Engineering Optimal Islets for Transplantation for Type 1 Diabetes

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Abstract

Islet transplantation offers an improvement in the quality of life for those suffering from type 1 diabetes. However, islet cells often die during or soon after transplantation. Our lab has shown that small islets are superior to large islets as they maintain viability and result in long-lasting insulin independence in diabetic rats. Large islets show areas of cell apoptosis and necrosis within 24 hours. In this study we engineered optimal islet tissue for transplantation as a means of curing type 1 diabetes.

Empirical modeling of the diffusion barriers in islets demonstrated that only the outer 4-5 layers of cells were exposed to glucose and sufficient oxygen tension. Core cell death occurred in 100% of the large islets with a radius larger than 5 layers, resulting in poor viability. Small islets (diameter < 100 μm) exhibited superior survival rates. Utilizing these observations, we sought to disperse large islets into single cells and then reaggregate them into smaller islet clusters that maintain viability. To engineer the optimal diameter, single cells were seeded onto an engineered mold. These designed molds contained conical-shaped recesses that allowed for reaggregation of cells into a defined size. Results showed that small islet aggregates maintained cell viability. Preliminary studies suggested that engineered islets were equivalent to, or better than, native small islets when comparing insulin secretion and oxygen consumption. Reaggregating tissue in an engineered mold has immense implication for both islet transplantation, and other forms of tissue regeneration.

Introduction

In an attempt to cure type 1 diabetes, islet transplantations were first performed with consistent success in 2000[2]. While transplants are beneficial to those with uncontrolled diabetic complications, the procedure is less than perfect. In order for the islet cells to produce an adequate amount of insulin, 1 million cells are needed, which is equal to two full donor organs. After transplantation, the functional capacity of islets in Type 1 diabetics is only a third of the function in a person without diabetes. The remaining two-thirds of islets are lost during the transplant or are unable to function adequately. Our work attempts to overcome the challenges of islet transplantation by studying early graft failure. We have determined that large islets (> 125 μm diameter) were unable to cure diabetes after transplantation[3], partially due to diffusion barriers present after isolation, which inhibited the viability of the large islets[4]. In contrast islet under 100μm diameter can survive better, produce more insulin and can cure diabetes in a rat model. We set out to engineer islets of optimal size and conformation for transplantation.

Methods

Micro-Mold Production: Glass micro-molds were formed using standard photolithography procedures. Briefly, metal and photoresist coated glass was exposed to UV light and then immersed in developer and etchants. Wet etching created small divots in the glass for reaggregation of the islets. Different designs for the molds allow for optimization of reaggregation. Divots depth is 60μm with holes that are 100-180μm in diameter with rounded bottoms. Each micro-mold, 1.5 inches in length contains 3000-12,000 divots (depending on the divot design).

Islet Reaggregation: Islets were isolated from Sprague-Dawley rats. Islets were dispersed into single cells via exposure to calcium-magnesium free HBSS and papain. The cells were transferred to the micro-molds and allowed to settle into the divots for subsequent cell culture.

Viability assays: After reaggregation, islets were incubated in live/dead fluorophores (YO-PRO-1 and Propidium iodide) for 30 min. Reaggregated islets were rinsed with PBS and placed in the Attofluor Chamber on a confocal microscope. All images were collected within 20 minutes of removal of the islets from the media.

Immuno-labeling: Antibody labeling was performed to detect insulin, glucagon, somatostatin, and proinsulin using standard procedures for immunofluorescence labeling. Images of serial sections (10 μm) were collected using confocal microscopy.

Insulin Secretion (Static Incubation): Large, small, and engineered islets of known islet equivalents are incubated in low-glucose (3 mM) and high-glucose (20 mM) medium. Aliquots of the culture medium are taken at specific time points during incubation and assayed for insulin content using ELISA techniques.

