A Study of Model B Cells in Diabetes Treatment

by

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Overview of Project

Oxygen availability in tissue engineered substitutes is a critical parameter that directly influences a construct's desired function. Previous investigations in our group have shown correlations between the function of cells encapsulated in calcium alginate beads and the dissolved oxygen (DO) concentration in the surrounding medium. However, these experiments measured only the DO upstream and downstream of a bioreactor within a perfusion system. To take the previous experiments one step further, and to initiate studies towards *in vivo* monitoring of DO levels in tissue substitutes, we utilize Fluorine-19 (^{19}F) nuclear magnetic resonance (NMR) spectroscopy to monitor the oxygen concentration directly available to the cells within calcium alginate-based beads. This is accomplished by co-encapsulating a perfluorocarbon (PFC) emulsion with the cells. The ^{19}F NMR studies are being performed at the University of Florida in a 500 MHz, vertical bore magnet. To perform the desired experiments in a controlled fashion, a perfusion system capable of maintaining the DO concentration at levels set by the user was developed and utilized. The perfusion system maintained temperature control, allowed for replenishment of nutrients, and enabled the performance of step changes in glucose and DO concentrations, as instructed by the operator. By collecting ^{19}F NMR spectra from the beads in the entire bioreactor volume, the average intrabead DO

concentrations (AIDOs), both axially over the bioreactor and radially through the alginate beads, were obtained.

The perfusion system was used to carry out controlled experiments with alginate-encapsulated mouse insulinoma cells, in which the DO concentration in the perfusion medium was subjected to prescribed decreases and increases. A mathematical model describing the cellular remodeling that occurs within the beads, and the changes in the intrabead cellular and DO distributions that occur with time, was developed and used synergistically with experiments to obtain additional information on the system at hand. This research is significant both fundamentally, in understanding the metabolic and secretory functions of the encapsulated system, and practically, in developing the necessary technologies for monitoring encapsulated systems non-invasively *in vitro* and *in vivo*.

There have been no changes in the Specific Aims of this subcontract. Graduate Research Assistant Jeff Gross graduated during last year, and this work is continued by Graduate Research Assistant Fernie Goh.

Work performed during the period 04/2006 to 05/2007

The work carried out at the Georgia Institute of Technology under this subcontract from the University of Florida focuses on developing, characterizing, and implementing the ^{19}F NMR methodology to monitor average DO levels in encapsulated cell systems *in vitro* and in small animals *in vivo*. During the above mentioned period we performed the following studies.

1. We completed the development and validation of a mathematical model of the encapsulated cell system that allows estimating the viable cell number and the radial cell density and DO concentration gradients from the value of average intrabead dissolved oxygen concentration (AIDO), where the latter can be measured experimentally by ^{19}F NMR. This work was published in Gross, J.D., Constantinidis, I., and Sambanis, A., "Modeling of Encapsulated Cell Systems," *Journal of Theoretical Biology*, **244**: 500-510 (2007).

2. We performed experiments with alginate-encapsulated mouse insulinoma βTC-tet cells placed in the perfusion bioreactor and subjected to step decreases of DO in the medium entering the bioreactor (DOin), followed by step increases of DOin to the initial levels. During the experiments, AIDO values were otbained by ^{19}F NMR spectroscopy, and cellular bioenergetics were evaluated by ^{31}P NMR. The experimentally obtained system dynamics were simulated by the mathematical model, and the agreement of the two was found to be good, thus providing further credence to the ability of the model to reliably predict aspects of the system behavior from a limited set of experimental measurements. This work is currently in press in Gross, J.D., Long, R.C., Jr., Constantinidis, I., and Sambanis, A., "Monitoring of Dissolved Oxygen and Cellular Bioenergetics within a Pancreatic Substitute," *Biotechnology and Bioengineering*.

3. Similar experiments were performed with alginate-encapsulated βTC-tet cells that were allowed to grow in the beads prior to being placed in the NMR-compatible perfusion bioreactor, and were then subjected to step changes in DOin or to a cytotoxic antibiotic. The latter was added to simulate a specific immune response against the encapsulated cell system. Results from this study are reported in the manuscript Gross JD, Simpson NE, Constantinidis I, and Sambanis A, "Monitoring a Pancreatic Substitute During Transient Hypoxia and Cell Death," in preparation.

3. Additionally, we developed a two PFC system that allows the simultaneous measurement of the average DO concentration in two populations of beads. The system was validated experimentally by encapsulating one of the PFCs in inflammatory alginate/PLL beads and the second PFC in non-inflammatory alginate only beads, implanting the two bead populations in the peritoneal cavity of mice, and demonstrating that the AIDO concentration in the alginate/PLL beads became lower than that in the alginate beads few days post-implantation. This was due to the fibrotic layer that developed around the inflammatory beads. This work was performed collaboratively with Dr. R.C. Long, Jr., Department of Radiology, Emory University. This methodology can be used to evaluate *in vivo* the biocompatibility of materials and to monitor the AIDO in a population of cell-containing beads, while a cell-free bead control is used to monitor the DO concentration at the implantation site. Measurements of the latter are significant, since the DO at particular *in vivo* locales can change significantly from animal to animal and with conditions, e.g., anesthesia. This work is in press in the same article cited in paragraph 2.

4. Lastly, we are in the process of evaluating experimentally and via mathematical modeling the effect of PFCs, if any, on the viability and metabolic and secretory function of encapsulated cells. This work will continue into the 2007-8 year of the grant.

Plans for 2007-8

For the next year of the subcontract, we plan to:

1. Continue the work on evaluating the effect of PFCs on encapsulated cells, both under normoxic and hypoxic conditions.
2. Initiating studies towards using the two PFC system to monitor the AIDO in an encapsulated cell implant simultaneously with the DO concentration at the implantation site.
3. Finalize and submit the manuscript in preparation described in paragraph 3 of the previous section.