Oxygen Releasing Biomaterials for Islet Transplantation

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Statement of Purpose Clinical islet transplantation (CIT) has shown significant promise in the treatment of Type I diabetes; however, the intraportal infusion of islets into the liver has been shown to lead to significant islet loss, primarily attributed to inflammatory responses, immune attack, and mechanical stresses during engraftment into the liver microvasculature. Numerous researchers have investigated the feasibility of using a device for housing transplanted pancreatic islets within an alternative site; however, designs have been plagued with problems such as poor engraftment, high islet cell loading, instability, and scalability to larger animal models. Due to the high metabolic demand of islets, the main source of these problems has been the poor nutrient delivery, primarily oxygen, to the transplanted islets. In this study, we sought to utilize a biomaterial as a means to enhance nutrient delivery and islet engraftment by fabricating oxygen releasing materials. We sought to use the oxygen releasing biomaterials as a bridge to supplement oxygen to the cells until the vascular bed fully develops. Previous publications using calcium peroxide illustrated the capacity of material to modulate oxygen release [1]. In this study, we evaluated the temporal release of oxygen from silicone biomaterials and assessed their capacity to enhance cell survival of insulin-secreting cells when cultured under oxygen tensions that mimic the *in vivo* environment.

Materials and Methods To fabricate the oxygen generating biomaterials, calcium peroxide (Sigma) was mixed with polydimethylsiloxane (PDMS), a medical grade silicone, at varying concentrations (5-50%), poured into molds (1mm height;10mm diameter) and incubated at 37ºC for 48hrs to complete silicone cross-linking. Oxygen release from the disks within a cell culture incubator at ~60mmHg O_2 was evaluated using a PreSens noninvasive oxygen sensing system. The ability of the disks to enhance cell viability was assessed by incubating 25%w/w peroxide/silicone disks with MIN6 cells, a continuous beta cell line, or rodent islets, isolated from Lewis rats. Cells were cultured under oxygen tensions designed to mimic in vivo conditions, 60mmHg, from 72hrs to 23days. Control cell contained silicone disks without peroxide. Cell viability was assessed via Live/Dead fluorescent staining & MTT Cell Viability Assay (Promega).

Results Silicone/peroxide disks demonstrated consistent oxygen release over the course of 1month when evaluated at 60mmHg. Three peroxide concentrations were tested: 5, 25, and 50% w/w. The 25% and 50% w/w peroxide/ silicone disks demonstrated a consistent release of oxygen over time, with maintenance of oxygen pressure at 150mmHg, or ~2-fold increase for over 1month. The 5% w/w disks also retained this level of oxygen release, but only for ~10days before reducing to the level of pure silicone controls, or 60mmHg.

In order to evaluate the ability of the peroxide/silicone scaffolds to improve cell viability via enhanced oxygen release, cells were incubated at 60mmHg for 3 days, with silicone disks with or without peroxide. MIN6 cells demonstrated a 2-fold increase in viable cell number, per MTT assay, from $400x10^3$ cells to $800x10^3$ cells, when co-incubated with a single 25% w/w peroxide/silicone disk. Rodent islets also demonstrated a significant increase in cell viability, where a 65% loss in islet IEQ was observed when rat islets were cultured at 60mmHg after 72hrs, but only a 20% loss was observed under identical culture conditions when islets were co-cultured with a single 25% w/w peroxide/silicone disk. The ability of peroxide/silicone disks to maintain cell viability long-term was evaluated via co-culture of disks and MIN6 cells for almost one month. In culture at 60mmHg, MIN6 cells co-cultured with a single 25% w/w peroxide/silicone disk demonstrated an 83% increase in viable cell number, per MTT, by Day 3 and a 142% increase by Day 7 over controls cultured with silicone only disks. From day 7 to day 23, MIN6 cells cultured at 60mmHg stabilized to $459\pm35x10^3$ cells/well to MIN6 cells, while MIN6 co-cultured with the peroxide/silicone disk stabilized to $1101\pm166x10^3$ cells per well. This represents an average of a 140% increase in viable cell number over the course of the 23 day culture period.

Discussion and Conclusions Through the incorporation of solid peroxide within the hydrophobic silicone polymer, we demonstrated the ability to modulate oxygen generation. Varying prototypes were evaluated for oxygen release over the course of 1month using noninvasive oxygen sensors. Results demonstrate the capacity of these inserts to generate a significant amount of oxygen over a period extending beyond 30days. In addition, cell co-culture experiments demonstrate the potential of these peroxide/ silicone scaffolds to significantly enhance oxygen availability, and, in turn, improve overall viable cell number. The significant improvement in islet cell viability during peroxide/silicone co-culture illustrates the strong potential of these disks to improve islet viability when placed in low oxygen environments, such as that seen *in vivo*. Given the flexibility of this platform, we envision that the peroxide/silicone inserts may be placed within numerous device prototypes to enhance oxygen availability. We currently are translating these promising benchtop results to in vivo experiments.

References

1.Harrison, B.S., et al., *Oxygen producing biomaterials for tissue regeneration.* Biomaterials, 2007. **28**(31): p. 4628-34.

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