Electronic supplementary Information

Three dimensional microwell arrays for cell culture

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Methods

Fabrication of microwells. Two dimensional templates composed of nickel panels and solder hinges were fabricated on silicon wafers using photolithography, thin film deposition and wet etching. Two sequential layers of photolithography were performed using photomasks that were designed using AutoCAD (Autodesk, Inc.) to pattern the porous frames and hinges. The templates were released from the substrates by dissolution of a polymeric sacrificial layer and heated above the melting point of the solder, wherein they spontaneously folded to form cubes. After assembly, the microwells were coated with an approximately 2 μm thick Au layer by electrodeposition using a commercial solution (Technic, In. TG-25E RTU) at a current density of 1.5 mA/cm^2. The Au-coated microwells were examined by optical microscopy to check for defects including uneven wall alignment, uneven Au deposition and monodispersity of pore size.

Fabrication of SU-8 holders and 3D microwell arrays. SU-8 50 (Microchem) was spin coated onto a silicon wafer at 1000 rpm and soft-baked in a two-step ramping process (65°C for 6 min then 95°C for 25 min) prior to template exposure. The wafer was then exposed, post-baked and developed in SU-8 Developer (Microchem). The holder thickness was measured with a profilometer; the SU-8 film and recessed slot thickness could be readily varied using different SU-8 formulations, spin speeds and photo exposure parameters.

β-TC-6 insulinoma cell culture. β-TC-6 cells were obtained from ATCC (CRL-11506) and cultured using the ATCC (www.ATCC.com) protocol. The β-TC-6 cells were cultured in 75 cm^2 culture flasks maintained in ATCC complete growth media containing Dulbecco's modified Eagle's medium with 4 mM L-glutamine. The media was supplemented with 1.5 g/L sodium bicarbonate and 4.5 g/L glucose, heat-inactivated fetal bovine serum (15%) and penicillin/streptomycin (1%). The cells were maintained in an incubator set to 37°C with a water-saturated 5% CO_2 atmosphere. The cells were subcultured weekly at a ratio of 1:3.

Cell loading. Prior to loading, the microwells were rinsed in 37°C media for one hour to remove any residual solutions. The β-TC-6 cells were passaged as described above and then concentrated to improve loading into the microwells. Concentrated cells (approx. 10^4 cells/ml β-TC-6) were tumbled along with microwells in a 250 µL microtube on a rotovap for approximately 45 minutes. Cell viability and packing density were verified periodically using the Live/Dead Assay (Invitrogen, www.invitrogen.com).

β-TC-6 cell functionality in microwells, *in vitro* insulin production. Experiments were performed in 96-well plates and data points were collected on days 1, 7, 14 and 28. The microwell arrays (with five microwells on each array) and controls (only beta cells and monolayer beta cells exposed to microwells) were placed in starvation medium (1.6 mM glucose) overnight prior to beginning the glucose stimulation experiment. To collect stimulated insulin samples, the starvation media was then removed and standard cell culture media containing stimulatory levels of glucose (200 µl of media containing 16.7 mM glucose) was added. Immediately after stimulation, 25 µl aliquots of the media were collected from both the microwell arrays and controls. The media was replaced and the 96-well plate was returned to the incubator to minimize the effects of temperature on insulin production. Aliquots were collected at 5, 10, 15 and 30 minutes and at 1, 2 and 4 hours. The data plotted in Fig. 4b corresponds to the average data collected at 4 hours (240 minutes); which represents a steady state. The samples were then analyzed using the insulin ELISA assay protocol (http://alpco.com/pdfs/80/80-INSRT-E01.pdf).

Detailed description of numerical simulation

Numerical simulations were carried out using COMSOL (COMSOL, Inc.).

Medium Geometry

The medium and microwell dimensions used in the model are shown in Fig. S1. The medium was modelled using an axially symmetric, cylindrical geometry with a radius of 3.25 mm and a height of 6.0 mm. These dimensions correspond to the typical medium volume used in our experiments. Due to the assumed axial symmetry of the problem, we modelled half of the cross-section (accordingly the left side of the schematics in both Fig. S1 a and b are shown with lines of a lighter shade).

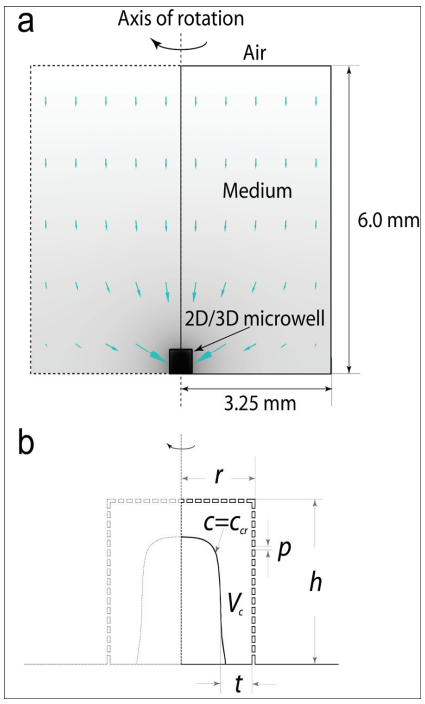

Figure S1. Schematic view of the numerical model used. (a) 2D or 3D microwells (represented by a black square) were positioned at the bottom center and assumed to be covered by 6.0 mm of medium. O_2 flux through the medium is shown with cyan arrows. (b) A zoomed-in view of the microwell shown in (a).

