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Label-free Detection of Antibody-Antigen Interaction by Si Nanowire MOSFET

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Abstract

We have fabricated Si nanowire transistors for protein detection using an SOI wafer and ultra-thin hafnium oxides as a gate dielectric. When a self-assembled monolayer is formed using a ProLinker on the gate dielectrics, the conductance of a transistor increases, and further attachment of hIgG protein on the surface does not noticeably change the conductance. By contrast, additional immobilization of anti-hIgG protein over the hIgG has reduced the conductance by antibody-antigen interaction and has exhibited the largest conductance change. These results confirm that this device has recognized a specific protein reaction through direct electrical detection.

Keywords: Si-NW MOSFET, Label-free detection, ProLinker, Nanobiosensor, Hafnium oxide

Introduction

Nanowire and carbon nanotubes have drawn a lot of attention for biosensor applications due to their high performances inherent to the high carrier mobility, their ultra small size, and their high surface to volume ratio[1-3]. When these are exposed to gas molecules, chemicals, and biomolecules such as protein and DNA, the adhered or bonded molecules to the surface change the surface potential or states and the intrinsic characteristics, for example, conductivity. A sensitive response makes the nanostructures suitable for electro-chemical sensing or direct detection of biomolecules.

The field effect transistor (FET) has been the most widely used device in modern electronic circuits; its current flow (drain current) from source to drain is controlled using a third terminal, called a gate. A gate of a FET is separated from the conducting region by insulating the oxide layers in a metal oxide FET structure. When voltage is applied on a gate terminal, the surface potential underneath the oxides where the carriers flow is varied due to an electric-field effect, and it renders a drain current change. The applied gate voltage is equivalent to extra charges on an oxide capacitor, or in other words the charges on a gate electrode induce opposite charges in a conducting region (called a channel) across the insulating layer. Thus, when biological molecules are immobilized on an insulating surface, charges of the species will induce extra carriers with opposite polarity in the conducting region and modify the current flow. For instance, the binding of a negatively charged protein to a p-type (positively-charged holes dominant) device enhances holes and increases conductance. The requirement of the insulating layer is that it should block any cross current flow and transfer the electric field from the gate effectively to the channel. The gate dielectric coating over a nanowire, or surface modification of a nanowire, can result in a FET-type operation after charged molecule attachment. The former is more conventional and is achieved by using polymer[4], photoresist[5], SiO_2[6], SiN[7], and the latter by PEG[8], PNA[9], etc. In the past decade of semiconductor manufacturing, high-k dielectrics such as hafnium, zirconium, and aluminum oxides have been successfully implemented to replace SiO_2 in order to reduce gate leakage current in nano-size Si transistors[10,11]. For the deposition of these materials, an atomic layer deposition (ALD) technique has been developed to cover a highly-stepped surface evenly at the vertical sides and flat area in monolayer precision. By employing ALD-deposited high-k gate dielectrics toward a nanotube FET, the device has exhibited on/off switching at a much lower voltage swing than that with a thick gate SiO_2[12].

Although the manufacturing of these nanowires has become relatively easier compared to the sophisticate downsizing process in Si, there are still drawbacks in their manipulation in order to realize electronic or biological sensing devices. The main obstacles are the reproducibility and positioning of nanowire(s) where a device design has been aimed. To avoid these obstacles, Silicon-On-Insulator (SOI) is one of the

alternative choices because the wafer has a single crystalline thin Si layer (100 nm or less) on the buried oxides and a thick Si wafer without major defects at the interfaces. This thin top Si layer can be made into nanowires using e-beam lithography or nanoimprint techniques, and thus biosensors have already been reported using SOI[5,13,14].

