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Current Applied Physics 6 (2006) 777–780
Current Applied Physics
An official journal of the KPS
www.elsevier.com/locate/cap
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Gene detection using Hoechst 33258 on a biochip

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Received 3 June 2004; received in revised form 8 September 2004
Available online 30 June 2005

Abstract

In this paper, a DNA chip with a microelectrode array was fabricated using microfabrication technology. Several probe DNAs consisting of mercaptohexyl moiety at their 5′-end were immobilized on the gold electrodes by using a DNA arrayer. Then target DNA molecules were hybridized and reacted with Hoechst 33258, which is a DNA minor groove binder and electrochemically active dye. Linear sweep voltammetry or cyclic voltammetry showed a difference between the target DNA and the control DNA in the anodic peak current values. This difference was derived from Hoechst 33258 concentrated at the electrode surface through association with the formed hybrid. We suggested that this DNA chip can recognize the specific gene sequences.

PACS: 82.45.Tv

Keywords: Microelectrode array; Mercaptohexyl moiety; Hoechst 33258; DNA minor groove binder; Cyclicvoltammetry; DNA chip

1. Introduction

DNA microarray technology using photolithography or stamping methods enables simultaneous analysis of thousands of sequences of DNA for genetic and genomic diagnostics and for gene expression monitoring. Affymetrix [1,2] has developed GeneChip® using a photolithography technique. DNA microarrays have been developed by using a DNA arrayer, which is an automated instrument to fabricate DNA chips [3,4].

Conventional DNA chip systems employ confocal fluorescence detection for highly sensitive imaging with high resolution and confocal fluorescence can detect more than ten thousands unique oligonucleotides in several square centimeters. Target DNAs are labeled with fluorescent dyes and are hybridized with complimental probe on the chip. DNA chips and microarray scanners using fluorescent detection are very expensive so they are used only at research institutes or large hospitals.

On the other hand, as for the electrochemical measurement method, there are advantages 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 [5]. Recently, some electrochemical DNA sensors [6,7] have been developed using electrochemically active DNA intercalators (metal coordination complexes, antibiotics, etc.). Thorp [6] used $Ru(bpy)_3^{2+}$ (bpy = 2,2′-bipyridine) as a detection marker for hybridization reaction and detected a single base-pair mismatch. Also, it is detecting with an indicator-free method, or redox material is modified to probe DNA or target DNA mainly. There are the problems that should be guanine (G) in a base-pair, or these methods introduce intercalator that reacts to DNA specifically, or redox material is modified to probe or target DNA [8,9].

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doi:10.1016/j.cap.2005.04.038

Hashimoto et al. [10,11] has reported sequence-specific gene detection using a gold electrode modified with probe DNA. They used 2′-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi-1H-benzimidazol-etri-hydrochloride (Hoechst 33258) as a detection marker for the hybridization reaction, which is a DNA minor groove binder [12] and electrochemically active dye. Hoechst 33258 is concentrated at the electrode surface through association with the formed hybrid and is irreversibly oxidized on the bare gold electrodes at about 550 mV. In this study, these electrochemical signals were measured to detect specific viral DNAs, for example, human immunodeficiency virus (HIV) or hepatitis C virus (HCV).

However, no integrated multichannel electrochemical DNA sensor has been developed. Microfabrication technology [13,14] is already established and enables us to advance miniaturization and mass production of DNA chips. In this study, microfabrication technology was utilized to fabricate an integrated microelectrode array. The main purpose of this study was to develop a device for multichannel electrochemical gene detection. In this paper, we describe the results of electrochemical gene detection using a microelectrode array on a DNA chip to develop a clinical gene diagnostic system.

2. Experimental

2.1. Materials and instrumentation

Probe DNA (29-mer deoxyoligonucleotide) having a mercaptohexyl group at the 5′-phosphate end and target DNA complementary to the probe were synthesized and purified. A DNA arrayer motion system was used to immobilize the probe DNA. HIV-1 SK38, 5′-ACCAACTATggCTCTCCCgggAgggggggg-3′, or SK39, 5′-gCATTCTggACATAAgACAAggACCAAA-3′, were the probe DNA. The target DNA was 5′-CCCCCCCTCCCgggAgAgCCATAgTTggT-3′.

The electrochemical measurements were carried out using an electrochemical analyzer manufactured by Bioanalytical Systems, Model BAS-50W, and a computer system with data storage. Voltammetric experiments were carried out in a Teflon, cell including a platinum wire as counter electrode, with Ag/AgCl as a reference.

2.2. Fabrication of microelectrode array on a DNA chip

Fig. 1 shows the fabrication process for the microelectrode array. A 200-nm gold layer was deposited over a 20-nm chromium adhesion layer on a glass chip by using a vacuum evaporation. Next, the chip was spin-coated with a photoresist and was then irradiated with UV light. Each metal layer was etched to form electrodes, lead wires, and their connections. The lead wires

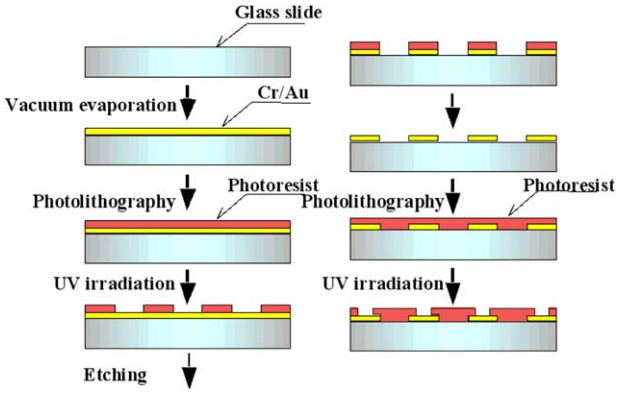

Fig. 1. Fabrication process of a microelectrode array.

were photolithographically covered with a photoresist for insulation. Thirty-two individually addressable gold electrodes (electrode area: $200 \times 200\ \mu m^2$) were arranged on the chip. Each microelectrode was connected to an external potentiostat by an insulated gold track. Probe DNAs consisting of mercaptohexyl moiety at their 5′-end were spotted on the gold electrode by using a micropipette or a DNA arrayer and were allowed to react at 10 °C for 12 h utilizing the affinity between gold and sulfur. The immobilized probe DNAs on the gold electrodes were confirmed by cyclic voltammetry in a 5-mM ferricyanide/ferrocyanide solution at 100 mV/s.

2.3. Immobilization of probe DNA

The gold electrodes were reversibly cycled in a 10 mM H_2SO_4 solution from 0 to 1.7 V (vs Ag/AgCl) at 100 mV/s until an ideal redox wave of H_2SO_4 was observed. Then, the electrodes were immersed in the solution of the DNA probe and the mismatched DNAs for 2 h at 25 °C, and allowed to react utilizing the affinity between gold and sulfur and washed with distilled water to remove probes which were not adsorbed.

2.4. Electrochemical gene detection with the DNA chip

Target DNA (complementary) or control DNA was hybridized at 37 °C for 1 h and was then allowed to react with 100-μM Hoechst 33258 for 10 min under dark conditions. After washing the electrodes, the electrochemical signals derived from Hoechst 33258 were measured by using a linear sweep voltammetry (LSV) or differential pulse voltammetry (DPV).

3. Results and discussion

Fig. 2 shows a 32-channel microelectrode array. Each gold electrode was formed with an electrode, a lead wire, and their connection. The lead wire was covered with a photoresist for insulation. Thirty-two (32) individually