Enzymatically degradable poly (ethylene glycol) hydrogels for long term maintenance and differentiation of human embryonic stem cell derived pancreatic precursor cells.

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Statement of Purpose: Type 1 diabetes is an auto-immune disorder that results in the destruction of insulin producing pancreatic β-cells, leading to a dysregulation of glucose metabolism. Human embryonic stem cells (hESCs) differentiated into insulin producing cells offers a potential therapy for this disorder. However, current *in vitro* approaches yield an insufficient number of cells capable of releasing insulin in response to glucose. One possible solution is a three dimensional (3D) culture platform that maintains cell-cell and cell-matrix contacts similar to the *in vivo* environment. This work investigates poly (ethylene glycol) (PEG) hydrogels and tests the hypothesis that encapsulation of pancreatic precursor cells derived from hESCs in PEG hydrogels improves viability long term and supports differentiation to β-cells. PEG hydrogels were developed with collagen type I to promote cell-matrix interactions and collagenase sensitive crosslinks to enable facile recovery of cells for analysis. Gamma secretase inhibitor, which promotes differentiation by inhibiting the notch signaling pathway, was also investigated.

Methods: HESCs that were differentiated into pancreatic precursor cells following a 13 day protocol ("T13") and aggregated [1] were received from the University of Toronto. HESCs or RIN-m5F aggregates were encapsulated by photopolymerizing 4-arm poly(ethylene glycol) tetranorbornene with a collagenase sensitive peptide (CVPLSLYSGC) [2]. Rat tail collagen type I was added to the pre-polymer solution at 0.25 mg/ml. Aggregates were cultured: i) as unencapsulated cells in suspension culture ii) in hydrogels with collagen (Pcol) and iii) in hydrogels with collagen and gamma secretase inhibitor (0.25 μM) (Pcol G6). HESCs were maintained in DMEM with 50μg/mL ascorbic acid, 50 U/mL penicillin, 50 μg/mL streptomycin, and 1X B27 and l-glutamine. At 2 ("T28"), 4 ("T42"), and 6 ("T56") weeks, hydrogels were enzymatically degraded by liberase TL (Roche) at 0.25 mg/ml for 45 minutes followed by treatment with trypsin. Recovered cells were analyzed by FACS for the pancreatic markers PDX1, NKX6.1, and C-peptide and qRT-PCR for insulin and glucagon, with TBP as the housekeeping gene.

Results: Initial studies employed aggregates of RIN-m5F cells to develop the enzyme degradable hydrogel platform that enabled encapsulation and subsequent recovery of viable aggregates. HESC-derived pancreatic precursor cells encapsulated in hydrogels remained viable and retained their original morphology, while unencapsulated cells formed larger aggregates concomitant with a decrease in cell number over time (Fig 1).

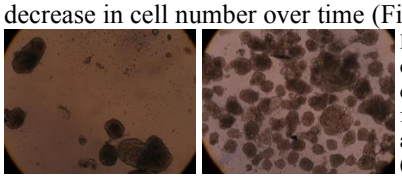

Figure 1 - Unencapsulated cell aggregates (left) and cell aggregates released from hydrogels (right) after 4 weeks in culture. (10X magnification).

At 2 weeks, the percent of positive cells was higher for pancreatic markers PDX1 and C-peptide, but lower for NKX6.1 compared to pre-encapsulation for all culture conditions (Fig 2). Aggregates in hydrogels with gamma secretase inhibitor had the highest number of C-peptide$^+$ cells along with double positive C-peptide$^+$/NKX6.1$^+$ and C-peptide$^+$/ PDX1$^+$ cells (Fig 2). However by 4 and 6 weeks, the percent positive cells for all pancreatic markers (PDX1+, NKX6.1, and C-peptide) declined.

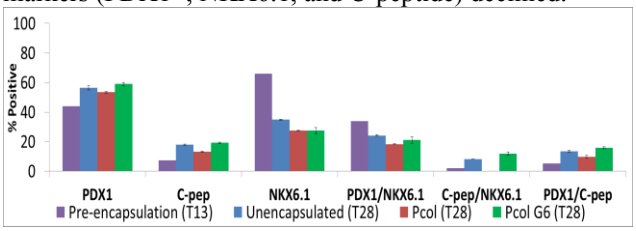

Figure 1 - FACS data for PDX1, C-peptide, and NKX6.1. Data are from 1 biological replicate and 1-2 technical replicates.

Insulin mRNA levels increased at 2 weeks, but decreased at 4 and 6 weeks for all conditions (Fig 3). Glucagon mRNA levels increased at 2 weeks and remained elevated for all conditions (Fig 3).

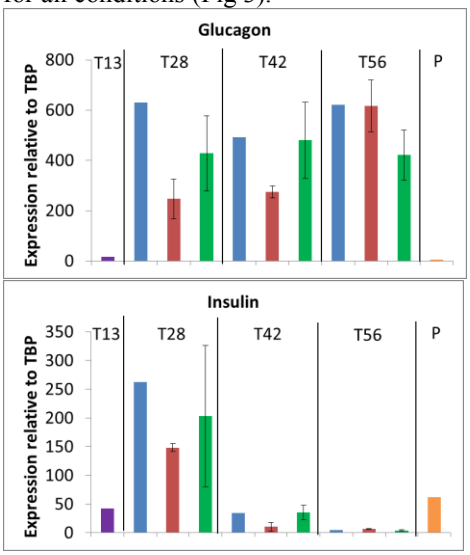

Figure 2 - qRT-PCR for insulin (INS) and glucagon (GCG). Purple – pre-encapsulation, blue – unencapsulated, red – Pcol, green – Pcol G6, orange – pancreatic mRNA. Data is from 1 biological replicate and 1-2 technical replicates.

Conclusions: We developed a collagenase sensitive hydrogel platform whereby cell aggregates can be encapsulated, maintained in culture, and then released. Cell viability and aggregate morphology were maintained within the hydrogels over six weeks. β-cell markers increased during the first two weeks in culture and appeared to be highest with gamma secretase inhibitor. However, differentiation was not maintained suggesting the need to identify additional factors to support β-cell maturation.

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References:

[1] M. C. Nostro *Development* 2011;138: 1445–1445.
[2] B. D. Fairbanks *AdvMat* 2009; 21: 5005–5010.