kitzmann[a], A.R. Pepper[b], B. Gala-Lopez[b], R. Pawlick[b], T. Kin[b], D. O'Gorman[b], K.R. Mueller[a], A.C. Gruessner[a], E.S. Avgoustiniatos[a], T. Karatzas[a,c], G.L. Szot[d], A.M. Posselt[d], P.G. Stock[d], J.R. Wilson[e], A.M. Shapiro[b], and K.K. Papas[a,*]

[a]Department of Surgery, University of Arizona, Tucson, Arizona, United States; [b]Clinical Islet Transplant Program, University of Alberta, Edmonton, Alberta, Canada; [c]Second Department of Propedeutic Surgery University of Athens, School of Medicine, Athens, Greece; [d]Diabetes Center, University of California, San Francisco, Calif, United States; and [e]Wilson Wolf Manufacturing Corporation, New Brighton, Minn, United States

ABSTRACT

Background. The shipment of human islets (IE) from processing centers to distant laboratories is beneficial for both research and clinical applications. The maintenance of islet viability and function in transit is critically important. Gas-permeable silicone rubber membrane (SRM) vessels reduce the risk of hypoxia-induced death or dysfunction during high-density islet culture or shipment. SRM vessels may offer additional advantages: they are cost-effective (fewer flasks, less labor needed), safer (lower contamination risk), and simpler (culture vessel can also be used for shipment).

Method. IE were isolated from two manufacturing centers and shipped in 10-cm^2 surface area SRM vessels in temperature- and pressure-controlled containers to a distant center after at least 2 days of culture (n = 6). Three conditions were examined: low density (LD), high density (HD), and a microcentrifuge tube negative control (NC). LD was designed to mimic the standard culture density for IE preparations (200 IE/cm^2), while HD was designed to have a 20-fold higher tissue density, which would enable the culture of an entire human isolation in 1–3 vessels. Upon receipt, islets were assessed for viability (measured by oxygen consumption rate normalized to DNA content [OCR/DNA)]), quantity (measured by DNA), and, when possible, potency and function (measured by dynamic glucose-stimulated insulin secretion measurements and transplants in immunodeficient B6 $Rag^{+/-}$ mice). Postshipment OCR/DNA was not reduced in HD vs LD and was substantially reduced in the NC condition. HD islets exhibited normal function postshipment. Based on the data, we conclude that entire islet isolations (up to 400,000 IE) may be shipped using a single, larger SRM vessel with no negative effect on viability and ex vivo and in vivo function.

ISLET cell replacement therapy has proved to be an effective treatment for type 1 diabetes [1–4]. However, the expense of establishing and maintaining large numbers of islet manufacturing centers is prohibitive [5]. To reduce transplant recipient burden and promote research at distant establishments, it is essential to be able to ship islets in a simple, cost-effective manner while maintaining cell viability and function upon receipt [6].

Traditionally, islet shipment vessels have been solid-bottom, gas-impermeable T-flasks or 50-mL conical tubes. These proved to be insufficient due to the lack of oxygen diffusion through the vessels and the tendency of the islets to form a pellet, resulting in a hypoxic or even anoxic environment [7–9]. Recently, islet shipment in gas-permeable cell culture bags (CCB) was shown to be an improvement over the traditional methods [7,8]. However, CCB may not completely prevent anoxia in cultured or shipped islets [9]. In

*Address correspondence to Klearchos K. Papas, PhD, Institute for Cellular Transplantation, Department of Surgery, University of Arizona, 1656 E Mabel St, Room 121, Tucson, AZ 85724. E-mail: kkpapas@surgery.arizona.edu

0041-1345/14/$–see front matter
http://dx.doi.org/10.1016/j.transproceed.2014.06.002

addition, the targeted islet surface density (measured in islet equivalent [IE]/cm^2) in CCB would require the use of multiple bags to ship an entire human islet preparation, especially for less-pure preparations. For example, using standard shipping protocols as established by the Integrated Islet Distribution Program, a highly purified clinical islet preparation of 400,000 IE would require 20 bags [10]. This is cost-prohibitive, increases the possibility of contamination, and requires significant time and effort to load the islets preshipment and recombine them postshipment.

