

차세대형 바이오칩의 개발 및 비수식화 표적 DNA를 이용한 유전자 검출

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Development of New Biochip and Genome Detection Using an Non-labeling Target DNA

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Abstract - This research aims to develop a multiple channel electrochemical DNA chip using micro-fabrication technology. At first, we fabricated a high integrated 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 by an electrical force. Redox peak of cyclic-voltammogram showed a difference between target DNA and mismatched DNA in the anodic peak current. Therefore, it is able to detect a various genes electrochemically after immobilization of a various probe DNA and hybridization of label-free DNA on the electrodes simultaneously. It suggested that this DNA chip could recognize the sequence specific genes.

1. Instruction

It is widely believed that thousands of genes and their products (i.e., RNA and proteins) in a given living organism function in a complicated and orchestrated way that creates the mystery of life. However, traditional methods in molecular biology generally work on a "one gene in one experiment" basis, which means that the throughput is very limited and the "whole picture" of gene function is hard to obtain. In the past several years, a new technology, called DNA microarray, has attracted tremendous interests among biologists. This technology promises to monitor the whole genome on a single chip so that researchers can have a better picture of the interactions among thousands of genes simultaneously.

Base-pairing (i.e., A-T and G-C for DNA; A-U and G-C for RNA) or hybridization is the underlining principle of DNA microarray.

An array is an orderly arrangement of samples. It provides a medium for matching known and unknown DNA samples based on base-pairing rules and automating the process of identifying the unknowns. An array experiment can make use of common assay systems such as

microplates or standard blotting membranes⁽¹⁾,⁽²⁾, and can be created by hand or make use of robotics to deposit the sample. In general, arrays are described as macroarrays or microarrays, the difference being the size of the sample spots. Macroarrays contain sample spot sizes of about 300 microns or larger and can be easily imaged by existing gel and blot scanners. The sample spot sizes in microarray are typically less than 200 microns in diameter and these arrays usually contains thousands of spots. Microarrays require specialized robotics and imaging equipment that generally are not commercially available as a complete system.

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 DNA, 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⁾.

Therefore, this research aims to develop the multi-channel type label-free DNA chip that has the above characteristics and be able to solve the problems. At first, we fabricated a high integrated type DNA chip array by lithography technology. It is able to detect a various genes electrochemically after immobilization of a various probe DNA and hybridization of label-free target DNA on the electrodes simultaneously.

2. Experimental

2.1 Materials and Instrumentation

SH-p72 (5'-HS-AGGCT GCTCC CCCC GGGCC-3')

MW: 6207.3, T_m : 80.5°C), SH-m72 (5'-HS-AAGCT GCTCC CCCC G TGGCC-3'; MW: 6191.3, T_m : 78.5°C) and SH-R72 (5'-HS-AGGCT GCTCC CCGCG TGGCC-3'; MW: 6247.3, T_m : 80.5°C) probe DNA having thiol group at 5' end and these target DNAs (p72: 3'-TCCGA CGAGG GGGGC ACCGG-SH-5', m72: 3'-TTCGA CGAG G GGGGC ACCGG-5' and R72: 3'-TCCGA CGAGG GCGC ACCGG-5'), which was complementary to the probe, were synthesized and purified. 1.0 μ l micropipette was used to immobilize probes DNAs.

The electrochemical measurements were carried out using an electrochemical analyzer manufactured by CH Instruments, Inc., Model 1030 and a computer system with data storage. Cyclic-voltammetric experiments were carried out in a conventional cell including a platinum as counter electrode and Ag/AgCl as reference electrode.

2.2 Fabrication of Microelectrode Array

Figure 1 shows fabrication process of microelectrode array. About 200nm gold layer was deposited over a 20nm titanium 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. 8 individually addressable gold electrodes (diameter : 0.7mm) 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 25°C for 2hr. utilizing the affinity between gold and sulfur. The immobilized probe DNA on the gold electrodes was confirmed by cyclic-voltammetry in 5mM $K_3Fe(CN)_6$ solution at 100mV/s.

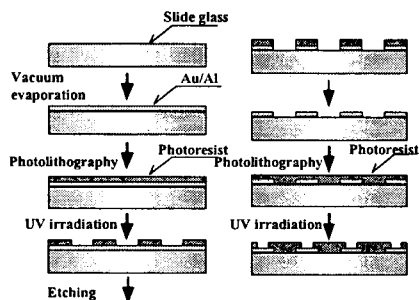


Figure 1. Fabrication process of microelectrode array for DNA chip.

2.3 Electrochemical Gene Detection

Figure 2 shows principle of microelectronic DNA chip array using electrochemical response. Target DNA (complementary) and mismatched DNA was hybridized by electrical force 300mV at 25°C for 3sec. After washing the electrodes, electrochemical signals derived from $K_3Fe(CN)_6$ solution were measured by cyclic-voltammetry.

DNA prevents a redox response of $K_3Fe(CN)_6$ ion after immobilization or hybridization on the Au surface.

