Deoxyribonucleic-acid-sensitive Polycrystalline Diamond Solution-gate Field-effect Transistor with a Carboxyl-terminated Boron-doped Channel

Yukihiro SHINTANI,^{*,†} Shoji IBORI,^{*} and Hiroshi KAWARADA^{*,**}

^{*}*School of Science and Engineering, Waseda University, 3-4-1 Okubo, Shinjuku, Tokyo 169-8555, Japan*

^{**}*Kagami Memorial Laboratory for Materials Science and Technology, Waseda University, 2-8-26 Nishiwaseda, Shinjuku, Tokyo 169-0051, Japan*

This paper describes a deoxyribonucleic-acid-sensitive electrolyte solution-gate field-effect transistor (SGFET) sensor utilizing a partial carboxyl-terminated boron-doped polycrystalline diamond surface as a linker to connect a deoxyribonucleic acid (DNA) probe. A high density of carboxyl termination on the polycrystalline diamond surface that was employed as a FET channel was achieved using a vacuum ultraviolet system with oxygen gas. A single-stranded DNA probe was immobilized on the polycrystalline diamond channel *via* amino coupling. The current-voltage characteristics of the polycrystalline diamond SGFET sensor was examined with bias voltages within its potential voltage window. The characteristics of the drain-source current versus the drain-source voltage showed a pinch-off, a shift voltage of up to 40 mV with a coefficient of variation of 4–11% was obtained between hybridization and denaturation. In addition, a single nucleotide mutation of DNA sequence was selectively recognized by the shift voltage up to *ca.* 10 mV.

Keywords Polycrystalline diamond, boron-doped diamond, carboxyl-termination, electrolyte-solution-gate field-effect transistor, deoxyribonucleic acid

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Introduction

A detection method of deoxyribonucleic acid (DNA) for medical diagnostics and biotechnology has been intensely researched in recent years.^{1–3} Genetic testing from a point-of-care vantage point requires miniaturized, cheap bio-analytical sensors. DNA hybridization is a well-known technology based on a single-stranded deoxyribonucleic acid (ss-DNA) sequence immobilized on the probe surface; the analyte ss-DNA (molecular target) is recognized by its specific binding affinity to the complementary nucleotide sequence. The hybridization is analyzed using various methods, including enzymatic, electrochemical, electroluminescence, microgravimetric, radioactive, and fluorescence methods.² In the field of genetic testing, a low-concentration DNA sample is amplified by a polymerase chain reaction (PCR), and then analyzed by electrophoresis. Typical disadvantages of this *de-facto* method include a long operation time and high cost. For the detection of leading-edge multi-spot DNA chips, a labeling step of either an analyte DNA or an immobilized catcher DNA probe is essential. Among DNA sensing methods developed recently, a silicon-based (Si-based) ion-sensitive field-effect transistor (ISFET) sensor has shown the following advantages for bio-analytical applications: the possibility of micro-fabrication and integration, non-labeling of biomolecule, and a potential fast response with high reliability.⁴

The output of a Si-based ISFET sensor responds according to the electrical potential changes at the dielectric/electrolyte interface; this modulates the conductance of its channel.⁵ The changes in the charge concentration and the presence of molecular dipoles within the Debye layer contribute to the shift of the FET I-V characteristic curve. This transducing mechanism avoids specific labeling analytes, and also leads to assay complexity and decreases in the time and cost. Since the gate channel of a Si-typed ISFET sensor is Si/SiO₂/metal oxide, an interface of cations, *e.g.*, sodium ion, potassium ion, calcium ion, could easily deteriorate the electrical properties. Therefore, encapsulation is a major concern in the design of Si-ISFET sensors to prevent the penetration of cations in the solution.

