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Electrochemical imaging device consisting of microelectrode arrays to induce local redox cycling for high-throughput cell analyses

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1. Introduction

Several kinds of electrode array devices have been developed for electrochemical imaging to achieve high-throughput bioassays. However, it is difficult to incorporate many individual measurement points into a single chip device because the space for the bond pads is insufficient on the chip border. To solve this problem, we have proposed a novel electrochemical system using local redox cycling, and the system has been designated as local redox cycling-based electrochemical (LRC-EC) system [1-4]. In the system, two arrays of band microelectrodes are arranged orthogonally to prepare an $n \times n$ electrochemical sensors with only $2n$ bond pads for external connection. By using the system, many electrochemical sensors can be incorporated into a single chip device beyond a conventional electrode array device, and comprehensive electrochemical imaging is achieved.

In this study, we applied the LRC-EC system to fabricate an electrochemical imaging device that can perform high-throughput cell analyses, such as detection of stem cell differentiation. Embryonic stem (ES) cells, which can differentiate into any body tissues, are detected. Since the ES cells can develop into cardiomyocytes by forming 3D tissue organs, such as embryoid bodies (EBs), the EBs were introduced into the electrochemical imaging device to evaluate their differentiation. The differentiation degree 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 electrochemical imaging device based on LRC-EC system.

2. Materials and methods

The outline and scheme are shown in Figure 1. Comb interdigitated array (IDA) electrodes were fabricated at individual crossing points to induce local redox cycling. The device fabrication process is described in our previous paper [1, 2].

The scheme for detection of ALP on EBs is described in Figure 2. *p*-Aminopheny phosphate (PAPP) was used as a substrate. PAPP was catalytically hydrolyzed by ALP to convert PAPP into *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 to PAP at the collector electrode (-0.30 V vs. Ag/AgCl). The scanning process is shown in Figure 3 and our previous paper [1, 2].

EBs were formed by using the hanging drop method. Briefly, ES cells were cultured in small droplets to form cell aggregates. The EBs were introduced into the electrochemical imaging device to detect ALP on the EBs, and the EBs were collected from the electrochemical imaging device with a capillary.

3. Results and discussion

We successfully incorporated 256 electrochemical sensors into a small area (Figure 4). The electrochemical imaging device consisted of IDA electrodes (10 fingers, 5 μm wide, 5 μm gap) and deep microwells (50 μm depth). There is an open space on the electrochemical sensors to introduce and collect EBs easily. As compared to conventional electrode array device, many electrochemical sensors can be dramatically incorporated into the electrochemical imaging device (Figure 5).

EB were successfully fabricated by using the hanging drop method (Figure 6). Figure 7 showed that an electrochemical image consisting of 256 pixels was acquired and the EBs were evaluated successfully through their ALP activity. The electrochemical signals depended on the culture period, which indicated that the EBs differentiated after the hanging drop culture. Although the interpretation on differentiation degree of the ES cells is complicated since the electrochemical signals depends on many parameters, the differentiation level of the EBs might be electrochemically evaluated.

4. Conclusions

In conclusion, the electrochemical imaging 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 cell analyses.

Acknowledgements

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References

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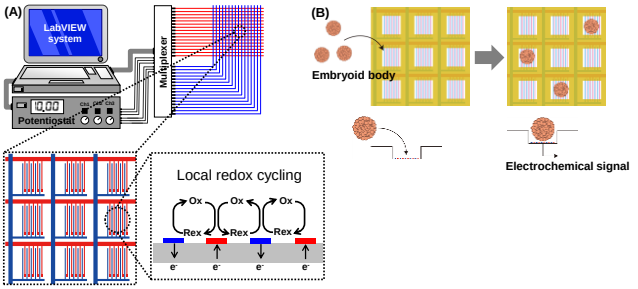

Fig. 1 Detection scheme using the present electrochemical imaging device. (A) The electrochemical imaging device has 16 row and 16 column electrodes to form IDA electrodes at the individual crossing points. The potentiostat is connected to these electrodes through the multiplexer and PC. Local redox cycling is induced only at the targeted IDA electrodes. (B) The EBs are introduced into the device.

