Detection of Streptavidin-Biotin Complexes Using a Highly Sensitive AlGaN/GaN-Based Extended-Gate MISHEMT-Type Biosensor

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Abstract

In this paper, we propose an AlGaN/GaN-based extended-gate metal-insulator-semiconductor high electron mobility transistor (MISHEMT)-type biosensor for detecting streptavidin-biotin complexes. We measure the drain current of the fabricated sensor, which varies depending on the antibody-antigen reaction of streptavidin with biotin molecules. To confirm the immobilization of biotin polyethylene glycol (PEG) thiol, we analyze the Au surface of a GaN sample using X-ray photoelectron spectroscopy (XPS). The proposed biosensor shows higher sensitivity than Si-based extended-gate metal oxide semiconductor field effect transistor (MOSFET)-type biosensor. In addition, the proposed AlGaN/GaN-based extended-gate MISHEMT-type biosensor exhibits better long-term stability, compared to the conventional AlGaN/GaN-based MISHEMT-type biosensor.

Keywords: Extended-Gate, AlGaN/GaN, MISHEMT, Biosensor, Streptavidin-Biotin

1. INTRODUCTION

In recent years, there has been a significant increase in the number of studies in the field of biosensors and biomolecular analysis methods. In particular, there has been considerable focus on protein molecules because the occurrence of diseases is at the protein level [1]. Conventional analysis methods such as optical measurements, electrochemistry and mass spectrometry require the use of various fluorescent materials and expensive equipment [2,3]. To overcome these problems, we propose an AlGaN/GaN-based metal-insulator-semiconductor high electron mobility transistor (MISHEMT)-type biosensor.

The devices that are based on AlGaN/GaN-based heterostructures exhibit unique electronic properties, such as spontaneous and piezoelectric polarization, which lead to high density and high mobility of a two-dimensional electron gas (2DEG) at the interface of the heterostructure without any additional gate voltage [4]. However, traditional semiconductor-based devices, such as silicon-based devices, require a supplied gate voltage to obtain an inversion layer channel. AlGaN/GaN-based heterostructure exhibit high mechanical hardness and chemical stability, because they are widely used in harsh environments [5]. They exhibit chemical stability also because AlGaN/GaN has high chemical inertness with biomolecules. This implies that they exhibit excellent biological compatibility [6]. On the other hand, AlGaN/GaN shows better immunity than Si with less limitation for biosensors. The conducting 2DEG channel of the AlGaN/GaN-based MISHEMT is very close to the surface. Therefore AlGaN/GaN-based biosensors are expected to exhibit high sensitivity. Moreover, we combined an AlGaN/GaN-based MISHEMT with an extended-gate structure. Extended-gate field effect transistor (FET)-type biosensors offer additional advantages such as long-term stability and a simple method for realizing passivation and packaging, as well as flexibility in the shape of the extended-gate [7-9].

In this study, we fabricated an AlGaN/GaN-based MISHEMT-type biosensor with an extended-gate structure for detecting streptavidin-biotin complexes. A gate voltage was applied to the fabricated devices using a commercial Ag/AgCl reference electrode. We measured the variation in the drain current of the fabricated sensors owing to the immobilization of a self-assembled monolayer (SAM) or by the reaction of streptavidin. The long-term stability of both the AlGaN/GaN-based extended-gate MISHEMT-type biosensor and the conventional AlGaN/GaN-based MISHEMT-type biosensor was measured in...
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Phosphate buffer saline (PBS) solution. X-ray photoelectron spectroscopy (XPS) was carried out to verify the immobilization of biotin polyethylene glycol (PEG) thiols on the Au-coated GaN surface.

