비수식화 바이오칩 및 유전자 검출

최 용성, 이 경섭
동신대학교 전기공학과

Genome Detection Using an DNA Chip Array and Non-labeling DNA

Yong-Sung Choi, and Kyung-Sup Lee
Department of Electrical Engineering, DongShin University

Abstract : This research aims to develop the multiple channel electrochemical DNA chip using microfabrication technology. At first, we fabricated a high integration type DNA chip array by lithography technology. Several probe DNAs consisting of thiol group at their 5-end were immobilized on the gold electrodes. Then target DNAs were hybridized and reacted. Cyclic voltammetry showed a difference between target DNA and control DNA in the anodic peak current values. Therefore, it is able to detect a plural genes electrochemically after immobilization of a plural probe DNA and hybridization of non-labeling target DNA on the electrodes simultaneously. It suggested that this DNA chip could recognize the sequence specific genes.

Key Words : Multi-channel, DNA chip, Microfabrication technology, Lithography technology, Target DNA

1. Introduction

The detection of a sequence-specific gene is of great significance in the biomedical field. Biosensor using DNA as the biosensing element is called DNA sensor, and integrated DNA sensor is particularly called DNA chip or DNA microarray. DNA microarray technology using photolithography or stamping methods enables simultaneous analysis of thousands of sequences of DNA for genetic and genomic diagnostics and gene expression monitoring. Affymetrix[1], [2] has developed GeneChip® using photolithography technique.

This research aims to develop the multiple channel electrochemical DNA chip that has the above characteristic and be able to solve the problems. At first, we fabricated a high integration type DNA chip array by lithography technology. It is able to detect a plural genes electrochemically after immobilization of a plural probe DNA and hybridization of non-labeling target DNA on the electrodes simultaneously.

2. Experimental

2.1 Fabrication of Microelectrode Array

About 100nm gold layer was deposited over a 20nm aluminum adhesion layer on a glass chip by vacuum evaporation. Next, the chip was spin-coated with photoresist and was irradiated with UV light. Each metal layer was etched to form electrodes, lead wires, and their connections. The lead wires were photolithographically covered with photoresist for insulation. Over 300 individually addressable gold electrodes (electrode diameter : 100㎛~1mm) were arranged on the chip. Each microelectrode was connected to an external potentiostat by insulated gold track. Probe DNAs consisting of thiol group at their 5-end were spotted on the gold electrode using micropipette and allowed to react at 5℃ for 24 hr. utilizing the affinity between gold and sulfur. The immobilized probe DNA on the gold electrodes was confirmed by cyclic voltammetry in 100mM KCl solution at 50mV/s.

2.2 Electrochemical Gene Detection

Target DNA (complementary), negative control DNA, control DNA or mismatch DNA was hybridized at 5℃ for 24 hr.. After washing the electrodes, electrochemical signals derived from the gold layer were measured by Cyclic-Voltammetry. DNA prevents a redox response of Au ion after immobilization on the Au surface.

3. Results and Discussion

Figure 1 (a) shows cyclic voltammograms (CV) in 100mM KCl solution with a bare electrode and a probe modified electrode (ss (single strabd)-DNA- electrode). The redox peak currents of gold could be observed and the peak decreased when the ss-DNA-electrode was immobilized compared with that of the bare electrode. This result shows that the DNA probe is immobilized on the gold electrode through the thiol group at the 5

end. The same results were obtained from Poly dT dG, dC probe DNAs and p-72 probe DNA.

Fig. 1 (b) shows cyclic-voltammograms of target DNA (complementary), negative control DNA or mismatched DNA on probe-modified electrodes reacted with 50μM target DNA and control DNA at 50mV/s. When the probe-modified electrodes were reacted with 50μM target DNA (Poly dT), the anodic peak current values was decreased to about 20nA. On the other hand, the anodic peak current values was same with the probe-modified electrodes when the electrodes were reacted with 50μM negative control DNA (Poly dA) and mismatched DNA. It is considered that the decreased current value is derived from the gold electrode surface due to hybridization. These results suggest that the microelectrode array specifically detected target DNA.

