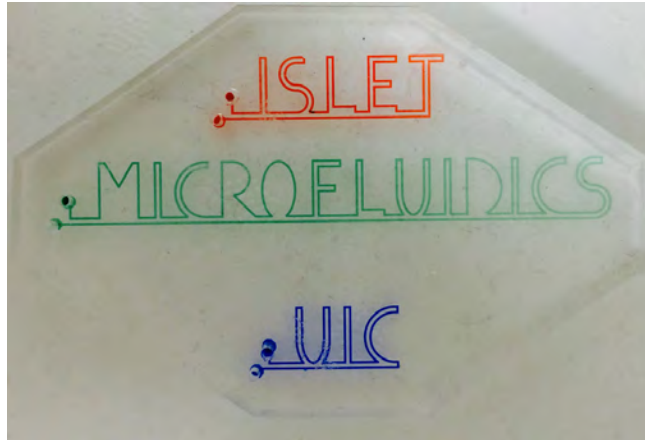# *The UIC Microfluidic-Based Beta-Cell Functional Analysis Facility*

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INTRODUCTION

The Microfluidic-based beta-cell functional analysis facility is located in the Department of Surgery, at the University of Illinois at Chicago (UIC) and is supported by the Juvenile Diabetes Research Foundation International (JDRF). This functional analysis facility will provide an analysis and characterization service for emerging islet cell derivatives including hESCs/hiPSCs, microencapsulated islets/hESCs/hiPSCs, as well as screening of potential pharmaceutical agents for diabetes treatment. This service will be available to the JDRF community as a whole and diabetes researchers who may not have such technology or access. The overall purpose is to assist the field of diabetes research in moving forward with therapeutic strategies for beta-cell replacement and beta-cell regeneration to cure Type I diabetes. Additionally, we are also willing to service those who are working on islet or beta-cell related research.

Islet cell derivatives will be analyzed to answer the following basic questions: (**1.**) Compared to human islets, do the biologics have similar physiological response profiles in the presence of glucose or other secretagogues? (**2.**) Physiologically, what are the functional similarities and/or dissimilarities of the biologics to human islets? (**3.**) Does the biologics have normal or altered insulin stimulator-secretion coupling factors and what, if any, is the impact of pathophysiological changes on insulin secretion? (**4.**) How does microencapsulation or hypoxia impact the function and survival of microencapsulated islet cells?

In 2009, we designed a three-layer microfluidic perifusion device specifically for pancreatic islets of Langerhans (Fig.1A). This initial design has evolved into a family of microfluidic devices that can be applied to the study of islet physiology and pathophysiology (Fig.1B-E).
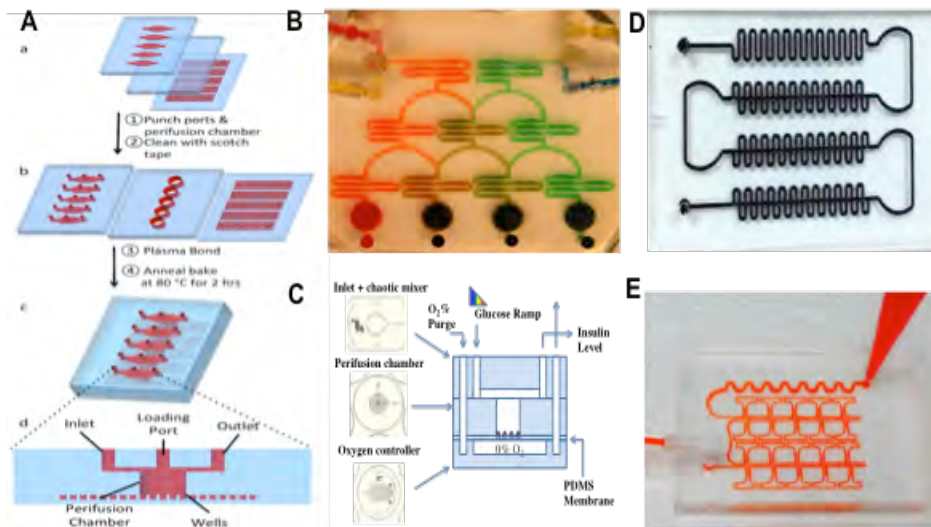
Fig.1. Microfluidic-based perifusion biochip. A: Schematic of the three-layer perifusion device with five identical chambers. **B:** Perifusion multiplexer integrated with concentration generator. **C:** Schematic of perifusion integrated with oxygen control. **D:** microcapsule islet array. **E:** islet array

