Biosensors and Bioelectronics 26 (2010) 1599–1604

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Aptamer-based biosensor for sensitive PDGF detection using diamond transistor

A. Rahim Ruslinda[a,*], Shinya Tajima[a], Yoko Ishii[a], Yuichiro Ishiyama[a], Robert Edgington[b], Hiroshi Kawarada[a]

[a] School of Science and Engineering, Waseda University, 3-4-1 Okubo, Shinjuku-ku, Tokyo 169-8555, Japan
[b] London Centre for Nanotechnology, University College London, Bloomsbury, London WC1H 0AH, England, United Kingdom

ARTICLE INFO

Article history:
Received 14 April 2010
Received in revised form 30 July 2010
Accepted 2 August 2010
Available online 7 August 2010

Keywords:
Aptamer
Diamond transistor
Platelet-derived growth factor
Biosensor

ABSTRACT

The detection of platelet-derived growth factor (PDGF) via a solution-gate field-effect transistor (SGFET) has been demonstrated for the first time using aptamers immobilized on a diamond surface. Upon introduction of PDGF to the immobilized aptamer, a shift of 31.7 mV in the negative direction is observed at a source-drain current of −50 μA. A shift of 32.3 mV in the positive direction is detected after regeneration by SDS solution, indicating that the static measurement returns to its original value. These SGFETs operate stably within the large potential window of diamond (>3.0 V), and hence the surface channel does not need passivating with a thick insulating layer. Thereof, the immobilized aptamer channels have been exposed directly to the electrolyte solution without a gate insulator. Immobilization is achieved via aptamers covalently bonding to amine sites, thereby increasing the sensitivity of the biosensors. Diamond SGFETs have potential for the detection of PDGF and show durability against biological degradation after repeated usage and regeneration.

1. Introduction

For the rapid diagnosis of genetic diseases and fabrication of analytical systems there has been a great deal of research efforts into the development of new devices for the detection of specific DNAs (Adrega et al., 2006). Aptamers are single-stranded DNA or RNA molecules that have been isolated from large pools of random-sequence oligonucleotides. They are capable of binding with small molecules (Baker et al., 2008; Radi et al., 2006), proteins (Cai et al., 2006; Le et al., 2006; Radi et al., 2006) and cells (Shangguan et al., 2007) with high affinity and specificity. In some cases, their selectivity is higher than that of an antibody (Nimjee et al., 2005). In addition to their considerable target diversity and tight-binding capability, aptamers are easy to synthesize and have a high stability and long shelf life, which makes them ideal alternative candidates for molecular recognition elements in bioassays as well as protein arrays (Hamula et al., 2006).

Platelet-derived growth factor (PDGF) is a protein that regulates tumor growth and division, and PDGF protein sensing has been reported using fluorescence (Yang et al., 2007; Fang et al., 2003), nanoparticles (Huang et al., 2005) and electrochemistry (Lai et al., 2007; Degefa and Kwak, 2008). The reported methods involve either labeling the aptamer with a reporter or using an aptamer–primer complex as part of the sensing element; however, in recent years label-free protein sensors have started to attract considerable attention (Willner and Zayats, 2007). In this approach the aptamer is assembled on an electrode surface to act as a sensing layer and the changes at the interface before and after binding of the protein allow protein sensing.

In this study we demonstrate a diamond solution-gate field-effect transistor (SGFETs) on a partially amine-terminated and conductive p-type hydrogen terminated diamond surface. The device is analogous to an FET-type sensor, such as a silicon-based ion-sensitive FET (ISFET), except that it does not require the gate insulator of a silicon-based ISFET (Si_3N_4 or Al_2O_3). Gate insulators prevent the intrusion of ions from an electrolyte solution onto a gate channel, and stop the generation of unwanted faradaic currents, however ions intrude into the gate passivation and deteriorate the electric properties of FETs. A major concern in the design of ISFETs is therefore the passivation, which must prevent ions from penetrating into the rest of the circuitry. Furthermore, a problem with current Si ISFETs multi-layered gate dielectrics such as $Si_3N_4/SiO_2/Si$, is that the sensitivity of surface potential change is reduced by the thick dielectrics. In contrast in SGFETs the diamond surface channel is directly exposed to the electrolyte solution (Kawarada et al., 2001), which leads to the higher sensitivity because electric double-layer capacitance plays a role for gate insulator. Additionally, the hysterisis characteristics of diamond SGFETs have proven to be lower than other gate dielectrics such as Si_3N_4 and Al_2O_3 (Sasaki and Kawarada, in press) meaning that no insulator is needed on the gate region due to the lack of faradaic

* Corresponding author. Tel.: +81 3 5286 3391; fax: +81 3 5286 3391.
E-mail addresses: ruslinda@toki.waseda.jp, ruslindarahim@gmail.com (A.R. Ruslinda).

