Electronic Supplementary Material (ESI) for Integrative Biology.

Supplementary Information

C-peptide and Zinc Delivery to Erythrocytes Requires the Presence of Albumin: Implications in Diabetes Explored with a 3D-printed Fluidic Device

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Fig.S1

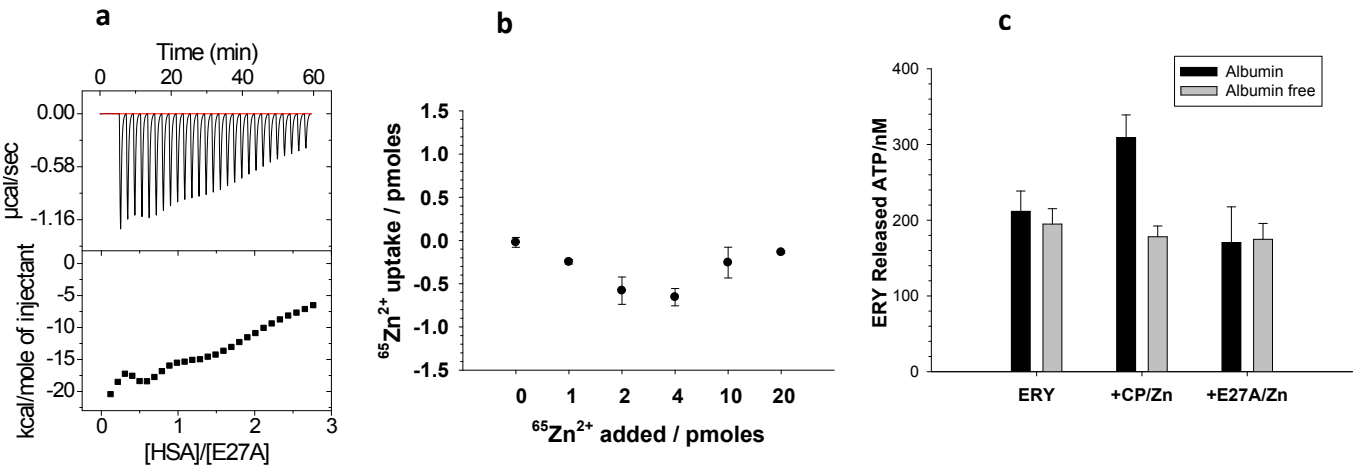
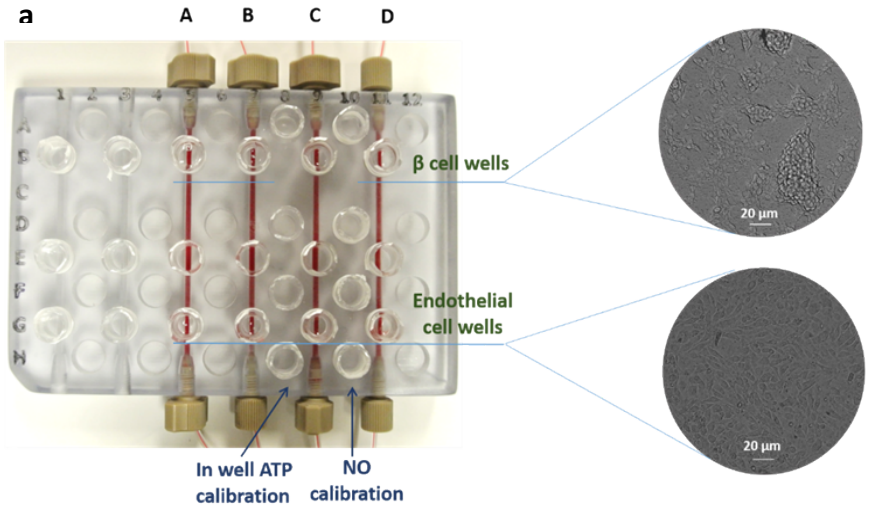
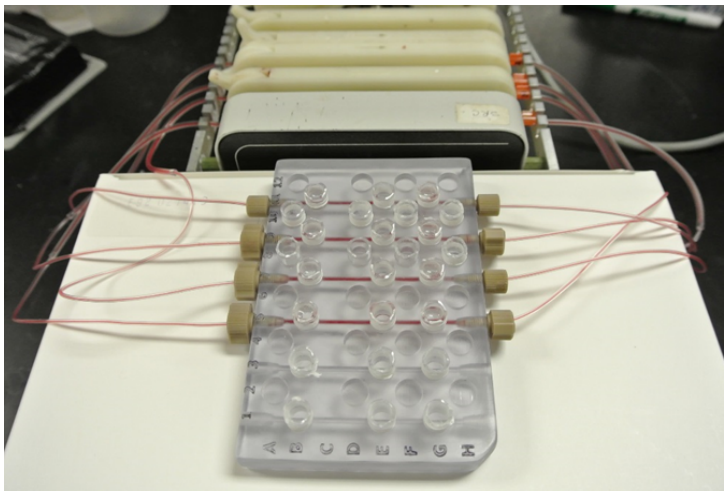

