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Label-free detection of alanine aminotransferase using a graphene field-effect biosensor

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ABSTRACT

In this paper, we developed a low operation voltage and a single reaction step of a graphene field-effect transistor (GFET) biosensor device to detect alanine aminotransferase (ALT). The device is fabricated using large-area graphene thin films by means of chemical vapor deposition. The GFET device operated at 0.1 V drain voltage and −0.1 to 0.3 V sweep gate voltage exhibited a high pH sensitivity of 23.12 mV/pH, a low hysteresis voltage of 1.2 mV, and a small drift rate of 4.74 mV/h. In this research, the hybrid configuration of L-alanine and α-ketoglutarate immobilized on the graphene membrane was proved to be able to detect ALT with good linearity (R^2 = 0.99) in the ALT concentration range of 10–100 U/L.

1. Introduction

Alanine aminotransferase (ALT), also called serum glutamate pyruvate transaminase (SGPT), is an enzyme that catalyzes the reversible transfer of an amino group from L-alanine to α-ketoglutarate. It normally resides in blood and body tissues, especially in the liver. It is released to the blood because of tissue injury; consequently, the level of ALT activity in the blood may be increased with acute damage to hepatic cells. Although not specific for liver disease, it can be employed in combination with other enzymes to diagnose the course of various liver disorders [1]. The normal concentrations of ALT in the blood are from 5 to 35 U/L. In addition, the blood of voluntary donation is carefully screened to protect patient safety in Taiwan. All donated blood was tested to be elevated in ALT levels by serological techniques [2]. The blood with high levels of ALT activity has to be discarded because they are unsuitable for the use in blood transfusion. Therefore, rapid and low cost devices for rapid blood screening of ALT activity in routine blood donation are required.

The search for label-free, high-sensitivity, and miniaturized sensors has recently caused a wide interest in nanoscale electrostatic and electrochemical sensors. Ion-sensitive field-effect transistor (ISFET) has been developed as an electronic sensor for rapid, real-time detection of ions and charged biomolecules in electrolytes [3–5]. The concept of such an ISFET device for electrical detection of chemical and biological species has also been shown to work using novel nanomaterial devices, such as silicon nanowires [6], carbon nanotubes [7] and graphene [8]. Methods for the electrical or electrochemical detection have provided highly sensitive detection of chemical and biological species for clinical genomics, diagnosis, and pharmacy applications, due to the surface–analyte or analyte–analyte bindings located close to the channel. An ISFET electrical measurement, by means of current–voltage (*I*–*V*) method, changes in its threshold voltage that can be correlated to the variation of surface charge density and potential induced by surface reactions with charged molecules at the gate oxide–electrolyte interface [9]. Graphene is attracting tremendous attention in nanoelectronics due to its excellent physical and electronic properties [10,11]. With its high carrier mobility, high saturation velocity, and large current density, graphene field-effect transistor (GFET) has been proposed as a promising candidate for sensitive and label-free detection of chemical and biological species [12]. GFET devices are a promising candidate to replace silicon-based semiconductor devices in integrated circuits because of their high carrier mobility and ability to maintain good performance low temperature [13,14]. In a GFET the graphene film acts as the semiconducting channel between the source and drain metal electrodes. When the charged biological molecules bind on the surface of the graphene film in the GFET, there is a measureable change in resistance. According to this detection principle, GFET device is able to act as a real-time biosensor for the detection of biological molecules [15–17]. In this

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paper, we developed a single reaction step GFET biosensor for ALT detection. We determined the sensing characteristics (pH sensitivity, hysteresis and drift rate) of the GFET pH sensor operated at very low drain and gate voltages. Finally, we demonstrate that the hybrid configuration of one-step biochemical reaction of GFET biosensor with an attached L-alanine + α-ketoglutarate-immobilized alginate hydrogel allows the detection of ALT.

2. Experimental

2.1. Preparation of GFET sensor

Large-area graphene monolayer film was grown by chemical vapor deposition (CVD) on 25 μm copper foil (Alfa Aesar) [18] and transferred onto the SiO_2/Si substrate (~300 nm-thick SiO_2). The chamber was evacuated to ~5 mTorr, and the temperature was increased from room temperature to 1030 °C. Before growth, a pretreatment step was performed under a H_2 atmosphere with 400 sccm flow at 500 Torr for 50 min. During the growth process, a gas mixture of methane and H_2 (CH_4/H_2 = 13 sccm/3 sccm) was introduced for 20 min, and the flow rate was kept at 300 mTorr. After 20 min of growth, the Cu foil was cooled to room temperature under Ar/H_2 environment. After the growth of CVD graphene films, a thin layer of polymethylmetha-acrylate (PMMA) was spin-coated on the Cu foils with as-grown graphene films. Subsequently, the samples were immersed into iron nitrate solution to remove the Cu foil. After etching off the Cu foil, the detached graphene film was transferred to a SiO_2/Si substrate, and then the PMMA was removed using acetone. Finally, the graphene on the SiO_2/Si substrate was washed using DI water and blow-dried gently with N_2 gas. The source–drain contacts to graphene were defined by lithography and subsequently metallization with 5 nm Ti/50 nm Au. GFET devices were then attached on the Cu lines of a printed circuit board by using a conductive Ag gel. A handmade epoxy package was used to encapsulate the GFET structure and the Cu line. The structure of the proposed GFET device is schematically shown in Fig. 1(a). Fig. 1(b) depicts the Raman spectra of the prepared single layer graphene with a laser excitation energy of 2.33 eV (532 nm). Two strong sharp peaks with a G band at ~1580 cm^{-1} and a 2D band at ~2700 cm^{-1} were observed, indicating the present studied graphene is single-layer graphene [19,20]. Small D-like peak that shows up at ~1400 cm^{-1} is originated from the carbon vacancies in the graphene films [21]. In this work, we used this single-layer graphene to fabricate GFET biosensor devices.