0956-5663/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2010.08.002

currents. This unique and simple structure of the diamond SGFET is only possible due to the exceptional electrochemical properties of diamond, such as its wide potential window and low background current, which are independent of the p-type surface accumulation layer on the functionalized diamond surface. By taking advantage of these properties, diamond SGFETs (Kuga et al., 2008; Song et al., 2003) can overcome passivation layer limitations and simplify biosensor design for electrochemical detection on solid surfaces (Sakata and Miyahara, 2005). It has been previously shown that DNA detection requires a close proximity to the gate surface of the transistor because the signal amplitude is dependent upon the distance between the DNA molecules and the gate surface (Uslu et al., 2004). Moreover, SGFET devices require the elimination of non-specific signals from the physical adsorption of DNA, which can be achieved by the tailoring of the hydrophobicity of the transistor surface (Allahvedyan et al., 2006). Diamond SGFETs can be easily fabricated and can be used to detect surface-charge changes, such as PDGF binding on aptamers with high sensitivity.

Most recently, we developed a process for using TiO_2 as an encapsulation layer for the source and drain regions. The titanium showed to be a good material on diamond because the titanium reacts with diamond to form a TiC layer which is an ohmic contact. Therefore, this advantage of titanium and diamond allows the fabrication an encapsulating layer on top and ohmic contact on bottom. In this study, we focused mainly on aptamer and protein reaction on functionalized diamond surface but the use of TiO_2 as an encapsulation layer is out of scope of this paper and will be reported separately.

2. Experimental

2.1. Chemicals and oligonucleotides

PDGF binding aptamer (5′-CAG GCT ACG GCA CGT AGA GCA TCA CCA TGA TCC TG-3′), PDGF-BB, PDGF-AB and PDGF-AA were purchased from Sigma Genosis Company (Hokkaido, Japan). PDGF can be assembled in atleast three isoforms: heterodimers PDGF-AB and homodimers PDGF-BB and PDGF-AA. The aptamer which has an affinity to B chain is used in this experiment (B-aptamer). The 5′ ends of the PDGF binding aptamer were terminated with a carboxyl group. This carboxyl group can covalently immobilize the DNA to an amine-terminated diamond surface directly without linker molecules. In the SGFET measurements, common probe DNA was immobilized on the surface channel, after which the SGFET I_{ds}–V_{gs} characteristics were measured (the change in the gate potential).

2.2. Synthesis of partially functionalized diamond surface

Polycrystalline diamonds purchased from Element Six Co. Ltd. were used in this study. These are free standing, transparent diamonds (optical grade) with a thickness of 300 μm and a large grain size (~100 μm) grown by the chemical vapor deposition method. Their surfaces were sufficiently flat for fabrication of high performance FETs with a high cut-off frequency (Hirama et al., 2008). These substrates were H-terminated using a hydrogen plasma. At room temperature, the sheet resistance and carrier concentration of these substrates were 10–20 kΩ/square and $1–3 \times 10^{13}$ cm^{-2} respectively as determined by direct current Hall effect measurements (Song et al., 2006; Kuga et al., 2008). Partial surface amination of the H-terminated diamond was performed by irradiation with UV light. These procedures were performed at room temperature and atmospheric pressure, allowing the modification to be performed in a short time. This did not cause damage to the diamond substrate, because the modification occurs very slowly. In this experiment, an amination of 18% of the diamond surface was achieved, as determined by X-ray photoelectron spectroscopy (XPS).

