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2014 J. Micromech. Microeng. 24 075023

(http://iopscience.iop.org/0960-1317/24/7/075023)

IOP Publishing Journal of Micromechanics and Microengineering
J. Micromech. Microeng. **24** (2014) 075023 (6pp) doi:10.1088/0960-1317/24/7/075023

Detection of prostate-specific antigen with biomolecule-gated AlGaN/GaN high electron mobility transistors

Jia-dong Li[1,2], Jun-jie Cheng[3], Bin Miao[1,2], Xiao-wei Wei[1,2,4], Jie Xie[1,2], Jin-cheng Zhang[5], Zhi-qiang Zhang[6] and Dong-min Wu[1,2]

[1] i-Lab, Suzhou institute of Nano-tech and Nano-bionics, Chinese Academy of Sciences, Suzhou 215125, People's Republic of China
[2] Key Laboratory of Nanodevices and Applications, Suzhou Institute of Nano-tech and Nano-bionics, Chinese Academy of Sciences, Suzhou 215123, People's Republic of China
[3] Heifei Institute of physical science, Chinese Academy of Sciences, Hefei 230031, People's Republic of China
[4] Suzhou Institute for Advanced Study, University of Science and Technology of China, Suzhou 215123, People's Republic of China
[5] Key Laboratory of Wide Band Gap Semiconductor Materials and Devices, School of Microelectronics, Xidian University, Xi'an 710071, People's Republic of China
[6] Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences, Suzhou 215163, People's Republic of China

E-mail: jdli2009@sinano.ac.cn and dmwu2008@sinano.ac.cn

Received 25 March 2014, revised 21 May 2014
Accepted for publication 28 May 2014
Published 23 June 2014

Abstract

In order to improve the sensitivity of AlGaN/GaN high electron mobility transistor (HEMT) biosensors, a simple biomolecule-gated AlGaN/GaN HEMT structure was designed and successfully fabricated for prostate specific antigen (PSA) detection. UV/ozone was used to oxidize the GaN surface and then a 3-aminopropyl trimethoxysilane (APTES) self-assembled monolayer was bound to the sensing region. This monolayer serves as a binding layer for attachment of the prostate specific antibody (anti-PSA). The biomolecule-gated AlGaN/GaN HEMT sensor shows a rapid and sensitive response when the target prostate-specific antigen in buffer solution was added to the antibody-immobilized sensing area. The current change showed a logarithm relationship against the PSA concentration from 0.1 pg/ml to 0.993 ng/ml. The sensitivity of 0.215% is determined for 0.1 pg/ml PSA solution. The above experimental result of the biomolecule-gated AlGaN/GaN HEMT biosensor suggested that this biosensor might be a useful tool for prostate cancer screening.

Keywords: high electron mobility transistor, biomolecule-gate, biosensor, prostate cancer screening

(Some figures may appear in colour only in the online journal)

1. Introduction

Prostate-specific antigen (PSA) is a protein produced by cells of the prostate gland and present in the serum of healthy men at a very low level [1]. This level is often elevated in the presence of prostate cancer or other prostate disorders; therefore, the detection of PSA is a good diagnostic tool for detecting early stage prostate cancer [2, 3]. In general, an average concentration of total PSA ranging from 0 to 4 ng/ml can be considered as normal by most doctors. However, there is evidence suggesting that a lower level of free PSA may be associated with more aggressive cancers, so the determination of the ratio of free to total amount of PSA (free plus bound) is required to improve the diagnostic accuracy. Furthermore, ultra trace detection enables us to monitor the recurrence of prostate cancer after treatment and assesses the quality of