Gas-permeable silicone rubber membrane (SRM) vessels allow oxygen transfer per unit area at a 100-fold higher rate than CCB at 22°C and have demonstrated the ability to maintain a higher islet viability while in culture at a high density (HD) surface coverage of 4000 IE/cm^2 compared to standard density. In addition, the design of SRM vessels allows for higher media depth, reducing the chance of nutrient deprivation during shipment [9,11]. We hypothesized that the use of SRM vessels would allow human islets to be shipped in an HD (4000 IE/cm^2) condition while maintaining viability and function upon receipt.

METHODS

Human islet cells from clinical-grade pancreata were isolated and purified using standard techniques [1,12,13] in two islet manufacturing centers (University of California San Francisco, Calif, United States and University of Alberta, Edmonton, Alberta, Canada). Islets utilized in shipping studies were deemed not fit for transplant, either due to a low yield and/or low purity (n = 5), or due to being the product of a research isolation (n = 1). After 2–3 days in standard culture, islets were shipped overnight to the University of Arizona in temperature- and pressure-monitored and controlled boxes [14]. Islets were shipped in 10-cm^2 surface area SRM vessels in two conditions: low density (LD) targeted at 200 IE/cm^2 (mimicking standard culture density), HD targeted at 4000 IE/cm^2, and a negative control (NC) of 5000 IE in a 1.5-mL microcentrifuge tube was included. All shipment densities were adjusted for purity (ie, 2000 IE/cm^2 for a 50% pure product in HD).

Upon receipt, islets were assessed for quantity by a fluorescent double-strand DNA (dsDNA) assay (Quant-iT PicoGreen dsDNA Assay Kit, Invitrogen, Life Technologies Corporation, Grand Island, NY, United States) and for viability by measuring oxygen consumption rate normalized to DNA content (OCR/DNA; nmol O_2/min • mg DNA) using a 175-μL fluorescence lifetime micro oxygen monitoring system (Instech Laboratories Inc, Plymouth Meeting, Penn, United States) and techniques described elsewhere [15–18]. Furthermore, one shipment of islets was returned the next day to the manufacturing center and assessed again using the aforementioned techniques, and additionally assessed by membrane integrity staining for viability, and by glucose-stimulated insulin secretion (GSIS), and immunodeficient B6 Rag$^{-/-}$ mice (B6.129S7-*Rag1*$_{tm1Mom}$/J; Jackson Laboratories, Bar Harbor, ME, USA) transplants using standard methods for function and potency [19,20].

Statistical Methods

Comparisons between conditions were examined using the Friedman test with $\alpha = .05$. Statistical analyses were completed using SAS statistical software package (v9.3, SAS Institute Inc, Cary, NC, United States) or GraphPad Prism software (v5.03, GraphPad Software Inc, La Jolla, Calif, United States).

RESULTS

No shipments were exposed to an extreme fluctuation of internal temperature (minimum: 11°C, median: 17°C, maximum: 24°C) as monitored by the temperature recorders within the containers (HOBO U12 4-Channel External Data Logger, Onset Computer Corp, Bourne, Mass, United States). The distance from the shipping to receiving center ranged from 750–3000 miles, and the purity of the islets ranged from 45% –65%. Aggregation of islets into large clumps that could be easily broken up with gentle pipetting was noted in most flasks.

The results of the assays are summarized in Table 1. In comparison to LD, on average HD had a 20-fold higher IE density based on counts and a 13-fold higher tissue density as measured by DNA. HD maintained viability as measured by OCR/DNA. Membrane integrity staining showed a comparable viability. NC was significantly lower in viability as measured by OCR/DNA ($P = .03$). One shipment included multiple LD and HD vessels, which allowed for a second return shipment to the manufacturing center. This provided an opportunity to assess OCR/DNA preshipment and postshipment, which showed comparable values for LD and HD (Table 1). After a total of 4 days in transit, HD islets exhibited normal GSIS and were able to reverse diabetes when transplanted in B6 Rag$^{-/-}$ mice (n = 1).

