Fabrication and Characterization of Cellular Devices for Electrochemical Biosensing

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The cell-based assays have been employed for studying cellular functions such as metabolism and gene expression, and recognized as an effective way in high-throughput screening for drug research. There have been various methods for monitoring the physiology changes of cells and microorganisms, e.g., the rates of uptake of glucose and oxygen, the production of heat, pH change, and enzymatic redox activity. We and other groups have recently applied scanning electrochemical microscopy (SECM) as a noninvasive monitoring of the cellular status, in which the tip of a microelectrode is scanned near the cells to map the local distribution of electroactive species such as oxygen. We report here our recent progress of fabrication and characterization of cellular devices using SECM systems.

Micropatterns of mammalian cells (HeLa cells, heart muscle cells, neuronal cells) were prepared on glass substrates, and the respiration of the patterned cells was studied by SECM [1]. The cellular patterns on a μm scale were prepared by microcontact printing of an extracellular matrix protein, such as fibronectin and laminin, onto a hydrophobic glass plate. The oxygen concentration in the vicinity of the patterned cells was mapped by SECM, indicating that that the cells in patterns were living with the uptake of oxygen. The respiratory activity of the collagen-embedded living cells can also be imaged by SECM [2]. Two cancer cells, the human erythroleukemia cell line (K562) and its adriamycin-resistant subline (K562/ADM) were immobilized at the array of micro-holes micromachined on a silicon wafer for the comparative characterization in their sensitivity to the anticancer drug, ADM. The results obtained by the SECM method showed correspondence to a conventional colorimetric assay (SDI assay).

The SECM-based assay was applied to cancer cells isolated from xenografts implanted into severe combined immunodeficiency (SCID) mice, as a model of a human tumor [3]. The assessment was completed under the condition of physiological drug concentrations, which is pharmacokinetically equivalent to anticancer drug plasma concentrations. Human promyelocytic leukemia (HL-60) cells were subcutaneously inoculated in SCID mice, and removed 31 days after the inoculation. The cells were embedded in a small volume (18 nL) of a collagen-gel matrix on a pyramid-shaped silicon microstructure for further cultivation. The respiration activity of the cells on the chip was measured by SECM. The proliferation behavior was continuously monitored for 6 days. It appeared that the proliferation rate of the cells removed from the mice was lower than that cultured in a flask and conformed to that in mice. The effects of cisplatin (CDDP) and etoposide (VP-16) on the HL-60 cultured in vivo were in good agreement with those obtained by a conventional colorimetric assay. Our results suggest that the SECM-based assay is appropriate for biopsy specimens in a relatively short-time evaluation. As an extension of the assay format, we fabricated a three-dimensional cell culture system with an array of cell panels (4 × 5) in a silicon chip, together with multi-channel drug containers. Human breast cancer (MCF-7) cells were embedded in a collagen-gel matrix and entrapped in a pyramidal-shaped silicon hole. Each cell panel can be isolated by a channel composed of a microfluid part and a reservoir. A cell panel was exposed to 200 mM KCN for 2 days to demonstrate that each cell panel could be independently evaluated under various stimulation conditions. Based on the cellular respiration activity, the proliferation behavior was continuously monitored on the silicon-based cell array for 5 days using SECM. The cells entrapped in the device (3-D culture) proliferated normally, and the proliferation rate was lower than that of cells grown in a monolayer cell culture (2-D culture). The effects of three anticancer drugs measured simultaneously on the cell chip were in good agreement with those obtained by a conventional colorimetric assay.

Similarly, a microbial chip for bioassay was fabricated and its performance was characterized by SECM [4]. A microbial chip was fabricated by filling the micropores on a glass substrate with collagen-embedded Escherichia coli (E.coli) cells, and characterized by SECM in a solution containing ferricyanide. The activity of the E.coli cells in the collagen gel microstructure was imaged and characterized with SECM by mapping the localized concentration of ferrocyanide produced by the respiration of the cells. The SECM-based activity measurement detected as low as approximately 100 E.coli cells. Furthermore, the optical-microscopic observation indicated that the E.coli cells on the chip proliferated during the incubation. The sequential SECM measurements were performed for the same E.coli chip to obtain the microbial growth curve for a small number of microorganisms.

We also detect an expression of a reporter-gene [5], lacZ encoding the Escherichia coli (E. coli) enzyme β–galactosidase,on a microbial chip based on a redox reaction. The activity of the expressed β-galactosidase can be determined by using p-aminophenyl β–D-galactopyranoside (PAPG) as a substrate. The product, p-aminophenol (PAP), can be oxidized by the electrode reaction. In the present study, we investigated β-galactosidase expression in a small number of E. coli cells by utilizing an electrochemical microdevice with on-chip incubation and on-chip monitoring. In the presence of IPTG, the oxidation current starts to increase at 2 h, while almost no response is observed without IPTG, indicating expression of galactosidase in the immobilized E. coli cells. Similarly, a microbial chip for mutagen screening can also be fabricated by immobilizing genetically-engineered microorganisms sensitive to carcinogen.

References

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