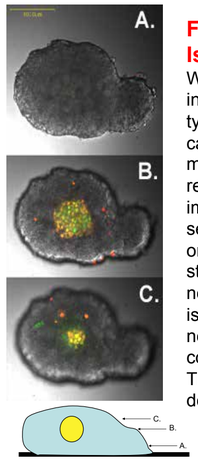

Figure 1: Isolated Large Islets Have Poor Viability. We have shown that large islets are inferior for transplantation to cure type 1 diabetes, while small islets can reverse diabetes in an animal model[3]. This observation has been reported in human islets[5]. The images illustrate different Z-sections through two islets (large on left, small on right). Islets were stained for apoptosis (green) and necrosis (red/orange). The large islet on the right shows extensive necrosis and apoptosis that was confirmed in Z-sections of the islet. The small islet had no core cell death.

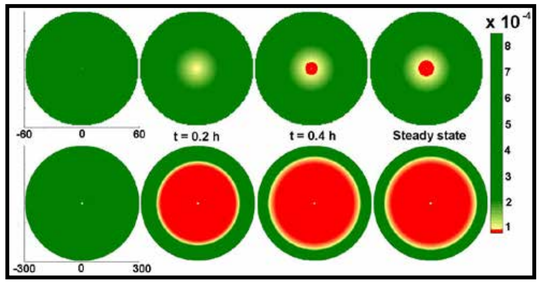

Figure 2: Modeling Islet Diffusion Barrier. Modeling of diffusion limitations in intact islets illustrate the rapid oxygen deprivation experienced by the core cells.[1] Red indicates critical oxygen concentration resulting in cell death, while green indicates oxygen concentration yielding viable cells. The upper panel provides an example of a small islet (60 μm radius). The lower panel provides a model of a large islet (300 μm radius) at steady state. (Coordinates are in μm)

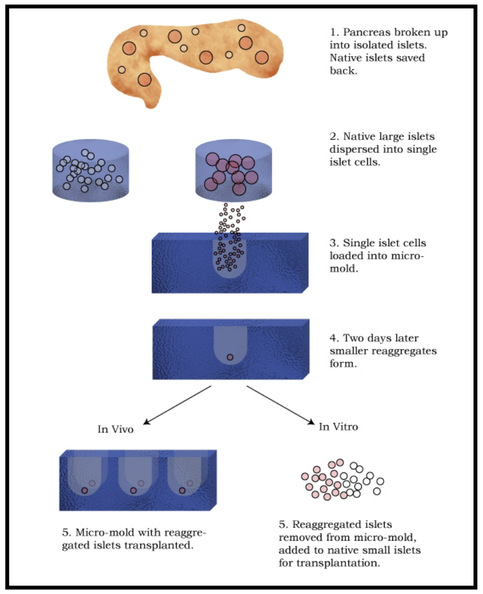

Figure 3: Flowchart of Islet Reaggregation into Micro-Molds. After islet isolation, large and small islets were separated, and large islets dispersed into single cells or small cell clusters to be loaded into the micro-molds for reaggregation into smaller islets.

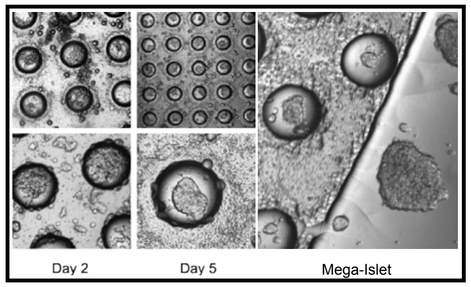

Figure 4: Reaggregated islets Growing in Micro-Molds. Cells were loaded into the micro-molds so that they covered the bottom of each divot. By day 5, the cells reorganized into 3D spherical shapes reminiscent of native small islets. When cell reaggregation is not limited by the micro-mold, mega-islets form. Divots filled with small reaggregated islets were present, while extra0large islets grew on the glass that was not etched.

