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Capacitive Immunoaffinity Biosensor by Using Diamond-like Carbon (DLC) Electrode

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Abstract

A new capacitive biosensor based on chronoamperometry was developed by using diamond-like carbon (DLC) film as an electric isolating layer. The electrochemical properties of the DLC layered electrode were analyzed by using a cyclic voltammetry. The immunoaffinity layer was prepared by physically adsorbing the anti-horseradish peroxidase (HRP) antibodies on the DLC electrode, and the feasibility of this capacitive biosensor was tested by using HRP as a model analyte. The limit of detection (LOD) was measured to be 0.01 pg/mL, and the detection range was determined to range from less than 0.1 pg/mL to more than 1 ng/mL. The applicability of the new capacitive biosensor was demonstrated by detecting C-reactive protein (CRP) which is known as a biomarker for the inflammatory diseases.

Keywords: Capacitive biosensor, Diamond-like carbon (DLC), Chronoamperometry, Immunoaffinity

Introduction

Immunoaffinity biosensors have been used to analyze a target analyte in a complex mixture, and applied for medical diagnosis, environmental monitoring, and so on[1]. Usually, such biosensors are composed of two major parts which consists of an immunoaffintiy layer and a transducer: (1) The target analyte in a sample binds to the immunoaffinity layer by the highly specific interaction between antigen and antibodies. (2) The transducer converts the amount of the bound analyte into a quantitative electric signal. Various kinds of transducers have been developed for immunoaffinity biosensors based on electrochemical[2], optical[3,4], piezoelectric[5], and acoustic principles[6,7]. Until recently, the immunoaffinity biosensors based on capacitive measurement have been investigated for the point-of-care applications because of the high sensitivity and the relatively simple instrumentation[8]. Basically, the capacitive biosensors measure the capacitance change, which occurrs because of the specific binding of the analyte to the immunoaffinity layer, by using transducers based on the principles of impedance spectroscopy[9,10] and pulse amperometry[11,12]. Generally, the capacitive biosensors could be classified into two types according to the types of electrodes: (1) Capacitive biosensors with two parallel metal plates were reported and the capacitance was described as the following: $C=\varepsilon \varepsilon_0 A/d$, where ε represents the dielectric constant of the material between the plates, ε_0 the dielectric constant in vacuum, A the area of the plate, and d the distance between the plates[8,13]. As the distance between the two plates decrease due to the adsorption of the analyte, the capacity changed proportional to the concentration of analyte. The capacitive biosensor with an electric isolation and a very thin immunoaffintiy layer provides a more sensitive measurement. (2) The three-electrode-system composed of working, counter, and reference electrodes was generally used to avoid the bulk solution effect and the mechanical limitation to control the electrode distance[14,15]. In this electrode system, a change in capacitance by a chemical modification of the electrode-solution boundary was measured and the amplitude of the signal was dependent to the nature and coverage of the modification material. Since the capacitive signal was determined by measuring the non-faradaic current after applying electric pulses, the faradaic current due to the redox reaction between the electrodes and sample solution should be avoided[16]. Therefore, the immunoaffinity layer should be prepared to have the ability to maintain the electric isolation of the electrode for the capacitive measurement. So far, various kinds of capacitive biosensors with self-assembled monolayer (SAM) based on thiolated hydrocarbon molecules have been reported[12,14,15,17].

In this work, a capacitive biosensor was presented by using a thin diamond-like carbon (DLC) film electrode in order to maintain an electric isolation with high charge transfer resistance. Diamond-like-carbon (DLC) is a non-crystalline carbon with many fractions of diamond-like (sp^3) bonds, and it is also known as amorphous hydrogenated carbon (a-C : H). Properties

of the DLC layers could be changed by adding other elements, and the modified DLC layers have been applied to various engineering fields, such as magnetic hard disk coatings, wear-protective coatings, engine parts, razor blades, and biomedical coatings[18,19]. Additionally, the DLC layers were characterized to have excellent physical properties (high hardness, high elastic modulus) as well as chemical inertness to any acids or alkaline solutions, and organic solvents[20,21]. In this work, a thin DLC film electrode was applied to a capacitive biosensor as an electric isolating layer on a highly p-doped silicon electrode. The feasibility of the DLC electrode to the capacitive biosensor was analyzed by using cyclic voltammetric analysis and impedance spectrometry. The applicability of the capacitive biosensor based on DLC electrode to medical diagnosis was demonstrated by detecting C-reactive protein (CRP) which is known as a biomarker for inflammatory diseases[22].

