Microchemistry- and MEMS-based Integrated Electrochemical Devices for Bioassay Applications
Kosuke INO*
Graduate School of Environmental Studies, Tohoku University, 6-6-11-604 Aramaki-Aza-Aoba, Aoba-Ku, Sendai 980-8579, Japan
* Corresponding author: ino.kosuke@bioinfo.che.tohoku.ac.jp

ABSTRACT
Here, we present an overview of our recent research progress in development of electrochemical devices/systems for bioassays. These devices/systems are based on microchemistry and micro-electromechanical systems (MEMS) for sophisticated analytical and electrochemical measurements. In particular, we exploit the unique chemical reactions occurring in micrometer-size spaces and/or involving micrometer-size structures for bioassays, including cell analyses. This paper addresses five topics: probe-, chip-, large-scale-integration chip-, and dielectrophoresis-based devices, and electrodeposition of hydrogels for bioassays.

Keywords: Electrochemical Device, Analytical Electrochemistry, Microchemistry, BioMEMS

1. Introduction
Microchemistry focuses on the unique chemical reactions occurring in micrometer- or submicrometer-size spaces and/or involving micrometer- or submicrometer-size structures. Microchemistry-based technologies are ideally suited for analytical devices, especially microchips and microfluidic devices for chemical and biochemical analyses, because microspaces have several unique beneficial features, such as short diffusion distances, a high surface area-to-volume ratio, and small heat capacity, compared to bulk-scale systems. Development of microchemistry-based devices has progressed rapidly due to advances in microfabrication techniques, and these devices have attracted considerable attention from scientists and engineers. Electrochemical devices incorporating microchemistry-based technologies have also been developed.

Sophisticated electrochemical analytical methods have been developed that incorporate micrometer- and submicrometer-size structures. Several unique features distinguish small-size microelectrodes from larger millimeter-size domains, such as the capability to perform localized measurements with microelectrodes, low double-layer charging currents, low ohmic drops, and rapid mass transport. These characteristics allow for more simplified measurements due to the steady-state conditions, enabling, for example, local detection of redox species, electrochemical measurements in highly resistive media, and monitoring of electrochemical processes over short time domains. In addition, the signal-to-noise ratio can be improved compared to that obtained with a large-size electrode when using a microelectrode under optimal conditions.

The unique features of microelectrodes are useful in many areas of modern electroanalytical chemistry, ranging from bioresearch to trace analysis and energy-related topics. Microelectrodes can be roughly categorized into two types: probe- and chip-based devices with microelectrodes (Fig. 1). Probe-based devices are usually fabricated by coating a fine metal or carbon wire with an insulating material such as glass or a polymer. Chip-based devices are typically fabricated using photolithography-based technologies. Small-size electrodes are also useful for signal amplification. For example, a comb-type interdigitated microarray electrode on a planar device (Fig. 2) provides a unique technology for signal amplification. In an IDA electrode, two sets of electrodes are located in close proximity to one another, a redox species generated at one array diffuses to the other array,
resulting in regeneration of the original species, thus enhancing the electrochemical response. Such reactions have been designated as “redox cycling”. When the arrays are sufficiently small, electrochemical signals can be amplified dramatically. In addition, microelectrode combinations can be used to manipulate microparticles. For example, electrophoresis and dielectrophoresis (DEP) can be induced using microelectrodes, and this technique has been used in biosensors16 and tissue engineering.17–19

