SUPPLEMENTARY MATERIALS

Oxygen concentration measurement in 3D cell culture using multifocal optical projection microscopy

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S1. CPOx-50-PtP bead cytocompatibility 2D cell culture experimental procedure

Cell culture

Commercial WI-38 human lung fibroblasts (Culture Collections, Public Health England, UK) were cultured in Dulbecco's Modified Eagle Medium (Gibco DMEM/F-12, USA), supplemented with 10% Fetal Bovine Serum (South American Origin, Biosera, Finland) and 0.5% Penicillin/Streptomycin 100 U/ml (Thermo Fisher Scientific, USA). The cells were incubated at 37°C in 5% CO_2. Tryple Select (Gibco, USA) was used in passaging the cells.

Viability assay

To evaluate cytotoxicity of the beads, a Live/dead Viability/Cytotoxicity Kit for mammalian cells (Invitrogen, USA) was used after 4, 7 and 14 days of WI-38 fibroblast cell culture. Cultures with cells only were used as positive controls. The beads were sterilized as described in the article's section 2.5. Each sample type had 4 parallel samples. 50 µl of bead solution was pipetted into each Nunclon Delta treated 96-well plate well (Thermo Scientific, USA) and 50 µl of cell suspension was added. For the positive controls, 50 µl of cell suspension was added first and then supplemented with 50 µl of medium. The cells were plated at density of 21 000 cells/cm^2.

For staining, the medium was removed from the wells followed by washing with 100 µl of PBS twice. Each sample was incubated at room temperature for 30 min on a rocker with 100 µl of PBS staining solution containing 0.1 µM fluorescent calcein-AM to stain live cells green and 0.5 µM fluorescent ethidium homodimer-1 to stain dead cells red. After incubation, the staining solution was replaced with 100 µl of PBS.