diagnosis and treatment [4]. At present, the most common technique for detecting and diagnosing prostate cancer is enzyme-linked immunosorbent assay (ELISA). ELISA is label-based detection method that determines the PSA level in a sample by the magnitude of a fluorescence signal [5–7]. However, this procedure has low sensitivity and/or is cumbersome and time consuming to perform in forensic laboratories, especially when only a few samples are analyzed per week [8]. For PSA detection, the development of real-time, highly-sensitive and label-free sensing techniques that overcome the drawbacks of the ELISA-based method remains a considerable challenge [9]. Many methods, such as micro-cantilevers, nanowires, nanobelt and carbon nanotube field effect sensors, and AlGaN/GaN heterostructure biosensors have been used in attempts to replace ELISA. The micro-cantilever biosensor enables the detection of PSA in solution at concentrations as low as pg/ml, but the microbalance method suffers from an undesirable resonant frequency change due to viscosity of the medium and cantilever damping in the solution environment [10]. Nanowire [11], nanobelt [12] and carbon nanotube field effect sensors [13] were believed to be promising devices for real time, highly sensitive detection of PSA. However, they suffered from long-term electrical drifting and instability due to the diffusion of ions from biological buffers into the gate oxides, and the requirement for a reference electrode ultimately limited their size [14]. AlGaN/GaN heterostructure biosensors are an excellent potential successor for PSA detection because of their higher chemical stability in physiological buffers [15, 16]. AlGaN/GaN HEMTs with thioglycolic acid-modified gold-coated gates were demonstrated for PSA detection. However, the deposited gold layer increases the processing cost and reduced the detection sensitivity of the sensors [17].

Herein, a simple ultrasensitive biomolecule-gated AlGaN/GaN HEMT sensor was developed for PSA detection. Fluorescence microscopy and contact angle measurements were used to characterize the self-assembled monolayer of the biomolecule on the gate surface. Different concentrations of PSA in solution have been detected using this device. Compared with the Au-gated AlGaN/GaN HEMT biosensor, the biomolecule-gated device exhibits a much lower detection limit.

2. Experimental procedures

2.1. Biomolecule-gated AlGaN/GaN HEMT sensor

Figure 1 shows a schematic cross-sectional view of the AlGaN/GaN HEMT heterojunction device with a biomolecule gate. The heterojunction structure consists of a 1.5 μm thick non-doped GaN layer, a 18 nm AlGaN barrier layer and 1.5 nm GaN cap layer. The two-dimensional electron gas (2DEG) is located at the interface between the non-doped GaN layer and the AlGaN layer. The electron mobility of the 2DEG in this device is measured to be 1672 $cm^2/V{\cdot}s$. Free electrons in the 2DEG channel of the AlGaN/GaN HEMT are induced by piezoelectric and spontaneous polarization effects, and are very close to the surface (19.5 nm). Any slight changes of the surface charge will be transduced into a change in the concentration of the 2DEG in the AlGaN/GaN HEMTs through the capacitive effect. The sensing area of this device was functionalized with APTES.

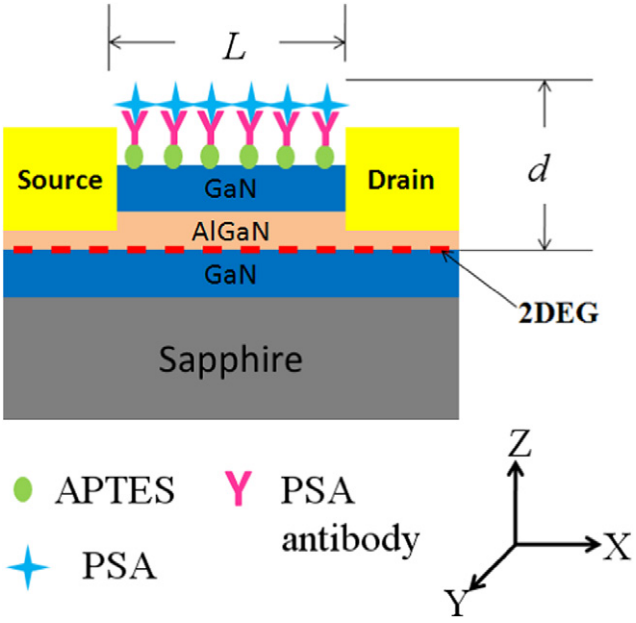

Figure 1. Schematic device cross section, anti-PSA is anchored to the sensing surface and function as specific recognition groups for PSA detection.

2.2. AlGaN/GaN HEMT fabrication

The AlGaN/GaN HEMT device fabrication processes are sketched in figure 2(*a*) and described below.

