



IGZO thin film transistor biosensors functionalized with ZnO nanorods and antibodies

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ARTICLE INFO

Article history:

Received 12 August 2013

Received in revised form

23 October 2013

Accepted 24 October 2013

Available online 12 November 2013

Keywords:

Biosensors

Thin film transistors

Nanorods

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.

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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 SiO₂ deposition by PECVD (plasma enhanced chemical vapor deposition) as the gate dielectric layer. Next, a 50 nm-thick IGZO (In₂O₃:Ga₂O₃: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 SiO₂ 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 nF 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 then 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

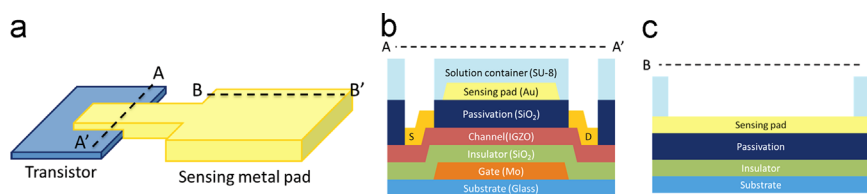


Fig. 1. (a) Schematic diagram of the biosensors. The device is divided to an IGZO TFT (b) for charge sensing and signal readout and a sensing pad (c) for biological solution. ZnO nanorods will be first disposed on the sensing pad before the application of antibodies and target solutions.

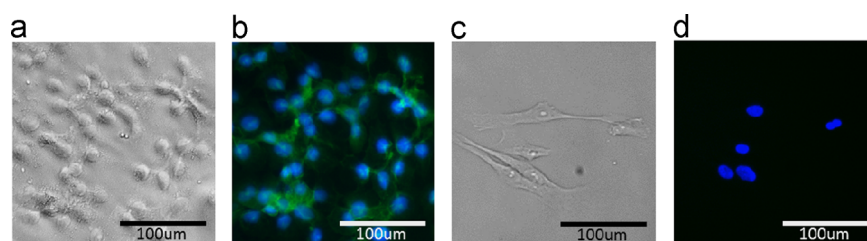


Fig. 2. The morphology (a) and the fluorescent image (after the application of FITC and DAPI) (b) of SCC. The green fluorescent emission implies the existence of EGFR and the blue fluorescent emission implies the nucleus of the cells. For control purpose, similar approaches were carried out on Hs68.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

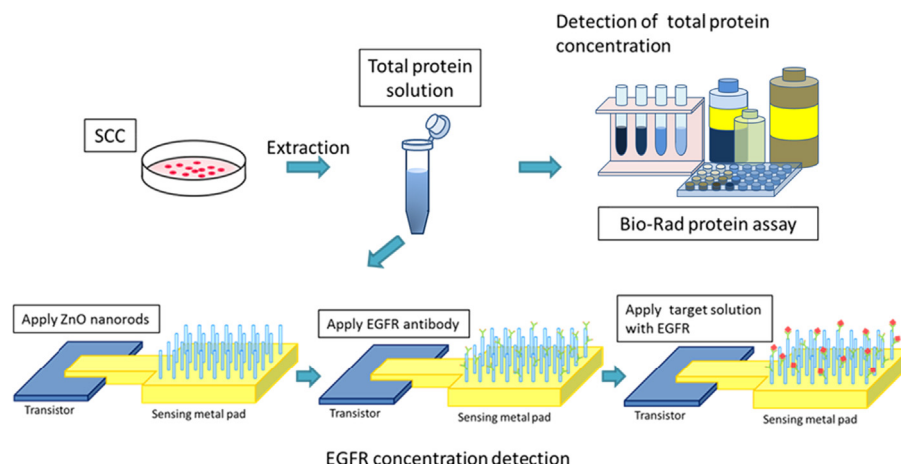


Fig. 3. Experimental flow of the biosensing. The protein solution of EGFR antibody was first extracted from SCCs. Bio-Rad protein assay was used to determine total protein concentration. The 100 $\mu\text{g/ml}$ SCC protein solution was decimally diluted to 10 $\mu\text{g/ml}$, 1 $\mu\text{g/ml}$, 100 ng/ml , ..., 0.1 ng/ml . The electrical responses after applying the total protein solution were then extracted from the functionalized TFT sensor.

nanorods on the pad is roughly 5×10^5 . The number is estimated by measuring the weight in a microbalance and determining the average size of the rods (assuming 80 nm in diameter and 1 μm in length) from SEM images. Next, the EGFR antibody was applied to the micro-sink. We wait for an hour to ensure the conjugation of the antibody with ZnO nanorods before the micro-sink was washed by phosphate buffered saline (PBS) for three times to remove the un-bonded EGFR antibody. Next, the crude protein solution was applied to the sink for 3 min and washed for three times to remove the unconjugated protein. The electrical signals were extracted before and after the application of nanorods, after the EGFR antibody binding, and after EGFR concentration test.

