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Fast Electrical Detection of Carcinoembryonic Antigen Based on AlGaN/GaN High Electron Mobility Transistor Aptasensor *

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As one of the most important tumor-associated antigens of colorectal adenocarcinoma, the carcinoembryonic antigen (CEA) threatens human health seriously all over the globe. Fast electrical and highly sensitive detection of the CEA with AlGaN/GaN high electron mobility transistor is demonstrated experimentally. To achieve a low detection limit, the Au-gated sensing area of the sensor is functionalized with a CEA aptamer instead of the corresponding antibody. The proposed aptasensor has successfully detected different concentrations (ranging from 50 picogram/milliliter (pg/ml) to 50 nanogram/milliliter (ng/ml)) of CEA and achieved a detection limit as low as 50 pg/ml at $V_{ds} = 0.5$ V. The drain-source current shows a clear increase of 11.5 μA under this bias.

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Malignant tumor or usually called cancer, as one of the greatest threats to human health, has shown an increasing trend year by year all over the world. Cancer is the second leading cause of death globally and accounted for 8.8 million deaths in 2015, nearly 1 in 6 of all global deaths.[1] Therefore, it has a far-reaching significance to realize the diagnosis and cure of cancer in the early stage. A tumor marker (TM) is synthesized and secreted through the gene expression progress; it is a kind of substance with an abnormal expression level that results from the body's reaction to tumors. The content of the marker in patients is much higher than that in healthy adults, playing an important role in early detection, diagnosis and prognosis of cancer.[2,3] A carcinoembryonic antigen (CEA) is an intercellular adhesion molecule, which is implicated in the hepatic metastasis from colorectal cancer. Currently, CEA is considered as a common clinical used tumor marker to screen and monitor colorectal cancer, breast cancer, lung cancer, gastric cancer and other cancers.[4–6] Thus rapid and accurate detection of CEA in serum has become increasingly important.

Aptamers are artificial single stranded nucleic acid molecules obtained by an in vitro selection and amplification technique, which is called systematic evolution of ligands by exponential enrichment (SELEX).[7] They can combine with the corresponding ligand molecular targets with high affinity and strong specificity and have become the most widely used DNA probe. Compared with traditional protein antibody, aptamers have many advantages, such as high affinity, strong specificity, simple preparation, easy proceeding progresses of chemical modification and separation, wide target molecular distribution range, controllable conditions during their combination with the target molecular targets and good stability. More information for the advantages of aptamers over antibodies has been reported in Refs. [8,9]. Therefore, they have a broader prospect in the biomedical field.

In recent years, the research and development of the third generation of wide bandgap semiconductor materials and devices represented by gallium nitride (GaN) have made a breakthrough and gradually approached maturity.[10–13] The particularly high two-dimensional electron gas (2DEG) density is closely related to the surface state of the GaN-based heterostructures, leading to a unique advantage in the field of sensors.[14,15] However, the existing GaN-based high electron mobility transistor (HEMT) biosensor in detecting the cancer antigen has adopted the traditional mechanism that requires antigen and antibody combined,[16–20] and it is difficult to achieve low limit concentration of detection. In the present study, we innovatively introduce CEA aptamers to the AlGaN/GaN HEMT and successfully detect different concentrations of CEA. The detection limit for CEA is below 50 pg/ml using an AlGaN/GaN HEMT aptasensor.

