**Supplementary Materials**

**Islet-on-a-chip: biomimetic micropillar-based microfluidic system for three-dimensional pancreatic islet cell culture.**

* 1. **Microfluidic system**

The geometry of the microstructure was designed using the CAD program (SolidWorks, Dassault Systemes). In the next step, a stamp that contained the designed geometry of the microstructures was formed. For this purpose, the geometry was reproducing to PMMA by micromilling using a CNC micromilling machine (Minitech Machinery Co.)

Milling was performed on a CNC micromilling machine from Minitech Machinery Corp. Mini-Mill model/3. Kyocera Series 1610 end mills with diameters of 500 µm, 150 µm and 1mm were used to mill the stamps/molds for PDMS casting. Stamps milled in PMMA Evonik GS, 2mm thick.

In the first step, the top layer 100 µm thick was removed from the entire work piece. It was done with a milling cutter with a diameter of 500 µm rotating at a speed of 12000RPM. Milling was performed with a feed rate of 500 mm/min and a plunge speed of 250 mm/min. Milling was performed in two steps, each time the tool was plunged into 50 µm. This stage was aimed at levelling the unevenness of the treated surface. In the next stage, proper microchannels with chambers were milled. They were made with a 500 µm cutter rotating at a speed of 12000RPM. Milling was performed with a feed of 300mm/min and a penetration speed of 150mm/min. The milling was performed in four steps, each time the tool plunged 50 µm. Then, in the made chambers, micropillars were milled. They were made with a 150 µm cutter. Milling was performed with a penetration speed of 100 mm/min and with a step of 15 µm. Finally, the molds were cut with a 1mm cutter to the dimensions of 62 x 26mm.

After this, the stamp with the microstructure was poured with a PDMS prepolymer and cross-linking reagent, prepared in a 9:1 ratio. The stamp with the mixture was then cured for 1 h in a 75°C oven. To obtain the top layer, the PDMS mixture was poured on

a flat stamp. Two PDMS layers were peeled off the stamps, and the inlet and outlet were drilled. Finally, the PDMS layers were bonded using oxygen plasma treatment (Plasma Preen System Inc. II 973).

**2.2 Three-dimensional pseudoislet cell cultures in the microfluidic systems**

Two cell lines were used in the experiments. Mouse pancreatic islet α-cells (α-TC1-6) were purchased from the American Type Culture Collection (catalog no. CRL-2934) and maintained in Dulbecco’s Modified Eagle Medium, low glucose, and pyruvate (Gibco, catalog no. 31885) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS; Gibco, catalog no.10270), 15 mM HEPES (Gibco, catalog no. 15630080), MEM non-essential amino acid solution (Gibco, catalog no. 11140035) to a final concentration of 0.1 mM, 0.02% bovine serum albumin (BSA), 100 µg/ml streptomycin, and 100 IU/ml penicillin (Sigma-Aldrich, catalog no. P4333). The rat INS-1E insulinoma β-cell line was a gift from Dr. Pierre Maechler (University of Geneva, Geneva, Switzerland). The medium that was used to culture INS-1E cells consisted of RPMI 1640 (Sigma Aldrich, catalog no. R0883) supplemented with 5% (v/v) inactivated FBS (Gibco, catalog no.10270), 50 µM 2-mercaptoethanol (Thermo Fisher, catalog no. 31350010), 10 mM HEPES (Gibco, catalog no. 15630080), 2 mM L-glutamine (Thermo Fisher, catalog no. 25030164), 1 mM sodium pyruvate (Thermo Fisher, catalog no. 11360070), 100 µg/ml streptomycin, and 100 IU/ml penicillin (Sigma-Aldrich, catalog no. P4333). Both cell lines were cultivated in a humidified atmosphere (37°C, 5% CO2, HERA-cell 150 incubator, ThermoScientific) and passaged every 3 days using trypsin (Sigma Aldrich, catalog no. 25200072).

**2.5 Immunofluorescence staining**

In the first step, the pseudoislets were washed with Dulbecco’s phosphate-buffered saline (DPBS; Thermo Fisher, catalog no. FB002). The cells were then fixed with 3.7% paraformaldehyde (PFA; Thermo Fisher, catalog no. FB002) in DPBS and incubated at room temperature for 10 min. In the next step, the pseudoislets were permeabilized with 0.5% Triton X-100 (Thermo Fisher, catalog no. A14288SA) in DPBS for 10 min. Afterward, the pseudoislets were washed and incubated at room temperature with 2% bovine serum albumin (Thermo Fisher, catalog no. A14289SA) in 0.1% Triton X-100 for 50 min. Primary antibodies for insulin (Cell Signaling Technology, catalog no. C27C9) and glucagon (Abcam, catalog no. ab10988) in a 1:200 dilution were introduced into the microfluidic system, and whole system was placed in a freezer at 4°C for the next 24 h. After overnight incubation, the pseudoislets were washed and incubated for 45 min with the following secondary antibodies: anti-mouse Alexa Fluor 594 (Thermo Fisher, catalog no. A-11032) and anti-rabbit Alexa Fluor 488 (Thermo Fisher, catalog no. A-11008). Finally, the DNA of the cells was stained with Hoechst (Sigma Aldrich, catalog no. B2261).

* 1. **Quantitative determination of insulin and glucagon secretion**

The glucose solutions were prepared in Krebs buffer (pH 7.4) that contained 135 mM NaCl, 3.6 mM KCl, 5 mM NaHCO3, 0.5 mM MgCl2×6H20, 1.5 mM CaCl2×2H2O, 10 mM HEPES, 0.1% BSA, and 0.5 mM Na2PO4×H20. The culture medium was first removed from the microfluidic system that contained pseudoislets by introducing PBS into the microchambers (10 μl/min, 3 min). Krebs buffer with 2.75 mM glucose was then introduced through both microchannels of the microfluidic system at a flow rate of 10 μl/min over 3 min and incubated in an incubator (37°C, 5% CO2) for 1 h. Afterward, Krebs buffer that contained 2.75 mM glucose was added (10 μl/min, 3 min) into one microchamber of the microfluidic system, and Krebs buffer that contained 16.5 mM glucose was added to the other microchamber (10 μl/min, 3 min) of the same microfluidic system. The microfluidic system with the introduced solutions was incubated at 37°C with 5% CO2 for 1 h. The solutions were then washed out of the microfluidic system at a flow rate of 10 µm/min, and the obtained samples were transferred to Eppendorf tubes.