ELECTROCHEMICAL DETECTION OF SECRETED ALKALINE PHOSPHATASE (SEAP) FROM TRANSFORMED HELA CELLS USING A LAB-ON-A-CHIP DEVICE BASED ON TARGET CONCENTRATION AND LOCAL REDOX-CYCLING

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ABSTRACT

This paper reports a lab-on-a-chip device having 256 individually addressable multi-detection points for high throughput single cell analysis. The chip is based on local redox-cycling and basically comprises 4 layers one on another. In order to fabricate individually addressable sensor points, two sets of microelectrode bands (row and column) were placed orthogonally to make 256 cross points where the sensor points are located. The chip device was then used to detect secreted proteins from single cells. First, the bottom surface of each detection point was modified with antibodies for the protein of interest and then single cells were captured in microwells at sensor points. The cells were incubated 4 h to secrete enough proteins to be immobilized. After which the cells were removed and the signal from the immobilized proteins were detected and amplified using redox cycling by setting the potential of electrodes to appropriate values. The results indicated that the secreted proteins were successfully detected using the chip device, which could be used for reporter gene assay and detection of some naturally secreted proteins.

KEYWORDS

Lab on a chip, Electrochemistry, Single Cell, Redox cycling, Secreted Alkaline Phosphatase, Biosensing.

INTRODUCTION

In the last decade, the focus of the micro-system research has shifted from electromechanical systems to bio-micromechanical systems and nanotechnology mostly because of the potential applications of micro-systems to biology, chemistry and medicine [1]. Using micro-technologies for biological applications has yielded many areas such as diagnostics, therapeutics, drug delivery and biosensing [2-4]. The incorporation of micro-system technology in biosensing devices provides many advantages like portability, low cost and rapidity due to small sensing area. Miniaturization also enables fabrication of multiple sensor points yielding high-throughput analysis. The most used method in biosensing devices is optical methods, which suffers from undesired fluctuation due to emission from non-target molecules. As an alternative to overcome such a problem, electrochemical methods that provide direct electrical signals have been introduced into biosensing devices. Electrochemical methods are more amenable to integration/miniaturization concepts compared to bulky optical detection setups, which makes them well suited for microdevices.

In this study, a local redox cycling based electrochemical (LRC-EC) chip device was fabricated and used for the detection of proteins secreted by single cells. The chip has 256 individually sensor points with ring-disc (generator-collector) electrode at the bottom of each sensor point. A narrow gap between the two electrodes yields a better redox cycling, hereby increasing the efficiency and the sensitivity of the device. Here, the concept of our previous study was applied to a LRC-EC chip device for a wider and rapid analysis [5]. Basically, the bottom surface of the sensor points was modified with anti-SEAP for immobilization of secreted proteins. Afterward, the secreted alkaline phosphatase transfected HeLa cells were introduced into the microwells placed on each sensor points. The cells were incubated for 4 h to secrete enough secreted alkaline phosphatase (SEAP) to be captured at the bottom of each sensor points and then the cells were removed. Subsequently, the immobilized SEAPs were detected electrochemically using ring-disc electrodes in the absence of the cells. This concept basically enables separation of the secreted proteins and the cells, which eliminates endogenous ALP and protects the cells from any possible harm of the detection conditions (pH 9.5). The results showed that information from single cells can be successfully obtained in 90 s using the device.

EXPERIMENT

The concept used in fabrication of LRC-EC device enables incorporation of n^2 individually addressable sensor points on a chip with 2n connection pads, and is particularly useful for fabrication of a chip having a large number of sensor points in a limited area [6]. In this study, the chip comprises 4 discrete layers. For the fabrication of the first layer, a Ti/Pt layer was sputtered on a piece of glass-slide. An insulator layer was used for the second layer to separate the row and column electrodes. The row electrodes were connected by means of sputtering of another Ti/Pt layer. In the final layer, all the electrodes were insulated along with fabrication of microwells at sensor points for the entrapment of single cell (Fig. 1). This fabrication approach allowed us to individually address each detection point on the chip. After fabrication of the chip, the signal based on varying *p*-aminophenol (PAP) concentration was acquired (Fig. 2). In addition, the collection efficiency and the amplification rate of the chip were determined as 64% and 3.1, respectively using 0.5 mM PAP solution.

