Detection of DNA recognition events using multi-well field effect devices

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Abstract

We proposed the multi-well field effect device for detection of charged biomolecules and demonstrated the detection principle for DNA recognition events using quasi-static capacitance–voltage (QSCV) measurement. The multi-well field effect device is based on the electrostatic interaction between molecular charges induced by DNA recognition and surface electrons in silicon through the Si$_3$N$_4$/SiO$_2$ thin double-layer. Since DNA molecules and DNA binders such as Hoechst 33258 have intrinsic charges in aqueous solutions, respectively, the charge density changes due to DNA recognition events at the Si$_3$N$_4$ surface were directly translated into electrical signal such as a flat band voltage change in the QSCV measurement. The average flat band shifts were 20.7 mV for hybridization and −13.5 mV for binding of Hoechst 33258. From the results of flat band voltage shifts due to hybridization and binding of Hoechst 33258, the immobilization density of oligonucleotide probes at the Si$_3$N$_4$ surface was estimated to be 10$^8$ cm$^{-2}$. The platform based on the multi-well field effect device is suitable for a simple and arrayed detection system for DNA recognition events.

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Keywords: DNA chip; Genetic field effect device; Capacitance–voltage; Molecular charge; DNA recognition event

1. Introduction

Combining biotechnology and semiconductor technology, various types of biochips and biosensors have been developed to detect and monitor specific binding of biomolecules on solid-state substrates (Cornell et al., 1997; Jain, 2001; Kajiyama et al., 2003). In particular, gene functional analyses have remarkably proceeded in the fields of molecular biology and pharmacetics based on the completion of the decoding of the human genome. In order to detect DNA hybridization, several detection methods such as fluorescence (Ziauddin and Sabatini, 2001; Zhao et al., 2003), chemiluminescence (Lockhart et al., 1996), surface plasmon resonance (SPR) (Jordan et al., 1997), quartz crystal microbalance (QCM) (Okahtah et al., 1992) and electrical current (Hashimoto et al., 1994) have been developed. Although fluorescent image analysis is suitable for parallel detection of dense arrays of oligonucleotides spots, it needs expensive optics and instrument with complicated data analysis algorithms. For the platform used in clinical diagnostics, a simple, reliable and inexpensive method and device would be required.

We have been investigating a new approach to realize a simple detection of molecular recognition events on a solid-state device. The concept of a genetic field effect transistor (FET) has already reported for potentiometric detection of single nucleotide polymorphism (SNP) based on temperature-controlled hybridization and binding of DNA binders (Sakata et al., 2003; Sakata and Miyahara, in press). Field effect devices have also been used as an electrochemical detection of hybridization events on a solid surface (Souteyrand et al., 1997; Fritz et al., 2002; Kim et al., 2003, 2004; Pouthas et al., 2004; Pouthas et al., 2004). Since DNA molecules are negatively charged in an aqueous solution, the amount of negative charges at the gate surface increases as a result of hybridization and the charge density change is transduced into electrical signal by the field effect. Binding of intercalators or DNA binders to double-stranded DNA is ef-
fective to distinguish hybridized double-stranded DNA from non-specifically adsorbed single-stranded target DNA, because they are ionized and positively charged in aqueous solutions and some of them bind specifically to double-stranded DNA (Sakata et al., 2003; Sakata and Miyahara, in press).

In this study, we propose multi-well field effect devices in which reaction chambers and detection devices for signal transduction are integrated in a single Si substrate. The proposed devices can be cost-effective, because they are fabricated with a simple fabrication process. For detection of DNA hybridization and binding of DNA binder, a quasi-static capacitance–voltage (QSCV) measurement was carried out using the multi-well field effect device and a reference electrode.

2. Materials and methods

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 Si3N4 surface. The base sequences of the oligonucleotide probes and target DNA for the wild-type gene at the R353Q locus of the factor VII gene, which was related to blood coagulation factor, were designed as shown in Table 1 according to Kajiyama et al. (2003).

The surface of the Si3N4 layer was cleaned with 1 M NaOH for 1 h at room temperature and silanized in toluene (Sigma-Aldrich) containing 2 wt.% 3-aminopropytriethoxysilane (Sigma-Aldrich). The amino-silanized Si3N4 surface was rinsed in toluene and dried in vacuo at 110 °C for 1 h. Reactive amino groups were then introduced at the Si3N4 surface.