In the current system, islet fixation is not required. Specific chemical gradients can be established and well maintained in the perifusion chamber (Fig.2A). The microfluidic perifusion system measures not only insulin secretion kinetics but also insulin stimulator-secretion coupling factors by integrating with multiparametric optical imaging technology, including measurement of calcium influx, mitochondrial potentials, NAD(P)H, ROS, glucose uptake (individually or combined two or three simultaneous fluorescence imaging) (Fig2B). In addition to insulin measurements, additional islet hormones can be measured with tested spatiotemporal resolution including; proinsulin, C-peptide, and glucagon (Fig.2C). Furthermore, various islet types, single beta-cells, and encapsulated islet cells can be analyzed (Fig.2D).
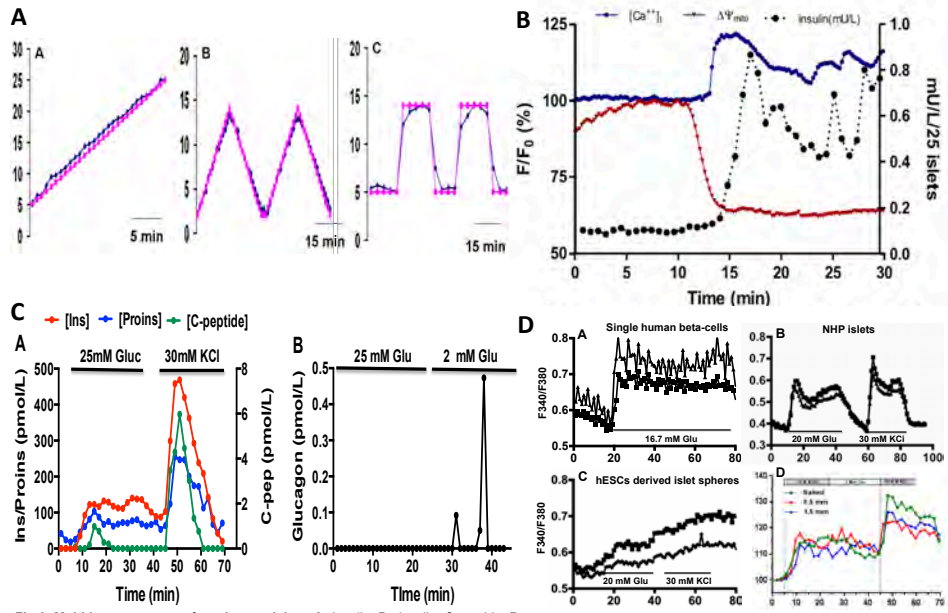
Fig.2. (A) Chemical gradients. **(B)** Simultaneous multiparametric assay. **(C)** Multi hormone assay **(D)** Suitability for various cell types

SERVICE

We propose the following array of multiple assays with their associated rationale, as well as a decision tree/order of the proposed assays.

Protocol 1. Testing Physiological Function and Viability by Pulsed Glucose and KCl Stimulation

Rationale: We first test the functionality of the cell sample by simple pulse stimulation with a high glucose concentration, followed by 30 mM KCl. From this protocol, we can characterize the physiological similarities or differences of cell-biologics compared to human islets. Furthermore, it acts as an initial test criteria to determine if further testing is needed. If the cell-biologic passes the initial test, the following systematic protocols may follow to further interrogate their insulin secretory pathway.

