Supplementary Figures and Tables

Oxygen consumption rate of tumour spheroids during necrotic-like core formation

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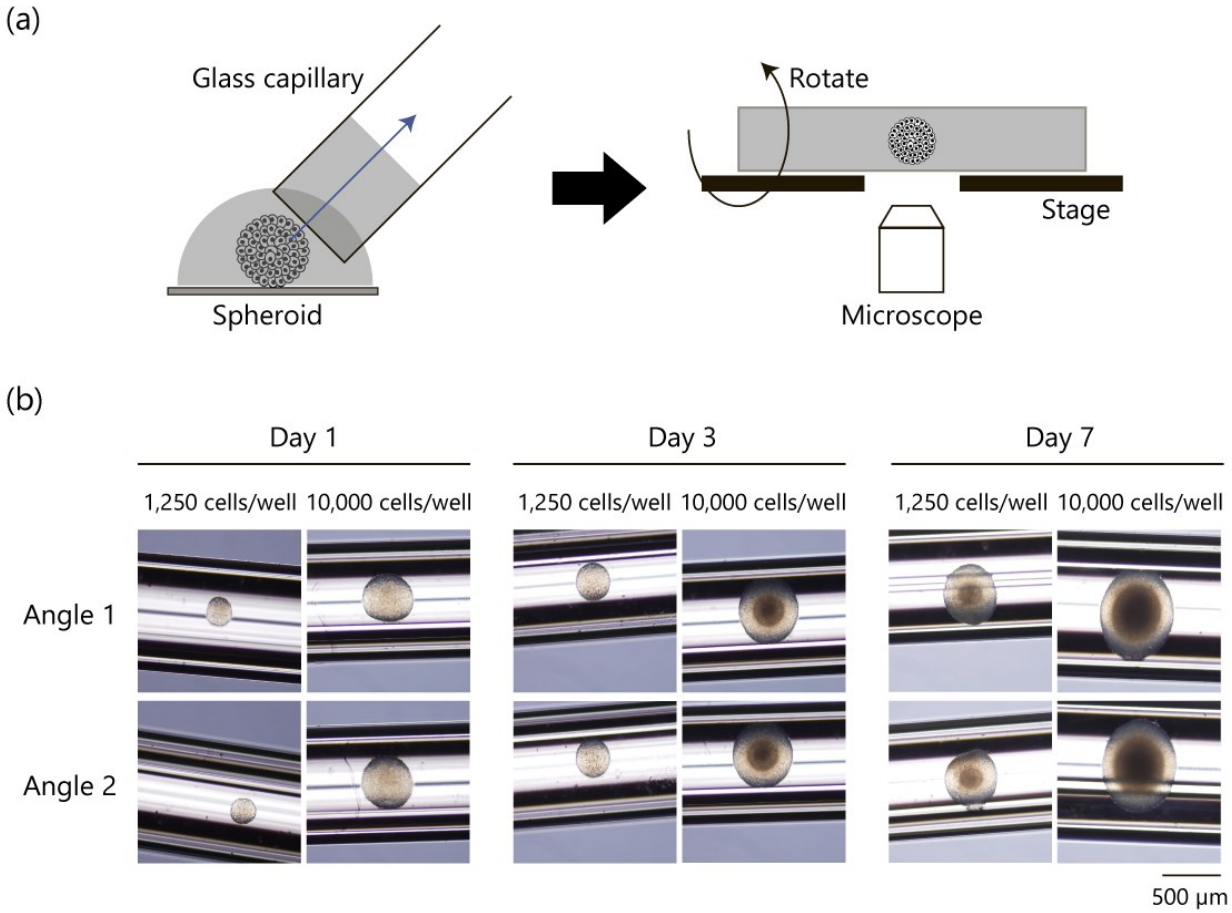


Figure S1: Confirmation of the spherical shape of MCF-7 spheroids. (a) Schematic of the observation using a glass capillary. The spheroid was collected into a glass capillary by capillary force. The morphology of the spheroid was observed using microscopy at multiple angles. (b) Phase contrast images of representative MCF-7 spheroids on days 1, 3, and 7.

