

# Effect of reference electrode in the nanoFET (field-effect transistor)-based biosensor experiment

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Published in Micro & Nano Letters; Received on 2nd July 2014; Revised on 16th October 2014; Accepted on 17th October 2014

The reference electrode in an electrolyte solution affects the surface potential of the nanoFET (field-effect transistor) channel which can result in a false signal to be measured in the FET channel during bio- and chemical sensing experiments. In this reported work, it was demonstrated that different sensing results that were obtained were affected by the choice of the reference electrode through a pH and ion concentration sensing experiment. Three types of reference electrodes were compared: the SCE (saturated calomel reference electrode), the Ag/AgCl tube and the integrated Ag/AgCl electrode. The pH sensitivity equivalent to the surface potential was evaluated: 32.3, 34.6 and 16.3 mV/pH in the case of the SCE, the refillable tube type Ag/AgCl and the integrated Ag/AgCl, respectively.

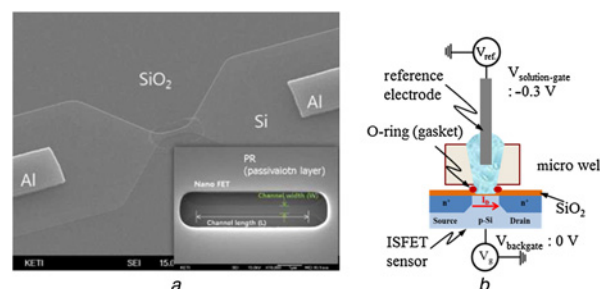
**1. Introduction:** The bio field-effect transistor (BioFET) is one of the most attractive sensors because of the advantages of system integration and direct electrical readout for charged biomolecule detection. Limitations exist in the development of practical applications of nanoFETs as biosensors, despite the simple detection principle to directly measure the field effects of the charges of biomolecules, because of the measuring conditions in which the sensors operate which is an electrolyte solution environment where many different ions exist, such as physiological saline [1]. Furthermore, the Debye screening effect, that the distance affected by the electrostatic force by the charge of biomolecules rapidly attenuates depending on the ion concentration in the buffer, is another topic of consideration for the application in biosensors. Also, the pH of the solution is an important factor because of its effect on the charge of the biomolecule, the isoelectric point (pI) [2]. Moreover, the electric potential exerted by the solution affects the channel of the nanoFET when the voltage is applied to the solution [3].

In this Letter, we confirm the effect of reference electrodes by measuring the pH and the ion concentration sensitivity by applying a bias voltage to the electrolyte solution with different types of electrodes: the SCE (saturated calomel reference electrode), the Ag/AgCl electrode (tube-type) and the microAg/AgCl electrode (paste-type) were used to compare characteristics. The solution gate voltage was applied to the FET channel by the reference electrodes to bias the nanoFET channel and to evaluate the pH sensitivity through the surface potential of the FET channel as well.

**2. Fabrication:** The proposed nanoFET device was fabricated on a SOI wafer [4]. The active silicone layer and the insulator layer were 20 and 145 nm, respectively. The channel length was 2  $\mu$ m and the thickness of gate insulator was 5 nm. The PR was applied onto the surface of the chip for passivation except the sensing area to detect biomolecules. Fig. 1 shows the field emission scanning electron microscopy (FESEM) image of the nanoFET and the schematics of the experimental setup used to measure  $I_{ds}$  including the reference electrode. The integrated reference electrode was made by applying and drying Ag/AgCl paste on the fabricated device. The proposed nanoFET biosensor consists of the PCB packaged chip and electrical interface allowing for convenient measurement and experimentation. The chip size is about 5  $\times$  7 mm and a PCB

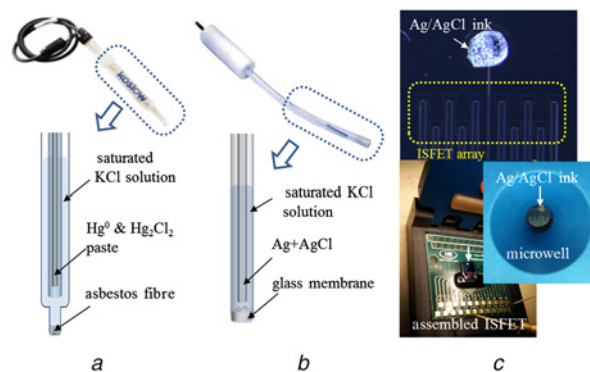
substrate of 30  $\times$  30 mm was used for the electrical interface with wire interconnection packaging.

