光環境センサを用いた三次元組織内酸素分布計測

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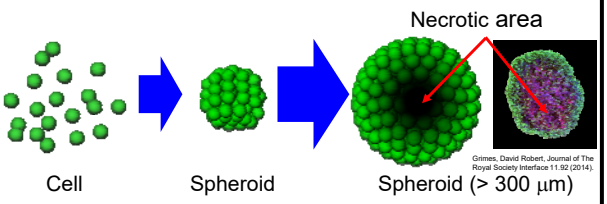
高品質の三次元組織を構築するには?

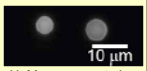
Background

Evaluation of physiological conditions in tissue



Deep part in large tissue: lack of oxygen

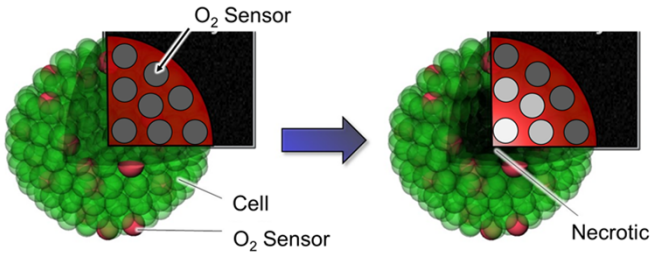
How to measure oxygen inside microscale?

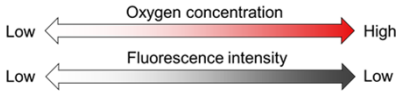
Sensor type	Fluorescence	Optode	Electrode
Image	 H. Maruyama, et.al., Trans. Mech (2012)a	 O_2 Microoptode (Unisense)	 O_2 Microsensor (Unisense)
Size	< 10 μm	50 μm	8 μm
Sensitivity	1.1 μmol/l	1.3 μmol/l	5.6 μmol/l
Cell migration	Possible	Difficult	Difficult
Influence of environment	Low	Low	High
Oxygen consumption	No	No	13.4 fmol/min

Non-contact sensing using fluorescence

Concept

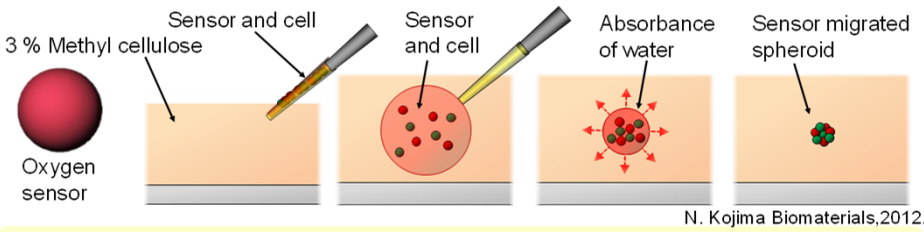
Direct measurement of oxygen in tissue by migration of fluorescence sensor





Ratio measurement of fluorescence
・Robust against photo-bleaching, light fluctuation, move of sensor

Migration process of fluorescence sensor into spheroid



N. Kojima Biomaterials,2012.

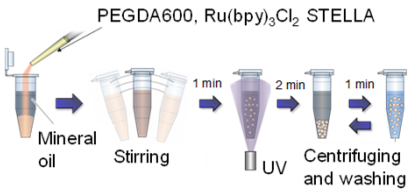
How to measure oxygen inside microscale?

Fabrication of Sensor

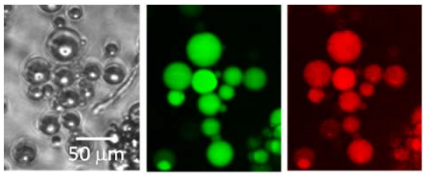
Sensor material

・Polyethyleneglycol diacrylate (PEG) 600 Biocompatible, hydrophilic
・$Ru(bpy)_3Cl_2$:High-sensitive to oxygen em. 488 nm, ex. 620 nm
・STELLA :Low-sensitive to oxygen ex. 561 nm, em. 610 nm

Fabrication process



Fluorescence images of sensor

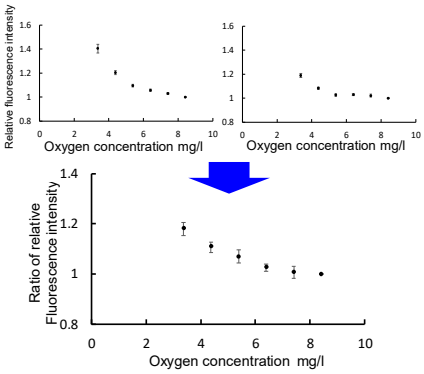


Optical image　Fluorescence of $Ru(bpy)_3Cl_2$　Fluorescence of STELLA

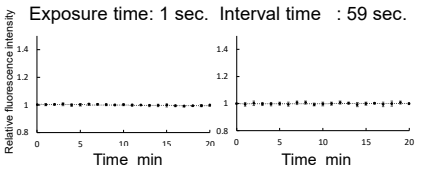
Biocompatible hydrogel oxygen sensor was fabricated.

Experiments

Calibration of sensor

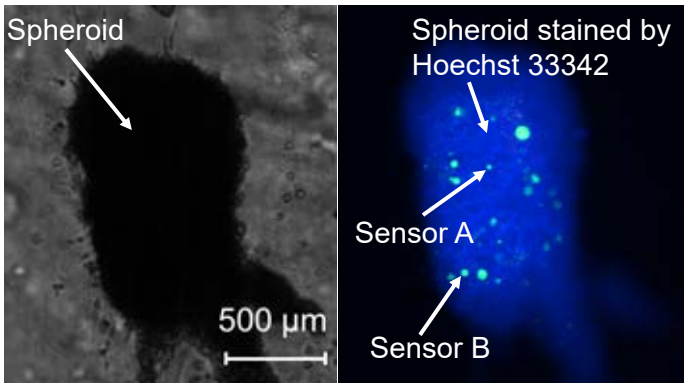


Stability of sensor (photobleaching)



Ratio measurement of oxygen using this sensor is possible.

Measurement of oxygen distribution inside spheroid (Cell: Osteoblast cell MC3T3-E1)



Optical image　Fluorescence image

Sensor	1 days	2 days	5 day
A	$2.6x10^2$ μM	$2.7x10^2$ μM	$1.1x10^2$ μM
B	$2.6x10^2$ μM	$2.6x10^2$ μM	$2.6x10^2$ μM

Measurement of oxygen distribution in spheroid using fluorescence sensor was succeeded.

Conclusions

1. Fabrication of fluorescence oxygen sensor and ratio measurement
2. Time-course variation of oxygen distribution inside spheroid was measured.

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Reference：
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