We utilized a synthesized diamond semiconductor as a FET sensor, the so-called electrolyte-solution-gate field effect transistor (SGFET) sensor.⁶ For the SGFET, a p-type diamond induced by hydrogen-termination was employed as a FET channel. For bio-applications, this diamond channel has many attractive characteristics, including chemical/physical stability, a low background signal, and biocompatibility. As a result of these advantages, several diamond-typed bio-FET sensors have been reported for the detection of various biological phenomena, such as antibody-antigen binding,⁷ DNA hybridization,^{8,9} and enzyme reactions.¹⁰

In our previous work, diamond SGFETs demonstrated significant advantages, including the presence of a p-type conductive layer on the diamond surface without boron-doping, a requirement of simple functionalization, and high sensitivity without an insulating layer.^{10–15} Significantly, a passivation layer

[†] To whom correspondence should be addressed.
E-mail: shintani@toki.waseda.jp

on the gate channel, which is essential to the conventional Si-ISFET, is unnecessary for diamond SGFETs. The diamond surface is directly exposed to the electrolyte solution. Partially amino-terminated and/or partially oxygen-terminated diamonds were successfully employed as SGFET biosensors with high sensitivity/repeatability.^{12,13} The change from a hydrogen-terminated surface has an influence on the SGFET current-voltage (SGFET I-V) characteristics. Surface modification processes were performed on the diamond surface to obtain partial amino-/oxygen-termination; this led to a loss of the hydrogen density and an increase in the drain-source resistance source. In order to overcome the above-mentioned problems, we proposed introducing a hole accumulation layer by boron-doping single-crystal diamond^{16,17} instead of using a hydrogen-terminated non-doped diamond. To advance the commercial viability of diamond sensors, a polycrystalline diamond with an oxygen-terminated surface was also applied in our previous work.^{14,18–21}

In this study, to verify the superiority and viability of an oxygen-terminated polycrystalline diamond surface with a boron-doped layer for biosensor, a partial carboxyl-termination was introduced for the polycrystalline diamond SGFET. A ss-DNA probe was immobilized on the polycrystalline diamond surface *via* amino coupling. The SGFET I-V characteristics and the DNA sensitivity of the developed polycrystalline diamond SGFET sensors were examined.

Experimental

Boron-doped layer deposition on the diamond

To fabricate boron-doped polycrystalline diamond layers, a 5-mm polycrystalline diamond and a 10-mm square polycrystalline diamond were purchased from Tomei Daiya Corp. and Element Six Inc. respectively, and were used for substrate material after pretreatment with acid. A boron-doping layer was deposited on the polycrystalline diamond by using a microwave-plasma-assisted chemical vapor deposition (MPCVD) reactor that was designed as the NIRIM (National Institute for Research in Inorganic Materials)-type. The typical conditions for deposition were as follows: methane (CH₄) concentration, 0.1–1.0%; boron source, 1–5 kppm trimethylboron (TMB) diluted in hydrogen (H₂); boron-to-carbon ratio (B/C ratio), 2.5–7.5 kppm; TMB flowrate, 1.0–2.0 standard cubic centimeters per minute (sccm), CH₄ flowrate, 2–5 sccm; microwave power, 400–700 W, pressure, 60–80 torr; and deposition time, 2–10 min. The B/C ratio and CH₄ concentration were calculated from the ratio of the TMB and CH₄ flowrates and the percentage of the total gas flowrate, respectively.

SGFET fabrication and surface modification

Polycrystalline diamond SGFETs were fabricated by a process described in our previous work.^{14,16,20–22} Following boron-doped polycrystalline diamond film deposition, two electrical contact pads of 200-nm gold on 30-nm titanium were deposited as a source/drain electrode using an electron beam evaporator (EB evaporation Model A500; Edwards). The electrical contact pads were positioned to give a 10-mm channel length and a 500-μm channel width. After EB evaporation, annealing was performed at 420 degrees C under 100-sccm H₂ flow to form an ohmic contact on the polycrystalline diamond surface.¹⁵ For a partial carboxyl-termination, a vacuum ultraviolet (VUV) treatment system (Model UER20-172, USHIO Corp.) was used under vacuum to radiate ultraviolet light in the presence of oxygen gas

Fig. 1 Schematic of fabricated deoxyribonucleic-acid-sensitive electrolyte solution-gate field effect transistor sensor and deoxyribonucleic acid sequence. Deoxyribonucleic acid probes were immobilized onto carboxyl groups of the polycrystalline diamond surface *via* amide-coupling.

