A high-throughput assay for evaluation of embryoid bodies using local redox cycling-based electrochemical chip device

Kosuke Ino*, Taku Nishijo*, Yusuke Kanno*, Hitoshi Shiku*, Tomokazu Matsue**
*Graduate School of Environmental Studies, Tohoku University, Japan
**Advanced Institute of Materials Research, Tohoku University, Japan
6-6-11, Aramaki, Aoba, Sendai 980-8579, Japan

We have previously developed a local redox cycling-based electrochemical (LRC-EC) chip device for high-throughput electrochemical detection [1-2]. In the LRC-EC chip device, row and column electrodes are arranged orthogonally and these electrodes are connected to interdigitated array (IDA) electrodes to form n² crossing points with only 2n bonding pads for external connection. Local redox cycling can be induced at the desired IDA electrode to acquire electrochemical signals at the IDA electrode. Many electrochemical sensors can be incorporated into a single chip by using the system. In this study, we applied the LRC-EC system to evaluate three-dimensional (3D) culture cells.

It is important to fabricate 3D culture cells since 3D culture cells are similar to natural tissues. Therefore, several kinds of cells are three-dimensionally cultured. For example, embryonic stem (ES) cells, which can differentiate into any body tissues, by forming 3D tissue organs, such as embryoid bodies (EBs) for developing the ES cells into cardiomyocytes. The degree of 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.

The scheme is shown in Figure 1. The device consisted of 256 (16×16) sensors by using 32 (16+16) connector pads. The electrochemical detection was performed after trapping the EBs into the microwells. The scheme for ALP detection using the LRC-EC device is described in our previous paper [1]. Briefly, p-Aminophenol phosphate (pAPP) was used for substrate. pAPP was catalytically hydrolyzed by ALP on the EBs and p-aminophenol (pAP) was yielded. The generator electrode (+0.30 V vs. Ag/AgCl) was used for oxidizing pAP and the oxidation product, p-quinone imine (QI), was then reduced back to pAP at the collector electrode (-0.30 V vs. Ag/AgCl). The scanning process is shown in our previous paper [1].

The device fabrication process is described in our previous paper [1]. The LRC-EC chip device had 256 sensors. Images of the IDA electrodes showed that the width of each electrode finger was approximately 5 μm and the gap between the fingers was approximately 5 μm and the number of the electrode fingers was 10.

Figure 3 showed that an electrochemical image consisting of 256 pixels and the image followed the position of the EBs on the LRC-EC chip device. The intensity was dependent on the ALP activity of the EBs. The electrochemical signals depended on the culture period and the size of the EBs also increased after culturing the EBs. We are interpreting these results for differentiation degree of the ES cells.

In conclusion, the LRC-EC chip device was applied for evaluating EBs. Since comprehensive electrochemical detection can be performed, we believe that the device can provide high-throughput electrochemical assays on EBs.

Figure 1 (A) Illustration of LRC-EC chip device. The potentiostat is connected to the row and column electrodes through the multiplexer and PC. Local redox cycling is induced only at the desired IDA electrodes. (B) Detection scheme.

Figure 2 Device images. (A) The device had 256 sensor points with only 32 connector pads.

Figure 3 Electrochemical imaging of EBs. (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.

References