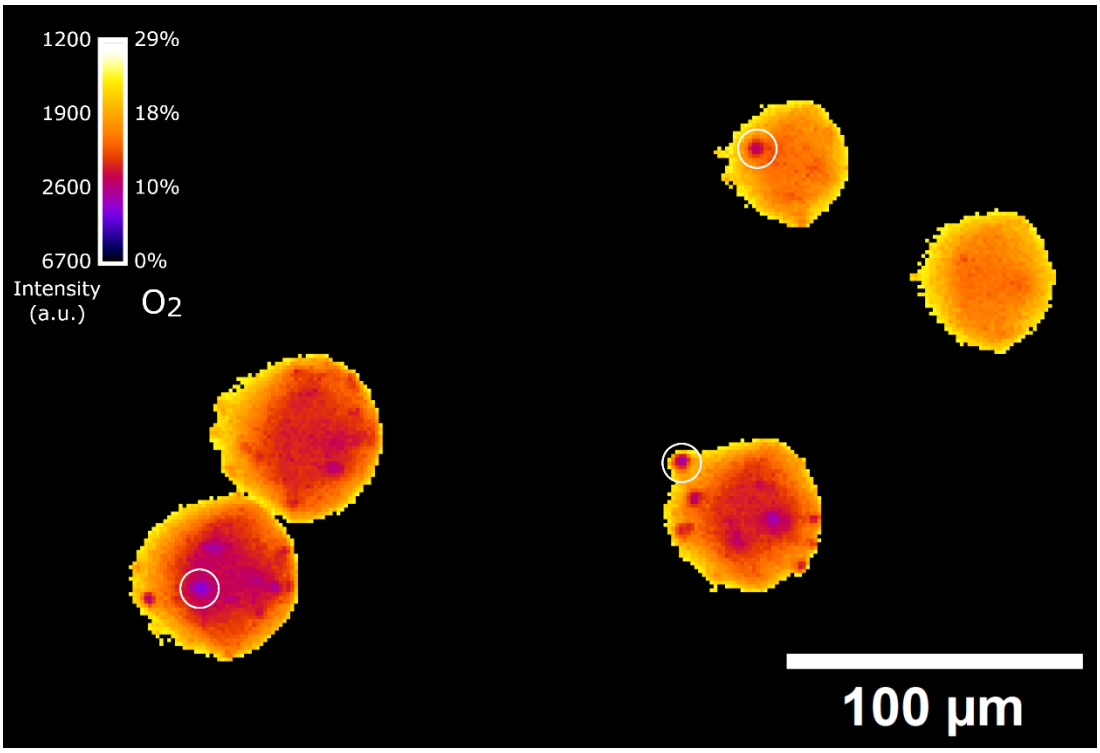
Imaging and analysis

An IX-51 wide field fluorescence microscope (Olympus, Japan) equipped with 4x magnification objective (Olympus, Japan) was used for evaluating cell viability. Excitation and emission wavelengths were 494 and 517 nm for live cells and 528 and 617 nm for the dead cells. A 488 nm wavelength filter was used for imaging the live cells and 568 nm filter for the dead. Images with 1360 x 1024 pixels were acquired using a DP30BW camera (Olympus, Japan). The images were processed using Fiji[1] software. Merged micrographs showing both live and dead cells were visually inspected for number dead cells and cell morphology. Viability percentage was calculated from the live/dead images with following equation (eq. 1) where the quantification is based on the ratio of surface area covered by the live and dead cells.

$$Viability\ \% = \frac{area\ of\ live\ cells}{area\ of\ live\ cells\ +\ area\ of\ dead\ cells} \qquad \text{(eq. 1)}$$

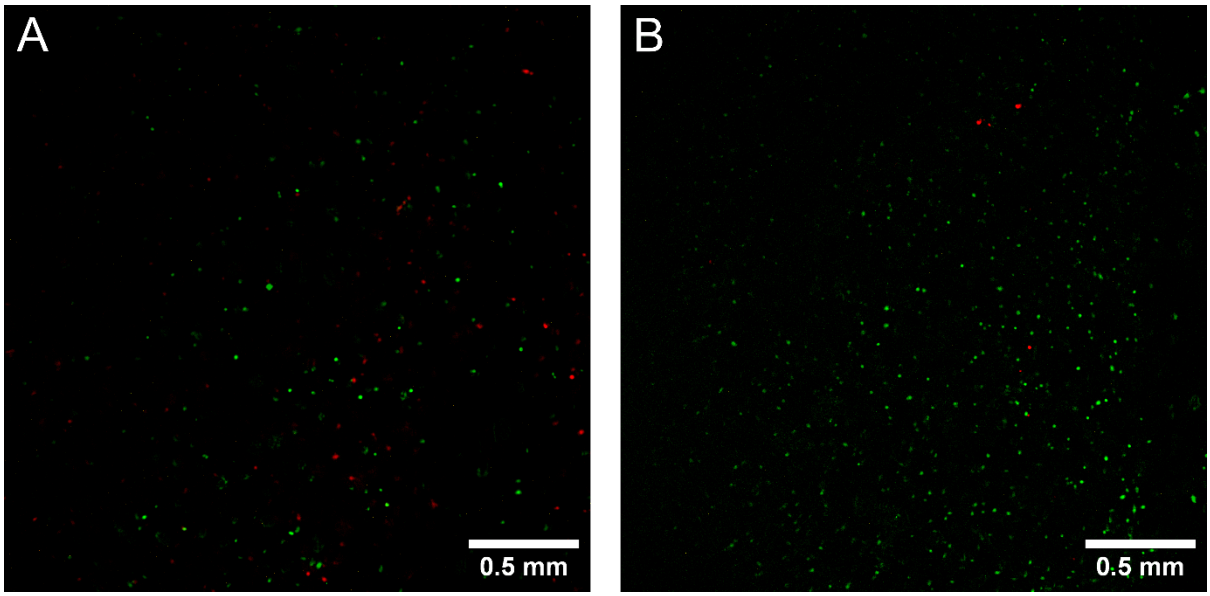
S2. Variability in CPOx-50-PtP bead coating

Figure S1 shows CPOx-50-PtP beads during the cell culture experiment described in chapter 2.5 of the article. The image is a maximum intensity projection (MIP) where fluorescence intensity is represented using a Fire LUT in Fiji[1]. Due to their close vicinity to each other, the beads are assumed to be in the same oxygen concentration. The image shows inhomogeneities in the oxygen sensitive coating within each bead and between different beads. There are also size variations between the beads.