The AlGaN/GaN HEMT structures in this work are grown by metal organic chemical vapor deposition (MOCVD) on a 2-inch *c*-plane sapphire substrate.[21,22] It was composed of a 2μm-thick undoped GaN buffer layer, a 1 nm AlN interlayer, a 22 nm $Al_{0.26}Ga_{0.74}N$ barrier layer with no intentional

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doping, and a 3 nm GaN cap layer. Hall measurements at room temperature yield a 2DEG density of $8.2 \times 10^{12}\,\mathrm{cm}^{-2}$ and an electron mobility of $1838\,\mathrm{cm}^2/\mathrm{V{\cdot}s}$. Ti/Al/Ti/Au (20 nm/100 nm/40 nm/60 nm) multilayers for ohmic contacts were sputtered on the source and drain areas with a gap of 30 μm between them, followed by rapid thermal annealing (RTA) under N_2 flowing at 870℃ for 30 s. Subsequently, mesa isolation was conducted by ICP dry etching with Cl_2/BCl_3. The specific contact resistivity of $3.89 \times 10^{-4}\,\Omega{\cdot}\mathrm{cm}^2$ was obtained by the transmission line method (TLM). A 10 nm thin Au film was sputtered and lifted off as the gate electrode to immobilize the thiol-modified aptamers, with length and width being 20 μm and 120 μm, respectively. A Ni/Au multilayer was then sputtered on top of the ohmic contacts to form the electrodes for source and drain. Afterwards, a 200 nm silicon nitride (Si_3N_4) passivation layer was used to package the AlGaN/GaN HEMT biosensor, with just the Au gate sensing area and pads of the electrodes for the measurement exposed. A schematic cross-sectional view of the AlGaN/GaN HEMT heterojunction device and a top view photomicrograph of the device are shown in Fig. 1

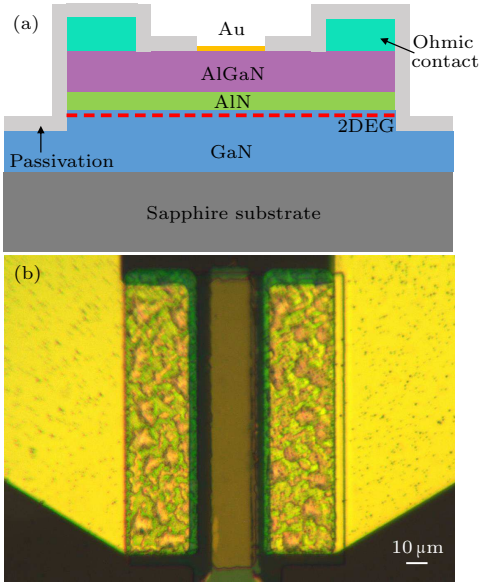

Fig. 1. (a) Schematic cross-sectional view of the AlGaN/GaN HEMT heterojunction device. (b) Top view photomicrograph of the device.

Figure 2 illustrates the current-voltage characteristics and the optical micrograph of the AlGaN/GaN HEMT test pattern. Figure 2(a) shows the output characteristics of the device, the drain-source voltage (V_{ds}) varied from 0 V to 8 V while the gate voltage (V_g) changed from −3 V to 2 V. The device exhibits a typical current saturation curve of HEMT as the drain-source current (I_{ds}) increases with V_g. The measured transfer characteristics curve of the HEMT at $V_{ds} = 0.5$ V is shown in Fig. 2(b). It is clear that the drain-source current is sensitive to the gate voltage, indicating that the 2DEG channel is quite sensitive to a slight change of the surface states, that is, induced by biomolecules, which makes it possible for the device used in the application of a biosensor. Figure 2(c) shows the optical micrograph of the test pattern, with $20 \times 120\,\mu\mathrm{m}^2$ ohmic contacts of source and drain with a gap of 10 μm and the Au gate area of $3 \times 120\,\mu\mathrm{m}^2$.

