POTENTIOMETRIC IMAGING OF STEM CELLS USING AN LSI-BASED ELECTROCHEMICAL CHIP DEVICE WITH 400 MICROELECTRODES

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ABSTRACT

In this study, we demonstrated a potentiometric imaging of enzyme activity in mouse embryonic stem (ES) cells using an LSI-based electrochemical chip device with 400 microelectrodes. Successful results on the detection of the alkaline phosphatase activity of ES cells show that the potentiometric imaging using our LSI-based device will be a widely applicable method for cell analysis.

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

INTRODUCTION

Electrode array chip devices are expected platforms for electrochemical bio-imaging. Previously, we developed an LSI-based electrochemical chip device with 400 microelectrodes [1,2,3]. Figure 1 and Table 1 shows a general architecture and a specification of the device, respectively. All signals from 400 electrodes can be acquired within 200 ms. In addition, we can select measurement modes; potentiostat and electrometer modes. The former is a potential-controlled and current-detection system for amperometric measurements. Although amperometry is a major electroanalytical method used in bioanalysis, it has some problems resulting from the electrochemical production and consumption of molecules, which potentially damage biological samples and cause undesired fluctuations of the measurement concentration themselves.

To overcome this problem, we used the electrometer mode of the device for evaluation of stem cells taking advantage of rest potential measurement which induces no production and consumption of molecules on the sensor electrodes. Rest potential depends on the concentration ratio of oxidants to reductants in a solution [1]. By measuring the change of rest potential, alkaline phosphatase (ALP) as an undifferentiation marker of embryonic stem (ES) cells was detected using the device.

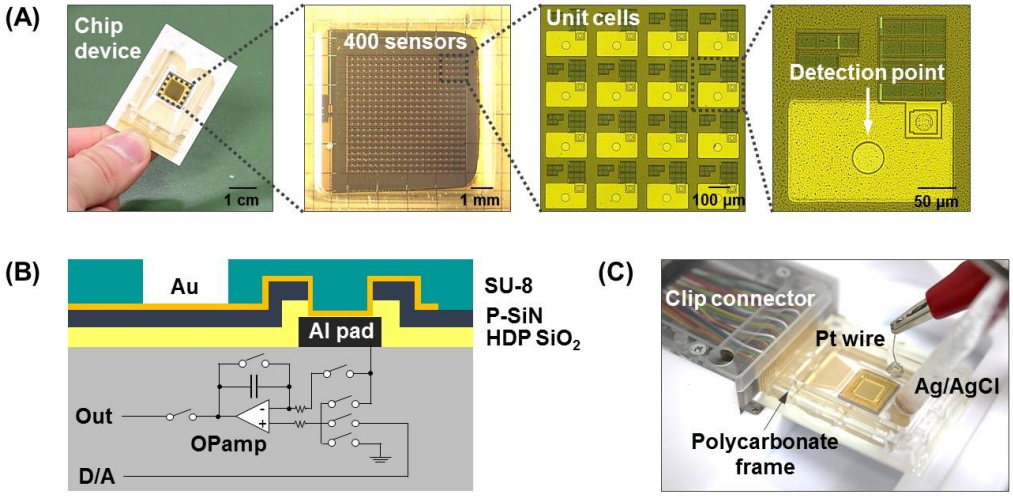

Figure 1: (A) General view and enlarged view of the sensors of LSI-based electrochemical chip device. (B) Schematic illustration of the cross-section of an unit cell. (C) Photograph showing the measurement setup.

Table 1 Specification of the device

Number of working electrodes	400
Electrode interval	250 μm
Electrode diameter	40 μm
Electrode material	Au
Sampling rate	125 ms

THEORY

Figure 2 shows a principle of potentiometric imaging of ALP. In the measurement, *p*-aminophenyl phosphate (PAPP) is dephosphorylated by ALP into *p*-aminophenol (PAP) which is known as a redox species (Figure 2(A)). ALP activity is imaged owing to reaction products, mainly PAP, which decreases rest potential in PAPP solution (Figure 2(B)). This principle has already confirmed in our previous experiments [4].

