Changes in Peri-Islet Extracellular Matrix Stiffness Regulate Insulin Secretion via Mechanotransduction Signaling

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Statement of Purpose: The pancreatic islets of Langerhans are multicellular micro-organs that regulate blood glucose. Specifically, β-cells are the most abundant cell type that secrete insulin in response to high blood glucose. Type 1 diabetes (T1D) is characterized by the destruction of insulin producing β-cells by autoreactive immune cells. Current therapies are aimed at controlling glucose levels by providing exogenous insulin. Unfortunately, these therapies do not cure the underlying disease and require lifelong maintenance. To fulfill the need for improved diabetes therapies, transdisciplinary approaches that merge tools and knowledge from multiple fields are needed to appropriately treat this complex disease [1].

Islet transplantation therapy is a promising treatment for diabetic patients. However, the restricted availability of donor islet supply and downfalls of long-term function and immunomodulation remain persistent [1]. Islet encapsulation in biomimetic polymers is a technique that can provide a microenvironment tailored for islet survival via immune protection, structural support and restored cell-cell and cell-matrix interactions, to further improve transplantation outcomes [1-3, 7]. Understanding the role of the islet microenvironment in cell survival might aid in overcoming challenges with long term graft survival and function [3]. Islets are surrounded by a specialized extracellular matrix (ECM) that regulates cell survival, insulin secretion and provides mechanical and biochemical cues to the β-cells [3,4,8]. ECM characteristics that closely resemble the native islet microenvironment might boost insulin secretion and viability; however, little is known about how the properties of the pancreas microenvironment, such as ECM stiffness, regulate islet function in both health and disease [3].

In a variety of systems, microenvironment stiffness is recognized to play a key role in cellular response and differentiation [3]. Previous studies have shown that tissue stiffness in muscle cells regulates phosphofructokinase (PFK) activity [4,5]. Nyitray et al. demonstrated that 0.1 kPa scaffolds increase glucose sensitivity in Min6-derived β-cell clusters [3]. The mechanisms underlying mechanotransduction regulation of insulin secretion have not been well studied in the β-cell nor has it been proposed to study islet function with intact islets. *Therefore, we hypothesized that increasing matrix stiffness from 0.1-10kPa in encapsulated, intact islets would increase islet glucose sensitivity by increasing PFK activity.*

We propose to use a 3D reverse thermal gel (RTG) culture system, poly(serinol hexamethylene urea)-*co*-poly(*N*-isopropylacylamide) or PSHU-PNIPAAm, that allows us customize the islet microenvironment to mimic the native pancreas and to investigate how the environment affects islet function and survival. Our goal is to use this 3D RTG encapsulation system to understand how scaffold stiffness influences function.

Methods: We synthesized a 3D RTG scaffold composed of PSHU-PNIPAAm as previously described [6]. The RTG forms a gel at body temperature and can easily be tailored to achieve scaffolds of varying stiffness by varying the polymer weight percentage (wt%). The storage modulus, indicating material stiffness, and the sol-gel transition of varied wt%'s of polymer were measured via rheology during a temperature sweep from 20-40°C by increments of 5°C. The 5, 10 and 15wt% RTG samples fell into the 0.1-10kPa [3] range of stiffness. We encapsulated isolated mouse (C57Bl/6) islets in 5, 10, and 15wt% RTG, as depicted in Figure 1, and measured glucose stimulated insulin secretion (GSIS) at 2mM and 20mM glucose and PFK activity based on RTG wt%. We also studied changes in intracellular calcium signaling dynamics as a measure of islet dysfunction to insulin secretion using confocal microscopy.

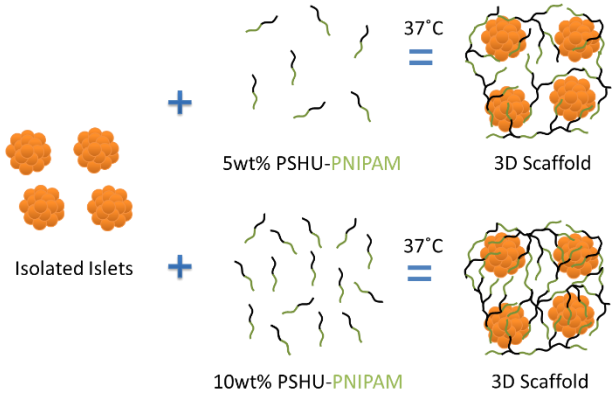

Figure 1: Graphical representation of varied wt% RTG encapsulation of isolated islets.

Results and Conclusions: We found that insulin secretion increased as the matrix stiffness increased in both basal (2mM) and high glucose (20mM) conditions. Insulin secretion at 20mM glucose normalized to 2mM glucose, or the stimulation index (SI), decreases with matrix stiffness indicating dysfunction to insulin secretion. PFK activity analysis showed an increase in PFK activity in islets encapsulated in stiffer RTGs.

The results from this work support a role for ECM mechanical properties in regulating islet function and define the relationship between mechanotransduction and metabolism in pancreatic islets. The results from this work will not only help drive scaffold design for islet encapsulation technologies to improve graft survival and function, but will also suggest a novel mechanism of islet dysfunction in T2D and pancreatitis where increases in matrix stiffness are prevalent.

References:

1. (Stabler CL. Nature Rev. Materials. 2019. 4: 429-450.)
2. (Papas KK. Adv. Drug Delivery. 2019. 139: 139-156.)
3. (Nyitray CE. Tissue Eng. 2014. 20: 1888-1895.)
4. (Alessandra G. Cells. 2020: 9: 1–24.)
5. (Park JS. Nature. 2020. 578: 621–626.)
6. (Peña B. Biomacromolecules. 2016. 17: 1593-1601.)
7. (Wu S. Adv. Healthcare Materials. 2021. 10: 1-17.)
8. (Stendahl JC. Cell Transplant. 2009. 18: 1-12.)