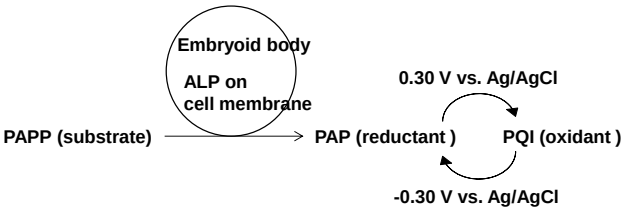

Fig. 2 Redox cycling-based electrochemical detection for ALP activity on EBs.

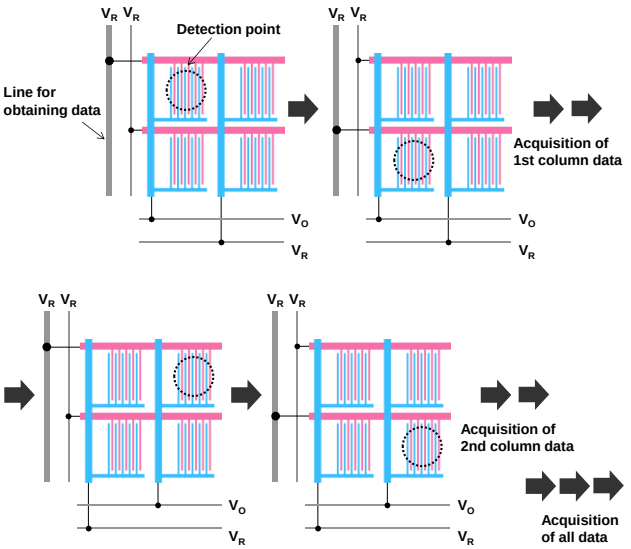

Fig. 3 Scheme of electrochemical imaging. V_R: Voltage for reducing PQI. V_O: Voltage for oxidizing PAP. The detailed detection process is described in our previous paper [1].

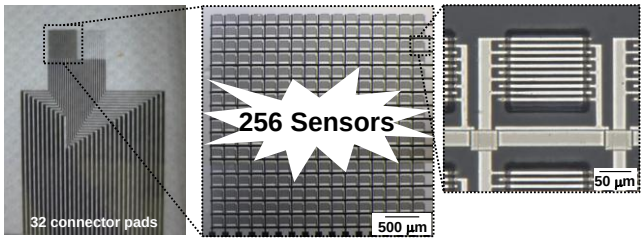

Fig. 4 Optical images of the electrochemical imaging device.

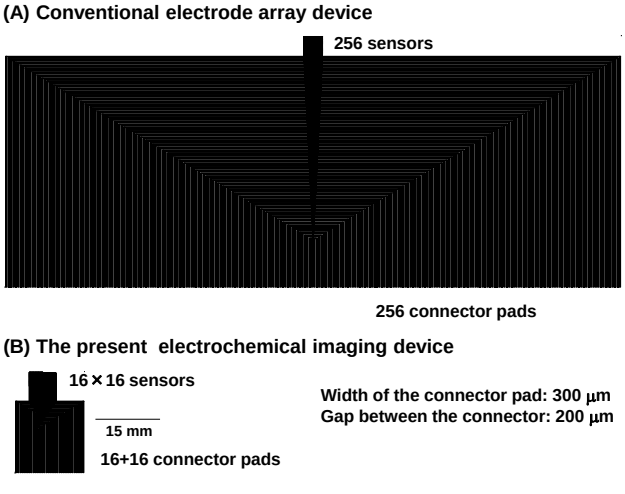

Figure 5 Convectional electrode array device (A) and the present electrochemical imaging device (B).

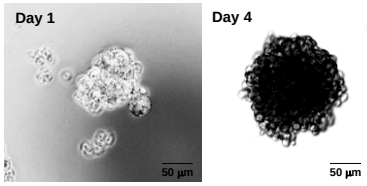

Figure 6 Optical images of EBs.

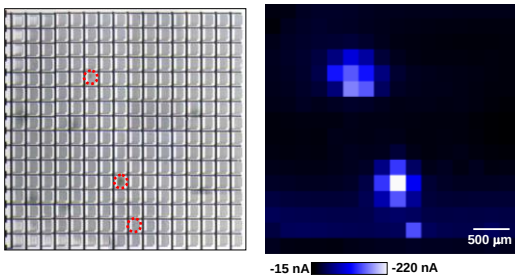

Fig. 7 Electrochemical imaging of EBs. (A) Optical image. (B) Electrochemical image consisting of 256 pixels. White pixels indicates high electrochemical signals.