PROT 1	Low glucose (2 mM)	High glucose (dependent on cell origin)	Low glucose (2 mM)	KCl (30 mM)	Low glucose (2 mM)
	10 min	20-30 min	30 min	15 min	10 min

Protocol 2. Determination of Optimal Glucose Sensitivity using Incremental Step-Up Glucose Pulse Stimulation and/or Glucose Ramp Stimulation

Rationale: It is known that some cell derivatives respond to glucose differently when compared to human islets, for example glucose sensitivity shift. This shift in glucose sensitivity is evidence that the cells are responsive to glucose either at lower or higher glucose concentrations when compared to human islets. It is expected that the iBCs derived from pluripotent stem cells might initially form cells that display a variation in glucose sensitivity, due in part to its position as a developmental intermediate. It is important not only to address how this varying threshold may change during maturation, but also to

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prevent potential complications such as hypoglycemia, which will have profound effects on clinical outcomes.

Step-up glucose pulse stimulation provides useful information on whether a shift in glucose sensitivity occurs. However, a detailed change in threshold/sensitivity can be further verified by a glucose ramp over a narrowed range of glucose concentrations determined by step-up glucose pulse experiments.

PROT 2.1	Glucose (2 mM)	Glucose (5 mM)	Glucose (2 mM)	Glucose (8 mM)	Glucose (2 mM)	Glucose (12 mM)	Glucose (2 mM)	Glucose (16 mM)	Glucose (2 mM)
	10 min	20 min	20 min	20 min	20 min	20 min	20 min	20 min	10 min

PROT 2.2	Glucose (2 mM)	Glucose ramp (2 mM – 12/15/18/25 mM)		Glucose (2 mM)
	20 min	Increase at interval of 1 mM/min		20 min

Protocol 3. Dissection of Insulin Stimulator-Secretion Coupling Factors

Rationale: Glucose-induced beta-cell insulin secretion is a cascade that is mainly mediated through the K_{ATP}-dependent pathway. This pathway involves several key stimulator-secretion coupling factors. Any defect or change in these coupling factors will significantly impact insulin secretion and, subsequently, *in vivo* function. Many iBCs are acknowledged to respond to glucose differently when compared to normal human islets. This difference may result from a complete absence of or immaturity of stimulator-secretion coupling factors through the differentiation protocols employed. Additionally, glucose can increase cytoplasmic calcium concentrations by inducing calcium release from the endoplasmic reticulum (ER) calcium stores without biphasic and pulsatile signatures. Often, the ER-induced calcium stimulates small quantities of insulin release in a non-controlled manner. Additionally, this can provide misleading information on iBCs glucose-responsiveness and functional maturity.

Protocol 3.1 Verification of K_{ATP} Channel Activity

Rationale: Since both plasma K_{ATP} channel existence and activity play an important role in glucose-induced insulin secretion, the K_{ATP} channel activity is assessed with dose responses of K_{ATP} channel closers (Tolbutamide and/or glyburide). Additionally, K_{ATP} channel activity can be further verified by K_{ATP} channel openers (Diazoxide) in response to either glucose or K_{ATP} channel closers.

PROT 3.1.1	Low glucose (2 mM)	Tolbutamide (50 mM) or Glyburide (0.1 µM)	Low glucose (2 mM)	Tolbutamide (100 mM) or Glyburide (0.5 µM)	Low glucose (2 mM)	Tolbutamide (150 mM) Or Glyburide (1.0 µM)	Low glucose (2 mM)
	10 min	20 min	20 min	20 min	20 min	20 min	10 min

PROT 3.1.2	Low glucose (2 mM)	High glucose (dependent cell origin)	High glucose (dependent cell origin) + Diazoxide(100/150 µM)	Low glucose (2 mM)
	10 min	20 min	30 min	10 min