2. EXPERIMENTAL DETAILS

2.1 Device Fabrication

Fig. 1 shows the structure of the AlGaN/GaN-based extended-gate MISHEMT-type biosensor. The AlGaN/GaN heterostructure was grown using metal-organic chemical vapor deposition (MOCVD) on a sapphire substrate, which consisted of a 2-μm-thick resistive GaN layer, an 80-nm-thick undoped GaN layer, and a 25-nm-thick Al$_0.3$Ga$_{0.7}$N layer with 30% Al. The grown heterostructure exhibited a sheet carrier concentration of $9.75 \times 10^{12}$ cm$^{-2}$ and a mobility of 1600 cm$^2$/Vs at room temperature. To fabricate the sensor, the active region of the sensor was defined by inductively coupled plasma reactive ion etching (ICP RIE) using a Cl$_2$/Ar gas mixture. A 30-nm-thick Al$_2$O$_3$ layer was deposited as a gate insulator using plasma-enhanced atomic layer deposition. Ti/Al/Ni/Au was deposited using electron beam evaporation after the contact hole opening for the source and drain. Then, ohmic contacts were formed using lift-off and rapid thermal annealing (RTA) at 850°C for 30 s in N$_2$ ambient. Ni/Au was deposited as a gate metal using an electron beam evaporator. Au, which has a chemical affinity to thiol, leads to the immobilization of a SAM [10]. A Ti/Al layer was deposited using electron beam evaporation and patterned using a lift-off process. The thicknesses of Ti and Al were 30 nm and 500 nm, respectively.

Fig. 2 shows a photograph of the fabricated biosensor. The gate length, width, and size of the extended-gate are 30 μm, 210 μm, and 200 x 200 μm$^2$, respectively. The fabricated sensor contains Ni/Au as a gate electrode. In electrochemistry, Au is widely used as the active surface of electrode with SAMs. Au and SAMs on Au are stable for a period of several months in air or when immersed in water [11]. Subsequently, Su-8 was coated as a passivation layer on a wafer at 4000 rpm (10-μm-thick Su-8) for 30 s, and it was patterned using a photolithographic process. The sensor was attached to a printed circuit board (PCB). Epoxy and silicon rubber were coated on bonding wires for electrical isolation, only the extended-gate of the biosensor was exposed to the solution. Fig. 3 shows a photograph of the packaged AlGaN/GaN-based extended-gate MISHEMT-type biosensor.

2.2 Measurements of the Fabricated Sensor

Fig. 4 shows the measurement setup that was used. In all the experiments, the electrical characteristics of the fabricated biosensor were measured using a semiconductor parameter analyzer (HP 4156). A commercial Ag/AgCl electrode was used to apply the gate bias for the fabricated biosensor, and as the reference electrode. The PBS solution, which has a pH of 7.4, was
used as the sample solution throughout the experiment for initial device stabilization and all subsequent SAM immobilizations and measurements. First, dark current was measured in the PBS solution without any biomolecules at a reference electrode voltage of -1 V.

23.64 mg of biotin PEG thiol was diluted in 30 ml of the PBS solution, and then the biosensor was exposed for 24 h to this solution, i.e., $1 \times 10^{-3}$ M biotin PEG thiol. The thiols were bound to the Au surface of the extended-gate region. After this binding, a SAM was formed on the Au surface. Subsequently, the biosensor was rinsed using a new PBS solution to prevent interference of non-combined biomolecules in the solution. Then the drain current of the biosensor was measured. Subsequently, a CRP (0.1 mg/ml) was used to confirm the selectivity of the sensor. The fabricated sensor was exposed to the CRP for 1 h. Finally, streptavidin, whose concentration varied from 0.1 μg/ml to 100 μg/ml, was dropped onto the device for 12 h. This was repeated three times for each concentration to obtain the standard deviation of the drain current of the fabricated sensor. When streptavidin was bound to the biotin of biotin PEG thiol, the biosensor was rinsed in a fresh PBS solution, after which the drain current was measured.

2.3 X-ray Photoelectron Spectroscopy

XPS was used to confirm the immobilization of biotin PEG thiol on the Au surface. Further, XPS spectra were obtained using a commercial XPS system (PHI Quantera SXM) with a monochromatic Al Kα source and a hemispherical electron energy analyzer. The Au 4f peak at 84.0 eV was used for data correction. XPS measurement provides information about the chemical structure within films and can be used to study samples prepared in the ambient environment, without additional in-situ treatment [10].

3. RESULTS AND DISCUSSIONS

The I-V electrical characteristics of the AlGaN/GaN-based extended-gate MISHEMT-type biosensor are shown in Fig. 5. Fig. 5(a) shows that the fabricated sensor exhibits depleted n-channel FET behavior. Its threshold voltage is -3.8 V, which is extrapolated from Fig. 5(b) in the saturation region ($V_{DS} = 7$ V). The maximum drain current and the maximum transconductance were measured as 15.6 mA and 4.1 mS, respectively.

