Single-Cell Analysis

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Electrochemical Gene-Function Analysis for Single Cells with Addressable Microelectrode/Microwell Arrays**

*Zhenyu Lin, Yasufumi Takahashi, Tatsuya Murata, Michiaki Takeda, Kosuke Ino, Hitoshi Shiku, and Tomokazu Matsue**

Cell arrays enable the simultaneous detection of single or grouped cells for the analysis of individual cellular functions under the same experimental conditions, which can greatly reduce experimental error, accelerate detection rates, and help elucidate cell heterogeneity.[1,2] Most cell array systems are based on fluorescence detection methods,[3,4] which usually have high sensitivity and for which many measurement tools are commercially available. An alternative electroanalytical detection method offers substantial advantages, including high compatibility with micro- and nanomachining technologies, system simplicity, small size, reliability, ease of use, and high sensitivity.[5] Among the various systems that are based on electrochemical detection,[6–9] individually addressable electrode arrays have been used recently for simultaneous multianalyte detection.[10–14] But all these methods were realized using individual addressing by connecting each electrode to a corresponding bond pad in the 1:1 mode for performing the electrochemical measurements. However, the integration of a large number of detection electrodes is difficult, since the space for bond pads on the chip border is limited.[15]

We recently communicated the basic principle of a novel multipoint addressable electrochemical device that consists of orthogonally arranged arrays of rows and columns of electrodes.[16] On the basis of redox cycling at the crossing points of the row and column electrodes, electrochemical responses at $n \times n$ crossing points were rapidly detected by using only $2n$ bonding pads for the external connection. Herein, a microwell array was incorporated into the addressable device to conduct high-throughput screening of bioparticles and genetically engineered cells accommodated in the wells. The detection device and system are shown in Figure 1. We demonstrate the rapid electrochemical detection of the reporter protein

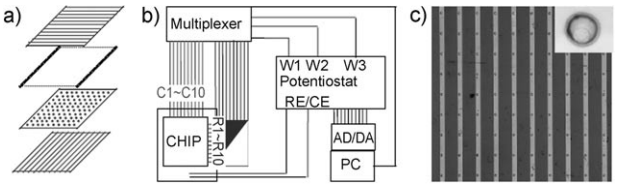

Figure 1. a) Layered structure of the device. Bottom to top: column electrodes (width: 50 μm), 15 μm thick SU-8 photoresist with 100 microwells (diameter: 30 μm), double-sided adhesive paper (thickness: 10 μm), row electrodes (width: 50 μm). b) Detection system of the addressable microelectrode array device. c) Optical photograph of the microwells (diameter: 30 μm) on the column electrodes; inset: magnified photograph of a single well accommodating a single HeLa-pSEAP cell.

(secreted alkaline phosphatase (SEAP)) from a single genetically engineered HeLa cell (HeLa-pSEAP) using the addressable microelectrode/microwell array device. The electrochemical signals initiated by the expressed protein from 10×10 measurement points were acquired within 22 s and displayed on a screen in real time to show the heterogeneity in the expression activity of individual cells.

Alkaline phosphatase (ALP) immobilized on polystyrene beads (ALP beads) or HeLa-pSEAP located in the well can hydrolyze 4-aminophenol phosphate (PAPP) to generate 4-aminophenol (PAP). PAP is oxidized at the row electrode set at 0.30 V to produce *p*-quinoneimine (QI), which can be reduced at the column electrode set at 0.0 V to regenerate PAP. Since the gap between the two electrodes is only 25 μm, redox recycling is expected between the row and column electrodes,[17,18] thereby amplifying the current responses.[19] The concentration of PAP generated in the well can also be determined through the amplitude of the current amplification.[20–22] By sequentially changing the potential applied to the row and column electrodes and the read-out column electrode, the current amplified at each well can be detected and displayed on a screen. It was found that the average reduction current for 100 microwells is proportional to the concentration of PAP in the range from 0.5 to 50 μM. Since the volume of a single microwell is approximately 1.8×10^{-11} L, the detection limit for PAP is found to be as low as 10^{-17} mol.

To test the performance of the established method, ALP beads were settled in the microwells, and the number of beads in each well was counted from a microscope image (Fig-

[*] Dr. Z. Lin, Y. Takahashi, T. Murata, M. Takeda, Dr. K. Ino, Dr. H. Shiku, Prof. T. Matsue
Graduate School of Environmental Studies, Tohoku University
6-6-11 Aoba, Aramaki, Sendai 980-8579 (Japan)
Fax: (+81)22-795-7209
E-mail: matsue@bioinfo.che.tohoku.ac.jp
Homepage: http://www.che.tohoku.ac.jp/~bioinfo/
Dr. Z. Lin
Ministry of Education Key Laboratory of Analysis and Detection Technology for Food Safety
Department of Chemistry, Fuzhou University
Fuzhou, Fujian 350002 (China)

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ure 2a). Then the microwell chip was filled with a 4.0 mM PAPP solution and the substrate was covered with a row electrode array to arrange a microwell at the crossing point of the row and column electrodes. The sequentially obtained

