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Isolated human islets require hyperoxia to maintain islet mass, metabolism, and function

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ABSTRACT

Pancreatic islet transplantation has been recognized as an effective treatment for Type 1 diabetes; however, there is still plenty of room to improve transplantation efficiency. Because islets are metabolically active they require high oxygen to survive; thus hypoxia after transplant is one of the major causes of graft failure. Knowing the optimal oxygen tension for isolated islets would allow a transplant team to provide the best oxygen environment during pre- and post-transplant periods. To address this issue and begin to establish empirically determined guidelines for islet maintenance, we exposed *in vitro* cultured islets to different partial oxygen pressures (pO_2) and assessed changes in islet volume, viability, metabolism, and function. Human islets were cultured for 7 days in different pO_2 media corresponding to hypoxia (90 mmHg), normoxia (160 mmHg), and hyerpoxia (270 or 350 mmHg). Compared to normoxia and hypoxia, hyperoxia alleviated the loss of islet volume, maintaining higher islet viability and metabolism as measured by oxygen consumption and glucose-stimulated insulin secretion responses. We predict that maintaining pre- and post-transplanted islets in a hyperoxic environment will alleviate islet volume loss and maintain islet quality thereby improving transplant outcomes.

1. Introduction

Pancreatic islet transplantation is an effective treatment for Type 1 diabetes and allows patients to be free from insulin injections and uncontrollable hypoglycemic episodes [1–3]. Currently, isolated islets are infused into the liver through the portal vein; however, more than 50% of injected islets are destroyed because of a hostile microenvironment caused by blood-mediated inflammation reactions, low oxygen tension, or high levels of glucose and toxins [3–5]. Transplantation of isolated islets at extrahepatic sites face similar issues, and these sites often present poor vascularity and hypoxia [6,7]. Improved oxygenation to the transplantation site might be a promising solution to prevent islet loss; however, the optimal oxygen tension for isolated islets remains unknown.

In the native pancreas, islets are exposed to a high partial oxygen pressure (pO_2: approximately 40 mmHg) and receive more blood flow than the rest of the pancreatic tissue [3,7], indicating that islets consume more oxygen because of their high metabolic activity [6]. Unlike whole organ transplants which largely preserve capillaries, islets used for transplantations are isolated by enzymatic and mechanical digestion which destroys the islet capillary network [8]. According to current practices, after isolation islets are cultured for a short period under 21% oxygen and 5% CO_2 at one atmospheric pressure, and the pO_2 of the media is equilibrated to a predicted 160 mmHg. Equilibration and the predicted pO_2 value are calculated mathematically without considering the oxygen consumed by active islets. Although the predicted pO_2 is four times higher than that reported in the native pancreas, islet central necrosis often develops. This suggests that the oxygen requirement for isolated islets differs from that in the native pancreas possibly due to environmental or structural changes. In this regard, it remains critically important to determine the optimal pO_2 that maintains the health of isolated islets pre-transplantation.

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Providing the optimal pO_2 is also crucial post-transplantation. Regardless of the transplant sites, islets have to rely on the oxygen within surrounding tissue until newly formed microcapillaries can begin to deliver oxygen [3,7,9]. However, similar to cultured islets, there is no available information that quantifies the pO_2 required for islets to survive post-transplantation. Keeping in mind the need to establish evidence based guidelines, the following *in vitro* studies were conducted to determine the optimal oxygen concentration for isolated human islets and to identify pO_2 levels that promote improved transplantation efficiencies and better patient outcomes.

2. Materials and methods

2.1. Isolation of human islets

Human islets were isolated by the Islet Manufacturing Team of the Southern California Islet Cell Resources Center (SC-ICRC) [8,10]. The use of human tissues in this study was approved by the Institutional Review Board of the Beckman Research Institute.

2.2. Human islet culture under different oxygen concentrations

Islets were cultured in a 24-well plate at 250 IEQ/well. Cells were maintained in 1000 μL of RPMI1640 medium containing 5 mmol/L glucose and 10% FBS at 37 °C for 7 days under various oxygen concentrations (10, 21, 35, and 50% oxygen) plus 5% CO_2 in Modular Incubator Chambers (Billups-Rothenberg, San Diego, CA).

