Approaches to reconstruct human islet physiology in vitro

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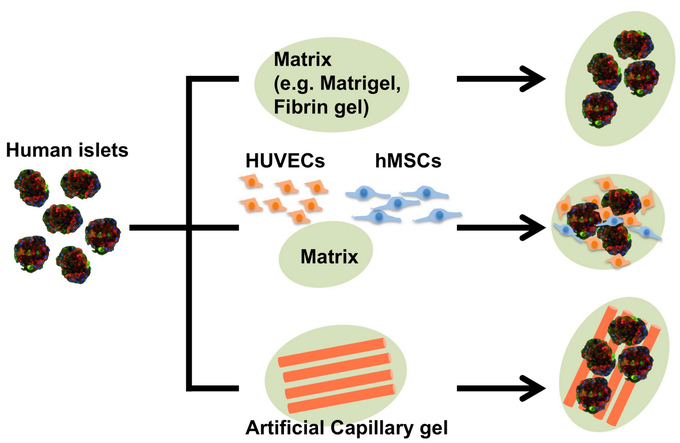
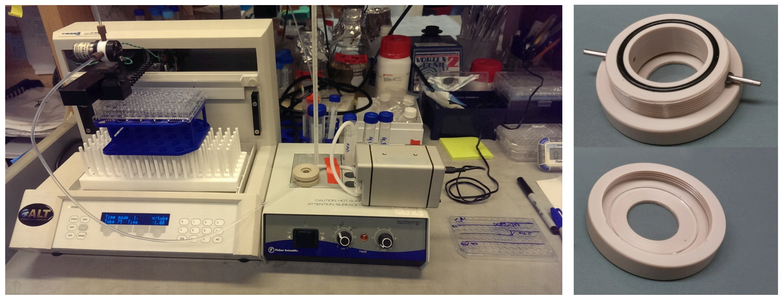
Abstract

Diabetes Mellitus is a worldwide healthcare challenge. The common outcome of diabetes is absence or insufficient production of insulin followed by hyperglycemia, which leads to the development of complications such as retinopathy, nephropathy, cardiovascular disease, and neuropathy. Transplantation of islets (Edmonton protocol) sheds light on curing the insulin-dependent diabetes mellitus. However, the progress is greatly hindered by limited source of human islets from cadaveric donors and inability to maintain long-term islet physiology in vitro. Despite advancements in culture techniques to prolong survival of post-isolated islets, an inexorable decline in islet function remains a challenge. By merging biological techniques and bioengineering tools, we aim to generate a biomimetic to reconstruct and maintain islet physiology in long-term culture. In their native environment, islets are vascularized and surrounded by extracellular matrix, which provides both mechanical and biological supports for tissue structure and functionality. We will investigate if the presence of artificial-matrix support and capillary structure in a biomimetic could preserve the functionality of long-term cultured human islets. To monitor the dynamic insulin secretion of islets and simultaneous fluorescence imaging of calcium influx, we built an in-house perifusion system. The perfusion chamber was designed to allow real-time imaging analysis and simple loading of gel and islets. With this perifusion system, we first examined human islet physiology in response to glucose stimulation on Day 0 and Day 6 in suspension cultures. In our preliminary data, without matrix support, human islets lost function coinciding with the loss of intra-islet capillary structure. Currently, the functionality and morphology of human islets encapsulated in artificial matrix (eg. Matrigel and Fibrin gel) with or without capillary structures is under examination. Additional work is required to determine if preservation of intra-islet capillary structure via matrix (scaffold) and extra-islet vasculature support in biomimetic could help to maintain islet functionality in vitro.

Approaches

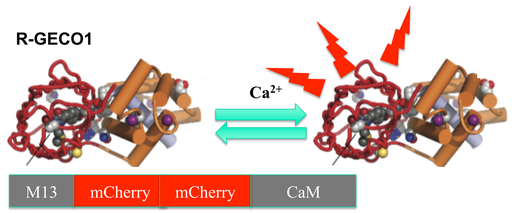


In-house perifusion system for dynamic functional assay coupled with real-time imaging analysis



Our laboratory has designed a prototypical perifusion system to monitor the real-time response of islet physiology. The first model of this system can incorporate hydrogels and microscopy for a multi-parametric approach to investigating islet physiology, while future improvements to the system will be multiple units in parallel and full automation for improved efficiency and reproducibility. The goal of this system is to standardize and improve the analysis of human islet physiology across human islet research centers.

