Encapsulating Hydrogels with Multi-Scale Porosity for Islet Transplantation To Treat Type 1 Diabetes

Rios, P.D., Zhang, X., Luo, X., Shea, L.D.
Simpson Querrey Institute for BioNanotechnology, Northwestern University (NU), Chicago, IL

Statement of Purpose: Islet transplantation is a potential cure for Type 1 diabetic (T1D) patients and islet encapsulation within hydrogels is employed to create a barrier that prevents immune cell contact with the islets. However, the barrier provided by the encapsulating hydrogels can also impart mass transport limitations. Herein, we present a novel approach that combines encapsulation with lithography techniques to create an encapsulating macroporous PEG hydrogel that minimizes mass transport limitations to promote islet engraftment upon transplantation to diabetic mice. The encapsulating PEG hydrogel has a nano-scale mesh size to protect islets from immune cells, while the macroporous architecture facilitates tissue and vascular ingrowth near islets to reduce hypoxia and aid in insulin delivery. A hydrogel-based platform that can support and protect transplanted islets may reduce the need for immunosuppressive drugs and enhance the utility of allogeneic and xenogeneic islets.

Methods: A photomask (CAD Art Services) and standard photolithography techniques were used to create PDMS molds. 4-arm PEG-Maleimide (10kDa MW, JenKem Technology USA) was suspended in HEPES buffer (pH 7.2) and functionalized with 2.5 mM CGRGDS (CelTek Peptides) via Michael-Type addition for 30 minutes at 37°C. Functionalized PEG precursor solution and approximately 10 µL of media containing islets were transferred to the PDMS mold and crosslinked with a YKNR nondegradable crosslinker at a 1:1 ratio (PEG-MAL: YKNR) for 5 minutes at 37°C. Final gels were ~30 µL in volume and 10% PEG. Poly(lactide-co-glycolide) (PLG) microparticles (10 mg) were used to coat hydrogels and promote adhesion to the fat pad of diabetic mice for transplantation. Hydrogels were formed in PDMS molds with approximately 1,000 islets in each gel. Upon removal from the mold, the hydrogel was cut into 4 equal quadrants with each quadrant containing ~250 islets. Each mouse received one gel quadrant per fat pad (left and right fat pad), a total of 500 islets/mouse. The fat pad site allows for a minimally invasive surgery and access to vasculature. Intraperitoneal glucose tolerance tests (IPGTT) were performed 8 weeks post-transplant to assess the efficacy of hydrogel grafts. Histology was performed to confirm islet function and hydrogel integration with host tissue.

Results: A non-degradable, PEG hydrogel with multi-scale porosity was developed using microfabrication techniques (Fig. 1). Swelling studies indicated a mesh size of approximately 9 nm, which is sufficiently large to support transport of metabolic factors and hormones, yet would prevent the infiltration of cells into the hydrogel. Mice transplanted with hydrogels containing 500 islets achieved normal blood glucose levels (< 200 mg/dL) within two weeks post-transplantation, as early as Day 10 post-transplant, and maintained normoglycemia over the two-month study (Fig 2). Upon graft removal at Day 60, all mice reverted to a diabetic state within 2-3 days, which confirmed maintenance of blood glucose levels was due to the hydrogel graft and not remaining endogenous islets. Histochemical staining revealed tissue ingrowth occurred through the macropores, and confirmed separation of the islets from the host tissue. Insulin and CD31 staining confirmed that the size and shape of encapsulated islets were retained in the hydrogel at 8 weeks post-transplant.

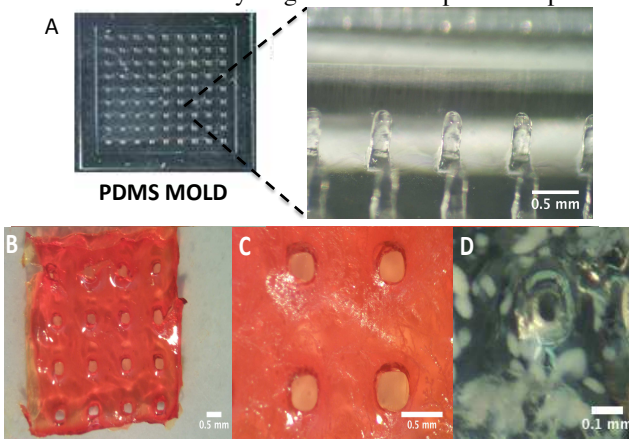

Figure 1. PDMS Molds and Macroporous PEG Hydrogels. (A) PDMS mold and inner structure features (B) Macroview of gel (Dimensions ~ 4mm x 4mm). (C) Close-up view of macropores. Macropores are ~200 µm diameter with 500 µm spacing between pore edges (D) Encapsulated islets surrounding macropore.

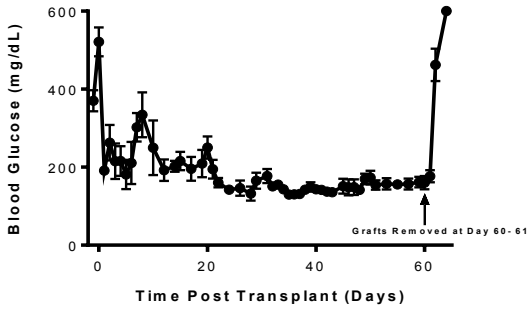

Figure 2. Graft Function Monitored in Diabetic Mice. Hydrogel grafts with 500 islets reversed diabetes in all recipient mice over a 2 month period. Normoglycemic levels (<200 mg/dL) were maintained until graft removal at Day 60-61, at which time all mice reverted to a diabetic state (>300 mg/dL) within 2 days (± SEM. n=6).

Conclusion: We present a non-degradable hydrogel-based platform and demonstrate the feasibility of this approach for long-term function and engraftment of encapsulated islets *in vivo*. The nanoporous regions of the gel provide protection from the host immune cells, while the macroporous regions allow vascular ingrowth near the islets. This design can be refined to modulate the host response at the transplant site to further protect islets, which are the focus of ongoing studies with allogeneic islet transplantation.