Facile fabrication of a silicon nanowire sensor by two size reduction steps for detection of alpha-fetoprotein biomarker of liver cancer

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Abstract
We present a facile technique that only uses conventional micro-techniques and two size-reduction steps to fabricate wafer-scale silicon nanowire (SiNW) with widths of 200 nm. Initially, conventional lithography was used to pattern SiNW with 2 μm width. Then the nanowire width was decreased to 200 nm by two size-reduction steps with isotropic wet etching. The fabricated SiNW was further investigated when used with nanowire field-effect sensors. The electrical characteristics of the fabricated SiNW devices were characterized and pH sensitivity was investigated. Then a simple and effective surface modification process was carried out to modify SiNW for subsequent binding of a desired receptor. The complete SiNW-based biosensor was then used to detect alpha-fetoprotein (AFP), one of the medically approved biomarkers for liver cancer diagnosis. Electrical measurements showed that the developed SiNW biosensor could detect AFP with concentrations of about 100 ng mL^-1. This concentration is lower than the necessary AFP concentration for liver cancer diagnosis.

Keywords: surface modification, GPTS, AFP detection, silicon nanowire, liver cancer, biosensor Classification numbers: 2.05, 4.08, 6.09

1. Introduction

Recently field-effect transistor (FET) biosensors based on silicon nanowire (SiNW) have emerged as promising devices for sensitive and rapid detection of biomolecules [1–3]. Alpha-fetoprotein (AFP) is a major plasma protein produced by the yolk sac and liver during fetal development. The normal range of AFP for adults and children is variously reported as under 50 ng mL^-1, 10 ng mL^-1, and 5 ng mL^-1, respectively. AFP can also be used as a biomarker to detect a subset of tumors in non-pregnant women, men, and children. The initial value of AFP in the range of 50–400 ng mL^-1 is significantly related to the development of hepatocellular carcinoma [4, 5]. Therefore, a SiNW-based biosensor has significant potential for sensitive and quick detection of AFP in liver disease diagnosis.

In this paper we present a facile technique using conventional micro-techniques and two size-reduction steps to fabricate wafer-scale SiNW with widths of 200 nm. Initially, conventional lithography was used to pattern SiNW with 2 μm width. Then the nanowire width was decreased to 200 nm by two size-reduction steps with isotropic wet etching. The fabricated devices were further investigated for being used as nanowire field-effect sensors. The electrical characteristics of the fabricated SiNW devices were evaluated and pH sensitivity was also investigated. Then a simple and effective surface modification process was carried out to modify SiNW surface for subsequent binding of a desired receptor. Electrical measurements of the complete SiNW-based biosensor were performed to detect AFP, one of the medically approved biomarkers for liver cancer diagnosis.
2. Facile fabrication and characterization of silicon nanowire by two size-reduction steps

2.1. SiNW device fabrication

Figure 1 shows a general procedure to fabricate SiNW by using the two size-reduction steps approach. In summary, a silicon-on-insulator (SOI) wafer (SOITEC, France) was oxidized by dried oxidation to create a SiO₂ layer of 44 nm. Then the wafer was deposited with an aluminum (Al) layer of 50 nm by sputtering in a high vacuum. Afterward, a photoresist coating and microlithography process were carried out to pattern a 2 μm line width in the photoresist.

The first step of size reduction: the resist was then used as a mask for isotropic etching of Al. In our work, we used our own made Al isotropic etch solution to precisely etch Al with an etch rate of ca. 100 nm min⁻¹. In the isotropic etching of Al, Al was etched both vertically and laterally. Therefore, after etching Al for ca. 7 min, we obtained the Al pattern lines with a line width of ca. 600 nm.

The second step of size reduction: the photoresist was then removed in acetone. The Al pattern was used as a masking layer for the second isotropic etching of SiO₂ in a diluted buffered hydrofluoric (BHF) solution with an etching rate of 40 nm min⁻¹. Similar to the isotropic etching of Al, the isotropic etching of SiO₂ also etched SiO₂ in both the vertical and lateral directions with an almost equal etching rate of 40 nm min⁻¹. Therefore, after etching of SiO₂ for ca. 5 min, we got the SiO₂ lines with the line width of ca. 200 nm. However, it should be noted that the sacrificial layer of Al we used should be realized in high vacuum to avoid Al being etched in the BHF solution.

Then the SiO₂ lines with the line width of ca. 200 nm was used as a masking layer for anisotropic etching of the Si layer in a diluted alkaline solution. In our work, we simply used a conventional and complementary metal-oxide-semiconductor (CMOS) developer solution to etch the Si device layer with an etch rate of ca. 2–3 nm min⁻¹. Etching of the 50 nm Si device layer was carried out for 25 min to make sure the 50 nm Si device layer was completely etched. After this step, we obtained SiNW with a line width of ca. 200 nm at the wafer scale.

