MICROCHIP ANALYSIS OF CELLULAR RESPIRATORY ACTIVITY ON PDMS MEMBRANE HAVING GAS PERMSELECTIVE PROPERTY

T. Shirai\textsuperscript{1}, T. Sakata\textsuperscript{1}, M. Takai\textsuperscript{1}, Y. Miyahara\textsuperscript{1,2}, K. Ishihara\textsuperscript{1,3}

\textsuperscript{1}Department of Material Engineering, School of Engineering and Center for NanoBio Integration, The University of Tokyo, JAPAN
\textsuperscript{2}Biomaterial Center, National Institute for Material Science, Tsukuba, JAPAN
\textsuperscript{3}Department of Bioengineering, School of Engineering and Center for NanoBio Integration, The University of Tokyo, JAPAN

ABSTRACT
A new microchip device for real-time analysis of cellular respiratory activity on poly(dimethylsiloxane) (PDMS) membrane with integration of carbon dioxide sensor based on pH-sensitive field-effect transistor (pH-FET) was developed. Controlling the thickness of PDMS membrane, response curves of the CO\textsubscript{2} gas under soda water flow and respiratory activity of HeLa cells on PDMS substrate were monitored as a pH-shift. Our results suggest that the pH-FET integrated microdevice having gas permselective PDMS membrane can contribute critically to basic understanding of biological mechanisms in the field of tissue engineering.

KEYWORDS: pH-FET, gas permselective property, poly(dimethylsiloxane), cellular respiratory activity

INTRODUCTION
Recently, especially in the field of tissue engineering, better control of cell responses on biomaterial surfaces plays a pivotal role. However, little is known about the physical-chemical mechanisms underlying cell biological events, such as growth and differentiation. For quantitative understanding of these events, microfluidic bioreactors are expected opportunities to study cells under simulated physiological microenvironments. Because microfluidic systems enable spatial and temporal control of cell behavior by complex biochemistry. In order to evaluate microenvironment of cell culture, it is necessary to integrate bioanalytical tools into the microsystems. The devices should be minimally cell-invasive, rapid, sensitive, and low cost. They must also be compatible with miniaturization and high sample throughput.

Cellular metabolic response assay is one of the cornerstones in cell biology. CO\textsubscript{2} is a common metabolic product closely related with cellular respiratory activity. Therefore, a microdevice monitoring cellular metabolic responses was developed by detecting the CO\textsubscript{2} gas passing through permselective PDMS membrane in this study. Here, we used CO\textsubscript{2} sensing system based on pH-FET and PDMS membrane. Compared to commercially available electrodes, there are several advantages of using pH-FET, like easier handling, higher mechanical stability, fast response time, simple construction and comparatively good value. Besides its high permselectivity of CO\textsubscript{2}, PDMS membrane has a role as a substrate for culturing cells. Using this device, cellular respiratory activity during cultivation of HeLa cells was observed.
EXPERIMENTAL

For all experiments, pH-sensitive field-effect transistors (BAS Inc., Japan) with Si$_3$N$_4$ as gate material were used for the monitoring of CO$_2$. The detection principle is shown in Fig. 1. This system works with the acceptor buffer containing $1 \times 10^{-2}$ mol L$^{-1}$ NaHCO$_3$ solution. Gas permselective PDMS membranes, located between sample and acceptor buffer. If there is a partial pressure difference between sample and acceptor buffer, a gas diffusion process starts through the gas phase of the membrane to compensate the concentration of dissolved CO$_2$. The pH-shift of the acceptor buffer, created during this process, is proportional to CO$_2$ concentration of the sample and can be detected by the pH-FET [1]. PDMS (Silpot184®, Toray-Dow Corning Asia Co., Japan) and its curing agent were mixed well in a mass ratio of 10:1. The mixture was degassed under vacuum for 1 h at 25°C before spreading, and it was cured at 25°C for 24 h in air after spreading. To determine the PDMS membrane thickness to obtain a gas permeable property, we fabricated it inside a cell-culture chamber (Transwell®, Corning, USA). The PDMS membrane was fabricated at its bottom of the chamber by spin-coating the PDMS mixture at 5000 rpm for 60 s. This chamber was fixed in the CO$_2$ sensing system. Calomel electrode was used as a reference electrode. HeLa cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% FBS, then the suspension (300 µl, $10^6$ cells ml$^{-1}$) was injected into the chamber and cultured at 37°C in 0% CO$_2$ atmosphere with maximum humidity. During the cultivation of HeLa cells on PDMS membrane inside the chamber, pH-shift of the acceptor buffer was monitored.

The design of the CO$_2$ sensor integrated into the micro-fluidics device having gas permselective PDMS membrane is shown in Fig. 2. To fabricate gas permselective membrane, the PDMS mixture mentioned above was spread on a clean flat polycarbonate plate, spun at 5000 rpm for 60 s, and cured at 25°C for 24 h in air. PDMS membranes having microchannel for sample cell or acceptor buffer cell were also fabricated. These three-types membranes were treated by oxygen plasma (85 W, 10 s) and sealed against each other. Using this microchip system, pH-shift of the acceptor buffer under soda-water flow was monitored. The measurement was performed at 37°C in 0% CO$_2$, and the rate of flow was 40 µl min$^{-1}$.
RESULTS AND DISCUSSION

Fig. 3 shows the time-course of the pH-shift of the acceptor buffer before and after injecting the HeLa cells suspension into the micro-chamber. To exclude this influence of CO₂ included in the cell-culture medium, the pH-shift of the medium as a control was subtracted from the pH of each sample. The result showed that the CO₂ concentration increased during cultivation of HeLa cells. The amount of CO₂ indicates CO₂ exhausted during the cellular-respiratory process. Therefore, that pH-shift was attributable to the respiratory activity of the culturing cells.

The response of the CO₂ sensor under the soda water flow is shown in Fig. 4. At the flow-start, the pH-shift was observed. As a control, pH-shift of the acceptor buffer under water flow was monitored. The increasing of CO₂ concentration of the sample cell caused the gas diffusion process, then the pH of the acceptor buffer was increased. After 10 minute-flow, CO₂ concentrations of the sample cell and acceptor buffer were balanced, then pH-shift stopped consequently. The result showed that the CO₂ sensing system integrated in the micro-fluidics worked adequately. This micro-chip device can contribute to the real-time analysis of cellular respiratory activity.

To fabricate much lower-volume platform for cell-respirometric screening, the system must be optimized. The volume of the acceptor buffer is one of the keys which have a decisive influence on the sensitivity of tested micro-system. Because the system depends on the CO₂ diffusion through the membrane. The thickness of the membrane is also important for a rapid response.

CONCLUSIONS

The concentration of CO₂ during cultivation of Hela cells was monitored using a PDMS membrane having gas permselective property. CO₂ sensor baced on pH-FET was integrated into micro-fluidic device and response during a flow of CO₂ was observed. The results indicate the fabricated micro-system provides real-time and non-invasive monitoring of cellular respiratory activity.

REFERENCES