Published in final edited form as:

Transplant Proc. 2008 March ; 40(2): 407–410. doi:10.1016/j.transproceed.2008.01.060.

Devices and Methods for Maintenance of Temperature and Pressure During Islet Shipment

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Abstract

Exposure to extreme temperatures and pressure fluctuations during shipment by air may have a detrimental impact on islet quality. In this study, we sought to assess the ability of methods and devices to provide better control of the internal environment of islet shipping containers in terms of temperature and pressure.

Methods—Experimental islet shipping containers were packed with 21 panels of commercially available TCP Phase 22 Phase Change Material (TCP). The containers were then exposed for at least 15 hours to three constant external temperature conditions, namely, -20°C , 4°C , and 40°C , and then evaluated for their ability to maintain an internal temperature close to the desired value of 22°C . Custom-designed pressure regulated gyroscopic shipping containers (PRGSC) placed in a vacuum chamber were exposed to an absolute pressure of 250 mm Hg (substantially lower than that experienced during shipment by air) for 25 minutes to assess their ability to control internal pressure under vacuum. Electronic data loggers were used to monitor internal and external temperatures and pressures under all conditions.

Results—Twenty-one TCP panels placed in a single islet shipping container were able to maintain the internal temperature between 17°C and 24°C for a minimum of 15 hours at all three external temperatures. The PRGSC tested were able to maintain a constant internal pressure of 760 mm Hg when exposed to vacuum.

Conclusions—Our results demonstrated that the use of containers equipped with TCP and PRGSC exert excellent environmental control over islet shipments by minimizing temperature and eliminating pressure fluctuations.

Islet preparations are sensitive to temperature extremes. Although the effects of pressure changes on islets have not been studied directly, it has been suggested that pressure fluctuations during islet shipment by air may have a negative impact on islet integrity and quality. In addition, some shipping vessels may not be able to withstand pressure fluctuations and thus either deform or break. Frequently, islets are shipped in a Styrofoam container with cooling packs.¹ However, cooling packs may accelerate freezing when islets are shipped to cold climates or during the winter. Overall, cooling temperatures may be detrimental to islet viability and therefore undesirable. Additionally, standard Styrofoam containers alone neither insulate well against temperature extremes (hot/cold) nor regulate internal pressures during shipment. Hence, there is a need to develop methods to control the internal environment of islet shipping

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containers while they are exposed to extreme external temperatures and pressure fluctuations, with the ultimate goal to make shipment an extension of islet culture.

MATERIALS AND METHODS

Temperature Control Investigation

Experimental container 1 consisted of a corrugated plastic package containing 21 TCP Phase 22 Phase Change Material panels (TCP, Part# FGEL00052, TCP Reliable, Edison, NJ) weighing 6.3 kg, Styrofoam walls 4 inches thick, and plastic bubble wrap to keep the culture vessel in place. Shipping containers were prepared for three separate temperature conditions, -20°C , 4°C , and 40°C . A Kenmore upright freezer (Model# 106.8263511, Sears Holdings Corp., Hoffman Estates, Ill) was used for all -20°C conditions. The freezer temperature oscillated between -20°C and -30°C at an approximate rate of 1 cycle/hour. A Fisher scientific refrigerator (Isotemp Model, Thermo Fisher Scientific, Inc., Waltham, Mass) was used for all 4°C conditions. A Heraeus water-jacketed incubator with 5% CO_2 was used for all 40°C conditions (6000 Model, Thermo Fisher Scientific). The TCP panels were appropriately primed for each temperature. Panels designated for -20°C and 4°C were fully liquified by incubation at 37°C for 6 hours before packaging. Panels designated for 40°C were fully solidified by cooling them at 4°C for 6 hours before packaging. Culture vessels were loaded with 50 mL of distilled deionized water and packed into the shipping containers (one vessel per container). Internal container temperatures were recorded using HOBO electronic temperature loggers (Part# H08-007-02, Onset, Pocasset, Mass) placed next to the culture vessels. The containers were closed and placed in the -20°C freezer, the 4°C refrigerator, or the 40°C incubator. A second set of HOBO electronic temperature loggers were placed outside each of the boxes and recorded the external surface temperatures of each shipping container. The containers were exposed to the experimental temperatures for 15 hours, which is the approximate time of an overnight shipment. After exposure, the containers were removed and the temperature data analyzed.

