Electronic Supplementary Material (ESI) for Analyst.

Three dimensionally printed nitrocellulose-based microfluidic platform for investigating the effect of oxygen gradient on cells

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Contents:

1. Materials and reagents

Ruthenium tris (2, 2’ -bipyridyl) dichloride hexahydrate (RTDP) was obtained from Sigma (St. Louis, MO). Zebrafish embryonic cell line ZF4 was purchased from China Zebrafish Resource Center (CZRC) (Wuhan, China). DMEM: F12 (1:1) medium, fetal bovine serum (FBS), and 0.25% trypsin solution were obtained from Gibco (Grand Island, NY). Matrigel was purchased from Corning (Tewksbury, MA). The primary antibodies Hif-1α, β-actin antibodies and secondary antibody conjugated to horseradish peroxidase came from Cell Signaling Technology (Boston, USA). Image-iT ™ Green Hypoxia Reagent was produced by Thermo Fisher Scientific (Waltham, MA). Protease inhibitor PMSF was from Solarbio Company of China. 10% SDS-polyacrylamide gels was manufactured by Bio-Rad. Easy Pure RNA Kit, Easy Taq PCR Super Mix, TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix Kit were brought from TransGen Biotech.

2. Cell culture

The cell line of zebrafish ZF4 was cultured at 28°C with 5% CO_2 in DMEM: F12 (1:1) supplemented with 10% fetal bovine serum, penicillin (100 U/mL) and streptomycin (0.1 mg/mL). When the cells had reached confluence, they were detached with 0.25% trypsin solution, then suspended and subcultured.

3. Cell cycle assays

ZF4 cells were exposed to different oxygen tensions for 12 h. The cells were harvested and washed with PBS, then the cells were thoroughly dispersed in 70% cold ethanol and fixed overnight at 4 °C. After washing in PBS, the cells were incubated with 200 μg/mL RNase A for 30 min at 37 °C and stained with 50 μg/mL Propidium Iodide (PI, Beyotime, China) for 30 min at 4 °C out of light. The cell cycle analyses were performed by a flow cytometer (BD, USA). The data were analyzed by Flow Jo software.