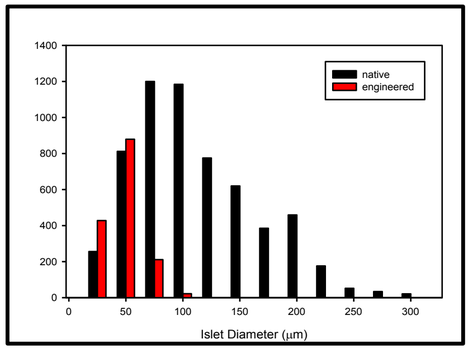

Figure 5: Change in Size Distribution of Islets After Reaggregation. After reaggregation in the micro-molds, all resulting islets were below 125μm in diameter, which is the critical diameter for optimal diffusion without vascularization.

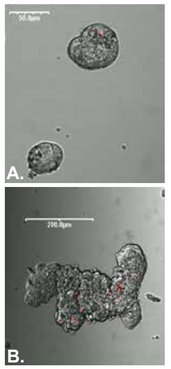

Figure 6: Viability Staining of Reaggregated Islets Compared to Mega-Islets. (A) Two islets reaggregated in the micro-molds are shown. The upper islet illustrates one of the few single dead cells identified in the small reaggregated islets. (B) A mega-islet formed on the surface of the micro-mold. The red indicates dead cells throughout the mass, which were present at all Z sections.

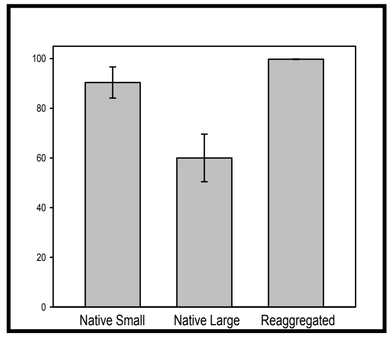

Figure 7: Viability of Native Compared to Reaggregated Islets. Five days after isolation native small and large, and reaggregated islets were assayed for viability. The reaggregated islets had a viability of 99.7% (N = 510 reaggregated islets). Native small and large islets had viability levels similar to those we have published previously[1,3].

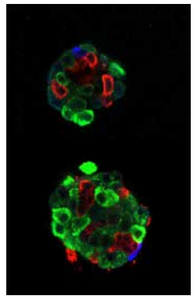

Figure 8: Cellular Composition and Organization in Reaggregated Islets. Immunostaining of reaggregated islets for beta cells (insulin-positive cells = green) alpha cells (glucagon-positive cells = red), and delta cells (somatostatin-positive cells = blue) shows that all three cell types were present in the reaggregated islets. Their organization is reminiscent of large native islets with a scattered distribution of cell types.

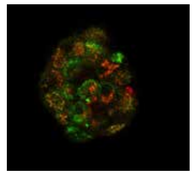

Figure 9: New Insulin Production in Reaggregated Islets. Six day old reaggregated islets were stained for insulin (green) and the precursor of insulin, proinsulin (red) to determine whether they were making new insulin. As expected, the beta cells are double labeled, indicating that new insulin is being synthesized in the reaggregated islets, even after six days in culture.

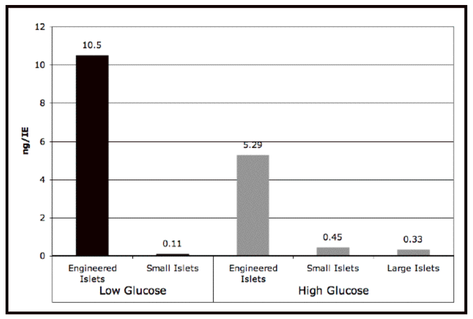

Figure 10: Insulin Secretion of Reaggregated Islets Compared to Native Islets. In conditions where reaggregated islets are exposed to low glucose (3 mM) or high glucose (20 mM), the reaggregated islets secrete more insulin compared to native islets.

Summary

- In this study we were able to demonstrate proof-of-concept for our approach to overcoming the inherent inferiority of large islets for transplantation as a cure for type 1 diabetes.
- Large islets were able to reform into smaller reaggregated islets in a relatively short period of time.
- Reaggregating large islets into smaller units prior to the initiation of core cell death lead to improved viability, which was even higher than native small islets.
- Current studies are focusing on the functional secretion properties, diffusion characteristics, and oxygen consumption of the reaggregated islets.

References

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