DEVELOPMENT OF LABEL-FREE BIOSENSOR BASED ON APTAMER-MODIFIED SI NANOWIRE FIELD EFFECT TRANSISTOR (FET) USING TOP-DOWN APPROACH AND SOL-GEL METHOD

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ABSTRACT

In this study, Si-NWs with the length of 200 μm, width of 200 nm, and height of 50 nm were fabricated using basic MEMS processes such as photolithography, RIE and KOH etching. They had high sensitivity and showed the FET characteristics due to high surface-to-volume ratio. Also, high affinity aptamers for prostate specific antigen (PSA) were immobilized on Si-NW using sol-gel method and then the real-time measurement of electrical signal of free-PSA and total-PSA which was guideline of prostate cancer was carried out according to PSA concentration.

KEYWORDS: Silicon Nanowire (Si-NW), Field Effect Transistor (FET), Sol-Gel, Aptamer, Prostate Specific Antigen (PSA)

INTRODUCTION

Nanowires (NWs) with high surface-to-volume ratio have excellently electrical characteristics and high sensitivity [1]. Si Nanowires (Si-NWs) have been studied as sensors especially for detecting biomolecules[1-2]. Si-NWs fabricated by top-down approach have several advantages comparing to ones by bottom-up method; (i) outstanding reproducibility, (ii) constant quality, (iii) excellent reliability of electrical characteristics and (iv) easy integration with CMOS process [2, 3].

Aptamers are short, single-stranded oligonucleotides which can fold into specific three-dimensional structures in order to recognize target molecules such as small chemicals, proteins, or even cells [4]. High affinity aptamers for specific target molecules can be isolated from a library of randomized sequences in vitro by using the SELEX process [5]. Due to their thermal stability, long shelf-life, and ease of chemical synthesis and modification, aptamer-based biosensors are considered as promising molecular diagnostics of the future [4]. Prostate cancer antigen (PSA) was selected for this study. The ratio of free-PSA (fPSA) to total-PSA (tPSA) is useful in assessing the risk of prostate cancer in patients with borderline or moderately increased tPSA and has been used to help select men who should have follow-up prostate biopsy [6]. Thus we selected aptamers both from fPSA and tPSA (unpublished data).

In this study, after Si-NWs are fabricated using MEMS processes, the aptamer is immobilized on it using sol-gel method and then the electrical signal is measured according to prostate specific antigen (PSA) concentration.

EXPERIMENTAL

Si-NW Field Effect Transistor (FET) devices (Figure 1(a)) were fabricated with SOI wafer of 50nm thick (100) single crystal Si layer and 150nm thick oxide layer. Basic MEMS processes were used such as photolithography, reactive ion etch (RIE) and KOH anisotropic etching [7]. The fabricated Si-NWs had width of 100nm, length of 180μm and height of 50nm (Figure 1(b),(c)). Si-NWs were doped with boron by ion implantation for electrical detection. Then, Cr/Au (50/200nm) electrode pads were formed for ohmic contact and the other areas except Si-NWs and electrode pad were covered with SU-8 for reducing noise signal (Figure 1(a),(b)). Finally, PDMS reservoir was bonded with the device and then the completed device was wire-bonded on PCB for electrical measurement (Figure 2(a),(b)).

PSA aptamers (fPSA and tPSA aptamers) were immobilized on the each Si-NW by Sol-Gel method (SolB Reagent, PCL Inc, Korea). The sol-gels with and without aptamers were individually dropped onto each Si-NW with 250pl/drop and 100μM concentration using Sciflexarrayer S1 (Scienion AG, Germany) (Figure 2(c)). To increase the reliability of measurement, when we measured the ratio of fPSA to tPSA, additional reference Si-NW were used to standardize the electrical signal and remove the background signal from each electrode.

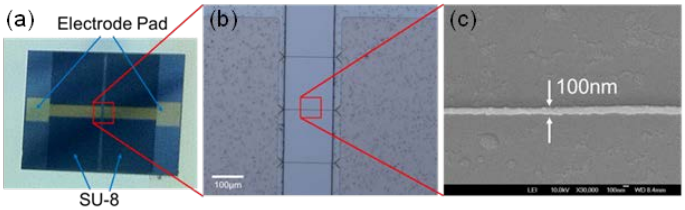

Figure 1: (a) Fabricated Si-NW FET device, (b) optical microscopy image of Si-NW, (c) SEM image of Si-NW (width of 100nm), (d) Sol-gel spotted on Si-NW array.

