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Multiple Reaction Analysis of Cancer with Different Markers Using Silicon Nanowire FET

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Abstract— **In this study, we have used newly developed Silicon nanowire (SiNW) arrays to evaluate their feasibility for the quantification of different markers of interests. We have quantified four different markers of PSA, EGF, IL-6, and VDBP. Each marker showed measurements in the range of 0.184 ~ 17.79 ng/mL (PSA), 10 pg/mL ~ 10 ng/mL (EGF), 10 pg/mL ~ 50 ng/mL (IL-6), and 10 pg ~ 5 ng/mL (VDBP), respectively. For the experiment, we collected 10 different serum samples, 5 prostate cancer patients and 5 breast cancer patients, and measured and compared the resulting signal from the SiNW FET to serum sample from normal patients. As a result, we observed a meaningful pattern of markers associated with each type of cancer. In addition, we have measured the response signal of SiNWs conjugated with Epithelial cell adhesion molecules (EpCAM) markers against tumor cells as they interacted with those markers.**

I. Introduction

The silicon nanowire field effect transistor (SiNW FET) sensor, one of the most rapidly growing research fields of nano bio-technology, has received much spotlight due to its potential for ultra-sensitivity, 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], oligonucleotides [3], pH detectors [4], and photodetection [5,6]. This approach is based on the direct detection of conductance change upon binding of charged molecules or receptors linked to the SiNW surfaces in real time. Our top-down based SiNW arrays showed simultaneous measurements against prostate specific antigen (PSA) and C-reactive protein (CRP) on a single chip in a previous study [8]. The prominent advantage of this top-down based fabrication lies in the mass production of SiNW chips in possession of uniform electrical characteristics [9,10].

In terms of multiplexed detection, numerous studies using various materials, such as nanobarcodes [9], silicon resonators [10], and quantum dots [11] have been performed. In this study, we have performed multiplexed assays using a single SiNW array chip wherein markers encapsulated in sol-gel materials were spotted on different position. There are a number of advantages to integrating sol-gel materials and SiNW arrays. First, markers of interest can be placed on specific locations of the SiNW surface. Secondly, entrapped proteins in sol-gel materials maintain their activity for months [12-14]. This allows the multiplexed assay to be much easier than conventional methodologies and suggests the possibility of sol-gel SiNW chip mass production.

Therefore, we prepared SiNWs arrays on which sol-gel droplets containing the target antibody proteins were fixed on the surface for the measurement. Specifically, the SiNW arrays were prepared using a fabrication method with some modification presented in previous work [15-17]. A plastic channel package was also fabricated and each chip was placed in it for the delivery of the target proteins to the sensing region with a custom-made fluidic system. The proposed sol-gel SiNW chip based multiplex assays showed meaningful quantification of each marker. For the pattern analysis, we have measured markers of PSA and interleukin-6(IL-6), in serum samples of male patients with prostate cancer using the chip. For the comparison, normal male serum samples were measured using the same protocol. With the same procedure, serum samples from female patients with breast cancer and normal serum samples were systematically measured and compared for levels of the markers Epithelial growth factor (EGF), Apolipoprotein A1 (ApoA1), and Vitamin D binding protein (VDBP). Finally, as a proof of concept study, breast cancer cells and normal cells were collected and a comparison of the measured signal differences was made to demonstrate the capability to detect circulating tumor cells using the SiNW array.

II. Experimental Methods

Fabrication method of silicon nanowire

A SiNW biochip was fabricated employing the thinning concept introduced in our previous study [8]. In brief, SiNWs obtain their nano-scale structure after oxidation on an hourglass-like silicon column with a width of approximately 1~2 μm. The overall size of the fabricated chip is 3 × 10 mm^2, including one sensing region where SiNW arrays are located. Our top-down method makes simultaneous fabrication of SiNWs feasible without the need of additional alignment. Fig. 1(a) shows an 8 inch wafer that contains a total of 768 SiNW chips. Fig. 1(b) shows an enlarged image of a chip with plastic package on the right and a size comparison between the previously fabricated SiNW chip shown on the left. The overall size is 1/12th of the originally fabricated SiNW chip.

