Correlating Regional Hypoxia to Reduction in Insulin Secretion in Encapsulated Pancreatic β-cell Aggregates

Matthew L. Skiles
Biomedical Engineering Program, University of South Carolina, Columbia, USA

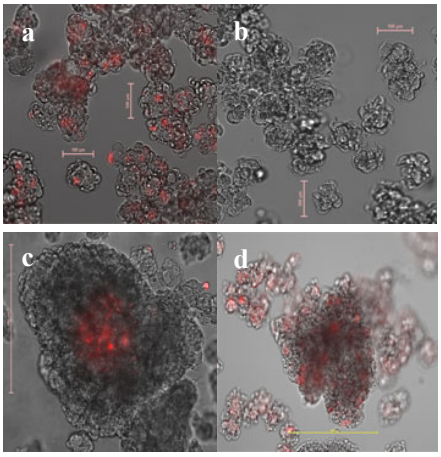
Statement of Purpose: Endeavors to create an artificial pancreas for the treatment of insulin-dependent diabetes have had minimal long-term success. Common device design includes functional pancreatic tissue encapsulated within a semipereable matrix. Though the cells are largely protected from immune responses and can retain normal function, inability of the tissue to revascularize places transport restrictions on essential nutrients and, notably, oxygen. As secretion of insulin from β-cells is highly dependent on oxygen availability, insufficiency of oxygen can, and often does, result in functional failure of the implanted device. The present work proposes a highly useful method for detecting hypoxia within living cells, which can be utilized concurrently with methods for quantifying cell function. Results employing this hypoxia detection technique highlight the sensitivity of β-cells to oxygen deprivation as well as the importance that tissue and matrix geometry play in development of regions of hypoxia.

Methods: Murine insulinoma (MIN6) cells (a mouse-derived, immortalized β-cell line) were genetically engineered using an in-house adenovirus system. Infected cells received a gene coding for the red fluorescent protein, DS-Red, under control of a minimal SV40 promoter and three copies of the hypoxia-responsive element (HRE) enhancer. In normoxia, the transcription factor, hypoxia-inducible factor-1α (HIF-1α), is expressed but rapidly degraded. When oxygen concentrations within the cell drop, HIF-1α is stabilized and dimerizes with HIF-1β to form a unit that binds to the HRE and initiates transcription of the downstream gene. Thus, hypoxic cells produce DS-Red and fluoresce.

MIN6 cells were aggregated by rotational culture in presence of the marker virus for 24 hours. Aggregates were then encapsulated in 40 µL of PEGDM which was photo-crosslinked by 10 minute exposure to UV light. Hydrogels were immediately imaged with a fluorescent microscope then placed in 20% O_2 incubation for 24 hours (denoted as Day 1) in 2 mM glucose media. After this time, media was replaced with a 16.7 mM glucose solution for one hour. At the end of the hour, the solution was removed for later analysis and media was replaced. Gels were then placed in either 20% or 1% O_2 incubation and glucostimulated in the same fashion every 24 hours for 2 weeks. During this time, gels were also periodically imaged for evidence of hypoxia signaling.

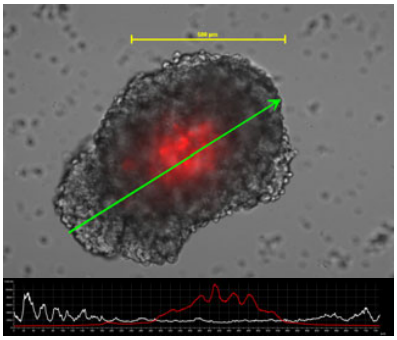
At the end of 2 weeks, daily samples were analyzed for insulin concentration by insulin ELISA. Marker signal intensity profiles were also constructed using imaging software.

Results: (Figure 1) Widespread hypoxic signaling was observed in all aggregates in 1% O_2 beginning at approximately 20 hours (a). Signal was not observed in small aggregates (<250µm) in 20% O_2 (b), but signal was observed in core regions of larger aggregates (>400µm, c).



Large aggregates in 1% O_2 initially displayed core signaling which became more widespread by 20 hours (d).

Figure 1.



(Figure 2) Intensity profiles for the fluorescent hypoxia signal confirmed a greater hypoxic response for aggregate core regions than the periphery.

Figure 2.

(Figure 3) Insulin secretion in 20% O_2 was well maintained over the 2 week trial period, while secretion was significantly reduced as early as day 2 in 1% O_2 and essentially absent by the end of the study.

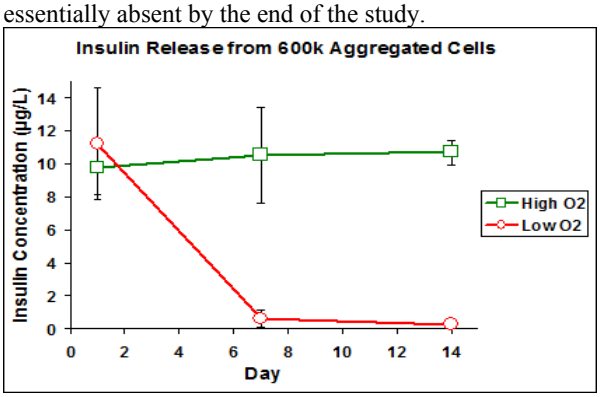


Figure 3.

Conclusions: Hypoxia severely reduces β-cell function. In low external oxygen conditions, the hypoxic effect is universal, but at higher external oxygen concentrations large aggregate cores could still produce a hypoxia response. This implies an optimal maximum aggregate size to ensure function throughout. Furthermore, the hypoxia marker was predictive of cell function and could be used to screen novel matrix materials or conformations.