Development of red-fluorescent calcium sensor to monitor dynamic calcium influx in cells



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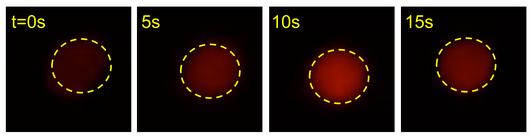
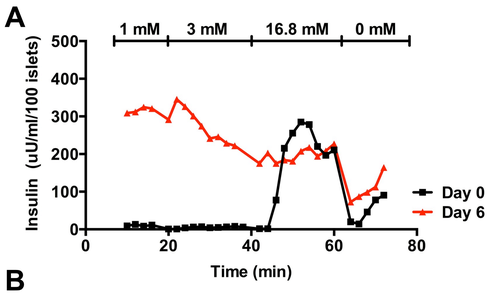


The R-GECO1 construct will be used in beta-cell lines and islets to optimize the perifusion system combined with epifluorescence microscopy analysis. Calcium influx is critical to insulin exocytosis in human and mouse islets. Using a fluorescent marker of calcium signaling along with real-time insulin secretion measurements will allow us to determine the glucose-sensing physiology in islets.

HeLa cells were transfected (Lipofectamine 2000) with 1 µg of CMV-RGECO1 plasmid DNA. One day later, cells were treated with 5µM histamine to induce Ca^{2+} cycling and imaged using time-lapse microscopy.

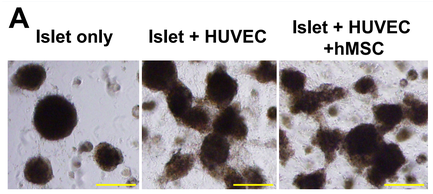
Functionality and capillary structure of human islets in suspension cultures

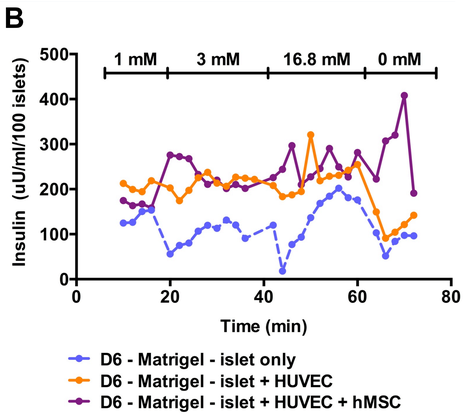


(A) Assessment of Insulin secretion in human islets by perifusion experiments. Human islets were received from IIDP and 100 islets with similar size (100-150 µm) were handpicked for 1 hour (Day0) or 6 days (Day6) culture followed by perifusion experiments to assess insulin secretion ability and morphology examination. On day 0, human islets display tight regulated-insulin secretion in response to 16.8 mM glucose. In contrast, after 6 days culture, the islets lost their insulin secretion ability in response to glucose stimulation.

(B) Intra-islet capillary structures were detected in Day0 but not Day6 cultured human islets. Human islets were immunostained with antibodies against C-peptide (red), human CD31/PECAM-1 (green, endothelial cell marker), and DAPI. Scale bars, 50µm.

Functionality and morphology of human islets when co-cultured with HUVECs, hMSC in matrigel





Human islets were mixed with HUVECs and hMSCs in GFR-Matrigel followed by 6 days culture at 37°C, 5%CO2. On day6, the gels containing islets and/or cells were transferred to perfusion chamber for perifusion experiments and morphology analysis.

(A)Images of islets co-cultured with HUVEC and hMSCs in 3D Matrigel culture on Day6. Scale bars, 100µm.

(B)Perifusion experiments to analyze islet functionality.

Future Directions

1. Form follows function. We observed that human islets lost function coincident with the loss of intra-capillary structure. We will investigate the effects of different parameters such as matrix compositions, co-culture with different cell types, and artificial capillary support on human islet functionality and morphology.
2. We will improve the efficiency and reproducibility of current perifusion system via multiple units in parallel and full automation design. The goal of this system is to standardize and improve the analysis of human islet physiology across human islet research centers.

Acknowledgment

❑ This work was supported by grant from NIH/NIDDK (UC4-DK104196-01), Integrated Islet Distribution Program (IIDP) and Penn Center for Islet Transplantation for access to human islets, Abramson Family Cancer Research Institute.
❑ We thank Ali Naji and Chengyang Liu at UPENN for providing some of the human islets; ChangHong Li at CHOP for helpful discussion; Dario Nicetto for providing HUVECs and hMSCs; Fabian (Leo) Cardenas for helping time-lapse imaging.