In order to make the nanobiosensor more applicable for analysis of biomarker proteins, it is necessary to improve both the surface chemistry for immobilization of captured antibodies on a solid surface, and an efficient detection technology for quantitative analysis of biomarker proteins. In regard to surface chemistry, it is noted that the correct orientation of captured antibodies immobilized on a solid surface is critical for the sensitivity and specificity of antigens on a nanobiosensor. ProLinkers are novel biofunctional molecular linkers, which have been developed by Proteogen, Inc. for stable immobilization of proteins on a solid matrix[18]. Particularly, antibodies are tightly and densely immobilized on a ProLinker surface with orderly orientation on a solid substrate[18,19]. In regard to detection technology, a non-labeling detection system is going to be useful for quantitative detection of biomarker proteins on a nanobiosensor. Label-free electronic detection of protein has focused on Biotin-streptavidin interaction due to an easy data comparison[1,3,4]; a few studies on human immuno-globulin-G (hIgG) and anti-hIgG protein interaction have been conducted[15,16]. In the interaction mechanism between these proteins, it is well known that anti-hIgG binds to the Fc region of IgG[19].

In this article, we report on the performance of a label-free nanowire protein sensor with hafnium oxides as a gate dielectric in an FET device structure using a SOI wafer. An individual Si nanowire has a rectangular cross-sectional shape with a width and height of 100 nm. On Proliker™ treated hafnium oxide, human immuno-globulin-G (hIgG) and anti-hIgG protein reaction has been clearly recognized by conductance change. These suggest that the implementation of the device is successful.

Results and Discussion

The feasibility of protein immobilization on a hafnium oxide surface is tested first before employing the oxide as a gate dielectric in a field effect transistor. The oxide layers are deposited using Atomic Layer Deposition (ALD) to a thickness of 0.1 μm on a Si wafer. A self-assembled monolayer (SAM) is formed on a hafnium oxide surface by immersing a sample in the ProLinker solution diluted by DMSO (dimethyl sulfoxides). The use of ProLinker for the surface treatment of a glass substrate has been used for a protein chip, the details of which can be found in the literature[18-20]. After drying, the samples are dipped in a human immunoglobulin-G (hIgG) solution (100 μg/mL) for 3 hrs at 37°C under 70% of humidity, and washed in PBS (phosphate buffered saline), followed by a rinse and dry process. To interact with hIgG immobilized on a SAM-modified hafnium oxide surface, we have used anti-human IgG (50 μg/mL) labeled with a fluorescence dye. Fluorescence labeling and light detection are employed to

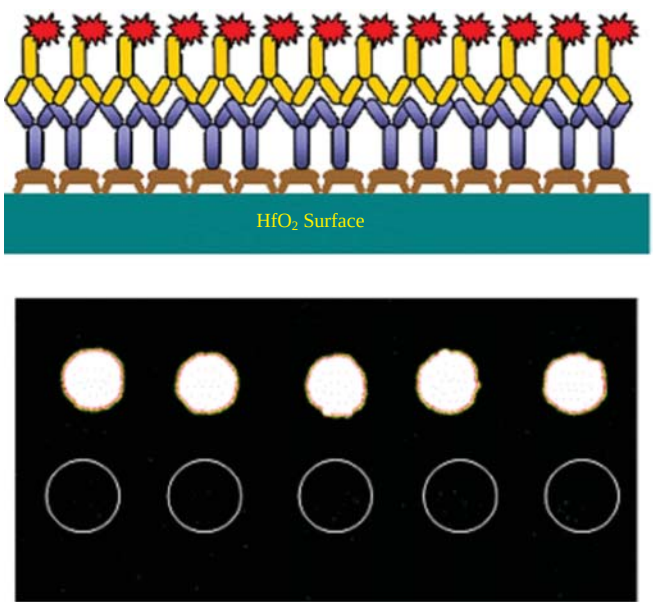

Figure 1. Fluorescence detection of dye-labeled anti-hIgG to hIgG interaction on a ProLinker-coated hafnium oxide surface.

analyze an antibody-antigen interaction on the hafnium oxide surface. Its fluorescence by external excitation is detected using a photomultiplier. The fluorescence intensity indicates the binding of anti-hIgG to hIgG in terms of protein-protein interaction. The result of luminescence detection is displayed in Figure 1; a rather strong light emission is observed from anti-hIgG attached to hIgG, but not from hIgG alone. If hIgG molecules were not attached to the hafnium oxide surface, the luminescence would not have been detected. Thus, this result confirms that the protein can be immobilized on a SAM-modified hafnium oxide surface by ProLinker.

