Title

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Journal

Transplantation Proceedings, 46(6)

ISSN

0041-1345

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Publication Date

2014-07-01

DOI

10.1016/j.transproceed.2014.06.002

Peer reviewed

Published in final edited form as:
Transplant Proc. 2014 ; 46(6): 1989–1991. doi:10.1016/j.transproceed.2014.06.002.

Human islet viability and function is maintained during high density shipment in silicone rubber membrane vessels

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Abstract

The shipment of human islets 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). Human islets(IE) were isolated from two manufacturing centers and shipped in 10cm^2 surface area SRM vessels in temperature and pressure controlled containers to a distant center following at least two days of culture (n = 6). Three conditions were examined: low density (LD), high density (HD), and a micro centrifuge tube negative control (NC). LD was designed to mimic the standard culture density for human islet 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.

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Upon receipt, islets were assessed for viability, measured by oxygen consumption rate normalized to DNA content (OCR/DNA), and quantity, measured by DNA, and, when possible, potency and function with dynamic glucose-stimulated insulin secretion (GSIS) measurements and transplants in immunodeficient B6 rag mice. Post-shipment OCR/DNA was not reduced in HD versus LD, and was substantially reduced in the NC condition. HD islets exhibited normal function post-shipment. 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.

Introduction

Islet cell replacement therapy has proven 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]. In order 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 addition, the targeted islet surface density (measured in islet equivalent (IE)/cm^2) in CCB would require the use of multiple bags for shipment of an entire human islet preparation, especially for less pure islet 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 pre-shipment and recombine them post-shipment. 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 4,000 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 a HD (4,000 IE/cm^2) condition while maintaining viability and function upon receipt.

Methods

Human islets from clinical-grade pancreata were isolated and purified using standard techniques [1, 12–13] in two islet manufacturing centers (University of California San Franciscoin the United States and University of Alberta Edmontonin 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) and high density (HD) targeted at 4,000 IE/cm^2. Additionally, a negative control of 5,000 IE in a 1.5 mL micro centrifuge tube was included. All shipment densities were adjusted for purity (i.e., 2,000 IE/cm^2 for a 50%pure product in HD).

Upon receipt, islets were assessed for quantity by a fluorescent dsDNA assay (Quant-iTPicoGreendsDNA Assay Kit, Invitrogen, Life Technologies Corporation, Grand Island, NY, USA) 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, PA, USA) and techniques described elsewhere [15–18]. Furthermore, one shipment of islets was returned the following 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 B6 rag mice transplants using standard methods for function and potency [19, 20].

Statistical methods

Comparisons between conditions were examined using the Friedman test with $\alpha = 0.05$. Statistical analyses were completed using SAS statistical software package (v9.3, SAS Institute Inc., Cary, NC, USA) or GraphPad Prism software (v5.03, GraphPad Software Inc., La Jolla, CA, USA).

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 Corporation, Bourne, MA, USA). The distance from the shipping to receiving center ranged from 750 to 3,000 miles and the purity of the islets ranged from 45 to 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 1a. In comparison to LD, HD had a 20-fold higher IE density based on counts and a 13-fold higher tissue density on average, 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 ($p = 0.03$).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 pre- and post-shipment, which showed comparable values for LD and HD (Table 1b). After a total of four 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 in the field of diabetes research. It will allow for hospitals which do not have the capabilities of maintaining 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 methods of islet shipment, including T-flasks and 50 mL conical tubes, have proven to be ineffective. While recently introduced gas-permeable cell culture bags (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, this method currently could not be applicable for clinical preparations, in addition to requiring an extra non-trivial step (encapsulation). 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 away 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 high density culture [11]. One limitation which applies to all shipping vessels considered is a change in orientation during shipment that can cause islets to sediment in one of the corners of a CCB or conical, 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 islet transplantation where the risk of a reduction in islet viability and function should be minimized, a gyroscope 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.

Islets shipped in a 20-fold higher density did not exhibit reduced viability in comparison to standard shipping density. However, we were able to observe a marked decrease in viability in the negative control, which demonstrates the capability of the OCR/DNA assay to measure differences. Perhaps more impressively, islets which were in transit for four days in the HD condition 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. In 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 cell culture bags using standard shipping protocols. Cell quality may be further retained by using gyroscopic devices to maintain vessel orientation during transit.

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Table 1

a) Comparison of three shipped human islet conditions for cell quantity as measured by counts (IE/flask) and nanograms of DNA (tissue/flask), and viability as measured by OCR/DNA and membrane integrity stain. Results are shown as mean ±standard deviation (SD) or standard error of the mean (SEM). 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 ($p = 0.03$). b) Comparison of pre-shipment to post-shipment

	Table 1a				Table 1b	
	IE/flask (counts)	**Tissue/flask (ngDNA)**	**OCR/DNA**	**Membrane integrity**	**Pre-shipment OCR/DNA**	**Post-shipment OCR/DNA**
10 cm^2 SRM Low Density	1,103±215	50,648±24,209	96.5±5.7	80%	114.1±4.8	107.0±11.0
10 cm^2 SRM High Density	22,061±4,298	681,077±347,433	112.0±6.6	79%	134.8±10.7	123.3±12.5
1.5 mL tube Negative Control	2,757±536	81,086±31,924	60.4±10.4	64%	49.4±8.3	88.0±1.6

OCR/DNA for all three conditions (n = 1).