Integrating human pancreatic islets within a vascularized, micro-organ platform

R. Hugh F. Bender[1], **Matthew Wortham**[2,3], Roberto Gaetani[4], Karen L. Christman[4], Maike Sander[2,3,5], Christopher C.W. Hughes[1]

[1]Department of Molecular Biology and Biochemistry, University of California, Irvine; [2]Pediatric Diabetes Research Center, Department of Pediatrics, [3]Sanford Consortium for Regenerative Medicine, [4]Department of Bioengineering, and [5]Department of Cellular & Molecular Medicine, University of California, San Diego

Introduction

Diabetes is estimated to affect over 387 million individuals worldwide, with this incidence expected to rise to more than 600 million by 2030. This disorder results from absolute or relative deficiency of the hormone insulin, which normally functions as a key regulator of blood glucose homeostasis. Insulin is exclusively produced by beta cells present in pancreatic islets, underscoring the importance of these structures for blood glucose regulation. In healthy individuals, nutritional cues stimulate insulin secretion to lower blood glucose levels following a meal. However, absolute or relative insulin deficiency leads to hyperglycemia, giving rise to chronic tissue damage. Current efforts to understand the mechanisms underlying defective beta cell function in diabetes have utilized in vitro techniques to maintain islets in the laboratory. However, existing methods cannot maintain islets in a functional state for longer than a few days. The Hughes Lab has previously developed a vascularized, micro-organ (VMO) device that offers a viable alternative to suspension culture techniques currently used to maintain human islets. Unique to this device is a 3D gel matrix that mimics the in vivo extracellular matrix and a perfused microvasculature capable of delivering nutrients directly to tissues within the 3D matrix. The purpose of this study is to utilize this VMO device to develop a biofunctional, pancreas-on-a-chip platform for the ex vivo study of human pancreatic islet function. Successfully cultivating these islets in a 3D, biomimetic environment will represent a significant step forward for the diabetes research field. As the incidence of diabetes continues to rise, development of this platform will enhance the duration, accuracy, and utility of islet studies within the laboratory for further understanding of this disease.

A Vascularized Micro-Organ Device

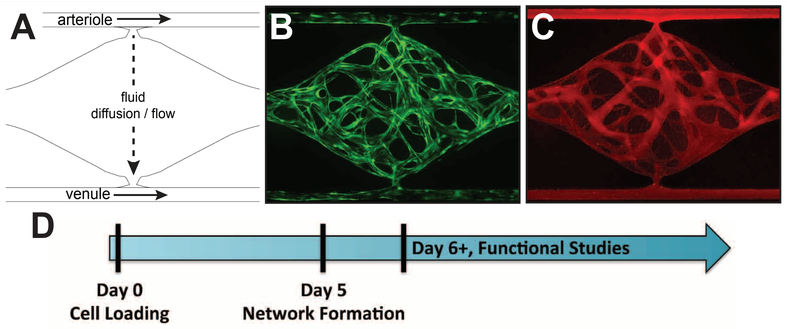


Figure 1. Vascular network formation within a microfluidics device for 3D cell culture. (**A**) A microfluidics device incorporating arteriole (high pressure) and venule (low pressure) fluid channels drives fluid flow across and (**B**) angiogenesis within a flanking cell chamber (green, ECs). (**C**) Functional anastomoses with these channels and physiological tightness of the vessels is shown by perfusion with 70 kDa rhodamine-dextran (red). (**D**) This device can be used for organ-on-a-chip studies by co-loading tissue-specific cells (islets) with vascular forming cells (day 0) and measuring biological function after vascular network formation (day 5).

