Controlled reaggegation of pancreatic ß-cells in PEG-based microwells

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Statement of Purpose: Islet transplantation is a promising treatment for type 1 diabetes, but survival and function of transplanted cells remain a barrier to the implementation of this treatment. Studies have shown that islet size is a major factor contributing to the survival and function of isolated islets.[1,2] Specifically, researchers have shown that smaller islets (<150µm) have higher viability and secrete more insulin on a per-cell basis than islets of larger sizes under transplant conditions.[1] It is suggested that, when vascularization is removed during isolation, diffusion of nutrients and other molecules becomes hindered in larger aggregates. Further, ß-cells are highly sensitive to hypoxic conditions. Inability to receive adequate oxygen due to these diffusion limitations may explain necrosis of central cells in large islets.[3]

Although it is established that smaller islets perform better than larger islets in vitro and in vivo, islet size has only been studied in bin fashion (i.e. islets <150µm or >150µm), so specific islet sizes that yield high viability and functional insulin secretion have not been identified. Here, we present a method to reproducibly control the reaggregation of pancreatic ß-cells using microwell devices. These aggregates can then be used to investigate the effect of aggregate size on cell viability and function.

Methods: Microwell devices were fabricated using photolithography with an ultraviolet light (365nm, ~35mW/cm^2). The devices were fabricated using a 15wt% PEG macromer solution containing 25mol% PEG-diacrylate (M_n~3000) and 75mol% PEG-monoacrylate (M_n~400). Methacryloxyethyl thiocarbamoyl rhodamine B at a concentration of ~300µM was incorporated for imaging purposes. Photomasks were used to control the diameters (d) of the microwells (100-300µm) and plastic spacers (150-250µm) inserted between the photomask and glass slide were used to control the depth (h) of the wells.

Mouse insulinoma 6 cells (MIN6), a pancreatic ß-cell line, were maintained in RPMI 1640 medium containing 10% FBS and treated with 1% Penicillin-Streptomycin, and 0.5% fungizone. For imaging, dissociated MIN6 cells were labeled with CellTracker Green^{TM} (CTG) and seeded (2x10^6 cells/mL) in microwell devices. Devices were centrifuged at 1200rpm for 2.5 minutes to accelerate cell settling into the microwells. After 3-4 days of culture within the devices, the cell aggregates were retrieved by gentle rinsing. The dimensions of the microwells and retrieved aggregates were measured using a confocal microscope (Nikon LSM 710).

Results: PEG-based microwell devices for aggregating pancreatic ß-cells were formed using photolithography (Figure 1A). Each device contained an array of 1000 (300µm diameter) to 1800 (100µm diameter) wells. MIN6 cells were seeded efficiently and uniformly within the microwells (Figure 1B). Further, cells formed aggregate structures after 3-4 days, aggregates were removed from the microwell devices by gentle rinsing and maintained their size and shape, confirmed with confocal microscopy (Figure 1C). Cells seeded into wells with the same dimensions formed uniform cell aggregates across several devices (n≥15 aggregates from at least three different devices) exhibiting reproducible control over cell aggregation.

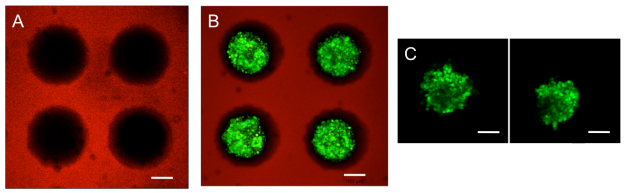

Figure 1: A: PEG microwell device. B: MIN6 cells stained with CTG seeded in a microwell device after 3 days. C: Aggregate of MIN6 cells maintained size and shape after removal from microwell device. (Scale bars: 100µm)

Changing well dimensions changed aggregate sizes proportionally. Wells with 200µm diameters and 200µm heights (d200h200) yielded aggregates of ~130µm diameter and ~50µm height while d300h250 devices yielded aggregates of ~170µm diameters and 65µm heights (Table 1). Device fabrication allowed the aggregate d and h to be changed independently of each other. For example, when the well diameter was kept at 200µm and the height was increased from 200µm to 250µm, the aggregates had roughly the same diameter but an increased height (Table 1). While the cell aggregates did not fill the wells completely, aggregate size is reproducible and can be changed by changing well dimensions. The volume of the removed aggregate was about 10% of the well volume in all cases.

Table 1: Well and removed aggregate dimensions

Diameter (µm)		Height (µm)		Volume (x10^{-6} µm^3)	
Well	**Aggregate**	**Well**	**Aggregate**	**Well**	**Aggregate**
200	130	200	45	2.0	0.19
200	140	250	70	2.5	0.34
300	170	250	65	5.6	0.47

Conclusion: We fabricated and characterized PEG-based microwell devices made by photolithography. Further, ß-cell aggregates of defined sizes were reproducibly prepared from single ß-cell suspensions seeded in the microwell devices. This contribution offers a facile means of creating defined ß-cell aggregates with high viability in vitro and these can be used to examine the effect of aggregate size on cell function.

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