(O₂).^{23,24} This instrument had a dielectric barrier discharge excimer lamp filled with xenon (Xe) and radiated light with a 172-nm central wavelength. A diaphragm vacuum pump was used for evacuating the chamber. A VUV treatment was performed at a 300-mbar chamber pressure for 45 min. Following the VUV treatment, the coverage of functional groups on the modified polycrystalline diamond surface was evaluated with an X-ray photoelectron spectroscopy (XPS: ULVAC-Phi Model 3300).

DNA immobilization

All chemicals used in this study were of analytical grade, and purchased from Kanto Chemical, Tokyo Kasei, and Wako. DNA oligonucleotides were obtained from Sigma-Genosys. In this study, a 5' amine-modified DNA probe with a base length of 21 mer was immobilized to the carboxyl group on the polycrystalline diamond surface *via* amide coupling by the following process:²⁷ (i) an ammonia-terminated DNA with the sequence 5'-NH₂-CCACGGACTACTTCAAACTA-3' was diluted to 1 nM with 0.1 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), 0.4 M *N*-hydroxysuccinimide (NHS) and 1 M sodium citrate, and then adjusted to pH 7 with NaOH; (ii) an amide coupling reaction occurred between the 5' DNA and carboxyl groups on the polycrystalline diamond; (iii) an immobilization reaction was incubated at 38°C for 2 h. The fabricated deoxyribonucleic-acid-sensitive SGFET sensor and its DNA sequences for DNA probe are illustrated in Fig. 1. The DNA-immobilized polycrystalline diamond surface was immersed in a urea solution for DNA denaturation.

SGFET I-V characterization of boron-doped polycrystalline diamond film

A SGFET I-V evaluation system comprised of two sets of source-measure unit instruments (Model 2400, Keithley Instruments) to obtain the drain-source voltage (V_{ds}) and gate-source voltage (V_{gs}). Device control and data acquisition were performed using a LabVIEW (Model 2010, National Instruments) to maintain a constant drain current by applying a compensating V_{gs} . The V_{gs} voltage was applied using a commercial silver/silver chloride (Ag/AgCl) reference electrode. All SGFET I-V measurements were performed at room temperature in two types of buffer solution: a 1 mM phosphate

Table 1 Synthesis condition for boron-doped layer on the surface of polycrystalline diamond

Sample No	Deposition time/min	CH ₄ concentration, %	B/C, ppm	Pressure/torr
A	3	0.1	6000	80
B	1	0.3	6000	80
C	10	0.3	6000	80

buffered saline (PBS) solution (pH 7.4) and a 1 × saline sodium citrate (SSC) (1 × SSC = 150 mM NaCl, 15 mM sodium citrate).

Results and Discussion

Mechanism of DNA detection of diamond SGFET and optimization of boron-doped polycrystalline diamond layer for deoxyribonucleic-acid-sensitive SGFET sensor

A diamond SGFET detects a characteristic shift due to the influence of charged molecules fixed on the channel surface. When charged molecules are immobilized on the gate surface, polar charges are localized near the gate. This electric-field-effect contributes to carrier change at the channel interface. As a result, the band in the diamond semiconductor is curved, and the carrier density changes. Since the carrier of the SGFET is a hole, the hole density decreases when positively charged molecules are fixed and, conversely, when a negatively charged molecule is fixed, the hole density increases. This change in hole density can be measured as a current shift or threshold voltage shift of FET. Since the polycrystalline diamond possesses physical/chemical stability and a high resistance to heat/pressure,²⁵ the boron-doped polycrystalline diamond SGFET sensor could operate stably even in harsh physical or chemical environments. A 0.37-eV acceptor level could lead the carrier density of a low-concentration boron-doped polycrystalline diamond to be one-digit smaller than the non-doped hydrogen-terminated diamond. The activation rate increases by raising the doped boron concentrations, and a percentage of the activation may reach *ca.* 100% at $3 \times 10^{20} \text{ cm}^{-3}$.²⁶ The carrier is induced by hydrogen-termination adjacent to the domain, which is less than 10-nm depth from the surface. In addition, an effective modulation is observed when the sheet carrier density is in the range from 1×10^{13} to $5 \times 10^{13} \text{ cm}^{-2}$. Modulation-free is observed when the sheet carrier density is above $1 \times 10^{14} \text{ cm}^{-2}$. Therefore, a carrier density above $3 \times 10^{20} \text{ cm}^{-3}$ and a sheet carrier density up to 1×10^{14} are required for a boron-doped polycrystalline diamond SGFET. The boron-doped layer thickness required for modulation to be observed and the percentage of activation to reach 100% is calculated as follows:

$$(1 \times 10^{14} \text{ cm}^{-2}) / (3 \times 10^{20} \text{ cm}^{-3}) = 3.3 \text{ nm}.$$