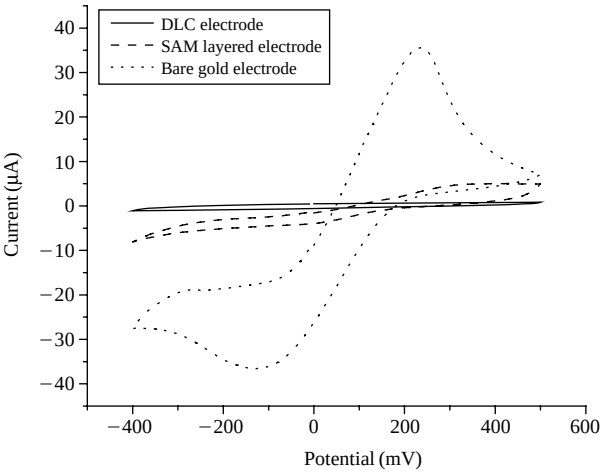

Figure 1. CV diagram of DLC electrode, SAM (UDT) layered electrode and bare gold electrode with the redox couple of 10 mM $Fe(CN)_6^{3-/4-}$. The scan rate was 10 mV/s. The redox peaks of $Fe(CN)_6^{3-/4-}$ can't be observed at the CV diagrams of DLC electrode and SAM layered electrode by the electric isolation effect through the DLC layer and SAM.

Results and Discussion

Electrochemical Property of DLC Electrode

As previously mentioned, the capacitive signal was determined by measuring the non-faradaic current after the application of electric pulses. Therefore, the faradaic current that might flow because of the redox reaction between electrodes and sample solution should be avoided[16]. For the investigation of such an electrochemical property of the DLC electrodes for capacitive measurement, CV analysis was performed by using DLC electrode, bare gold electrode, and SAM layered electrode with the redox couple 10 mM $Fe(CN)_6^{3-/4-}$. As shown in Figure 1, the CV diagram of bare gold electrode shows the redox peaks.

However, in the case of DLC electrode and SAM layered electrode, such redox peaks can't be observed, resulting from the electric isolation by the DLC layer and SAM. Additionally, the CV diagram showed that the DLC electrode itself carried out no redox reaction at the applied potential range. For the DLC electrode and the SAM layered electrode, the total charge transfer was calculated to be 11.9 mC, 19.5 mC by integrating the CV diagrams, respectively. In the case of bare gold electrode, the total charge transfer was calculated to be 50.5 mC. These parameters showed that the DLC film on the highly P-doped Si-electrode could isolate the electrode surface more efficiently than the SAM layered electrode as well as the gold electrode.

Capacitive Measurement by Using DLC Electrode

The chronoamperometric method was utilized to measure the capacitance change of the DLC electrode. In this method, the current profile was recorded as a function of time after an excitation potential (E2), and applied to the working electrode from a starting potential (E1) for a few milliseconds, and then returned to the starting potential (E1)[16]. In this work, a potential step of 50 mV was applied from 0 V for 10 ms and then returned to 0 V. The current decay was measured every 0.05 ms just after the potential step. The capacitance was analyzed by fitting the decaying current curve of chronoamperometry during the first 10 measurements based on the following formula: $i(t)=u/R_s \cdot \exp(-t/R_sC_d)$, where i(t) represents the current at time t, u the amplitude of the potential pulse applied, R_s the resistance of the solution, C_d the total capacitance measured at the electrode/solution interface, and t the time elapsed after the potential pulse was applied. To estimate the parameters, the formula was converted to have a linear relationship of ln i(t) and t as follows : $\ln i(t)=\ln(u/R_s)+(-t/R_sC_d)$. From this linear correlation, the capacitance value was evaluated by extrapolating the slope and y-intercepts[8,23]. According to the adsorption of analyte to the working electrode, only the C_d should change while the other parameters should be constant. As the immunoaffinity biosensors measured the amount of the adsorbed analyte to the immunoaffinity layer, several washing steps are usually followed with the incubation of the sample solution to remove the non-specifically bound proteins. In this work, the measurement was performed at the end of the washing step under the same solution, and the sig-

