ZnO thin film transistor immunosensor with high sensitivity and selectivity

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A zinc oxide thin film transistor-based immunosensor (ZnO-bioTFT) is presented. The back-gate TFT has an on-off ratio of 10^8 and a threshold voltage of 4.25 V. The ZnO channel surface is biofunctionalized with primary monoclonal antibodies that selectively bind with epidermal growth factor receptor (EGFR). Detection of the antibody-antigen reaction is achieved through channel carrier modulation via pseudo double-gating field effect caused by the biochemical reaction. The sensitivity of 10 fM detection of pure EGFR proteins is achieved. The ZnO-bioTFT immunosensor also enables selectively detecting 10 fM of EGFR in a 5 mg/ml goat serum solution containing various other proteins. © *2011 American Institute of Physics*. [doi:10.1063/1.3582555]

The ion-selective field effect transistors (ISFETs) has been used popularly as a sensitive *p*H sensor and various biochemical sensors.[1–3] Recently the ISFET structure has been integrated with poly-Si thin film transistors (TFTs) and GaN/AlGaN high electron mobility transistors for detection of DNA, penicillin, and cellular potentials.[4,5] However, the sensing procedure using the ISFET can be invasive as its entire gate serves as the sensing area which contains both the analyte solution and the reference electrode. Another class of FET-type biosensors is based on organic field-effect transistors (OFETs).[6–9] The general structure of an OFET consists of a back-gate metal-oxide semiconductor field-effect transistor (MOSFET) with the conducting channel made of organic semiconductors. The OFET has the advantage of being easily controlled through biasing due to the back-gate configuration. However, OFETs require high bias voltages, and suffer from low channel mobility. Currently, nanowire-based FET sensors are demonstrated with high sensitivity reaching the order of fM.[10,11] However, these prototypes of sensors generally involve a complex fabrication process as they are constructed individually by manipulating and aligning a single strand of semiconducting nanowire such as TiO_2 or Si as the FET channel between the source and drain patterns. It is difficult to achieve repeatability and manufacturability in fabrication and integration of these devices for larger sensor arrays.

ZnO is emerging as a wide band gap semiconductor oxide with multifunctional properties that makes it an attractive sensor material. ZnO and its nanostructures are compatible with intracellular material and ZnO–based sensors have been demonstrated for detection of biochemicals such as enzymes, antibodies, DNA immobilization, and hybridization.[12–15] In this letter, we report the highly sensitive and selective immunosensing ability of a ZnO based TFT biosensor (ZnO-bioTFT). The epidermal growth factor receptor (EGFR) is used as the example because the sensing of EGFR-antibodies reacting with EGFR proteins has its implications in cancer related studies and drug screening for cancer, as EGFR is well-known to be overexpressed in solid tumors, especially breast cancers. The ZnO-TFT devices possess excellent and repeatable characteristics. It can be fabricated using conventional microelectronic process and can be integrated into a large-scale at sensor arrays low cost, which will benefit further development of a device platform not only for diagnosing cancers, but also for monitoring a patient's response to therapy in real-time.

The device schematic is shown as the inset of Fig. 1(a). It follows a back-gate inverted-staggered configuration. A Si substrate was covered with 1 μm layer of SiO_2 through wet oxidation followed by e-beam deposition of a layer of Au (50 nm)/Cr (100 nm) that serves as the gate electrode. A 70 nm layer of SiO_2 serving as the gate oxide was then deposited through plasma enhanced chemical vapor deposition with substrate temperature of 250 °C and using SiH_4 and N_2O as the source gases. A 50 nm ZnO thin film was grown using metalorganic chemical vapor deposition on the top of the

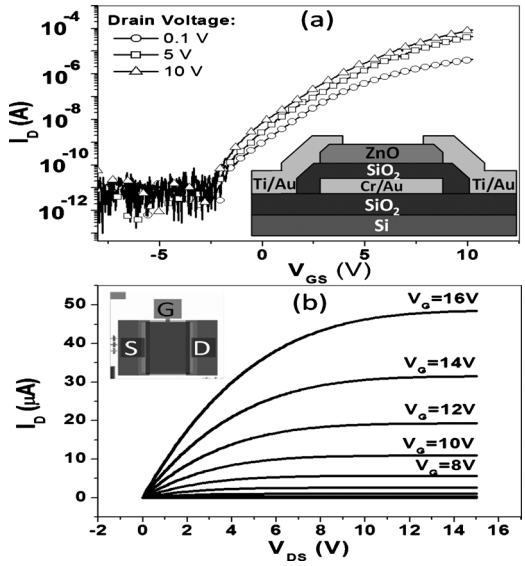

FIG. 1. (a) Transconductance curve of the ZnO-bioTFT and its vertical structure schematic (inset); (b) transistor characteristic curves for various gate bias, and the top view of the device (inset).

