Islet on a Chip: Bottom-up Islet Engineering

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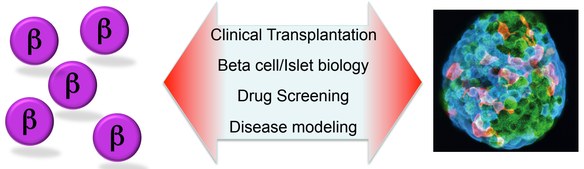
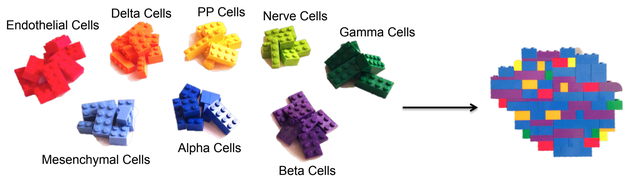
Type 1 Diabetes

Diabetes Mellitus results from failure of pancreatic islets leading to an increase in morbidity and mortality. Mechanistic studies of this disease are hindered by low availability, high variability and the cost of human islets. Our recent advances have led to the first successful method to generate mature, glucose sensing-insulin secreting β cells from human embryonic stem (ES) cells *in vitro*. This method, and its application using human iPS cells, provides a virtually unlimited supply of standardized human β cells. Moreover, as the β cells can be prepared from patient iPS cells, normal and diseased states can be analyzed. This advance provides a renewable source of β cells for cell replacement therapy for insulin dependent diabetics and the opportunity to perform rigorous disease modeling to identify novel therapeutic targets for all diabetics.



Despite these advances, challenges remain. Robust, sensitive and routine technologies to assess b cell function are lacking. Further, it is unlikely that b cells by themselves will recapitulate the complex biology involved in islet function. As such, the proposed research aims to combine approaches in stem cell and islet biology with tissue engineering to design, build and test new technologies for generating human islets *in vitro* and assessing their function in microfluidic devices. We seek to generate whole islets *in vitro* using a bottom-up tissue engineering approach that incorporates multiple endocrine cell types. Here we report progress towards the generation of α and δ cells from ES sources.

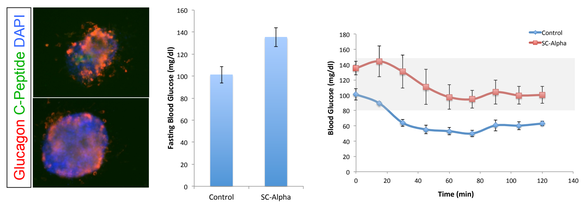


Using a combinatorial screening approach we identified protocols for differentiating embryonic stem cells (ES) first to pancreatic progenitors (PP1) and subsequently to each of the pancreatic endocrine cell types.

Stem cell derived α cells

ES → DE → PP_1 → EP → α

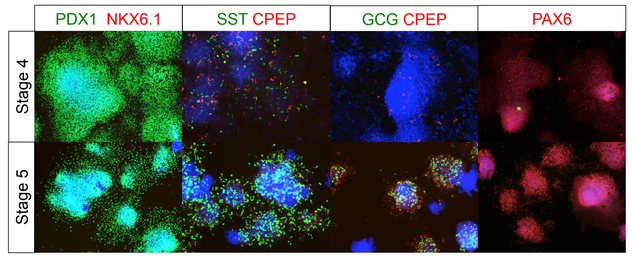
Directed differentiation of ES cells towards glucagon expressing alpha cells progresses through similar developmental stages resulting in cells that express and secrete glucagon in response to low glucose, raise fasting blood glucose when transplanted under the kidney capsule of mice and prevent insulin induced hypoglycemia. The resulting SC-alpha cells comprise of ~40% of the cell population.