To evaluate the FET characteristic of Si-NWs, PDMS reservoir was filled with DI water and drain current (I_d) variation was measured by applying V_{gate} voltage to DI water, as shown in Figure 3(a). Figure 3(b) is the schematic view of PSA real-time measurement.

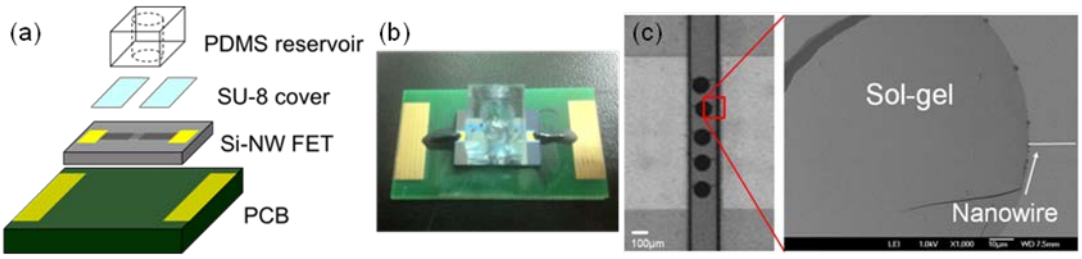

Figure 2: (a) Schematic view of Si-NW FET package, (b) After wire-bonding on PCB, (c) Sol-gel spotted on Si-NW array

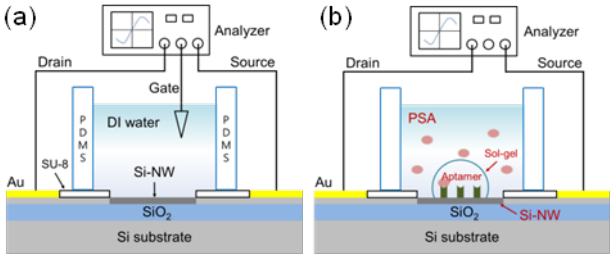

Figure 3: (a) Schematic view of FET characteristic measurement, (b) PSA real-time measurement.

RESULTS AND DISCUSSION

Figure 4 shows changes of I_d with V_{gate}. This makes it possible to obtain the concentration value of PSA according to charge change which is created by reaction of PSA and PSA aptamer at Si-NW surface. Figure 5 shows the resistance variations of Si-NW before and after PSA aptamer immobilization with sol-gel method when the gate voltage is 0 V. The resistance of Si-NW increased for fPSA aptamer immobilization, but it decreased for tPSA immobilization. It is thought that the fPSA aptamers carry a positive charge but tPSA aptamers carry a negative charge due to the characteristics of p-type Si-NW FET.

Figure 6 shows the real-time measurement results for Si-NW's resistance change by varying PSA concentration from 4pg/ml to 400pg/ml. Although the frequency range was high due to the noise of measurement equipment, constant resistance gap was shown according to PSA concentration. After PSA reacted with fPSA aptamer, the electrical resistance increased according to the PSA antigen concentration. Whereas the electrical resistance decreased after the reaction with tPSA aptamer. In Figure 6(a), there was noise around equipment and environment but the fPSA real-time measurement showed the change of electrical signal. The average change of electrical signal was 8.25% for 1pg/ml of PSA concentration. In tPSA real-time measurement, we applied the gate voltage (V_{gate} = 0 V), so the noise dramatically decreased (Fig. 6(b)). This is due to the P-MOSFET characteristics of the fabricated Si-NW device. That is, when FET is on at V_{gate} = 0 V, the resistance between source and drain decreases, so its noise also decreases.

The electrical signal change for tPSA aptamer was smaller than that for fPSA aptamer, so it was 0.42% for 1pg/ml of PSA concentration. Because there is difference between fPSA and tPSA in PSA and PSA-aptamer binding, it cannot be said that the concentration of tPSA is lower than that of fPSA although the resistance variation of tPSA is numerically lower than that of fPSA. In conclusion, Si-NW sensor with fPSA aptamer responded to PSA more sensitively than that with tPSA aptamer. This means that the small amount under 4pg/ml in the case of fPSA can be detected more easily when compared with that of tPSA.

