

# FLEXIBLE PHOSPHORESCENT OXYGEN MICROSENSOR ARRAY DEVICES FOR NONINVASIVE MONITORING OF CELLULAR OXYGEN METABOLISM DURING CULTIVATION

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## ABSTRACT

A novel flexible sheet-type sensor device was developed for the *in situ* and noninvasive oxygen metabolism measurement of cultured cells and tissues. The combined use of the device and an automated optical measurement system enabled the simultaneous measurement of oxygen consumption rate ( $R_{ox}$ ) and optical observation of local groups of cells on a cultured dish. We monitored oxygen metabolism of the human breast cancer cell line MCF7 on Petri dish and evaluated the  $R_{ox}$  to be  $1.43 \pm 0.24$  fmol/min/cell. Furthermore, we demonstrated mapping of the  $R_{ox}$  of rat brain slices and succeeded in visualizing the clear difference between layer structures of hippocampus.

## KEYWORDS

Oxygen Sensor, Cellular Oxygen Metabolism, Brain Slice, Optical Sensor, Phosphorescence Lifetime, MCF7

## INTRODUCTION

For the advancement of cell therapy and/or tissue engineering, cell quality control is essential. Oxygen consumption is an important parameter for evaluating cell metabolism due to its direct correlation with ATP production. Microfluidic devices integrated with oxygen sensors were previously reported to be capable of analyzing cell-level oxygen consumption rate ( $R_{ox}$ ) [1]. However, as petri dishes are currently used as a standard format for cell culture, the use of a microfluidic format is anticipated to be limited in practical medical use. To resolve this issue, we have developed a flexible sensor device that has good compatibility with standard cell cultivation techniques.

## EXPERIMENT

### Device Design

Figure 1 shows a schematic of the device and experimental setup. The device comprised a transparent ethylene-vinyl-alcohol (EVOH)/ polydimethylsiloxane (PDMS) sheet and an array of microwell structures ( $\phi$  90  $\mu$ m and 50  $\mu$ m depth) with a 1- $\mu$ m-thick sensing layer at their bottom. The oxygen sensor layer consisted of platinum octaethyl porphine (PtOEP) mixed into polystyrene, an oxygen-permeable polymer. In contrast, EVOH is impermeable to gases ensuring a tight seal during measurement. A change in oxygen concentration ( $C_{ox}$ ) in the vicinity of cells can be evaluated by measuring the phosphorescence lifetime. In the presence of oxygen, the lifetime is shortened as a result of quenching by the collision of oxygen molecules with the phosphor which deactivates the excited triplet state. The phosphorescence lifetime and dissolved oxygen concentration has a linear relation in accordance with the Stern-Volmer equation.

$$\frac{\tau_0}{\tau} = 1 + K_{SV} [O_2]$$

Here,  $\tau_0$ : unquenched emission lifetime,  $\tau$ : emission lifetime in the presence of different oxygen concentration,  $K_{sv}$ : Stern-Volmer constant, and  $[O_2]$ : dissolved-oxygen concentration.

Using an automatic measurement system, the  $R_{ox}$  measurement and imaging of cultivated cells were conducted simultaneously.

### Cellular oxygen consumption measurement of MCF7

The human breast cancer cell line MCF7 was cultured at 37°C in 5% CO<sub>2</sub>, 95 % air in RPMI 1640 medium supplemented with 10% fetal calf serum, 10 mg/L kanamycin sulfate, 10,000 unit/ml penicillin and streptomycin. Cells were cultured to confluency in 30-mm glass-base dishes. During cellular oxygen consumption measurement, the cultivation dishes were set in a compact cell incubator mounted on a microscope and the devices were attached onto the dish with the pressure 10 kPa.

### Oxygen consumption rate mapping of the acute brain slice of rat

An anesthetized 16-day-old rat pups were decapitated and the hippocampus was rapidly removed and immersed in 37°C artificial cerebrospinal fluid (ACSF) medium. The brain was cut transversly through hippocampus by tissue chopper at an

interval of 350  $\mu\text{m}$ . The device-applied cultivation dish submerged with ACSF medium was set in a compact cell incubator mounted on a microscope. The brain slice was attached onto the polyvinylidene difluoride membrane to ease handling, followed by oxygen metabolism measurement using the device. The oxygen consumption rates are analyzed for 4 $\times$ 4 microchambers to conduct oxygen consumption mapping of the brain.

