199a Non-Invasive Monitoring of Cellular Oxygenation within a Pancreatic Substitute

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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. Nuclear magnetic resonance (NMR) has been shown by our group to be a potent tool in monitoring cell functionality, cell viability, and bioenergetic status within a construct non-invasively. Previous research from our group utilizing 1H nuclear magnetic resonance (NMR) imaging and spectroscopy demonstrated the ability to assess structural features and the number of viable cells within a tissue engineered pancreatic substitute in vitro and post-implantation in vivo. 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 19F NMR spectroscopy constitute robust tools for monitoring dissolved oxygen concentration within a construct in vitro and in vivo. However, due to constraints especially in vivo, these measurements represent an average of the oxygen concentration over the entire construct volume and do not reflect local differences due to concentration gradients, which also change as the construct remodels. Therefore, we have developed a mathematical model to account for such regional differences within a spherical geometry. Utilizing the model, the weighted average pO2 measurements—as would be acquired by 19F NMR—were mapped to both cellular and pO2 profiles within the construct for a variety of initial cell densities and external oxygen concentrations. The mathematical model in conjunction with an NMR compatible perfusion system was then used to characterize the response of perfused, APA-encapsulated bTC-tet mouse insulinoma cells to normoxic and hypoxic conditions. The perfusion system used for the above characterization is designed to be utilized in a 500 MHz vertical bore magnet. The system meets the following six design criteria: (1) the system fits on a cart giving it maximum portability for entering and exiting an NMR facility; (2) the NMR-compatible bioreactor can accommodate the necessary amount of calcium alginate/poly-L-lysine/alginate (APA)-encapsulated insulin secreting cells; (3) medium entering the bioreactor is held at a constant 37°C temperature; (4) medium oxygen level can be set to any desired value by the operator and is held constant at the set point by computer control; (5) concentrations of all other nutrients are maintained at proper values for cellular nourishment; (6) the system delivers step changes in glucose concentrations when needed. A calibration curve using the PFC T1 relaxations has been developed, utilizing the perfusion system's ability to maintain oxygen concentrations at desired levels. The expansion of this methodology to in vivo situations and the possibility that in vivo NMR spectroscopy can be used to elucidate the mechanism of implant failure will be discussed.