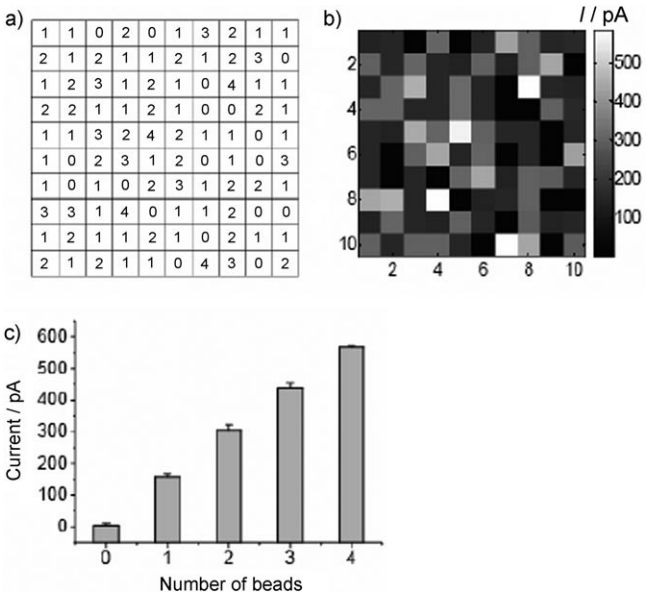

Figure 2. a) Scheme of microbeads in the microwells. The numbers represent the number of beads in each microwell. b) Imaging of the current responses in 10×10 microwells. This image was taken 20 min after the injection of PAPP. c) Relationship between the current responses and number of beads in the microwell.

reduction current for 10×10 wells is displayed in Figure 2b. The magnitude of the response increases linearly with the number of beads in the well, with only a small deviation (Figure 2c). The PAP concentration produced by single ALP beads in the microwell was estimated to be 1.8×10^{-16} mol. Control experiments were also performed to confirm the catalytic effect of the immobilized ALP. When bare beads were set in the wells, no current amplification was detected. These results indicate that the number of ALP-immobilized bioparticles and their catalytic activity can be quantitatively characterized using the addressable microelectrode/microwell array device. The electrochemical image was formed from the response at the column electrode (collector), and therefore the background level was quite low.

HeLa-pSEAP cells that secrete ALP were randomly seeded in the microwells, and the amplified current was detected. The reduction current for microwells with single HeLa-pSEAP cells increased with time, while no meaningful response was detected for empty wells. These results indicate that SEAP secreted from the cell catalyzes the hydrolysis of PAPP to produce PAP, which accumulates in the well.

Figure 3a,b shows an image of the reduction current of each microwell after 20 min incubation. The response in the empty microwells is (0.754 ± 0.996) pA, while in cell-occupied microwells, the response is (16.3 ± 5.49) pA. Figure 3c shows a histogram of the distribution of current responses from the cells; unlike the case for ALP beads, each single HeLa-

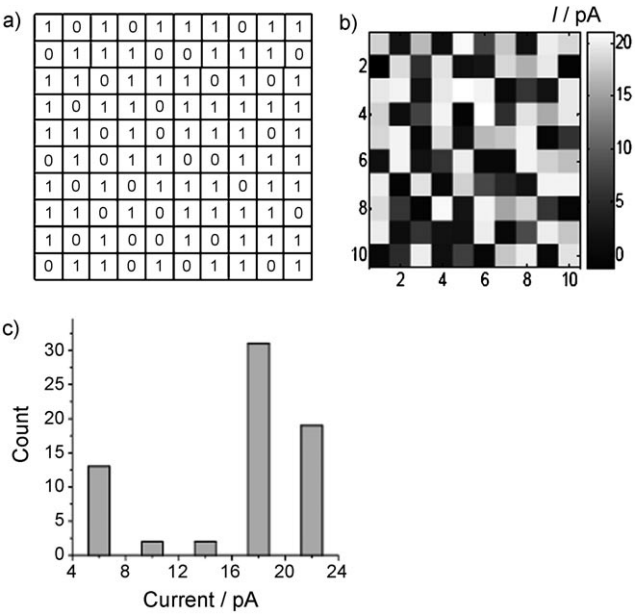

Figure 3. a) Scheme of cells in the microwells. The number represents the number of cells in each well. b) Imaging of the current responses at 10×10 microwells. This image was taken 20 min after the injection of PAPP. c) Histogram of the current response distribution of the cells.

pSEAP cell showed a different current response, and approximately 20% of the cells show responses similar to those observed for wild-type cells. These variations in the response are due to the different expression levels of ALP from individual cells and to the size variations of single cells. The average amount of PAP generated from the catalytic reaction of a single HeLa-pSEAP cell was calculated from the current responses to be 3.6×10^{-17} mol for an incubation time of 20 min. These results demonstrate that the present device can be used for highly sensitive and high-throughput screening to detect the protein expression activity of genetically engineered cells at the single-cell level.

In summary, based on the current amplification arising from redox cycling at the crossing point, the amperometric responses initialized by ALP on beads or secreted from single cells in 100 microwells were detected sequentially in as little as 22 s. Since ALP has been widely used as a labeling enzyme and reporter protein, the present device can be used for the screening of a comprehensive analysis of DNA, proteins, and cells.

Experimental Section

The details about the principle of detection, device fabrication procedures, amperometric scanning procedure, preparation of ALP beads, cell culture, and transfection can be found in the Supporting Information. ALP (Wako Pure Chemical Industries, Ltd. Japan) was physically adsorbed on the polystyrene beads (diameter: 6 μm; Polysciences Inc.). The microwell diameter was optimized to accommodate only a single HeLa-pSEAP cell. The ALP beads and cells settled down into the microwells and on the chip surface by gravitational force. Then, the ALP beads and cells that adhered to

the chip surface were removed by gentle washing with a phosphate-buffered saline solution.

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