LSI-BASED AMPEROMETRIC CHIP DEVICE WITH 400 SENSORS FOR DETECTION OF ALKALINE PHOSPHATASE AND RESPIRATION ACTIVITIES OF EMBRYONIC STEM CELLS

Y. Kanno[1*], K. Ino[1,3], K. Y. Inoue[1.3], A. Suda[2], R. Kunikata[2], M. Matsudaira[3], H. Shiku[1] and T. Matsue[1,3,4]

[1]Graduate School of Environmental Studies, Tohoku Univ., Japan,
[2]Japan Aviation Electronics Industry, Ltd., Japan,
[3]Micro System Integration Center, Tohoku Univ., Japan and
[4]WPI-Advanced Institute for Materials Research, Tohoku Univ., Japan

ABSTRACT

In this present study, we fabricated an LSI-based amperometric chip device with highly temporal and spacial resolution for electrochemical imaging, applied to detection of alkaline phosphatase (ALP) activity and respiration activity of embryonic stem (ES) cells.

KEYWORDS: Electrochemical imaging, Electrode array, LSI-based amperometric chip device

INTRODUCTION

Many kinds of electrochemical chip devices have been developed, and some of them contain electrode arrays for bioanalytical applications. We have previously developed local redox cycling-based electrochemical (LRC-EC) chip devices for incorporating of many sensors into a small area [1]. However, there is a problem that the device can only measure redox cycling-inducing molecules.

To solve the problem, we have developed an LSI-based amperometric chip device with highly spacial and temporal resolution [2-3]. The device is based on a technology of complementary metal-oxide-semiconductor (CMOS). Each sensor contains an operational amplifier with a switched-capacitor type *I-V* converter for in-pixel signal amplification [2]. The device has 400 sensor points which are addressable for getting electrochemical signals individually.

In this study, we used the device to detect alkaline phosphatase (ALP) activity and respiration activity of embryonic stem (ES) cells cultured on a culture plate. ALP is one of markers for differentiation of stem cells. Respiration activity is used for detection of cell activity.

THEORY

The cross-section of a sensor point onto the device is shown in Figure 1. Pt electrodes were fabricated onto an LSI chip and connected with LSI circuit through Al pads. An SU-8 layer was fabricated to determine the sensor area. To acquire current signals at individual sensor points, an operational amplifier with a switched-capacitor type *I-V* converter was incorporated into each unit cell of the device. Current signals are calculated by monitoring the capacitor at the sensors using equation (1).

$$V = Q/C = \int I \, dt / C \qquad (1)$$

V is the voltage between the capacitor (V), Q is the amount of charge (C), C is the electric capacitance (F), I is the current signal from electrodes (A).

The device was applied to detection of ALP and respiration activities of embryoid bodies (EBs) fabricated using mouse ES cells. To avoid fouling of electrode surfaces, EBs were cultured on a culture plate modified

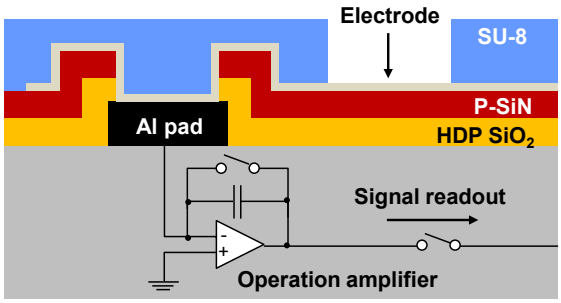

Figure 1: Cross-section of the sensor point onto the device.

with gelatin and the culture plate was mounted onto the device (Figure 2). To evaluate ALP activity, *p*-aminophenyl phosphate (PAPP) was used as a substrate for ALP. PAPP was hydrolyzed by ALP into *p*-aminophenol (PAP), resulting in the generation of the oxidation current on Pt electrodes at 0.30 V vs. Ag/AgCl [1, 3, 4]. Respiration activity was monitored as oxygen reduction current with Pt electrodes at −0.50 V vs. Ag/AgCl [4].

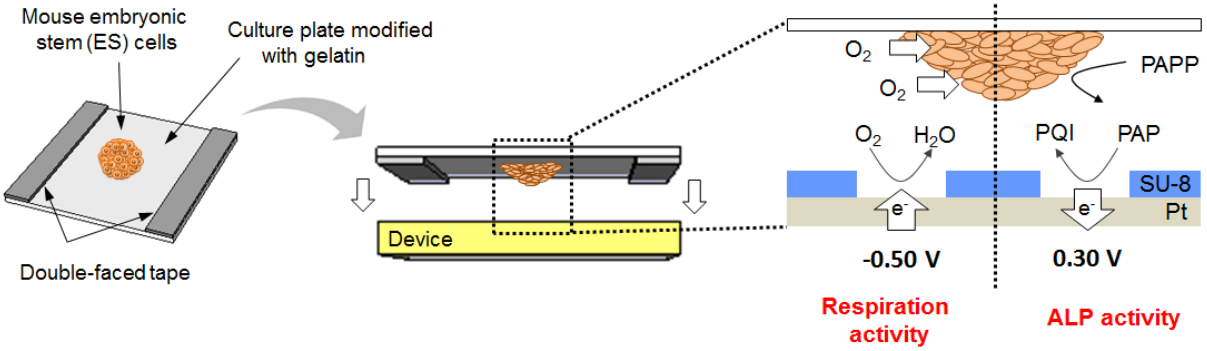

Figure 2: Measurement for ALP and respiration activities of ES cells cultured on glass substrates. (PQI: p-quinone imine)

EXPERIMENTAL

The bare LSI chip was modified using microfabrication techniques including photolithography to be used as a amperometric sensor. In order to check the operation of the device, the concentration-dependent amperometric signals were detected by the device using ferrocenemethanol ($FcCH_2OH$) solutions.