**3. Experiment:** To minimise external noise and to simplify solution exchange, an electrical interface with a microwell socket for the



**Figure 1** FESEM image of nanoFET, and the schematics for measuring the readout current for stabilisation of the sample solution potential with a reference electrode

a FESEM image of nanoFET  
b Schematics for the measuring



**Figure 2** Three kinds of reference electrodes used to compare the effecting of the dependency on the ion concentration of the buffer solution

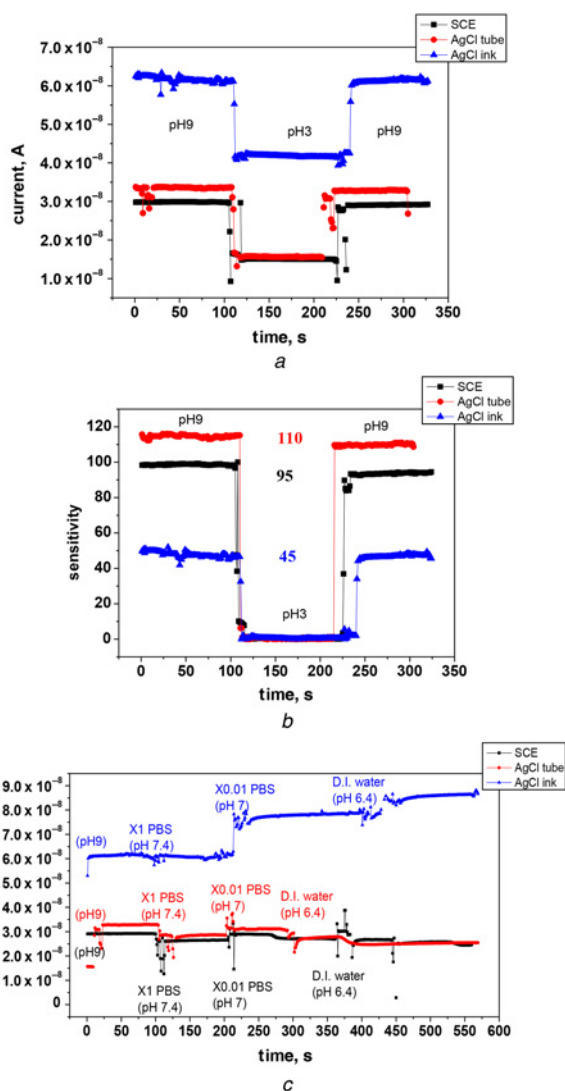
a SCE  
b Ag/AgCl reference electrode in saturated KCl solution tube  
c Integrated reference electrode on chip with Ag/AgCl ink

printed circuit board packaged chip was developed and the reference electrode was fixed to a mechanical stand as shown in Fig. 2. A maximum of 100  $\mu\text{l}$  of solution volume can be injected into the microwell. Also, Fig. 2 shows the three kinds of reference electrodes used for the experiments. As shown in Figs. 2a and b, a commercial SCE (Koslow Scientific Co., USA) and refillable miniature Ag/AgCl (eDAQ Co. Ltd, Japan) used, were [5, 6]. Fig. 2c shows the integrated micro reference electrodes made using Ag/AgCl ink (ALS Co. Ltd, Japan) on the surface of the device [7]. Before the pH sensing experiment, the conductance change of the nanoFET channel according to the solution voltage applied by the reference electrode was measured from 0 to  $-0.3$  V in  $-0.1$  V steps. The surface potential of the FET channel was directly affected by the applied voltage to the electrolyte solution gate. Evaluation of pH sensitivity was performed for each

reference electrode by comparing the equivalent surface potential induced by a pH 9/pH 3 change.