DISCUSSION

Having the capacity to effectively transport human islets for clinical and research purposes is an important consideration for clinical islet transplantation [5] and for the field of diabetes research. It will allow hospitals that do not have the capability to maintain an islet manufacturing center to receive high-quality products. It is essential to be able to maintain islet viability and function during shipment while using a simple, cost-effective, and safe method of transportation. Traditional

Table 1. Comparison of 3 Shipped Human Islet Conditions for Cell Quantity and Viability and Preshipment/Postshipment Comparison

	Cell Quantity Comparison (n = 6)		Viability Comparison (n = 6)		Preshipment/Postshipment Comparison (n = 1)	
	IE/flask (counts)	Tissue/flask (ng DNA)	OCR/DNA	Membrane integrity	Preshipment OCR/DNA	Postshipment OCR/DNA
10 cm^2 SRM low density (LD)	1103 ± 215	50,648 ± 24,209	96.5 ± 5.7	80%	114.1 ± 4.8	107.0 ± 11.0
10 cm^2 SRM high density (HD)	22,061 ± 4298	681,077 ± 347,433	112.0 ± 6.6	79%	134.8 ± 10.7	123.3 ± 12.5
1.5 mL tube negative control	2757 ± 536	81,086 ± 31,924	60.4 ± 10.4	64%	49.4 ± 8.3	88.0 ± 1.6

Results are shown as mean ± SD (counts and DNA) or SEM (OCR/DNA). There was a 20-fold higher density between LD and HD as measured by counts and a 13-fold higher density on average as measured by DNA. There was comparable viability as measured by OCR/DNA between LD and HD and a significant decrease in viability between LD and NC ($P = .03$).

methods of islet shipment, including T-flasks and 50-mL conical tubes, have proven to be ineffective. While recently introduced gas-permeable CCB are an improvement, up to 20 CCB may be required for the shipment of an entire clinical preparation (400,000 IE) based on current standard protocols [10], and they may not prevent anoxia during shipment [7–9]. Other methods investigated have included alginate encapsulation prior to shipment [21]. However, in addition to requiring an extra nontrivial step (encapsulation), this method currently is not applicable for clinical preparations. Using vessels with highly gas-permeable SRM bottoms (which allow a 100-fold higher rate of oxygen transfer than currently used CCB and for higher nutrient availability during shipment) may be a way to easily ship a high quantity of islets while maintaining quality. In addition, the vessels can function as a culture device and were shown to maintain islet viability in HD culture [11]. One limitation that applies to all shipping vessels being considered is a change in orientation during shipment that can cause islets to sediment in one of the corners of a CCB or conical tube, or in the cap of an SRM if upside down. In the current study, we did not observe a decline in viability during shipment with SRM vessels, which suggests that this issue was not encountered. However, in transport for clinical IT where the risk of a reduction in islet viability and function should be minimized, a gyroscopic device can be used to maintain vessel orientation. Gyroscopic devices will be investigated with future human islet shipments and in controlled laboratory experiments in which device orientation will be purposely changed with or without a gyroscope to investigate any negative effects on islet viability and function.

Islet cells shipped in a 20-fold higher density did not exhibit reduced viability in comparison to standard shipping density. However, we observed a marked decrease in viability in the NC, which demonstrates the capability of the OCR/DNA assay to measure differences. Perhaps more impressively, islets that were in transit for 4 days in the HD condition returned to the manufacturing center exhibited normal GSIS, which demonstrates that they were able to retain function. In addition, these islets were able to successfully reverse diabetes in B6 $Rag^{-/-}$ mice transplants, considered the gold standard in assessing islet efficacy.

CONCLUSION

Shipping islets in SRM vessels may allow for a 20-fold higher density than standard methods, while maintaining viability, function, and efficacy. This may allow the transport of an entire clinical islet isolation (400,000 IE) using a single vessel. In contrast, it would require 20 T-175 flasks or gas-permeable CCB using standard shipping protocols. Cell quality may be further retained by using gyroscopic devices to maintain vessel orientation during transit.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the NIH/NIDDK Phase II SBIR DK069865.

REFERENCES

[1] Shapiro AMJ, Ricordi C, Hering BJ, et al. International trial of the Edmonton protocol for islet transplantation. N Engl J Med 2006;355:1318–30.

