

LOCAL REDOX CYCLING-BASED ELECTROCHEMICAL CHIP DEVICE FOR HIGH-THROUGHPUT ASSAY TOWARD EVALUATING EMBRYOID BODIES

Kosuke Ino¹, Taku Nishijo¹, Yusuke Kanno¹, Hitoshi Shiku¹, Tomokazu Matsue^{1,2}

¹Graduate School of Environmental Studies, Tohoku University, Japan,

²Advanced Institute of Materials Research, Tohoku University, Japan

ABSTRACT

We have previously developed a local redox cycling-based electrochemical (LRC-EC) chip device to achieve high-throughput electrochemical detection for cell analysis. In the device, two arrays of band microelectrodes are arranged orthogonally to fabricate an $n \times n$ array of crossing points with only $n+n$ external bonding pads. The electrochemical signal at the individual crossing point can be obtained by inducing local redox cycling at the desired crossing points. By using the system, 256 electrochemical sensors can be incorporated into a single chip in high density. In this study, we applied the LRC-EC system to evaluate three-dimensional (3D) culture cells.

KEYWORDS

Electrochemical detection, Cell analysis, Microelectrode array, Embryoid body, Cell differentiation

INTRODUCTION

We have previously developed a novel electrochemical system based on redox cycling for high-throughput electrochemical detection, and designated the system as a local redox cycling-based electrochemical (LRC-EC) system [1-4]. In the LRC-EC chip device, n row electrodes and n column electrodes are arranged orthogonally and these electrodes are connected to comb-type interdigitated array (IDA) electrodes or ring-ring electrodes to form $n \times n$ crossing points with only $n+n$ bonding pads for external connection. By applying proper potential to these electrodes, local redox cycling can be induced at the desired electrodes, and the comb-type IDA electrodes [1, 3, 4] or the ring-ring electrodes [2] can be used as individual electrochemical sensors. Therefore, many electrochemical sensors can be incorporated into a single chip by using the system. In this study, we applied the LRC-EC chip device to perform cell analysis, such as screening of three-dimensional (3D) culture cells.

Since 3D cell culture is a similar microenvironment to natural tissues, several kinds of cells are three-dimensionally cultured to prepare *in vivo*-like tissue organs. For example, embryonic stem (ES) cells, which can differentiate into any body tissues, can develop into cardiomyocytes by forming 3D tissue organs, such as embryoid bodies (EBs). The degree of their differentiation can be evaluated through their activity of alkaline phosphatase (ALP) on the EBs. In this study, the EB activity was evaluated via their ALP activity using the LRC-EC chip device.

EXPERIMENT

The general architecture is shown in Figure 1. The device consisted of 256 sensors in a small area. The EBs were trapped into the microwells and the electrochemical detection was then performed. *p*-Aminopheny phosphate (PAPP) was used as a substrate for detecting ALP activity (Figure 2). PAPP was catalytically hydrolyzed by ALP on the EBs to yield *p*-aminophenol (PAP). The PAP was oxidized at the generator electrode (+0.30 V vs. Ag/AgCl). The oxidation product, *p*-quinone imine (PQI), was then reduced back to PAP at the collector electrode (-0.30 V vs. Ag/AgCl). The scheme for the scanning process is shown in Figure 3 and our previous paper [1-4]. EBs were prepared by using a hanging drop method [3].

The device fabrication process is described in Figure 4 and our previous paper [1, 3, 4]. Figure 5 showed that the LRC-EC chip device consisted of 256 band-type IDA electrodes (10 fingers, 5 μ m wide, 5 μ m gap) or 256 ring-type IDA electrodes (18 fingers, 5 μ m wide, 5 μ m gap). At the IDA electrodes, deep microwells (50 μ m depth) were placed for trapping 3D culture cells. In the LRC-EC chip device with band-type IDA electrodes, the distance of the center-to-center of the electrochemical sensors was 200 μ m. The density of the electrochemical sensors was the highest in the field of electrochemistry for multi-detection.

The band-type IDA electrodes were used for evaluating small EBs (diameter: less 150 μ m). The ring-type IDA electrodes were used for evaluating large EBs (over 300 μ m). The LRC-EC chip device had 256 sensors and there was an open space on the sensors to introduce and collect EBs easily.

Figure 6 showed that an electrochemical image consisting of 256 pixels was obtained and the small EBs were evaluated successfully through their ALP activity. The electrochemical signals depended on the culture period. Since the size of the EBs increased after culturing the EBs and the interpretation on differentiation degree of the ES cells is complicated, the EBs may be differentiated during the culture.