Table S1: Correlations between spheroid radius (r_S) and necrotic-like core radius (r_N) in the conditions of 20,000, 5,000, 2,500, 625, and 300 cells/well. The red line indicates the threshold for the appearance of the necrotic-like core in MCF-7 spheroids (n = 3 for each culture condition).

[μm]

Initial cell #	Day 1		Day 2		Day 5		Day 6	
	r_S	r_N	r_S	r_N	r_S	r_N	r_S	r_N
20,000 cells/well	391	0	419	293	461	349	498	360
	435	0	424	291	464	338	486	342
	415	0	415	267	471	346	488	354
5,000 cells/well	240	0	272	88	348	189	369	222
	232	0	268	73	356	188	376	230
	241	0	266	71	354	194	375	219
2,500 cells/well	183	0	212	0	289	138	329	171
	185	0	213	0	314	142	331	173
	184	0	212	0	311	140	327	155
625 cells/well	117	0	149	0	234	0	273	110
	127	0	145	0	240	0	260	91
	120	0	139	0	239	0	264	84
300 cells/well	98	0	112	0	205	0	232	0
	86	0	112	0	206	0	217	0
	93	0	110	0	202	0	234	0

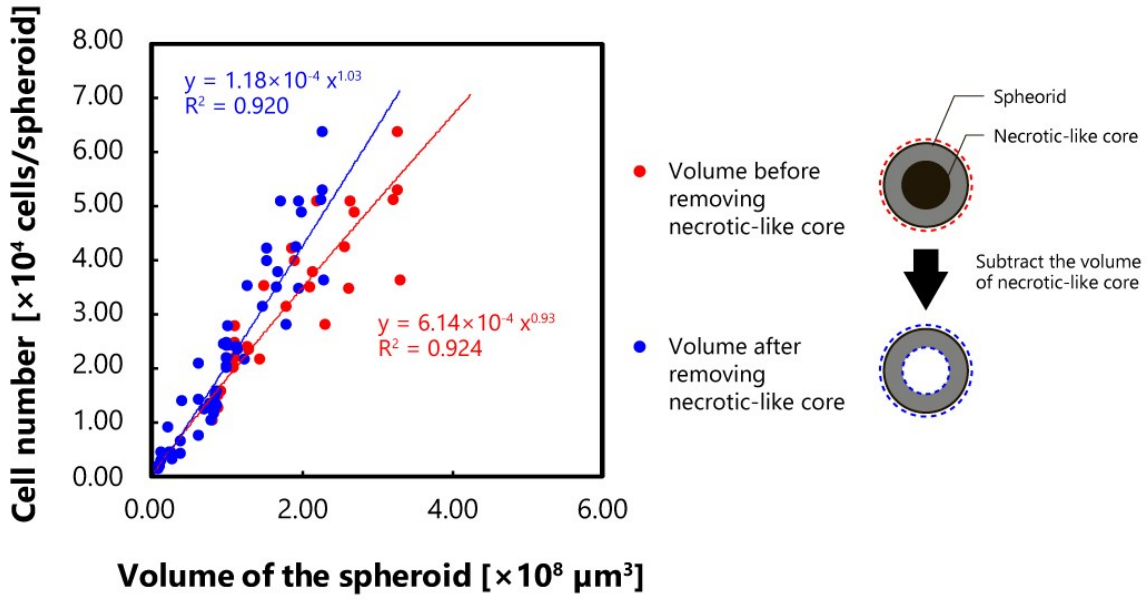


Figure S2: Relationship between cell numbers and spheroid volume. The horizontal axis indicates the volumes before (red line) and after (blue line) removing necrotic-like cores in spheroids. The figure on the right is a schematic of the spheroid volume before and after removing a necrotic-like core. The power approximation trendlines are also indicated in the graph.