For the characterization of CVD-deposited boron-doped polycrystalline diamond films, three samples (samples A, B, C) were prepared under different conditions shown in Table 1. The boron layers of the samples were examined by secondary ion mass spectroscopy (SIMS). According to the SIMS charts of samples A, B, C, the boron-doped polycrystalline diamond film of the sample A was not well-synthesized; the boron-doped layer of samples B and C was *ca.* 5 nm depth and above 50 nm depth, respectively. The carrier concentrations of samples B and C were *ca.* $1.5 \times 10^{19} \text{ cm}^{-3}$ and *ca.* $2 \times 10^{19} \text{ cm}^{-3}$,

Fig. 2 C1s XPS spectra of diamond surface treated by VUV oxidation.

respectively, so that the sheet carrier density of samples B and C was calculated to be $3.3 \times 10^{13} \text{ cm}^{-2}$ and $4.0 \times 10^{13} \text{ cm}^{-2}$, respectively. For DNA FET sensor, sample B was used.

VUV treatment to obtain a high-density carboxyl surface on the boron-doped polycrystalline diamond

In our previous studies, the hydrogen-terminated diamond surface was transferred to partially oxygen-terminated diamond using hot mixed acid (sulfuric acid:nitric acid = 1:1) or ultraviolet (UV) irradiation in the presence of O₂ gas,²² where the density of oxygen-functional groups reached *ca.* 30% calculated by XPS. The results suggested that the major functional group was a hydroxyl group, and the percentage of reactive carboxyl-groups available to bind ss-DNA was less than 1%. In the case of using a UV treatment with a mercury lamp (185/254 nm) under the 100% O₂ gas condition, O₃ gas was generated by the emission of 185-nm UV. However, the O₃ was re-decomposed by the absorption of 254-nm UV light. By using a VUV system, the formed O₃ gas could exist for a relatively long period because of no 254-nm emission line in the chamber,^{15,16} and a higher density of carboxyl groups could be formed due to the stronger oxidizing conditions; therefore, VUV was, in this study, applied for oxidation of the boron-doped polycrystalline diamond surface. C1s XPS spectra of diamond surface treated by VUV oxidation is shown as Fig. 2. The calculated density of carboxyl-groups was *ca.* 2.3%, which was sufficient for DNA immobilization.

Characterization of boron-doped polycrystalline diamond SGFET

The device properties of partially carboxyl-terminated boron-doped polycrystalline diamond SGFET was characterized as shown in Fig. 3. The SGFET with a Ag/AgCl reference electrode was immersed in a 1 × PBS buffer solution (pH 7.4), and its I-V characteristics was obtained in two ways: $V_{\text{gs}}-I_{\text{ds}}$ characteristics shown in Fig. 3(a) and $V_{\text{ds}}-I_{\text{ds}}$ characteristics shown in Fig. 3(b). The obtained I_{ds} correspond to $(V_{\text{gs}} - V_{\text{th}})^2$, conforming to the metal-oxide semiconductor field-effect transistor (MOSFET) theory, where V_{th} is the threshold voltage. Both I-V profiles clearly indicate that the drain current was pinched off, and also indicate that I_{ds} increased as V_{ds} increased. The on-off ratio was 10^3 in the case of varying the gate voltage from 0 to -1.4 V. The repeatability of FET I-V was also examined as shown in Fig. 4. The average and coefficient of variation (C.V.) at $V_{\text{ds}} = -0.2$ and -0.5 V were 0.8 $\mu\text{A/mm}$ and 11%, and 1.9 $\mu\text{A/mm}$ and 4%, respectively.