3. Results and discussions

The electrical response of the biosensors was next investigated. Fig. 4(a) and (b) demonstrates the drain-source current (I_{DS}) vs. gate-source voltage (V_{GS}) transfer characteristic and drain-source current (I_{DS}) vs. drain-source voltage (V_{DS}) modulation curves, respectively, of the TFT prior to the biosensing measurement (bare device). The transfer curves were measured at the drain voltage of 10 V. The threshold voltage, V_{T} , of the TFT is 2.56 V. The sensing process starts from functionalizing the device by ZnO nanorods and EGFR antibody. The ZnO nanorods were bonded to the sensing pad through van der Waals force. Next, EGFR antibody was diluted ten times with PBS then applied to the sensing sink for an hour to ensure the binding of EGFR with ZnO nanorods. The transfer and modulation curves of the device are shown in Fig. 4(c) and (d), respectively. The transfer curves were measured at the drain voltage of 10 V and the modulation $I_{\text{DS}} - V_{\text{DS}}$ curves were measured at the gate bias of 8 V. The drain current increases tremendously as the ZnO nanorods were applied since they provide a significant amount of negative charges. At $V_{\text{GS}} = 8 \text{ V}$ and $V_{\text{DS}} = 15 \text{ V}$, the current increases from 0.02 to 0.63 mA after nanorods were applied. The application of EGFR antibodies further shifts the current higher to 0.68 mA due to the existence of negative charges on the antibodies.

In the next step, protein detection was performed by applying target solution (for 3 min) to the functionalized device. As the TFT was biased at V_{GS} of 8 V and V_{DS} of 15 V, the corresponding drain currents at various total protein concentrations are demonstrated in Fig. 4(e). The currents were extracted from the same device with the electrical properties shown in Fig. 4(a)–(d). Furthermore, the

current increment is defined to benchmark the sensitivity of the devices. It is the percentage increase of the drain current after the target protein solution is applied, as compared to the drain current of functionalized device (the device with only ZnO nanorods and EGFR antibody). In Fig. 4(e), we plot the statistical current increment by measuring three samples for each total protein concentration. The average and standard deviation were shown. The current increment is increased linearly with the logarithm of the protein concentration. The correlation coefficient is about 0.99.

The advantage of adding ZnO nanorods on the sensing pad is discussed next. We measured the current increments after applying EGFR antibody from the devices with and without ZnO nanowire modification. In such an experiment, no total protein solutions were added. In the case without ZnO nanorods, the EGFR antibody was applied directly to the micro-sink to functionalize the device. From Fig. 5, the sensitivity curves show correlation coefficients of 0.99 and 0.95, respectively, for devices with and without applying ZnO nanorods. The slopes of the linear regression curves are nearly the same, indicating that the sensitivity of the biosensors is almost the same for both cases. With the same concentration of EGFR antibody, the current increment of the device with ZnO nanorods is, in average, 110.86% higher than that without rods, which suggests that ZnO nanorods help to attract proteins due to the possession of more static charges and larger surface-to-volume ratio. Furthermore, Fig. 5 shows that the standard deviation of the device without nanorods is higher than that with nanorods, which is attributed to the excellent binding capability of the ZnO rods to the EGFR antibody. For the device with only the Au pad on the micro-sink, antibody can be easily washed off, leading to the electric charge variations for different samples. The above discussion again implies that ZnO nanorods help on improving detection of limit and reducing sensing variations.

