Controlled reaggegation of pancreatic ß-cells promotes viability and functional expression

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Statement of Purpose: Allogeneic islet transplantation is a promising treatment for type 1 diabetes, but graft failure of transplanted cells remains a problem. Studies have shown that islet size is a major factor contributing to the survival and function of isolated islets.[1,2] Specifically, under hypoxic transplantation conditions, smaller islets (<150µm) show higher viability and more per-cell insulin secretion than islets of larger sizes.[1] This is perhaps due to the fact that, when the dense vascularization native to islets is removed during isolation, transportation of nutrients and other small molecules within the cell aggregate is controlled by diffusion which is less effective in larger aggregates. Inability to receive adequate oxygen due to these diffusion limitations may explain necrosis of central cells seen in large isolated islets.[3]

Although smaller islets perform better than larger islets in vitro and in vivo, a method to systematically control islet size in order to form viable, functional, and implantable cell aggregates has not been shown. Here we present a method to reproducibly control the reaggregation of pancreatic ß-cells using microwell devices which yield aggregates of defined sizes that exhibit functional capabilities and improved survival compared to single ß-cells.

Methods: Microwell devices were fabricated using photolithography and a 15wt% PEG macromer solution (25mol% PEG-diacrylate (M_n~3000) and 75mol% PEG-monoacrylate (M_n~400)). Chrome photomasks were used to control the widths of the microwells (50-300µm).

Mouse insulinoma 6 (MIN6) single cell suspensions ($3x10^6$ cells/mL) were seeded in the microwells using centrifugation at 1200rpm for 2.5 minutes. After five days of culture in the wells, the cell aggregates were removed and encapsulated in a 10wt% PEG-diacrylate (M_n~10,000) hydrogel capsule. For imaging, dissociated MIN6 cells were dyed with CellTracker Green CMFDA™ prior to seeding and aggregate sizes were measured using confocal microscopy (Nikon LSM 710) and ImageJ (NIH) software.

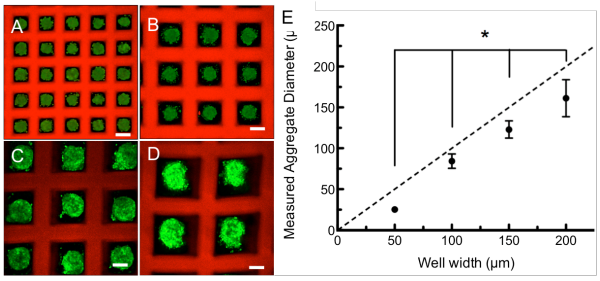

Figure 1: MIN6 aggregates formed with PEG microwells of widths (A) 100µm, (B) 150µm, (C) 200µm, and (D) 300µm. (E) Average measured aggregate diameter plotted against well width. Dashed line indicates theoretical fill line. All averages significant p<0.001. Error bars represent standard deviation.

Cell viability was evaluated by staining encapsulated MIN6 aggregates and single cells with LIVE/DEAD viability dye. Cell functional expression was assessed by immunostaining encapsulated ß-cell aggregates with antibodies against E-cadherin and insulin. The antibodies were then tagged with specific fluorescent secondary antibodies and cell nuclei were counterstained with DAPI.

Results: PEG-based microwell devices can be used to control ß-cell reaggregation and aggregate size scales with well dimensions (Figure 1). After five days of culture in the microwell devices, the aggregates can be removed and encapsulated for further studies.

Cell clusters of MIN6 retain their cell-cell contact as demonstrated by the appearance of aggregates within the hydrogel capsule as well as positive staining for E-cadherin, a transmembrane protein related to cell-cell contact (Figure 2A). These aggregates also stained positive for murine insulin (Figure 2B) indicating that the cells maintain their functional abilities during the reaggregation and encapsulation processes. MIN6 ß-cell aggregates also display higher viability than encapsulated single cells (Figure 3) indicating that by aggregating the cells we can promote viability in an encapsulation system.

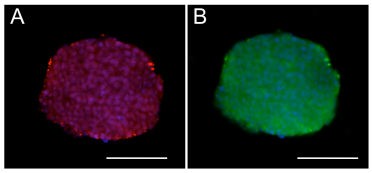

Figure 2: MIN6 aggregates stained for (A) E-cadherin (red) and (B) insulin (green). Nuclei are stained blue. 100µm scale bars.

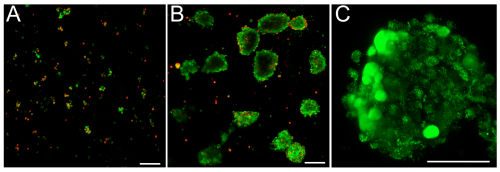

Figure 3: MIN6 single cells (A) and aggregates (B-C) stained with a viability stain that labels live cells green and dead cells red. Scale bars represent 100µm (A-B), and 50µm (C).

Conclusion: MIN6 ß-cell aggregate size can be controlled using PEG-based microwell devices. During reaggregation, the ß-cells form the necessary cell-cell contacts to preserve viability and insulin content during encapsulation. This platform may be used to investigate the effect of ß-cell aggregate size on cell viability and function in transplant conditions.

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References:

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