Device Fabrication Process

Nanowire bio-sensor devices are fabricated using a SIMOX SOI (Silicon On Insulator) wafer that consists of n-type (P-doped, resistivity of 1-20 ohm-cm) 100 nm thick top Si layers, and 150 nm thick buried oxides on an n-type Si thick body. The fabrication of the device starts with photolithography in order to define the active area on the top Si layers followed by reactive ion etching to remove the non-active area. Then e-beam direct writing is also utilized to make a narrow channel to a width of 50 nm and a length of 3 μm in the middle of the channel area. Sacrificial oxides up to a thickness of 18 nm are deposited, and photoresist dummy gates are additionally formed to protect the narrow channel area prior to ion implantation for the p-type source, drain. BF^+ (70 keV, $2 \times 10^{15}/cm^2$) is used for an implant species in the source and drain, and As^- (70 keV, $1 \times 10^{16}/cm^2$) is used for n-type bottom body contact. For the activation of the implanted species, a thermal anneal is performed at 950°C for 1 min. During annealing, borons are expected to diffuse from the source/drain into a wide channel region, converting it into a p-type. After removing the dummy gate and sacrificial oxides, 10 nm thick hafnium oxide layers for a gate dielectric are deposited by an atomic layer deposition (ALD) method at 350°C. As the entire surface is covered by hafnium oxides during the ALD process, only the contact area is therefore open by wet etching before metal deposition. As-deposited Mo is used without high-temperature contact annealing to avoid the quality deterioration of low-temperature deposited hafnium oxides. Finally, Al is vacuum evaporated on the back side for the bottom gate contact metal. Thus, the device has a narrow channel, t=100 nm, W=50-90 nm, and L=3 μm, which is covered by ultra-thin hafnium oxides at its top and also at the sides, and it will operate as a p-channel field effect transistor by negative bottom gate voltage and by attached protein charges on the hafnium oxides.

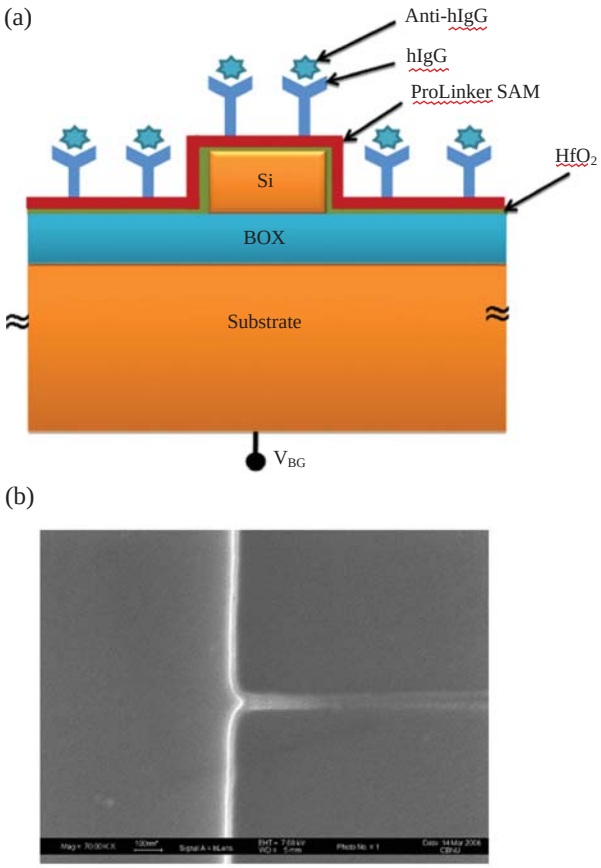

Figure 2. MOSFET for protein analysis. (a) A schematic drawing of a protein detection biosensor (A cross-sectional view), (b) Scanning Electron Microscopy (SEM) observation of the fabricated 50 nm wide nanowire channel.