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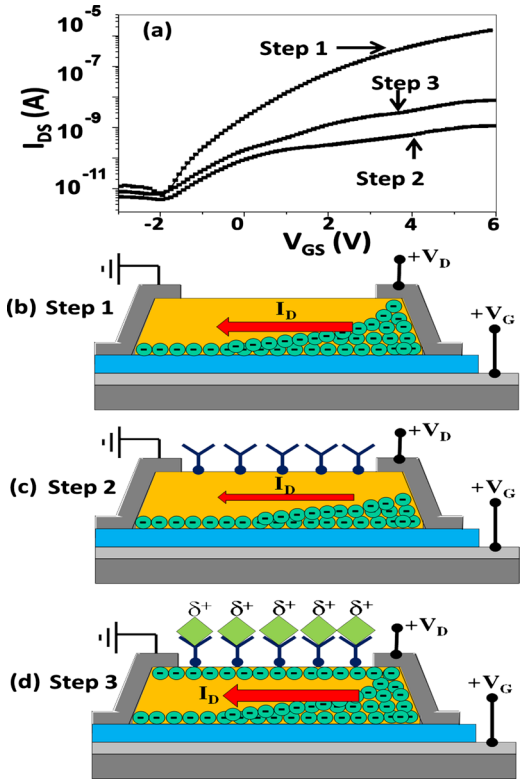

FIG. 2. (Color online) (a) Drain current vs gate bias for fixed drain bias of 10 V. Step 1: bare device, step 2: EGFR-antibody immobilization, and step 3: EGFR protein detection; [(b)–(d)] schematic of the carrier modulation mechanism for steps 1 to 3, respectively.

SiO_2 to serve as the *n*-type conduction channel, with substrate temperature at 350 °C and using diethyl zinc (DEZn) as the metal precursor and ultrahigh purity O_2 as oxidizer. Au (50 nm)/Ti (100 nm) was deposited through e-beam evaporation for the source and drain Ohmic contacts. The exposed ZnO channel acts as the sensing area and has a dimension of 200 μm × 400 μm, giving a W/L ratio of 2. Shown in the inset of Fig. 1(b) is the top view of the TFT device. The electrical characteristics of the ZnO-bioTFT are shown in Figs. 1(a) and 1(b). The transconductance curve [drain current (I_D) versus gate voltage (V_{GS})] in Fig. 1(a) shows that the bioTFT is a normally-OFF enhancement mode transistor with a threshold voltage of 4.25 V and an ON-OFF ratio of $\sim 10^8$. The high ON-OFF ratio of the device provides the high sensitivity of the device to the charge modulation within the ZnO channel. Figure 2(b) shows the transistor characteristic curves with drain current versus drain voltage for various gate-biasing of the device.

To realize the immunosensing ability of the ZnO-bioTFT, the exposed ZnO channel was functionalized using linkage chemistry, which involves three basic steps. First, the ZnO channel was functionalized with trimethoxysilane aldehyde (having a reactive aldehyde end group) by incubating the device in 1% v/v solution of the silane-aldehyde in 95% ethanol for 30 min. The device was then cured at 120 °C for 15 min. Second, the aldehyde groups were coupled to the amine groups of the monoclonal EGFR antibodies (1:50) through reductive amination in the presence of 4 mM sodium cyanoborohydride in PBS (*p*H 7.4) for two hours. Third, unreacted aldehyde groups were blocked using 100 mM ethanolamine in a similar manner to prevent nonspecific interactions of proteins. Finally, the device was rinsed in a continuous flow of PBS, *p*H 7.4 for 10 min.

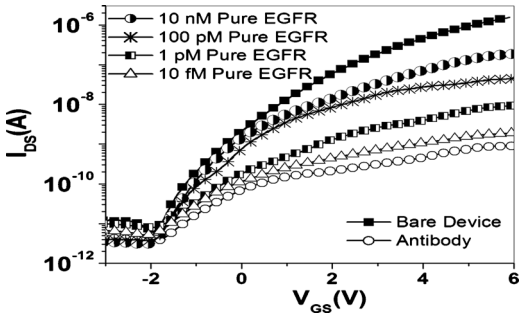

FIG. 3. Drain current vs gate bias for various Molar concentrations of pure EGFR proteins detected by the ZnO-bioTFT to demonstrate sensitivity.