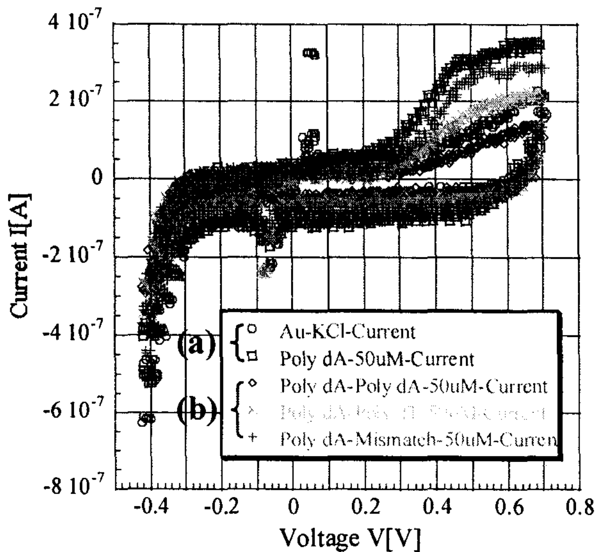

Figure 1. Cyclic-Voltamogra of Poly dA in KCl solution (sweep rate: 50mV/s, 25℃).

Figure 2 shows concentration dependence of target DNA, negative control DNA and mismatch DNA against probe DNA from 50μM to 1aM. When target DNA was detected using cyclic-voltammetry, there was a difference and decreased according to the concentration of target DNA in the anodic peak current values. However, almost no difference in the anodic peak current values was observed for negative control DNA and mismatched DNA. The result suggests the DNA microarray can detect the target DNA almost quantitatively. The same results were obtained from Poly dT dG, dC probe DNAs and p-72 probe DNA. However, the slope of Poly dC and p-72 probe DNAs were reverse due to guanine.

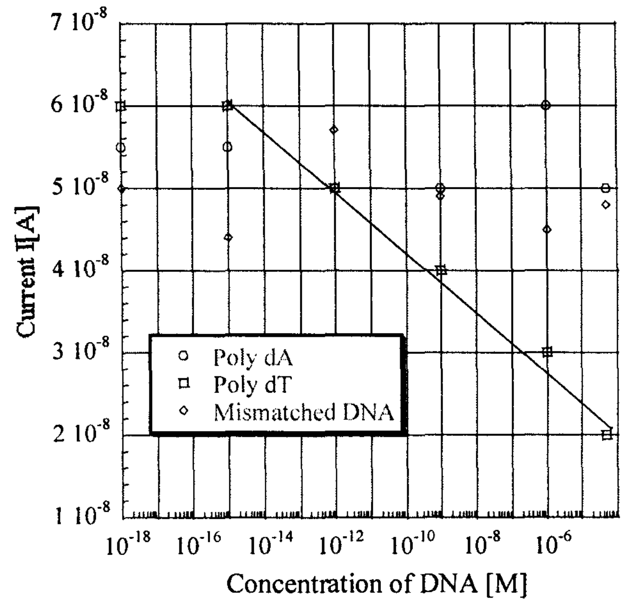

Figure 2. Concentration dependence of target DNA, negative control DNA and mismatch DNA against probe DNA.

4. Conclusions

In this study, an integration type microelectrode array was fabricated on slide glass using microfabrication technology. Probe DNAs consisting of thiol group at their 5-end were spotted on the gold electrode using micropipette utilizing the affinity between gold and sulfur. Cyclic-voltammetry in 100mM KCl solution at 50mV/s confirmed the immobilization of probe DNA on the gold electrodes.

When several DNAs were detected electrochemically, there was a difference between target DNA and control DNA in the anodic peak current values. It was derived from Au ion due to hybridization of target DNA. The detection sensitivity was fM or aM.

감사의 글

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[참 고 문 헌]

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