Once the drain currents of a biosensor are measured by applying the bottom gate voltage, the device is immersed in a ProLinker solution for SAM modification. The measurements are carried out after each step of SAM treatment, capture protein immobilization, and interacting protein binding. A schematic drawing of the device and its microscopic observation are illustrated in Figure 2.

Characterization of Biosensor

The current-voltage characteristic of a virgin device prior to SAM treatment is illustrated in Figure 3. As the bottom gate voltage becomes more negative, the drain current increases due to an accumulation of a hole in the channel. At small drain voltages, the drain current linearly increases with the increase of drain voltage, and shows a slight current saturation at a high large-drain voltage. This is typical behavior of a p-channel MOSFET in the triode region. The same device is measured at a bottom gate voltage of −1 V

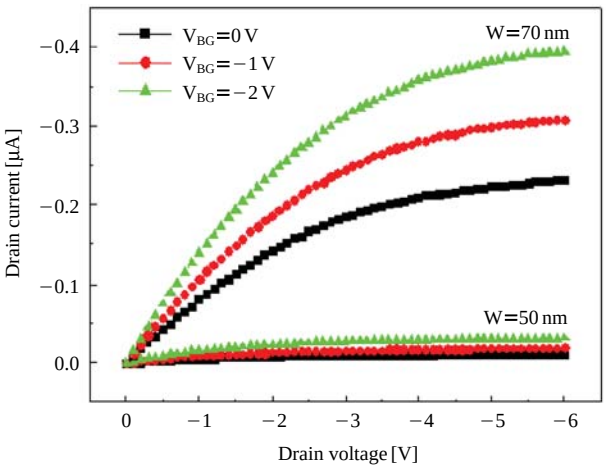

Figure 3. Drain current vs. drain voltage characteristics taken from a virgin surface.

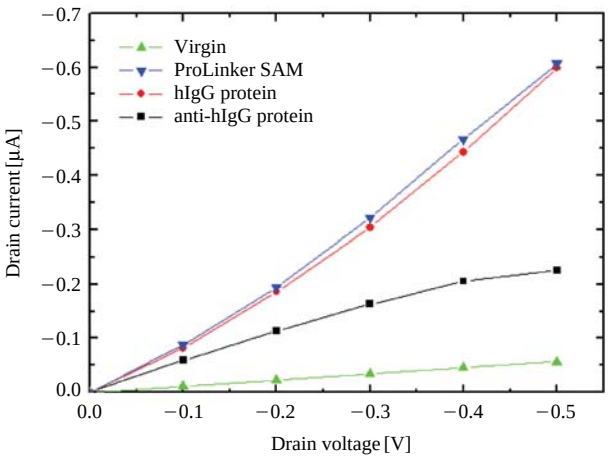

Figure 4. I-V characteristics in the low gate voltage region.

after every step of the SAM treatment, hIgG as capture protein immobilization, and anti-hIgG as interacting protein binding. The I-V characteristics at the small-drain voltage region are summarized in Figure 4. From an overall view of the results, the current is in order of the largest by SAM treatment, probe-protein immobilization, and target-protein attachment, and in order of the least when untreated. The conductance, the slope of current vs. voltage calculated at −0.3 V, is 0.11 μS for a virgin surface, and it increases to 1.37 μS after SAM formation. Immobilization of hIgG protein reduces it slightly to 1.29 μS and further attachment of anti-hIgG results in a tremendous change of conductance, showing 0.45 μS. We may neglect the small difference of conductance between the SAM and hIgG protein immobilization data. From this observation, the protein-protein interaction is very clearly sensed, and we conclude that the fabricated nanowire device performs as a direct electrical detection of proteins.

As there have been only a few studies on label-free anti-hIgG and hIgG interaction, a direct comparison is not available; however, a surface plasmon resonance (SPR) as small as about 10 pg/mL has been recognized, and a surface acoustic wave (SAW) device has 50 μg/mL, which is the same as what we have obtained[15,16]. In the present study, we have only tested at a fixed protein concentration, but we anticipate that our device is eligible to sense a conductance change within the tens of nS range, which is two orders of magnitude lower than that presented in this study.