Microwell Geometry

To generate Fig.1e, microwells of three different sizes were modelled as cylinders with heights of $h = 500\mu m$, $250\mu m$ and $100\mu m$. We note that the important geometric variables that affect O_2 diffusion, namely surface area to volume ratio ($5/h$; in the model we have assumed $h=2r$ *i.e.* the cylinder diameter and height are equal to the side length of the cube) and the ratio of the top face area to side face area (1:4) are the same. Hence, utilizing cylindrical geometry is a good approximation and greatly simplifies the analysis.

Porosity was introduced using eight uniformly-spaced slit shaped pores created on the top of the microwells along with 16 uniformly spaced pores on the sides. The pores had

widths of 10 μm, 5 μm and 2 μm for microwells with heights of 500 μm, 250 μm and 100 μm respectively. The microwell wall thickness was assumed to be equal to the pore width for each of the cases above. Porosity of a face ϕ was defined as the ratio of the total pore area to the total area of the corresponding face: $\phi = \frac{NA_f}{\pi r^2}$ (top face) or $\phi = \frac{NA_f}{2\pi rh}$ (circumference of the cylinder), where N is the number of pores per face and A_f is the area of an individual pore. The geometry and pore distribution were chosen so that these two expressions gave values within a few percent of each other.

The dependence on the decreasing pore size was studied by varying the diffusion coefficient, which was scaled linearly with pore area. This approximation is justified by Fick's law, wherein the O_2 flux across the pore is proportional to the product of the area of the pore and the diffusion coefficient. It was verified that at distances large compared to the pore size varying the actual pore size or alternatively the diffusion coefficient gives essentially the same result. Our approach of varying the pore diffusion coefficient allowed us to utilize the same model and mesh for all the data points, thereby increasing the consistency of the simulations.

Modeling of O_2 consumption by β-TC-6 cells

The pancreatic β-TC-6 cells were assumed to completely fill the microwell. Accordingly, their O_2 consumption was modelled by assigning a uniform volume consumption rate to the volume within the microwell using a Michaelis-Menten type of expression as described in the main text. Several different values can be found in the literature for the value of the maximal O_2 consumption R_{max} by primary pancreatic β-TC-6 cells. The O_2 consumption rate for the specific cell line β-TC-6 used in the experiments was estimated using indirect experimental data (1). Numerical and analytical estimates (data not shown) suggest that it is possible to get better fits to experimental data when an R_{max} value of

34 μM/s (2) or higher is used as opposed to lower values. The low value of 16 μM/s, which favors cell survival (3), was used merely for comparison.

Solving the diffusion equation

The diffusion equation was set up as described in the main text. To improve convergence of the solution and to avoid the appearance of negative concentration values, the logarithm of the concentration $C=\ln(c)$ was used as the dependent variable (4). Accordingly, the diffusion coefficient D was replaced by $D\exp(C)$ along with the appropriate numerical value changes in the boundary conditions.

Cell viability estimates

As shown in Fig. S1, the region where O_2 concentration is below the cell survival threshold c_{cr} was then approximated by a cylinder with living cells confined to a region of thickness t within the microwell walls. The fraction f of the viable cells was calculated as $f=\frac{t}{h}$ for the 2D microwell case (see Fig. 1c of the main text) and $f=1-(1-\frac{t}{r})^2(1-\frac{t}{h})$ for the 3D microwell case. The last expression was obtained by subtracting the volume of necrotic cells approximated by a cylinder from the total cylinder volume.

Modelling cell density variation

The variation of cell density inside the microwells was modelled by varying the O_2 consumption rate according to $R=\rho\times R_{\max}\times\frac{c}{c+c_{mm}}\times\theta(c>c_{cr})$. In accordance with our estimates of O_2 consumption by the β-TC-6 cell line, $R_{\max}$ = 34 μM/s was used. ρ was defined relative to the cell density in intact islets of Langerhans ($1.1\times10^9\,\mathrm{cm}^{-3}$) with the assumption that they were composed only of β-TC-6 cells.

Supplementary Table 1.

Variable	Definition	Expression/Value
r	Radius of the microwell	{250 μm, 125 μm, 50 μm}
h	Microwell height / diameter (We have assumed h=2r)	{500 μm, 250 μm, 100 μm}
t	Thickness of the surviving cell layer	
V_t	Total microwell volume	$\pi r^2 h$
V_c	Volume within the microwell occupied by β-TC-6 cells (i.e. volume of the region inside the microwell where O_2 concentration is higher than the critical concentration c_{cr} and thus β-TC-6 cells can survive)	
f	Fraction of the microwell volume where β-TC-6 cells can survive (O_2 concentration higher than critical)	V_c / V_t
ϕ	Porosity of a face, ratio of the total pore area to the area of the corresponding face	$\phi = \frac{NA_f}{\pi r^2}$ (top face) & $\phi = \frac{NA_f}{2\pi r h}$ (circumference)
ρ	Ratio of cell density in the microwell to the cell density in islets of Langerhans (the latter assumed to be 1.1×10^9 cm^{-3})	
D_{med}	Diffusion coefficient of O_2 in the medium (outside of the region occupied by β-TC-6 cells)	3×10^{-9} m^2/s
D_{cells}	Diffusion coefficient O_2 in the tissue (inside the region occupied by β-TC-6 cells)	2×10^{-9} m^2/s
$R_{\max}$	Maximal O_2 consumption rate by β-TC-6 cells	{16 μM/s, 34 μM/s}
c_{mm}	Michaelis-Menten constant	1.0 μM
c_{cr}	Critical O_2 concentration for the onset of necrosis	0.1 μM
c_{O2}	Maximal O_2 concentration in the medium	0.2 mM

Supplementary material references

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