Microfluidic Encapsulation of Human Pancreatic Islets in Synthetic Polymer Hydrogel Microspheres

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Statement of Purpose: Hydrogel microencapsulation of cells is a promising strategy for immunoprotection after transplantation. The ease of alginate microencapsulation, along with alginate's inherent biotolerance *in vivo*, have led to its prevalence, even though the ability to control local cellular environment and microparticle size is limited. Although some groups have reported synthetic polymer encapsulation of cells, control of cellular microenvironment is limited [1]. Previously we have developed a synthetic PEG-MAL hydrogel system that allows for incorporation of protease degradable or non-degradable crosslinks, as well as functionalization with bio-active ligands and growth factors in a plug-and-play fashion [2]. Building from this platform, we have established a flow-focusing microfluidic scheme for generating cell-laden microgels, which can be customized to provide desired biochemical and mechanical microenvironment for cells. For this study, human pancreatic islets were microencapsulated, were maintained in culture, and were assayed for functional activity. Not only was functional activity retained after encapsulation, but microgel molecular release studies indicated that immunoprotection potential is high. The control provided by this system represents a necessary, and long overdue, step forward in cell microencapsulation technology, which can be applied to many cell therapy and regenerative medicine applications.

Methods: *Microgel Generation and Islet Encapsulation.* Soft lithography was used to generate PDMS microfluidic flow focusing devices in which microgels were generated and crosslinked. For the crosslinker phase, a 20 mg/mL solution of dithiothreitol (DTT) in PBS was emulsified in 2% SPAN80/mineral oil solution in a 1:10 ratio. Media containing a 4% 4-arm PEG-MAL (20kDa) solution and human pancreatic islets (obtained from IIDP*) was isolated from the crosslinker phase through the flow focusing nozzle by a co-flowing a 2% SPAN80/mineral oil solution. After droplet generation, emulsified DTT diffused into droplets, rapidly forming islet-laden microgels. Microgels were washed 5 times in media before being cultured. Cells were stained for viability (Calcein AM, TOTO3 iodide) on days 1, 2 and 5 after encapsulation, and were then imaged.

Glucose Stimulated Insulin Secretion. Encapsulated and bare islets were washed and challenged with either low (1.67mM) or high (16.7mM) glucose for 30 minutes (n=5). Supernatant samples were assayed for insulin using ELISA, followed by normalization to DNA content in each sample. Glucose stimulation index is the ratio of insulin secretion in high glucose to low glucose secretion.

Molecular Release Studies. Molecules of interest, containing fluorescent tags, were encapsulated following the same procedure as cell encapsulation. After washing, microgels were suspended in known volumes of PBS (n=5). Supernatant samples were taken for 3 days, and their fluorescent intensity was measured.

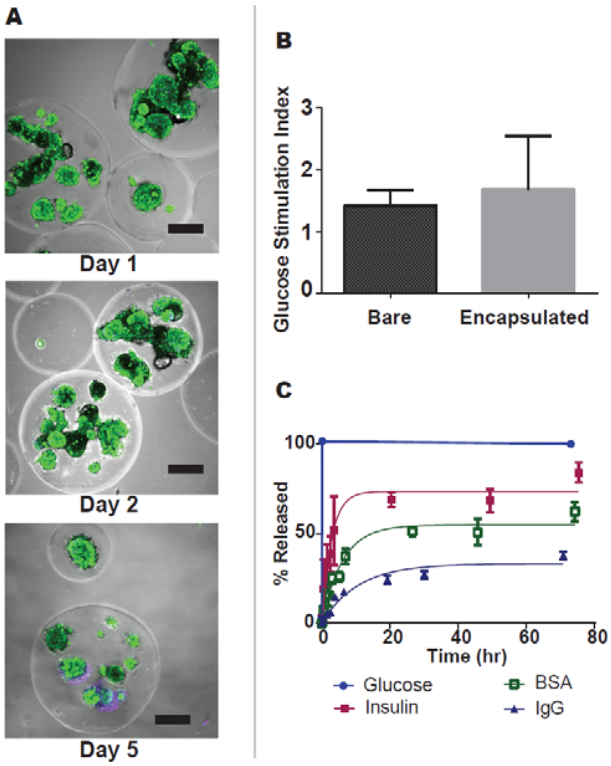

Figure 1. PEG-4MAL microencapsulation of human pancreatic islets is a promising immunomodulation method for transplantation. **(A)** Representative fluorescence images for live (calcein AM, green) and dead (TOTO-3 iodide, purple) cells indicate that PEG-4MAL microencapsulation allows sustained viability in culture. Scale bars are 200 µm. **(B)** Glucose stimulated insulin secretion assay shows no significant functional difference between bare and encapsulated islets (n=5) **(C)** Studies of molecular release from microgels suggest microencapsulation allows rapid equilibration of glucose and insulin across the capsule while hindering IgG from reaching surface epitopes (n=5).

Conclusion: Microencapsulation holds promise for cell delivery applications, but well-defined, synthetic hydrogels have not been fully utilized for this application. In our scheme, a plug-and-play, cytocompatible hydrogel network allows for biochemical and mechanical tuning of cellular microenvironment without significantly affecting cell viability or function in vitro. Tunable network structure can also supply immunoisolation, expanding regenerative medicine applications for our platform.

References:

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