The above result states that the intrinsic negative charges of the SAM draw the highest current by accumulating more holes in the channel. When hIgG protein is attached to the SAM, the protein charges are screened by SAM charges, and thus the current does not change noticeably. While anti-hIgG is immobilized to the underlying hIgG, the protein-protein interaction draws the negative charges from hIgG, and thus the effective charges seen at the channel of the transistor become less than those of the SAM. Therefore, the drain current is less than that of hIgG attached on the SAM, but it is still larger than that of the virgin sample.

We may estimate the equivalent attached charge density attached to the gate dielectric using the conductance in the MOSFET linear region assuming that a single channel is already formed by the bottom gate and an additional current is due to the top gate charges. The conductance is expressed by surface charges, which have an opposite sign but the same magnitude as the channel charges, as in the following relation:

$g_d=\mu_p(W/L)Q_p$ (1), where g_d, μ_p, W, L, and Q_p are the conductance, channel hole mobility, channel width, channel length, and effective charge density, respectively. When we use the channel hole mobility value of $\mu_p=15\ cm^2/V\text{-}s$ taken from the data of the virgin surface, we can obtain an effective charge density from the conductance difference between two sequential steps. Those values are as follows:

$Q_{SAM}=-5.0\times10^{-6}\ C/cm^2$, $Q_{hIgG}=0\ C/cm^2$, $Q_{anti-hIgG}=+3.4\times10^{-6}\ C/cm^2$ for the SAM, hIgG, and anti-hIgG protein, respectively. These values can be used for further device modeling and for later sensing the circuit design.

Conclusions

We have fabricated and tested a nanowire protein

biosensor by utilizing HfO_2 as the gate dielectrics of a MOSFET device and also as a protein immobilization surface. The nanowire channel has a width of 50 nm and a length of 3 μm formed on a SOI wafer. By conventional fluorescence labeling, we have confirmed the protein (anti-hIgG, hIgG) attachment to the HfO_2 surface after SAM formation using a ProLinker. When the surface is modified by a ProLinker treatment, the conductance of the MOSFET increases by more than 10-fold from 0.11 μS to 1.37 μS in the linear region of the transistor operation and stays almost at the same value by further attachment of 100 μg/mL hIgG protein. On one hand, when 50 μg/mL of anti-hIgG is immobilized over hIgG protein, the conductance drops to 0.45 μS due to protein-protein interaction, and the conductance change is the largest among three events. Thus, we are able to distinguish the protein interaction using label-free direct electrical detection utilizing the fabricated Si nanowire transistor. The MOSFET system will require further work on its validation for quantitative analysis of specific biomarker proteins in biological samples, and for a trial on other biological applications such as proteomics and drug discovery.

Materials and Methods

The sample is first immersed in 5% ATMS (3-amino-propyl-trimethoxysian) in an ethanol solution followed by a diluted ProLinker solution to form SAM on an oxide surface. The dried sample is then immersed in a human immuno-globulin-G (hIgG) solution (100 μg/mL) for 3 hrs. at 37°C under 70% of humidity, and washed in PBS (phosphate buffered saline), followed by a rinse and dry process. Thus, the antibody is immobilized to the SAM-modified hafnium oxide surface. To react with hIgG, we used anti-human immuno-globulin G (anti-hIgG) (50 μg/mL). The process of anti-hIgG reaction to hIgG occurs by immersing the sample in an anti-hIgG solution. The sample is washed again in a buffered 0.5% PBS solution, rinsed in water, and finally blow dried under N_2 gas. The anti-hIgG has a Y-type structure with 5 immune antigens; IgG has a molecular wight of 150,000 Da. The conventional IgG molecule has two heavy chains and one light chain. The upper two parts are called Fab, and the lower part is called an Fc fragment. The antibody, anti-hIgG, will be attached to the Fab part of hIgG.

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