2.3. Detection of PDGF using the SGFET diamond transistor

SGFETs were fabricated as follows: source and drain electrodes were deposited onto H-terminated diamond with a metal mask using a 150 nm thick film of Au by thermal evaporation. Then Ar ion was implanted (acceleration voltage 25 keV, ion density 2×10^{-14} cm^{-2}) through another metal mask to form an insulating region outside the metal electrodes and channel/gate region. The dosed region was highly resistive and did not form graphitic defects (that can be found at ion densities greater than 10^{16} cm^{-2}) and exhibited as expected electrochemical properties. Wires were bonded to the drain and source electrodes using electroconductive paste and were covered with insulating epoxy resin to protect them from the electrolyte solution. In common silicon devices we normally used the back gate structure. The bulk electrode is not used in this experiment because diamond is an insulating material and the surface channel SGFET is electrically isolated from the bulk like silicon on insulator (SOI). The channel was exposed directly to the electrolyte solution. The length and width of the gate channel were 500 μm and 8 mm, respectively. The static characteristics of the SGFET were determined in 1 mM PBS (pH 7) using an Ag/AgCl reference electrode as the gate electrode. Drain current (I_{ds}) as a function of gate voltage (V_{gs}) was determined at a constant drain-source voltage (V_{ds}) of −0.1 V. The B-aptamer was immobilized on surface channel at 38 °C for 2 h in a humidity-controlled chamber to prevent the droplets drying out. PDGF was bound to the aptamer via a 1 h incubation at 25 °C. Regeneration was carried out by sodium dodecyl sulfate (SDS) solution. In each step, the static characteristics measurement was determined and compared.

3. Results and discussion

3.1. Detection of PDGF using SGFETs

The structure of a SGFET is similar to that of a Si MOSFETs except that the inversion layer of the MOSFET is replaced by a p-type surface accumulation layer (holes) in a SGFET of a H-terminated (conductive) diamond surface. In the SGFETs, a space-charge region is only composed of accumulation layer of the holes, unlike Si MOSFET where the inversion layer coexists with the depletion region. Electric double-layer capacitance plays a role for gate insulator. The SGFET detects the electrical charge of the molecules near the surface channel and since the dominant carrier is holes in diamond SGFETs, the hole concentration decreases or increases near the surface with the approach of positively or negatively charged molecules, respectively. In principle the carrier density of the surface channel is affected by the electrical charge of the molecules in its proximity. When negatively charged molecules such as DNA approach the surface the I_{ds}–V_{gs} characteristics shift in the positive direction (Song et al., 2006; Kuga et al., 2008) and the result is reversed for the binding of positively charged molecules (Fig. 3).

The charge distribution in parallel capacitances in diamond SGFETs is discussed as shown in Fig. 1. The equivalent circuits of the FET with electric double-layer capacitance (C_{dl}) and surface channel capacitance on the solid side (C_i) are shown. When target biomolecules are bound with the aptamer the charge decrease ($+\Delta Q_{PDGF}$) appears between two capacitances and is reflected in the positive charge decrease on the two sides according to the ratio between two capacitances C_i and C_{dl} in a solution. The decreased charge carrier density from aptamers binding with PDGF is shown

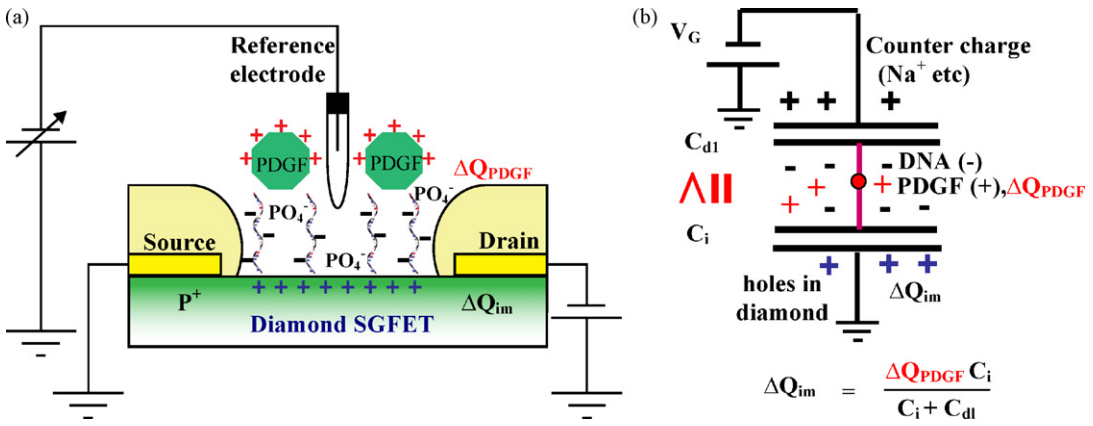

Fig. 1. (a) The static device characteristics of diamond SGFET by PDGF binding with aptamer and the mechanism of detecting signal depending on the surface-charge change. (b) C_i represents the surface capacitance of solid surface, C_{dl} represents the electric double-layer (liquid side) capacitance, ΔQ_{im} represents a decreased carrier density by PDGF reaction with aptamer and ΔQ_{PDGF} represents a decreased negative charge by PDGF reaction with aptamer.