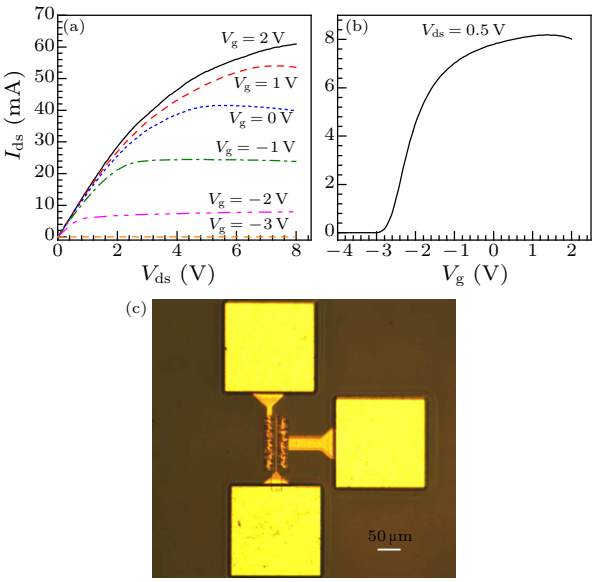

Fig. 2. (a) Output characteristics of the HEMT: V_g changed from −3 V to 2 V. (b) Transfer characteristics curve of the HEMT at $V_{ds} = 0.5$ V. (c) Optical micrograph of the test pattern.

Before the functionalization of the sensor, to remove the surface oxides and contaminants, the HEMT devices were cleaned successively with 50% hydrochloric acid, carbon tetrachloride, acetone, ethanol and deionized (DI) water in an ultrasonic bath and dried with a nitrogen stream. To ensure that the single stranded aptamers were fully immobilized onto the Au-gated region, the sensing surface was immersed in 50 μM thiolated carcinoembryonic antigen (CEA) aptamers of 5'-HS-$(CH_2)_6$-ATACCAGCTTATTCAATT-3' in the TE buffer solution (10 mmolL^{-1} Tris-HCl, 1 mmolL^{-1} EDTA, 0.1 molL^{-1} NaCl, pH=7.40, purchased from Sigma Aldrich) for 12 h at room temperature, and was successively washed off the unbound aptamers. The CEA aptamers were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China).[23,24] The aptamers can specifically recognize carcinoembryonic antigen and have also been experimentally validated in Ref. [24]. To avoid nonspecific adsorption, the residually active sites were blocked with 100 μl of 1 mM mercaptohexanol (MCH, Solarbio) for 1 h followed by a washing step. The AlGaN/GaN HEMT aptasensor for CEA detection was thus obtained. The device was immediately immersed in the TE buffer solution until it was used for CEA detection. Figure 3 represents the functionalization process on the Au-gated area of the sensor and the detection of CEA. After attaching the aptamers onto the sensing area, the HEMT biosensors were used to detect CEA in phosphate buffer solution (PBS) (0.1 molL^{-1}, pH=7.40) with different concentrations. The CEA full length protein from human

colon carcinoma liver metastatic tissue was supplied by Abcam Trading Co., Ltd. (Shanghai, China). The drain-source current characteristics of the HEMT sensor was measured at a dark condition using an Agilent B2900A Precision Source.

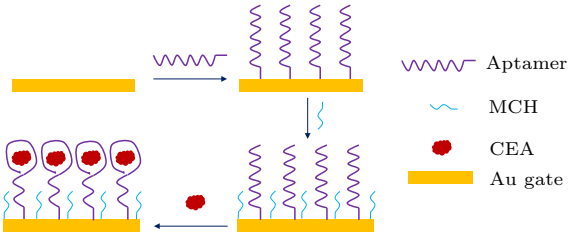

Fig. 3. Schematic illustration of the functionalization process on the Au-gated area of the HEMT and the selective detection of CEA.

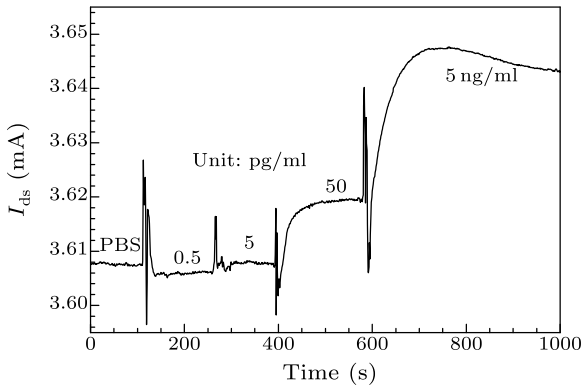

Fig. 4. Current response of AlGaN/GaN HEMT sensor for different CEA concentrations at a constant bias of 0.5 V.