Supplementary Figure S1: CPOx-50-PtP sensor beads in 3D agarose during cell culture experiments showing non-uniform oxygen sensitive coating. Selected coating artefacts are highlighted with white circles.

S3. Cell viability in 3D cell cultures



Supplementary Figure S2: Viability assessment in 3D fibroblast culture. Representative fluorescence live/dead projection images from (A) 200 000 cells/ml and (B) 700 000 cells/ml samples. Live cells are shown in green and dead cells in red, demonstrating high cell viability throughout the sample. Scale bars indicate 0.5 mm.

S4. Validation of oxygen concentration measurements in 3D

Table S1: Comparison of oxygen concentrations measured at different depths in 3D fibroblast cultures (200,000, 400,000, and 700,000 cells/ml), comparing bead-based MF-OPM measurements with the PreSens PM-PSt7 fibre-optic oxygen sensor. For the beads, mean and median results are shown with respective standard deviation (SD) and interquartile range (IQR) values. The SD/IQR ratio shows the data to have normal distribution type characteristics. Z-score shows acceptable (<±2) statistical agreement between the PM-PSt7 probe and CPOx-50-PtP bead (mean) values.

200 000 cells/ml

	CPOx-50-PtP beads				PM-PSt7 probe (control)		
	Oxygen concentration (%)				Oxygen concentration (%)		
Position	Mean	SD	Median	IQR	Single measurement	SD/IQR ratio	z-score
1	20.8	4.5	20.7	7.0	15.4	0.64	-1.20
2	20.1	4.1	20.0	6.2	13.5	0.66	-1.60
3	19.9	4.9	19.8	7.3	12.2	0.67	-1.57
4	19.6	4.6	19.3	7.1	11.8	0.65	-1.68
5	17.4	4.0	17.2	6.1	11.4	0.65	-1.49
6	13.4	2.6	13.6	3.3	11.4	0.78	-0.78
7	14.9	3.1	15.0	4.4	11.4	0.71	-1.14

8	15.3	2.9	15.3	4.3	11.4	0.67	-1.35

400 000 cells/ml

	CPOx-50-PtP beads				PM-PSt7 probe (control)		
	Oxygen concentration (%)				Oxygen concentration (%)		
Position	Mean	SD	Median	IQR	Single measurement	SD/IQR ratio	z-score
1	16.8	3.9	16.8	6.0	15.4	0.65	-0.37
2	15.1	3.8	15.3	5.8	10.4	0.65	-1.25
3	12.5	3.6	12.3	5.6	6.9	0.64	-1.59
4	11.8	4.0	11.7	6.1	5.0	0.66	-1.70
5	9.4	3.2	9.4	4.9	3.8	0.66	-1.76
6	9.4	3.7	9.3	5.7	3.0	0.65	-1.71
7	8.2	3.0	8.1	4.5	2.6	0.67	-1.84
8	8.0	3.2	8.0	5.0	2.6	0.65	-1.70

700 000 cells/ml

	CPOx-50-PtP beads				PM-PSt7 probe (control)		
	Oxygen concentration (%)				Oxygen concentration (%)		
Position	Mean	SD	Median	IQR	Single measurement	SD/IQR ratio	z-score
1	12.0	2.9	12.0	3.8	10.8	0.75	-0.41
2	9.5	2.6	9.8	2.8	8.5	0.92	-0.40
3	11.3	4.0	11.2	6.0	6.0	0.66	-1.33
4	7.4	2.5	7.6	3.9	3.5	0.64	-1.54
5	5.9	2.8	5.8	4.6	2.3	0.61	-1.27
6	5.2	2.8	5.2	4.5	1.4	0.62	-1.37
7	3.4	2.1	3.2	3.5	0.7	0.59	-1.28
8	2.5	1.6	2.4	2.6	0.3	0.60	-1.40

REFERENCES

1 J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J.-Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak and A. Cardona, *Nat Methods*, 2012, **9**, 676–682.