PROT 3.1.3	Low glucose (2 mM)	Tolbutamide/Glyburide	Tolbutamide/Glyburide + Diazoxide(100/150 µM)	Low glucose (2 mM)
	10 min	20 min	30 min	10 min

Protocol 3.2 Verification of VDCCs Activity

Rationale: Glucose-induced calcium influx mainly occurs through the voltage-dependent calcium channels (VDCCs). We have observed that glucose-induced calcium may be independent of VDCCs in some iBCs. This may explain why iBCs often have abnormal insulin secretion patterns and lower insulin release in response to the glucose challenge. Here, we apply Nifedipine (L-type Ca^{2+} channel closer) pre or post glucose administration to determine the spatiotemporal relationship between $[Ca^{2+}]_I$ and insulin secretion in cells to be tested. Additionally, we can determine the origin of cytoplasm calcium by using a calcium-free Krebs-Ringer buffer and assessing $[Ca^{2+}]_i$ in response to glucose.

PROT 3.2.1	Low glucose (2 mM)	High glucose (dependent cell origin)	High glucose (dependent cell origin) + Nefedipine (1, 20,50 nM)	Low glucose (2 mM)
	10 min	20 min	20 min	10 min

PROT 3.2.2	Low glucose (2 mM)	Nefedipine (1, 20,50 nM)	High glucose (dependent cell origin) + Nefedipine (1, 20,50 nM)	Low glucose (2 mM)
	10 min	20 min	20 min	10 min

PROT 3.2.3	Low glucose (2 mM)	High glucose (dependent cell origin)	Low glucose (2 mM)	Low glucose (2 mM) w/o calcium	High glucose (dependent cell origin) w/o calcium	Low glucose (2 mM)
	10 min	20 min	20 min	30 min	20 min	10 min

Protocol 3.3 Determination of Intracellular Calcium Source

Rationale: In addition to activation through the VDCCs, cytoplasm calcium can also be induced by glucose through calcium release from the ER. This in turn then triggers insulin secretion; however, such calcium signaling often does not have normal biphasic and oscillatory patterns, similar to VDCC-regulated calcium signaling. In this protocol, thapsigargin (an ER calcium ATPase pump blocker) is applied to deplete ER calcium before glucose stimulation in order to determine the role, if any, that ER calcium plays in insulin secretion and intracellular signaling of test cell derivatives.

PROT 3.3	Low glucose (2 mM)	Thapsigargin (1, 2.5, 5 µM)	High glucose (dependent on cell origin)	Low glucose (2 mM)
	10 min	30 min	30 min	10 min

Protocol 4. Determination of Maximal Insulin Secretion Capability and Exhaustion.

Rationale: Beta-cell insulin granules populate two different pools: the readily releasable pool (RRP) and the reserved pool (RP). The RRP pools are associated with phase I secretion. In contrast, phase II

secretion requires the trafficking of the RP to the plasma membrane. Normal beta-cells contain excess amounts of insulin granules and only a small percentage (1-5%) is utilized for insulin secretion under each stimulation. We apply repetitive glucose stimulation to mimic *in situ* glucose homeostasis to determine maximal insulin secretion capabilities and identify insulin secretion defects in phase I/II or beta-cell exhaustion. This pulse stimulation protocol may provide useful information on insulin granule distribution and release capability (RRP pools vs. reserved pools) by analyzing phase I vs. phase II or phase I or II vs. total insulin release. If repeated stimulation elicits a significant reduction of acute insulin release with each pulse then the beta-cells are considered exhausted. This in turn indicates that the cell derivative may not provide long-term *in vivo* function.

