Extended Abstracts of the 2005 International Conference on Solid State Devices and Materials, Kobe, 2005, pp.454-455

H-5-3

Immobilization of DNA Probes onto Gold Surface and its Application for a Fully Electric Detection of DNA Hybridization by Field Effect Transistor Sensor

Yu Ishige[1], Maki Shimoda[2] and Masao Kamahori[1]

[1] Central Research Laboratory, Hitachi, Ltd.
1-280 Higashi-Koigakubo, Kokubunji-shi, Tokyo 185-8601, Japan
Phone: +81-42-323-1111; E-mail yishige@crl.hitachi.co.jp
[2] Hitachi ULSI Systems Co., Ltd.

1. Introduction

DNA chips have played an important role in the fields of molecular biology, pharmaceutical research, and in clinical applications. Most DNA chips used fluorescence detection [1], which is the most popular method for DNA analysis. Other several methods, such as electrochemical detection [2], surface plasmon resonance [3], and quartz crystal microbalance [4] have also been used increasingly. Most of the methods require large and expensive instruments.

Recently, field effect transistor (FET) sensors have been applied to DNA chips. FET sensors are well-known as pH-sensors and can potentiometrically detect bio-molecules. FET sensors do not require expensive instruments and reagents. Fully electric DNA chips can be produced using FET sensors, which enabled the detection of hybridization between probe DNA on the sensing area and target DNA as the potential change (a shift of the threshold voltage) [5]. The efficiency of DNA hybridization depends on the density and physical structure of DNA probes. Therefore, it is important to control the density of these probes. However, it is difficult to control this density with the silane coupling method, which is widely used for immobilizing probes onto DNA chips. Fortunately, it has been reported that the surface density of DNA probes can be controlled using Au-S binding as the immobilization technique [6].

In this research, we used an extended gate FET sensor with a gold electrode sensing area, where the Au-S binding could be applied. We developed the surface density control method and the probe counting method by voltammetry in a strong alkaline solution. In addition, the hybridization efficiency of the DNA probes immobilized on the gold surface was directly measured by the single base extension reaction method [7]. DNA hybridization was successfully detected with the extended gate FET sensor by optimizing the surface density of the DNA probes.

2. Principles and methods

Structure of extended gate FET sensor

The extended gate FET is composed of two parts, one is the gold electrode that generates the surface potential change caused by the DNA hybridization, and the other is the FET structure that transduces the surface potential change into electrical signals (Fig. 1). The FET is an n-channel depletion type with a gold electrode (0.4 mm x 0.4 mm) as the extended gate.

Control of surface density of DNA probes

The surface density of the DNA probes on a gold electrode was controlled by introducing competition reaction between the DNA probes and alkanethiols. The DNA probe was synthesized with an alkanethiol linker ($HS-(CH_2)_6$) at 5' end and its sequence was 5'- CACAC TCACA GTTTT CACTT -3' (partial sequence of ALDH2 gene). First, several mixing ratios of the number of the DNA probes to 6-hydroxy-1-hexanethiol (6-HHT) were prepared in mixture solutions. Second, the immobilization of the DNA probes was carried out within 15 min after dropping each mixture solution onto the gold electrode. Finally, the gold electrodes were washed out a few times with a buffer solution.

Bioluminescence detection of hybridization efficiency

The hybridization efficiency of immobilized DNA probes was measured by the single base extension reaction combined with bioluminescence detection.

The DNA probe immobilized gold electrode was incubated with complementary target DNA (5'-GCATA CACTA AAGTG AAAAC TGTGA GTGTG-3') for 30 min at 50°C for hybridization. After the hybridization reaction, a mixture solution of polymerase and luminescence reagent was added on the DNA probe immobilized gold electrode. Bioluminescence that accompanied the single base extension reaction by adding the dNTP solution was detected using our original detection system. In this experiment, a 4 mm x 4 mm square gold electrode was used instead of the 0.4 mm x 0.4 mm square gold electrode of the FET sensor.

Measurement using extended gate FET sensor

The extended gate FET sensor was immersed in a buffer solution together with an Ag/AgCl reference electrode, with a saturated KCl solution (Fig. 2). The electrical characteristics of the FET sensor were measured using a semiconductor parameter analyzer (Agilent 4155C). A constant voltage was applied to the reference electrode and the gate voltage (V_G) - drain current (I_D) characteristics were obtained. For DNA detection, superimposed high-frequency voltage was applied to the reference electrode for the stabilization of the FET sensor.

3. Results and discussion

The electrical characteristics of the FET sensor were measured as the V_G-I_D curve shown in Fig. 3. This curve indicates that the FET sensor operated normally in an aqueous solution within a stable electrochemical window (applied voltage of -0.5 to 0.5 V).

The increase in the number of immobilized DNA probes leads to a high sensitivity in detection of DNA hybridization. However, the hybridization efficiency

depends on the density and the physical structure of the DNA probes. When the DNA probes are immobilized close together, the adjacent DNA probes interfere with the hybridization. Therefore, it is necessary to optimize the surface density of the DNA probes. Since the diameter of a double-stranded DNA (dsDNA) is about 2.4 nm, high performance of the DNA hybridization theoretically requires the DNA probes to be at a distance of about 5 nm ($4 \times 10^{12}/cm^2$), which is approximately twice the diameter of dsDNA. When only single-stranded DNA (ssDNA) probes were immobilized without alkanethiols, the counting data by voltammetry showed the surface density of $4 \times 10^{13}/cm^2$, which is about 10 times the most optimized density. The surface density of the DNA probes immobilized on a gold electrode was controlled using a competition reaction (Fig. 4). The ratio of the DNA probes to 6-HHT on the gold surface corresponded to the ratio in the mixture solution.

