Design and Development of a Highly Macroporous, Protein Coated Silicone Scaffold as a Bioartificial Pancreas for Type 1 Diabetes

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Statement of Purpose: Clinical islet transplantation, the intraportal infusion of allogeneic pancreatic islets into a diabetic recipient, is a promising treatment for type 1 diabetes; however, the success of clinical islet transplantation is hindered by the location of the implant site, which is prone to mechanical stresses, inflammatory responses, exposure to high drug and toxin loads, and irretrievability of the transplanted islets. The development of devices to house islets at alternative transplant sites may alleviate many of these issues. Structural support to the islets in the form of a scaffold is critical to reducing pelleting and agglutination of the islets, which results in decreased availability of nutrients to the islets, leading to cell death. In this study, we sought to design and develop a highly porous silicone scaffold with the goals of maximizing nutrient delivery by creating a structure that supports and spatially distributes the islets, as well as promotes vascular infiltration.

Methods: *Fabrication and Characterization:* Macroporous silicone sponges were fabricated using the solvent casting and particulate leaching technique (SCPL). The silicone molds are created by combining varying ratios (50-90% v/v) of sodium chloride crystals (Mallinckrodt Baker, NJ) (250 to 425 μm diameter) and silicone polymer. The salt/silicone mixture is loaded into prefabricated, stainless steel molds (10 mm diameter, 3 mm height), pressurized to 1500 psi and incubated at 37 ºC for 48 hrs to complete silicone cross-linking. The NaCl is then leached out from the scaffolds over 72 hrs. Pore size and degree of porosity were individually controlled by varying the particle size and polymer to particle ratio, respectively. For enhanced cell adhesion, scaffold surface was modified with adhesion proteins.The macroporous structure of the scaffold was visualized by scanning electron microscopy (SEM). Final porosity was measured using gross measurements and weights. Uniformity of protein-modified scaffold surface was visualized through confocal imaging of fluorescently stained proteins. In vivo biocompatibility was assessed for via subcutaneous implantation into Lewis rats and histological analysis (H/E) at 7, 14, and 30 days.

Islet Loading: Pancreatic islets from male Lewis rat, non human primate (NHP) baboon, and human sources were used. Islets were loaded at desired islet equivalent (IEQ) density into the scaffolds by pipeting them onto the scaffolds and applying a light pressure gradient to distribute the islets into micro-sized pores. Two-dimensional cultures were used as control.

Preliminary findings in our laboratory evaluated spatial distribution, viability, and function of both rodent, non-human primate, and human islets within the scaffolds. In vivo studies assessed biocompatibility and stability of the silicone scaffolds, as well as vascular infiltration. Islet-seeded scaffolds were inspected for spatial distribution by phase light microscopy. Viability of islets was determined via live/dead dye (Invitrogen, CA) and confocal microscopy. Viability of human islets was quantified by MTT assay (Promega, WS). Functional insulin secretion rates were determined by collecting insulin samples from low and high glucose stimuli and quantifying by insulin ELISA assay (ALPCO, NH). Vascular infiltration was assessed via lipophilic dye infusion prior to explantation. In vivo studies were performed to determine the efficacy of scaffolds loaded with 500 IEQ at restoring euglycemia when implanted into the epididymal fat pad of STZ-diabetic mice.

Results: SEM images illustrate a highly porous with pore size representative of the salt crystal diameter. Moreover, the pores are interconnected and tortuous. Porosity of scaffolds manufactured with 90% porosity was determined to be 85% ± 5%. Fluorescence imaging demonstrated a homogenously modified scaffold surface with fibronectin coating. Histological cross-sections from in vivo biocompatibility showed silicone (with or without coating) to be similar to blank control and superior to Dachron material, with positive remodeling and matrix deposition within pores and absence of fibrotic tissue. Viability functionality of islets within the scaffolds were found to be statistically identical to controls. Vascular infiltration into scaffold was observed. Islet containing scaffold implants illustrate function within diabetic mice.

Conclusions: Biocompatible, silicone scaffolds were successfully fabricated with intended porosities and a controllable pore size range (250-425 μm), which is conducive to housing typical 150μm diameter islets. The results of the biocompatibility test demonstrate that scaffolds are suitable for application in vivo, given their high biocompatibility after extended implantation. Islets, regardless of species origin, consistently distribute evenly throughout the scaffold. Viability and functionality of islets was not adversely affected by loading within silicone scaffold. Since the experiments were conducted under normal oxygen tension, they are not reflective of the low oxygen tension of in vivo conditions. Therefore, the results of this experiment understate the potential of the scaffold at enhancing islet viability. These results are promising because they indicate the potential for transplanting islets into alternative sites by addressing the issues of spatial distribution of islets, mechanical protection, and intra-device vascularization.

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