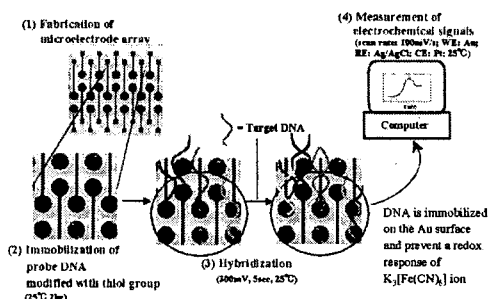


Figure 2. Principle of microelectronic DNA chip array using electrochemical response.

3. Results and Discussion

A probe DNA having a mercaptohexyl group was immobilized on a gold electrode. Cyclic-voltammograms (CV) of a ferrocyanine/ferricyanine redox couple with the bare electrode and the electrode modified with the DNA probe (ssDNA-modified) are shown in Figure 3. The redox peak currents of ferrocyanine/ferricyanine observed at 0.26 V and 1.46V, respectively (Figure 3 (a)). Then, the peak currents (I_{pa}) of ferrocyanine/ferricyanine decreased and the peak-to-peak separation (E_p) increased when the ssDNA-electrode was used compared with that of the bare electrode (Figure 3 (b)). On the other hand, a gold electrode treated with the DNA probe not having a mercaptohexyl group made no changes in the current and E_p (data not shown). This suggested that the DNA on the electrode blocked the electrochemical reaction between DNA and the anionic redox couple ions. This result shows that the DNA probe is immobilized on the gold electrode through the mercaptohexyl group of the 5 end. The ssDNA-electrode was used for the detection of a specific gene.

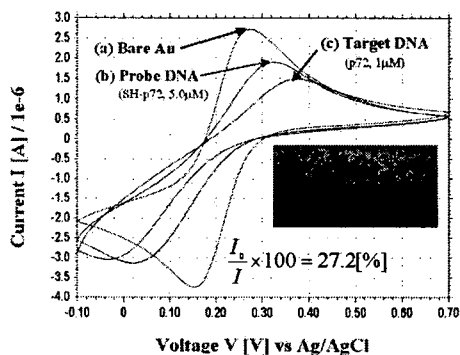


Figure 3. Cyclic-voltammograms of 5mM ferricy ferrocyanide in 100mM KCl at 100mV/s using bare gold electric and (b) probe-modified electr (SH-p72) and (c) after hybridization with ta DNA.

The ssDNA (SH-p72) was used for specific

gene detection. The ssDNA-electrodes were reacted with single stranded p72 by applying an electric field in the hybridization buffer. After hybridization, voltammetric experiments were carried out in the 5mM ferricyanide/ferrocyanide in 100mM KCl buffer at 100mV/s. When the bare gold electrode was reacted with 1 μ M p72, the I_{pa} values were almost same with the bare gold electrodes (data not shown). The voltammetric data (Figure 3 (c)) showed that when the ssDNA-electrode was reacted with 1 μ M p72, the I_{pa} value was decreased, and the change ratio of peak current (I_0/I) is 27%. It is considered that the decreased value is derived from ferricyanide/ferrocyanide ion. These results suggest that p72 can be detected specifically by using ferricyanide/ferrocyanide and ssDNA-electrode.

On the other hand, the peak currents of ferricyanide/ferrocyanide decreased and the E_p increased when the ssDNA-electrodes (SH-R72) were used compared with those of the bare electrodes (Figure 4 (a) and (b)). The ssDNA (SH-R72) were used for non specific gene detection. The ssDNA-electrodes were reacted with single stranded p72 and measured the voltammetric experiments through the same method mentioned Figure 3. After hybridization, the voltammetric data (Figure 4 (c)) showed that when the ssDNA-electrode was reacted with 1 μ M p72, the I_{pa} value was almost same values, and the change ratio of peak current (I_0/I) is 80%. It is considered that the ssDNA probes (SH-R72) were not reacted with the targeted gene. It is considered that the new label-free DNA chip array can detect the targeted gene.

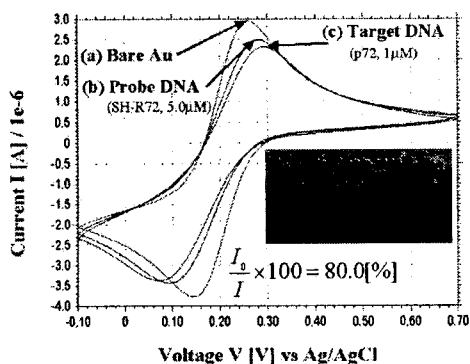


Figure 4. Cyclic-voltammograms of 5mM ferricy ferrocyanide in 100mM KCl at 100mV/s using bare gold electric and (b) probe-modified electrod (SH-R72) and (c) after hybridization with ta DNA.

4. Conclusions

In this study, an high integrated 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 of 5mM ferricyanide/ferrocyanide in 100mM KCl solution at 100mV/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 mismatched DNA in the anodic peak current values. It was derived from ferricyanide/ferrocyanide ion due to hybridization of target DNA.

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.

It suggested that multichannel electrochemical DNA microarray is useful to develop a portable device for clinical gene diagnostic system.

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