Experimental container 2 was modeled after a model recently evaluated for standardization of shipments of human research islets by the Islet Cell Resource (ICR) Center Consortium. It consisted of a ThermoSafe EPS Insulated Shipper (Model# 355, Arlington Heights, Ill), 2 ThermoSure Temperature Stabilizer gel packs (TS, Model# 1290, Sebra, Tucson, Ariz) weighing 1.6 kg, and plastic bubble wrap to keep the culture vessel in place. The containers were primed according to the manufacturer's instructions, loaded, and exposed to the above environmental temperatures. Data were recorded in the same manner as for the TCP containing packages.

A ThermoSafe EPS Insulated Shipper (Model# 413) with Styrofoam walls was packed with plastic bubble wrap, a culture vessel, and a HOBO electronic temperature logger. It was used as a reference container to simulate shipping containers routinely used for distribution of islets in the past. This reference container was exposed to the three experimental temperatures for 8 hours. A second HOBO electronic temperature logger was placed outside the reference container to record external temperatures for each condition. The reference container was also used to record temperatures and pressures during actual islet shipments from the University of Minnesota (UMN) to five investigators around the United States. The data from these shipments are presented in Fig 1.

Pressure Control Investigation

The pressure regulated gyroscopic shipping container (PRGSC, Part# PR-AY-11-0007, Wilson Wolf Manufacturing Corporation, New Brighton, Minn) was designed in a collaboration between the UMN and Wilson Wolf Manufacturing Corporation. The PRGSC was loaded with

the same culture vessel used in the temperature investigation and a HOBO absolute pressure logger (Part# HPA-0015), sealed, and placed in a custom-designed acrylic vacuum chamber (Donovan Specialties, Osseo, Minn). A second HOBO pressure logger was placed on top of the PRGSC to measure external pressure. The vacuum chamber was sealed and an attached Cole-Parmer vacuum pump (Cat. # K-07061-40, Cole-Parmer Instrument Co., Vernon Hills, Ill) was used to reduce the absolute pressure to 250 mmHg (about 150 mmHg lower than the lowest pressure observed during shipment, as shown in Fig 1B) for 25 minutes. After vacuum exposure, the PRGSC was removed for analysis of the pressure data.

RESULTS

Internal temperatures and pressures recorded over the course of actual islet shipments with the reference container varied substantially (Fig 1). The variations in temperature depended on several factors such as the climate of the shipping and receiving centers, the season, and the conditions of air transit, ground transit, and warehouse storage. Pressure fluctuations depended on the number of connecting flights used per shipment and the use of pressurized versus nonpressurized flight environments.

When experimental container 1 containing TCP was exposed to temperatures between -20°C and -30°C for 15 hours, it maintained an internal temperature between 21°C and 24°C (Fig 2A). In comparison, the internal temperature of experimental container 2 with TS dropped from this range within 2 hours and reached 0°C after 12 hours of exposure (Fig 2A). Experimental container 1 with TCP maintained a stable internal temperature of 22°C for longer than 24 hours when exposed to 4°C (Fig 2B). Experimental container 2 with TS exposed to 4°C was unable to maintain an internal temperature close to 22°C . The temperature fell as low as 15°C before equilibrating at 17°C after 6 hours (Fig 2B). Experimental container 1 containing TCP exposed to 40°C for 15 hours maintained an internal temperature between 17°C and 22°C (Fig 2C). In contrast, experimental container 2 was able to maintain this temperature range for less than 4 hours and the internal temperature was within 3 degrees of the ambient by the end of 15 hours (Fig 2C).

During the PRGSC trial, the pressure inside the vacuum chamber was reduced from 760 to 250 mmHg within 1 minute and maintained at that level for over 25 minutes. Despite the large drop in external pressure, the PRGSC maintained a constant internal pressure of 760 mmHg (Fig 3).

DISCUSSION

Data obtained from actual islet shipments in reference containers during March of 2004 indicated that shipped islets were exposed to temperatures that ranged between 0°C and 32°C (Fig 1A). Additionally, the reference containers experienced pressure drops as much as 200 mmHg during these shipments (Fig 1B). The environmental conditions used in this study simulate conditions that may occur during overnight islet shipments by air. Ground transit on trucks during summer or in warmer climates can result in exposure of shipped islets to external temperatures at least as high as 40°C . At the opposite extreme, nonpressurized flights or warehouses without temperature control during winter can expose shipped islets to subfreezing temperatures. Islet shipments on nonpressurized flights can also experience low pressures (Fig 1B).