as follows.

$$\Delta Q_{im} = \frac{\Delta Q_{PDGF} C_i}{C_i + C_{dl}} \quad (1)$$

In diamond SGFETs, the effective thickness of the gate insulator is very thin because the surface channel is exposed directly to the solution without an insulating layer, and so the charge of PDGF has a larger effect on the diamond surface channel. Here we estimate the capacitance of the solid side (C_i) on the diamond surface channel based on the current–voltage characteristics of metal oxide semiconductor field effect transistor (MOSFETs). This is because the characteristics of the SGFETs are equivalent to those of MOSFETs. The saturated drain current of MOSFET is shown in Eq. (2).

$$IDS = \frac{1}{2}\mu C_{Tot}\frac{W}{L}(V_{gs} - V_T)^2 \quad (2a)$$

$$C_{Tot} = \frac{C_i C_{dl}}{C_i + C_{dl}} \quad (2b)$$

As shown in Eq. (2b), C_{Tot} is a serial sum of capacitance an electric double-layer capacitance (C_{dl}) on the liquid side and an insulating layer on the solid side (C_i) of the surface channel in solution, as shown in Fig. 1. From the device characteristics (I_{ds}–V_{gs}) as shown in Fig. 3, the capacitance is calculated for the solid side (C_i) of the diamond SGFETs using Eq. (2). When the mobility (μ) of the polycrystalline diamond surface is ~10 $cm^2\ v^{-1}\ s^{-2}$, channel width (W) is 8 mm and channel length (L) is 500 μm, solid side capacitance C_i is calculated to be about 5 $\mu F/cm^2$ from Eq. (2). Assuming C_{dl} = ~5 $\mu F/cm^2$ (Swain and Ramesham, 1993), C_{Tot}, is 2.5 $\mu F/cm^2$. Furthermore, the value of $\Delta Q_{im}/\Delta Q_{PDGF}$ from Eq. (1) is 0.5. Therefore, the charge of the aptamer binding with PDGF on the surface channel of diamond SGFET is changed by at least 50%.

Aminated diamond surfaces (NH_3^+) are positively charged. As shown in Fig. 2, a shift of 13.2 mV in the positive direction was observed when the aptamer is immobilized on an aminated diamond surface. Therefore based on the surface-charging effect, we can expect that the negatively charged DNA molecules are selectively adsorbed on the positively charged surface.

The positively charged PDGF was successfully detected by the SGFET. The static I_{ds}–V_{gs} characteristics of the SGFET during three steps are shown in Fig. 3, namely, (1) aptamer immobilization on the channel surface, (2) PDGF binding to the aptamer, and (3) SDS regeneration. Following the introduction of PDGF, a shift of 31.7 mV in the negative direction was observed at a source-drain current of −50 μA. After regeneration by SDS, a shift of 32.3 mV in the positive direction was detected. This indicates that the static characteristic returned to the original position even though the slope seems to change within experimental error. Since the isoelectric point of PDGF is 9.8, PDGF is positively charged under this experimental condition (pH 7). It is reasonable that PDGF binding is observed as a shift in the negative direction of the static I_{ds}–V_{gs} characteristic. Moreover, the device showed stable operating characterization of PDGF detection by aptamers.

3.2. Confirmation of reusability of diamond SGFETs

The measurement was reproducible and was performed repeatedly to confirm the high selectivity and sensitivity of detecting PDGF on the partially aminated diamond surface. Fig. 4 shows the sequential shift in gate potential at I_{ds} = −40 μA upon PDGF binding to the aptamer. The gate voltage shifted repeatedly from 27 to 32 mV in four cycles of PDGF binding and releasing. This reversible shift of gate potential is paramount for a reusable biosensor. The effective reflecting of biochemical potential changes indicated that the partial functionalization and direct immobilization of probe aptamers on the diamond surface without linker molecules yielded a SGFET suitable for detecting PDGF. The sensitivity of FET-based sensors is strongly related to the properties of the surface channel, such as channel mobility, capacitance on the gate surface, and threshold voltage.

As the Debye length of buffer solution of 1 mM/L NaCl solution was 9.6 nm and the length of the PDGF is 2.5 nm, and PDGF on immobilized aptamer (~4 nm) were within the Debye length.