Figure 4 illustrates the real time detection of CEA by the aptamer functionalized Au-gated HEMT sensor. The sensor was biased at 0.5 V and I_{ds} was measured at an interval of 1 s. Firstly, the sensing surface was exposed to the 0.1 M PBS solution. After 100 s, 0.5 pg/ml CEA in the PBS solution was dropped onto the sensing area, an abrupt peak appeared and then quickly recovered to the original baseline. The appearance of the abrupt peak can be explained by a mechanical disturbance which can cause the piezoelectric polarization.[19,25] Then, the current tended to be stable, without any significant change. A similar situation also appeared when the 5 pg/ml CEA was added onto the sensing area. However, when 50 pg/ml CEA was dropped, an obvious current change was observed. It implies that the detection limit can be down to the order of a few tens of pg/ml at least.

The I_{ds}–V_{ds} characteristics of the Au-gated HEMT before and after sensing surface functionalization, and after being exposed to different concentrations of CEA (ranging from 50 pg/ml to 50 ng/ml) in PBS solution were measured, as shown in Fig. 5. Here I_{ds} of the sensor acts as a function of V_{ds} from 0 V to 7 V. At V_{ds} = 2.5 V, the drain-source current was increased by 500 μA after being functionalized with aptamers, and it was further increased after being exposed to different concentrations of CEA solution. The current was increased by 300 μA for a CEA concentration as low as 50 pg/ml. As a result of the limited number of modified CEA aptamers, the drain-source current tended to saturate when the sensor was exposed to 50 ng/ml CEA solution. The mechanism of the increase in the source-drain current of the sensor can be ascribed to the effect of the surface modification and the charge transfer mechanism at the interface. An equivalent model for the effect of the Au-S bond on GaN-based HEMT has been proposed in our previous work, which has explained the reason for the rise of current when the thiol-modified single stranded DNA immobilized on the Au gate area.[26] When the negatively charged CEA in PBS solution was added onto the sensing area, positive charges were attracted to the Au-gate surface to maintain neutrality.[27,28] These changes in the surface charge are transduced into an increase in the concentration of the 2DEG in the AlGaN/GaN HEMTs, thus resulting in a positive response of the drain-source current.

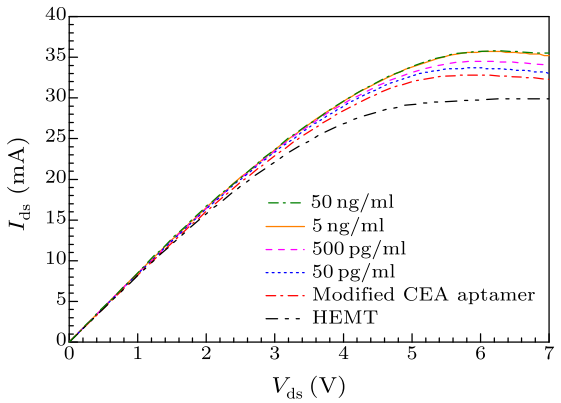

Fig. 5. The I–V characteristics of AlGaN/GaN HEMT sensor before gate functionalization, after being modified with CEA aptamer, and after applying different concentrations of CEA (ranging from 50 pg/ml to 50 ng/ml).

In summary, we have fabricated a CEA aptasensor using AlGaN/GaN HEMT. Based on the functionalization of the Au-gate by the thiol-modified aptamer, the sensor shows a sensitive and rapid response for the CEA detection. The detection limit for CEA is as low as a few tens of pg/ml. GaN-based aptasensors therefore exhibit an immense potential, and they are not only suitable for developing a broader application of diverse aptamers, but also promising candidates for the determination of a variety of cancers in clinical diagnostics.

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