2.2. Fabrication of GFET ALT biosensor

To evaluate the performance of the GFET biosensor, ALT sensing was performed based on a conventional immobilization method. Briefly, 1X phosphate buffered saline (1× PBS) containing L-alanine (0.56 mM) and α-ketoglutarate (0.41 mM) was added to alginate suspension to form a L-alanine + α-ketoglutarate/alginate suspension. The mixed solution was then loaded onto a clean glass slide and followed by covering with another glass slide with a 1 mm thick spacer in between, allowing the definition of the thickness of alginate thin layer. In the gelatinization process, the sandwich-like structure was incubated in 102 mM $CaCl_2$ solution for 3 min. Finally, the alginate gel containing L-alanine and α-ketoglutarate was shaped according to the sensing zone of graphene film and subsequently attached onto the surface of grapheme film. ALT standard solutions with varied concentrations (10–100 U/L) and 0.1 M of PBS, then the pH level of each analyte was checked to be equal by a pH meter. In the sensing experiments of ALT, GFET sensor with a layer of L-alanine and α-ketoglutarate membrane was incubated in the analyte and then *I–V* plot was immediately recorded. The L-alanine and α-ketoglutarate immobilized on the GFET sensors were rinsed with RO water after each detection event.

3. Results and discussion

3.1. Sensing characteristics

The dependence of the conductance characteristics of GFET sensors on pH was evaluated. Fig. 2 shows the conductivity (σ) plot of the GFET device immersed into solutions at values of pH ranging from 2 to 12. The operation condition is that the drain voltage (V_{ds}) held constant at 0.1 V and the gate voltage (V_{gs}) is swept between −0.1 V and 0.3 V. The σ–V_{gs} curves exhibit a V-shaped notch and the dip in the V-shaped notch corresponds to ~$4e^2/h$ at the Dirac point. Both types of carriers in the GFET device can be continuously tuned

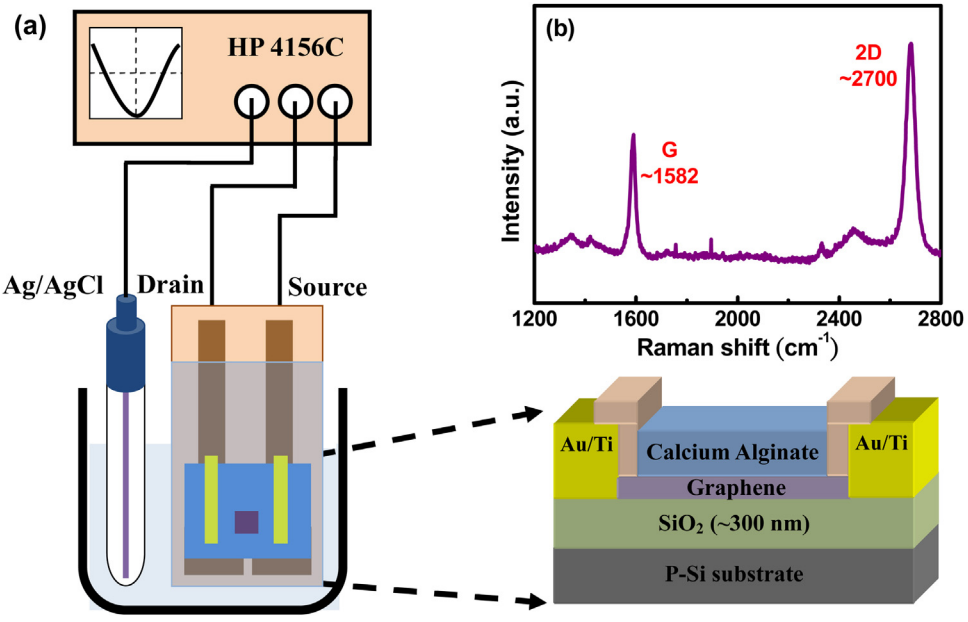

Fig. 1. (a) Schematic representation of the preparation of a GFET ALT biosensor. (b) Raman spectrum of single-layer graphene.