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IGZO thin film transistor biosensors functionalized with ZnO nanorods and antibodies

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ABSTRACT

We demonstrate a biosensor structure consisting of an IGZO (Indium–Gallium–Zinc–Oxide) TFT (thin film transistor) and an extended sensing pad. The TFT acts as the sensing and readout device, while the sensing pad ensures the isolation of biological solution from the transistor channel layer, and meanwhile increases the sensing area. The biosensor is functionalized by first applying ZnO nanorods to increase the surface area for attracting electrical charges of EGFR (epidermal growth factor receptor) antibodies. The device is able to selectively detect 36.2 fM of EGFR in the total protein solution of 0.1 ng/ml extracted from squamous cell carcinoma (SCC). Furthermore, the conjugation duration of the functionalized device with EGFR can be limited to 3 min, implying that the biosensor has the advantage for real-time detection.

1. Introduction

Quantification and analysis of biological processes are of great interest for biomedical applications. Measurement of proteins in human body fluids provides an important tool in disease diagnosis and drug prescription (Ninfa et al., 2009). Several technologies have been established to determine the concentration of proteins, which include, for example, colorimetric protein assay (Sapan et al., 1999), Western blotting (Ashley et al., 1988), spectrophotometric assay (Smeltzer et al., 1992) and surface plasma resonance (SPR) (Stenberg et al., 1990). Despite the availability of those methods, there are still demands for more accurate, real-time and simplified biosensors. The above properties can be fulfilled by field effect devices (FEDs) because of their ability to quickly translate the electrostatic binding phenomena to a readable signal (Poghossian et al., 2007). Biosensors based on FEDs have been demonstrated using various semiconductor materials such as Si (Poghossian et al., 2007), GaAs (Kirchner et al., 2002), ZnO (Reyes et al., 2011), and In–Ga–Zn–O (IGZO) (Kim et al., 2013). Furthermore, to improve the sensitivity, transistors with one dimensional (1-D) nanowire channels are employed due to their effective induction of channel currents when the external charges are applied (Allen et al., 2007; Chen et al., 2011; Wanekaya et al., 2006). However, the 1-D structure is limited by the sensing area and difficulties of aligning nanowires with source/drain contacts.

In this paper, we propose a biosensor structure using an IGZO TFT (thin film transistor) as the sensing and readout device. The IGZO TFTs offer several advantages as the transistors sensors. For example, they can be fabricated on the glass substrates using sputtering technique, which makes low-cost mass production possible. Because IGZO channel possesses high optical transmission under visible light, the TFT is immune to the ambient light induced current. As compared with typical amorphous or organic TFTs, IGZO devices have high carrier mobility, high device stability in air under bias stress, and high spatial uniformity on key parameters such as the threshold voltage. In our sample, an extended sensing pad on which ZnO nanorods were disposed ensures the isolation between biological solution and the transistor channel layer, and meanwhile increases the sensing area. In this work, the target protein is epidermal growth factor receptor (EGFR) which is commonly used in cancer related studies and drug screening for cancer (Kim et al., 2006; Wang, 1998). With the proposed structure, the IGZO TFT protein sensor is able to selectively detect 36.2 fM of EGFR with the total protein solution of 0.1 ng/ml extracted from squamous cell carcinoma (SCC).

2. Material and methods

A schematic diagram of the bio-sensors is shown in Fig. 1. The device consists of two parts; the electrical signal readout transistor

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and the extended sensing gold pad. Since most biological substances carry electric charges, as they are applied to the gold pad, an electric field will be induced across the metal sensing pad on the channel. As a result, the electrical charges of the proteins on the sensing pad will induce corresponding charges in the TFT channel, resulting in the changes of the channel currents. The concentration of proteins can thus be benchmarked by the variation of transistor currents.

2.1. Fabrication of the biosensors

A bottom-gate IGZO TFT on the glass substrate is employed for signal extraction of the biosensor. The fabrication started from depositing a patterned Mo gate contact electrode by DC sputtering and followed by a 80 nm-thick SiO_2 deposition by PECVD (plasma enhanced chemical vapor deposition) as the gate dielectric layer. Next, a 50 nm-thick IGZO (In_2O_3:Ga_2O_3:ZnO=1:1:1) channel was RF sputtered and then patterned by wet chemical etching to define the mesa area. The width and length of the channel are 100 and 50 μm, respectively. Mo source and drain contacts were then coated by a DC sputter. To passivate the channel, a 200 nm-thick SiO_2 layer was sputtered via holes opened by reactive ion etching. Next, the sensing gold pad was evaporated with a thickness of 300 nm and then baked at 250 °C for an hour. Finally, SU-8 was patterned around the sensing pad. The thickness of SU-8 is about 100 μm, leading to a 72.25 nl micro-sink capacity for the sensing pad.