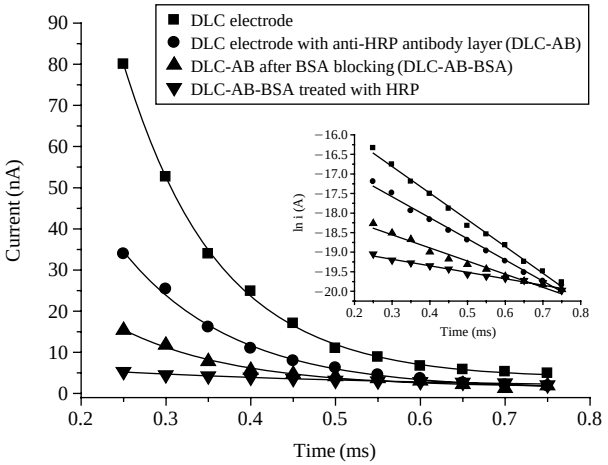

Figure 2. Chronoamperometric signal by the sequential protein layer formed on the DLC electrode. The protein layers of the anti-HRP antibodies, BSA blocking of non-specific binding and the analyte (HRP) were sequentially formed on the DLC electrode.

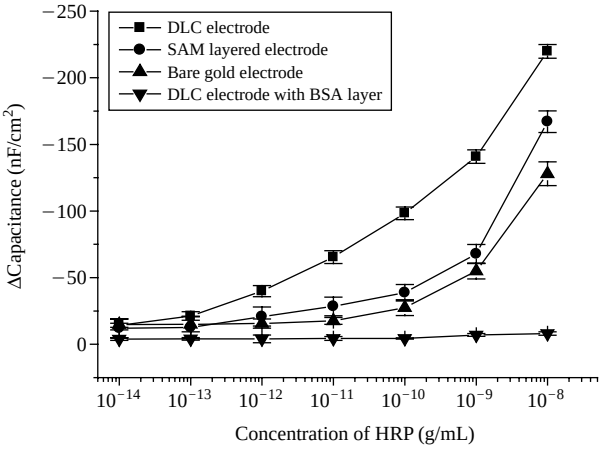

Figure 3. Capacitive detection of horseradish peroxidase (HRP) as a model analyte. The anti-HRP antibody layer was prepared on the DLC electrode, SAM (1-undecanethiol) layered electrode, bare gold electrode and BSA coated DLC electrode (negative control) by incubating the anti-HRP antibody solution, followed by BSA blocking. The error bar indicated the standard deviation of measurement (n=5).

nal was calculated as the difference with the previous measurement. Therefore, the capacitive signal difference could be considered to have resulted from the net-adsorption of the analytes binded to the immunoaffinity layer. Therefore, the capacitive change from the chronoamperometric measurement could correlate to the amount of adsorbed analytes to the working electrode.

The capacitive measurement was demonstrated during the preparation of the immunoaffinity layer on the DLC electrode by using chronoamperometry. As shown in Figure 2, the current decay curves were recorded during the immunoaffinity layer preparation composed of the sequential adsorption of anti-HRP antibodies, BSA blocking of non-specific binding, and analyte (HRP) binding. The decay curves were observed to be smoother as the adsorption increased, which could also have a correlation to the capacitance change. These results indicate that the DLC electrode could be effectively applied to the measurement of adsorption.

HRP was used as a model analyte to demonstrate the quantitative capacitive measurement. To compare the sensing parameters, the anti-HRP antibody layer was prepared on the DLC electrode, SAM (1-undecanethiol) layered electrode, bare gold electrode and BSA coated DLC electrode (negative control) by incubating anti-HRP antibody solution (10 μg/mL) followed by BSA blocking. The BSA coated DLC electrode was prepared as a negative control to measure the non-specific binding. Then, HRP samples with the concentration ranging from 0.01 pg/mL to 10 ng/mL were treated to each electrode, and the capacitive measurement was carried out by chronoamperometry.