Subsequently, a lift-off technique was carried out to fabricate metal contact pads (Pt/Ni) for the previously patterned SiNW.

Finally, a plasma-enhanced chemical vapor deposition (PECVD) SiN isolation layer of 200 nm was deposited on the whole wafer, followed by a lithography and etching process to open sensing windows (to reveal only the SiNW) and partial areas of contact pads. After this step, the SiNW was ready to be used as a biosensor for further investigation.

SEM images of the fabricated SiNW by the two size-reduction steps method are shown in figure 2. Figure 2(b) shows a dual SiNW with metal contact pads used as electrodes, and the metal contact pads are covered with a SiN isolation layer. The SiNW has a desired width of ca. 200 nm with smooth surface, which is suitable for use in a SiNW-based biosensor for biological detection.

Apparently, the strong point of our developed fabrication approach is that we only utilized conventional microfabrication technology to fabricate the SiNW. Moreover, the whole process is completely CMOS compatible and thus allows the SiNW to be integrated directly with the CMOS controls made previously in the same wafer. The mask was designed so that we could fabricate many devices on a wafer. Each device had several arrays of SiNW, and some arrays included one SiNW (single wire) and some arrays included more SiNW such as 3,
5, or 10 SiNW. This helped us ensure diversity in the devices. Another advantage of this design was that each array could be separately functionalized to detect different kinds of bio-
markers. Therefore, one device could detect several bio-
markers at the same time, hence increasing the sensitivity and speci-
ficity of diagnosis.

2.2. Electrical characterization of the fabricated SiNW

The electrical characteristics of the fabricated SiNW devices were characterized to identify a practical sensing regime in which the SiNW device exposes the best sensor character-
izations. The current–voltage (I–V) measurements were performed on a probe station (SUSS EP6) connected with a semiconductor parameter analyzer (HP/Agilent 4155C).

The output I–V characteristics of the SiNW devices were recorded by sweeping the source-drain voltage $V_{ds}$ from $-2 \text{V}$ to $+2 \text{V}$ with a step of $0.05 \text{V}$, while a biasing voltage, $V_{bg}$, was varied from $-5 \text{V}$ to $+5 \text{V}$ with a step of $1 \text{V}$. The measured I–V curves are shown in figure 3. As can be seen in the figure, the devices exclusively have a sensing regime at $V_{ds} = 1.8 \text{ V}$ and $V_{bg} = -4 \text{ V}$.

3. pH sensing characteristics of SiNW and detection of alpha-fetoprotein (AFP) biomarker

3.1. pH sensitivity of the SiNW

To investigate pH sensitivity of the SiNW devices, the SiNW were exposed to different pH buffer solutions and I–V mea-

urements were performed. The measurements were first started with pH 3 and then the pH value was step-wisely increased with an increment of 2 to a maximum of pH 11. In between solution exchanges, the devices were cleaned by deionized water and dried by argon gas and then the new pH solution was introduced. The I–V result is shown in figure 4. As can be seen in the figure, the current increases with increasing pH value of the solution. This effect can be explained by the change of the surface charges. With p-type SiNW, when pH increases or the hydroxyl group is rich in the solution, negative charge is accumulated and therefore the current of the SiNW increases [6, 7]. When measuring the SiNW in a pH range of 5 to 9, the current change was not as large, but when the pH increased from 9 to 11 or from 3 to 5 the current changed drastically. Thus, we conclude that the fabricated SiNW are sensitive to pH, and the best working condition is neutral or near neutral pH values. Therefore, in the subsequent experiments we did not investigate the devices at extreme pH values such as 3 or 11.

The electrical characteristics of the SiNW device mea-

sured with different pH solutions are shown in figure 5. As can be seen in the figure, the current increased with an increase in pH in the range of back-gate voltage $V_{bg}$ of $-5 \text{ V}$ to $-3 \text{ V}$. This was in good agreement with the results in figure 3, which show that the SiNW devices had the sensing regime exclusively at $V_{ds} = 1.8 \text{ V}$ and $V_{bg} = -4 \text{ V}$.

3.2. Surface modification of silicon nanowire and receptor immobilization

3.2.1. Materials. 3-glycidoxypropyl trimethoxysilane (GPTS) and bovine serum albumin (BSA) were purchased from Sigma–Aldrich and other chemicals were purchased

Figure 3. Measured current-voltage (I–V) curves of the fabricated SiNW device with varying back-gate voltage.
from Merck. The alpha-fetoprotein (AFP) and the monoclonal antibody to AFP were purchased from Insight Genomics.