(1) The GaN cap layer was spin coated with photoresist AZ5214 with a thickness of 2 microns. Then the photoresist was baked at 95 °C for 1.5 min. This was followed by photolithography using an EV620 aligner to define the mesa structure. Mesa isolation was performed by inductively coupled plasma etching with Cl_2/BCl_3. The etching rate is about 0.1 μm/min. A rinsing step followed.

(2) The mesa structures were then spin coated with a thick layer of photoresist AZ4620 and the ohm contact window was opened after photolithography and development. The GaN surface of the window area was then treated with HCl: H_2O=1:8 solutions to remove the native oxide layer. A Ti/Al/Ni/Au multilayer for the ohm contact was then deposited by using an e-beam evaporation system. To remove the resist, the wafer was cleaned in acetone, then alcohol, and dried with nitrogen. After that, the samples were introduced into a rapid thermal processing system (RTP-500, at 880 °C for 45 s) for annealing under a flow of nitrogen ambient.

(3) The overlay layer mask was transferred into the ohmic contact layer by photolithography (with a Shipley AZ5214 photoresist). The Ti/Ni/Au multilayer was then deposited by an e-beam evaporation system to form the source and drain electrode (as shown in figure 2(*b*)).

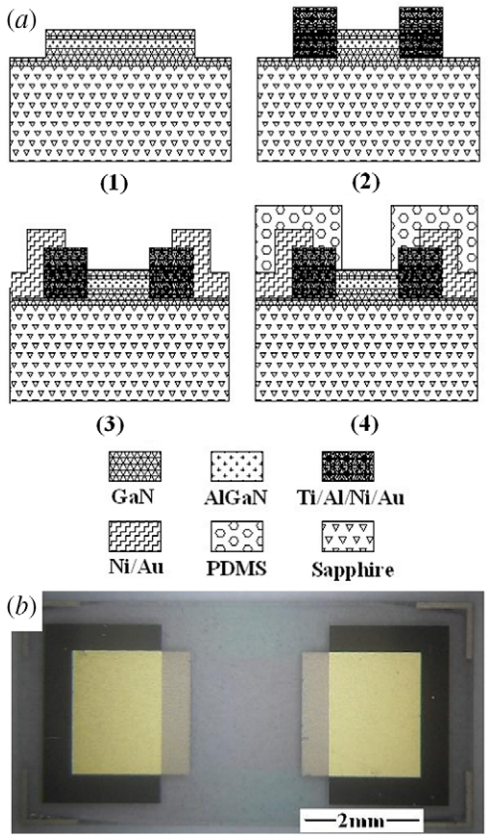

Figure 2. (*a*) Fabrication process of the AlGaN/GaN HEMT structure. (*b*) Photograph of an AlGaN/GaN HEMT device without PDMS packaging.

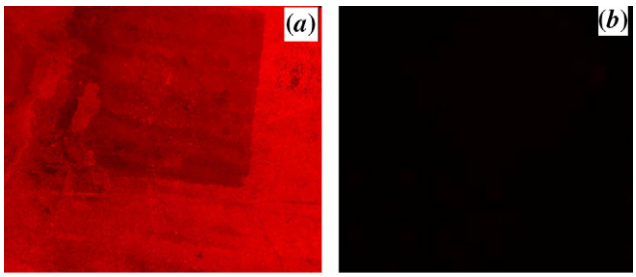

Figure 3. The fluorescence images of the GaN surface in the process of APTES hybridization. (*a*) Image after Cy5-labeled single-stranded DNA immobilization on APTES-functionalized GaN surface. (*b*) Image of Cy5-labeld single-stranded DNA immobilization on GaN surface without APTES modification.

(4) Finally, 3 mm thick PDMS was used to package the AlGaN/GaN biosensor, with a $2 \times 1.5\,mm^2$ detection window to allow the liquid solutions to access the sensing surface.