4. The outline diagram of 3D printed microfluidic chip

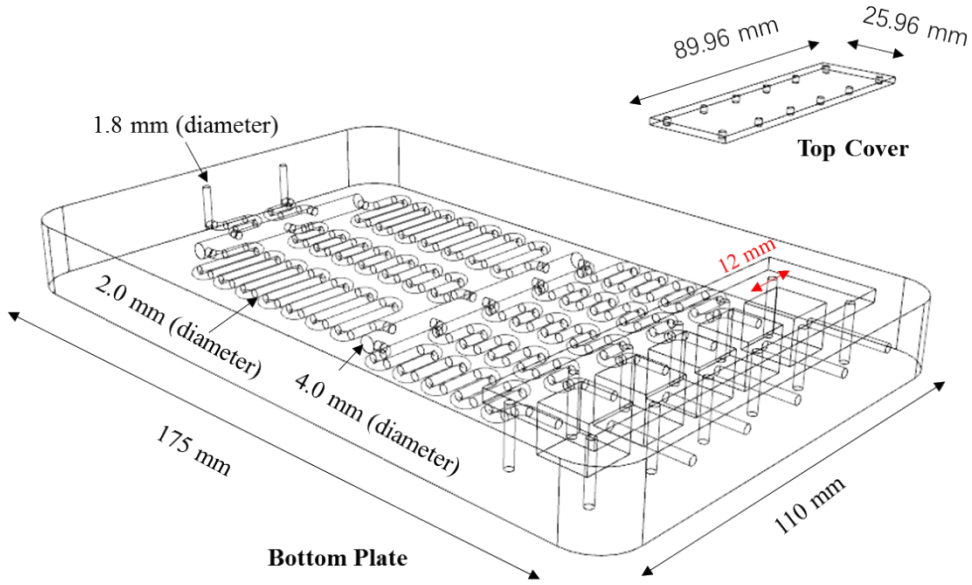

Figure S1. The outline diagram of 3D printed microfluidic chip

5. The real product photo show of 3D printed microfluidic chip

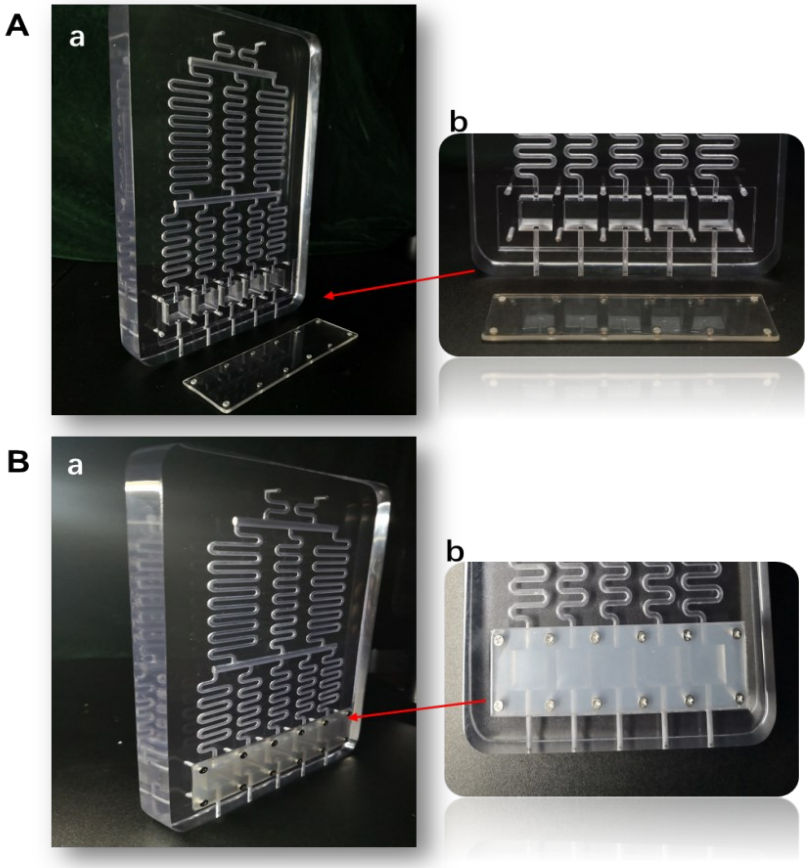

Figure S2. Pictures of 3D printed microfluidic chip. (A) The bottom plate and top cover of 3D printed microfluidic chip. (B) The two pieces of the chip and soft silicone pad were assembled with screws.

6. The working photograph of oxygen gradients-generating chip

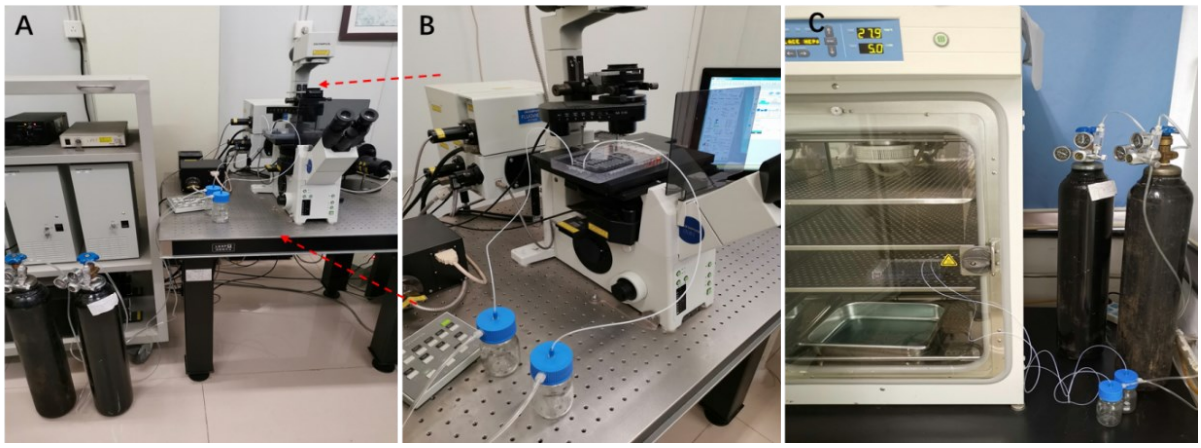

Figure S3. **The working photograph of oxygen gradients-generating chip.** (A) The working photograph of detecting oxygen concentration in the cell culture chambers with laser scanning confocal microscope. (B) is partial graph of (A). (C) The working photograph of oxygen gradients-generating chip for cell culture.

7. Zero percent oxygen equilibration studies

In order to determine the time required for gas equilibrium in the cell culture chambers, we used solution of oxygen-sensitive fluorescent probe whose emitted fluorescent intensity is inversely proportional to the ambient oxygen concentration as oxygen quenches the fluorophore of the probe[1]. We added 300 μL solution of oxygen-sensitive fluorescent probe into them, then N_2 was infused into two inlets of the device, both gas lines were set at the pressure of 80.0 psi, and images were captured every 2 min for 30 min at the same position of culture chamber with confocal laser scanning microscope. As shown in Fig. S3, the fluorescent intensity reached a stable value in about 23 min, which suggested that the gas in the cell culture chamber equilibrated to a steady state.

