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Multiplexed detection of protein markers with silicon nanowire FET and sol-gel matrix

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Abstract— **A new modified top-down based fabrication method has been developed to obtain highly ordered silicon nanowire (SiNW) arrays. With this method, we could produce as many as 500 chips (size dependable) in an 8 inch wafer. The immobilization of multiple proteins on each chip was performed by spotting sol/gel materials encapsulating antibodies on the different regions. The most commonly used protein markers in clinics, such as C-reactive protein (CRP) and prostate specific antigen (PSA), were systematically entrapped in sol-gel materials and used for multiplexed testing. Upon formation, the electrical signal of different concentrations of CRP and PSA could be simultaneously determined in the range of 0.12 ~ 10 ng/mL and 0.18 ~ 8.87 ng/mL, respectively.**

I. Introduction

Silicon nanowire field effect transistor (SiNW FET) sensor, one of the most rapidly growing research fields of nano bio-technology, has been intensively studied due to its potential for ultra-sensitivity, possible label free detection, and size miniaturization. Technologies based on SiNWs have been found in many applications such as biochips used for the detection of disease markers, [1,2] pH detectors and photodetection [3,4]. This promising approach is based on the direct electrical detection of conductance change upon binding of charged molecules or receptors linked to the SiNW surfaces. However, a number of studies have been performed on SiNW arrays fabricated by bottom up method which requires series of complex processes, such as chemical vapor deposition technique, transfer, and aligning of an individual nanowire, rendering them not suitable for mass production. In order to minimizing variations and increasing uniformity, dominant fabrication method has been changed to top down approach. This method provides high density nanowire allowing reliable and uniform electrical characteristics. [5,6] Previously, we developed a novel fabrication method that is optimal for mass production of SiNW arrays. [7] We have demonstrated the highly sensitive detection of antigen-antibody binding on these top down etched SiNW arrays. [8,9]

In terms of wet chemistry for the surface modification described in most studies related to SiNW FET sensor, the standard protocol does need covalent bonding and blocking processes to immobilize capture reagents or protein antibodies. [10,11] However, those protocols often lead to the loss of protein activity and binding site due to the difficulties in maintaining native conformation of antibody. In order to maintain the protein activity, we used sol-gel materials for the immobilization of proteins in this study. Main advantages of using sol-gel materials can be addressed as follows. First, they can be placed on a specific region of SiNW arrays accounting for immobilizing different antibodies in a precise way on a single chip and the spotted sol-gels can be measured simultaneously. Second, entrapped proteins in sol-gel materials maintained their activity for months. [12,13] Third, sol-gel materials are relatively inexpensive and easy to integrate with SiNW arrays making sol-gel SiNW FET chips mass producible. [14,15]

Therefore, we prepared SiNWs arrays on which sol-gel droplets containing the target CRP and PSA antibodies were fixed for the measurement. Specifically, the SiNW arrays were prepared using the previous fabrication method with some modification. [9] Polydimethylsiloxane (PDMS) micro-channel was placed on the sol-gel SiNW arrays for the delivery of the target proteins to the sensing region with the custom-made fluidic system. The proposed sol-gel SiNW chip showed simultaneous measurements of CRP and PSA in clinically used standard serum samples in the ranges of 0.12 ~ 10 ng/mL and 0.18 ~ 8.87 ng/mL, respectively, demonstrating its capability to detect multiple target proteins.

II. Experimental

Fabrication method of silicon nanowire

A SiNW biochip was fabricated employing the thinning concept introduced in our previous paper.[9] In brief, SiNWs obtain their nano-scale structure after oxidation on a hourglass-like silicon column with a width of approximately 1~2 μm. The overall size of each chip is 2 × 2 cm^2, including eight detection regions which have electrodes for probing. Our top-down method makes simultaneous fabrication of SiNWs feasible without the need of additional aligning processes compared to the VLS grown SiNWs. Fig. 1A shows an 8 inch wafer that contains a total of 64 SiNW chips and Fig. 1B shows an enlarged image of a chip with sensing region marked with four triangles. In the sensing area of each chip located five hundred highly-ordered SiNWs (Fig 1C) and each SiNW is 150 nm in width and 15 μm in length. Fig. 1D shows two neighboring spotting region of sol-gel materials with anti-CRP and anti-PSA encapsulation. The silicon wafer used to deposit the SiNWs was *p*-doped and had a resistivity of

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1~10 Ω·cm. We utilized two regions for the measurements of the CRP and PSA in sample (Fig. 1C and 1D).