2.3. Surface modification of the AlGaN/GaN HEMT sensors

Before the surface modification, each of these devices was prepared by cleaning in acetone, ethanol and de-ionized water respectively, then drying in a nitrogen stream. The sensing area of the AlGaN/GaN HEMT was then oxidized by UV/ozone treatment (Model UV-1, Samco International Inc.). A 5% (by volume) APTES solution in ethanol solvent was used to treat the device sensing area. Those

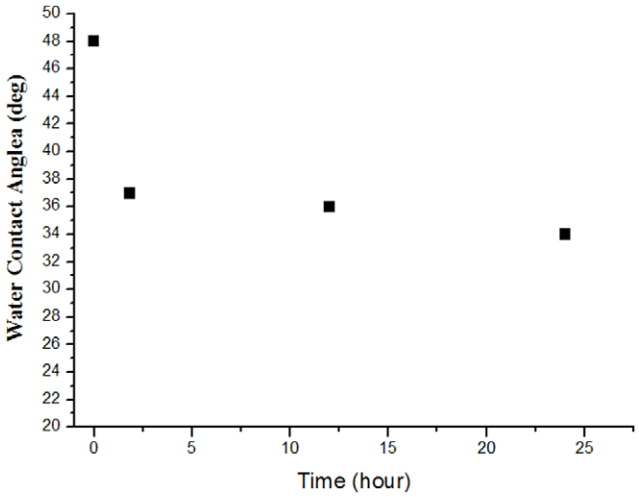

Figure 4. Water contact angle after immersion in 0.1 M PBS for 0, 12 and 24 h.

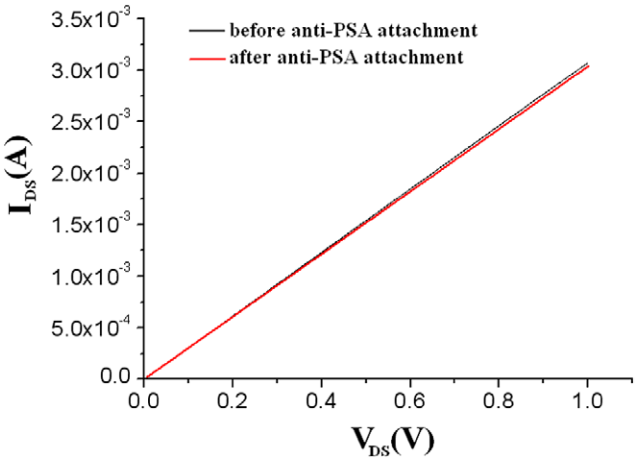

Figure 5. I-V characteristics of the biomolecule-gated AlGaN/GaN HEMT sensor before and after PSA antibody incubation.

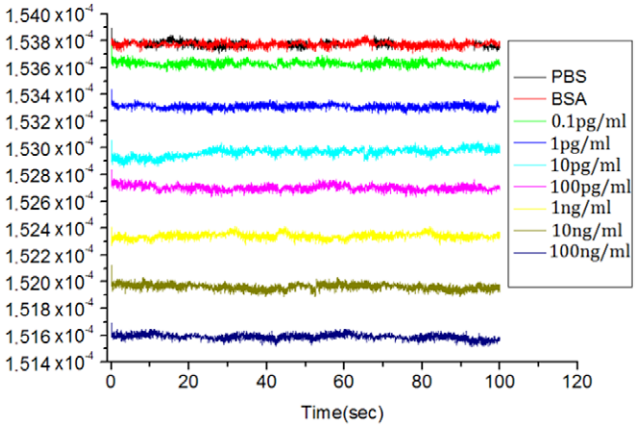

Figure 6. Current response of an AlGaN/GaN HEMT sensor for different PSA concentrations from 0.1 pg/ml to 100 ng/ml.

