SUPPORTING INFORMATION FOR:

A dual detection system for simultaneous measurement of intracellular fluorescent markers and cellular secretion

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The materials and reagents, fabrication procedure of the microfluidic device, islet procurement, perfusion system, and data analysis are described in detail. A typical insulin calibration curve produced from the microfluidic device is shown in Figure S-1. Figure S-2 demonstrates simultaneous measurement of intracellular [Ca^{2+}] using Fura-2 PE3 with insulin secretion from a single islet.

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Experimental Design

Materials and reagents

Ethylenediaminetetraacetic acid (EDTA), ammonium hydroxide (NH_4OH), sodium hydroxide (NaOH), and bovine serum albumin (BSA) were from EMD Chemicals (San Diego, CA). Sulfuric acid (H_2SO_4), nitric acid (HNO_3), hydrogen peroxide (H_2O_2), and hydrofluoric acid (HF) were from Avantor Performance Materials (Center Valley, PA). Dextrose was obtained from Fisher Scientific (Pittsburgh, PA). Pluronic F-127 and Fura 2-acetoxymethyl ester were from Life Technologies (Grand Island, NY). Fura-PE3 acetoxymethyl ester was from Cayman Chemical (Ann Arbor, MI). Cy5 monofunctional N-hydroxysuccinimide ester was from GE Healthcare Bio-Sciences (Piscataway, NJ). A monoclonal antibody to human insulin C-terminal (Ab) was obtained from Meridian Life Science, Inc. (Saco, ME). RPMI 1640 was purchased from Mediatech (Manassas, VA). Gentamicin sulfate was purchased from Lonza (Basel, Switzerland). Cosmic Calf Serum was from HyClone Laboratories (South Logan, UT). Collagenase P (from *Clostridium histolyticum*) was purchased from Roche Diagnostics (Indianapolis, IN). All other chemicals were obtained from Sigma-Aldrich (Saint Louis, MO), unless otherwise stated. Labeling of bovine insulin with Cy5 (Ins*) was performed as previously described.[1] All buffers were prepared using ultrapure deionized water (NANOpure Diamond™, Barnstead International, Dubuque, IA) and filtered using 0.2 μm nylon syringe filters (Pall Corporation, Port Washington, NY).

Fabrication of microfluidic devices

A photomask from Digidat, Inc. (Pasadena, CA) was used to fabricate EOF channels on one glass layer, and another photomask from Fineline Imaging (Colorado Springs, CO) was used to fabricate perfusion channels on the other glass layer. Each photomask was aligned on top of separate borofloat photoresist blanks (Telic Co., Valencia, CA) that were pre-coated with a layer of AZ1500 positive photoresist and a chrome layer. After exposing with 18 mW cm^{-2} collimated UV radiation for 15 s, the exposed photoresist and underlying chrome were removed with AZ 400K Developer (AZ Electronic Materials Corp., Sommerville, NJ) and a chrome etchant solution (CR-7S, Cyantek Corp., Fremont, CA), respectively. The exposed glass was then etched in a 5:1:3 (v:v:v) mixture of H_2O:HNO_3:HF to a depth of 6 μm (EOF channels) or 25 μm (perfusion channels). The channel dimensions were measured with a portable surface roughness tester (Mitutoyo, Aurora, IL). The access holes were drilled on the EOF channel layer with a 300 μm diameter drill bit (islet chamber), a 600 μm diameter drill bit (EOF channel access holes), or a 1.1 mm diameter drill bit (perfusion channel access holes). After removing the remaining

photoresist and chrome, the etched glass slides were cleaned in a 3:1 (v:v) solution of H_2SO_4:H_2O_2 for 30 min, and subsequently in a 5:1:1 (v:v:v) solution of H_2O:NH_4OH:H_2O_2 heated to 60°C for another 30 min. After rinsing, the cleaned glass slides were aligned under a microscope by hand and bonded at 640°C for 8 hours. Reservoirs (IDEX, Oak harbor, WA) were then attached to the devices above the drilled access holes.

Isolation and culture of islets of Langerhans

Pancreatic islets were isolated from 20-40 g male CD-1 mice (Charles River Laboratories Internal, Inc., Wilmington, MA) using collagenase P digestion as previously described.[2] The isolated islets were incubated at 37°C, 5% CO_2 in RPMI 1640 media containing 11 mM glucose, 10% calf serum, 100 units mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, and 10 μg mL^{-1} gentamicin. All islets were used within 4 days after isolation.