Fig. 6 shows the $I_D$-$V_{DS}$ characteristics of the fabricated sensor that were attributed to the interactions of biotin PEG thiol and streptavidin with the Ag/AgCl reference electrode. At $V_{GS} = -1$ V and $V_{DS} = 9$ V, the drain current decreased by 0.59 mA, because of the negative charges that were produced when the thiol was injected into the solution and immobilized on the Au sensing gate. The net negative charges of the reactive thiol head group
introduce a negative surface potential on the gate electrode of a MISHEMT [12]. In addition, the drain current decreased by 2.81 mA when streptavidin was immobilized by the antibody-antigen reaction. The high-affinity streptavidin-biotin complexes (\(K_a = 1.3 \times 10^{15} \text{ M}^{-1}\)) are characterized by an extensive hydrogen-bonding network [13]. The drain current of the fabricated sensor decreased because of the net negative charges of streptavidin. Therefore, the variations in the drain current of the proposed biosensor could detect the conjugation of the streptavidin and biotin protein complexes. In addition, the drain current of the AlGaN/GaN-based extended-gate MISHEMT-type biosensor decreased by 2.81 mA and that of the Si-based extended-gate MOSFET-type biosensor increased by 20 μA when streptavidin was immobilized [8]. From these results, we confirmed that the sensitivity of the AlGaN/GaN-based extended-gate MISHEMT-type biosensor increased approximately 120 times, since the AlGaN/GaN-based MISHEMT has high electron mobility, which is induced by piezoelectric and spontaneous polarizations [5].

As shown in Fig. 7, the change in the drain current of the biosensor was measured as a function of streptavidin concentration to quantify the limit of detection (LOD) for streptavidin. The device was exposed to streptavidin concentrations ranging from 0.1 μg/ml to 100 μg/ml. Only a small change was observed when 0.1 μg/ml streptavidin was injected. Therefore, the LOD of this device is 0.1 μg/ml streptavidin in the PBS solution.

Fig. 8 shows a comparison between the long-term stability of the AlGaN/GaN-based extended-gate MISHEMT-type biosensor and the conventional AlGaN/GaN-based MISHEMT-type biosensor. The long-term stability of the proposed biosensor was attributed to good isolation between the sensor device and the measuring solution because the measuring solution was in contact with only the extended-gate of the sensor device [8,14]. For the purpose of comparison, the AlGaN/GaN-based extended-gate MISHEMT-type biosensor and the conventional AlGaN/GaN-based MISHEMT-type biosensor were fabricated using the same process, with the same device dimensions. From this figure, we confirmed that the output current of the AlGaN/GaN-based extended-gate MISHEMT-type biosensor is more stable than that
XPS spectra were measured to examine biotin PEG thiol immobilization on the Au surface. An SAM of biotin PEG thiol is adsorbed on the Ni/Au extended-gate by reaction with thiol modification owing to a strong interaction between Au and the thiol group [15]. To confirm that the modification was successful, XPS measurements were performed under the same conditions as those for the electrical measurements of the fabricated extended-gate MISHEMT-type biosensor. Fig. 9 shows the measured high resolution 2p and S 2s XPS spectra after biotin PEG thiol modification of the Au-coated GaN sample. The S 2p observed at binding energies of 162 eV and 163 eV indicate an Au thiolation sulfur species [1]. The S 2s peak at 226 eV was observed for the biotin PEG thiol immobilized sample. Therefore, the results of the XPS measurements support the SAM formation on the Au surface.

4. CONCLUSIONS

The AlGaN/GaN-based extended-gate MISHEMT-type biosensor was fabricated and its characteristics were investigated. Au, which exhibits chemical affinity to thiols, was used as the gate metal to immobilize biotin PEG thiol. The AlGaN/GaN-based extended-gate biosensor employing a commercial Ag/AgCl reference electrode was investigated for detecting streptavidin-biotin complexes. The drain current decreased because of the negative charges that were produced when the thiol was injected into the solution and immobilized on the Au sensing gate. The drain current increased when streptavidin was immobilized by the antibody-antigen reaction owing to the positive charge of the amine group in streptavidin. The XPS measurements verified the SAM formation on the Au surface. Experimental results showed that the AlGaN/GaN-based biosensor exhibited approximately 120 times higher sensitivity than the Si-based extended-gate MOSFET-type biosensor. In addition, the long-term stability of the biosensor was demonstrated in the PBS solution.

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