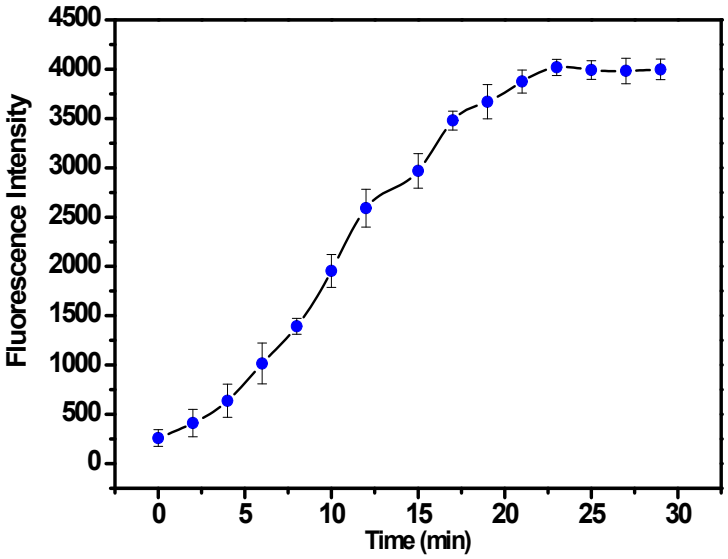

Figure S4 Zero percent oxygen equilibration studies.

8. Fluorescence intensity of channels in N_2 or air

According to Stern–Volmer equation, in order to calibrate [O_2], the fluorescence intensities, I_{air} and I_0, in 21% and 0% oxygen tension were needed[2]. We added the solution of oxygen-sensitive fluorescent probe into the grow chamber, then two inlets of the 3D printed microfluidic chip were fed N_2 or air for 30 min. The cell culture chamber of each channel was imaged on the laser scanning confocal microscope and quantified using image processing software. The fluorescence intensity was shown in Table S1

Table S1 Fluorescence intensity of 5 channels in N_2 or air

Channel	Fluorescence Intensity	
	N_2	Air
1	4132	364
2	4086	319
3	4107	307
4	4081	216
5	3982	335
Average	4077.6	308.2

9. Measured oxygen concentration in the channels

Table S2 Measured oxygen concentration in the channels

Channel	Calculated [O_2]
1	0.07%
2	3.61%
3	11.1%
4	16.51%
5	21.2%

10. Nitrocellulose-based chips

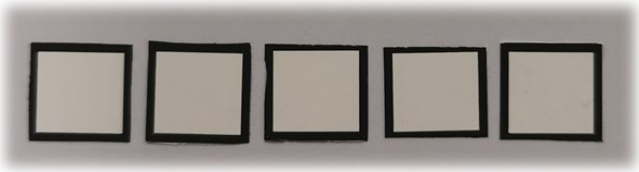

Figure S5 Photograph of paper-based chips in nitrocellulose membrane. The side length of the square hydrophilic area was 10 mm**.**

11. Fish cells culture and identification on the NC paper-based chip

To check whether the zebrafish ZF4 cells could grow on the NC paper-based chip, the NC paper-based chips were pre-coated with Matrigel (diluted 1:40) at 4 °C for 12 h, then incubated at 37 °C for 1 h to solidify the Matrigel[3]. Put the NC paper-based chips into a cell culture plate of 24 wells, then we seeded zebrafish ZF4 cells on the NC paper-based chips at 1 $\times 10^4$ cells/cm^2. After 12 and 24 hours of cultivation, the cells on the NC paper-based chips were stained with NucBlue Live Cell Stain Ready Probes reagent (Invitrogen, USA) to test the growth viability of cells. The cells on the chips were rinsed three times with phosphate buffer saline, then imaged on laser scanning confocal microscope.

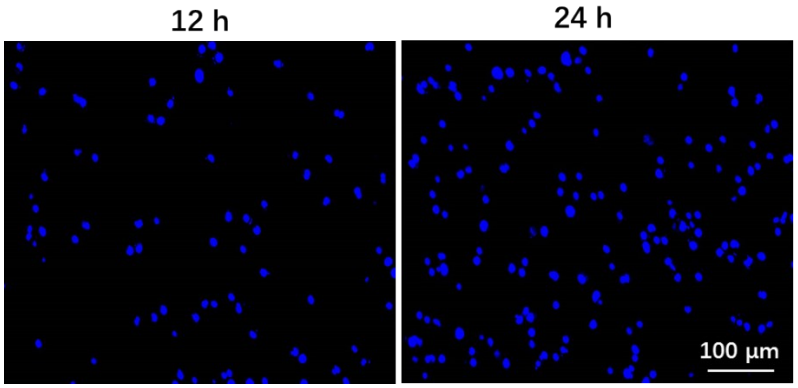

Figure S6. Live cells grown on the NC paper-based chip. The fluorescent images of cells stained with live cell stain reagent after the cells incubated for 12 h (a) and 24 h on the NC paper-based chip.

12. References

1. S. C. Oppegard, A. J. Blake, J. C. Williams and D. T. Eddington, *Lab Chip*, 2010, **10**, 2366-2373.
2. K. Funamoto, I. K. Zervantonakis, Y. Liu, C. J. Ochs, C. Kim and R. D. Kamm, *Lab Chip*, 2012, **12**, 4855-4863.
3. L. Wang, C. Xu, Y. J. Zhu, Y. Yu, N. Sun, X. Q. Zhang, K. Feng and J. H. Qin, *Lab Chip*, 2015, **15**, 4283-4290.