Dual-Layer PEG Hydrogels Promote Islet Engraftment and Function in an Alternative Transplant Site

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Statement of Purpose: Type 1 diabetes affects millions worldwide, and treatment is typically limited to exogenous insulin injections. While exogenous insulin improves life expectancies and quality of life, diabetic patients face high rates of secondary complications. Islet transplantation is a promising alternative therapy that can potentially restore native insulin signaling, but its widespread application is hindered by compounded immune and autoimmune responses that result in short graft lives and necessitate life-long systemic immunosuppression, which comes with a host of adverse side effects.[1] Islet encapsulation aims to modulate the host immune response, and thereby reduce or eliminate the need for systemic immunosuppression, by preventing the cell-to-cell contact required to initiate the direct antigen recognition pathway. Encapsulation of allogeneic islets has previously demonstrated promise by prolonging graft rejection in diabetic rodent models; however, traditional microcapsule diameters (600-1000 μm) impart additional barriers to transplantation. These large capsule sizes result in an increase in graft volume imparted by the encapsulation material, which can produce a diffusional delay to nutrients and insulin, and limits potential transplant sites. These complications, combined with a poorly vascularized transplant site, necessitate larger numbers of islet equivalents (IEQ) for diabetes reversal, an additional barrier to the translation of this therapy.[2] We have engineered a microfluidics-based islet encapsulation platform that produces smaller capsules than traditional methods.[3] Herein, we demonstrate that PEG encapsulation with our microfluidic platform has minimal impact on islet viability and function. Additionally, we demonstrate that an encapsulated marginal islet mass of 500-600 IEQ, delivered in a vasculogenic, degradable construct, is sufficient for diabetes reversal within the alternative epididymal fat pad (EFP) transplant site.

Methods: In vitro evaluation of microfluidic islet encapsulation: 48 hr after isolation, 500–600 mouse IEQ were encapsulated as previously described[3] in an RGD (1 mM) functionalized PEG-maleimide hydrogel (20kDa, 4-arm) crosslinked with dithiothreitol. Islet viability and function were evaluated 48 hr post-encapsulation by Alamar Blue, glucose-stimulated insulin response (GSIR) assay, and Live/Dead imaging. Syngeneic marginal islet mass transplantation studies: Degradable, vasculogenic PEG hydrogel constructs containing 500-600 IEQ, free or encapsulated, were fabricated as previously described.[4] Briefly, prior to transplant, 4-arm 20kDa PEG-maleimide macromer is functionalized with RGD (1mM) and VEGF (10 μg/mL), mixed with cells, and crosslinked with a proteolytically cleavable peptide. Islet-containing gels were placed on the exposed EFP of diabetic mice, completely enclosed in the EFP, and sealed with a small volume of degradable PEG hydrogel. Blood glucose was monitored continuously for up to 100 days. An intraperitoneal glucose tolerance test (IPGTT) was performed on the final day of the experiment. Explanted grafts were evaluated by immunofluorescence staining for islets (insulin), blood vessels (CD31), and infiltrating cells (CD68).

Results: Mouse islets were encapsulated using our microfluidic platform, resulting in PEG capsules in the range of 200-400 μm in diameter. In vitro evaluation of microfluidic PEG encapsulation demonstrates no significant impact on islet viability, as evaluated by Alamar Blue metabolic assay and live/dead confocal imaging. Encapsulated islet function was evaluated by GSIR, where temporal insulin secretion was comparable to unmodified controls, with no evidence of diffusional delay.

Next, we sought to determine the marginal islet mass required to restore euglycemia in a syngeneic diabetic mouse model. Vasculogenic, proteolytically degradable PEG hydrogel constructs containing 500-600 IEQ, either free or encapsulated, were transplanted in the EFP of diabetic mice. Both groups exhibited the characteristic delay of diabetes reversal observed in marginal islet mass transplants (Fig. 1A), and IPGTT demonstrated comparable glucose responsiveness between groups. Explanted grafts exhibit insulin-positive islets, as well as CD31+ blood vessels in close proximity to islets (Fig. 1B).

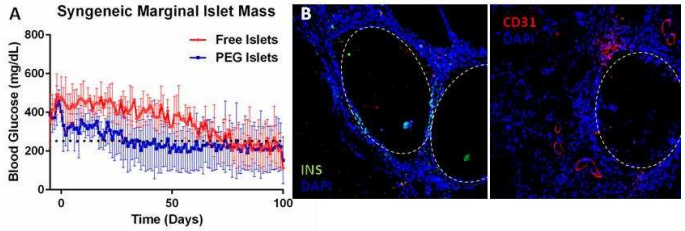

Figure 1. Syngeneic marginal islet mass transplantation demonstrating diabetes reversal (A) in both PEG and free groups. Explanted grafts exhibit insulin positive islets and CD31+ blood vessels proximal to the graft.

Conclusions: This microfluidic encapsulation platform results in significantly smaller capsules than traditional encapsulation techniques, allowing for the use of alternative transplant sites, as well as a reduction in the marginal islet mass required for diabetes reversal in a syngeneic mouse model. Future studies will explore the potential of microfluidic-generated PEG capsules to prevent or delay graft rejection in an allogeneic murine model.

References: 1. Korsgren et al. Curr Opin Organ Transplant. 2009; 14: 683-7. 2 de Groot et al. J Surg Res. 2004; 121: 141-50. 3 Headen et al. Adv Mater. 2014; 26:3003-8. 4. Phelps et al. Biomaterials. 2013; 34: 4602-11.

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