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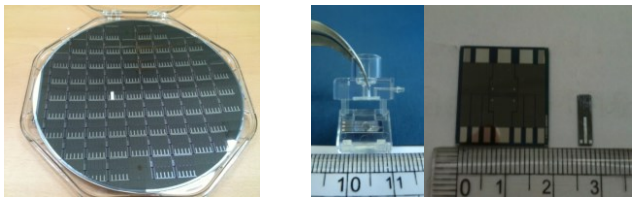


(a) (b)

Figure 1.
Images of fabricated SiNW array chip in 8 inch wafer. (a) Each wafer contains a total of 768 chips, (b) enlarged image of each chip placed in a plastic channel package and their size comparison with previously fabricate SiNW chip

Preparation of sol/gel material

We utilized the same protocols for immobilizing protein markers in sol-gel according to the manufacturer's recommendation (silicate monomer mixed with buffer solution) provided by PCL Inc [14]. 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 proteins 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 3 hours for gelation according to the manufacturer's recommendation. Multiple antibodies could be dispensed individually so that different targets may be spotted on different locations of the SiNW arrays. Fig. 2 shows sol-gel droplets formed on the SiNW array region.

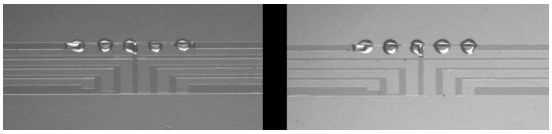
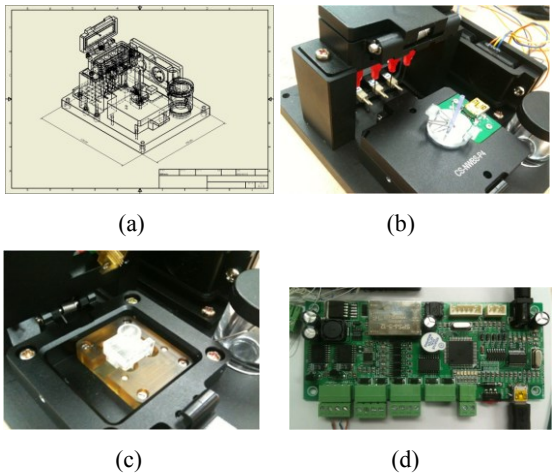


Figure 2.
Optical images of sol-gels wherein protein markers are encapsulated on SiNW arrays in chip

III. Results and Discussion

Preliminary measurements were performed to quantify the electrical response of the SiNW array using the custom made prototype system shown in Fig. 3. The system is composed of four different small solenoid valves for the control of fluids and an electrical circuit board for data acquisition and control. The system is capable of monitoring changes of resistance from 0.1 MΩ to 10 GΩ and displays the current levels in the SiNW FET. By using four solenoid valve and two piezoelectric pumps, control of four different fluids could be achieved without the use of separate pressure gas. Additionally, the system includes probes for collecting data, components for data storage and a display window. The potential voltage applied between the electrodes of SiNWs was $1V$ and the average current flowing through the SiNW arrays without the addition of any solution was estimated to be $\sim 10^{-8} A$, and the resistance $\sim 10^{9}\ \Omega$.

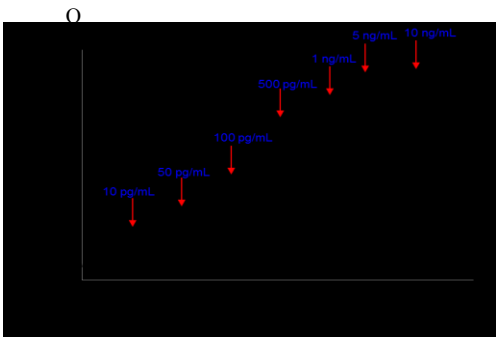


(a) (b)

(c) (d)

Figure 3.
(a) Design of prototype system, (b) Prototype system used for measurements, (c) enlarged image of SiNW chip with plastic channel package, and (d) control circuit board.

