Label-Free Protein Nano-Biosensor using Top-Down Fabrication

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ABSTRACT

A nanosensor of n-type Si field-effect transistor (FET) via top-down method was employed to monitor PSA with great selectivity and high sensitivity up to 10 pg/mL. When a biomaterial, negatively charged PSA, is introduced into a fluidic channel, the conductivity of FET is lowered than that in pure buffer solution from the n-type nature of nanochannel as a nanobiosensor. The Si FET nanobiosensor offers a label-free and real-time monitoring, which explains the origin of electrical property resulting from the specific binding between antibody and antigen.

Keywords: Si FET, nanosensor, top-down, PSA, electrical detection, protein

1. INTRODUCTION

When the size of electronic devices reaches to nanometer ranges, the circuits become extremely sensitive to a minute change of external environment, implying that nano-sized sensors will be at high demand that requires the lowest detection limit for the high sensitivity. That is, the nano-scaled device is a promising candidate in extremely sensitive detection of biomolecules. Great interest for device development which runs in nanometer size has been focused in terms of its scientific and industrial importance [1-8]. Especially, Si field-effect transistor (FET) nano-bio electronic device for the detection of biomolecules have been a subject of many studies due to its high sensitivity and detection limit to very low concentration. The detection of Streptavidin molecule at nanomolar concentration using biotin-bound Si nanowires FET, and that of DNA molecules of femtomolar concentration using PNA-coated Si nanowires FET has been explored for this research [5,6]. However, since they are fabricated by bottom-up method, there are still considerable problems in positioning the Si nanowires FET at defined position on chips properly and integrating the Si nanowires FET with high density while maintaining their physical and electrical characteristics. In this paper, we have demonstrated highly sensitive, selective, real-time, and label-free direct electrical detection of PSA by Si FETs fabrication from top-down approach.

2. EXPERIMENTAL

Si FET devices were fabricated using a silicon-on-insulator (SOI) wafer (p-type, (100), 14-22 Ω·cm) with a 100 nm top silicon layer and a 200 nm buried oxide (BOX) layer. To form the heavily doped channel of Si-FET, phosphorous ions were implanted into the top silicon layer and the dose concentration was $3\times10^{13}/cm^2$. Implanted ions were dispersed uniformly within the top silicon by the thermal annealing process at 950℃. To make a thin

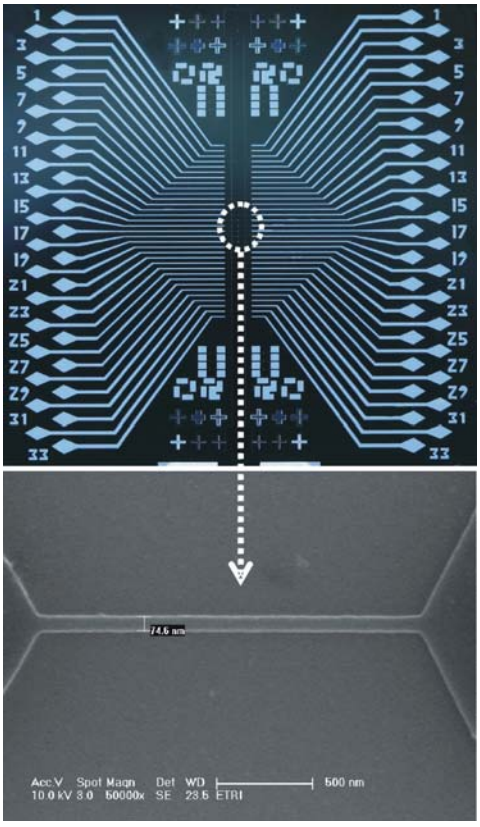

Figure 1: Structure of a Si FET nanosensor (2 cm × 2 cm). Right panel shows a SEM image of one out of 33 individual nanochannels, height: 40 nm, width: 75 nm.

conducting channel highly sensitive to biomolecules, the top Si layer with 100 nm thickness was narrowed down to the 40 nm by wet thinning process. The conductive nanochannels were formed after defining patterns using the e-beam lithography, the photolithography processes and etching the top silicon by Cl_2-based dry etching. Finally, the metal electrode was formed as a stack of Au/Cr/Al (50 nm/5 nm/50 nm), followed by annealing at 400^{o}C for ohmic contacts between metal and silicon.