Oligonucleotide probes were immobilized on the modified Si3N4 surface using glutaraldehyde as a bifunctional cross-linking agent. The amino-silanized Si3N4 surface was soaked in a 25 wt.% glutaric dialdehyde (Sigma-Aldrich) solution with 0.5 g of sodium cyanoborohydride (Sigma-Aldrich) per 50 ml for 4 h at room temperature, followed by rinsing in deionized water and drying in vacuo at room temperature for 1 h. Oligonucleotide probes were dissolved in a TE buffer (pH 8.0, Nippon Gene) at a concentration of 100 μM. To couple amino-modified oligonucleotides with the glutaraldehyde-treated Si3N4 surface, the sensing area was kept at 50 °C in the oligonucleotide solution with 0.5 g of sodium cyanoborohydride per 50 ml overnight to complete the coupling reaction. The sensing area was then soaked in a phosphate buffer solution (0.04 M Na2HPO4 and 0.03 M K2HPO4, pH 7.0, Wako) with 1 M glycine (Wako) at 50 °C for 1 h to block any remaining glutaraldehyde groups. The sensing area was washed with the phosphate buffer solution (pH 7.0) and with deionized water and dried in vacuo at room temperature for 1 h. The surface-modified field effect device was ready for use in hybridization and binding of DNA binder studies.

2.2. Hybridization and binding of DNA binder

Target DNA used for hybridization was prepared by dissolving complementary target oligonucleotides in a hybridization buffer solution, which was composed of 4 × SSC + 0.1% SDS (Invitrogen), at a concentration of 100 μM. The field effect device with immobilized oligonucleotide probes was kept in the hybridization buffer solution containing target oligonucleotides for 12 h at 25 °C. Following hybridization, the field effect device was washed with 1 × SSC + 0.03% SDS, 0.2 × SSC, 0.05 × SSC, and deionized water at room temperature in order to remove non-hybridized oligonucleotides.

DNA binder used in this work is 5'-[4-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bis-1H-benzimidazole trihydrochloride, Hoechst 33258 (H-258) (Dojindo). The DNA binder was dissolved in deionized water at a concentration of 100 μM. After hybridization, the field effect device was immersed in the DNA binder solution for 12 h at 25 °C and washed with deionized water.

2.3. Measurement of electrical characteristics

The capacitance of the field effect device has been measured using quasi-static capacitance–voltage (QSCV) technique. The electrical characteristics of the field effect device such as the capacitance–voltage (C–V) curve were measured at a constant frequency of 150 Hz in the voltage range from 3 to −3 V using a precision impedance analyzer (4156C, Agilent). The shift of the flat band voltage $V_F$ was calculated after hybridization and binding of DNA binder. The $V_F$ shift was defined as a difference of the C–V characteristics at a constant capacitance which corresponds to the flat band capacitance ($C_F$).

Ten sensing areas, of which diameters are 4 mm, are integrated in a silicon substrate in multi-well format (Fig. 1a). For the fabrication of the field effect device, n-type silicon with the resistivity of 10 Ω cm was used as a substrate. The thicknesses of the Si3N4 layer and the SiO2 layer are 50 and 30 nm, respectively. The fabricated multi-well field effect device was encapsulated with glass rings using an epoxy resin (ZC-203, Nippon Pelnox) except for the sensing areas.

The sensing spots were soaked in a phosphate buffer solution (0.025 M Na2HPO4 and 0.025 M K2HPO4, pH 6.86, Wako) was kept at 50 °C in the oligonucleotide solution with 0.5 g of sodium cyanoborohydride per 50 ml overnight to complete the coupling reaction. The sensing area was then soaked in a phosphate buffer solution (0.04 M Na2HPO4 and 0.03 M K2HPO4, pH 7.0, Wako) with 1 M glycine (Wako) at 50 °C for 1 h to block any remaining glutaraldehyde groups. The sensing area was washed with the phosphate buffer solution (pH 7.0) and with deionized water and dried in vacuo at room temperature for 1 h. The surface-modified field effect device was ready for use in hybridization and binding of DNA binder studies.