Figure 7 showed the scheme for preparing large EBs to check their ALP activities and differentiation with microscope observation. We prepared long-term and short-term cultured EBs that were same size, and detected ALP activity by using the LRC-EC chip device containing ring-type IDA electrodes. The ALP activity of the short-term cultured EBs was higher than that of the long-term cultured EBs, indicating that the long-term cultured EBs differentiated. After ALP detection, the EBs were collected and reseeded onto gelatin-coated dishes to culture the EBs for further 3 days. The long-term cultured EBs beat spontaneously while the short-term cultured EBs did not

beat, which also indicated that the long-term cultured EBs differentiated.

In conclusion, the LRC-EC chip device was applied for evaluating EBs. Since electrochemical signals from each of the 256 sensors can be acquired, we believe that the device can provide high-throughput electrochemical assays on EBs.

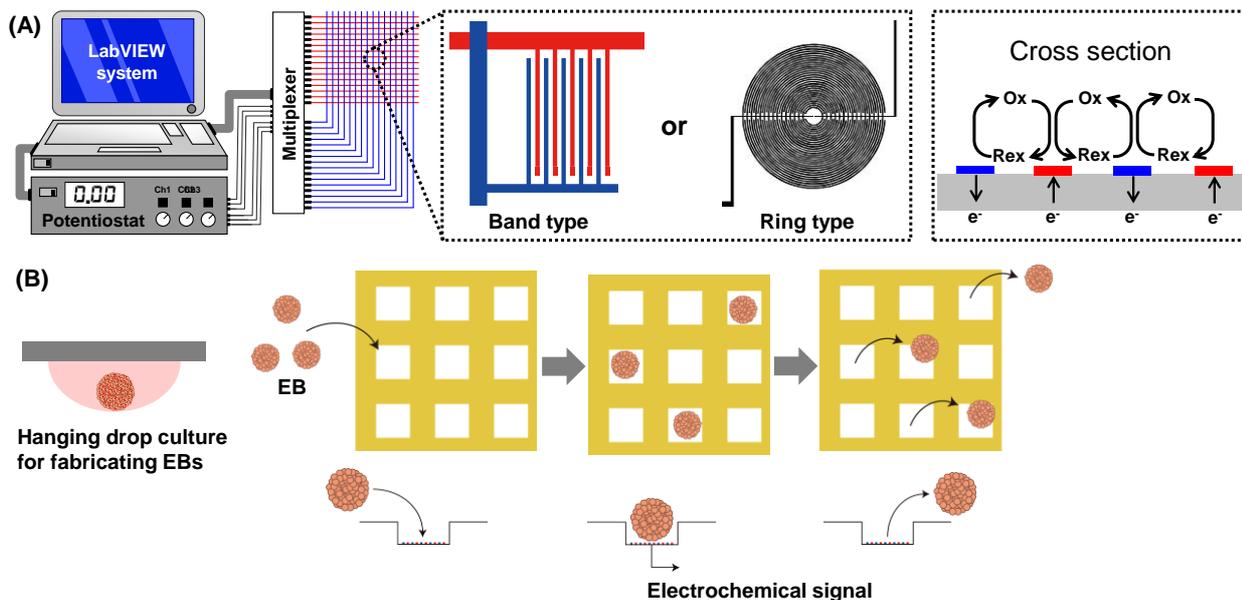


Figure 1. Detection scheme using LRC-EC device. (A) The chip device comprised 16 row and 16 column electrodes to form band or ring-type IDA electrodes at the individual crossing points. A potentiostat was connected to these electrodes through a multiplexer and a PC. Potential at these electrodes and data acquisition were controlled with a LabVIEW program. Local redox cycling was induced only at the desired the IDA electrodes by applying proper potential at electrodes. (B) The EBs were randomly introduced into the device and collected after the electrochemical detection to reset the EBs [3].

