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Glucagon measurement from islet populations using a TR-FRET assay

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TR-FRET homogenous assay method using a microfluidic device for quantitation of glucagon secretion from human islet populations is described. A PDMS microfluidic device was used to perfuse a population of islets and collect 10µL fractions of perfusate every two minutes. The method was used to measure glucagon secretion from human islet populations from 5 donors.

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Chemicals and Reagents

Polydimethylsiloxane (PDMS) prepolymer (Sylgard 184) -Dow Corning (Midland, MI)

Dextrose -Fisher Scientific (Pittsburgh, PA)

TR-FRET glucagon assay -Cisbio (Walthan, MA)

All other reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless noted otherwise. All solutions were made with ultrapure DI water (NANOpure Diamond System, Barnstead International, Dubuque, IA). A balanced salt solution (BSS) was used for islet experiments which contained 125 mM NaCl, 5.9 mM KCl, 1.2 mM MgCl2, 2.4 mM CaCl2, 25 mM tricine, and brought to pH 7.4 before addition of 0.1% BSA.

Materials and Instrumentation

HTRF 96 well low volume plates

Spectramax iD5 plate reader

PDMS device was fabricated using previously described methods[1]

Chemical reagents

1 TR-FRET glucagon assay -Cisbio (Walthan, MA)

1.1 Dextrose -Fisher Scientific (Pittsburg, PA)

1.2 Polydimethylsiloxane (PDMS) prepolymer (Sylgard 184) -Dow Corning (Midland, MI)

1.3 All other reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless noted otherwise. All solutions were made with ultrapure DI water (NANOpure Diamond System, Barnstead International, Dubuque, IA). A balanced salt solution (BSS) was used for islet experiments which contained 125 mM NaCl,

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5.9 mM KCl, 1.2 mM MgCl2, 2.4 mM CaCl2, 25 mM tricine, and brought to pH 7.4 before addition of 0.1% BSA.

Materials and Instrumentation

2 HTRF 96 well low volume plates

2.1 Spectramax iD5 plate reader

2.2 PDMS device was fabricated using previously described methods[1]

Calibration Curve

3 10,000** pg/ml stock glucagon was diluted following manufacturer's protocol for a serial dilution.

3.1 Final concentration was 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.6 and 0 pg/mL

3.2 The table below shows how a typical well plate was arranged for incubation. Rows A,B and C are shown in blue and are for the calibration to be run in triplicate. Values are given for pg/mL.
The yellow and orange portion are marked for the fractions from the first and second islet experiment and the numbers correspond to the time stamp of the fraction. For example, the "0" corresponds to the perfusate from 0-2 min.

	1	2	3	4	5	6	7	8	9
A	2000	1000	500	250	125	62.5	31.3	15.6	0
B	2000	1000	500	250	125	62.5	31.3	15.6	0
C	2000	1000	500	250	125	62.5	31.3	15.6	0
D	0	2	4	6	8	10	12	14	16

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E	24	26	28	30	32	34	36	38	40
F	48	50	52	54	56	58	60	0	2
G	10	12	14	16	18	20	22	24	26
H	34	36	38	40	42	44	46	48	50
I	58	60	---	---	---	---	---	---	---

96 well plate sample arrangement

3.3 Open wells (- - -) can be used for the lysate dilutions described below

Preparation for on chip measurements

4 Islets were incubated in PIM(S) media (Prodo Labs).

4.1 Chip had been conditioned with BSS for at least 30 minutes.

4.2 Islets washed by transferring them to a petri dish with prewarmed BSS with desired concentration of glucose (we used 20 mM) and let sit in BSS for a few minutes (~2 min)

4.3 Islets were then transferred to the device in a 10 µL pipet and were positioned so that they could sediment into the islet chamber on the device

4.4 Once islets are loaded the top of the chip is dried and chamber is sealed with PCR tape

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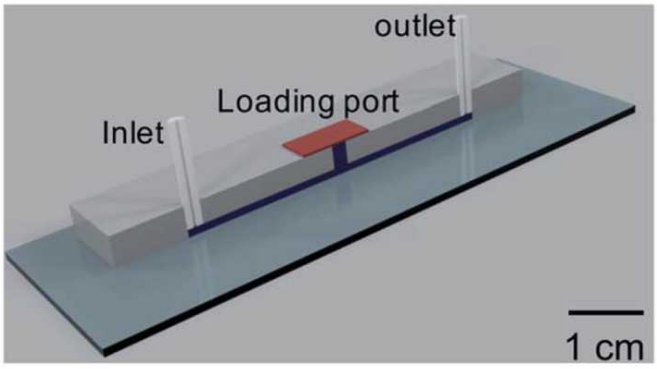
4.5 Inlet and outlet tubing are connected to the device

4.6 Flow from syringe pumps is started and run for ~10-15 minutes or at least until the volume of the chip and outlet tubing has been displaced twice. We used a flow rate of 5 uL/min. ***Note-*** times will change depending on flow rate.

4.7 Collect fractions every 2 min into low volume Eppendorf tubes and seal once fraction is collected.

4.8



Chip design

Islet Lysis

5 Remove islets from device with a 200 µL pipet by vacuum and transfer to a small centrifuge tube

5.1 Remove islets with a 10 µL pipet after centrifugation

5.2 Lysis is performed using an acid ethanol mixture as described previously[2]

5.3 Transfer lysate to a low adsorption vial

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5.4 Dilute lysate appropriately (we used 10:1 and 20:1) with BSS.

5.5 10 uL of TR-FRET reagents were added to each well, covered, and incubated overnight for 12-14 hours at room temperature in the dark.

5.6 The Spectramax ID5 plate reader was used to measure the TR-FRET signal according to the manufacturer's protocol.
Excitation light (360 nm) was set for 50 us, followed by a 100 us delay, and a 600 us recording time of the two FRET channels (620 and 665 nm). This timing protocol was repeated every 2 ms for 100 cycles.

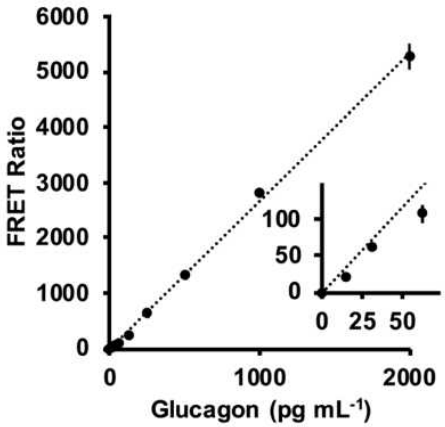
Data Analysis

6 FRET ratios are presented as the ratio of the emission at 665 nm to that at 620 nm multiplied by 10,000

6.1 The average FRET signal from the 0 pg mL1 standard solution was subtracted from all FRET measurements

6.2 Calibration curves were generated by averaging the FRET ratio from the three replicates of each standard glucagon solution and plotting these values vs. the concentration of glucagon

6.3



An example calibration is shown

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6.4 Using calibration convert FRET ratio to pg/mL glucagon for all fractions and diluted lysate samples

6.5 Use lysate dilution that fell within the calibration range to quantify the amount of glucagon in the lysate

6.6 To report data as a percent of glucagon secreted, the amount of glucagon from each fraction was summed and added to the total glucagon in the lysate.[3]

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