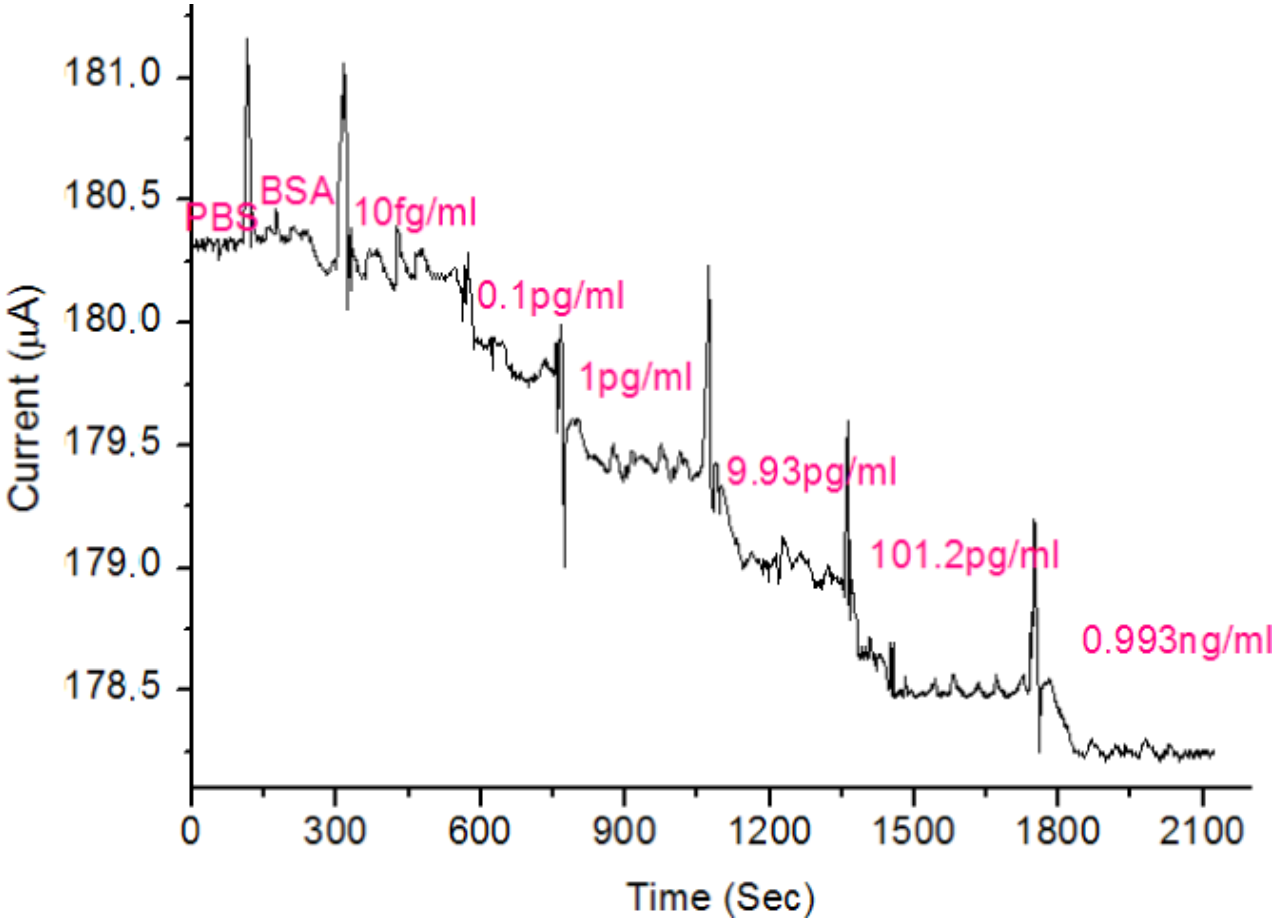

Figure 7. Real-time detection of the PSA from 10 fg/ml to 1 ng/ml at a constant bias of 50 mV.

devices were immersed in the solution for 2 h. APTES self-assembled monolayer deposition occurs through a reaction between the methoxy group on the silane molecule and the hydroxyl group on the substrate [18]. All samples were washed in ethanol and distilled water, respectively, to remove the unbounded APTES, then dried under a nitrogen flow, and baked at 120 °C for 2 h. After that, the sensing region is further modified with amine groups. A 2.5% (by volume) glutaraldehyde aqueous solution is applied onto the silanized sensing region. After 1h, the samples were washed in distilled water to remove unlinked glutaraldehyde molecules. This activation step resulted in the formation of Schiff base groups on the sensing region of the AlGaN/GaN HEMT biosensor and can improve the sensitivity of the biosensor. Finally, the samples were immersed into anti-PSA solution at a concentration of 10 μg/ ml for 24h, and the anti-PSA was attached to the sensing region of the device. The non-specific reactive sites were blocked by using 1% bovine serum albumin (BSA) solution. Each step was rinsed by distilled water and dried with nitrogen. After attaching the anti-PSA onto the sensing area, the HEMT biosensors were used to detect PSA solutions with different concentrations by monitoring the current change (at constant source-drain voltage) of the device using a source meter (KEITHLEY 2636A).