As a demonstration of the electrochemical imaging, ALP and respiration activities of the EBs were detected with the device. Mouse ES cells were cultured according to our previous report. EBs of the mouse ES cells were formed by the hanging drop method [4]. The EBs were introduced onto a culture plate modified with gelatin and cultured for 2 days. To keep a distance between the EBs and electrodes, double-faced tapes were affixed on the culture plate. After the mounting of the culture plate onto the device, we applied 0.30 V and −0.50 V vs. Ag/AgCl to the electrodes on the chip to detect ALP and respiration activities, respectively .

RESULTS AND DISCUSSION

Optical images of the device are shown in Figure 3. A polycarbonate frame was set onto the device to prevent the leakage of solutions. The specification of the device is shown in Table 1. The device composed of 400 sensors. The pitch among the sensors is 250 µm. The temporal and spacial resolution of electrochemical imaging are 200 ms and 1.6×10^3 pixels/cm^2, respectively. Figure 4 shows the calibration curve for $FcCH_2OH$, indicating that the device can be applied for a quantitative assay. Figures 5 shows that ALP and the respiration activities of the EBs on the substrate were successfully detected. The electrodes were not modified with proteins for cell culture, which prevents signal decrease due to the electrode fouling and which is useful for the device reuse.

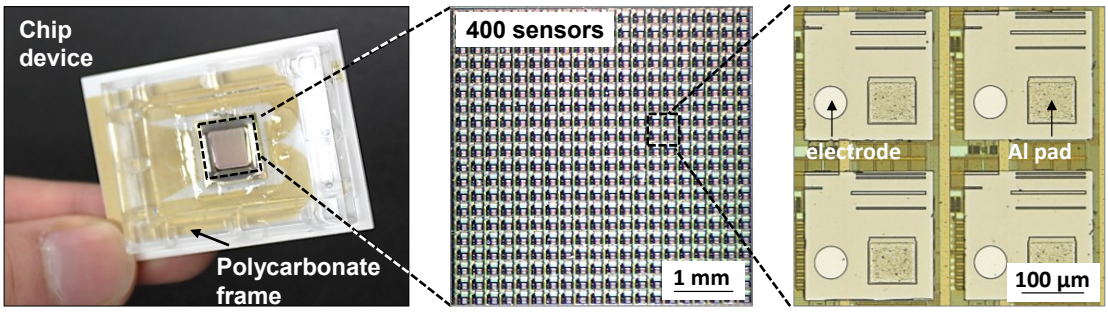

Figure 3: Optical images of the device.

Table 1: Specification of the device

Number of working electrodes	400
Electrode interval	250 μm
Electrode diameter	50 μm
Electrode surface materials	Pt
Current detection range	±0.1- 100 nA
Sampling rate	200 ms

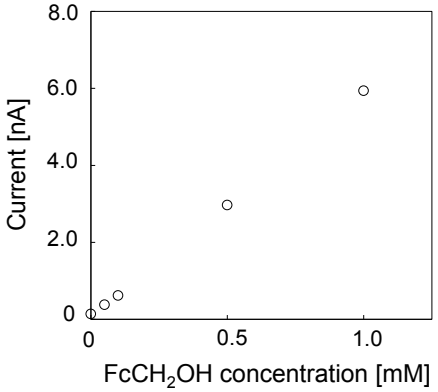

Figure 4: Dependence of the current at 0.50 V on the $FcCH_2OH$ concentration (0, 0.050, 0.10, 0.50 and 1.0 mM) using the device.

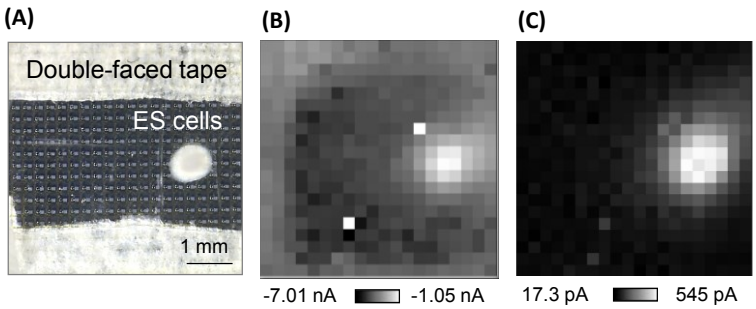

Figure 5: Electrochemical imaging of the ES cells. (A) Optical image of ES cells on the sensor point. Electrochemical images of respiration activity (B) and ALP activity (C).

CONCLUSION

ALP and respiration activities of ES cells were successfully detected by using the LSI-based amperometric chip device. We believe that the device is useful for further bioanalytical applications.

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CONTACT

* Y. Kanno; phone: +81-22-795-7281; kanno@bioinfo.che.tohoku.ac.jp