The demand for micro-electromechanical systems (MEMS)-related technologies in biological applications such as diagnostics, therapeutics, drug delivery, and biosensing has increased rapidly.20–23 The incorporation of MEMS-related technology in biosensing devices enables the integration and miniaturization of actuators/sensor elements that can be used for many purposes, including analyses of single cells.24,25 Miniaturization of the chip also facilitates the quantification of multiple analytes. For example, compartmentalization of individual samples in microenvironments (such as microwells) is now widely used in high-throughput assays in chemistry and biology research, including techniques for cell analysis.26,27 Thanks to recent advances in the integration and miniaturization of biosensors, it is now possible to create inexpensive, rapid, and responsive biosensors, including sensors with very small sample volume requirements. Furthermore, miniaturized and integrated biosensors enable the detection of signals from multiple small-volume samples, resulting in increased measurement accuracy.28 Electrochemical technologies have been widely integrated into miniaturized biosensors. The incorporation of micromachining and MEMS technologies has facilitated the fabrication of complex microelectrodes on planar substrates. Microstructures (such as microwells) are also useful for electrochemical bioanalysis. For example, a single cell can now be easily trapped in a microwell for subsequent electrochemical analysis (Fig. 3). In this case, a microelectrode is incorporated into each microwell, enabling multi-electrochemical detection. In addition, the ability to accumulate cell secretions in microwells allows for signal amplification.

Based on these technological advances, we have developed integrated electrochemical devices based on microchemistry and MEMS for use in bioassays. This review summarizes our recent research in the development of highly sophisticated electrochemical devices for use in electrochemical assays. The following topics are described in detail: chip-, probe-, large-scale-integration (LSI)-, and DEP-based devices, as well as hydrogel deposition.

2. Chip-based Devices for Electrochemical Bioanalysis

Chip-based devices containing microelectrodes, microwells, and microfluidics are useful for bioanalysis applications. The development of several types of electrochemical devices for bioassay applications, including analyses of yeast and mammalian cells, has been reported.29–44 IDA electrodes were incorporated to amplify signals from cells and provide highly sensitive assays. Yeast cell–based IDA electrodes were utilized for the detection of hormone active chemicals.30 Also, microwell arrays were incorporated into a small chip-based device for trapping single mammalian cells31 and for accumulation of redox active molecules for signal amplification. In addition, an electrode array device for high-throughput bioanalysis and bioimaging applications was developed. Electrode array devices are widely used for cell analyses and DNA arrays in bioimaging and high-throughput bioassay applications. However, it is difficult to incorporate a large number of electrode sensors into a small chip-based device when electrodes are arranged simply. To overcome this difficulty, we developed integrated electrode array devices. Our integrated electrode array devices for high-throughput bioanalysis and bioimaging are described in the following section.

2.1 Electrochemical imaging and high-throughput analysis using a local redox cycling–based electrochemical (LRC-EC) system

Fluorescence detection is widely used in array-based biosensing applications26,27,45 because it generally provides high sensitivity, and the necessary reagents are widely available commercially. However, fluorescence detection does have some disadvantages, such as undesired fluctuations due to quenching or emission from non-target materials, and shielding of the signal in highly turbid solutions. Electrochemical detection has been proposed as an alternative to fluorescence-based assays because electrochemical signals can be acquired inexpensively and rapidly. Furthermore, electrochemical detection systems can be easily miniaturized using conventional microfabrication technologies. Chip-based devices containing electrode arrays have been reported for high-throughput electrochemical detection and electrochemical imaging. These electrochemical array devices provide several important advantages, including rapid response time and the ability to perform both qualitative and quantitative analyses.46,47 Electrochemical sensors are incorporated into theses devices in order to achieve multipoint detection. However, as mentioned in the previous section, it is difficult to incorporate a large number of electrochemical sensors into a small device. To overcome this difficulty, we proposed a novel electrochemical approach based on redox cycling, the local redox cycling–based electrochemical (LRC-EC) system.33–44 In the LRC-EC system, two arrays of electrodes (n rows and m columns of electrodes) are orthogonally placed to produce n * m crossing points (Fig. 4). By applying proper potential at these electrodes, redox cycling can be induced at the desired crossing points, such that the crossing points can be used as individual electrochemical sensors.

![Figure 3. (Color online) General illustration of cell analysis using a simple electrode array.](Image)

![Figure 4. (Color online) Schematic illustration of the LRC-EC system with simple band electrodes. The LRC-EC device consists of n columns and m rows of electrodes. The column electrodes are arranged perpendicular to the row electrodes. A potentiostat is connected to these electrodes through a matrix system, and the instruments are controlled via computer. Redox cycling is induced only at the desired crossing points of the column and row electrodes.](Image)
Therefore, \( n^2 \) electrochemical sensors can be easily incorporated with only 2 \( n \) connector pads to produce a small device containing many electrochemical sensors.