Islet Loading

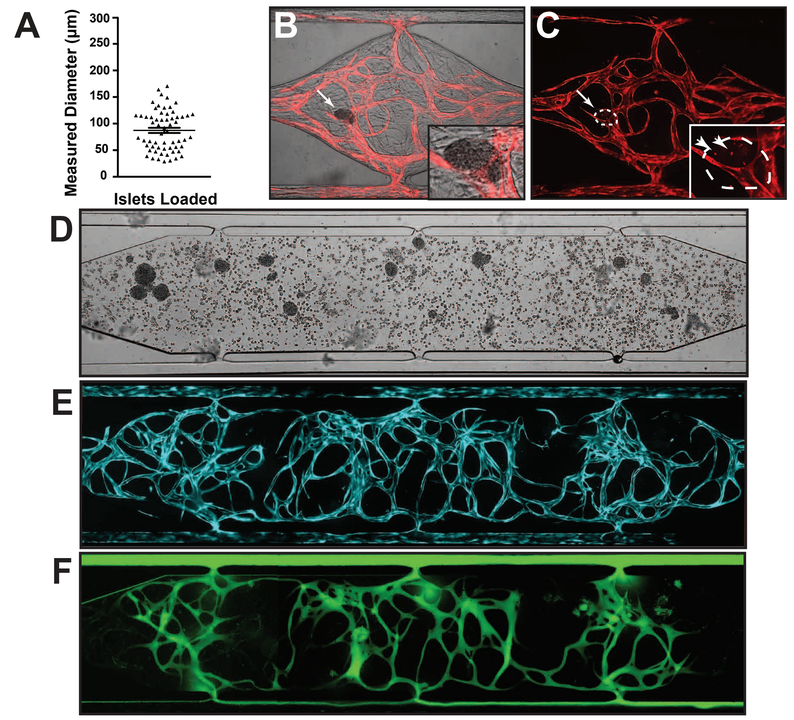


Figure 2. Incorporation of human islets within a vascularized micro-organ device. (**A**) Islets from 25-175µm in diameter can be loaded into this platform (n=4, 3-chamber devices) prior to (**B**) vessel formation in the proximity of these islets (arrow; red, ECs; inset shows enlarged islet). (**C**) Due to these neighboring vessels, islets show a low rate of cell death (EthD-1 punctate [red] within inset [arrowheads]). (**D**) More islets can be consistently loaded within a modified, elongated cell chamber (21.7 ± 1.8 islets per chamber). (**E**) The resulting vascular network (blue) is (**F**) readily perfusable with 70kDa FITC-dextran (green).

Pancreas Extracellular Matrix

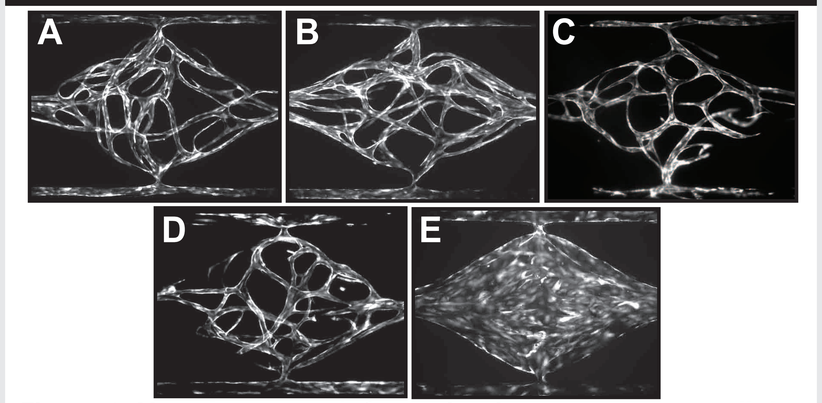
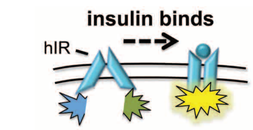


Figure 3. Vascular network formation in pancreas extracellular matrix (pECM). Relative to fibrin gel alone (**A**), a comparison of porcine pECM with fibrin gel mixtures demonstrates that vascular networks form in (**B**) 40%, (**C**) 50%, and (**D**) 60% pECM. However, (**E**) no network formation occurs in 75% pECM.

A Bioluminescent Reporter for Insulin



B

Luciferase	Luciferin (substrate)	Peak Emission	Signal Half-Life (min)	Size
Firefly luciferase (Fluc)	D-luciferin	612 nm	62 ± 5	61 kDa
Renilla luciferase (Rluc)	coelenterazine	480 nm	86 ± 5	36 kDa
NanoLuc (Nluc)	furimazine	460 nm	160 ± 18	19 kDa

Adapted from: Prescher and Contag, 2010, *Curr. Opin. in Chem. Biol.*, 14: 80-89

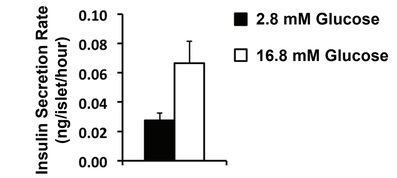


Figure 4. Planned design of bioluminescent reporter tools for detecting insulin secreted from islets within the device. (**A**) Split luciferase constructs will be fused to the intracellular portion of human insulin receptor (hIR). In the absense of insulin, the halves will remain separated, and no light will be generated. However, upon insulin binding, conformational changes in the hIR allow for recombination of the luciferase holoenzyme, leading to the generation of light signal. (**B**) Several split forms of luciferase enzymes may be conducive for this application and will be tested in parallel. (**C**) ELISA measurement following static insulin secretion assays indicate that glucose-stimulated islets produce an average of 0.07 ng insulin/islet/hour (n=10), providing an approximate, functional detection range necessary for this reporter system.

Key Findings & Work in Progress

Key Findings:

- A modified, elongated cell chamber improves the loading consistency and optimizes the number of islets when loading this microfluidics device.
- This device still forms functional, perfusable vascular networks despite the altered design.
- Pancreatic extracellular matrix (pECM) can be used to mimic the native pancreas without inhibiting vascular network formation.

Work in Progress:

- Thorough assessment of islet survival, metabolism, and biological function (insulin secretion) upon incorporation within the device.
- Determination of optimal pECM concentration for consistent, functional vascular network formation.
- Validation and comparison of bioluminescent reporter construct function in response to increasing insulin concentrations.

UCI Ayala School of Biological Sciences

HiRN Human Islet Research Network

CIRM California's Stem Cell Agency