PROT 4	Low glucose (2 mM)	Pulse 1 glucose	Low glucose (2 mM)	Pulse 2 glucose	Low glucose (2 mM)	Pulse 3 glucose	Low glucose (2 mM)	Pulse 4 glucose	Low glucose (2 mM)
	20 min	30 min	30 min	30 min	30 min	30 min	30 min	30 min	20 min

Protocol 5. Determination of the Impact of Microencapsulation

Rationale: Microencapsulation is a promising approach to prevent islet graft immunorejection. It is important to understand the impact of the encapsulation process, capsule material, and capsule size on the nutrient/insulin diffusion, glucose metabolism, and function of encapsulated cells. Additionally, hypoxia is considered another important factor responsible for the functional loss of encapsulated islets. Encapsulation aggravates islet hypoxia by preventing revascularization and increases the oxygen diffusion distance. Hypoxia may also facilitate the attraction of macrophages and, subsequently, causes cell overgrowth on the microcapsule.

The function of microencapsulated islets, impact of capsule materials, and capsule sizes are assessed using previously described protocols (1-4). For the hypoxia study, microencapsulated islets or islet-like cells are assessed using the perifusion device integrated with an oxygen controller shown in Figure 1C.

If required by interested researchers, we can further provide new experimental protocols focused on the following areas: (*please note that all protocols are well established and fully verified*).

(1) Simultaneous measurement of $[Ca^{2+}]_i$/NAD(P)H and insulin.
(2) Simultaneous measurement of ROS using DCFDA/MitoSox, $[Ca^{2+}]_i$, and insulin.
(3) Investigation of insulin secretion independent of K_{ATP} channels and amplification of K_{ATP}-dependent pathways through the application of GLP-1, carbachol, glucose + arginine, or glucose + amino acid mix.
(4) Investigation of long-term dynamic and dose-response of pharmaceutical agents on islet survival and function using the biochip shown in Figure 1C.
(5) High-throughput, high-content imaging, and screening of iBCs and pharmaceutical agents using the array biochip (Fig. 1D and E) that can hold up to 200 islets or encapsulated islets in an array.

The UIC Human Islet Transplant Program has a certified cGMP isolation facility and has conducted more than 600 human islet isolations. The program is one of only six centers funded through the IIDP for the distribution of human islets. Additionally, we have archived data from over 150 human islet preparations using this microfluidic assay, as well as *in vivo* animal transplant data, histological imaging/analysis, and other *in vitro* data from each preparation that is useful as an historical control. Since we isolate human islets on a weekly basis, freshly isolated human islets can be used as a suitable control whenever required by cell providers.

Suitable testing materials, parameters, and quantification are listed in the following table

Cells/Materials	Parameters	Characterization and Quantification	Control
• Human islets • Islet-like spheres • iBCs • Single beta-cells • Pharmaceutical agents • Animal islets/beta-cells • Cell-lines	Calcium signaling ($[Ca^{2+}]_i$)	• Basal $[Ca^{2+}]_i$ • Starting time, delay times, slopes, peak level of $[Ca^{2+}]_i$ and percentage changes under stimulation • Preservation of physiological biphasic pattern (Y/N) and oscillatory pattern (Y/N; slow/fast oscillation) • AUC under stimulation • Returning of $[Ca^{2+}]_i$ into basal level post stimulation (Y/N) and time needed to return to basal level • Spatiotemporal relationship with $\Delta\Psi_M$ and insulin secretion (Y/N and degree of dependence)	Human islets
	Mitochondrial potential ($\Delta\Psi_M$)	• Basal Ψ_M • Starting time, delay times, slopes, peak level of $\Psi_{M,}$ and $\Delta\Psi_M$ under stimulation • Returning of $[Ca^{2+}]_i$ into basal level post stimulation (Y/N) and time needed to return to basal level • Spatiotemporal relationship with $[Ca^{2+}]_i$ and insulin secretion (Y/N and degree of dependence)	Human islets
	Hormone secretion kinetics	• Basal [Insulin], [Proinsulin],[C-peptide], [Glucagon] • Starting time, delay times, slopes, peak level of $[Ins]_,$ and percentage changes under stimulation • Preservation of physiological biphasic pattern (Y/N) and oscillatory pattern (Y/N; slow/fast oscillation) • Returning into basal level post stimulation (Y/N) and time needed to return to basal level. • Glucose-induced [Insulin], [Proinsulin], [C-peptide], [Glucagon] • KCl-induced [Insulin], [Proinsulin], [C-peptide], [Glucagon] • Total released [Insulin], [Proinsulin], [C-peptide], [Glucagon] • Phase I [Insulin], [Proinsulin], [C-peptide], [Glucagon] • Phase II [Insulin], [Proinsulin], [C-peptide], [Glucagon] • Phase I [Insulin], [Proinsulin], [C-peptide], [Glucagon] / Total released [Insulin], [Proinsulin], [C-peptide], [Glucagon] • Phase II [Insulin], [Proinsulin], [C-peptide], [Glucagon] / Total released [Insulin], [Proinsulin], [C-peptide], [Glucagon] • Total released [Insulin], [Proinsulin], [C-peptide], [Glucagon] / Total content [Insulin], [Proinsulin], [C-peptide], [Glucagon] • Spatiotemporal relationship with $\Delta\Psi_M$ and $[Ca^{2+}]_i$ (Y/N and degree of dependence) • [Insulin], [Proinsulin], [C-peptide], [Glucagon] under repetitive glucose stimulation	Human islets
• Encapsulated islets • Encapsulated iBCs	$[Ca^{2+}]_i$ $\Delta\Psi_M$ Hormone kinetics	Same as above	• Human islets • Various $[O_2]$ • Capsule sizes • Capsule materials