2.4. Fluorescence and contact angle measurement

The surface modification was verified by fluorescence analysis (LuxScan 10 K microarray Scanner, CapitalBio Corporation, Beijing, China). The modified GaN surface stability was investigated by measuring water contact angles (Dataphysics OCA20, Data Physics Instruments GmbH, Germany) with the sessile drop method (2.0 μL)

3. Results and discussions

3.1. Characteristic of the self-assembled monolayer -treatedGaN surfaces

Fluorescence imaging of the modified GaN surface (figure 3) was implemented on a microarray scanner. Figure 3(*a*) shows the fluorescence image after Cy5-labeled single-stranded DNA immobilization on an APTES-functionalized GaN surface. Figure 3(*b*) shows the fluorescence image of the GaN surface treated with the same Cy5-labeled single-stranded DNA, but without APTES functionalization. By comparing the fluorescence intensity of those two images, we concluded that the GaN surface had been successfully modified with the APTES self-assembled monolayer.

The stability of APTES self-assembled monolayer on GaN surface is important for their future applications. It was

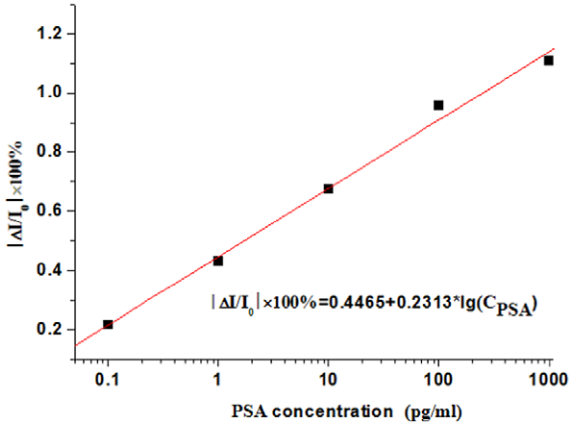

Figure 8. Normalized I_{ds} change versus the logarithm of PSA concentrations.

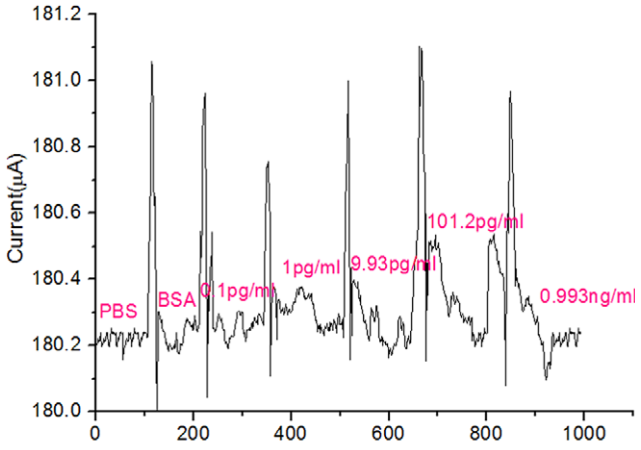

Figure 9. The control experiment with different concentration of the PSA at a constant bias of 50 mV.

Table 1. Comparison of the linear dynamic range and limit of detection of PSA biosensors based on AlGaN/GaN HEMTs.

Methods	Linear range	Limit of detection	Ref
Au-gated AlGaN/GaN HEMTs	10 pg/ml to 10 ng/ml	10 pg/ml	[16]
Biomolecule-gated AlGaN/GaN HEMTs	0.1 pg/ml to 1 ng/ml	0.1 pg/ml	This work

previously reported that an APTES self-assembled monolayer on silicon may be desorbed upon immersion in an aqueous solution [19]. Here, the stability of the APTES self-assembled monolayer on the GaN surface (heating treatment at 120 °C for 2h) in the 0.1 M PBS solution was investigated using contact angle measurements. As shown in figure 4, just after heating treatment, the water contact angle of the sensing surface is 48° and then decreases to 37° after immersion into 0.1 M PBS, and almost does not change with the increasing immersion time. It is shown that the APTES self-assembled monolayer has a good stability in buffer solution over 24 h.