As shown in Figure 3, the baseline drift of the measurement was evaluated to be less than 4.95 nF/cm^2 for the BSA coated DLC electrode (negative control). The LOD's of the DLC electrode, SAM layered electrode, and bare gold electrode was calculated to be less than 0.01 pg/mL, 0.1 pg/mL, and more than 10 pg/mL, respectively. The sensitivity of the DLC electrode was estimated to be higher than the other electrodes. These results illustrate that the capacitive detection of protein adsorption to the immunoaffinity layer could be effectively measured by using the DLC electrode at the analyte (HRP) concentration ranging from picogram to nanogram level.

Application of DLC Electrode for the Detection of CRP

The capacitive biosensors have been investigated for the point-of-care applications because of the high sensitivity and the relatively simple instrumentation[8]. In this work, the applicability of the capacitive biosensor to medical diagnosis was demonstrated by detecting C-reactive protein (CRP) which is used for the medical diagnosis of inflammatory diseases[22]. The immunoaffinity layer to detect CRP was prepared by incubating anti-CRP antibodies (10 μg/mL) for 2 hrs at room temperature, and then BSA blocking was performed as previously described in the method. To compare the sensing parameters, the same immunoaffinity layers were prepared on the SAM layered electrode, bare gold electrode and BSA coated DLC elec-

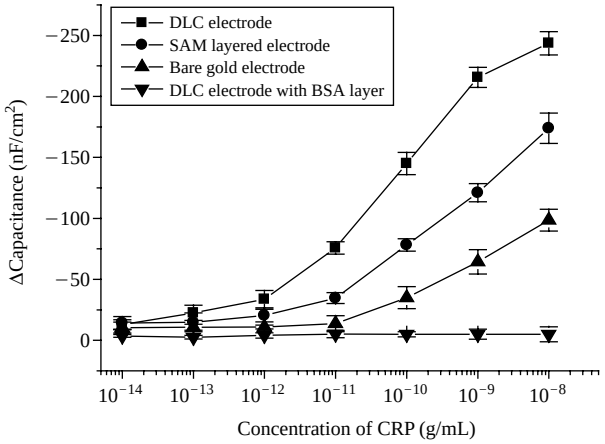

Figure 4. Capacitive measurement of CRP by using DLC electrode. The anti-CRP antibody layer was prepared on the DLC electrode, SAM (1-undecanethiol) layered electrode, bare gold electrode and BSA coated DLC electrode (negative control) by incubating the anti-CRP antibody solution, followed by BSA blocking. The error bar indicated the standard deviation of measurements (n=5).

trode (negative control).

As shown in Figure 4, the standard curves according to the CRP sample concentration were obtained through the capacitive measurement. The baseline drift of the measurement was evaluated to be less than 6.02 nF/cm^2 from the BSA coated DLC electrode (negative control). The LOD's for the DLC electrode, SAM layered electrode, and bare gold electrode was calculated to be less than 0.01 pg/mL, 0.1 pg/mL and more than 10 pg/mL, respectively. As shown in the detection which used HRP as a model analyte, the sensitivity of the capacitive biosensor based on the DLC electrode was estimated to be higher than the other electrodes at the tested CRP concentration. These results show that the capacitive biosensor with the DLC electrode could be effectively applied to detect protein analytes at the concentration of picogram level by using a suitable immunoaffinity layer.