[2] Bellin MD, Barton FB, Heitman A, et al. Potent induction immunotherapy promotes long-term insulin independence after islet transplantation in type 1 diabetes. Am J Transplant 2012;12:1576–83.

[3] Barton FB, Rickels MR, Alejandro R, et al. Improvement in outcomes of clinical islet transplantation: 1999-2010. Diabetes Care 2012;35:1436–45.

[4] Posselt AM, Szot GL, Frassetto LA, et al. Islet transplantation in type 1 diabetic patients using calcineurin inhibitor-free immunosuppressive protocols based on T-cell adhesion or costimulation blockade. Transplantation 2010;90:1595–601.

[5] Papas KK, Karatzas T, Berney T, et al. International workshop: islet transplantation without borders enabling islet transplantation in Greece with international collaboration and innovative technology. Clin Transplant 2013;27:E116–25.

[6] Kaddis JS, Hanson MS, Cravens J, et al. Standardized transportation of human islets: an islet cell resource center study of more than 2,000 shipments. Cell Transplant 2013;22:1101–11.

[7] Ichii H, Sakuma Y, Pileggi A, et al. Shipment of human islets for transplantation. Am J Transplant 2007;7:1010–20.

[8] Ikemoto T, Matsumoto S, Itoh T, et al. Assessment of islet quality following international shipping of more than 10,000 km. Cell Transplant 2010;19:731–41.

[9] Avgoustiniatos ES, Hering BJ, Rozak PR, et al. Commercially available gas-permeable cell culture bags may not prevent anoxia in cultured or shipped islets. Transplant Proc 2008;40:395–400.

[10] Integrated Islet Distribution Program, City of Hope. Standard operating procedure (SOP) standardized packing of IIDP shipping container used for research islet distribution and the return of packaging materials. Version: SHP-001–008. Available at: http://iidp.coh.org/docs/SOPs/Shipping/SHP%20001.pdf. Accessed April 10, 2013.

[11] Papas KK, Avgoustiniatos ES, Tempelman LA, et al. High-density culture of human islet on top of silicone rubber membranes. Transplant Proc 2005;37:3412–4.

[12] O'Gorman D, Kin T, Imes S, et al. Comparison of human islet isolation outcomes using a new mammalian tissue-free enzyme versus collagenase NB-1. Transplantation 2010;80:255–9.

[13] Barbaro B, Salehi P, Wang Y, et al. Improved human pancreatic islet purification with the refined UIC-UB density gradient. Transplantation 2007;84:1200.

[14] Rozak PR, Weegman BP, Avgoustiniatos ES, et al. Devices and methods for maintenance of temperature and pressure during islet shipment. Transplant Proc 2008;40:407–10.

[15] Papas KK, Pisania A, Wu H, et al. A stirred microchamber for oxygen consumption rate measurements with pancreatic islets. Biotechnol Bioeng 2007;98:1071–82.

[16] Papas KK, Colton CK, Nelson RA, et al. Human islet oxygen consumption rate and DNA measurements predict diabetes reversal in nude mice. Am J Transplant 2007;7:707–13.

[17] Colton CK, Papas KK, Pisania A, et al. Characterization of islet preparations. In: Halberstadt C, Emerich DF, editors. Cellular Transplantation: From Laboratory to Clinic. Waltham, Massachusetts: Academic Press; 2007. pp. 85–134.

[18] Papas KK, Suszynski TM, Colton CK. Islet assessment for transplantation. Curr Opin Organ Transplant 2009;14:674–82.

[19] Barnett MJ, McGhee-Wilson D, Shapiro AM, et al. Variation in human islet viability based on different membrane integrity stains. Cell Transplant 2004;13(5):481–8.

[20] McCall M, Pawlick R, Kin T, et al. Anakinra potentiates the protective effects of etanercept in transplantation of marginal mass human islets in immunodeficient mice. Am J Transplant 2011;12:322–9.

[21] Vaithilingam V, Barbaro B, Oberholzer J, et al. Functional capacity of human islets after long-distance shipment and encapsulation. Pancreas 2011;40(2):247–52.