3.2. Electrical characterization of the AlGaN/GaN HEMT biosensor

The voltage-current curve of the AlGaN/GaN HEMT biosensor was measured before and after anti-PSA attachment, as shown in figure 5. It is shown that the chemical modification of the sensing area of the AlGaN/GaN HEMT will slightly influence the concentration of the 2DEG in the AlGaN/GaN HEMT sensor.

We have performed the PSA detection in 0.1 M PBS solution with an AlGaN/GaN HEMT biosensor. The electrical current of the biosensor was monitored by using a Keithley 2636A with constant bias at 50 mV. 0.1 M PBS buffer solution and nonspecific bovine serum albumin (BSA) in 0.1 M PBS were injected onto the sensing region respectively (timings for adding solutions are indicated in figure 6). Figure 6 shows that the current reading from the biosensor does not change after the adding of the 0.1 M PBS buffer solution, further proving the high stability of this biosensor; upon the addition of BSA in 0.1 M PBS, the current still does not change, indicating that the nonspecific binding of BSA was successfully suppressed [20, 21]. By contrast, target PSA with different concentrations in buffer solution (0.1 pg/ml, 1 pg/ml, 10 pg/ml, 100 pg/ml, 1 ng/ml, 10 ng/ml, and 100 ng/ml) was detected. The measured result shows a current change when target PSA was added to the surface, which is consistent with previous observations [16].

Further tests to quantify the detection limit of PSA were carried out at real time as shown in figure 7. Firstly, 0.1 M PBS solutions were added onto the sensor surface. When 1% BSA solution was dropped onto the sensing area, an abrupt peak appeared and then quickly recovered to the baseline. It was due to the mechanical disturbance resulted from adding the BSA solution with a micropipette by hand (because the mechanical disturbance can cause the piezoelectric polarization phenomenon, a peak may appear). After the current recovered to the original baseline, there was no change observed for the time tested (150 s), further confirming the excellent stability of the biosensor devices. When the 10 fg/ml PSA was added onto the sensing area, there was no significant current change. When the 0.1 pg/ml PSA was dropped, a clear current change was observed at steady state. We define the sensitivity in percentage as $S=\Delta I/I_0\times100\%$. A sensitivity of 0.215% is determined for 0.1 pg/ml PSA solution. The biosensor response showed logarithmic dependence on the target protein concentrations (as shown in figure 8), which was also presented in the Au-gated AlGaN/GaN HEMTs biosensors [16]. Table 1 summarizes PSA biosensors based on AlGaN/GaN HEMTs. From table 1, the biomolecule-gated AlGaN/GaN HEMTs biosensor achieves a quite wide linear dynamic range and a relative low detection limit for the detection of PSA with respect to previous reports.

The control test of the sensor was investigated with the similar surface modification procedure (APTES immobilized and BSA blocking) but no antibodies were immobilized on the sensor. As shown in figure 9, the control experiment was conducted with different concentrations of the PSA showing no significant current change. It demonstrated that there was no non-specific binding on the sensor. Therefore, the signal

generated from the sensor was attributed to the specific antibody–antigen interaction.

4. Conclusions

A simple biomolecule-gated AlGaN/GaN HEMT structure were designed and successfully fabricated to detect prostate-specific antigen. UV/ozone was used to oxidize GaN surface, and the APTES self-assembled monolayer was bound to the sensing region for attachment of anti-PSA. Fluorescence imaging confirms the APTES has been bonded to the sensing area. The biosensor showed a sensitive and rapid response for the PSA protein with a logarithm relationship between the current and the PSA concentration (from 0.1pg/ml to 1ng/ml). The sensitivity of 0.215% is determined for 0.1 pg/ml PSA solution. The high sensitivity and stablility of the biomolecule-gated AlGaN/GaN HEMT biosensor suggested that this biosensor might be a useful tool for the prostate cancer screening.

Acknowledgements

This work has been supported by the NSFC project under grant number 61104226,the National Basic Research Program of China (2010CB934700) and partial support from CAS Instrumentation Project (#YZ201152). We thank the Nano-Characterization & Nano-Fabrication Platforms at the Suzhou Institute of Nano-Tech and Nano-Bionics, Chinese Academy of Sciences for the fabrication supporting and thank Professor Jincheng Zhang for offering AlGaN/GaN samples.

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