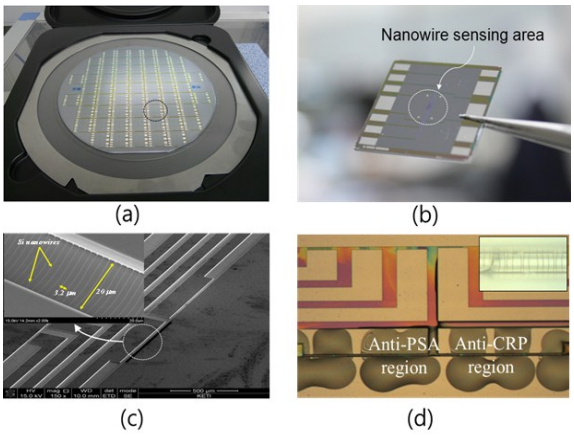


Figure 1.
Images of fabricated SiNW array chip in 8 inch wafer. (a) Each wafer contains a total of 64 chips, (b) enlarged image of each chip indicated by circle in (a), (c) enlarged image of nanowire sensing region marked by 4 triangles with inlet image of SiNW arrays, and (d) SiNW biochip with dried sol-gel material

Preparation of sol/gel material

We utilized the different sol-gel materials for immobilizing anti-CRP and anti-PSA according to manufacturer's recommendation (silicate monomer mixed with buffer solution) provided by PCL Inc.[16] In detail, using sol-gel formulation #2 (F-II: SolB I 25%, SolB II 7.5%, SolB III 5%, SolB H 12.5%, SolB S 12.5%), 0.6 *mg/ml* of anti-CRP and anti-PSA were mixed with SolB reagent mixture, and 5 sol-gel droplets were arrayed onto SiNWs using the non-contact dispensing instrument (sciFLEXARRAYER S11, Scienion AG, Germany). After arraying, sol-gel droplets were dried for more than 3hrs for gelation according to manufacturer's recommendation. Multiple antibodies could be dispensed individually so that they could be spotted on different locations on SiNW arrays, as shown in Fig. 1D. Figure 2 shows schematic of sol-gel preparation and the process of sandwich assay performed for the fluorescent images of antigen-antibody interactions on SiNW arrays.

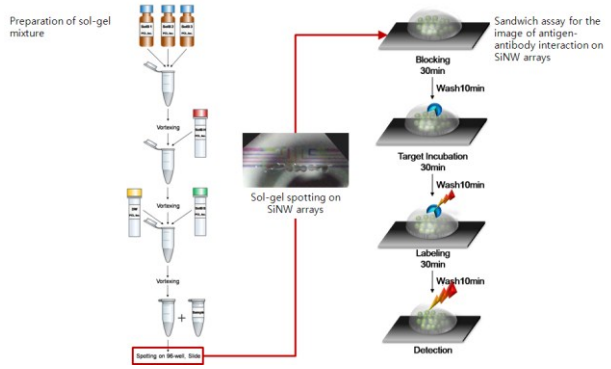


Figure 2.
Schematic diagram of sol-gel preparation and process for the fluorescent image of antigen-antibody interaction on SiNW arrays