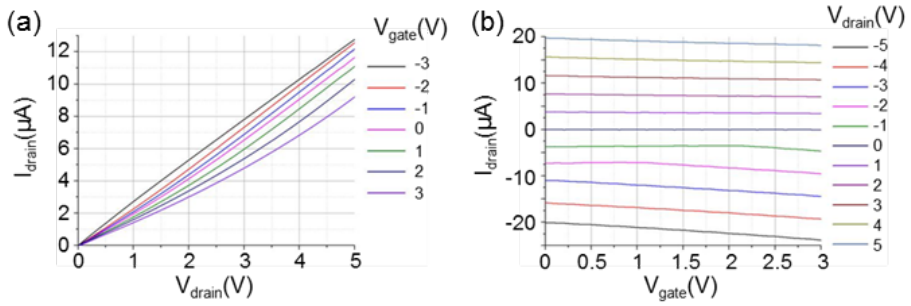

Figure 4: (a) I_d-V_d characteristics for the Si-NW FET, (b) I_d-V_{gate} characteristics for the Si-NW FET

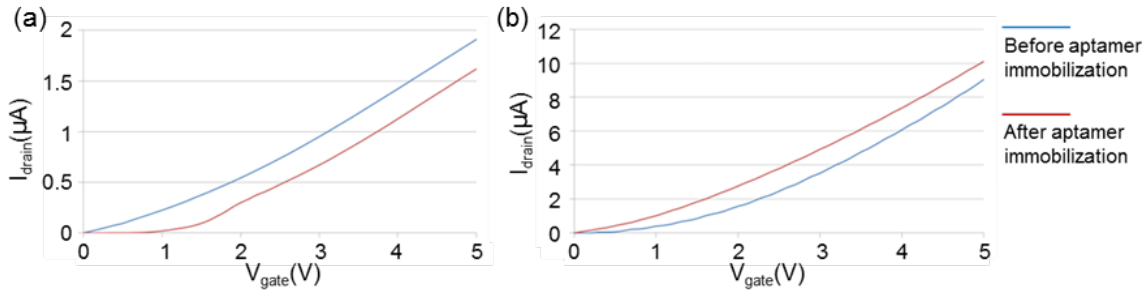

Figure 5: Resistance variation of Si-NWs before and after (a) fPSA aptamer immobilization, (b) tPSA aptamer immobilization when applying the gate voltage (V_{gate} = 0 V).

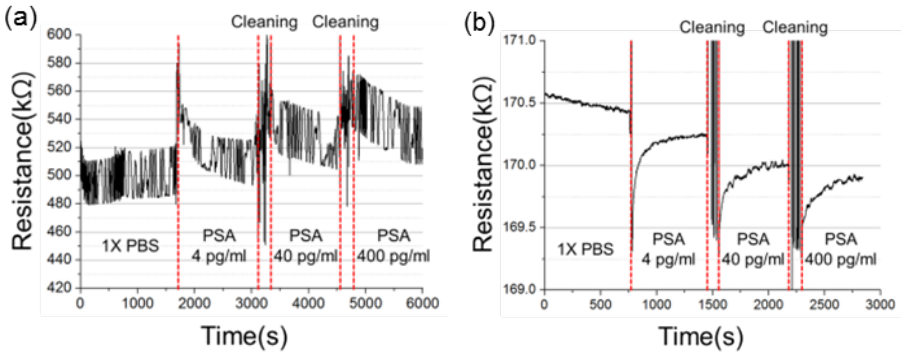

Figure 6: Real-time electrical measurement of (a) fPSA according to PSA concentration without the gate voltage, (b) tPSA according to PSA concentration with the gate voltage (V_{gate} = 0 V)

CONCLUSION

In this paper, we presented the novel fabrication process of Si-NWs and easy immobilization of aptamer using sol-gel method. Simple processes including photolithography, RIE and KOH etching have benefits of mass production and low cost manufacturing. Aptamer immobilization method using sol-gel drop has some advantage such as long time preservation of aptamer and chemical stability. The electrical signal of fPSA and tPSA was measured according to the their concentration. The electrical resistance of Si-NW with fPSA aptamer increased but that with tPSA aptamer decreased when PSA concentration increased. It is expected that it enable more accurate prostate cancer diagnosis through comparing the measurement values of fPSA with that of tPSA.

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