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Quasi-Static Capacitance-Voltage Measurement for DNA Hybridization Using Integrated Field Effect Devices

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1. Introduction

Combining biotechnology and semiconductor technology, detection methods and fabrication technologies have been advanced in the field of biochips that can detect and monitor specific binding of biomolecules in a parallel way on solid-state substrates [1,2]. In particular, various gene functional analyses using solid-state devices have remarkably proceeded based on the completion of the decoding of the human genome. We have been investigating a new approach to realize a simple detection of molecular recognition events on a solid-state device. In this study, we show the novel concept of an integrated field effect device for DNA hybridization using quasi-static capacitance-voltage (C-V) measurement.

2. Experimental procedure

2.1. Immobilization of oligonucleotide probes

Oligonucleotides were synthesized using phosphoramidite method and purified by HPLC. The 5'-end of the synthesized oligonucleotide was modified with an amino group for attachment to the Si_3N_4 surface. Oligonucleotide probes were immobilized on the modified Si_3N_4 surface using glutaraldehyde as a bifunctional cross-linking agent after reactive amino groups were introduced by amino-silane treatment. The sensing area was then soaked in a phosphate buffer solution with glycine to block any remaining glutaraldehyde groups.

2.2. Hybridization and intercalation

Target DNA used for hybridization was prepared by dissolving complementary target oligonucleotides in a hybridization buffer solution at a concentration of 100 μM. The integrated field effect device with immobilized oligonucleotide probes was kept in the hybridization buffer solution containing target oligonucleotides for 15 h at 25 ℃.

Intercalator used in this work is Hoechst 33258. The intercalator was dissolved in deionized water at a concentration of 100 μM. After hybridization, the integrated field effect device was immersed in the intercalator solution at room temperature for 12 h and washed with deionized water.

2.3. Measurement of electrical characteristics

The n-type silicon was used as the substrate for the field effect device. The charge density change on the integrated field effect device was measured using quasi-static capacitance-voltage (QSCV) technique. The sensing spots were soaked in a phosphate buffer solution with a Ag/AgCl reference electrode (**Fig. 1**).The electrical characteristics of the capacitors such as the capacitance-voltage (C-V) curve were measured at 25 ℃ and a constant frequency of 150 Hz in the voltage range from 3 to -3 V using an impedance analyzer. The flat band voltage V_F shift was calculated after hybridization and intercalation. The V_F shift was defined as a difference of the C-V characteristics at a flat band capacitance (C_F).

3. Results and Discussion

The integrated field effect device was fabricated as shown in **Fig. 2**. The integrated field effect device is composed of a Si substrate with Si_3N_4/SiO_2 thin layer, on which oligonucleotide probes are immobilized and hybridized with target DNA in sample solutions. Since DNA molecules are negatively charged in an aqueous solution, the amount of negative charges at the Si_3N_4/SiO_2 surface increases as a result of hybridization and the charge density change is transduced into electrical signal by the field effect (**Fig. 3**). One of the unique features of our method is to utilize intercalators as charged species because they are ionized and positively charged in aqueous solutions. After hybridization with target DNA and intrioduction of intercalator, the charge density change due to hybridization and intercalation was measured using quasi-static C-V measurement tequnique. **Figure 4** shows an example of the quasi-static C-V characteristics of a field effect device. In order to observe the flat band voltage (V_F) shift in the C-V characteristics in detail, the local area shown in **Fig. 4(a)** was magnified (**Fig. 4(b)**). When the complementary target DNA was introduced to the Si_3N_4 surface and hybridized with oligonucleotide probes, the V_F shifted in the positive direction by the amount of 10 mV. This is due to increase of negative charges of the target DNA by hybridization. After hybridization, intercalator, Hoechst 33258 was introduced to the Si_3N_4 surface. The V_F shifted in the negative direction by the amount of -13 mV. The negative shift of the V_F indicates increase of positive charges at the gate surface and is due to intercalation of Hoechst 33258 into the double-stranded DNA. Thus, the charge density change at the Si_3N_4 surface after each molecular recognition event on genetic FETs could be successfully detected using quasi-static C-V measurement tequnique. The proposed field effect device is suitable for a simple and minituarized detection system for DNA molecules.

3. Conclusions

We demonstrated the basic principle of the integrated field effect device based on the electrostatic interaction between

molecular charges induced by DNA recognition events and surface electrons in silicon crystal through the Si_3N_4/SiO_2 thin layer using quasi-static capacitance-voltage (QSCV) measurement. Since DNA molecule and intercalator have intrinsic charges in aqueous solutions respectively, the charge density changes due to hybridization and intercalation were directly translated into electrical signal using the field effect device..