In the next step, the chip was prepared for single cell analysis. The bottom surface of each microwell was modified with anti-SEAP using 3-glycidoxypropyltrimethoxysilane (GPTMS) diluted in pure ethanol (1:100) after creating hydroxyl groups on the surface using oxygen plasma. The HeLa cells were transfected with pSEAP2-control

plasmid using Lipofectamine™ 2000 for constant secretion of SEAP. After 24 h incubation, the transformed cells were harvested and introduced into microwells on the sensor points. The cells were incubated 4 h at 37 ^{0}C in microwells filled with medium RPMI-1640 to secrete enough SEAP to be immobilized. Subsequently, the cells were removed and 4.7 mM *p*-aminophenol phosphate (PAPP) in Tris-HCl solution (pH 9.5) was introduced into microwells, which is converted into PAP by the immobilized SEAPs (Fig. 3A). The ring-disk electrodes (generator/collector) were used as working electrodes for the scanning process to induce redox cycling, and the signal was acquired from the disk electrode as a reduction current. Additionally, an Ag/AgCl electrode and Pt wire were used as reference and counter electrodes, respectively. Redox cycling was induced by applying 0.25 V vs. Ag/AgCl through a row (generator) connector to oxidize PAP into *p*-quinoneimine (PQI) and -0.30 V vs. Ag/AgCl through column (collector) connector to reduce PQI back to PAP (Fig. 3B). The electrochemical response from all sensor points was acquired within 90 s. According to the results signal from multiple single cell can be successfully obtained using the LRC-EC device (Fig. 4).

