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 dissolved oxygen (DO) concentration within a construct in vitro and *in vivo*. We have established a calibration curve for perfluorotributylamine entrapped in calcium alginate bead constructs. 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, a calibration curve was created for measuring dissolved oxygen (DO) concentrations within a pancreatic bead construct. Specifically, cell-free beads, containing a homogeneous distribution of a PFC emulsion, were placed into an NMR tube bioreactor that was continuously perfused with medium using an NMR-compatible perfusion circuit. The circuit is capable of computer-controlling the DO concentration in the perfusion medium, hence the T_1 relaxation of the entrapped PFC was acquired at several DO concentrations.

To determine the effect of intrabead DO concentration on cellular bioenergetics, experiments were performed with bTC-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.

Results and Discussion

The calibration curve for the entrapped PFC emulsion is shown in Figure 1, where the inverse T_1 relaxation is plotted against the DO concentration in the perfusion medium, the latter measured by a fiber optic DO sensor. A linear correlation is seen over the range of 0% to 100% air saturation with an R^2 of 0.99.

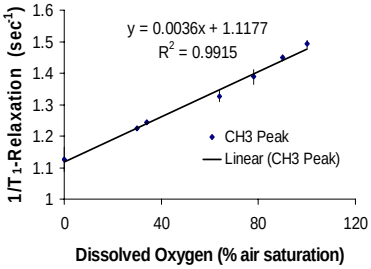

Figure 1: Calibration curve for perfluorotributylamine entrapped in alginate.

Figure 2 depicts the changes in β-NTP—attributed mostly to intracellular ATP—with changing DO concentration in the perfusion medium. A net increase in NTP was observed over the course of 50 hours with a decrease seen only at a DO of 40% AS or lower. The AIDO at each external DO concentration was also acquired (data not shown).

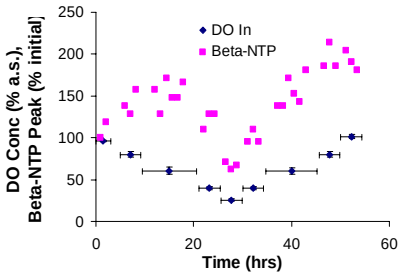

Figure 2: Change in the beta-NTP peak with respect to external DO levels.

Conclusions

A calibration curve relating the inverse T_1 relaxation of alginate entrapped perfluorotributylamine with the DO concentration within an alginate construct has been developed. The calibration curve provides the foundation for quantifying the DO concentration profiles in cell-containing alginate constructs. 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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