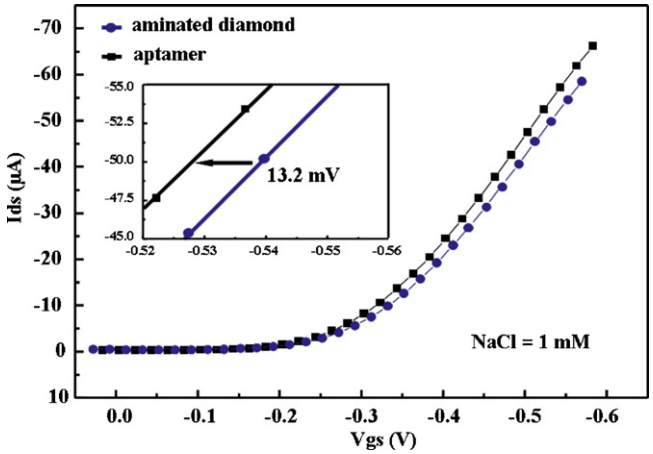

Fig. 2. The comparison of I_{ds}–V_{gs} characteristics between aminated and aptamer immobilization on diamond surface.

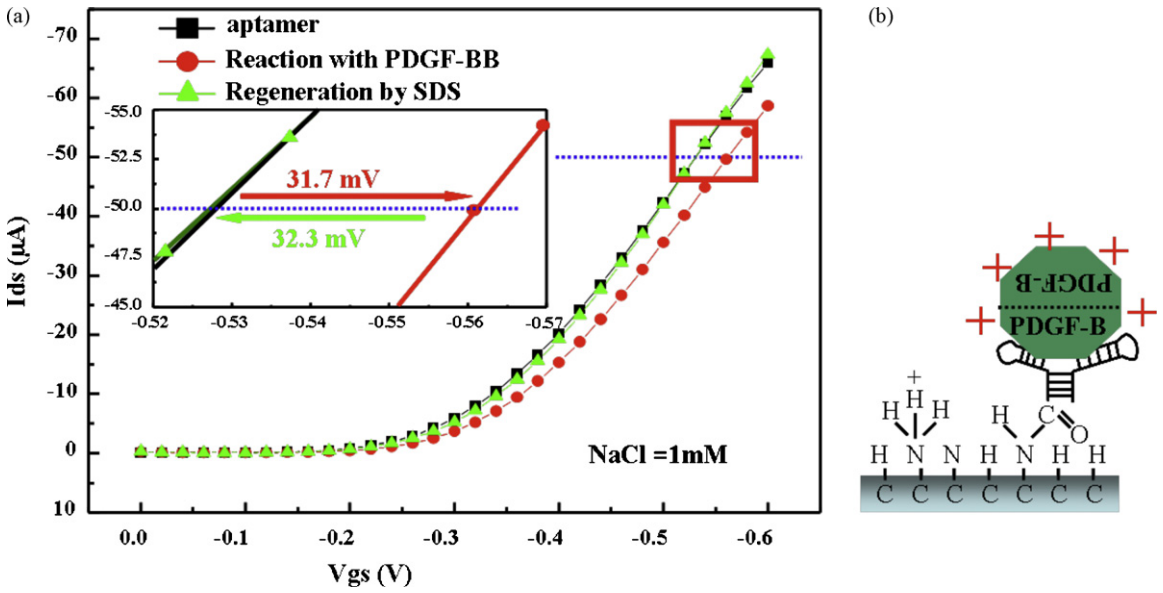

Fig. 3. (a) The directly exposed surface channel is very sensitive to surface charge on diamond SGFETs. After aptamer binding with PDGF on the functionalized surface channel, the surface negative charge decrease and hole carrier density also will decrease due to the decreased negative charge on the surface channel. With the introduction of PDGF, a 31.7 mV shift in the negative direction was observed. After the removal of PDGF (regeneration) by SDS, the voltage shift was 32.3 mV in the positive direction. (b) The schematic showing the procedure for detecting PDGF-BB on functionalized diamond surfaces by potentiometric method.

The SGFET could detect the positive charge of PDGF where the shift voltage was observed. However, when the measurement was carried out in a buffer solution of 100 nM/L NaCl with a Debye length 0.96 nm smaller than the size of PDGF, the gate voltage remained unchanged (data not shown). In addition, the detection method using the aptamer is suitable for FET-based biosensors. The typical size of an antibody is relatively large such as 10 or 15 nm. Thus, when an antigen is used as a probe of the sensor, the antigen antibody complex can easily exceed the Debye length. Since the target aptamer complex is located in a relatively fixed position within the Debye length precise aptamer-based detection can be realized.