2.2. Synthesis of ZnO nanorods

ZnO nanorods were synthesized by a two-step hydrothermal method. First, ZnO nanoparticles were prepared by dissolving zinc acetate powder into ethanol. The solution was than spin-coated on the sapphire substrate and then baked at 200 °C for 2 h to form the seed layer. Second, ZnO nanorods were synthesized in the mixture of zinc acetate and hexamethylenetetramine (HMT) solution at 90 °C for 2 h. The nanorods are about 50–100 nm in diameter and 1 μm in length.

2.3. Materials for biosensing

EGFR will be employed as the sensing target in this work. It was extracted from SCC (see Fig. 2(a)). To verify the existence of EGFR, we applied the fluorescein isothiocyanate (FITC) secondary antibody on the sample. First, EGFR antibody (Abcam, Cambridge, MA) was applied overnight to ensure the specific conjugation to the EGFR on the membrane of the cells. They were then washed to remove the non-conjugated EGFR. The FITC secondary antibody was applied to stain EGFR antibody. FITC emits fluorescent light at the peak wavelength of 528 nm when excited by the photons at the wavelength of 495 nm (Majone et al., 1992). Therefore, the green fluorescent emission of FITC suggests the existence of EGFR. Finally, dihydrochloride (DAPI) was applied to stain the nucleus. DAPI emits fluorescent light at the wavelength of 488 nm when excited at 340 nm (Sigma-Aldrich). The fluorescent image in Fig. 2(b) indicates EGFR highly expresses in SCC.

For control purpose, the protein solution from Hs68 was extracted (see Fig. 2(c)). Hs68 is a cell line derived from human foreskin fibroblasts, in which the expression of EGFR is significantly low. The application of FITC and DAPI indicates a low expression of EGFR in Hs68 (see Fig. 2(d)). From Fig. 2, it suggests that EGFR protein is abundant in SCC rather than in Hs68. In the biosensing experiment, cells were cultured on the 6-inches culture dish. Trypsin was applied to harvest cells. Next, with centrifuging the cells with Lysis Reagent at 13,000 *g* for 5 min, the total solution including EGFR was extracted.

2.4. Measurement

Fig. 3 illustrated the experimental flow. We first extracted the total protein solutions from the SCC cell line. The concentration of total protein solution was determined by Bradford protein assay (Bio-Rad, Hercules, CA) and then diluted to various concentrations as target samples for subsequent detection. Bradford protein assay quantifies the concentration by staining the total protein solution. The absorptions of the solutions were read by the Enzyme-linked immunosorbent assay (ELISA) reader. By comparing the absorption of the total protein solution to that of the bovine serum albumin (BSA) solution of known concentration, we are able to determine the concentrations of total protein solutions extracted from SCC.

To functionalize the biosensor, first, ZnO nanorods were lifted-off using a razor blade in the ethanol solution. The ZnO nanorod solution was disposed on the sensing pad (micro-sink) and then baked at 120 °C for 5 min to remove ethanol. The number of ZnO

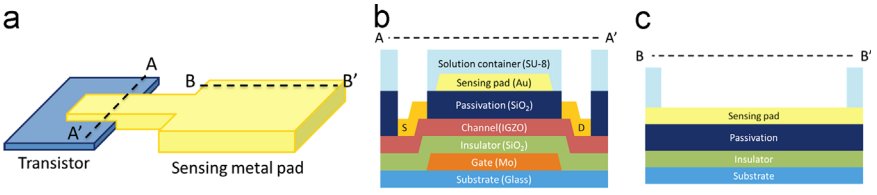

Fig. 1. (a) Schematic diagram of the biosensors. The device is divided to an IGZO TFT (b) for charge sensing and signal readout and a sensing pad (c) for biological solution. ZnO nanorods will be first disposed on the sensing pad before the application of antibodies and target solutions.

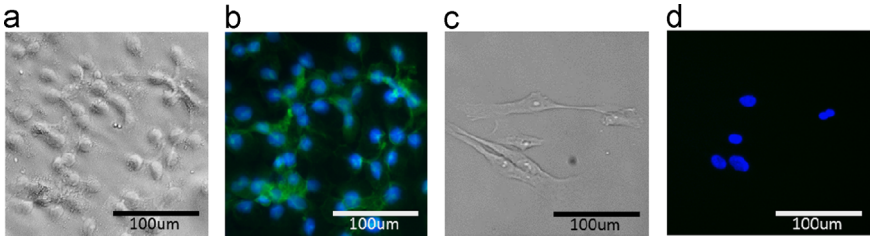

Fig. 2. The morphology (a) and the fluorescent image (after the application of FITC and DAPI) (b) of SCC. The green fluorescent emission implies the existence of EGFR and the blue fluorescent emission implies the nucleus of the cells. For control purpose, similar approaches were carried out on Hs68. The morphology and the fluorescent images are shown in (c) and (d), respectively. Lock of green emission suggests that the expression of EGFR is significantly low.