Assessment of Tissue-Engineered Islet Graft Viability by Fluorine Magnetic Resonance Spectroscopy

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ABSTRACT

Introduction. Despite significant progress in the last decade, islet transplantation remains an experimental therapy for a limited number of patients with type 1 diabetes. Tissue-engineered approaches may provide promising alternatives to the current clinical protocol and would benefit greatly from concurrent development of graft quality assessment techniques. This study was designed to evaluate whether viability of tissue-engineered islet grafts can be assessed using fluorine magnetic resonance spectroscopy (^{19}F-MRS), by the noninvasive measurement of oxygen partial pressure (pO_2) and the subsequent calculation of islet oxygen consumption rate (OCR).

Methods. Scaffolds composed of porcine plasma were seeded with human islets and perfluorodecalin. Each graft was covered with the same volume of culture media in a Petri dish. Four scaffolds were seeded with various numbers (0–8000) of islet equivalents (IE) aliquoted from the same preparation. After randomizing run order, grafts were examined by ^{19}F-MRS at 37°C using a 5T spectrometer and a single-loop surface coil placed underneath. A standard inversion recovery sequence was used to obtain characteristic ^{19}F spin-lattice relaxation times (T1), which were converted to steady-state average pO_2 estimates using a previously determined linear calibration ($R^2 = 1.000$). Each condition was assessed using replicate ^{19}F-MRS measurements ($n = 6–8$).

Results. Grafts exhibited IE dose-dependent increases in T1 and decreases in pO_2 estimates. From the difference between scaffold pO_2 estimates and ambient pO_2, the islet preparation OCR was calculated to be 95 ± 12 (mean $\pm$ standard error of the mean) nmol/(min · mg DNA) using theoretical modeling. This value compared well with OCR values measured using established methods for human islet preparations.

Conclusions. ^{19}F-MRS can be used for noninvasive pre- and possibly posttransplant assessment of tissue-engineered islet graft viability by estimating the amount of viable, oxygen-consuming tissue in a scaffold.

WIDESPREAD UTILIZATION of islet transplantation (ITx) in the treatment of type 1 diabetes has yet to be achieved[1–3] despite significant progress over the last decade.[4–9] Intraportal delivery of islets may not be the optimal approach. The development of tissue-engineered strategies for extrahepatic ITx may present a promising long-term opportunity.[10,11] Ultimately, the liver may not provide the best environment for healthy engraftment of

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transplanted islet tissue for a number of reasons that include but are not limited to: (1) higher concentrations of orally administered immunosuppressants,[12,13] which are known to impair normal insulin secretory function[14,15] and islet revascularization[16,17]; (2) intraportal thrombus formation and the associated inflammatory reaction, which are believed to contribute to early islet loss[18–20]; (3) poor reestablishment of the surrounding extracellular matrix, which is believed to adversely affect islet survival[21,22]; and (4) poor oxygenation due to the mixed arterial and portal circulation[23] and the presence of significant oxygen gradients within the hepatic parenchyma,[24] which are believed to be particularly important due to the poor capacity of islets to undergo anaerobic metabolism and their general susceptibility to hypoxic stress.[25-27]

Current efforts in islet graft tissue engineering have been accompanied by inconsistent outcomes when translated to small- or large-animal transplant models. These efforts may have been stunted by an inability to accurately assess graft quality both pre- and posttransplantation. Improved methods for quality assessment of islet products prior to transplantation have enabled more accurate characterization of islet viability and potency.[28] Many of the most promising methods involve quantification of oxygen consumption.[29–35] However, these techniques have yet to be extended to assessing the quality of tissue-engineered islet grafts. Herein we have presented preliminary data on the use of fluorine magnetic resonance spectroscopy (^{19}F-MRS) to assess tissue-engineered islet graft viability by noninvasively measuring oxygen partial pressure (pO$_2$ or P), and using these measurements to estimate oxygen consumption rate (OCR).