3.3. High selective detection of PDGF by SGFETs measurement

Platelet-derived growth factor (PDGF) is a dimeric glycoprotein composed of either two A (-AA) or two B (-BB) chains, or a combination of the two (-AB). The aptamer has very high specificity toward PDGF-BB (a dimer composed of two different types of monomer – A and B chains), and therefore the three variants were investigated using SGFETs. It has been shown that the aptamer used here binds to these variants with different shift voltages and Fig. 5 shows the different shift voltages for the different types of PDGF. A smaller shift in voltage was seen in PDGF-AA (an isoform of PDGF-BB) than that in PDGF-BB (Fig. 5(c)) which shows this sensor can distinguish

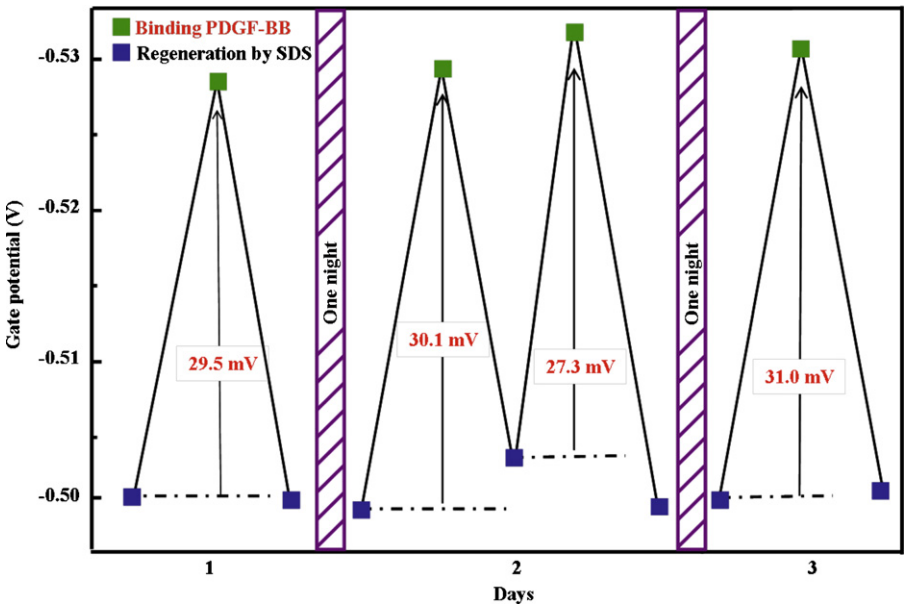

Fig. 4. Transfer characteristics of a SGFET over four cycles in PDGF binding to the aptamer and regeneration were performed repeatedly on functionalized diamond surfaces. The gate potential shift was observed at I_{ds} = −40 μA. The reproducible measurement yielded a SGFET is suitable for FET-type protein sensors.