We have designed and fabricated several LRC-EC devices. We first developed an LRC-EC device containing simple band electrodes (Fig. 4).\(^{41-44} \) Although the device enabled high-throughput electrochemical detection, it had to be carefully assembled for each measurement by precisely aligning two different glass substrates with the row or column electrodes, which was time-consuming and led to poor reproducibility. Furthermore, because the sensor areas were surrounded by glass substrates with electrodes, no open space was available for accommodating samples such as cells or cell aggregates. To address these problems, planar comb-type IDA electrodes were incorporated into the LRC-EC device, which allowed all electrodes to be arranged on a single substrate.\(^{34-40} \) One of the LRC-EC devices has 1,024 sensors/\( 41 \text{mm}^2 \),\(^{39} \) making the device well-suited for high-throughput amperometric detection and rapid electrochemical imaging. In addition, vertically separated electrodes were incorporated into the LRC-EC device to further increase the sensor density.\(^{33} \) These LRC-EC devices have been used in enzyme activity analyses, DNA arrays, and imaging of droplet-containing biosamples. Furthermore, the LRC-EC system has been used to detect enzymes secreted from single cells.

Thus, the large number of sensors incorporated in our LRC-EC device enables the simultaneous analysis of a large number of samples on a single chip. The system is also useful for gene reporter assays and for the detection of a variety of secreted proteins. In addition, the system can be used to measure the alkaline phosphatase (ALP) activity of embryonic stem (ES) cells in the evaluation of cell differentiation (Fig. 5).\(^{35,36} \) Therefore, the proposed LRC-EC system is useful for cell screening in tissue engineering and is ideal for high-throughput biosample assays and imaging applications.

3. Probe-based Devices for Bioanalysis

A probe-based device has been developed for scanning electrochemical microscopy (SECM). In SECM, a micro- or nano-electrode is scanned across the sample surface to induce chemical changes and collect electrochemical information.\(^{45-50} \) SECM is a powerful tool for studying structures and processes in micrometer- and submicrometer-size systems because it enables probing of electron, ion, and molecular transfers and other reactions at solid-liquid, liquid-liquid, and liquid-air interfaces,\(^{51} \) thus permitting a diverse range of applications, from analysis of metal corrosion to examination of cells.

We reported the development of several types of probe-electrode systems for bioanalysis.\(^{52-58} \) For instance, we fabricated a probe consisting of a carbon nanoelectrode and Ag/AgCl reference electrode (carbon-Ag/AgCl probe) for use in SECM.\(^{56} \) Because the tip of the carbon-Ag/AgCl probe is very small, it is useful for electrochemical analyses in microenvironments. Furthermore, we proposed a new electrochemical assay involving the combination of SECM and microwell arrays for the detection of proteins secreted by cultured cells (Fig. 6).\(^{57} \) This assay allowed the detection of protein secretion at the single-cell level, demonstrating the usefulness of the system for single-cell analyses.

4. LSI-based Chip Devices for Bioanalysis

As described above, it is difficult to incorporate a large number of sensors in an addressable microelectrode array. To overcome this problem and incorporate more sensors into a chip-based device, semiconductor technology has been employed.\(^{59-68} \) For instance, Lindau and colleagues reported the development of a complementary metal oxide semiconductor (CMOS)-based chip containing 10 \( \times \) 10 microelectrodes for use in SECM.\(^{68} \) Because the tip of the carbon-Ag/AgCl probe is very small, it is useful for electrochemical analyses in microenvironments. Furthermore, we proposed a new electrochemical assay involving the combination of SECM and microwell arrays for the detection of proteins secreted by cultured cells (Fig. 6).\(^{57} \) This assay allowed the detection of protein secretion at the single-cell level, demonstrating the usefulness of the system for single-cell analyses.
cell. The Bio-LSI chip has a dynamic range of 1 pA to 100 nA, with lower electronic noise compared with other CMOS-based sensors. The Bio-LSI chip uses 400 sensors to produce an electrochemical image that can be acquired within 200 ms. We have used the device for real-time monitoring of the activities of enzymes and cells (Fig. 7B).70,71 The Bio-LSI device has also been used for time-course electrochemical analyses, demonstrating the usefulness of the device for real-time electrochemical imaging of biosamples.