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References

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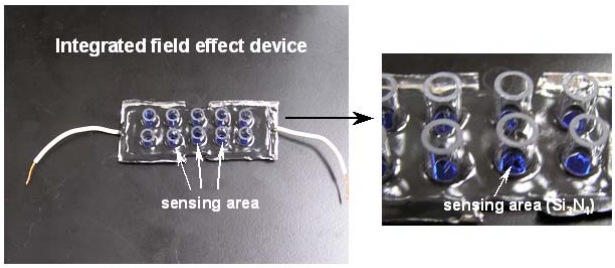

Figure 2 Photograph of integrated field effect device in a microplate format. Ten sensing areas are composed of Si_3N_4, on which molecular recognition events can be detected using quasi-static C-V measurement.

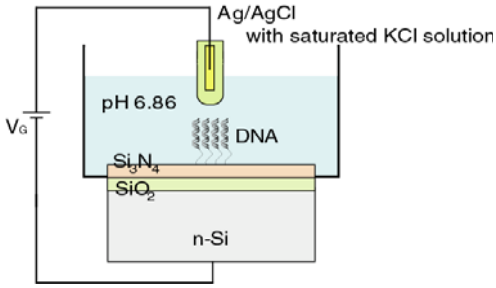

Figure 1. Schematic diagram for measurements of electrical characteristics of field effect device.

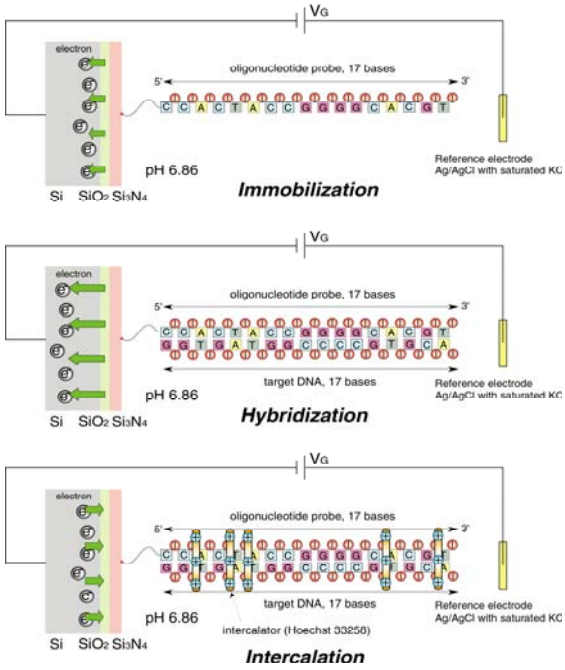

Figure 3 Scheme for a quasi-static C-V measurement of DNA molecules and intercalators using genetic FET.

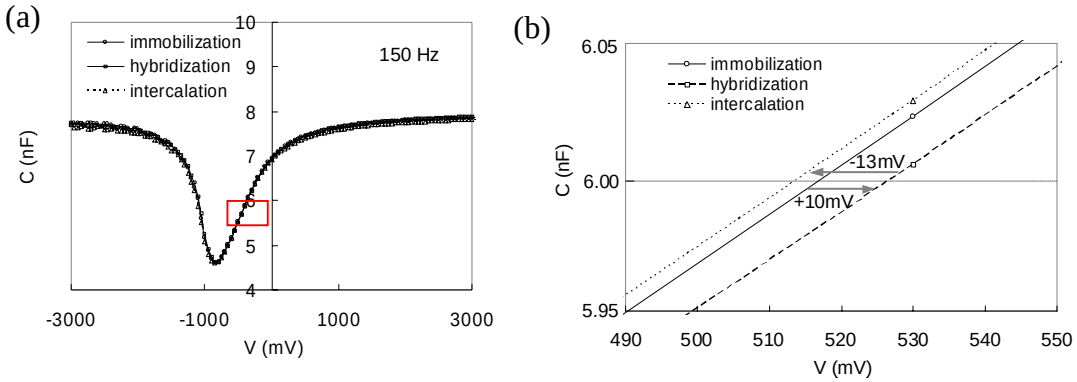

Figure 4 Quasi-static C-V characteristics of genetic FETs after immobilization of oligonucleotide probes, hybridization with target DNA and intercalation of Hoechst 33258. The surrounded area shown in (a) was magnified as shown in (b).