Monitoring Activity of Living Cells Entrapped in a Membrane-Sealed Microcapillary with Scanning Electrochemical Microscopy

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The respiration- or photosynthesis-induced change of oxygen concentration was monitored with scanning electrochemical microscopy (SECM). Cell suspension was put into a glass capillary tube and then one edge was sealed with an oxygen permeable membrane. The total volume of the cell suspension in the capillary was as small as 10 μL . The edge of the sealed capillary was imaged with SECM based on oxygen reduction current. The images indicated the respiration activity as low as 2.0×10^3 HeLa-S3 cells. This method was also applied for imaging the respiration and photosynthesis activities of a single alga (*Bryopsis plumosa*) protoplast.

Key Words : Oxygen Electrode, Living Cell, Scanning Electrochemical Microscopy, Small Volume Analysis

1 Introduction

Clark-type oxygen electrode has been widely used to monitor oxygen concentration changes generating from living cells, tissue or microorganism suspensions.^{1–3)} The reliability and selectivity of the Clark-type electrode are mainly guaranteed by an oxygen permeable membrane, which permeates oxygen selectively and separates biological materials from the measurement solutions to prevent contamination on the electrode surface. However, at least 10^5 to 10^7 cells are generally required to measure their respiration activities. Furthermore, spatial resolution can not be expected with the conventional technique.

Recently, microamperometric detection of biologically important chemicals has been recognized as a useful method to characterize cellular functions with single-cell level.⁴⁾ Unfortunately, the intra- and extra-cellular detection schemes reported until now have been carried out in the solutions that the perturbing components such as proteins or electrochemically active species are excluded. Otherwise microelectrode probe has to be covered with membrane for monitoring cell functions in the culture medium. However, the fabrication of such membrane-covered microelectrode is very difficult.

We report here a novel method for monitoring oxygen concentration in small volumes with biological cells using scanning electrochemical microscopy (SECM).^{5–7)} Glass capillary tube was used as a tool to entrap the limited volume of cell cultures. The SECM images of sealed end of the glass capillary have clearly shown the respiration and photosynthesis activities of the entrapped cells.

2 Experimental

HeLa-S3 cells (Japanese Cancer Research Resources Bank) were cultured in T-25 culture flask (10^6 cells) with a growth medium which contained RITC 80-7 (Asahi Techno Grass. Co.) and 5% fetal bovine serum (FBS; ICN Biomedical. Co.) at 37°C in a humidified atmosphere of 5% CO_2 in air. A glass capillary tube (Narishige, GD-1 or G-1, inner diameter 0.55 mm) was filled with cell suspension and one edge of the capillary was sealed with an oxygen permeable membrane (Yellow Springs Instrument Co., Ohio). The total volume of the inner suspension was 10 μL . The protoplasts of the marine algae *Bryopsis plumosa* were prepared according to the method reported by Tatewaki and Nagata.⁸⁾

Figure 1 shows a schematic view of the measurement

Fig. 1 Schematic view of the experimental setup.

Fig. 2 SECM images ($1020\ \mu\text{m} \times 1020\ \mu\text{m}$) of the sealed end of the capillary tube containing 1.4×10^4 (A) and 2.0×10^3 HeLa-S3 cells (B) with $10\ \mu\text{L}$ of the culture medium.

system. A Pt microdisk electrode (tip radius, $2.0\ \mu\text{m}$) was fixed at the bottom of a measuring chamber filling with sodium phosphate buffer (pH 7.4). The probe potential was controlled with a potentiostat (Hokuto Denko, HA1010mM4) and held at $-0.6\ \text{V}$ vs. Ag/AgCl to monitor the reduction current for oxygen. The membrane-sealed capillary was attached to a motor-driven XYZ stage (Chuo-Seiki, M9103) and scanned in XY directions above the probe in the buffer solution.

The distance between the sealed edge of the capillary and the tip microelectrode was typically $100\ \mu\text{m}$ which was far enough to ignore negative feedback effects due to topographical changes. Single SECM image was captured within 20 min for $1020\ \mu\text{m} \times 1020\ \mu\text{m}$ square and the current was normalized to that recorded at the most left pixel for each scanning line. The simultaneous observation with an optical microscope indicated that the cells fell down onto the membrane and did not move during the measurements.

3 Results and Discussion

Figure 2 shows SECM images of the sealed edge of

glass capillaries with the 1.4×10^4 and 2.0×10^3 HeLa-S3 cells in $10\ \mu\text{L}$ of the medium. The images were captured 4 hours after putting the cell suspension into the capillary. The dark area with low oxygen reduction current indicates that the oxygen concentration in the capillary decreases due to the respiration activity of the cells. The shapes of the dark region are identical with the inner-circle of the capillary.