Practicalities

(1) All data gathered from performed analysis will be held and treated as highly confidential. The data will only be communicated between "cell providers" and "test performers" as required by JDRF. "Test performers" have absolutely no rights to publish or publicly communicate the data without prior written permission from "cell providers".

(2) This facility is established based on a pay-per service agreement with JDRF for JDRF sponsored research projects. We are willing to work together with interested researchers to adapt the microfluidic technology and develop any new protocol for optimal analysis of their cells and purposes.

(3) Cell derivatives tested should preferably be shipped under the cold shipping protocol (~8ºC) adopted from NIH-sponsored IIDP at a maximal density of 250 islets or equivalent/mL. Given the different cell-

biologic cell source origins, it is the responsibility of cell providers to determine optimal culture media, as well as the antibiotics/growth factors to be used and communicate with test performers. The Cell-biologic providers should provide extra culture media to "test performers". Cell providers should provide any necessary information on cell origin, type, cell amount, and protocols to be performed. It is also the responsibility of cell providers to provide all necessary biohazard information. All cell derivatives are preferably shipped in suspension. If shipped in adherent status, cell providers must provide the necessary information and protocols for cell handling. The detailed information of shipping protocol and cell quantity will be provided upon request by researchers.

(4) The cost of analysis for the interested researchers outside JDRF sponsored projects is depended on the protocol selected and extent of the analysis requested. For detailed information, please contact the facility director prior to analysis.

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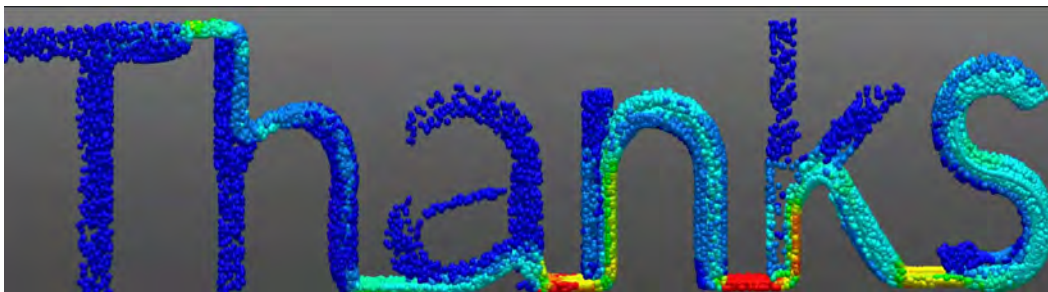
Simulation of flow dynamic and velocity in a microchannel