Supporting Online Material
Dishinger, J. F., Reid, K. R., Kennedy, R. T.

SUPPORTING ONLINE MATERIAL

Quantitative Sampling of Biphasic and Pulsatile Insulin Release from Pancreatic Islets with High Throughput Immunoassays on a Microfluidic Chip

John F. Dishinger[†], Kendra R. Reid[†], Robert T. Kennedy[†‡]
[†]Department of Chemistry and [‡]Department of Pharmacology, University of Michigan, Ann Arbor, Michigan 48109

CONTENTS

I. COMPARISON OF EOF AND PRESSURE DRIVEN PERFUSION

Solution compositions for data in Figure 3B & C are as follows (concentrations in mM): KRB, 120 NaCl, 5.4 KCl, 1.4 KH_2PO_4, 1.4 $MgSO_4$, and 20 HEPES; RPMI, media used as purchased from Gibco; KSOM, 92 NaCl, 2.5 KCl, 0.35 KH_2PO_4, 0.15 $MgSO_4$, 21.7 $NaHCO_3$, 1.24 $CaCl_2$, and 0.02 EDTA; HBSS, 134 NaCl, 5.2 KCl, 0.4 KH_2PO_4, 0.4 Na_2HPO_4, and 10 HEPES; aCSF, 140 NaCl, 2.4 KCl, 1 $MgSO_4$, and 1.2 $CaCl_2$; DPBS, 137 NaCl, 2.7 KCl, 1.5 KH_2PO_4, and 8.2 Na_2HPO_4; HEPES, 128 NaCl, 0.8 Na_2HPO_4, and 25.2 HEPES.

II. MIXING OF INSULIN ACROSS SAMPLING CHANNEL

Before the insulin sampling stream enters the flow-split (with the sampling channel emerging from one side as shown in Figure 1D) it was imperative that perfusate be adequately mixed to avoid bias from laminar flow segregation of secreted insulin (i.e., sampled insulin needs to be of uniform concentration across the channel before being split). To determine the appropriate length of channel between islet chamber and flow-split that allowed for complete mixing of sample by lateral diffusion, the distribution of FITC-ins across two laminar streams was imaged at various points on a straight microchannel with the same dimensions as those used on the 15-islet chip as summarized in Figure S1. It was found that laminar flows of 50% FITC-ins solution and 50% buffer flowing at a combined rate of 500 nL min^{-1} mixed completely via diffusion at a point ~0.6 cm downstream of the convergence point. To allow mixing of insulin leaving the islet chamber, 2.4 cm (a length four-times what was needed for two-stream mixing) of channel was used to link the islet and flow-split portions of the chip.