a
Time (min)
0
20
40
60
0.00
-0.58
-1.16
µcal/sec
0
-5
-10
-15
-20
kcal/mole of injectant
0
1
2
3
[HSA]/[E27A]
b
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
$^{65}Zn^{2+}$ uptake / pmoles
0
1
2
4
10
20
$^{65}Zn^{2+}$ added / pmoles
c
400
300
200
100
0
ERY Released ATP/nM
Albumin
Albumin free
ERY
+CP/Zn
+E27A/Zn

Fig.S2


a
A
B
C
D
β cell wells
Endothelial cell wells
20 µm
20 µm
In well ATP calibration
NO calibration

b



c

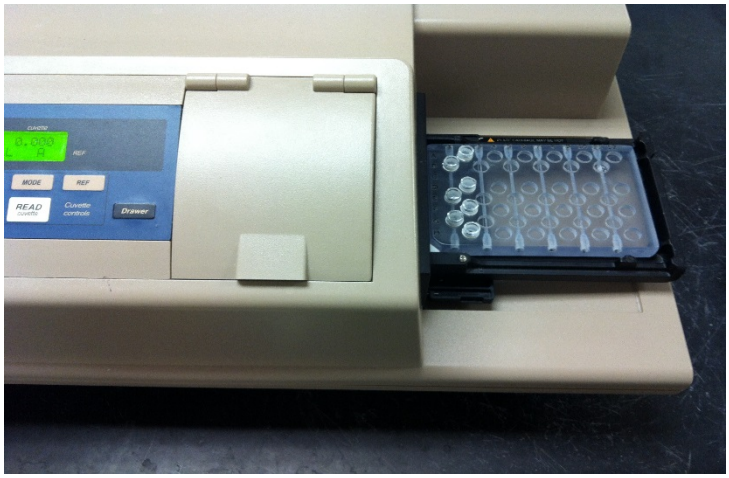

MODE
REF
READ
Drawer

Fig. S3

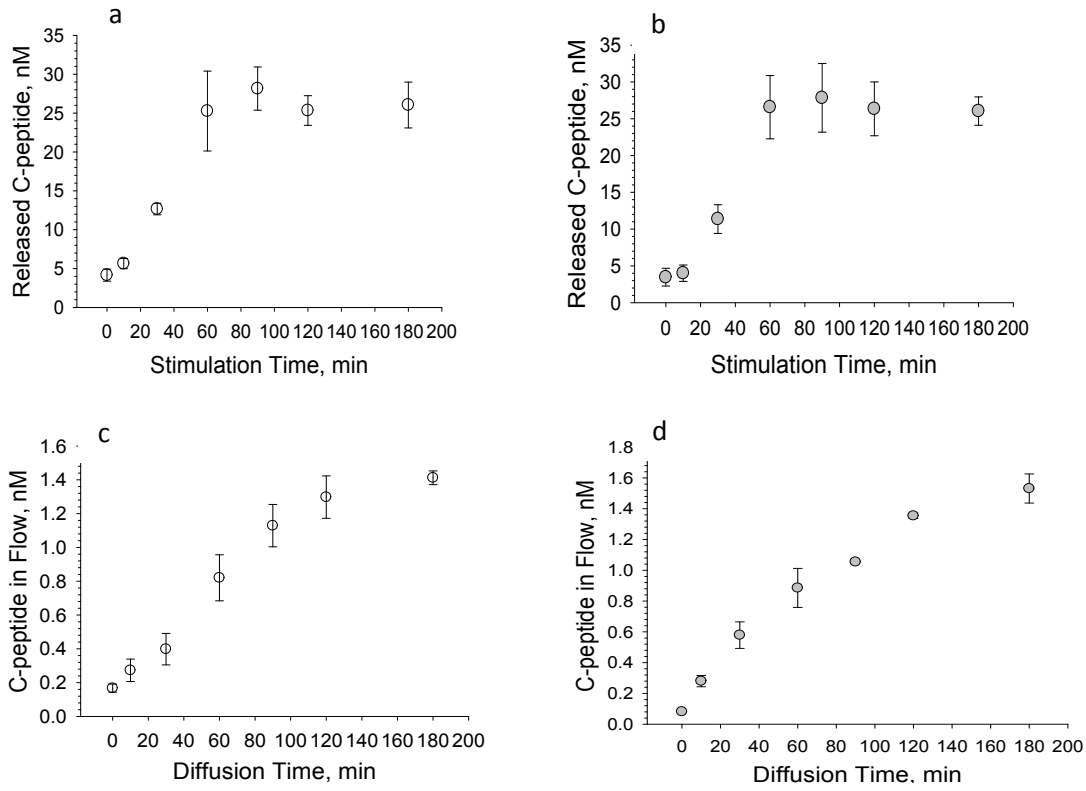

a
Released C-peptide, nM
Stimulation Time, min
b
Released C-peptide, nM
Stimulation Time, min
c
C-peptide in Flow, nM
Diffusion Time, min
d
C-peptide in Flow, nM
Diffusion Time, min

Figure Captions

Fig. S1

a. Isothermal titration calorimetric analysis of the interaction of E27A and HSA. Correction was applied for the heat of dilution. No specific binding was observed. **b.** Scintillation counting determination of $^{65}Zn^{2+}$ uptake by the ERY with C-peptide mutant, E27A, in PSS containing albumin. No uptake of $^{65}Zn^{2+}$ by the ERY was observed. n = 4, error=S.E.M. **c**. ATP released from ERYs treated with C-peptide/Zn^{2+} and E27A/Zn^{2+}. In albumin containing PSS, C-peptide/Zn^{2+} increased ATP release significantly, while E27A/Zn^{2+} did not show such an effect. In albumin free PSS, however, the role of C-peptide/Zn^{2+} on increasing ATP release from ERYs was not observed. n=5, error=S.E.M.

Fig. S2

Images of the setup of the inter-tissue circulation mimic platform.

a. Four channels of the device was used for four trials A, B, C and D. There are three inserts above each channel, of which the top one was used for β-cell culture, the middle one for ATP collection from underneath flowing and the bottom one for endothelial cell culture. Trial A was a positive control that containsβ-cells and albumin in the blood flowing. In Trial B, ERYs were flowing in albumin free PSS in the channel. β-cells were absent in Trial C. In Trial D, the endothelial cells were inhibited by PPADS to block ATP binding to them. There are static wells on the device for quantification purpose. The wells on column 10 were for NO calibration. NO standards were pipetted into these wells that would be measured simultaneously with sample wells (those containing endothelial cells) by the plate reader, which accomplished simultaneous calibration and sample detection on the same device. The wells on column 9 were designed for ATP standards to calibrate the amount of ATP that diffused into the inserts (the amount of ATP that directly affected endothelial cells).