5. DEP-based Devices for Bioanalysis

Several rapid and simple methods for manipulating bioparticles on chips are used in the fabrication of biosensors and in tissue engineering.18,19 Array patterns of microparticles or cells have been utilized in the design of biosensors, as scaffolds for patterning proteins, and in cell cultivation. In recent years, various techniques for manipulating bioparticles on chips have been developed using hydrodynamics,1,73 magnetic forces,74–80 and DEP.5,17,21–25 DEP was first described by Pohl as the motion of dielectric particles under the influence of a non-uniform electric field.56 DEP techniques can be roughly categorized into three types, according to the way the electric field interacts with the microparticles: positive DEP (pDEP), negative DEP (nDEP), and a combination of the two. Both pDEP and nDEP can be used to manipulate microparticles toward the direction of stronger and weaker electric fields, respectively. As DEP can be used to manipulate several types of particles, including non-charged particles, DEP techniques are often used for separating, concentrating, or aligning target particles.81–85 For these purposes, the electrode design is very important.

We developed several electrode devices for manipulating microparticles using DEP.87–89 We reported the development of a novel DEP-based chip device containing IDA electrodes (DEPIDA) for producing a cell culture array with paired cells.97 The chip-based device consists of an array of 900 gourd-shaped microwells and IDA electrodes. Different types of cells are trapped sequentially in the microwells by inducing pDEP, resulting in pairing of single cells of different types. The ability of the device to pair a large number of cells easily and rapidly makes it a highly attractive tool for controllable cell pairing in a range of biological applications (Fig. 8).
6. Hydrogel Deposition Using an Electrochemical Approach\textsuperscript{90,91}

Hydrogels, such as alginate hydrogel, have attracted considerable attention for use in biosensing and tissue engineering applications. In the presence of Ca\textsuperscript{2+}, alginate forms a gel that can subsequently be used to entrap and immobilize proteins and cells. Cheng et al. demonstrated an excellent method for fabricating alginate gels using anodic electrodeposition.\textsuperscript{92,93} In their study, electrochemical acidification (formation of H\textsuperscript{+}) with the accompanying release of Ca\textsuperscript{2+} was employed to deposit calcium alginate on the electrode surface.

Based on the work of Cheng et al., we employed the method for preparing 3D tissue structures of tubular hydrogels by anodic electrodeposition of calcium alginate.\textsuperscript{91} The method involves electrodeposition of hydrogel around a wire electrode. The general architecture is shown in Fig. 9. Deposition can be achieved by simply applying potentials, with no requirements for specific chemicals, equipment, or sophisticated techniques.

We also developed a novel method for fabricating microwell arrays constructed of alginate gels for 3D cell culture.\textsuperscript{90} The alginate gel microwells were fabricated on a patterned electrode. ES cells or hepatocellular carcinoma cells (HepG2) were cultured in alginate gel microwells containing 3T3 cells. During culture, embryoid bodies (EBs) of ES cells or HepG2 spheroids are formed in the alginate gel microwells (Fig. 9). In general, hydrogel microwell arrays are fabricated using molds made of SU-8 or PDMS.\textsuperscript{94,95} However, the procedures are not suitable for the fabrication of fragile hydrogels such as alginate gel because they can be easily broken when the molds are removed. In contrast, our method is ideal for fabricating fragile hydrogels because it requires no mold. Thus, the method is well-suited for use in tissue engineering applications.

7. Conclusions

This article presents an overview of recent progress made by my research laboratory in the development of microchemistry- and MEMS-based electrochemical devices/systems for use in bioassays. Our results demonstrate that these electrochemical devices/systems are ideal for use in various areas of analytical electrochemistry research.
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