집적형 DNA칩 미소 전극 어레이 및 비수식화 표적 DNA를 이용한 유전자 검출

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Genome Detection Using an Integrated type DNA Chip Microelectrode-array and Non-labeling Target DNA

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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.

1. Instruction

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.

Conventional DNA chip systems employ conforcal fluorescence detection for highly sensitive imaging with high resolution. It detects more than ten thousands of unique oligonucleotides in several square centimeters. Target DNAs are labeled with fluorescent dyes and hybridized with complimental probe on the chip. Fluorescent detection style DNA chips and microarray scanners are too expensive to use only a part of research institute or large hospital.

On the other hand, as for electrochemical measurement method, there are the advantage such as the low cost of an analysis device, the simplification of the whole equipment and the analysis time, and the development to the portable DNA chip in comparison with fluorescence measurement method, and the research is carried out[3]. It is detecting with an indicator-free method, whether or not these introduce intercalator that reacts to DNA specifically, or redox material is modified to probe DNA or target DNA mainly[4]. There are the problems that should be guanine (G) in a base pair, whether or not these method introduce intercalator that reacts to DNA specifically, or redox material is modified to probe or target DNA[5].

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 Materials and Instrumentation

Poly dA, dT dG, dC probe DNAs (50-mer deoxyoligo- nucleotide acid) and p-72 probe DNA (5`-HS-AGGCT GCTCC CCCCG TGGCC-3`) having thiol group at 5` end and these target DNAs, which was complementary to the probe, were synthesized and purified. 0.5$\mu\ell$ micropipette was used to immobilize probes DNAs.

The electrochemical measurements were carried out using an electrochemical analyzer manufactured by Bioanalytical Systems, Model BS-1 and a computer system with data storage. Cyclic-voltammetric experiments were carried out in a Teflon cell including a platinum wire as counter electrode and Ag as reference.

2.2 Fabrication of Microelectrode Array

Figure 1 shows fabrication process 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.

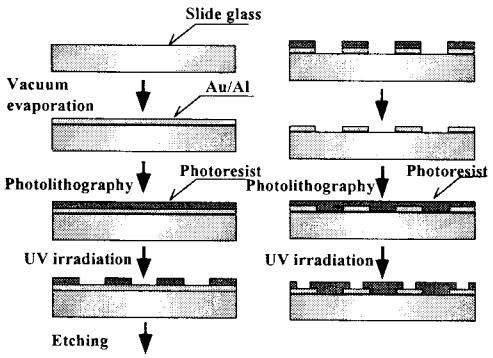

Figure 1. Fabrication process of microelectrode array for DNA chip.

2.3 Electrochemical Gene Detection

Figure 2 shows principle of microelectronic array DNA chip using electrochemical response. 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.

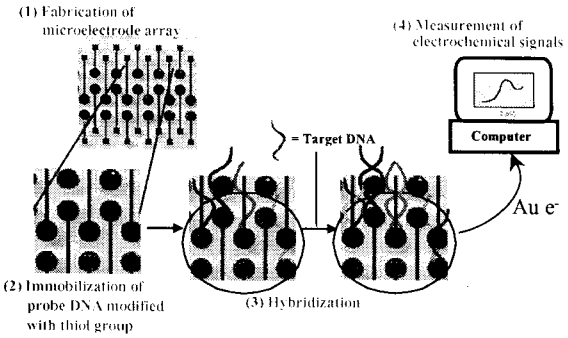

Figure 2. Principle of microelectronic array DNA chip using electrochemical response.

3. Results and Discussion

Figure 3 (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. 3 (b) shows cyclic-voltammograms of target DNA (complementary), negative control DNA or mismatched DNA on probe-modified electrodes reacted with 50㎛M target DNA and control DNA at 50mV/s. When the probe-modified electrodes were reacted with 50 ㎛M 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 50㎛M 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.

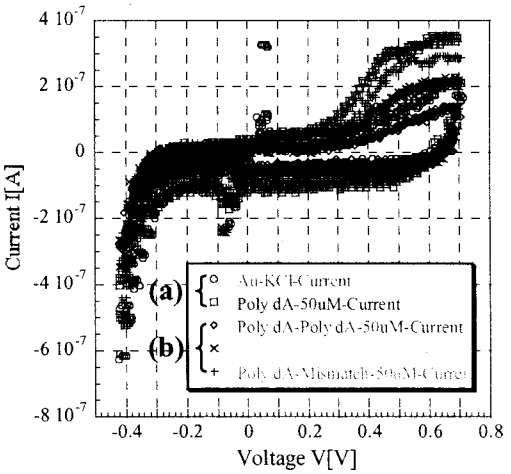

Figure 3. Cyclic-Voltamogra of Poly dA in KCl solution (sweep rate: 50mV/s, 25℃).

Figure 4 shows concentration dependence of target DNA, negative control DNA and mismatch DNA against probe DNA from 50㎛M 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.

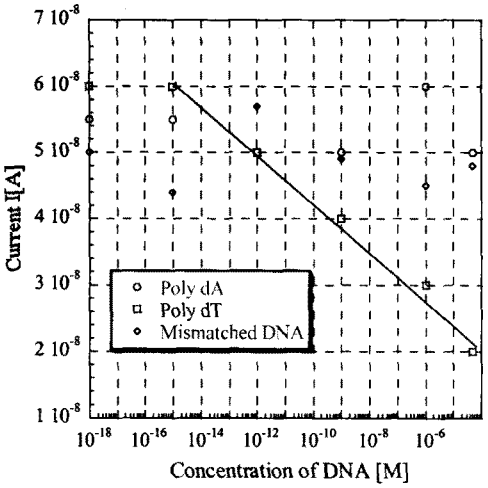

Figure 4. Concentration dependence of target DNA, negative control DNA and mismatch DNA against probe DNA.

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.

These results suggest that target DNA can be detected specifically by using this microelectrode array. In principle, the method requires no labeling of target DNA. This feature provides simple pretreatment of target DNA.

Single channel electrochemical biosensor was already commercialized and its size was reduced to palm-top size. Some electrode arrays of more than several hundred channels were already developed and their size was also miniaturized. It suggested that multichannel electrochemical DNA microarray is useful to develop a portable device for clinical gene diagnostic system.

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