More recently adopted packaging, such as experimental container 2 with 2 TS units, is better than previously used Styrofoam reference containers with or without cooling packs, but it is still inadequate for maintaining internal temperature under realistic shipping conditions from various islet isolation centers. During extreme heat exposure, the internal temperature of

experimental container 2 came within 3 degrees of the external temperature after 15 hours. Furthermore, when exposed to extreme cold, the internal temperature of experimental container 2 fell below -10°C . It is undesirable for investigators to receive tissue exposed to extreme temperatures; freezing can lead to reduced viability, whereas overheating can lead to increased metabolic demand, nutrient depletion, increased accumulation of toxic metabolic byproducts, and ultimately cell death.

It should be noted that an exact comparison between the two phase-change materials, TS and TCP, was not conducted in this investigation. The number of TS packs used was chosen to simulate test containers evaluated by the ICR Center Consortium. Increasing the number of TS packs used could further improve the performance of a shipping container based on this phase-change material. The density, compactness, and pricing of TS will determine in part the practicality of increasing the number of TS for achieving better temperature control.

The data suggest that the target shipping temperature of $22 \pm 2^{\circ}\text{C}$ can be maintained using corrugated plastic shipping containers packed with properly primed TCP. Even when exposed to extreme external temperatures, experimental container 1 with TCP maintained moderate internal temperatures. These stable temperatures approximated the target culture temperatures for human islets 24 hours postisolation.

Future temperature control studies should focus on simultaneously protecting against both cold and hot temperature extremes. To account for seasonal differences in external temperatures, it has been suggested that liquified packs should be used in the winter and solidified packs in summer. However, this approach of priming the phase-change materials based on season is undesirable because of ambiguity during the spring and fall seasons and, more important, because temperatures can vary significantly across the route of a shipment (e.g., Minneapolis to Miami in January). Two approaches for better addressing this problem have been proposed. The first approach is to prime all packs by bringing them into a slushy (part liquid/part solid) state. However, priming may be poorly controlled with this approach as the fraction of the material in each phase cannot be accurately quantified and reproduced in all laboratory environments and situations in terms of ambient temperature, heat transfer, and timing. The second approach is to liquify a fraction of the packs to protect against cold temperatures and solidify the rest for protection against hot temperatures. The shipping container is then loaded with liquified as well as solidified packs. The fraction of each group could be defined based on the magnitude and duration of the possible temperature gradients in each direction.

The experimental results from the pressure control investigations suggest that the PRGSC can be used to maintain a stable internal pressure during islet shipment. When the external pressure was reduced to 250 mmHg, a constant atmospheric pressure of 760 mmHg was maintained internally. The PRGSC can reduce the stress upon both the culture vessels and the shipped islets, providing one more measure of protection for islet shipments.

Based on the results presented in the current manuscript we concluded that, when TCP phase-change material was used in conjunction with the PRGSC for islet shipments, greater environmental control was achieved as pressure variations were eliminated and temperature fluctuations were minimized over long periods of time. This level of environmental control is expected to result in improvements in the quality of shipped islets received by investigators in the United States and worldwide. These improvements are particularly important when shipment of islets for clinical transplantation is implemented on a larger scale.

Acknowledgments

Funded by grants from the National Institutes of Health (National Center for Research Resources, U42 RR016598, and National Institute for Diabetes and Digestive and Kidney Diseases, R43 DK069865), the Schott Foundation, and the Carol Olson Memorial Diabetes Research Fund.

The authors acknowledge Kurt Albrecht of Donovan Specialties (Osseo, Minn), Denice Dudero, Laurie Macleod, Dr Kristen Maynard, Heather Nelson, William E. Scott III, and Christine Vincent from the Diabetes Institute for Immunology and Transplantation at the UMN.

References

1. Ichii H, Sakuma Y, Pileggi A, et al. Shipment of human islets for transplantation. *Am J Transplant* 2007;7:1010. [PubMed: 17391141]

Fig 1.

Shipments using the reference container sent to five research centers from the University of Minnesota, Minneapolis, Minnesota, on March 10, 2004. The vertical dashed lines indicate one group of flight departures and arrivals. **(A)** Temperature data recorded every 4 minutes. The horizontal dashed line indicates target islet vessel temperature (22 °C). **(B)** Pressure data recorded every 4 minutes.

Fig 2. Comparison of internal temperatures for various shipping containers plotted over time under exposure to external temperatures of (A) -20°C , (B) 4°C , and (C) 40°C . The horizontal dashed lines indicate the targeted islet vessel temperature (22°C).

Fig 3.
Internal pressure for pressure regulated gyroscopic shipping container (PRGSC) when exposed to 250 mmHg absolute pressure vacuum.