Perfusion system

A balanced salt solution (BSS) was used as the perfusion solution for all islet experiments. The BSS contained 125 mM NaCl, 5.9 mM KCl, 1.2 mM $MgCl_2$, 2.4 mM $CaCl_2$, 25 mM tricine, and either 3 or 13 mM glucose (pH 7.4) with an additional 1 mg mL^{-1} BSA. Two 60 mL plastic syringe tubes, that contained BSS with either 3 or 13 mM glucose, were connected to the perfusion inlets on the microfluidic device by Tygon tubing (0.03 in. o.d., 0.01 in. i.d., Cole-Parmer, Vernon Hills, IL). The final glucose concentration delivered to the islet chamber was determined by the flow rate ratios of the two solutions, which was controlled by the relative heights of the two syringes. A program written in LabView software was used to control the heights of the two syringes. The mixing ratio of the two perfusion solutions as a function of syringe height was calibrated as previously described.[3]

For the characterization of the perfusion system, 1 μM fluorescein was added to the syringe containing 13 mM glucose and the CMOS camera (50 ms exposure time at 0.5 s intervals) was used to image the fluorescence intensity in the perfusion channel before the islet chamber. The perfusion flow rate was determined by measuring the time required to deliver a new perfusion solution to the detection point.[4]

Data analysis

The B/F ratios from electropherograms were analyzed using an automated program.[5] For the calibration curve of insulin immunoassay, the average B/F ratios of 15 consecutive

electropherograms were plotted against the insulin concentrations and fitted with a weighted four-parameter logistic function. Detection limits were calculated by using the calibration curve to determine the concentration of insulin that would decrease the B/F ratio of a blank solution by an amount equal to three times the standard deviation of the B/F ratio of a blank solution. For the islet experiments, insulin concentrations were quantified with a calibration curve that was obtained daily, converted to mass, and normalized to the perfusion flow rate.

The oscillatory periods of $[Ca^{2+}]_i$ and insulin profiles were assessed by performing a Fast Fourier transform without smoothing or detrending the data. Phase offset (ϕj) between each oscillation of $[Ca^{2+}]_i$/insulin and glucose was calculated by normalizing the difference in times of each $[Ca^{2+}]_i$/insulin oscillation peak ($t_{i,j}$) to the closest glucose oscillation peak ($t_{g,j}$) to the period of the glucose wave (5 min), as previously described.[3]

$$\phi_j = \frac{360° \times (t_{i,j} - t_{g,j})}{5} \quad (1)$$

Insulin calibration curve

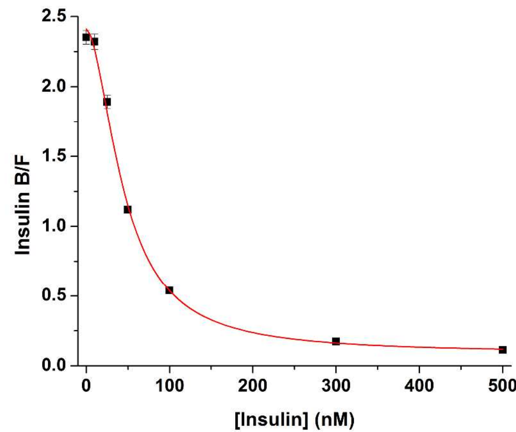

Figure S-1. Calibration curve of insulin immunoassay. Immunoassay reagents were 50 nM Cy5-insulin and 50 nM anti-insulin antibody. The average peak height ratio of bound to free (B/F) from 15 consecutive runs of insulin immunoassay was plotted against the concentration of perfused unlabeled insulin as shown in Figure 2B. Error bars represent ±1 standard deviation.

Islet traces using Fura-PE3

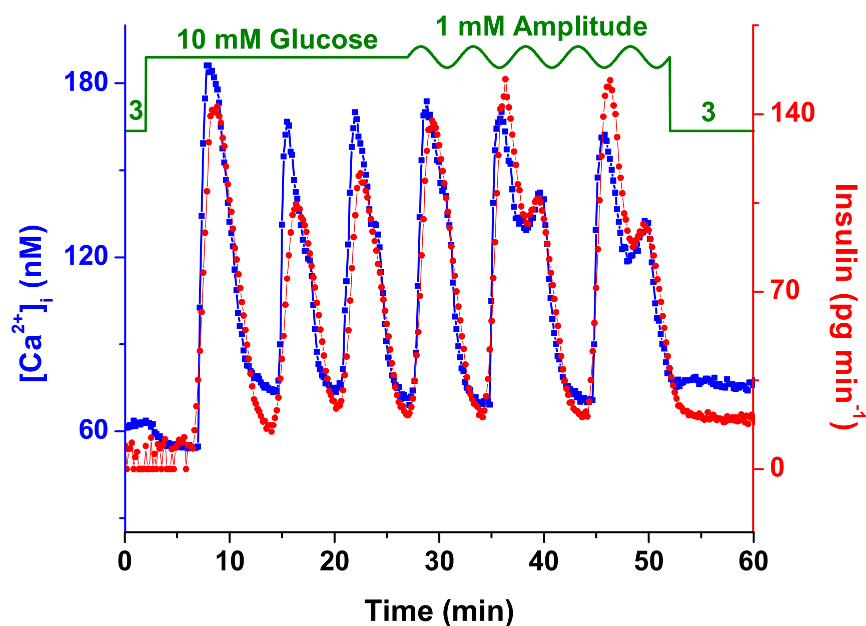

Figure S-2. Simultaneous measurement of $[Ca^{2+}]_i$ using Fura-PE3 and insulin secretion. The peak areas of the $[Ca^{2+}]_i$ oscillations increase during exposure to oscillatory glucose levels whereas the amplitudes do not. Peak insulin levels also increase during exposure to the oscillatory glucose levels.

References

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