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<table>
<thead>
<tr>
<th>Probe</th>
<th>Target</th>
</tr>
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<tbody>
<tr>
<td>5′-amino group-CCCTACCG-GGCACGTG-3′ (17mer)</td>
<td>5′-ACGTCGCCGGTACGTG-3′ (17mer)</td>
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3. Results and discussion

The novel concept of a multi-well field effect device is proposed in the present study for direct detection of DNA hybridization (Fig. 2). The field effect device is composed of a silicon substrate with a Si$_3$N$_4$/SiO$_2$ thin layer, on which oligonucleotide probes are immobilized (Fig. 2a). The charge density change at the Si$_3$N$_4$ surface can be detected by measuring the capacitance–voltage ($C$–$V$) characteristics between the silicon substrate and a reference electrode in a sample solution. When complementary DNA molecules are contained in a sample solution, hybridization occurs at the Si$_3$N$_4$ surface. Since DNA molecules have negative charges derived from the phosphate group in an aqueous solution, electrons are expelled from the surface of the silicon substrate by electrostatic force through the thin insulator (Fig. 2b). As a result of this interaction, the surface electron density in silicon decreases and hybridization event can be directly detected in principle by measuring the shift in the $C$–$V$ characteristics of the field effect device. One of the unique features of our method is to utilize DNA binders as charged species, while DNA binders are usually used as fluorescent dyes in the field of molecular biology (Sriram et al., 1992; Boger et al., 2001). When DNA binders are introduced into the double-stranded DNA after hybridization, electrons are induced and attracted to the surface of silicon (Fig. 2c), because DNA binders such as Hoechst 33258 (H-258) are positively charged in an aqueous solution. Since some of DNA binders such as intercalators react specifically with double-stranded DNA, an undesirable background noise caused by non-specific adsorption of single-stranded target DNA can be eliminated and more precise and reliable detection of hybridization event would be realized.

Oligonucleotide probes, of which the base sequence is 5’-CCACTACCGGGGCACGT-3’, were immobilized on the sensing areas according to the immobilization process including cleaning, silanization, glutaraldehyde treatment, immobilization of oligonucleotide probes and blocking with glycine. Fig. 3 shows an example of the QSCV characteristics of the field effect device at 150 Hz after immobilization, hybridization and binding of H-258. The accumulation, depletion and inversion regions can be clearly observed in the $C$–$V$ curve (Fig. 3a). Since the $C$–$V$ curve shifts along the voltage axis depending on the charges at the Si$_3$N$_4$ surface, the hybridization response caused by molecular charges on the Si$_3$N$_4$ surface can be detected by measuring the shift of the $C$–$V$ curve at
Fig. 2. Scheme for electrostatic interaction between molecular charges induced by (a) immobilization, (b) hybridization and (c) binding of DNA binder and surface electrons in silicon through Si$_3$N$_4$/SiO$_2$ thin layer.

In order to measure the flat band voltage $V_F$ shift precisely, the local area shown in Fig. 3a was magnified (Fig. 3b). When the complementary target DNA was introduced to the Si$_3$N$_4$ surface and hybridized with oligonucleotide probes, the $V_F$ shifted in the positive direction by the amount of 10.4 mV. This is due to increase of negative charges of the target DNA by hybridization which expels electrons from the surface of silicon. After hybridization, DNA binder, H-258 was introduced to the Si$_3$N$_4$ surface. The $V_F$ shifted in the negative direction by the amount of $-13.1$ mV. The negative shift of the $V_F$ indicates increase of positive charges at the Si$_3$N$_4$ surface and is due to binding of H-258 to the hybridized DNA.

The reproducibility of electrical signals due to DNA recognition events was evaluated based on QSCV measurement data using the multi-well field effect device. When the measurements of the $V_F$ shifts after hybridization were performed for 10 sensing areas as shown in Fig. 4a, the average $V_F$ shifts were calculated to be 20.7 mV with the standard deviation of 2.2 mV, resulting in the coefficient of variation of 10.9%. The average $V_F$ shifts after the introduction of H-258 were calculated to be $-13.5$ mV with the standard deviation of 3.7 mV resulting in the coefficient of variation of 27.4% (Fig. 4b). The amount of $V_F$ changes due to the DNA recognition events was similar to the amount of threshold voltage $V_T$ changes obtained using generic field effect transistor (FET) (Sakata et al., 2003, 2004; Sakata and Miyahara, in press). The experimental reproducibility of the $V_F$ changes will be improved
Fig. 3. Capacitance–voltage (C–V) characteristics of multi-well field effect device after hybridization with target DNA and binding of Hoechst 33258 (H-258). The surrounded area shown in (a) was magnified as shown in (b).

Fig. 4. Change of flat band voltage \( V_F \) at the 10 Si3N4 sensing areas of integrated genetic field effect device. (a) Change of flat band voltage \( V_F \) after hybridization. The QSCV measurement was performed based on the diagram shown in Fig. 1b. The \( V_F \) was shifted in the positive direction because of negative charges of target DNA. (b) Change of flat band voltage \( V_F \) after binding of Hoechst 3258 (H-258). The QSCV measurement was performed based on the diagram shown in Fig. 1b. The \( V_F \) was shifted in the negative direction because of positive charges of H-258.