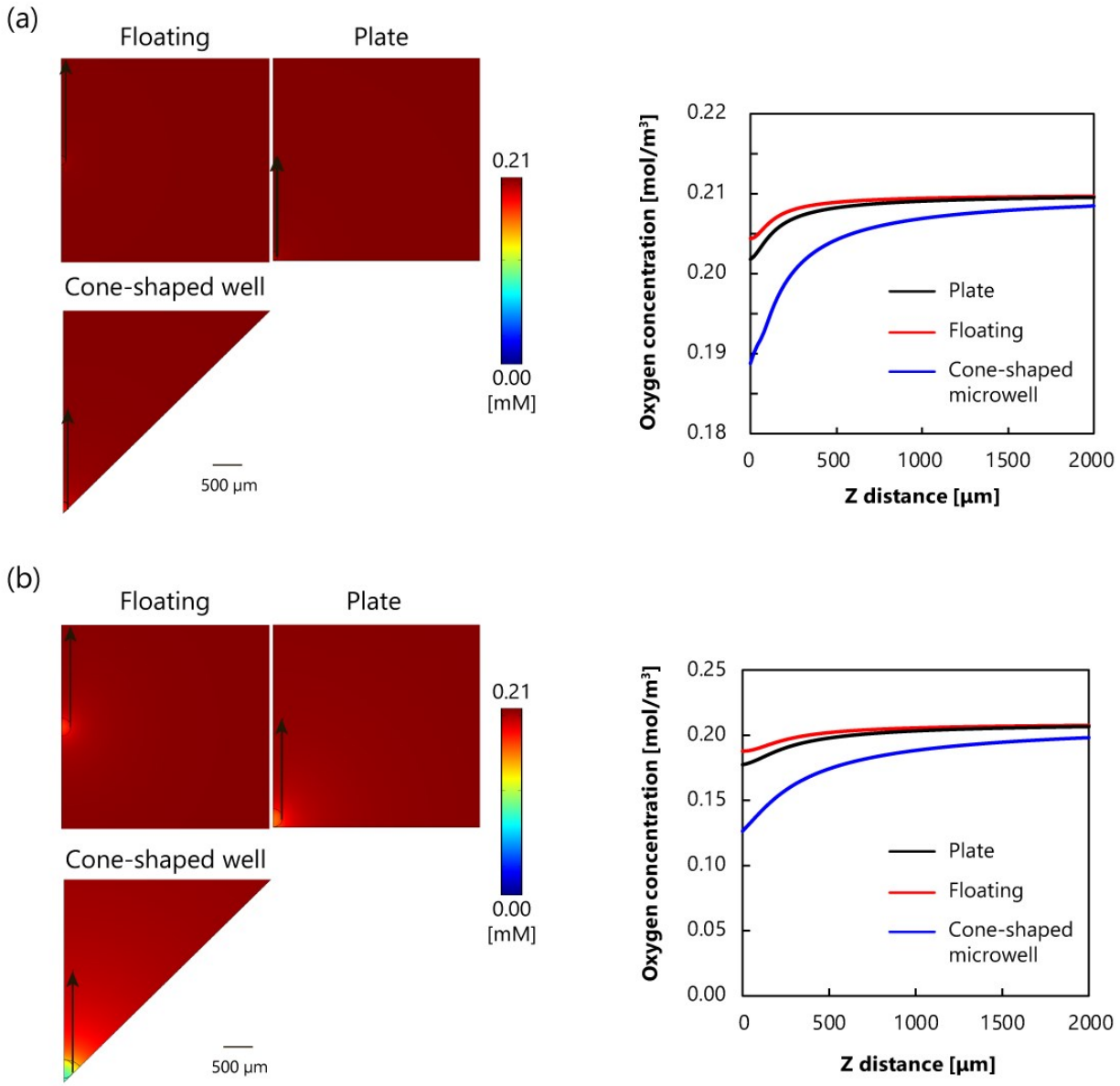


Figure S3: Numerical simulation of oxygen concentration near spheroids with a radius of (a) 93 µm and (b) 185 µm. Black arrows represent the z direction. The figures on the right are the line plots of the oxygen concentration along the paths of the black arrows in the figures on the left.