2.3. Simulation and absolute oxygen tension in media under various oxygen settings

We simulated the pO_2 around islets placed in different volumes of culture medium in a 24-well plate under 21% oxygen. We assumed an islet oxygen consumption rate (OCR) of 200 pmol/min/well. The pO_2 shift corresponding to various OCR in 1000 μL of media was also calculated. The finite-element simulation was performed using COMSOL 5.0 (COMSOL, Los Angeles, CA) based on the reaction–diffusion equation:

$$\frac{\partial[O_2]}{\partial t} = D_{OX}\nabla^2[O_2] - Q_{OX}$$

where $[O_2]$ is the concentration of dissolved oxygen, and D_{OX} and Q_{OX} represent the diffusion coefficient for oxygen in water and islet OCR, respectively. ∂ and ∇ denote partial derivative and the del operator, respectively. After culturing islets for 24 h, the absolute pO_2 in the media was measured at the bottom of each well using an oxygen microsensor (Unisense, Aarhus N, Denmark). A total of 18 wells were analyzed for each oxygen setting.

2.4. Single human islet culture

Single islets measuring 200–300 μm in diameter were hand-picked and cultured individually in 96-well plates. Micrographs were taken under bright filed (IX50, OLYMPUS, Tokyo Japan) on day 0, 3 and 6. FDA staining was performed on day 6 as previously described [11]. A total of 24 single islets from three different islet batches were analyzed.

2.5. Islet area and volume measurement

For each micrograph, the islet area was converted to the islet volume using a conversion curve created in the following manner (Supplemental Fig. 1). Human islets were collected in PE50 tubing and packed by centrifugation at 160 × *g* for 2 min. Tubing containing packed islets was cut 1.25–15 mm of length. Islets from a single cut tube were placed in a well of a 24-well plate, and the islet area was measured using imaging software (cellSens OLYMPUS). The packed volume was calculated as the product of cross sectional area and length of each cut tube segment. The data were plotted to examine the relations between islet area and packed volume. The experiments were performed in triplicate and repeated three times using different batches of the isolated islets. The islets area was well-correlated with the packed volume ($R = 0.9607$).

2.6. Assessment of islet viability

Islets, cultured for 7 days, were collected and stained with fluorescein diacetate (FDA)/propidium iodide (PI) and micrographs were taken [11]. Islets 150–250 μm and 250–500 μm in diameter were manually selected for viability analysis. The average of 55 islets (ranging 44–70) was analyzed in each pO_2 from three independent batches.

2.7. Assessment of islet metabolism

OCR of the human islets was measured on day 7 as previously described [12]. Basal OCR was calculated from the average of three consecutive measurements and normalized to islet volume. Experiments were performed in duplicate using three different islet batches.

2.8. Islet function; response to glucose stimulated insulin release

Islet function on day 7 was examined, and the stimulation index (SI) was calculated as previously described [13]. The data were normalized using the islet volume. The assay was duplicated and repeated using three different islet batches.

2.9. Statistical analysis

Data are reported as mean ± standard error (SEM). Statistical analysis was performed using JMP 9.0.0 (SAS Institute, Cary, NC). For statistical comparison, Student's t-tests were performed for islet area, islet volume, and islet viability assessment. A nonparametric Wilcoxon tests were performed for islet OCR and islet function analyses. $p < 0.05$ denotes statistical significance.

3. Results

3.1. Simulated and measured values of pO_2 in culture medium under various oxygen settings

Simulation results predicted a pO_2 gradient within the media as a result of islet oxygen consumption. The simulated pO_2 around islets under air containing 21% oxygen were calculated as 152.6, 147.4 and 134.3 mmHg in 300, 500 and 1000 μL of media, respectively (Fig. 1A). In the simulation, OCR also acted to decrease the pO_2 (Fig. 1B). The measured average pO_2 in 1000 μL of culture media on day 1 under 10, 21, 35 and 50% oxygen was 90, 160, 270 and 350 mmHg, respectively (Fig. 1C).

3.2. Cultured islets gradually degrade under 21% oxygen

The physical appearance of single human islets cultured for 6 days under 21% oxygen ($pO_2 = 160$ mmHg) are shown (Fig. 2A). The number of small clusters around islets increased on days 3 and 6. These small clusters did not stain with FDA, indicating that they have no intracellular esterase activity (Fig. 2B).