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Evaluation of Human Islet-Specific Functional Quality Cultured on Different Gas-Permeable Membranes

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ABSTRACT

The aim of this study was to investigate different gas-permeable membranes for culturing human islets. Dynamic insulin release was used to assess islet functional quality. Islets isolated from cadaveric pancreata ($n = 8$) using standard isolation methods were stained with dithizone, counted, and cultured on five different commercially available medical-grade membranes reported to have high permeability to O_2, CO_2, and other gases. Fraction 1 (20,000 islet equivalents [IEQ] purity >70%; viability >85%) was cultured using serum-free medium in nonadherence tissue culture flasks (group I) and custom-made chambers with membranes (group II). Each vessel contained 5000 IEQ at a density of 30 IEQ/cm^2 and 69 IEQ/cm^2 for groups I and II, respectively. Islets were cultured for 48 to 90 hours at 37°C in 5% CO_2. In vitro dynamic insulin response to low glucose (3 mmol/L), high glucose (16.7 mmol/L), and 25 mmol/L KCI was measured. Stimulation indices were calculated by dividing average of initial response over basal insulin release; basal insulin release defined as average of the first seven values. Islets cultured on MG7 ($n = 3$) showed a higher stimulation index (3.49 ± 0.37) compared with flasks (2.44 ± 0.22), indicating better specific functional quality. Islets cultured on other membranes proved to show similar or worse functional quality than those cultured in flasks. In fact, islets cultured on MG6 ($n = 2$) were not tested owing to complete disintegration. Islet functional quality was improved when cultured on selected biocompatible gas-permeable membranes; however, finding the best membrane requires further investigation before clinical application.

ISLET CULTURE before transplantation has become a standard procedure, currently used globally by many centers after the successful establishment of the Edmonton protocol for reversing type 1 diabetes.[1,2] Culturing islets before transplantation has a great advantage; it reduces islet immunogenicity, decreases acinar tissue volume, and allows for product release testing before transplantation.[3–5]

Currently, islets cultured in serum-free medium with no animal products are supplemented with human serum albumin, insulin, transferin, selenium, nicotinamide, and Trolox.[3–5] They are usually cultured in nontissue culture flasks at a density of approximately 500 islet equivalents (IEQ) per milliliter. However, islets are micro-organ particles with an average size of 50 to 400 μm that readily settle to the bottom of flasks with limited access to air (O_2 and CO_2), which leaves them vulnerable to hypoxia.[6] Islets, particularly the large ones, often suffer from central necrosis, possibly resulting in lower islet functional quality.

Herein we have examined islets cultures on gas-permeable membranes as a way to reduce hypoxia and potentially improve islet quality. Specifically, we investigated the quality and function using dynamic insulin secretion of islets grown on five different medical-grade membranes purported to be highly permeable to O_2, CO_2, and other gases. Dynamic insulin release was used to assess islet function.

MATERIALS AND METHODS

Human Islet Isolation

Islets were isolated from cadaveric pancreata ($n = 8$) after informed consent was obtained from a close relative of the donor.

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Table 1. Stimulation Indices for Islets Cultured on Five Gas-Permeable Membranes

Group (n)	Stimulation Index
Control (7)	2.44 ± 0.58
MG2 (3)	1.68 ± 0.47
MG3 (4)	2.00 ± 0.39
MG4 (1)	2.35
MG6 (2)	ND*
MG7 (3)	3.49 ± 0.64

*Perfusion was not done because the islets did not survive the culture period.

Approval was also obtained from our Institutional Review Committee. Isolation was performed following the protocol at our center using a modified semiautomatic method.[7]

Islet Culture

Fraction I (20,000 IEQ; purity >70%; viability >85%) counted using DTZ was divided into four aliquots, which were cultured in two standard nonadherence tissue culture flasks (group I) and two custom-made chambers containing replaceable membranes reported to have high permeability to O_2, CO_2, and other gases (group II). The membranes were designated MG2 (CS Hyde company cat no. 71-MED-DSP), MG3 (Bentec Medical cat no PR72034-04N), MG4 (Specialty Silicone Products cat no SSPM823), MG6 (Biorep Technologies Infusion Bag), and MG7 (Baxter Lifecell Tissue Culture Bag, cat no R4R2111). Each vessel contained 5000 IEQ in serum-free medium at a density of 30 IEQ/cm^2 and 69 IEQ/cm^2 for groups I and II, respectively. Islets were cultured for 48 to 90 hours at 37°C in 5% CO_2.

Dynamic Insulin Secretion

From each group, 200 IEQ were placed in columns containing Bio-Gel P4 polyacrylamide beads in Hepes-Krebs buffer. The islets were perifused with 3 mmol/L then 16.8 mM glucose (plus 25mM KCL) for a total of 76 minutes. Eluted samples were collected insulin was measured using an ELISA kit (ALPCO, Salem, NH).

RESULTS

Basal insulin release was determined as the average of the first seven values from islets perifused in low glucose (3 mmol/L). The average high glucose response (16.8 mmol/L) for the first seven insulin release values were used to calculate the stimulation index by dividing this value over the basal insulin.

Table 1 shows the stimulation indices for islets cultured on the five gas-permeable membranes. MG7 showed the highest index compared with control islets cultured in standard nontissue culture flasks. Islets cultured on the MG6 membrane did so poorly that none remained for postculture evaluation.

DISCUSSION

Islets were placed on medical-grade, gas-permeable membranes at approximately double the concentration to test the likelihood that such cultures would allow them to remain functionally viable compared with those exposed to lower concentrations in flasks. One of five membranes tested yielded islets of superior functional quality postculture. The functional quality of islets cultured on the other three membranes was similar to or worse than those cultured in standard flasks. In fact, one membrane resulted in complete disintegration of islets, leaving nothing to be evaluated by dynamic insulin secretion.

Islets are hypoxia sensitive and metabolically active; therefore, having optimal nutrients and increased access to oxygen is of prime importance to achieve maximum islet function without compromising quality. A previous study also reported the benefit, as measured by improved oxygen consumption.[4] Therefore, cultures with a biocompatible membrane may have an advantage over flasks to maintain islet quality and minimize hypoxia, allowing greater numbers of islets to be cultured in an even smaller surface area.

In conclusion, culturing islets on gas-permeable membranes has several advantages. First, a minimal number of vessels can be used for culture of an entire fraction, thus reducing the likelihood of contamination. Second, islets may be cultured for longer periods with minimal loss of function and integrity, which would allow pooling of islets from multiple donors for transplantation. Third, long- term culture reduces islet immunogenicity and tissue volume. Additional work remains to identify the best biocompatible membrane before clinical application.

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