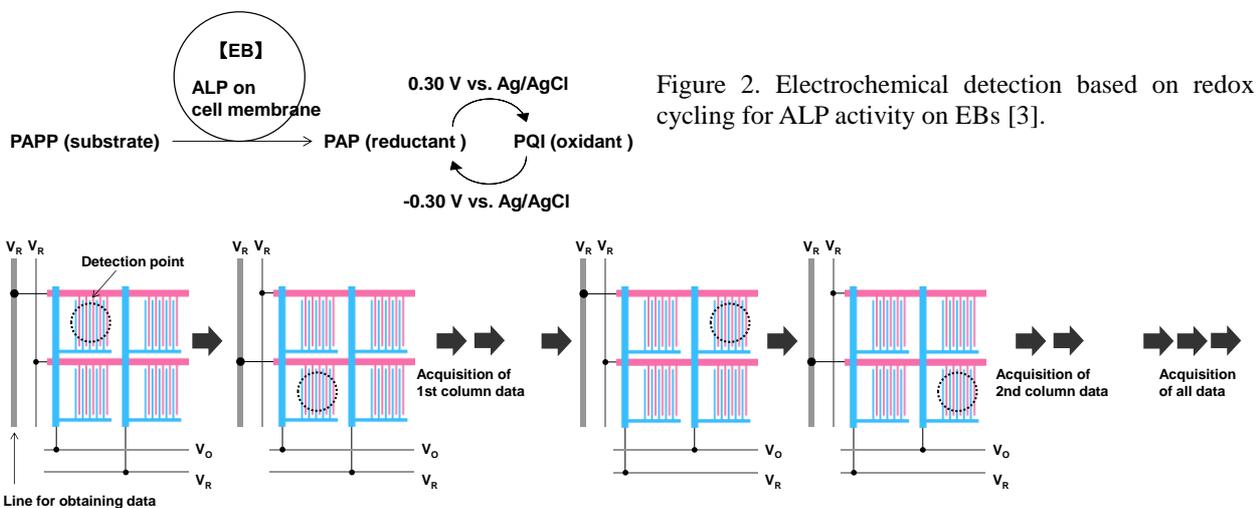


Figure 3. Scheme of the scanning procedure. V_R : Voltage for reducing QI. V_O : Voltage for oxidizing PAP. The detailed scanning process is described in our previous paper [1, 3, 4].

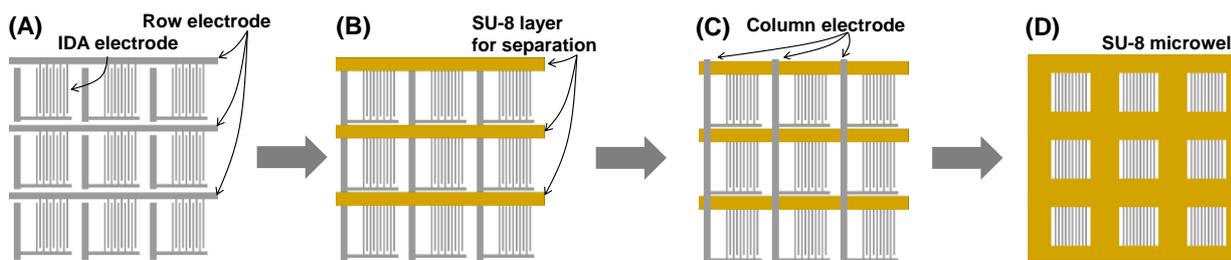


Figure 4. Device fabrication [1, 3]. Pt electrodes for IDAs and row and column electrodes were fabricated with a conventional lithography method. SU-8 layers were then covered on the row electrodes. The column electrodes were fabricated on the SU-8 layers to complete the electrodes. Finally, SU-8 microwells (depth: 50 μm) were fabricated.

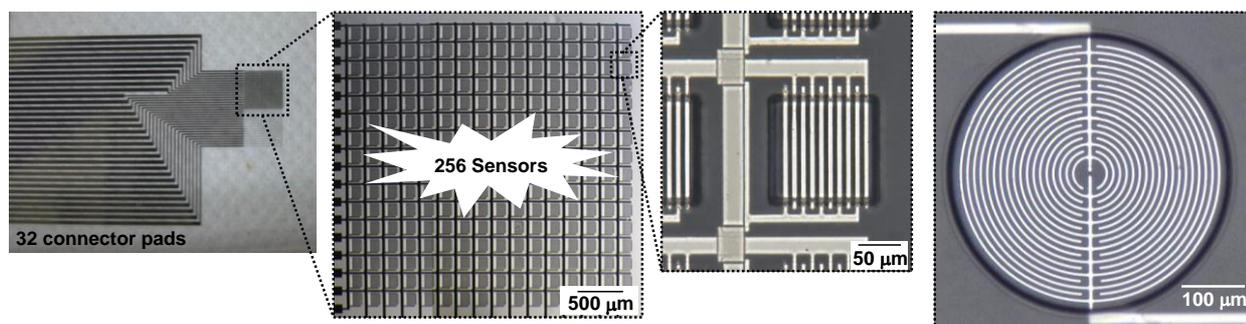


Figure 5. Device images. The device had 256 sensor points with only 32 connector pads. The band or ring-type IDA electrodes were incorporated into the sensor areas [3].