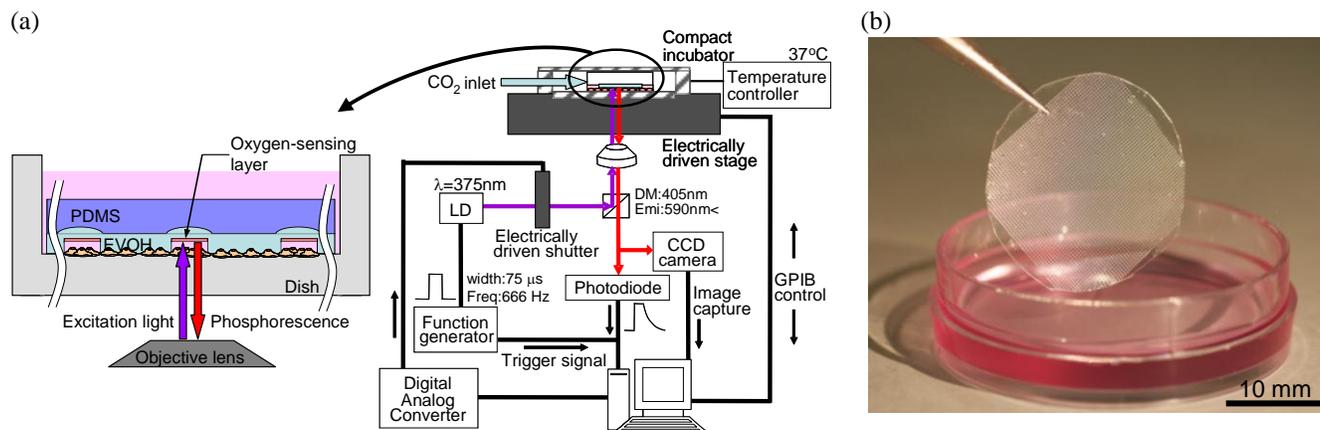


Figure 1. (a) Schematic diagram of flexible sensor sheet and experimental setup for in situ measurement of cell oxygen consumption. During the measurement, the device is attached to the bottom of the culture dish to form a temporarily closed microspace around the target cells, hence enabling the short-time evaluation of oxygen consumption rate. (b) Photograph of flexible sensor sheet. Flexible sensor sheet has good compatibility with practical cell cultivation formats. The three-dimensional microstructures of the sensor were fabricated by the self-aligned hot embossing method that was newly developed to enable a simple and low-cost production[2].

## RESULTS AND DISCUSSION

Prior to the cellular measurement, we checked the fundamental characteristics of the device, namely, sensitivity calibration, sealing performance, and cell viability (Figures 2(a,b,c)).  $R_{\text{ox}}$  measurement was conducted with minimum cell damage by attaching the device for measurement within less than 10 min at a pressure of 10 kPa.

Cellular measurements were conducted by attaching the measuring device to MCF7 breast cancer cells cultivated on petri dishes as shown in Figure 3. The linear decrease in oxygen concentration was measured as a result of cell respiration. Furthermore, the increase of the number of cells lead to the sharp slope, which means the oxygen consumption increased according to the number of the cells. By dividing the  $C_{\text{ox}}$  slope in Figure 3(d) by the number of cells,  $R_{\text{ox}}$  of MCF7 was evaluated to be  $1.43 \pm 0.24$  fmol/min/cell ( $N=22$ ), which was in agreement with previously reported values [3]. This suggests that the device is applicable to evaluation of the oxygen metabolism of the cells cultured on the Petri dish.

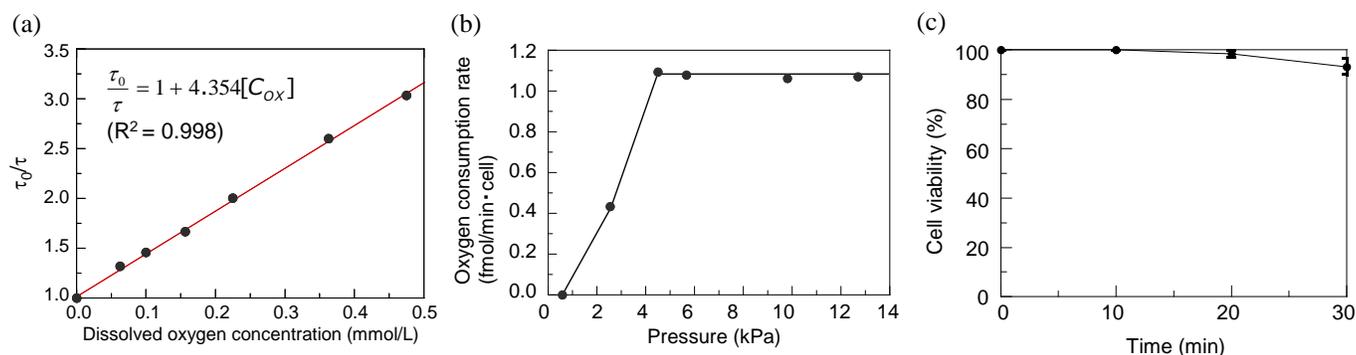


Figure 2. (a) Oxygen sensor calibration.  $\tau$  and  $C_{\text{ox}}$  exhibited a linear relationship in accordance with the Stern-Volmer equation. (b) Oxygen consumption rate ( $R_{\text{ox}}$ ) depends on pressure.  $R_{\text{ox}}$  is convergent at pressure 4.5-12.7 kPa because of the high seal performance of the microchamber. (c) The viability of cells outside the microchamber by trypan blue cell viability assay. Cell viability slightly decreased after 20 min but there was no change before 10 min.