Conclusions

A new capacitive biosensor based on chronoamperometry was developed by using diamond-like carbon (DLC) film as an electric isolating layer. For the capacitive measurement by using chronoamperometry, the electric isolation of electrodes from the liquid sample should be maintained during the immunoaffinity layer formation as well as during the repeated measurements. In this work, the diamond-like carbon (DLC) film layered on highly P-doped electrode was used for the electric isolation. The electrochemical properties of the DLC electrode were analyzed by using a cyclic voltammetry in comparison to SAM (1-undecanethiol) layered electrode and bare gold electrode. The immunoaffinity layer of anti-horseradish peroxidase (HRP) antibodies was prepared by physical adsorption and the feasibility of such capacitive biosensor was tested by using HRP as a model analyte. The limit of detection of the DLC electrode was determined to have improved more than 10-fold and 1000-fold higher than the SAM layered electrode and the bare gold electrode, respectively. The detection range was determined to range from less than 0.1 pg/mL to more than 1 ng/mL. The applicability to medical diagnosis was demonstrated by detecting C-reactive protein (CRP) in serum. The limit of detection and the sensitivity were determined to have improved far more compared to the SAM layered electrode and bare gold electrode. These results indicate that the capacitive biosensor with the DLC electrode could be effectively applied to detect a protein analyte having the concentration of picogram per mL by using a suitable immunoaffinity layer.

Materials and Methods

Materials

C-reactive protein (CRP), anti-CRP antibodies (polyclonal) were bought from AbCam (Cambridge, UK). Bovine serum albumin (BSA), horseradish peroxidase (HRP), anti-HRP antibodies (polyclonal), 1-undecanethiol and all of the other chemicals (of analytical grade) were purchased from Sigma-Aldrich Korea (Seoul, Korea).

Electrode Preparation

The diamond-like carbon (DLC) film with the thickness of 20 nm was deposited on a 4-inch highly P-doped Si wafer ($< 10\ m\Omega \cdot cm$) at Korea Institute of Science and Technology (KIST, Seoul). The Si wafer was cleaned by Ar ion plasma before the DLC deposition, and an a : Si buffer layer was deposited with the thickness of 2 nm using silane (SiH_4). The deposition of the DLC film with the thickness of 25 nm was carried on using 13.56 MHz radio frequency plasma-assisted chemical vapour deposition (RF-PACVD). The pressure gas used C_6H_6 with the flow rate of 20 sccm in order to make the working pressure to be 10 mtorr. The electrical contact made by the thin gold film layer having the thickness of 100 nm was deposited on the other side of the wafer with the DLC film. Then, the wafer was cut into electrodes with the area of $5 \times 10\ mm^2$ by using a wafer sawing system (Disco

Corp., Tokyo, Japan). The exposed surface area of the working electrode was adjusted to be approximately 5 mm^2 by using a passivation layer. A counter electrode with a gold layer with the thickness of 100 nm was made on the highly P-doped silicon wafer (< 10 $m\Omega \cdot cm$) which was cut into the size equal to that of the working electrode.

Instrumentation

A flow-cell type electrode-holder with an internal volume-capacity of 20 μL was made with flexi-glass. A sintered Ag/AgCl electrode with the diameter of 1 mm and the length of 5 mm from Warner instruments (CT, USA) was integrated on the electrode-holder as a pseudo reference electrode. The working electrode with DLC film and the counter electrode were combined into the electrode-holder. The liquid reagents were supplied to the electrode by using a peristaltic pump with the flow rate of 1 mL/min, and the sample was injected to the electrode by using a manual injection value from Rheodyne Co (CA, USA). For the chronoamperometric measurement, the electrodes were connected to a commercial potentiostat called compatstat from IVIUM Co (Netherlands).

Preparation of Immunoaffinity Layer

To estimate the applicability of the DLC electrode as a capacitive biosensor, an immunoaffinity layer to detect horseradish peroxidase (HRP) was prepared by physically adsorbing the anti-HRP antibodies (10 μg/mL). Then, BSA (1 mg/mL) was incubated to block any non-specific binding. As the DLC layer has a hydrophobic surface with the contact angle of 80° which is suitable for the physical adsorption of proteins, the immunoaffinity layer was prepared on the DLC electrode by physical adsorption. For the preparation of SAM layered electrode, 1-undecanethiol was chosen. The SAM was layered on the gold surface by incubating it in 10 mM 1-undecanethiol solution in ethyl alcohol overnight. Then, the anti-HRP antibodies were adsorbed to the SAM layer by the same procedure with that of the DLC electrode. To compare the capacitive signal, the same immunoaffinity layer was prepared by physical adsorption on a bare gold electrode, and then the electrode was treated with BSA solution (1 mg/mL) to block any non-specific binding.

Acknowledgements

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