The immobilization density of oligonucleotide probes can be estimated from the \( \Delta V_F \) shift caused by hybridization with target DNA. The \( \Delta V_F \) shift after hybridization, \( \Delta V_{F1} \), can be expressed in Eq. (1), where \( Q_{ds-DNA} \) is the charge per unit area of the double-stranded DNA after hybridization, \( Q_{ss-DNA} \) the charge per unit area of the oligonucleotide probes, \( \Delta Q_{hyb} \) the charge difference per unit area after hybridization, and \( C_i \) the insulator capacitance per unit area.

\[
\Delta V_{F1} = \frac{Q_{ds-DNA} - Q_{ss-DNA}}{C_i} = \frac{\Delta Q_{hyb}}{C_i} \quad (1)
\]

Since \( \Delta V_{F1} = 20.7 \text{ mV} \) (the average \( V_F \) shifts) and \( C_i = 6.6 \times 10^{-4} \text{ F/m}^2 \) for the field effect device, the amount of charges increased after hybridization is calculated to be \( 1.4 \times 10^{-5} \text{ C/m}^2 \). The base lengths of the oligonucleotide probe and the target DNA used in this study are both 17 bases, which corresponds to 5.78 nm in length. Negative charges derived from phosphate groups are distributed along the double-stranded DNA from the Si3N4 surface to the bulk of the sample solution. We assume that these negative charges along the DNA molecules contributed to the \( V_F \) shift equally and that all the oligonucleotide probes were hybridized with the target DNA. Under these assumptions, the number of oligonucleotide probes on the Si3N4 surface can be calculated to be \( 6.2 \times 10^{13} \), which corresponds to \( 5.0 \times 10^8 \text{ cm}^{-2} \).

The negative shift due to binding of H-258 is related not only to the immobilization density of oligonucleotide probes but also charges and chemical structure of the DNA binder, base sequence of the DNA and so on. The \( \Delta V_F \) shift after binding of H-258, \( \Delta V_{F2} \), can be expressed in the same equation as (1). Since \( |\Delta V_{F2}| = 13.5 \text{ mV} \) (the average \( V_F \) shifts), the amount of charges increased after binding of H-258 is calculated to be \( 8.5 \times 10^{-6} \text{ C/m}^2 \). H-258 was reported to show selectivity toward AT-rich sites of the minor groove in DNA molecule (Sriram et al., 1992; Boger et al., 2001). If H-258 is presumed to be bound to only AT sites of double-stranded DNA, five H-258 molecules can be bound to a double-stranded DNA with the present base sequence described before (Fig. 2c). In addition, a H-258 molecule has three positive charges in an aqueous solution. Considering these features, the numbers of H-258 molecules and hybridized DNA molecules on the sensing region can be calculated to be \( 2.3 \times 10^{14} \) and \( 4.6 \times 10^{13} \), respectively. From this result, the immobilization density of oligonucleotide probes was estimated to be \( 3.7 \times 10^8 \text{ cm}^{-2} \). This value is in good agreement with that obtained by hybridization only.

The above results demonstrate that the DNA recognition events such as hybridization and binding of DNA binder can
be directly transduced into the $\Delta V_F$ signal using the multi-well field effect devices. Since the field effect device is based on the electrostatic interaction between electrons in silicon and charged DNA molecules in an aqueous solution through the thin gate insulator, this principle can be applied for detecting other charged biomolecules such as proteins. It is possible to integrate more sensing areas easily by optimizing the reaction volume and arranging more glass rings on the Si$_3$N$_4$ surface. Simultaneous analyses of different base sequences of DNA molecules can therefore be realized based on the multi-well field effect device. Since the $V_F$ shift of the multi-well field effect device is generated with the intrinsic charges of DNA molecules and DNA binders, no labelling is necessary for the target DNA.

4. Conclusions

In conclusion, we demonstrated the basic principle of the multi-well field effect device based on the electrostatic interaction between molecular charges induced by DNA recognition events and surface electrons in silicon through the Si$_3$N$_4$/SiO$_2$ thin layer. Since reaction chambers and detectors for signal transduction are integrated in a device, online detection of electrical signal can follow reaction such as hybridization and specific binding. Furthermore, fabrication process of the multi-well field effect device is very simple, cost-effective assay for DNA molecules can be realized. The platform based on the multi-well field effect device is suitable for a simple, inexpensive and arrayed system for genetic analysis in clinical research.

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