The induced charges can be calculated from the TFT current following the equations (Sedra and Smith, 2003):

$$Q_{\text{t}} = \frac{2}{3} WLC(V_{\text{GS}} - V_{\text{T}}) \quad (1a)$$

$$I_{\text{D}} = \frac{1}{2} \mu C \frac{W}{L} (V_{\text{GS}} - V_{\text{T}})^2 \quad (1b)$$

Where W and L are the width and length of the channel respectively, C is the capacitance per unit gate area, and μ is the mobility of carriers in the channel. From the current-voltage curves in Fig. 4, for the device with ZnO nanorods, equivalent charges of 45.22 fC (femto coulomb) are induced by the nanorods

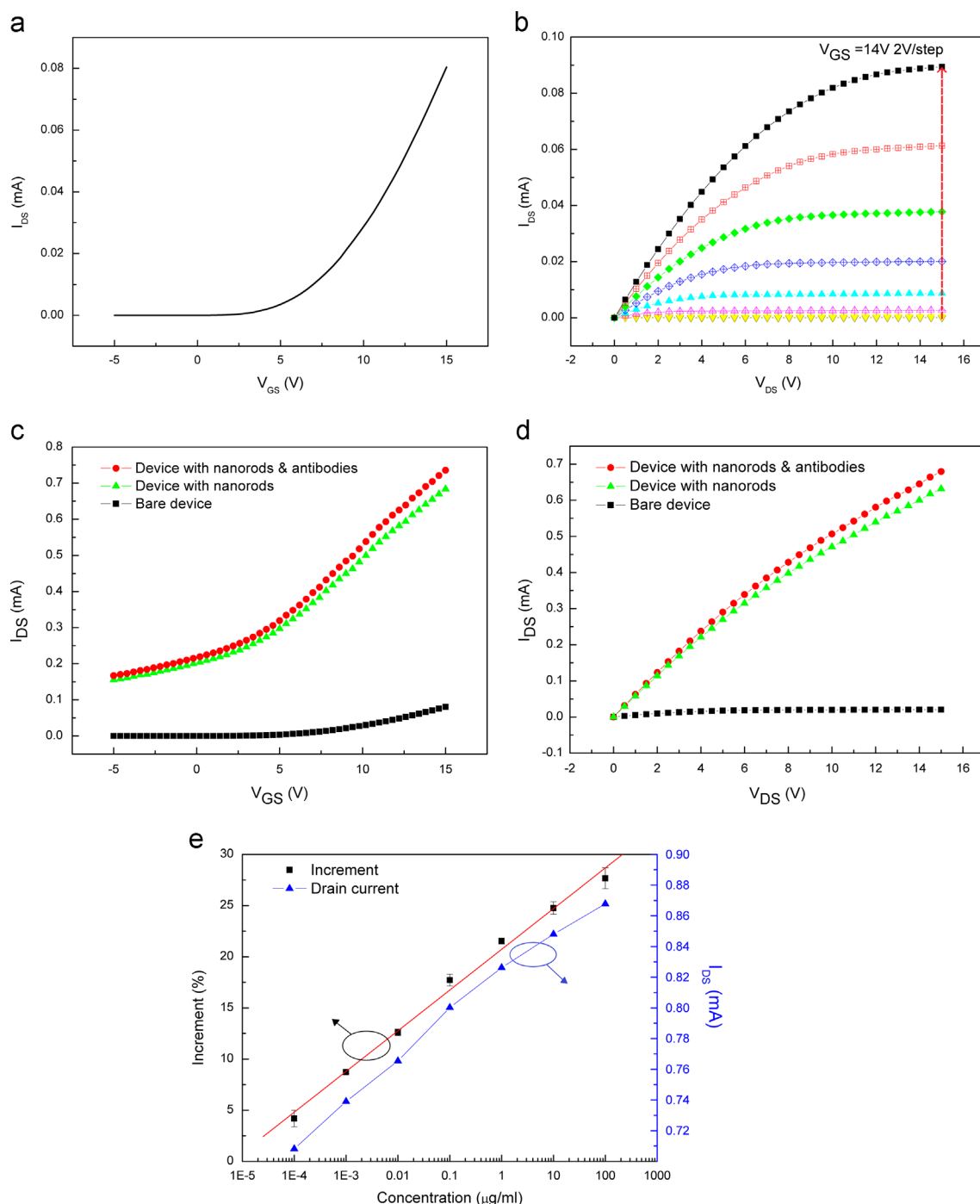


Fig. 4. Electrical properties of the biosensor. Prior to functionalizing the device, the transfer and the modulation curves of the TFT are shown in (a) and (b), respectively. The biosensor was then functionalized by applying ZnO nanorods and EGFR antibodies. The corresponding I_{DS} - V_{GS} and I_{DS} - V_{DS} curves of the device with ZnO rods and ZnO rods/EGFR antibodies are shown in (c) and (d), respectively. And (e) demonstrates the corresponding drain currents and current increments at various total protein concentrations.

and 2.02 fC by the diluted EGFR antibodies. On the other hand, for the device without nanorods, only 0.44 fC is induced by the EGFR antibody. From the above results, a device with ZnO nanorods can attract 4.7 times the static charges of EGFR antibody as compared to one without rods.