Finally, the  $R_{ox}$  mapping of rat brain slices was achieved using the device developed. Oxygen consumption rate mapping of rat hippocampus slice is shown in Fig. 4. It is known that oxygen consumption rate of the brain is 10 times faster than the other tissue, and indeed we observed short-time measurement. As shown in Figure 4, differences in  $R_{ox}$  among brain layers such as CA1, CA3, and DG were observed. Thus, the device developed has enabled the real-time mapping of the metabolic activity of cultivated tissues, and is expected to be widely used in the field of drug development and neuroscience research.

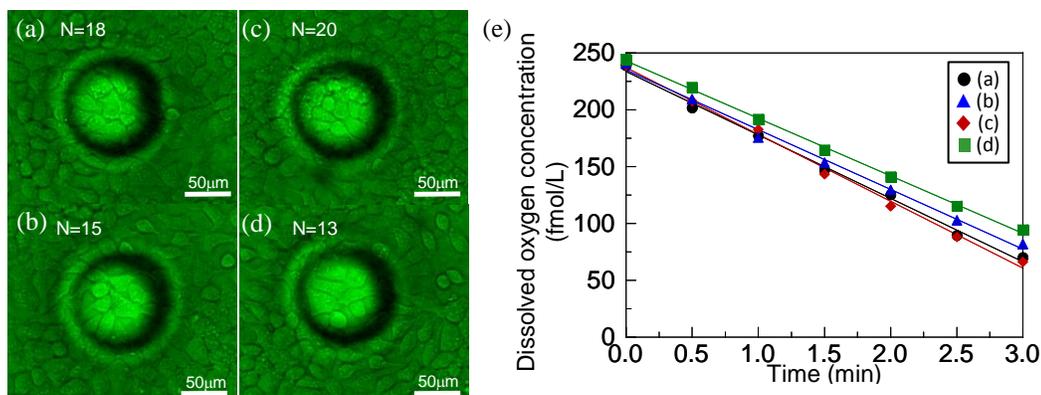


Figure 3. (a)-(d) Bright-field images of MCF7 and flexible sensor sheet. (e)  $R_{ox}$  measurement performed using the device. The linear decrease in oxygen concentration was measured as a result of cell respiration.

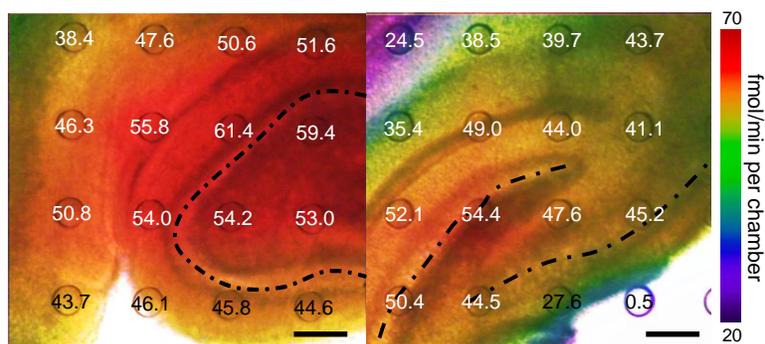


Figure 4.  $R_{ox}$  mapping of rat hippocampus slice. CA1 and CA3 are pyramidal cells of the hippocampus and DG is the dentate gyrus. Broken lines show neuronal cell bodies. This result indicates that the  $R_{ox}$  of DG is higher than the  $R_{ox}$  of CA3 and CA1. Scale bar: 200  $\mu$ m.

## CONCLUSION

In this research, we have developed a novel flexible sensor device for in situ and spatiotemporal monitoring of oxygen consumption of cultivated cells and tissues with fmol/min resolution. The device comprised a transparent sheet and an array of microwell structures with an oxygen sensing layer at their bottom. By the combined use of the device and an automatic optical measurement system, we successfully measured the oxygen consumption rate of local groups of cells cultivated on a dish, and the real-time mapping of the metabolic activity of cultivated tissues. The present technology can be further extended to include other types of extracellular analyte sensing such as pH,  $CO_2$ , and NO by incorporating different phosphor materials as the sensing layer.

## REFERENCES

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