METHODS
Engineered Scaffold Design and Fabrication

Human islets isolated at the University of Pennsylvania were shipped to our institution (as part of the Integrated Islet Distribution Program) following 3 days of postisolation culture. Upon arrival, islets were transferred into silicone rubber membrane culture flasks (Wilson-Wolf Manufacturing Corp, New Brighton, Minn, USA) for culture at 22°C in 5% CO$_2$ for an additional 2 days. On the day of experimentation, approximately 14,000 islet equivalents (IE) aliquoted into a 15-mL conical tube (BD Biosciences, Franklin Lakes, NJ, USA) were allowed to settle by gravity. After decanting the supernatant, the islet pellet was reconstituted in fresh CMRL 1066 culture media (Mediatech, Inc, Manassas, Va, USA) supplemented with HEPES buffer, heparin, and human serum albumin at a total suspension volume of 180 μL. Engineered scaffolds were constructed by combining fresh commercially available porcine plasma (Sigma-Aldrich, St Louis, Mo, USA) dissolved in calcium-free Krebs buffer solution, 30% v/v perfluorodecalin (PFD; Fluoromed, L.P., Round Rock, Tex, USA), and varying volumes of well-mixed islet suspension (amounting to 2000, 4000, and 8000 IE) in 3.5-cm-diameter Petri dishes (~10 cm^2 area), mixing, and then cross-linking using 5% v/v topical bovine thrombin solution (King Pharmaceuticals, Bristol, Tenn, USA; 1000 U/mL). Each scaffold had an approximate thickness of 0.3 cm and was covered with 1 mL of culture media. An additional control scaffold was constructed in the same manner only without the

addition of an islet suspension. The grafts were cultured at 37°C for a few hours prior to experimentation to allow the oxygen profiles to reach steady state.

Fluorine Magnetic Resonance Spectroscopy

^{19}F-MRS spectra were obtained at 5T using an APOLLO spectrometer (Tecmag Inc, Houston, Tex, USA) with a custom-built single-loop surface coil. Each sample was placed on top of the surface coil and centered within the bore of the magnet. Temperature was controlled using a custom-built water jacket; all measurements were performed at 37°C. A standard inversion recovery sequence was used to estimate ^{19}F spin lattice relaxation time (T1) values corresponding to inversion of the singlet peak in the PFD spectrum. Data were obtained using the NTNMR software (Tecmag Inc) and inversion recovery curves were fit using a custom routine generated in Matlab R2008a v 7.6.0 (Mathworks, Natick, Mass, USA). Equation 1 represents the generalized solution to the Bloch equation for relaxation of longitudinal magnetization (M_z), where A, B, and T1 are fitting constants that are obtained from the analysis and t is the time.

$$M_z = A \cdot \left(1 - 2e^{-t/T1}\right) + B \qquad (1)$$

The characteristic T1 for each of the conditions was converted to a steady-state average pO$_2$ estimate (in the scaffold) using a previously determined linear calibration (pO$_2$ (mm Hg) = 8.96 · 10^5/T1 (ms) −213; R^2 = 1.000). Each scaffold was assessed in replicate (n = 6–8).

Mathematical Modeling

The graft was modeled at steady state and as a diffusion-reaction system seeded with a known volume fraction of homogeneously distributed oxygen-consuming tissue. Oxygen was assumed to diffuse from the ambient air at the surface of the media layer (P_A = 160 mm Hg at x = 0 cm). Since the Petri dish is impermeable to gas transport, a zero, flux boundary condition was imposed at the bottom of the scaffold material (dP/dx = 0 at x = 0.4 cm). Each graft was modeled as a one-dimensional slab of engineered tissue with constant thickness. It was assumed that the islet tissue consumed oxygen with zero-order kinetics, which has been shown to be a reasonable assumption for most conditions involving pO$_2$ >> Michaelis-Menten constant, Km ~ 0.5 mm Hg.[36,37]

Assuming that no anoxic core develops at the bottom of the scaffold (which was warranted by the tissue volume fractions used), the generalized solution to estimate the volumetric OCR (mol/(cm^3 · s)) is shown by Equation 2, where P_A (mm Hg) is the ambient (surface) pO$_2$; P_s (mm Hg) is the average steady-state scaffold pO$_2$ (obtained from ^{19}F-MRS); L_M (cm) is the thickness of media; L_S (cm) is the thickness of the scaffold; ε (dimensionless) is the islet volume fraction in the scaffold; $(\alpha D)_M$ (mol/(cm · s · mm Hg)) is the permeability of oxygen in culture media at 37°C, and $(\alpha D)_s$ (mol/(cm · s · mm Hg)) is the effective permeability of oxygen in the scaffold at 37°C.[36]

$$OCR = \frac{P_A - P_S}{\left(\dfrac{L_S \cdot L_M}{(\alpha D)_M} + \dfrac{L_{S^2}}{3(\alpha D)_S}\right)\varepsilon} \qquad (2)$$

The effective permeability of oxygen in the scaffold was estimated using the Maxwell relationship,[36] which accounts for the contribution of the dispersed phase (ie, PFD) to the overall permeability. This adjustment is important given the high oxygen solubility of perfluoro-