We measured three different markers of EGF, IL-6, and VDBP for the quantification. As shown in Fig. 4, the range measured by the SiNW is 10 pg/mL ~ 10 ng/mL in EGF, 10 pg/mL ~ 50 ng/mL in IL-6, and 10 pg/mL ~ 5 ng/mL in VDBP, respectively. All the measurements were performed with the use of our prototype system shown in Fig. 3. After the addition of EGF samples of 10 pg/mL, the flow was paused for a moment until the signal became stabilized. After stabilization, different concentrations of EGF sample were injected onto the surface with a flow control stage following each injection. The same procedure was applied to measure other protein concentrations. The plots showed linear relationships in the concentration ranges of each marker. As is well described in a previous study [8], there was no conductance change from the baseline level observed when any non-matching antigen and antibody interacted in the sensing region.



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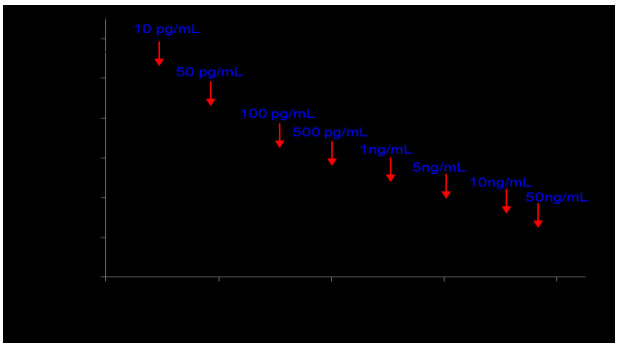

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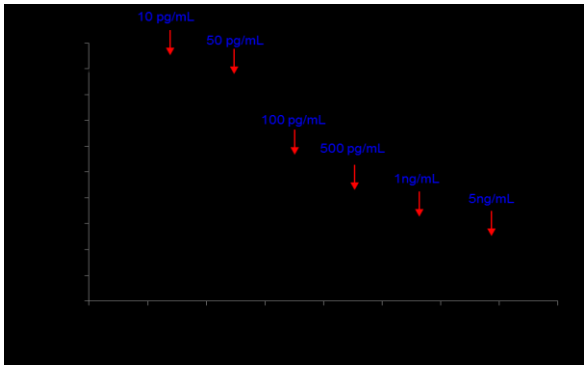
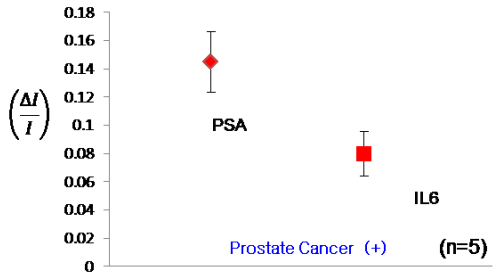


(c)

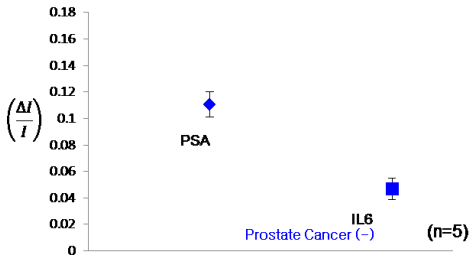
Figure 4.
Quantification of three different protein markers (a) EGF, (b) IL-6, and (c) VDBP.

Fig. 5 shows the signal patterns of the measured electrical conductance changes for different markers. Fig. 5(a) shows the pattern of patients with prostate cancer and Fig. 5(b) the pattern of normal male serum. PSA and IL-6 is well known protein markers for diagnosis of prostate cancer [18-20]. Fig. 5(c) and (d) show the patterns for breast cancer patients and normal female serum. In this case, we have selected the three markers of EGF, apolipoprotein A1 (ApoA1), and Vitamin D binding protein (VDBP). These three markers were selected based on the previous study of [21]. The signal level for a particular marker was calculated as the ratio of conductance change upon injection to the initial conductance of the buffer solution. The sensitivity was determined using the mean and standard deviation of the signal level across the five different patients. The resulting pattern of markers for each cancer type when compared to the pattern from normal subjects agreed with results obtained for each marker using conventional methods.

