Differentiation and containment of derived pancreatic beta cells
Caden Duffy, Alonzo Cook
Brigham Young University

Introduction
Type 1 Diabetes (T1D) is an autoimmune disease that affects 1.25 million people in the United States, according to the American Diabetes Association. Currently, the only permanent cure for T1D is a pancreatic or islet transplant. With a large shortage of available donors, our work is progressing research towards alternative therapies by differentiating induced pluripotent stem cells (iPSCs) into derived pancreatic β-cells that can be transplanted and used as treatment for T1D. No established protocol has yet been described for a recoverable device with fully differentiated iPSCs. We are investigating methods to increase the yield of fully derived, insulin producing pancreatic β-cells and are researching the use of hydrophilized expanded polytetrafluoroethylene (ePTFE) containment devices as a future alternative for islet transplantations in human patients.

Materials and Methods
For our study, we use human iPSCs that are generated from peripheral blood monocytes. These are cryopreserved cells at -190°C that are thawed and seeded in 6-well plates coated with vitronectin, with growth and expansion performed in Essential 8 medium. StemPro Accutase is then used as a cell dissociation reagent to detach the iPSCs from the plates. Rho-associated protein kinase inhibitor is added to the cell suspensions for the first 24 hours after each passage to improve the survival rate. The human iPSCs are then differentiated into pancreatic progenitor cells using the STEMdiff Pancreatic Progenitor kit, which includes a series of 6 medias. We are currently researching the exposure of various proteins and metabolites, such as retinoic acid and IGF-1, in progressing the differentiation process beyond the progenitor stage. We test for correctly differentiated cells by running three wells through a fluorescence-activated cell sorting (FACS) machine to determine if our protocol has been successful. We will then determine if the cells produce insulin by treating the remaining three wells with retinoic acid, dorsomorphin, and SB431542, and run these through a solid phase sandwich ELISA test. If insulin is found, it would signify that some percentage of cells are producing insulin.

The ePTFE membrane is being hydrophilized through a protocol that consists of washes with isopropyl alcohol, polyvinyl alcohol, and glutaraldehyde. Glucose and insulin concentrations are then being measured and analyzed with either a glucometer or ELISA test.

Results and Discussion
We have successfully completed the STEMdiff protocol for differentiating iPSCs into pancreatic progenitor cells, but encountered problems in the disassociation of these cells for a run through the FACS machine. We are currently undergoing our second round of proliferation, with an updated procedure on minimizing the aggregation of progenitor cells. We have a third study planned which will focus on differentiating these cells into fully functioning β-cells.

In addition, after experimenting with various densities of ePTFE membranes, we have successfully completed our glucose tests. With our preliminary data, we have found that the lighter densities of ePTFE have increased diffusion of glucose. We are currently testing the diffusion of insulin, and have planned trials for utilizing β-cells to ensure that they will be contained by the ePTFE membrane.

Conclusion
We have successfully shown that facilitated diffusion of glucose can be achieved through a hydrophilized ePTFE membrane, improving its probability as a viable material for a retrievable device. We plan on continuing our study of ePTFE as a containment device for our derived pancreatic progenitor cells, and eventually our fully derived, insulin producing β-cells. Furthermore, we are improving the differentiation protocol for derived insulin producing β-cells, and expect to have fully functioning cells by the end of next year.