III. Results and Discussion

Preliminary measurements were performed to estimate the pH response of the SiNW biochip, as shown in Fig. 3A. We modified the surface of SiNWs with 3-aminopropyltriethoxysilane (APTES) in order to obtain a response according to the concentration of H^+ and OH^-, which is well described in previous study.[1] The potential voltage applied between the electrodes of SiNWs was 1 V and the average current flowing through the in SiNW arrays without the addition of any solution was estimated to be ~10^{-8} A, and the resistance ~10^9 Ω. Standard solutions with a pH of 2, 4, and 6 were subsequently injected through a PDMS channel. All the measurements, including the pH, CRP, and PSA detection, were performed with the use of our custom-made fluidic system shown in Fig. 3B. The system is composed of its own probes for collecting data, components for data storage and a display window. The SiNW arrays modified by APTES were exposed to a pH standard solution and they demonstrated stable responses for multiple flow cycles. These responses to pH are in good agreement with previous studies,[11,17] suggesting the performance of the SiNW as a field effect transistor is suitable for protein detection applications. Figure 4 shows measurements of CRP and PSA levels in samples using our SiNW arrays. After the addition of 0.12 ng/ml of CRP serum sample, the flow was paused for a moment until the signal became stabilized. After stabilization, different concentration of CRP sample was injected and each addition was followed by flow control. Same concept was applied to measure PSA concentrations. The binding between CRP and anti-CRP as well as that between PSA and anti-PSA are equivalent to applying a positive potential voltage on the gate because the iso-electric-point (pI) of both CRP (7.4) and PSA (6.9) are larger than the pH of the buffer solution.[18,19] In the case of p-doped SiNWs, a positive gate voltage results in the depletion of SiNWs, which ultimately decline the conductance of SiNW. Therefore, decreases of the conductance were observed upon the injection of the CRP solution, as shown in Fig. 4A. The plots showed linear relationships in the concentration ranges of 0.12 – 10.00 ng/ml (CRP) and 0.18 – 8.87 ng/ml (PSA), respectively. There was no conductance change from the baseline level observed either when different CRP solutions were injected to anti-PSA conjugated SiNW arrays or when PSA solutions were injected to anti-CRP conjugated SiNW arrays (Fig. 4B). This technique makes possible high sensitive, rapid, and multiplexed assays on a small chip. The sensitivity was calculated as the ratio of the conductance change to the initial conductance of the buffer solution and was proportional to the logarithmic concentration of both CRP (Fig.5A) and PSA (Fig.5B).

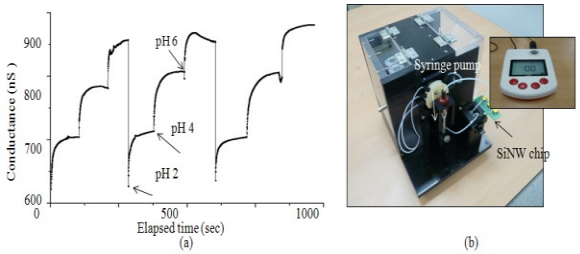
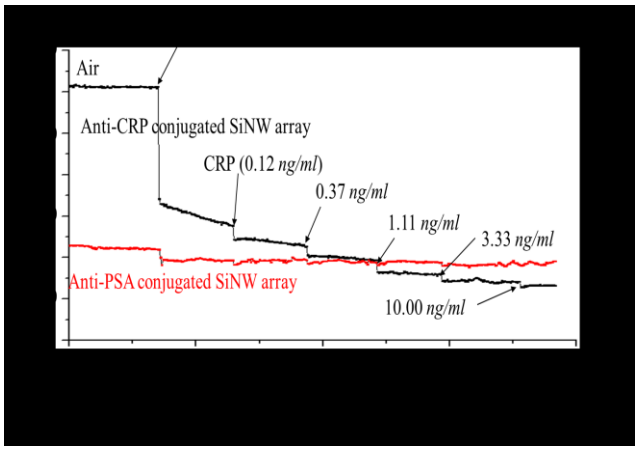


Figure 3.
(a) SiNW conductance responses versus pH with APTES modified surface, (b) custom-based fluidic system with a portable measurement system