**4. Experimental results:** Fig. 3 shows the real-time measurement of the conductance change of the nanoFET channel according to the solution gate voltage applied by the reference electrodes in 1X PBS solution (pH 7.4), pH 9/3/9 exchange and ion concentration dilution with deionised (DI) water in sequence. The reference electrodes used were the SCE, the Ag/AgCl electrode (tube type) and the integrated Ag/AgCl (ink) electrode. During the pH sensing measurement, the bias voltage of the solution gate through the reference electrode was found to be  $-0.3$  V. The sensitivity ( $\Delta I/I_0$ ) for different pH solutions was normalised as shown in Fig. 3b. The sensitivity of  $\Delta I/I_0$  is necessary to compare real-time detection values. The pH sensitivity equivalent to the surface potential, which corresponds to the solution gate voltage, was deduced to be 32.3, 34.6 and 16.3 mV/pH in the case of SCE, the refillable tube type Ag/AgCl and the integrated Ag/AgCl, respectively. The integrated Ag/AgCl electrode (ink) case showed deteriorated pH sensitivity. We thought that the result was caused by the surface potential variation between the integrated Ag/AgCl reference electrode and the electrolyte solution during the pH 9/3 solution exposure. In addition, the response of the FET channel, according to the ion concentration dilution of  $\times 0.01$  phosphate buffered saline (PBS) and DI water solution, showed different results in the pH sensing tendency for the integrated Ag/AgCl electrode when compared to the SCE and the tube-type Ag/AgCl electrode in Fig. 3c. This was analysed as the result of the change in the Cl-ion concentration because of the dilution of the salt ion concentration, thus hindering the function of the reference electrodes (SCE, the refillable tube type Ag/AgCl and the integrated Ag/AgCl). It was confirmed that the reference electrode had a significant impact on the measurement when the ion concentration was diluted. From the results, it was found that as the equilibrium of the oxidation–reduction reaction changes, the nanoFET sensor was more sensitive to the reference potential changes than the pH of the sample solution.

Fig. 4 shows that the slope of the  $I_{ds}$ – $V_{sg}$  plots represents the sensitivity of the device. The sensitivity to different pH solutions confirmed that the change in the current level relates to the change in sensitivity ( $\Delta I/I_0$ , mV/pH). As the previous results in Fig. 3, the solution gate voltage was modulated from 0 to  $-0.3$  V in steps of  $-0.1$  V to compare the characteristics of the device in the buffer solution  $1 \times$  PBS for each reference electrode. Reed's group at Yale University suggested that it was possible to quantify the concentration of charged protein molecules in liquid solution. Their trial to



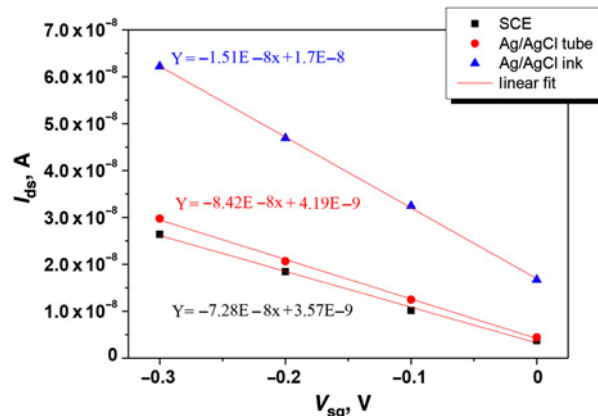
**Figure 3** Results of  $I_{ds}$  real-time measurement with corresponding pH concentration and electrolyte solution

Reference electrode used were SCE, Ag/AgCl electrode (tube type) and integrated Ag/AgCl (ink) electrode while current was being measured a According to the variation in pH concentration, real time of  $I_{ds}$  detected average value from two sensors of each device

b Measured current level in each device with normalised sensitivity value ( $\Delta I/I_0$ )

Values of the voltage per changed pH 1 are calculated from the  $I_{ds}$ – $V_{sg}$  plots in Fig. 3a

c Sensitivity real-time plots of nanoFET biosensor by measuring ion concentration with same device



**Figure 4**  $I_{ds}$ – $V_{sg}$  plots of current change ( $\Delta I_{ds}$ ) according to the change of surface potential in nanoFET from the results of  $I_{ds}$  real-time measurement Sensitivity of device can be deduced from the slope and mV/pH

quantify and calibrate the current change rate for the concentration of the immobilised negatively charged biomolecules on the channel such as protein in liquid solution was reported in [8]. The  $I_{ds}$ - $V_{sg}$  plots of current change ( $\Delta I_{ds}$ ) according to the change of the surface potential in the nanoFET are from the results of  $I_{ds}$  real-time measurement. The sensitivity of the device can be deduced from the slope and mV/pH.

**5. Conclusions:** The fabricated nanoFET is sensitive to the ion and pH concentration of the operating environment such as buffer solutions. The Debye screening effect is a cause of difficulty in applications such as a biosensor in an environment with a low ion concentration, such as the buffer solution environment. It was confirmed that the reference electrode had a significant impact on the measurement when the ion concentration was diluted. Therefore, careful selection of the reference electrode is needed to accurately control the reference voltage of the solution when measuring the potentials of biological materials in biosensor applications. Further research is under way to determine the characteristics of reliability and reproducibility in measurements for commercialisation.

**6. Acknowledgments:** This work was supported by the R&D programme of the Ministry of Trade, Industry and Energy

(MOTIE, Korea) and the Korea Evaluation Institute of Industrial Technology (KEIT, Korea).

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