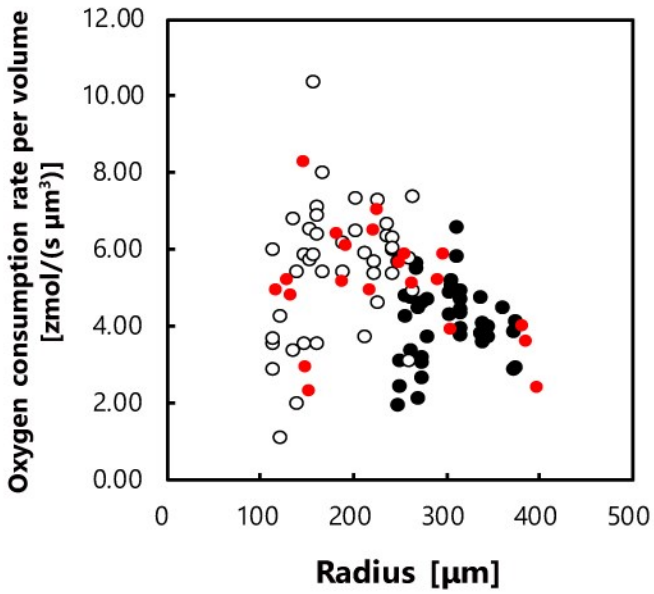


Figure S4: Oxygen consumption rate according to spheroid volume depending on the spheroid radius. Filled and open circles represent the data of the spheroids using 10,000 and 1,250 cells/well, respectively. The average values were calculated each day (they are plotted in Figure 4b). The red filled circles indicate the data from 20,000, 5,000, 2,500, 625, and 300 cells/well after 3 days in suspension culture.

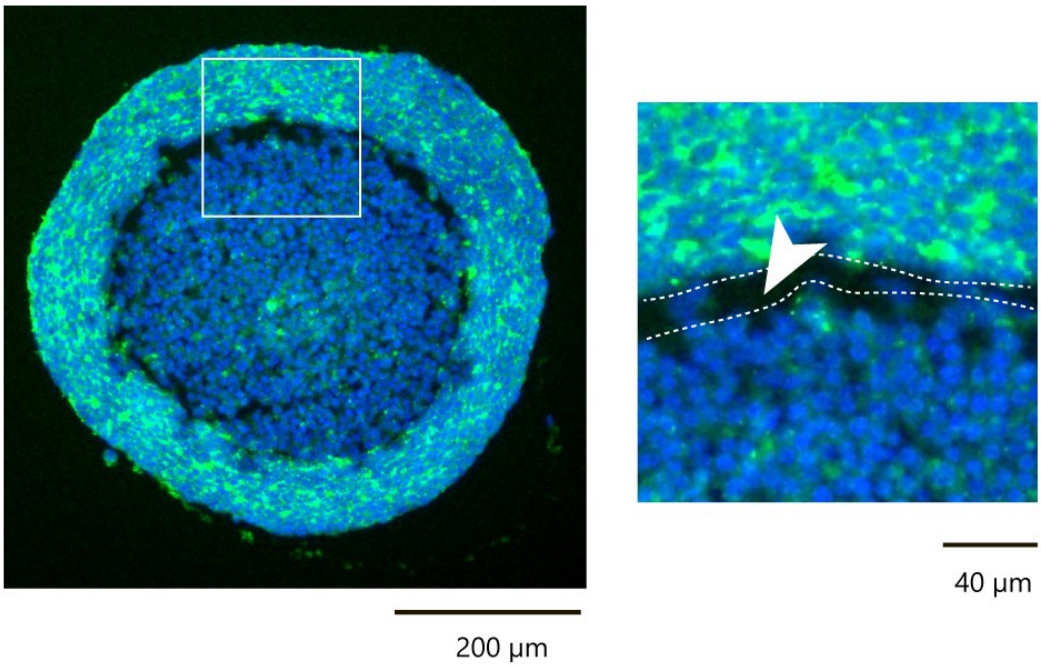
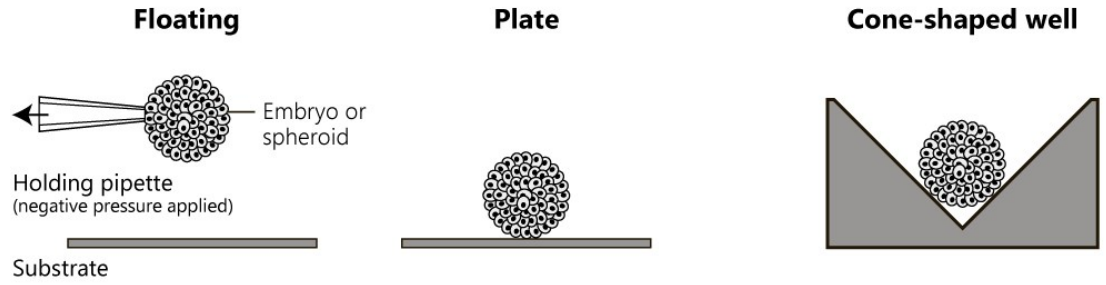