In order to modify the fabricated Si-FET surfaces with anti-PSA, first, oxygen plasma treatment (42 Pa, 25 W, 5 min) was performed to produce hydroxyl-terminated Si surface. The hydrophilic Si-FET surfaces were exposed to 1% ethanol solution of 3-aminopropyltriethoxysilane (APTES, Sigma-Aldrich) for 30 min, followed by rinsing with ethanol and heating at 120°C in N_2 atmosphere for 10 min. To make the surface bio-activate for anti-PSA immobilization, the amine-functionalized Si-FET was immersed in a 25 wt. % glutaraldehyde solution with 0.2 g sodium cyanoborohydride for 4 h, followed by rinsing with deionized water. Surface thickness of the molecular layer after each reaction step was measured by ellipsometer

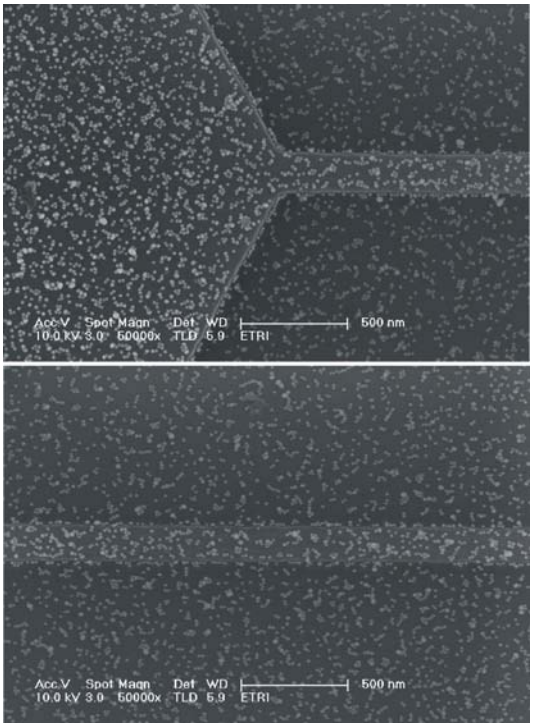

Figure 2: SEM images of nanochannels labeled by DNA derivatized-13 nm Au NPs. Particle density of nanochannels is about 1100/μm^2.

(Gaertner Scientific L116S). The thickness of the molecular layers measured by ellipsometer is 5.2 ± 0.2 Å (APTES) and 10.6 ± 0.2 Å (glutaraldehyde plus APTES), where the thickness using density functional theory (DFT) is calculated to be 6.72 Å (APTES) and 12.86 Å (glutaraldehyde plus APTES), suggesting the monolayer formation of APTES and gluatraldehyde on Si surface. Immobilization of anti-PSA was achieved by exposing the aldehyde-modified Si-FET to 120 μg/ml anti-PSA (clone 6915780, Cortex Biochem) in 10 mM phosphate buffer solution containing 4 mM sodium cyanoborohydride ($NaBH_3CN$) at pH8.4 overnight. To block the unreacted aldehyde group, the device was immersed in 0.5 mM ethanolamine solution (10 mM phosphate buffer, pH 8.4) with 4 mM $NaBH_3CN$ for 1h.

Alternate injection of 1μM phosphate buffer solution with 2 μM KCl (pH 7.4) and a PSA solution dissolved in the buffer was performed through a PDMS microfluidic channel. The highly-diluted buffer solution was used to reduce the counterion screening of the negatively charged PSA. Real-time electronic measurements of our sensor were performed by ac lock-in technique (frequency: 31.47 Hz, voltage amplitude: 20 mV).