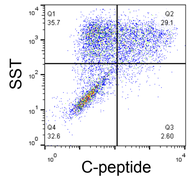
SC-alpha cells express and secrete glucagon in vitro, raise fasting blood glucose in mice and prevent insulin induced hypoglycemia

Interaction of β and δ cells

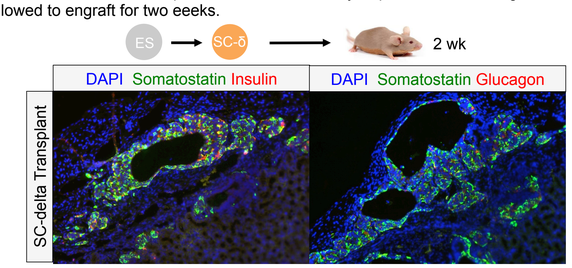
ES → DE → PP_1 → EP → δ



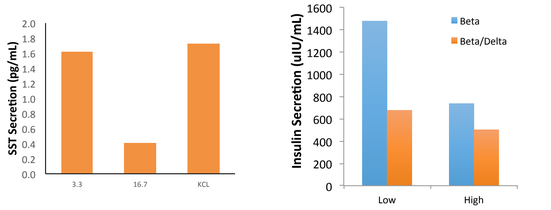
Directed differentiation of ES cells towards somatostatin expressing delta cells progresses through several developmental stages. First, ES cells are induced to become definitive endoderm (DE) followed by pancreatic progenitors (PP), endocrine progenitors (EP) and finally delta cells. At later stages, cells express markers of delta cells and lack markers for beta and alpha cells. The resulting SC-delta cells comprise of ~30% of the cell population.



SC-delta cells were transplanted under the kidney capsule of SCID-beige mice and allowed to engraft for two eeeks.



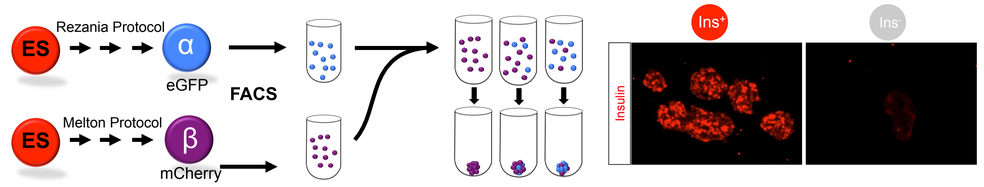
SC-delta cells persist *in vivo* for up to two weeks



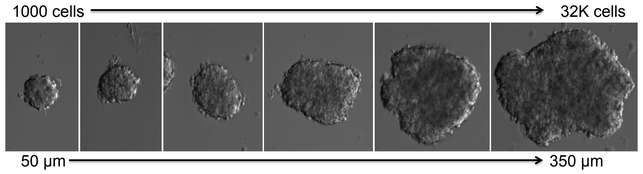
Engineering islet organoids

With protocols established and validated for the generation of each these endocrine cell type, we will build islet organoids composed of alpha, beta and delta cells. Using reporter lines to sort pure populations of each endocrine cell type, multiple cell types are mixed into organoids. Once optimized, these organoids will be incorporated into microfluidic devices for evaluation of endocrine function and for use in disease modeling and drug screening (See Posters 16 and 17).

An insulin reporter line allows for sorting of SC-beta cells and reaggregation into homogeneous cell clusters. Insulin negative cells can also be collected and reaggregated as a control.

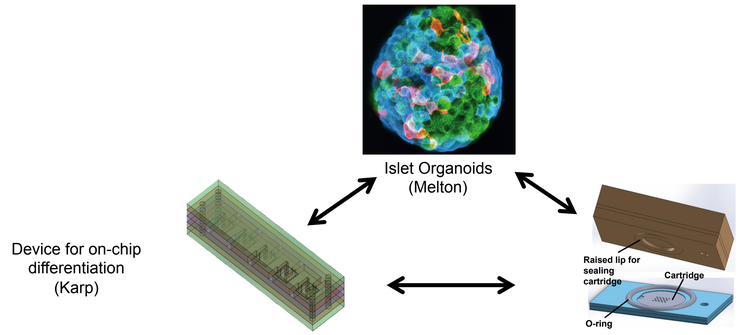


By modulating the ratios of each cell population during reaggregation we can generate islet organoids of defined size and composition with as few as 1000 cells per organoid, and as many as 32,000 cells. Multiple organoids can be generated with consistent sizes. Organoid sizes range from 50 μm in diameter to 350 μm in diameter.



By fusing smaller cell aggregates, organoids composed of multiple cell types can be generated with control over the orientation of the cells. Shown here is the fusion of two smaller cell aggregates over time. The cell aggregate in red is expressing insulin as marked by mCherry. The dark aggregates are insulin negative and somatostatin positive.

Endocrine cell types can be isolated and assembled with control over size, composition and orientation of the cells within the organoid



Acknowledgments

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