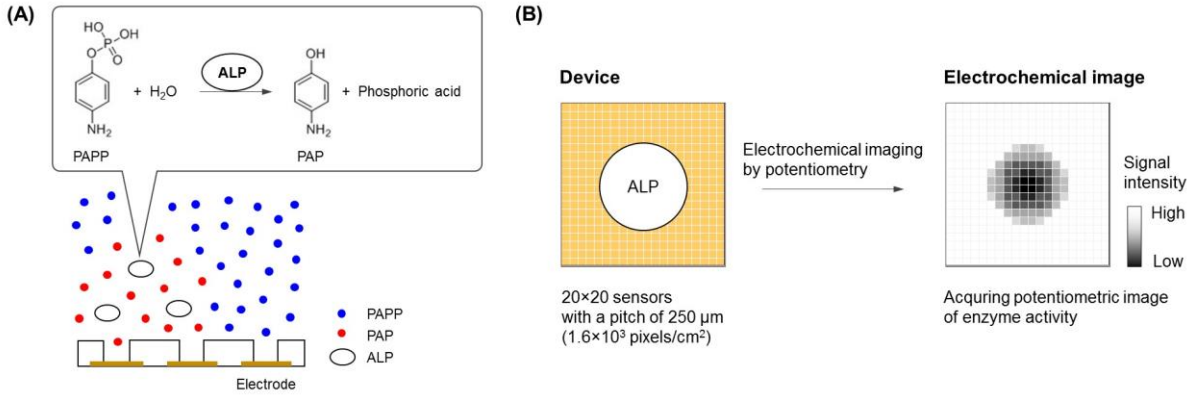

Figure 2: Scheme of the principle for measuring ALP. (A) PAPP dephosphorylation reaction by ALP. (B) Overview of potentiometric imaging of ALP using the device. The spatial resolution of an electrochemical image is 1.6×10^3 *pixels/cm*^{2}.

EXPERIMENTAL

Detailed information on the fabrication process of the device was described in our previous papers [1,2]. Briefly, 400 Au electrodes were fabricated at the bottom of each microwell (40 μm diameter, 5 μm deep) made of SU-8 photoresist. A polycarbonate sample chamber was bonded on the device.

Mouse ES cell aggregates were prepared as samples for potentiometric imaging of their ALP activity (Figure 3(A)). First, 20 μL droplets of the cell suspension containing 500 cells were hung from a dish cover and incubated for 2 days to form cell aggregates. Then, the cell aggregates were introduced into the 4.7 mM PAPP solution (pH 9.5) on the device using a micropipette. Ag/AgCl electrode and Pt wire were inserted into a sample solution on the device as reference and counter electrodes, respectively (Figure 1(C)). The rest potential was monitored using the device.

RESULTS AND DISCUSSION

Figure 3(B) shows a potentiometric image of ALP activity in ES cell aggregates obtained by the device. Rest potential at electrodes nearby the cell aggregates decreased due to generation of PAP from PAPP through ALP activity in ES cells. In consequence of the diffusion of PAP, small decrease of the potential was observed at electrodes a little near the cell aggregate. At electrodes far from the cell aggregates, no change of the potential was observed. Consequently, the device can detect ALP activity in ES cell aggregates by potentiometric imaging [4].

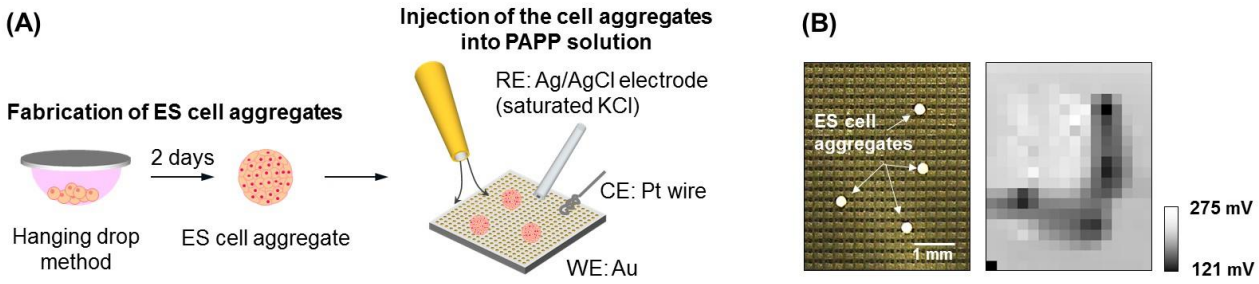

Figure 3: Experimental scheme and result of measuring ALP activity in ES cell aggregates. (A) Flow chart of fabricating ES cell aggregates and preparing the measurement system. (B) Photograph of the cell aggregates onto the device (left) and potentiometric image of ALP activity in the cell aggregates (right). In the experiment, 20×16 sensors were used.

CONCLUSION

The potentiometric imaging using the LSI-based electrochemical chip device can detect enzyme activity in ES cells. The potentiometric imaging using LSI-based device is useful and will be a widely applicable method for bio-imaging without the influence of the sensor electrode reactions.

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