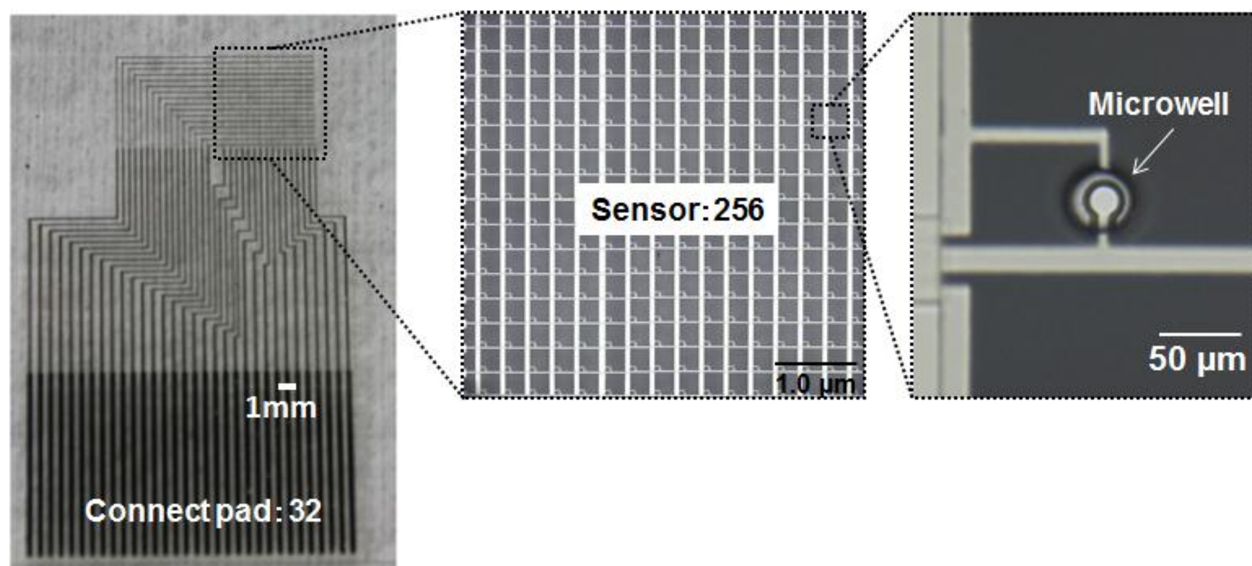

Figure 1. Optical images of the LRC-EC device

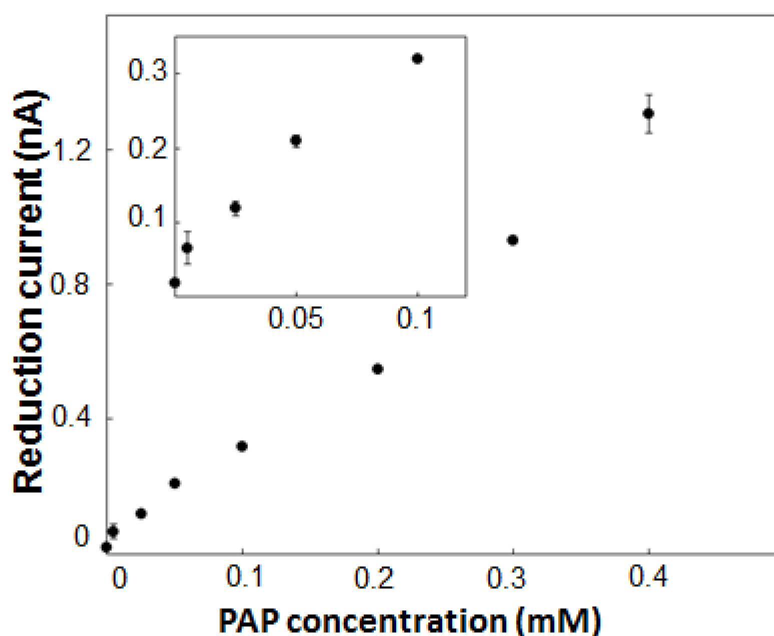

Figure 2. The dependence of electrochemical signal on varying PAP concentration

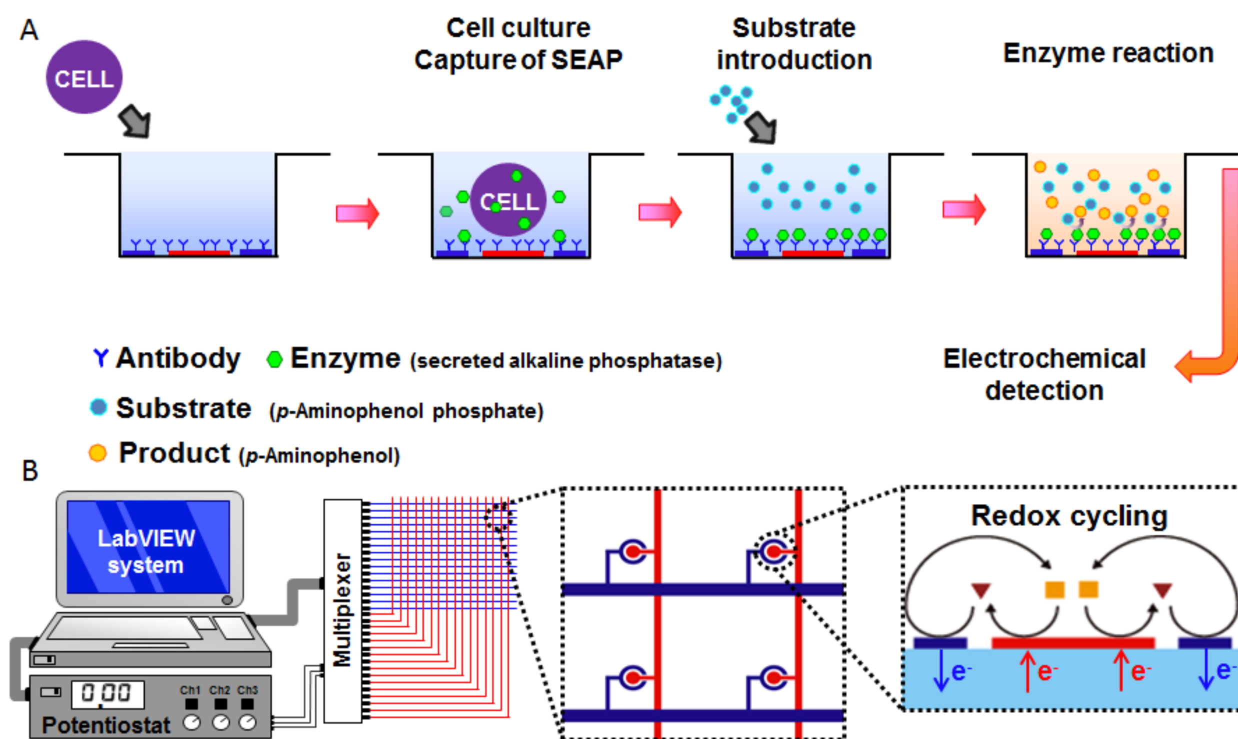

Figure 3. A schematic illustration of the electrochemical assay (A) and using the LRC-EC device (B).

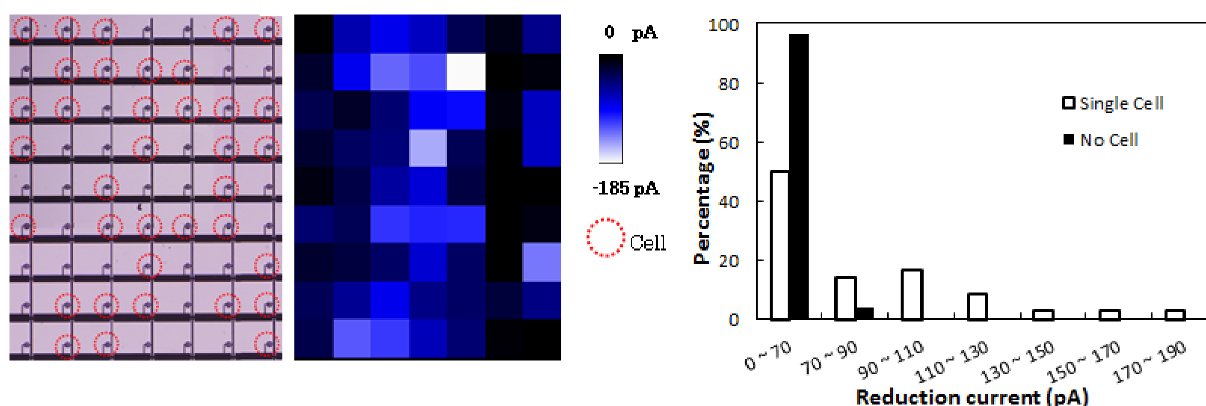

Figure 4. Optical and corresponding electrochemical images. A histogram of the results obtained from the electrochemical image.

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