Finally, the specificity of EGFR is investigated. As in Fig. 6, with the same total protein concentration applied to the functionalized devices for 3 min, the SCC protein solution has a greater percentage current increment than the Hs68. At 100 $\mu\text{g/ml}$, the current increment of the SCC extracted protein is 4.24 times higher than that extracted from Hs68. Furthermore, the smaller slope of the linear regression of the protein extracted from Hs68 implies that it is less

expressed to EGFR antibody. It also suggests that the physisorption on ZnO surface is mostly blocked by the EGFR antibody; otherwise a large current increment will be observed due to the binding of the ZnO nanorods and the protein from Hs68. The results indicate that our bio-TFTs functionalized with ZnO nanorods and EGFR antibodies provide a good specificity to identify EGFR.

4. Conclusions

A biosensor structure that consists of an IGZO TFT and an extended sensing pad with ZnO nanorods were demonstrated.

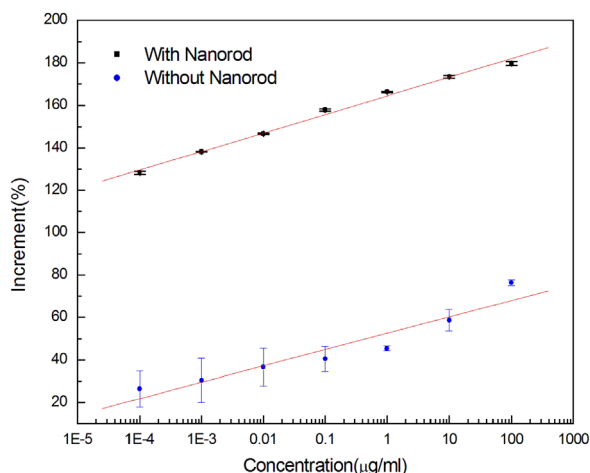


Fig. 5. Comparisons of the current increments after applying EGFR antibody from the devices with or without ZnO nanorods. In this experiment, no total protein solutions were added on the devices. The currents were extracted at $V_{GS}=8$ V and $V_{DS}=15$ V. The statistical data were extracted from three samples at each concentration.

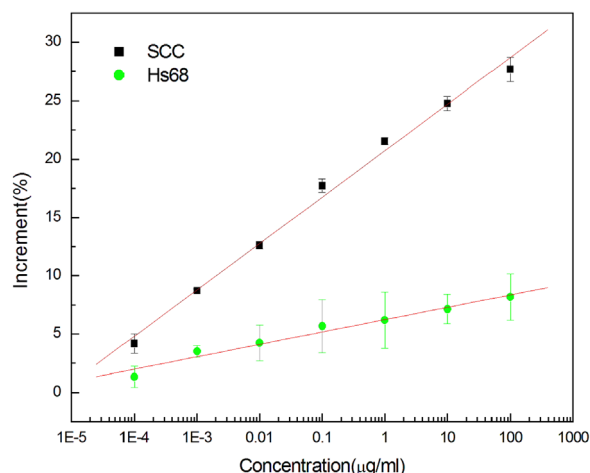


Fig. 6. Specificity testing of the biosensor. The selectivity of the sensor is demonstrated by verifying the extracted protein from either SCC or Hs68. At the same concentration of total protein solution, the current increment of adding Hs68 protein solution is much lower than that of SCC protein solution. The statistical data were extracted from three samples at each concentration.

The biosensor is functionalized by applying ZnO nanorods and then EGFR antibodies to the sensing pad. 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). The disposal of ZnO nanorods can significantly increase the current increment of the sensor and reduce the detection variation; thus the limit of detection can be improved. From the experiment, a device with ZnO nanorods can attract 4.7 times the static charges of EGFR antibody as compared to one without rods. Also, the 3-min conjugation duration of the total protein solution implies that our biosensor can be employed for real-time detection. Finally, the specificity of the functionalized biosensors was studied. Clear distinction of the current increments was observed from the bio-TFTs with total proteins extracted from either SCC or Hs68.

Acknowledgment

This work was supported by the National Science Council of Taiwan under the Grants NSC 100-2628-E-002-030-MY3 and NSC 101-2218-E-002-009.

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