Additionally, we performed a study on detection of tumor cells in a serum sample. In this case, the SiNW surfaces were modified with epithelial cell adhesion molecules (EpCAM) and a positive cell line (MCF7, 10 cells/50 uL) and a control cell line (HeLa, 10,000 cells/50 uL) was prepared to be injected onto the surface. As can be seen in Fig. 6(a), the electrical signal gradually decreases upon injection of positive cells. However, the signal did not recover to the normal range after washing. Comparitively, in the control case, as shown in Fig. 6(b), the electrical signal gradually returns to normal range after washing. This suggests that tumor cells systematically attached to the surface of modified SiNWs while control cells did not.



(a)



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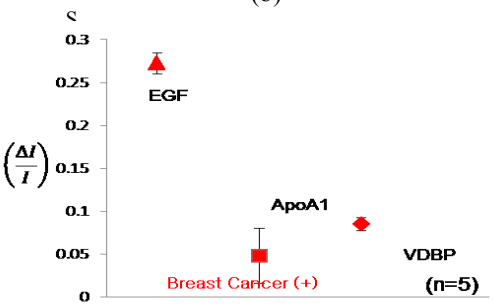

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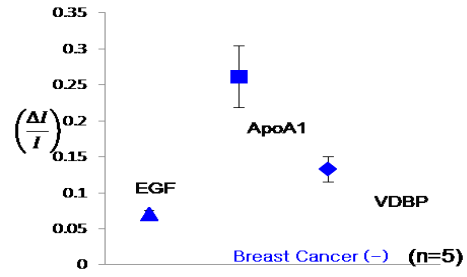
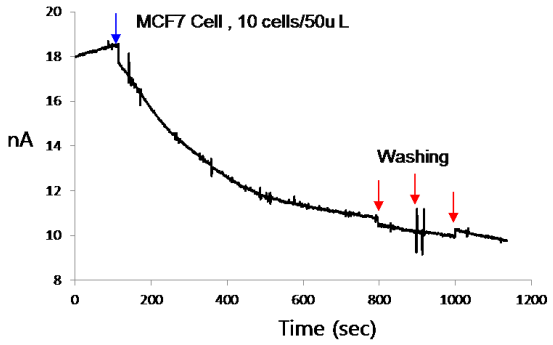


(d)

Figure 5.

Electrical signal patterns of (a) patients with prostate cancer, (b) normal subjects, (c) patients with breast cancer, and (d) normal subjects. Signals were ratio of conduction change upon injection to baseline conductance (no unit). Measurements using samples from five patients for each type of cancer and normal subjects were performed separately.



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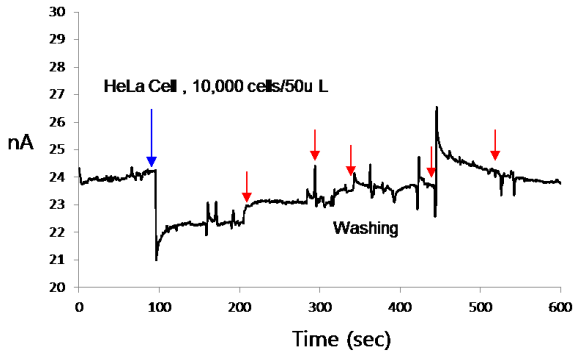

(b)

Figure 6. Electrical signal changes of EpCAM modified SiNW arrays with the injection of (a) tumor cells and (b) control normal cells. After washing, signals become stable in tumor cells (a) while signals are recover to initial range in control cells (b).

IV. Conclusions

This study is an extension of the study presented in previous work [8] and demonstrates that miniaturized sol-gel SiNW arrays are capable of providing high immunoassay performance when detecting multiple protein targets. Each chip size is reduced by $1/12^{th}$ of previous work resulting in over 700 chips per wafer. The prototype system is capable of providing a more efficient and simple method of detecting proteins by controlling fluid injection and measuring current levels.

Our findings show that the proposed system can detect multiple disease markers and electrical signals patterns identical to those measured by other conventional methods. In addition, we have detected tumor cells selectively using SiNWs arrays indicating that SiNW arrays could be an efficient candidate for the detection of circulating tumor cells.

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