The biofunctionalization enables the exposed ZnO channel direct interaction with the biochemical species being detected. The mechanism of detection of antibody-antigen reaction is illustrated in Figs. 2(a)–2(d). In the first step [Fig. 2(b)] the unfunctionalized ZnO-bioTFT is positively biased at the drain and gate electrode. The positive voltage at the gate causes the majority carriers of the *n*-type ZnO channel to accumulate near the base of the ZnO layer to facilitate a conduction path for the current flow from drain to source. The positive voltage at the drain causes some of the carriers to also accumulate near the side of the drain electrode forming a wedge-shaped conduction path. The bias at the drain also acts as the electron pump to drive the current to flow. For the second step [Fig. 2(c)], the exposed ZnO channel is functionalized with EGFR monoclonal antibodies (mAbs) having free lysine groups. The immobilized antibody molecules caused significant decrease in conductivity of the ZnO surface layer, thus, reducing the drain current. In the third step [Fig. 2(d)], the EGFR protein captured by the EGFR mAbs forms a polarized molecule with a dominant partially-positive charged tip[16] which led to the accumulation of negative carriers within the ZnO channel to accumulate near the exposed surface where the antibody-protein pairs were present. This carrier accumulation was in addition to the conduction path created near the gate. The combined amount of accumulation layer caused an increase in the current flow. The top molecule layer (reacted protein) acted as a virtual top gate and the antibody layer acted as a virtual insulator layer, thus forming a pseudodouble gated field-effect conduction scheme for the ZnO-bioTFT. The actual measured drain currents that confirmed each step of the detection process are shown in Fig. 2(a). The drain voltage is fixed to 10 V and the gate voltage is varied from −5 to +15 V, and the drain current is measured using an HP4156C semiconductor parameter analyzer and Cascade Microtech probe station.

To demonstrate the high sensitivity of the ZnO-bioTFT, solutions of pure EGFR (in PBS) were prepared with four different Molar concentrations using serial dilutions, namely, 10 nM, 100 pM, 1 pM, and finally 10 fM. Each EGFR solution (2 μl) was introduced to a separate but similar ZnO-bioTFT fabricated on a single chip that were simultaneously functionalized with EGFR mAbs. The drain current was monitored as a function of gate voltage with a fixed drain voltage of 10 V, for each concentration. Figure 3 shows the measured drain current versus gate voltage of the bioTFT. An increase in drain current was measured as the EGFR concen-

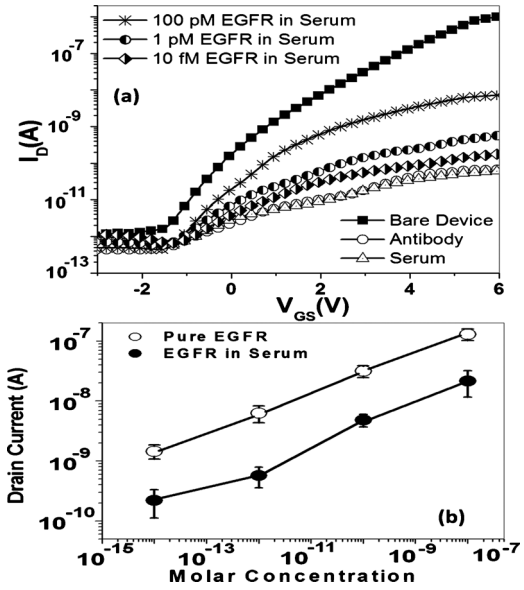

FIG. 4. (a) Drain current vs gate bias for various molar concentrations of EGFR-proteins in a serum solution containing many different proteins. (b) Sensitivity plot of the device for pure protein and protein in serum detection.

tration was increased and the graph also shows that the device was able to detect as low as 10 fM of EGFR concentration. The trend in the current readings agrees with the hypothesis provided by the pseudodouble gating effect discussed above.

The highly selective sensing of EGFR using the ZnO-bioTFT was also demonstrated. In this experiment, a 5 mg/ml (in PBS, *p*H 7.4) goat serum solution was prepared, which contains many different species of proteins. As mentioned above, different EGFR solutions were prepared, namely, 100, 1, and 10 fM, using this serum solution as the solvent and not pure PBS. For all the concentrations, the total amount of serum present remained approximately the same. Each of the different solutions (2 μl) was introduced onto a chip containing multiple similar bioTFT devices that were biofunctionalized with EGFR mAbs. The drain current of each device was measured as a function of gate voltage, with a fixed drain voltage of 10 V. As a control, we first introduced serum solution without the EGFR proteins to the ZnO-bioTFT. Figure 4(a) shows no change in the drain current for the pure serum confirming that there were no EGFR molecules in the solution. The drain current increased as a function of EGFR concentration. The bio-TFT detected only the EGFR proteins out of the many different proteins present in the serum solution introduced onto the sensing area of the device. Moreover, the device was able to discern as low as 10 fM of EGFR protein concentration in the serum solution. The sensitivity plot of the device for both pure and in-serum detection is shown in Fig. 4(b) which exhibits linearity in the x-y logarithmic scale.

In summary, we have demonstrated a ZnO bioTFT that has the ability to perform immunosensing with high sensitivity and selectivity. The channel of the bioTFT is functionalized with amine-terminated EGFR mAbs. EGFR proteins with the lowest concentration of 10 fM were detected by the device in both pure state and selectively in a concentration serum solution containing various other protein species. The ZnO-bioTFT enables bias-controlled operation though its bottom gate configuration. The high sensitivity of the device is attributed to its high on-off ratio, and the output current trend is explained by the pseudo-double gating electric field effect. The realization of the ZnO-bioTFT functionalized with EGFR mAbs reacting with EGFR proteins has potential applications in cancer diagnosis and treatment.

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