The DNA hybridization was directly observed by the single base extension reaction combined with bioluminescence detection. The single base extension reaction was caused by the incorporation of the dNTP complementary to the DNA probes when a DNA probe perfectly matches a DNA target. This produces only one pyrophosphate (PPi) for one target DNA and PPi is converted into bioluminescence by luminescence reagent. Thus, the hybridization efficiency of DNA probes can be measured by bioluminescence detection. Since this reaction does not occur with a nonspecific adsorption or mismatch of the DNA hybridization, we can precisely estimate the amount of DNA hybridization.

The result of the bioluminescence detection is shown in Fig. 5. The intensity of the bioluminescence increased with a higher mixture ratio of the DNA probes to 6-HHT. However, the intensity decreased with a mixture ratio of more than 1:5. The decrease in the efficiency of the DNA hybridization was caused by increasing interaction between the adjacent DNA probes. As above, it found that the optimized mixture ratio was 1:5. This result indicates that the optimized surface density of the immobilized DNA probes was achieved using the competition reaction between the DNA probes and 6-HHT.

We successfully measured the potential change from the ssDNA to the dsDNA with the FET sensor with the optimized probe density. The drain current decreased when the complementary target DNA was introduced to the gold electrode modified and hybridized with DNA probes (Fig. 6). This means the surface potential was decreased by increased minus charge because of the DNA hybridization.

4. Conclusion

We designed an extended gate FET sensor with a gold electrode. The control method for the surface density of immobilized DNA probes on the gold electrode was established with the competition reaction between the DNA probes and alkanethiols. In addition, the efficiency of the DNA hybridization was directly measured by the single base extension reaction combined with bioluminescence detection and the optimized condition for immobilized DNA probes was clarified. Using the extended gate FET sensor combined with the optimized density of DNA probes, a fully electric detection of DNA hybridization was achieved. The proposed extended gate FET sensor for detection of DNA hybridization is useful for realizing a miniaturized and cost-effective system.

Acknowledgement

This work was performed as a part of the research and development project of Industrial Science and Technology Program supported by New Energy and Industrial Technology Development Organization in Japan.

[1] S. P. Fodor et al., Nature **364** (1993) 555
[2] K. Hashimoto et al., Anal. Chem. **60** (1994) 3830
[3] K. Nakatani et al., Nat. Biotechnol. **19** (2001) 51
[4] M. A. Cooper et al., Nat. Biotechnol. **19** (2001) 833
[5] E. Souteyrand et al., J. Phys. Chem. B **101** (1997) 2980
[6] M. H. Tonya et al., J. Am. Chem. Soc. **119** (1997) 8916
[7] M. Kamahori et al., Meas. Sci. Technol. **13** (2002) 1779

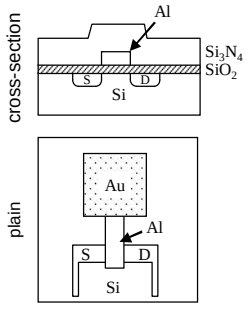

Fig. 1 Structure of extended gate FET Gold electrode and FET is connected with conductive wire.

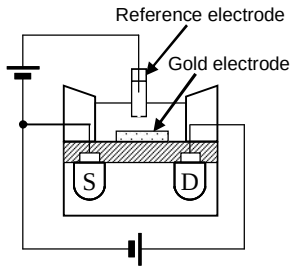

Fig. 2 Diagram of measurement Voltage is applied through aqueous solution by reference electrode.

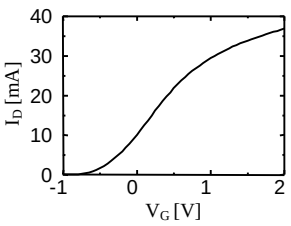

Fig. 3 V_G-I_D curve of FET sensor Characteristic of FET sensor in aqueous solution

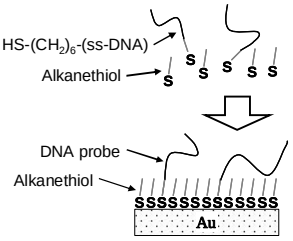

Fig. 4 Density control method Competitive reaction between DNA probes and alkanethiols

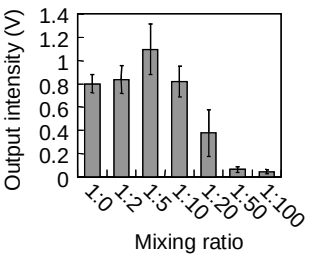

Fig. 5 Bioluminescence detection The number of hybridized DNA probes was maximized at 1:5.

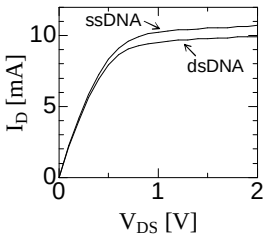

Fig. 6 Hybridization detection Hybridization was detected by extended gate FET sensor as the change of drain current.