Improved Islet Culture on Top of Gas-Permeable Membranes

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Abstract

Background. Current islet culture practice requires islet seeding at surface densities below 200 islet equivalents (IE)/cm^2 and medium dilutions of no more than 1 µl/IE to prevent oxygen diffusion limitations. These conditions require more than 20 flasks to culture a porcine or human islet preparation and media to be changed every two days. Islet culture on top of gas-permeable (GP) membranes could alleviate the problem of limited oxygenation and thus allow culture at higher surface densities and media depths without frequent media changes.

Methods. Culture vessels with gas-permeable 100 µm thick silicone rubber membrane bottoms were constructed and compared to standard flasks for culturing porcine and human islets for 2 days. Seeding islet surface density was varied. Increased medium dilutions and 7-day culture were also tested with the GP devices. Islet fractional viability was assessed using oxygen consumption rate per DNA content (OCR/DNA), viable tissue recovery by total OCR, and potency with the nude mouse bioassay (2000 IE per mouse).

Results. Islets cultured in GP devices at surface densities between 1000 and 4000 IE/cm^2 had statistically similar fractional viability, higher viable tissue recovery (p=0.04), and similar (100%) diabetes reversal rates in nude mice as control islets (T-flask, 200 IE/ cm^2, 1 µl/IE). In contrast, islets cultured at surface densities higher than 1000 IE/cm^2 in non-GP devices had lower fractional viability (p=0.02) and viable tissue recovery (p=6·10^{-4}) relative to control, and failed to reverse diabetes in nude mice. Dilutions up to at least 4 µl/IE, media heights up to at least 4 cm, and 7-day culture without media change did not have a negative effect on islets cultured in GP devices.

Conclusions. Islet surface density in GP devices was increased 20-fold relative to standard conditions with no detrimental effects in islet fractional viability, viable tissue recovery, or potency. Duration of culture without media change was extended to at least one week, and culture of an entire isolation in a single device was made possible.

Objective

To compare the quality, potency, and recovery of high-purity porcine and human research islets when cultured with standard methods or in GP devices.

Hypotheses

- Islets can be cultured in gas-permeable (GP) devices at higher seeding density and dilution without loss of viability or function relative to standard culture at 200 IE/cm^2 and 1 µL/IE (1000 IE/mL).
- 7-day culture in GP devices can be carried without media changes.

Theoretical Background

N = Oxygen Transfer Rate (mol/s)

A = Area (cm^2)

D = Oxygen Diffusivity (cm^2/s)

α = Oxygen Solubility (mol/cm^3·mm Hg)

$D{\cdot}\alpha$ = Oxygen Permeability (mol/cm·mm Hg·s)

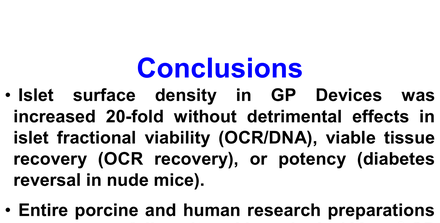
pO_2 = Oxygen Partial Pressure (mm Hg)

x = Distance (cm)

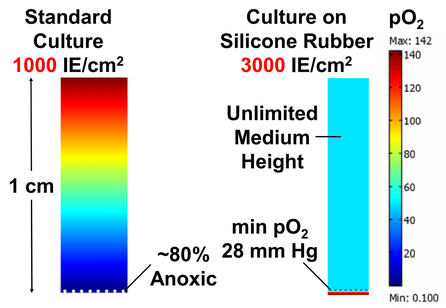
$$N = A \cdot D\alpha \cdot \frac{dpO_2}{dx} \cong A \cdot D\alpha \cdot \frac{Air\ pO_2}{x}$$

Standard Culture Large x => Low N

GP Membrane Small x => High N



Simulation Results



Methods

Islet Surface Density:
Varied from 200 IE/cm^2 to 4000 IE/cm^2 (~confluent islet monolayer).
Results expressed using DNA IE (equivalent to 11.4 ng DNA).

Islet Volume Density:
1000 IE/mL (1 µL/IE) for non-GP culture
250-1000 IE/mL (1-4 µL/IE) for culture on GP membranes

Culture Duration:
2 or 7 days. 2 days between media changes for culture in non-GP devices, no media changes for culture on GP membranes

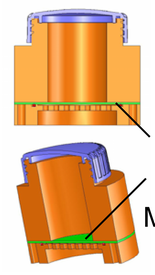
Quality Assessment (QA):
Oxygen consumption rate (OCR) per DNA content – Fractional viability
Total OCR recovery – Viable tissue recovery
Nude mouse bioassay - Potency

Statistical Analysis:
Results scaled with day 2 non-GP control (200 IE/cm^2)
Two-sided paired t-test for OCR/DNA, OCR Recovery
Fisher's exact test for nude mouse bioassay

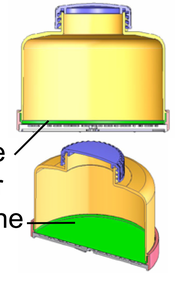
Non-GP T-Flask

Device with GP Silicone Rubber Bottom

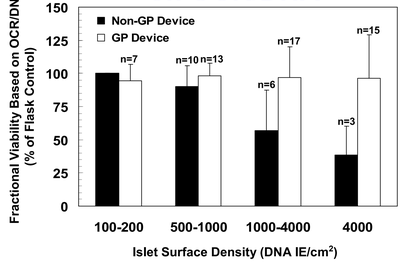
10 cm^2 GP Device



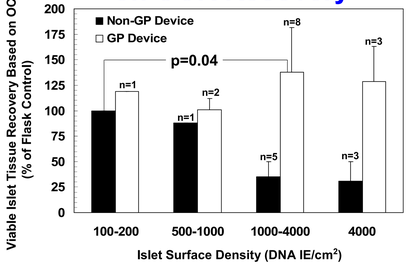
100 cm^2 GP Device



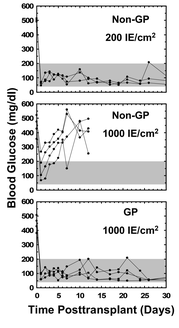
Effect of Islet Surface Density on OCR/DNA



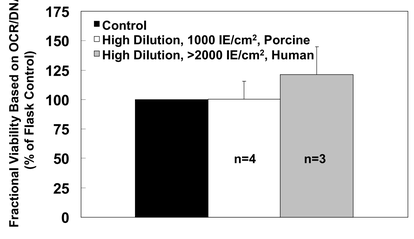
Effect of Islet Surface Density on OCR Recovery



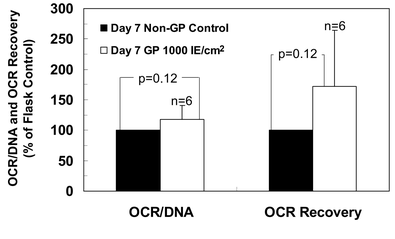
Nude Mouse Bioassay



OCR/DNA not Affected by Dilutions Higher than 1 µL/IE



7-Day Culture is Possible on GP Membranes with No Media Changes



Conclusions

- Islet surface density in GP Devices was increased 20-fold without detrimental effects in islet fractional viability (OCR/DNA), viable tissue recovery (OCR recovery), or potency (diabetes reversal in nude mice).
- Entire porcine and human research preparations were successfully cultured in a single 100 cm^2 GP device.
- Islets can be cultured in GP devices at dilutions at least as high as 4 µL/IE without detrimental effects.
- Higher dilutions allowed 7-day culture in GP devices without media changes.

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