NON-INVASIVE MONITORING OF OXYGEN AVAILABILITY WITHIN PANCREATIC SUBSTITUTES

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Introduction

Non-invasive monitoring of pancreatic substitutes, as well as other tissue engineered constructs, is imperative to establishing better clinical treatments and determining if and when a substitute will fail. Oxygen availability within a tissue engineered substitute is a critical parameter that directly influences the functionality of the cells in the construct. The use of perfluorocarbons (PFCs) and ^{19}F NMR spectroscopy constitute robust tools for monitoring the dissolved oxygen (DO) concentration within a construct *in vitro* and *in vivo*. We established a system that utilizes two PFC emulsions for measuring DO concentrations both within a pancreatic substitute and external to the substitute. Additionally, the effect of the average intrabead dissolved oxygen (AIDO) concentration on the bioenergetic status of the encapsulated mouse insulinoma cell line βTC-tet is being investigated.

Experimental

Using the 500 MHz NMR magnet at the University of Florida, the DO concentration within a pancreatic substitute and external to the substitute was quantified using ^{19}F NMR acquired T_1 relaxations from perfluorotributylamine (PFTBA) and perfluoro-15-crown-5-ether (PFCE), respectively. Cell-free beads containing PFCE equilibrated with the perfusing medium, giving a measure of the medium DO concentration, while PFTBA, placed in cell containing beads, measured a lower DO concentration within the beads due to cellular oxygen consumption. Puromycin was added to the medium at 10ng/ml to kill the encapsulated cells, consequently bringing the DO concentration within the bead to the same concentration in the medium.

To determine the effect of intrabead DO concentration on cellular bioenergetics, experiments were performed with βTC-tet mouse insulinoma cells and a PFC emulsion co-encapsulated in calcium alginate beads. The DO concentration in the perfusion medium entering the NMR bioreactor was first decreased from 100% to 20% air saturation (AS) in increments of 20% AS, then was returned to 100% AS. by the same increments. At each DO concentration, the AIDO was acquired by ^{19}F NMR and the phosphorous spectrum was acquired by ^{31}P NMR. By measuring the area of the β-nucleotide triphosphate (β-NTP) resonance in phosphorous spectrum, the level of intracellular NTP was assessed.

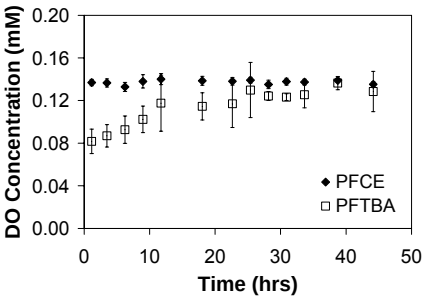

Figure 1: DO concentration measured in cell-free beads (PFCE) and in cell-

Results and Discussion

Figure 1 shows the DO concentrations measured by PFCE and by PFTBA. As cells died within the beads containing the PFTBA, the DO concentration approached the medium DO concentration, as quantified by the cell-free beads containing PFCE.

Figure 2 depicts the changes in β-NTP—attributed mostly to intracellular ATP—with changing DO concentration in the perfusion medium. The net decrease of NTP observed over the course of 50 hours is likely due to cell death occurring under hypoxic conditions. The AIDO at each external DO concentration was also acquired (data not shown).

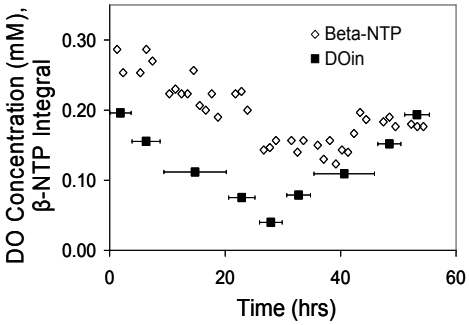

Figure 2: Change in beta-NTP with respect to external DO concentrations.

Conclusions

A two PFC system has been developed to measure the DO concentration from two distinct bead populations. Studies relating the external DO concentration to the average intrabead DO concentration and the bioenergetic status of the cells have been initiated.

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