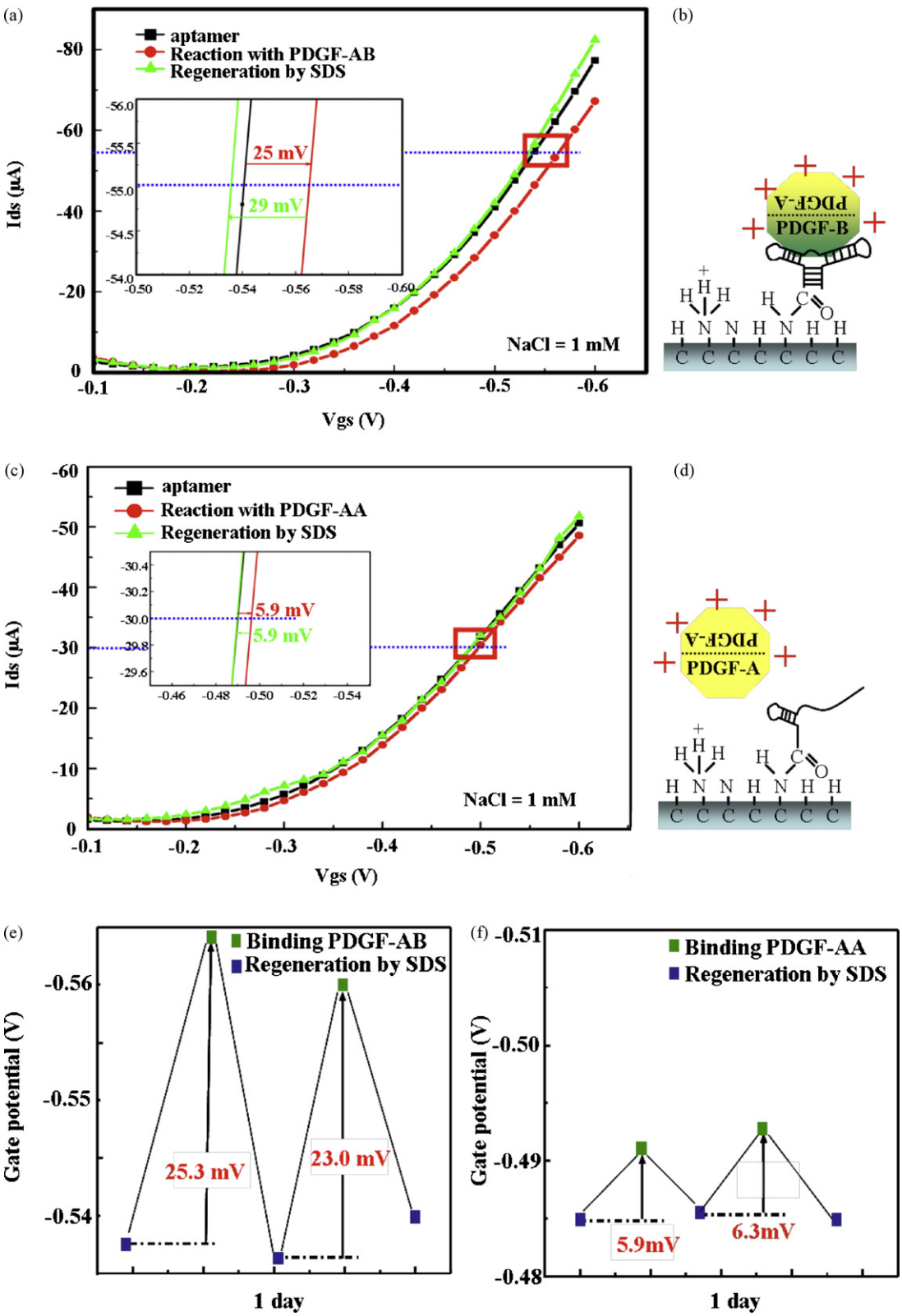

Fig. 5. Comparison the sequential changes of gate potential and drain current in PDGF binding with immobilized aptamer of the PDGF-B chain (PDGF-B) with other growth factors, such as the PDGF isomers PDGF-AB (a) and PDGF-AA (b). To determine the non-specific binding probability, the transfer characteristics of the SGFETs on diamond surface over two cycles detection was observed in reproducibility shown in (e) and (f), respectively, which shows that non-specific binding is minimal.

isoforms with high selectivity; however, in the case of PDGF-AB, a shift of 25 mV relative to PDGF-BB was observed. This shift voltage value is closer to PDGF-BB. PDGF-AB consists of A and B; it has one site that binds to the aptamer. In the case of B site attached to the probe aptamer, PDGF is confirmed to bind with aptamer as shown in Fig. 5(b). In contrast, the A site (Fig. 5(d)) has no affinity towards the probe aptamer and the shift voltage is caused by B site only. Since the structures of A and B chains have 40% mismatched part,

the shift voltage of 20–30 mV cannot be obtained (Fig. 5(c)). The fact that the A chain is more acidic than the B chain may cause its lower affinity.

The isoforms have similar structures to our target however the SGFET exhibits selectivity between different PDGF isoforms, which shows promise for selectively detecting other proteins. The alternating measurement of PDGF-BB and PDGF-AA is due to be further investigated. The concentration of PDGF has been varied from 100 to 1 nM and the shift voltage has not been observed, however it is too early to say what is the detection limit of PDGF by using diamond SGFET. To determine the probability of non-specific binding, the reproducibilty of PDGF-AB and AA has been performed as shown in Fig. 5(e) and (f). It shows that the reversible shift of gate potential in PDGF-AB and PDGF-AA is observed and stable, which demonstrates that non-specific binding is minimal.