The SECM images have clearly shown that the oxygen concentration changes on the outside surface of the membrane sealing the cell suspension are homogeneous. The contrast of the image lowered as the number of the cell in the sample decreased. No image contrast was observed for the solution inside the capillary without cells (data not shown).

This measurement procedure is also applicable for monitoring oxygen concentration change induced by cellular activity of single cells. We have imaged the cellular activity of a single alga protoplast in the capillary. Figure 3 shows SECM images of the sealed edge of a capillary with a protoplast in sea water, together with an optical photograph of the protoplast in the capillary. The protoplast, about $200\ \mu\text{m}$ diameter, located at the upper side of the capillary cross section as seen in Fig. 3 C. The image obtained 20 min after the light irradiation indicates that the oxygen concentration of the solution inside the capillary was remarkably high compared with that of outside solution. The photosynthetic oxygen generation from the single cell increases the oxygen concentration of the entrapped solution in the capillary. In the dark, the oxygen concentration of the entrapped solution became lower due to the respiratory uptake of oxygen by the protoplast. It is worth to mention that the region with high or low oxygen concentration does not correspond to the shape of the protoplast⁹⁾ but to the shape of inner-circle of the capillary.

As both inner-capillary wall and the oxygen permeable membrane effectively entrap the limited solution with different oxygen concentration, the concentration distribution around the single cell has drastically changed. This experimental system enables to collect total oxygen con-

Fig. 3 SECM images ($1020\ \mu\text{m} \times 1020\ \mu\text{m}$) of the sealed end of the capillary tube containing a protoplast in sea water in the dark (A) and under light irradiation (B). A photograph (C) is also shown. A protoplast was isolated within a capillary tube. Outer- and inner-diameters are 1.00 and 0.55 mm, respectively.

centration changes occurred within the small volume with high efficiency. The measurement system will be widely applicable to monitor oxygen concentration changes of various type of cells, for example, even mobile cells or microorganisms.

We also carried out time course measurements after light irradiation. When the light was irradiated, the reduction current gradually increased and reached steady state after ~ 100 s. On turning light off the current tailed down on the same time scale. The response was at least 10 times slower than that observed in direct microamperometry.¹⁰⁻¹²⁾ Further studies are in progress for improving response time and forming smaller sample volume with tapered capillaries. Multi-sample monitoring system will be easily achieved by utilizing multi-barrel capillary tubes.

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References

- 1) D. C. Fork, *Methods Enzymol.*, **26**, 113 (1972).
- 2) H. Suzuki, E. Tamiya, and I. Karube, *Anal. Chem.*, **60**, 1078 (1988).
- 3) H. Suzuki, H. Ozawa, S. Sasaki, and I. Karube, *Sensors and Actuators*, **B53**, 140 (1998).
- 4) T. Matsue and I. Uchida, *Chemical Sensor Technology*, Vol. 3, Kodansha Ltd. p.263 (1991); R. M. Wightman, T. J. Schroeder, J. M. Finnegan, E. L. Ciolkowski, and K. Pihel, *Biophys. J.*, **68**, 383 (1995); T. K. Chen, G. Luo, and A. G. Ewing, *Anal. Chem.*, **66**, 3031 (1994); H. Lu and M. Gratzl, *Anal. Chem.*, **71**, 2821 (1999); S.-K. Jung, W. Gorski, C. A. Aspinwall, L. M. Kauri, and R. T. Kennedy, *Anal. Chem.*, **71**, 3642 (1999).
- 5) R. C. Engstrom and C. M. Pharr, *Anal. Chem.*, **61**, 1099 A (1989).
- 6) A. J. Bard, F.-R. F. Fan, J. Kwak, and O. Lev, *Anal. Chem.*, **61**, 132 (1989).
- 7) M. V. Mirkin, *Anal. Chem.*, **68**, 177A (1996).
- 8) M. Tatewaki and K. Nagata, *J. Phycol.*, **6**, 401 (1970).
- 9) T. Yasukawa, T. Kaya, and T. Matsue, *Chem. Lett.*, **1999**, 975.
- 10) T. Matsue, S. Koike, T. Abe, T. Itabashi, and I. Uchida, *Biochim. Biophys. Acta*, **1101**, 69 (1992).
- 11) T. Matsue, S. Koike, and I. Uchida, *Biochem. Biophys. Res. Comm.*, **197**, 1283 (1993).
- 12) T. Yasukawa, I. Uchida, and T. Matsue, *Biophys. J.*, **76**, 1129 (1999).