Supporting Information

of "High-glucose 3D INS-1 cell model with microfluidic circular concentration gradient generator for high throughput screening of drugs against type 2 diabetes"

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1. Experimentals of INS-1 cells in 96 well plate

Culture of INS-1 cells in 96 well plate

INS-1 Cell culture in BME

We trypsinized the INS-1 cells, centrifuged them, and resuspended them at 30000 cells/well in the 96 well plate. Then, the plate were then cultured at 37°C with 5% CO2 and 95% relative humidity in RPMI 1640 medium supplemented with 15% fetal bovine serum and 100 U/mL penicillin and 100 U/mL streptomycin.

Characterization of high-glucose 2D INS-1 cell model

The INS-1 cells in the 96 well plate were treated with the RPMI 1640 media containing 5.6mmol/L and 25.5mmol/L glucose for 24h, 36h, 48h, and 72h, respectively. 5.6mmol/L and 25.0mmol/L corresponded to control and high-glucose experimental groups, respectively. Then, we carried out MTT experiment, Calcein-AM/PI staining experiment, and insulin secretion measurement.

MTT experiment: dissolve the MTT powder in PBS buffer at a concentration of 5 g/L before use. Add 10-μL 5g/L MTT solution and 100 μL INS-1 cell culture medium to each well of a 96-well plate, place the plate on a plate shaker (700-900 rpm) for 20s to mix the solution well. Place the plate in a constant temperature incubator for 5h. The temperature inside the incubator was 37 °C, the CO2 concentration was 5%, the humidity was 95%, and the gas exchange was good. The wells were aspirated carefully, and the liquid inside the well was discarded. Add 150 μL DMSO to each well and incubate at 37 ° C for 15 min on a plate shaker (700-900 rpm). Detect the light absorption at 490 nm. The entire experiment should be operated in dark.

Calcein-AM/PI staining experiment: 1 μL of Calcein-AM solution and 1 μL of PI solution were dissolved in 2 mL of PBS solution to prepare the working solution. The 96-well culture plates were aspirated and the culture medium inside was discarded, and 50 μL of Calcein-AM / PI working solution was added into each well. The cells were incubated in an incubator for 30 min. The temperature in the incubator was 37°C. the concentration of CO2 was 5 %, humidity was 95%, and keep a good gas exchange. The cells were washed with phosphate buffer stock solution (PBS buffer) for three times, and fluorescent images were taken under a fluorescence inverted microscope. The entire experiment should be operated in dark.

Insulin secretion assay: Discard the culture medium off from the well plate, and washed the cells with 150 μL sugar-free KRBH buffer (containing 135 mM NaCl, 3.6 mM KCl, 1.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.5 mM NaH_2PO_4, 5mM $NaHCO_3$, 10 mM HEPES, 0.1% BSA, pH=7.4) for three times, and then in each well was added with 200 μL sugar-free KRBH buffer, the plate was incubated in an incubator for 1 h. The temperature of incubator was 37℃, CO_2 concentration 5%, humidity 95% and keep good gas exchange. Discard the solutions in the well plate. 200μL of KRBH buffer containing 16.8 mM glucose was added in the well, and the well plate was placed in

a cell incubator for 2 h. Collect the supernatant. Insulin was assayed according to the instruction of Ultrasensitive Rat Insulin ELISA Assay kit.

Effect of glipizide on 2D high-glucose INS-1 cell model

Culture the INS-1 cells for 72h in the 96 well plate in the presence of 25.0mmol/L glucose, followed by loading of RPMI 1640 medium containing 0.24μM glipizide and 25.0mmol/L glucose in the plate for another 48h. At 24h and 48h after addition of glipizide, carry out MTT experiments and insulin secretion assays as described previously.

2. Images of INS-1 cells in the 96 well plate with and without high glucose environment

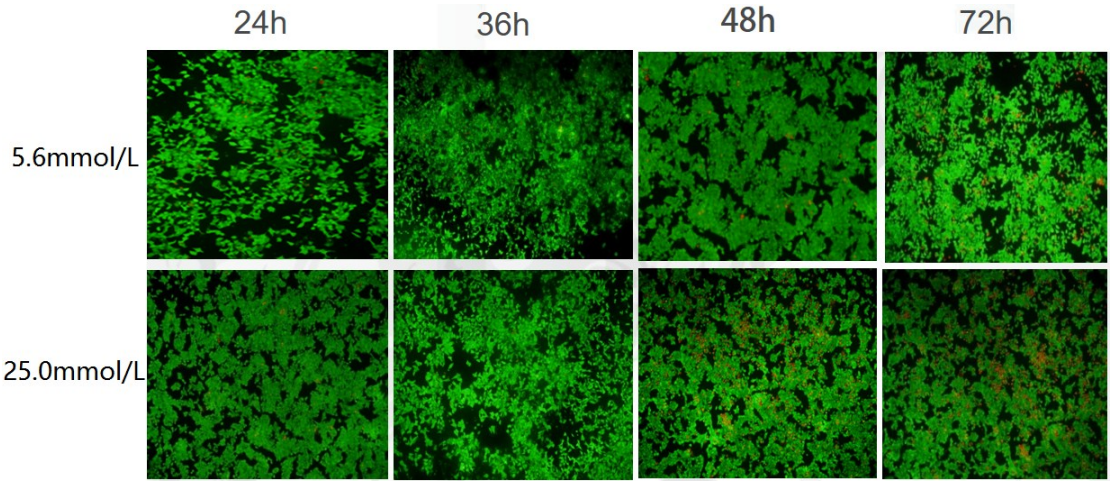

Figure s1 Time-resolved fluorescence images of INS-1 cells cultured in the well plate. The green and red colors indicated live and dead cells, respectively.

We observed the effect of high concentration of glucose on the viability of 2D INS-1 cells in the 96 well plate by Calcein-AM/PI staining experiment. As shown in Figure S1, at 24h and 36h, there was no observable difference between the control and experimental groups; however, at 48h and 72h, obvious apoptosis of INS-1 cells in the experimental group occurred, while the control still had a good viability.