3.2.2. Methods. Although the SiNW-based FETs are sensitive to the surrounding environment, they do not have the desired molecular recognition properties. The surface of the sensing element (NW) thus needs to be modified so that the device acquires specific recognition of a desired analyte [8, 9]. In this work, a simple process of surface modification and subsequent immobilization of receptor on the silicon surface was carried out. First, the SiNW device was cleaned with a piranha solution ($\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2 = 3:1$) in 3 min and then was attached to a glass well by epoxy (figure 6). The cleaned SiNW device was then treated with a piranha solution ($\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2 = 3:1$) in 3 min and then was attached to a glass well by epoxy (figure 6). The cleaned SiNW device was then treated with 2% ($\nu/\nu$) 3-glycidoxypropyltrimethoxysilane (GPTS) in toluene for 16 h. The SiNW device was rinsed three times in toluene (each time for 5 min) and incubated at 120 °C for 15 min. The monoclonal antibody to AFP was used for receptor immobilization on the SiNW by incubating in a receptor solution of 10 $\mu$M at 4 °C for 5 h. After that the SiNW was rinsed three times in 1 mM phosphate-buffered saline (PBS) solution (pH 7.4) for 5 min each time. Unreacted epoxy surface groups were subsequently passivated by a reaction with 5 mg ml$^{-1}$ BSA and rinsed with 1 mM PBS. The freshly prepared chips were used immediately for AFP detection by electrical measurements. The method used to functionalize the SiNW with an oxide coating is illustrated in figure 7 [10–13].

3.2.3. Evaluation of modification efficiency. Throughout the surface modification, we checked the modification efficiency of each step by measuring the I–V characteristics of the SiNW device. Each SiNW device was measured in detail four times: after being cleaned, after being activated with GPTS, after
being attached to the monoclonal antibody to AFP, and finally after capturing the AFP.

As can be seen in figure 8, the current change after the chip was activated with GPTS was negligible, which can be explained by the fact that GPTS carries no charge. In contrast, the AFP has negative charge at pH 7.4 so the current increased in this step.

3.3. AFP detection

AFP (pH 5.48) has an isoelectronic point that is less than the pH of the used assay buffer (pH 7.4) and thus AFP carries a net negative charge in solution [6, 13]. After the immobilization of the monoclonal antibody to the AFP molecules onto the SiNW surface, the current flow before and after the binding of the AFP through the SiNW devices was recorded.

Since the SiNW devices used in our experiments are p-type, the presence of a negative surface charge on the device causes carrier accumulation in the device, which increases the SiNW’s conductance and decreases its resistance [12–15]. Figure 9 shows the binding that occurred between the AFP receptor of the monoclonal antibody to AFP, which is indicated by the increase in the observed current [16]. In this figure we see that the binding between the AFP receptor and the AFP occurred and the increased current was about 55 nA (from 3.485 μA to 3.54 μA) after the chip was exposed to AFP solution for about 300 s. After a period of 600 s the increasing signal was clearly observed.

The current response with a lower AFP concentration of 100 ng mL$^{-1}$ is shown in figure 10. As seen in figures 9 and 10, the real-time conductance response of SiNW upon injection of various AFP concentrations of 100–500 ng mL$^{-1}$ was observed. The conductance decreased along with the decrease of the concentrations of AFP, and it was found that the current increased about 55 nA at 500 ng mL$^{-1}$ AFP and was about 20 nA at 100 ng mL$^{-1}$ AFP.

The fabricated SiNW device had many arrays with single or multiple SiNW as mentioned above. In this study we measured and detected AFP on different arrays. Figure 11 shows the measurement results of AFP at 400 ng mL$^{-1}$ concentration on the array including 10 SiNW.

The final purpose of AFP detection is to quantify AFP concentration. However, measuring the devices at many different concentrations is complicated and time- and labor-consuming and thus in this study AFP was only detected quantitatively with the SiNW devices. Building the calibration curve to quantify AFP concentrations is currently being carried out.
4. Conclusions

In this paper we presented the facile and CMOS-compatible approach for fabrication of SiNW devices by using two size-reduction steps. Our method is simple and only utilizes conventional microfabrication techniques. Thus, it can be performed in many laboratories. The electrical property of the SiNW was characterized and the pH sensitivity was investigated at different pH values. The developed SiNW device could detect the AFP biomarker of liver cancer with low concentration of ca. 100 ng mL$^{-1}$, which is lower than the required AFP concentration used in the liver cancer diagnosis. Currently, detection of AFP at lower concentrations is ongoing in our laboratory with direct utilization of the SiNW devices in the diagnosis of liver cancer.

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Figure 10. Measured current–time (I–t) curve shows binding of AFP at 100 ng mL$^{-1}$.

Figure 11. Measured current–time (I–t) curve shows binding of AFP at 400 ng mL$^{-1}$. 