Engineering Pseudo-Islets of Defined Sizes from Primary Murine Islets

Kelly MT Shekiro[1,2], Thomas H Hraha[4], Abigail B Bernard[1,2], Richard KP Benninger[4,5], Kristi S Anseth[1,2,3]
[1]Department of Chemical and Biological Engineering, [2]Biofrontiers Institute, and [3]Howard Hughes Medical Institute University of Colorado, Boulder, CO 80303 [4]Department of Bioengineering, University of Colorado, Anschutz Medical Campus and [5]Barbara Davis Center for Childhood Diabetes, Aurora, CO 80045

Statement of Purpose: Islet transplantation is a promising therapy for the treatment of Type 1 Diabetes, however current protocols have failed to establish extended graft function. One cause of graft failure may be islet death due to stresses experienced at the transplant site. It has been well demonstrated that smaller islets, <125 μm in diameter, display increased viability and insulin secretion (1). When islets are prepared for transplantation, they are isolated from the extensive vasculature that delivery oxygen and nutrients, which does not get reestablished post-transplant (2), leading to central cell necrosis in large islets (3). Given that β-cell-β-cell contact is necessary for normal, responsive insulin secretion (4,5), creating functional β-cell clusters with desired dimensions is an important step towards a cellular therapy for type 1 diabetes.

3-dimensional β-cell clusters have been made through a variety of techniques (5,6). However neither of these methods provide for the creation of aggregates of defined dimensions over a range of size. Here, we report on the use of hydrogel microwell arrays to create pseudo-islets from primary murine islets that show improved functional properties over age-matched wild-type islets.

Methods: Hydrogel poly(ethylene glycol) microwell arrays were fabricated using contact photolithography as previously described (7). Wells were either 100 or 200 μm wide and 100 μm tall. Pancreatic islets were isolated from female Balb/cByJ mice, dissociated into a single-cell suspension and seeded into microwell devices using a combination of centrifugation and orbital shacking (7). After 5 days of culture, pseudo-islets were removed from the microwell devices and cultured in suspension. Pseudo-islet size was quantified using ImageJ and viability was measured using Live/Dead (Life Technologies) staining and confocal microscopy. Pseudo-islet composition and cellular organization was evaluated with immunohistochemistry against insulin (β-cells), glucagon (α-cells) and somatostatin (δ-cells). Pseudo-islet and age-matched wild-type islet function was evaluated by glucose challenge and subsequent insulin secretion measured through the use of a sandwich-ELISA. Insulin secretion dynamics were measured by monitoring calcium flux using Fluo-4 AM (Life Technologies) dye and real-time fluorescent microscopy.

Results: Robust pseudo-islets that remained instact in suspension culture after removal from devices were formed. They were spherical in shape with average diameters of 50 μm and 100 μm for pseudo-islets from 100 μm and 200 μm wide devices. Compared to whole rodent islets, which generally range from 50 μm to 300 μm in diameter, these pseudo-islets are smaller and much more consistent in size than wild-type islets. Through Live/Dead staining and imaging, high levels of viability were observed in pseudo-islets over at least 14 days in culture, while age-matched whole islets began to display increased ethidium staining.

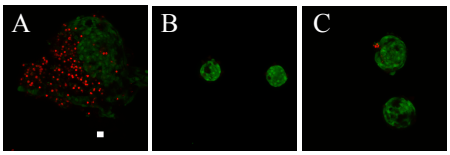

Figure 1. Live/Dead staining of (A) whole islets, (B) 50 μm pseudo-isletes and (C) 100 μm pseudo-islets on day 14. Live cells stain green and dead cells stain red. 100 μm scale bar.

Insulin secretion in response to 20mM glucose and total insulin content was measured. Similar levels of insulin secretion were observed for freshly-isolated whole islets and pseudo-islets on day 7 and 14. However, at the same time points, insulin secretion by age-matched whole islets increased.

Pseudo-islets maintained regular, robust calcium oscillations through day 14, while the oscillations of whole islets deteriorate into chaotic oscillations by day 7. Pseudo-islets are also highly coordinated; on day 14, 100 μm pseudo-islets displayed higher levels of coordination compared to whole islets. In addition, when observed under low glucose, 100 μm pseudo-islets were better able to suppress spontaneous calcium activity than age matched whole islets on day 14.

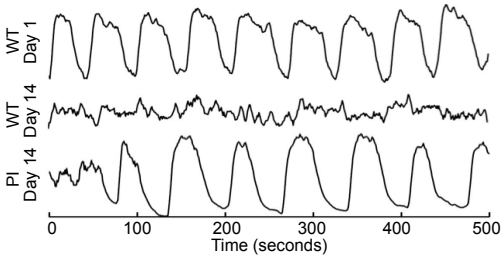

Figure 2. Calcium oscillations of freshly-isolated wild-type islets (top), and day 14 whole islets (middle) and pseudo-islets (bottom).

Conclusions: Microwell devices can be used to create defined pseudo-islets from primary cells. Pseudo-islets maintain viability and functional glucose resonse over 14 days in culture. In addition, the pseudo-islets show superior dynamic response to insulin than age-matched whole islets. Future studies will focus on testing these pseudo-islets under hypoxic culture and *in vivo*.

References: **(1)** MacGregor RR. Am J Physiol-Endoc M. 2006; 290:E771-E779. **(2)** Lau J. Transplantation. 2009; 87(3):322-325. **(3)** Vasir B. Diabetes. 1998; 47(12):1894-1903. **(4)** Hopcroft DW. Endocrinology. 1985; 117(5):2073-2080. **(5)** Pipeleers D. Proc Natl Acad Sci USA. 1982; 79:7322-7325. **(6)** O'Sullivan ES. Diabetologia. 2010; 53(5):937-945. **(6)** Cavallari G. Transpl P. 2007; 39(6):2018-2020. **(7)** Bernard AB. Tissue Eng Pt C-Meth. 2012; 18(8):583-592.

Acknowledgements: Funding for this work was provided by NIH (R01DK076084) and HHMI.