3. RESULTS AND DISCUSSION

A nanobiosensor fabricated by using silicon on insulator (SOI) substrate comprises 33 individual nanochannels respectively, ranging from 50 to 200 nm in width and 2 to 20 μm in length with the height of 40 nm (Figure 1). The antibody surface density was indirectly quantified by hybridizing probe DNAs-stabilized Au NPs with capture DNAs chemically coupled with aldehyde group on nanochannels, and imaging the modified nanochannels by scanning electron microscopy (SEM), which provides the coverage density of antibodies per unit square of nanochannels to be estimated (Figure 2). Figure 2 shows that the Au-DNA conjugates are immobilized with a high density of 1100 NPs per μm^2.

Figure 3a shows the time-dependent conductance change of an n-type Si FET nanochannel induced from the specific binding between anti-PSA and PSA antigen, which was measured by alternately delivering PSA solution and pure buffer through a microfludic channel to the devices. Time-dependent conductance measurements exhibit a drop (to 148 nS) after addition of PSA-antigen solution followed by an increase (to 185 nS) in the conductance of pure buffer solution, showing the reversibility of the conductance changes that nonspecific, irreversible protein binding does not occur. When the PSA antigen specifically bound to its anti-PSA on the Si FET nanochannels, the increase of negative charges introduced by the PSA at the pH 7.4 (pI of PSA ~ 6.8) reduced the carrier concentration in the n-type Si FET nanochannels, resulting in the observed decrease of the Si FET nanochannel conductance. The response of the sensors for n-type nanochannels was 15 %. We further investigated the specificity in binding experiments with bovine serum albumin (BSA) (Figure 3b). Addition of 1 μg/mL BSA, which is not specific for anti-PSA, does not result in any change in conductance, indicating that the

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conductance change of Si FET nanochannels originating from the specific binding between anti-PSA and PSA.

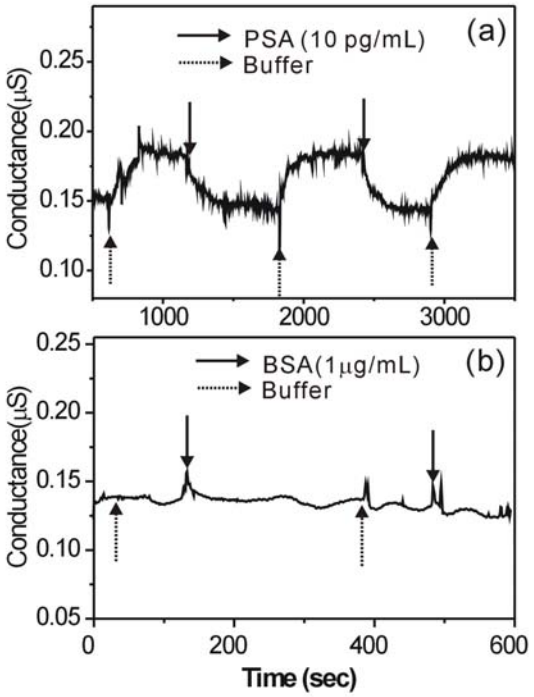

Figure 3: Conductance-verse-time data recorded anti-PSA modified Si FET nanochannels after alternate delivery of the following protein and pure buffer solution. (a) 10 pg/mL PSA (solid arrow) and pure buffer solution (dotted arrow). (b) 1 μg/mL BSA (solid arrow) and pure buffer solution (dotted arrow). The buffer solutions used in all measurements were 1 μM phosphate buffer containing 2 μM KCl, pH 7.4.

4. CONCLUSION

The immobilization of anti-PSA on the Si FET surface with very effective and good coverage has been achieved via the procedure of O_2 plasma ashing, silanization and coupling reaction between aldehyde group and amino group. We have demonstrated highly sensitive, selective, real-time, and label-free direct electrical detection of protein cancer marker using Si FETs fabricated by top-down method. Si FET nanosensors from the top-down approach could be fabricated as sensor arrays with extremely high density to be multiplexed, and exploited to improve the diagnosis and treatment of cancer and other complex diseases. This Si FET is believed to be an alternative to Elisa Kits as a next generation antibody-antigen analysis technology provided quantitative analysis of Si FET chips are successfully achieved.

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