4. Conclusions

The high resolution and label-free potentiometric sensing of PDGF using an aptamer immobilized on a diamond SGFET has been described. The SGFET platform does not require light sources, optics, high-voltage power supplies or other potentially heavy and cumbersome equipment. Using the diamond SGFET, the shift in the gate potential caused by the intrinsic charge of PDGF is observed by evaluating static electrical characteristics measurements. The stability of functionalized the SGFET is also confirmed by the stable oscillations of gate potential through repeated detection. The detection of PDGF at high resolution was achieved through controlling the surface chemistry of the diamond SGFET. This method of biosensing provides a very simple and promising detection technique for both protein and small molecules.

Acknowledgements

This work was supported in part by a Grant-in-Aid from a Global Center of Excellence (GCOE) Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, and a Grant-in-Aid for Challenging Exploratory Research (21655071) of Japan Society for the Promotion of Science (JSPS).

References

Adrega, T., Prazeres, D.M.F., Chu, V., Conde, J.P., 2006. J. Non-Cryst. Solids 352, 1999.
Allahvedyan, A.E., Gevokian, Z.S., Hu, C.K., Nieuwenhuizen, T.M., 2006. Phys. Rev. Lett. 96, 98302.
Baker, B.R., Lai, R.Y., Wood, M.S., Doctor, E.H., Heeger, A.J., Plaxco, K.W., 2008. J. Am. Chem. Soc. 128, 3138–3139.
Cai, H., Lee, T.M.H., Hsing, I.M., 2006. Sens. Actuators B 114, 433–437.
Degefa, T.H., Kwak, J., 2008. Anal. Chim. Acta 613, 163–168.
Fang, X.H., Sen, A., Vicens, M., Tan, W.H., 2003. ChemBioChem 4, 829.
Hamula, C.L.A., Guthrie, J.W., Zhang, H.Q., Li, X.F., Le, X.C., 2006. Trends. Anal. Chem. 25, 681–691.
Hirama, K., Takayanagi, H., Yamauchi, S., Yang, J.H., Kawarada, H., Umezawa, H., 2008. Appl. Phys. Lett. 92 (11), 112107.
Huang, C.C., Huang, Y.F., Cao, Z.H., Tan, W.H., Chang, H.T., 2005. Anal. Chem. 77, 5735.
Kawarada, H., Araki, Y., Sakai, T., Ogawa, T., Umezawa, H., 2001. Phys. Status Solidi A 185, 79–83.
Kuga, S., Yang, J.H., Takahashi, H., Hiirama, K., Iwasaki, T., Kawarada, H., 2008. J. Am. Chem. Soc. 130 (40), 13251.
Lai, R.Y., Plaxco, K.W., Heeger, A.J., 2007. Anal. Chem. 79, 229.
Le, F.F., Ho, H.A., Leclerc, M., 2006. Anal. Chem. 78, 4727–4731.
Nimjee, S.M., Rusconi, C.P., Sullenger, B.A., 2005. Annu. Rev. Med. 56, 555.
Radi, A.E., Sanchez, J.L.A., Baldrich, E., O'Sullivan, C.K., 2006. J. Am. Chem. Soc. 128, 117–124.
Sakata, T., Miyahara, Y., 2005. ChemBioChem 6, 703.
Sasaki, Y., Kawarada, H., in press. J. Phys. D: Appl. Phys. 43.
Shangguan, D.H., Cao, Z.H.C., Li, Y., Tan, W.H., 2007. Clin. Chem. 53, 1153–1155.
Song, K.S., Sakai, T., Kanazawa, H., Araki, Y., Umezawa, H., Tachiki, M., Kawarada, H., 2003. Biosens. Bioelectr. 19, 137.
Song, K.S., Song, K.S., Zhang, G.J., Nakamura, Y., Furukawa, K., Hiraki, T., Yang, J.H., Funatsu, T., Ohdomari, I., Kawarada, H., 2006. Phys. Rev. E 74 (4), 041919.
Swain, G.M., Ramesham, R., 1993. Anal. Chem. 65, 345.
Uslu, F., Ingebrandt, S., Mayera, D., Bocker-Meffert, S., Odenthal, M., Offenhauser, A., 2004. Biosens. Bioelectr. 19, 1723.
Willner, I., Zayats, M., 2007. Angew. Chem. Int. Ed. 46, 6408–6418.
Yang, L.T., Fung, C.W., Cho, E.J., Ellington, A.D., 2007. Anal. Chem. 79, 3320.