Sustained, Controlled Delivery of Oxygen from Hydrolytically Activated Silicone Scaffolds

E. Pedraza[1-2], C. Fraker[1-2], M. Coronel[2], and C.L. Stabler[1-2]

[1]Department of Biomedical Engineering, [2]Diabetes Research Institute, and [3]Department of Surgery, University of Miami Miller School of Medicine, Miami, FL, USA

Introduction

The fabrication of tissue engineered constructs for highly vascularized and metabolic organs is a significant challenge, particularly in meeting the critical demand of oxygen. Poor nutrient delivery is confounded by additional parameters such as: implant geometry; high metabolic demands of the cells; and high cell loading densities. Furthermore, most tissue engineered constructs experience a delay in vascular-ization following implantation on the order of 5 to 14 days, leading to exacerbation of hypoxia during this crucial engraftment period. With this in mind, it is critical to identify and develop methods to increase oxygen availability within scaffolds, particularly to serve as a bridge between implantation and the development of an adequate vascular network. In this study, we sought to design and develop an oxygen generating scaffold in the form of solid peroxide encapsulated in silicone, for the continuous delivery of oxygen, with the goals of supplemented oxygen during the critical engraftment window. The scaffold is hydrolytically activated, producing oxygen upon contact with water through the decomposition of calcium peroxide, and this activation is modulated via the hydrophobicity of the polymer. The oxygen generator was integrated into the scaffold in either of two ways: (1) as a disk trapped within the center of the scaffold or (2) directly incorporated within the scaffold structure. Key parameters studied included the kinetics of oxygen generation, protection from detrimental by-products, and the ability of this oxygen generating biomaterial to enhance cell survival and function.

Materials and Methods

Scaffold Fabrication: Macroporous silicone scaffolds with 85% ± 5% porosity, interconnected/ tortuous pores, and coated with fibronectin were generated using methods previously described[1]. The oxygen generating biomaterial was fabricated by encapsulating 10-25% w/w calcium peroxide (CaO_2, Sigma Aldrich) in silicone. This material was integrated during scaffold fabrication as either: a solid disk (10 mm diameter, 1 mm thickness) in the middle of the scaffold, or as a component of the scaffold itself. *Material Assessments:* Materials were assessed for long-term release of oxygen via a sealed, titanium chamber (Instech) in dPBS at 37 °C and oxygen sensing fluorescent probes. Hydrogen peroxide and hydroxyl radical concentrations in the surrounding milieu were assessed daily for 30 days via colorimetric assay (Assay Designs) and HPF probe (Calbiochem), respectively. *In Vitro Assessments:* The oxygen generating material was incubated with either mouse insulinoma cells (MIN6) or nonhuman primate pancreatic islets in either a suspension or loaded within the 3-D scaffold. Cells were cultured at either normal (20%) or hypoxic (5%) oxygen tensions and cell viability (MTT, Promega) and function (glucose stimulated insulin secretion, Mercodia ELISA) were assessed following 3 day culture periods.

Results

The oxygen generative capacity of the biomaterial could be modulated by changing the peroxide concentration, total polymer volume, and material total surface area. For example, macroporous scaffolds containing 25% w/w CaO_2 produced oxygen for over 2 weeks at a minimum rate of 100 mmHg per day, while macroporous scaffolds containing a single 25% w/w CaO_2 oxygen generating disk produced oxygen for over 40 days at a minimum rate of 100 mmHg per day, within sealed oxygen chambers. Following an initial 1hr wash of the disk with buffer, hydrogen peroxide and hydroxyl radical production from the disks were found to remain below negligible levels during the study duration. Under hypoxic conditions, co-culture of MIN6 cells or nonhuman primate islets with the oxygen generating biomaterial resulted in a nearly a two-fold increase in MTT viability over controls, with viability levels comparable to controls at 20% oxygen ($p<0.05$). Islets cultured in hypoxic conditions also demonstrated significant insulin dysfunction, while islets incubated with the oxygen generating biomaterial illustrated insignificant changes in insulin function.

Conclusions

We have illustrated the ability to fabricate a biomaterial capable of providing optimal, sustainable, and controlled delivery of oxygen for a time period > 14 days. Encapsulation of solid peroxide within hydrophilic silicone shielded the surrounding milieu from detrimental by-products of peroxide degradation, as well as the peroxide itself. This oxygen generating biomaterial was successfully incorporated into a 3-D scaffold without inhibiting its performance. In vitro studies illustrate enhanced cell viability and function for both beta cell lines and islets when incubated with the oxygen generating biomaterial at hypoxic conditions. Given the ubiquitous needs for optimal oxygen delivery within implants, we believe this material can provide benefit to numerous tissue engineered systems, particularly for highly vascularized and metabolic organs.

References

1. Brady, A-C, et al *Transplant*, 90(2S), pp.1007, (2010).

Acknowledgements

The authors would like to acknowledge funding sources from the Juvenile Diabetes Research Foundation International and the Diabetes Research Institute Foundation. Pedraza is supported by an National Institutes of Health NIBIB predoctoral fellowship.