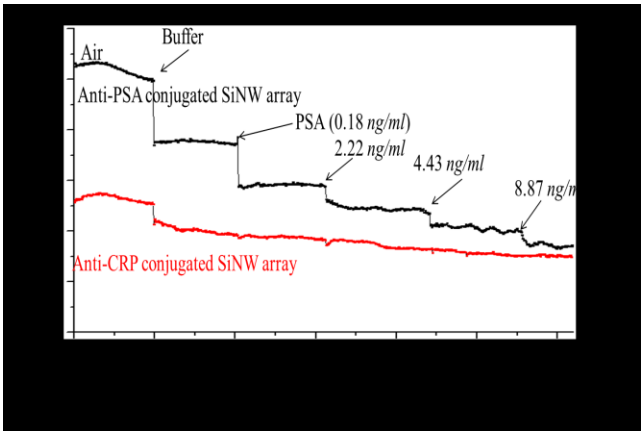
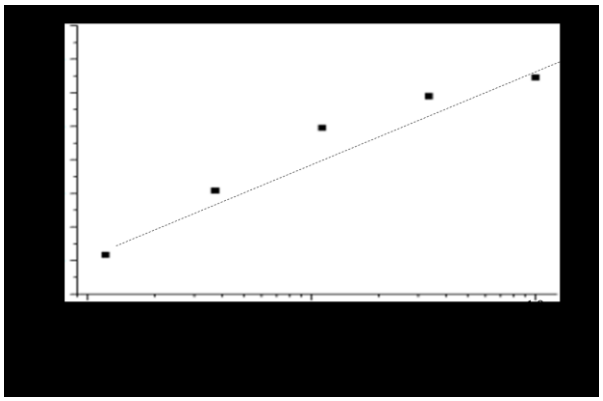


Figure 4.
Simultaneous and multiplexed detection of CRP and PSA. Conductance of SiNW arrays after the injection of solutions of varying concentration of (a) CRP and (b) PSA. Both figures show that independent detection is possible with a presence of other protein marker at the same time.



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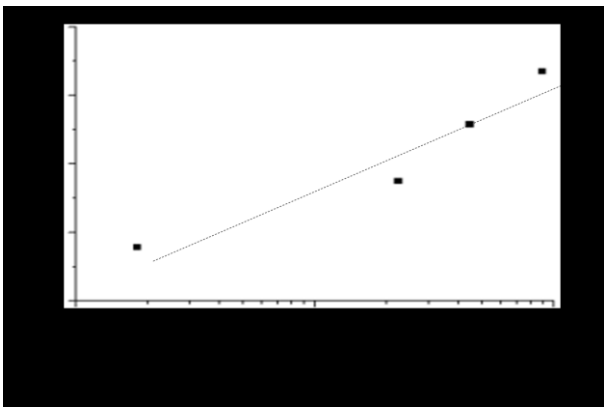

Figure 5.

Sensitivity versus concentration of (a) CRP and (b) PSA (log scale)

IV. CONCLUSIONS

The main demonstration of this study is that sophisticated spotting of sol-gel-antibodies allows the full integration with SiNWs FET sensor with high immunoassay performances. We have demonstrated that this integration enables high-sensitivity and label-free immunoassays with small amount of serum within a few minutes. In our SiNW FET based assays, detection is performed by measuring electrical signal changes based on a gating effect which requires precise control of fluid, light, and injection of proteins. In addition, the functionalization of SiNW surface with various proteins is accompanied by precise and repetitive techniques. Our simple assay is attributable for efficient and solid capture of antibodies by sol-gel materials, no chemistry involved for the conjugation, and positioning of multiple proteins on small area. Another important advantage is that the sol-gel-antibody is well integrated with our mass producible SiNW arrays which lead to the production of the cost effective packages in large numbers feasible.

Our findings show that the proposed system can detect multiple disease markers simultaneously as well as provide stable measurement during the injection of fluids. By optimizing the chip size and sol-gel dropping method, the current detection system could also be applied to a portable biochip for the detection of more markers simultaneously.

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