Supporting Information for:

A passively operated microfluidic device for stimulation and secretion sampling of single pancreatic islets

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Additional Experimental Details

**Microfluidic device fabrication.** The microfluidic channel layout ([Figure 1A in main text](#)) was designed in Adobe Illustrator software and sent to Fineline Imaging (Colorado Springs, CO) for photomask printing at 65,024 DPI resolution (negative image). The SU-8/silicon master was fabricated using standard photolithography with two different thicknesses of photoresist (Microchem), as previously described for islet trapping.\(^1\) Deeper islet-loading channels were 156 µm deep, 600 µm wide, and 1.5 mm in length. Shallow sampling channels were 14.8 µm deep, 60 µm wide, and 10.8 mm long. Using standard soft lithography\(^2\), PDMS precursors were mixed thoroughly in a 10:1 ratio of elastomer:curing agent, poured over the master and additional plastic insert (see below), degassed in a vacuum chamber, then cured overnight at 70 °C. Cured PDMS was peeled from the master, and access holes were punched. Prior to use, the channeled PDMS surface and a glass cover slip were treated with an air plasma (Harrick Plasma; Ithaca, NY) for 50 s, then permanently bonded. The channel pattern is shown in [Figure 1A](#) in the main text.

The 11-mm diameter plastic insert was fabricated using a two-part epoxy resin (Smooth-Cast 310; Smooth-On plastics).\(^3\) To create support pegs on the insert, a 1 mm thick PDMS slab (thin PDMS mold), with three holes punched in a triangular pattern, was first plasma oxidized onto a glass coverslip. Using a cork borer, an 11-mm hole was created in a separate PDMS slab that was ~2 cm thick (thick PDMS mold). Silicone oil was used as a pretreatment for the thin PDMS mold to ensure the cured smooth-cast would remove easily. Smooth-cast was mixed per manufacturers instructions, pipetted into the three small holes, and the excess was removed. A blunt tip needle was used to ensure all air bubbles had been removed. After 5 min, the thick PDMS mold was bonded reversibly to the thin PDMS mold to form the completed mold. This mold was filled with Smooth-Cast, which was
allowed to cure overnight. The resulting plastic insert was used to define the modified reservoirs during standard soft lithography, as shown in Figure 3B in the main text.

**Insulin Adsorption.** Two sets of mono-labeled fluorescent insulin standards (“FITC-Ins”; Invitrogen) were diluted into imaging medium at 100 nM, one into BSA-free imaging medium and the other into standard imaging medium (with 0.1% BSA). Following plasma bonding of devices, a pre-treatment solution of either BSA-free imaging medium or standard imaging medium (0.1% BSA) was added directly to the central input reservoir, ensuring that each channel was filled. After this 1-hour pre-treatment, each device was thoroughly washed with the respective medium for 10 min. The device was placed into a microscope stage-top humidified incubator (Tokai Hit) held at 37 °C to mimic experimental conditions. FITC-Ins standards were passed through each device at 0.011 mm³ s⁻¹ (~40 μL h⁻¹) for 30 min, and eluted samples were collected. Fluorescence was measured using a NanoDrop 3300 spectrofluorometer (Thermo Scientific), with LED excitation at 490 nm and emission measurement at 520 nm. To determine the percent recovery of insulin, measured FITC-Ins concentrations were quantified using one of two linear calibration curves for the BSA-free medium (R² = 0.998) or the standard imaging medium (R² = 0.999). This insulin adsorption study was carried out on 2 batches (separate PDMS mixing and curing), 2 chips per batch, and 3 channels per chip to ensure a representative sampling.

**Stimulation Timing.** Stimulation timing was characterized using a step change in solution conductivity, a method modified from previous reports.¹⁹-²¹ Briefly, the voltage drop across a 20 MΩ resistor placed in series with the microfluidic channel was measured during introduction of the step change in conductivity within the channel (see apparatus, Figure S-1). This step change was defined
by switching from standard imaging medium (0.125 M NaCl, etc.) to imaging medium with 1.0 M NaCl, or vice versa. Devices were filled with imaging medium and held at 37 °C. A vacuum was applied to initiate flow, then a step change in conductivity (~7-fold change in ionic strength) was introduced into the large reservoir to mimic a change in glucose concentration. Upon reaching the entrance to the microfluidic channel, the newly introduced solution resulted in a significant increase (or decrease) in the electrical resistance of the channel. This resulted in a significant decrease (or increase) in the measured voltage drop across the 20 MΩ resistor in series. A 9-V battery served as the source, and a single-channel analog input of the voltage drop across the 20 MΩ resistor was acquired (NI-USB 6008 interface to desktop; National Instruments). An in-house written LabVIEW application acquired data at 10 Hz over time.