Figure S5: Cell free area in a spheroid with a necrotic-like core. Cryosections of the MCF-7 spheroid after 5 days in suspension culture (10,000 cells/well). F-actin and nuclei were fluorescently labelled using Alexa Fluor 488 (green) and Hoechst 33342 (blue), respectively. The figure on the right is the enlarged view of the white rectangle in the figure on the left. The area that the white arrow heads indicate is the cell free area.

(a)



$$\mathrm{OCR}_{\mathrm{float}} = 4\pi r_s D\Delta C \quad (3)$$

$$\mathrm{OCR}_{\mathrm{plate}} = 2.76\pi r_s D\Delta C \quad (4)$$

$$\mathrm{OCR}_{\mathrm{cone}} = 2\pi\left(1-\frac{1}{\sqrt{2}}\right)(h+r_s)D\Delta C \quad (5a)$$

$$h = \left(\frac{1}{\sin\theta}\right)r_s \quad (5b)$$

(b)

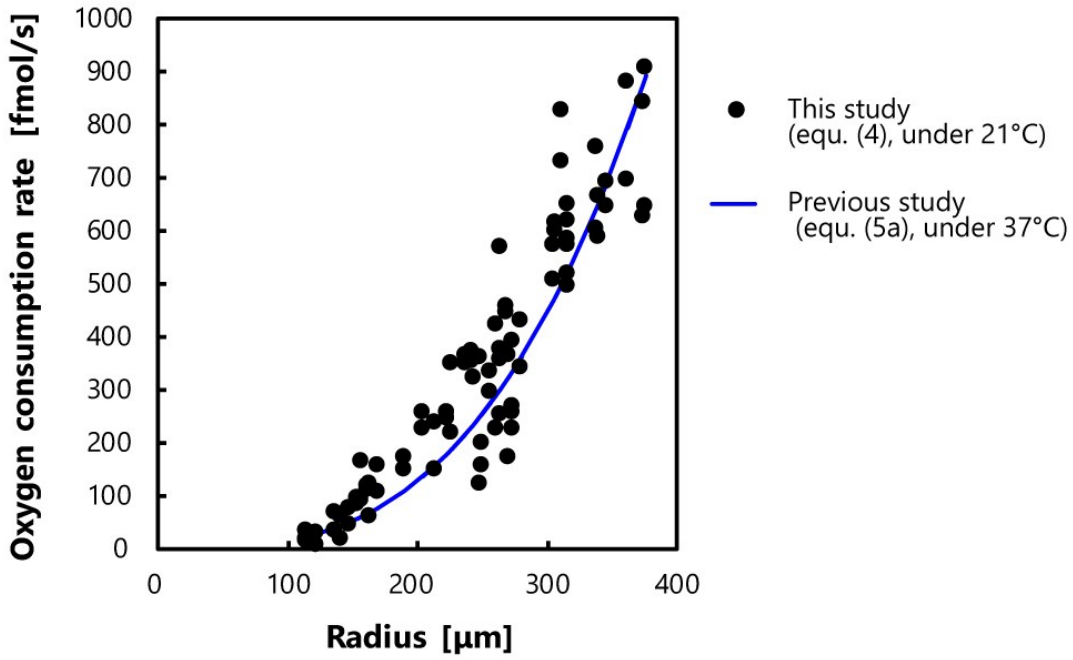


Figure S6: Comparison of the oxygen consumption rate (OCR) calculated using equation (4) with our previous results using the cone-shaped microwell. (a) Schematic of the culture plate and the equation for OCR calculations. (b) Filled circles represent data calculated by equation (4) in this study (10,000 and 1,250 cells/well, day 1~7). The blue line is the power trendline from our previous study (19) using the cone-shaped microwell ($y = 1.18 \times 10^{-6}\, x^{3.06}$, the power trendline in the previous study was multiplied by 0.7). The Pearson's correlation coefficient between the data in this study and in our previous study was 0.924.