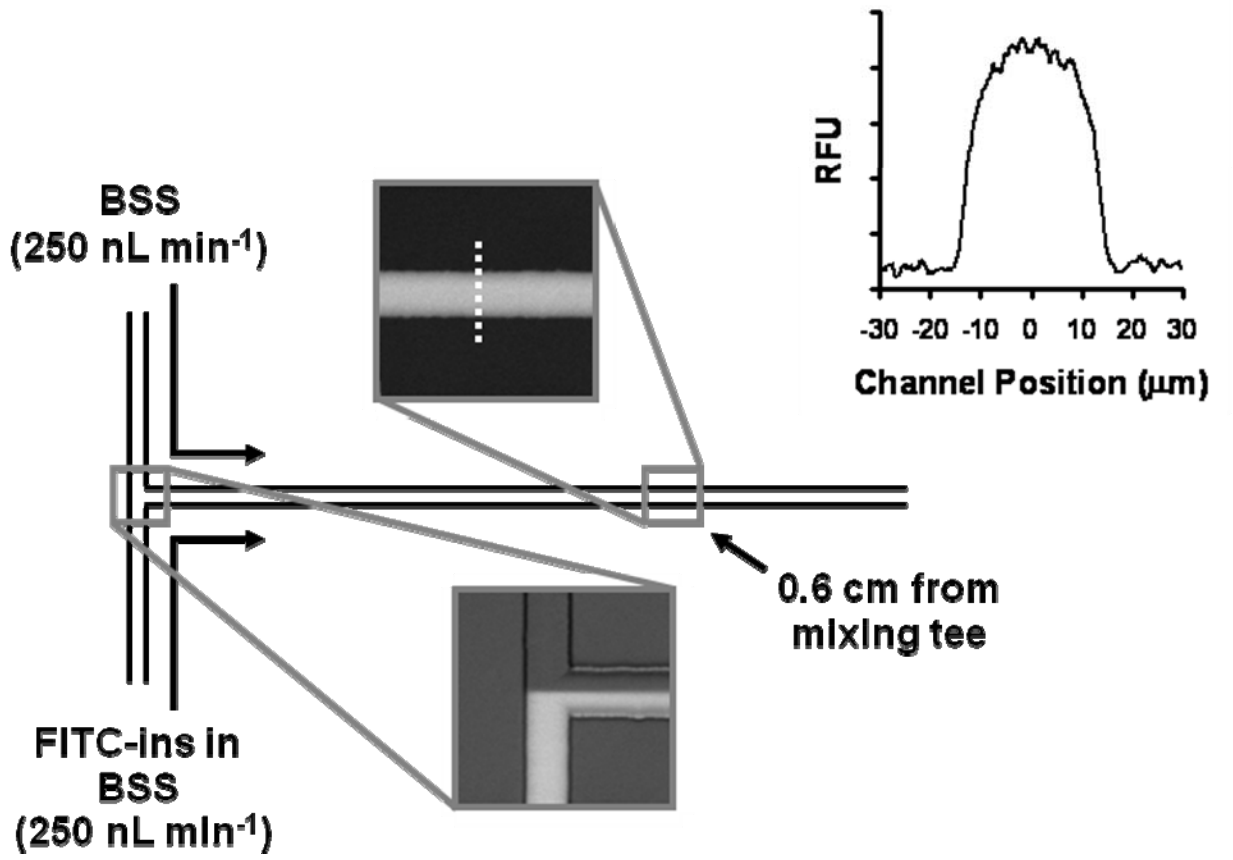

Figure S1. Summarized setup and results for determining appropriate length of channel between islet and flow-split allowing adequate sample mixing. Black lines in the figure represent a microfluidic channel design (mixing tee) and arrows indicate direction of flow. Solutions of BSS and FITC-ins in BSS were mixed on the chip (shown by the lower fluorescence CCD image) by flowing both solutions at 250 nL min^{-1} with a syringe pump. An image taken 0.6 cm from the mixing tee (upper expansion) shows complete mixing of the two streams. Line-scan data (upper right) taken from the dotted line drawn on this image confirms complete mixing. Channel dimensions were identical to those used on the 15-islet chip. To allow adequate mixing of secreted insulin, 2.4 cm of channel (a distance 4-times that which was observed for two-stream mixing) was used to link the islet chamber with the flow split.

III. CHARACTERIZATION OF DEVICE TEMPORAL RESOLUTION

The limit of temporal resolution that can be obtained with this device is dependent upon the dispersion of insulin in transit from the islet to the detection area. The dispersion of sampled insulin across the chip was estimated by perfusing a FITC-ins solution into an operating device and taking fluorescence images at various positions of interest. Results from these experiments are shown in Figure S2. The upper plot, produced by monitoring the fluorescence intensity of an islet chamber with a loaded islet, shows a rise time (10% - 90%) of 3.5 s. This suggests the capability of fluid exchange and sampling of all insulin secreted by an islet within this time. The bottom plot was produced from images taken at the injection cross and shows an overall delay time of 153 s and a maximum response time of 22 s for the chip (this analysis included perfusion dispersion prior to reaching the islet chamber). These times were verified by monitoring B/F on the device after a step-change in perfused insulin standard concentrations (data not shown). A response time of 22 s was found to be adequate for monitoring insulin secretion dynamics and oscillations from islets with a typical period of several minutes; however, it would not be suitable to monitor secretory pulses of 10-20 s that are presumed to accompany such pulses of intracellular Ca^{2+} in islets.

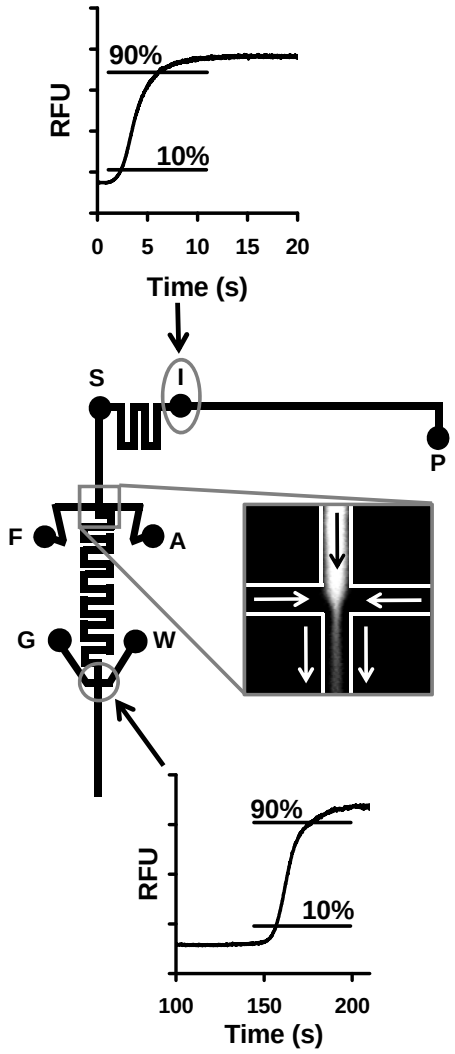

Figure S2. Illustration of a single network of the 15-sample chip with flow characterization plots from throughout the device. Fluidic reservoir abbreviations as follows: P, perfusion inlet; I, islet chamber; S, flow-split; F, FITC-ins; A, Ab; G, gate; W, waste. The upper plot shows fluorescence intensity vs. time produced from images of the islet chamber (with a loaded islet) while perfusing FITC-ins in BSS at 500 nL min^{-1}. A CCD image of the point of reagent mixing (middle expansion) shows perfusate and immunoassay reagent flows entering the reaction channel (FITC-ins is only present in the BSS; FITC-ins and Ab reservoirs contain only immunoassay reagent buffer). White lines represent channel walls, and arrows indicate direction of flow. The lower plot, produced from images of the injection cross, shows the delay and response time (10% - 90%) for the device up to the separation channel.