**Islet Staining and Imaging Protocols.** For calcium imaging, isolated islets were stained with 4 µM Fluo-4 AM in imaging medium at room temperature for 1 h. Islets were loaded onto the microchip in the stage-top incubator (37 °C) and perfused with imaging medium at ~0.011 µL min⁻¹ (~40 µL h⁻¹). Fluo-4 emission (525 ± 25 nm) was recorded at 1.0 Hz using an inverted fluorescence microscope (Nikon Ti-E) during excitation at 470 ± 20 nm. Photobleaching was limited using a software-controlled shutter.

**Islet volume measurements.** All confocal images of single islets were obtained post-sampling using an A1si laser scanning confocal microscope (Nikon) with 488-nm laser excitation, using a Plan Fluor 20× objective (0.75 NA). Z-scans were captured by delineating the top and bottom of the islet, then slicing at 2 µm increments, collecting reflectance (485 nm bandpass) and transmitted light images of each slice. Reflectance images were reconstructed in Nis Elements (Nikon) for 3-D renderings of
each islet, and the volume of each islet was determined. Images collected from confocal reflectance Z-scans were reconstructed in Nis Elements (Nikon) to generate 3-D renderings of each islet. 3D renderings and two individual images representing cross-sections of islets are shown in Figure 5A of the main text (reflectance intensity represented in blue). Each image was converted to a binary image using an automated thresholding function, and these binary images (red in Figure 5A) were used to reconstruct a volume rendering of each islet. The volume of each islet was then determined using an automated volume measurement tool in Nis Elements, which functions by integrating the total interpolated volume and correcting for dimensions. Final volumes of each islet are represented in nanoliters. By manual comparison of the reflectance and binary images, it was observed that the most significant error in this method occurred near the solution-glass or solution-PDMS interfaces, a result of stray reflections at these interfaces. Errors are estimated to be less than 5% of the total volume.

**Vacuum Manifold Fabrication.** The eight-plex vacuum manifold (Figure 1B, main text) was used to interface the single hand-held syringe with eight tubes, which were interfaced to the outlets of the eight microfluidic channels. This manifold was fabricated in-house using cured PDMS, two glass microscope slides, and PEEK tubing. Higher magnification images of the manifold are included in Figure S-5, below. A 10-mm thickness, pre-cured PDMS layer was sliced into a rectangular section with dimensions of 75 mm × 25 mm. A scalpel was then used to remove a smaller rectangular section in the center, with dimensions of 40 mm × 10 mm. To accommodate PEEK tubing, eight 1-mm interface holes were punched into one face of the PDMS, and a 1-mm hole was punched into the opposite face. The patterned PDMS was plasma bonded to two glass slides in a sandwich configuration, shown in Figure S-5. PEEK tubing was then inserted into each hole and sealed with uncured PDMS by curing overnight at 70 °C. This formed an airtight, eight-plex manifold.
Figure S-1. Experimental apparatus used to measure stimulation timing ($t_{\text{stim}}$). A simple voltage divider was assembled, using the microchannel as one resistor in series with a 20 MΩ resistor. The voltage drop across the 20 MΩ resistor was used to monitor changes in the electrical resistance of the microchannel after switching to a higher or lower conductivity solution. This allowed determination of the average $t_{\text{stim}}$ encountered by islets in the trapping region of the channel. Example data and further analysis details are included in the main text (discussion and Figure 2). The electrical circuit, including a 9-V battery, resistor, and microchannel, was completed by soldering a connection to a blunt-ended steel needle attached to the tubing, which was attached to the syringe for flow control.
**Figure S-2.** A pressure sensor was used to determine the precision of vacuum levels generated using the handheld, 100-mL syringe filled with air. Each point represents a separate starting and stopping of the syringe plunger, using spacers to generate a vacuum of 8 kPa. As shown by others, this approach is consistent, with approximately 2% relative standard deviation.
**Figure S-3.** Fluidic resistance ($R_{\text{sampling}}$) of microchannels. The expected linear response of flow rate versus pressure was observed, and the inverse of the slope gave a fluidic resistance of $700 \pm 20 \text{kPa s mm}^{-3}$ at 37 °C. A vacuum of -7.7 kPa achieves the target flow rate of 0.011 mm$^3$ s$^{-1}$ (40 µL h$^{-1}$). Error bars represent standard deviations of at least 3 measurements from 3 chips.

**Figure S-4.** Location of cross-section shown in **Figure 2A** in the main text is depicted here by referencing the channel layout pattern. Reservoirs are shown as blue circles, and the cross-sectioning location is shown as a red line.
Figure S-5. Higher magnification images of the eight-plex vacuum manifold, which was fabricated from PDMS, glass slides, and PEEK tubing. Fabrication details are included above. Outer dimensions of the rectangular manifold were 75-mm in length, 25-mm in width, and 10-mm in depth.
References