Fig. 3 Device properties of boron-doped polycrystalline diamond electrolyte-solution-gate field-effect transistor in a 1 M phosphate buffered saline (pH 7.4). (a) V_{gs} - I_{ds} characteristics at V_{ds} in the range of -0.2 to -1.0 V. (b) V_{ds} - I_{ds} characteristics at V_{gs} in the range of 0 to -1.4 V.

Characteristics of a DNA-sensitive polycrystalline diamond SGFET and detection of a single nucleotide mutation of DNA sequence

A probe DNA was immobilized on the surface of the partially carboxyl-terminated boron-doped polycrystalline diamond. In this study, direct immobilization was achieved *via* the carboxyl-group to simplify the process. The I-V characteristics of the DNA-immobilized SGFET were examined as shown in Fig. 5. Although DNA probes were immobilized on the polycrystalline diamond channel, the drain current was successfully pinched off, just the same as no-DNA-immobilized diamond SGFET described above. I_{ds} increased as V_{gs} increased, according to MOSFET theory. Next, a deoxyribonucleic-acid-sensitivity was examined using 1-, 10-, 100-nM complementary strand DNAs (comp.-DNA) and a single nucleotide mutation of the DNA sequence (1NM-DNA). The change of SGFET I-V between hybridized and dehybridized states was evaluated, as shown in Fig. 5. The shift voltages of 1-, 10-, and 100-nM DNA were 19, 39, and 42 mV, respectively. The DNA-immobilized SGFET sensor *via* carboxyl termination on the diamond surface was applied for the detection of a 1NM-DNA. The results are shown in Fig. 6. The voltage changes when 10-nM comp.-DNA was retained was 38 to 42 mV, and the voltage change due to single base mutation DNA was 5 mV. The surroundings in the DNA involved by Na^+ ions (counter ions) in the solution that neutralized phosphate groups (negative ions) in the DNA strand. When the amount of Na^+ in the vicinity of the surface was increased by hybridization of DNA, the surface density of the hole in the surface conduction layer was lowered, because a repulsive force on the surface of the carboxyl terminal surface was generated. Therefore, it is considered that a shift in the

Fig. 4 Repeatability of the current-voltage characteristics between $V_{ds} = -0.2$ and -0.5 V. The average and coefficient of variation of $V_{ds} = -0.2$ and -0.5 V are $0.8 \mu A/mm$ with 11% and $1.9 \mu A/mm$ with 4%.

threshold voltage in the negative direction can be obtained on a surface where the surface is negatively charged, such as a partial fluorine-terminated surface or a carboxyl-terminated surface.

For a stability evaluation, changes in the DNA retention characteristics after 2 weeks were evaluated. After maintaining 10-nM comp. DNA for 2 weeks, the comp. DNA was dissociated. The voltage shifts due to the retention of comp. DNA and 1NM DNA after 2 weeks were 37 and 11 V, respectively. From comparisons at the time of manufacture and after 2 weeks, stable sensor characteristics were shown with no decrease for practical use.

Conclusions

In this study, we developed a DNA-sensitive polycrystalline diamond SGFET sensor utilizing partial carboxyl groups on a polycrystalline diamond surface as a linker to connect the DNA probe. The hydrogen-termination on as-grown polycrystalline diamond was successfully transferred to partial carboxyl-termination that was coupled with the N-terminal of cc-DNA. The shifted FET voltage in response to hybridization/denaturation of complementary strand DNA in the concentration of 1-, 10- and 100-nM was successfully detected. In addition, the single nucleotide mutation of DNA sequence was selectively recognized. Future challenges will be addressed in the miniaturization of SGFET and the thinning of the thickness of the boron-doped polycrystalline diamond layer to be higher sensitivity/stability.

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Fig. 5 Output profiles of the detection of DNA hybridization/dehybridization. The shift voltages of 1- (a), 10- (b), and 100- (c) nM DNA were 19, 39, and 42 mV, respectively.

Fig. 6 Stability of developed DNA-sensing SGFET. Even after 2 weeks, no appreciable decrease in DNA-detection sensitivity was observed for boron-doped carboxyl-terminated SGFET.

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