b. Each channel was connected by a soft tubing to form a closed loop, which was driven by a peristaltic pump to mimic a blood circulation. The flowing rate was set to 200 μL/min, which meant it took about 2 min to finish one circulation. The whole setup was placed in a 37 ºC incubator for 2 hours, during which, theβ-cells elicited an effect on ERYs in the circulation, which in turn, exert a downstream effect on the blood barrier mimic, endothelial cells.

c. The device was modeled following a 96-well plate, with all the wells aligning up with the internal detectors in a plate reader, for direct endpoint measurement in a high throughput manner. The image shows an measurement on the device.

Fig. S3

C-peptide secretion profiles from stimulated INS-1 cells cultured in membrane inserts were measured as a function of time using ELISA. **Fig. S3 (a)** shows C-peptide release from INS-1 cells stimulated by albumin-containing (0.5%) stimulation buffer (Kreb's buffer with 12 mM glucose). For **Fig. S3 (b)**, INS-1 cells were stimulated with albumin-free stimulation buffer. It is concluded that albumin did not affect C-peptide secretion from stimulated INS-1 cells. After 60 min stimulation, the amount of C-peptide reached a maximum at around 27 nM (N=4 inserts, error=S.E.M.). 60 min after the INS-1 cells cultured in membrane inserts were stimulated, the inserts were plugged into the device above different channels. For the inserts that had albumin containing stimulation solution, albumin-containing PSS was circulating underneath. For INS-1 cells stimulated with albumin free solution, albumin-free PSS was pumped in the underlying channel. C-peptide flowing in the channels was determined at different time points. **Fig. S3 (c, d)** show the diffusion profiles of C-peptide from stimulated INS-1 cells to the flowing channel under the membrane. Each data point was the mean C-peptide concentration of different channels, in triplicate. The small error bars (S.E.M.) ensured the uniformity of C-peptide diffusion across channels. The diffusion profiles did not show significant difference between albumin containing **(c)** and albumin free **(d)** trials.