S1: Numerical simulation of oxygen profile

To help us to design the geometry and determine the dimensions of the microfluidic device, we simulated the oxygen transport inside the PDMS and cell medium using COMSOL Multiphysics. Using the 'transport of Diluted Species' in Comsol, the diffusion equation without convection is

$$\frac{\partial c}{\partial t} = D\nabla^2 c$$

where c is the oxygen concentration and D is the diffusion coefficient. The diffusivity and solubility of oxygen in PDMS and cell media were chosen as the following table[1, 2]

Material	Solubility	Diffusivity
	(mmol/L·atm)	(cm^2/s)
PDMS	1.62	3.4×10 ⁻⁵
Culture media	0.22	1.9×10 ⁻⁵

As comparison, 20% oxygen in air means the concentration of oxygen is \sim 9mmol/L·atm and the diffusion coefficient of oxygen in air is 0.176 cm²/s at 25°C.

The interface conditions between the liquid and PDMS are

$$D_1 \frac{\partial c_1}{\partial n} = D_2 \frac{\partial c_2}{\partial n}$$
 and $\frac{c_1}{S_1} = \frac{c_2}{S_2}$

where the subscript '1' represents PDMS phase and '2' represents liquid phase, respectively. The interface conditions were calculated by the stiff spring method in COMSOL.

To study the characteristics of circular hypoxic region, the simulation domain was set as a $5 \times 5 \times 0.25$ mm block where the height of the media is 0.1mm and the height of PDMS is 0.25mm as shown in Fig. S3. There was a 1.5mm in diameter circle in the middle of the top surface. The boundary condition at the circle was set c = 0 (the blue circle in Fig. S3a). Outside of this circle, there was a 0.3mm ring which has zero flux of oxygen. Then there was another 0.25mm ring where the oxygen concentration is $c = s_1 p_{o_2} =$ $8.1 \times 20\% = 1.62 \left[\frac{mmol}{L}\right]$ (the red ring in Fig. S3a). Other boundaries have zero flux as shown in grey in Fig S3a. Fig. 1d shows the oxygen concentration in the PDMS domain and Fig. 1e show a section view at the slide in the middle. The oxygen concentration along a line which passes the center of the bottom surface was shown in Fig. S3b.

S2: Calibration of oxygen sensing dye

A single microfluidic channel made by PDMS was bond to the PtTFPP coated glass slide. A continuous gas flow (0%, 1%, 2.5%, 5% or 21% O_2 in N_2) was subsequently introduced in the channel. The PtTFPP emission intensity was taken once the fluorescent intensity became stable (normally after 5min) by the Olympus microscope with the equipped TRITC filter. All images were analyzed using the NIH ImageJ software. The fluorescent intensity was supposed to follow the Stern-Volmer equation [3]

$$\frac{I_0}{I} = 1 + K_{SV} P_{o_2}$$

where I_0 is the intensity in the absence of oxygen (pure N2) and I is fluorescent intensity at the O₂ partial pressure, P_{o_2} . K_{SV} is the Stern-Volmer constant. To determine the sensor response, K_{SV} was calculated using a linear regression in MATLAB. The calibration curve was shown in Figure S5.

Movie 1: Time-course of hypoxic pattern in the two-chamber device at the initial 45 min. the two thin lines shows where the barrier and the thick line on the left shows the left chamber's boundary.

Movie 2: Hypoxic pattern in the two-chamber device over 24h.



Fig. S1: Main steps of fabrication of the NOA O₂ mask.



Fig. S2: A photo image of assembled microfluidic device.



Fig. S3: The geometry for the numerical simulation of hypoxic circle. a) dimensions and boundary conditions for COMSOL simulation. b) oxygen concentration at the bottom surface along a line in the middle of the block.



Fig. S4: Main dimensions of the two-chamber microfluidic device (the schematic is not draw in real scale to show fine structures). There was an array of microgrooves $(5 \times 20 \times 100 \mu m, height \times width \times length with 30 \mu m spacing)$ between the two chambers.



Fig. S5: Calibration curve of the PtTFPP-PDMS sensing film.



Fig. S6: Schematics of metabolic symbiosis in the two-chamber microfluidic device.

Reference:

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