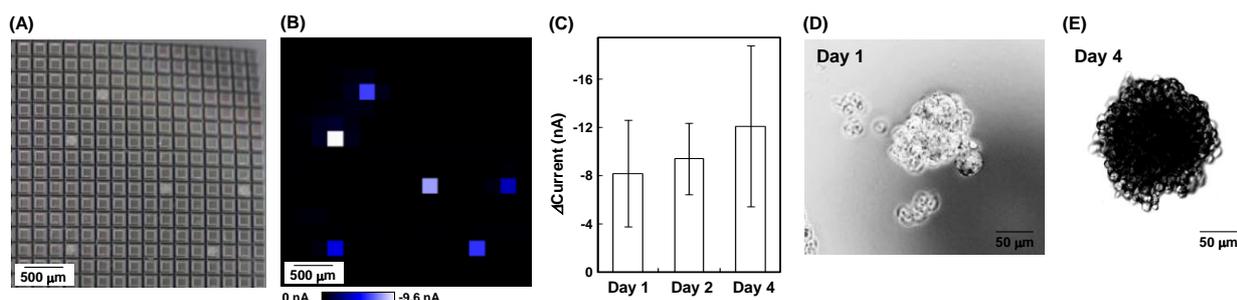


Figure 6. Electrochemical imaging of EBs (diameter: less 150 μm). (A) Optical image. (B) Electrochemical image consisting of 256 pixels. (C) The electrochemical signals were plotted into a graph. (D, E) Optical images of EBs [3].

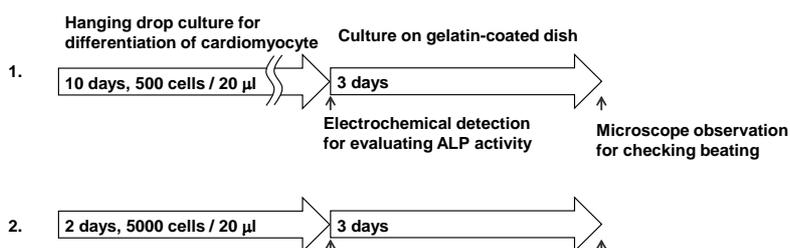


Figure 7. Scheme for preparing large EBs, ALP detection and check of differentiation level.

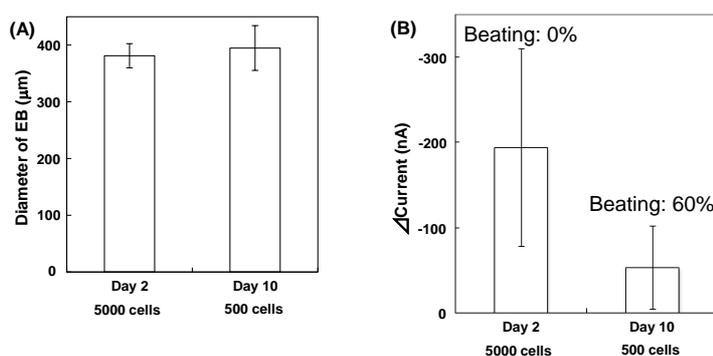


Figure 8. EB evaluation. (A) Size of long-term and short-term cultured EBs. (B) ALP activity on long-term and short-term cultured EBs. After the EB evaluation, the EBs were observed to check whether EBs beat spontaneously.

REFERENCES

- [1] Ino K, Saito W, Koide M, Umemura T, Shiku H, Matsue T. Addressable electrode array device with IDA electrodes for high-throughput detection. *Lab Chip*. 11, 385-8, 2011.
- [2] Takeda M, Shiku H, Ino K, Matsue T. Electrochemical chip integrating scalable ring-ring electrode array to detect secreted alkaline phosphatase. *Analyst*, 136, 4991-6, 2011.
- [3] Ino K, Nishijo T, Arai T, Kanno Y, Takahashi Y, Shiku H, Matsue T. Local redox-cycling-based electrochemical chip device with deep microwells for evaluation of embryoid bodies. *Angew Chem Int Ed*. 51, 6648-52, 2012.
- [4] Ino K, Kanno Y, Nishijo T, Goto T, Arai T, Takahashi Y, Shiku H, Matsue T. Electrochemical detection for dynamic analyses of a redox component in droplets using a local redox cycling-based electrochemical (LRC-EC) chip device. *Chem Commun*, 2012, in press.

CONTACT

Kosuke Ino +81-22-795-7281 or ino.kosuke@bioinfo.che.tohoku.ac.jp