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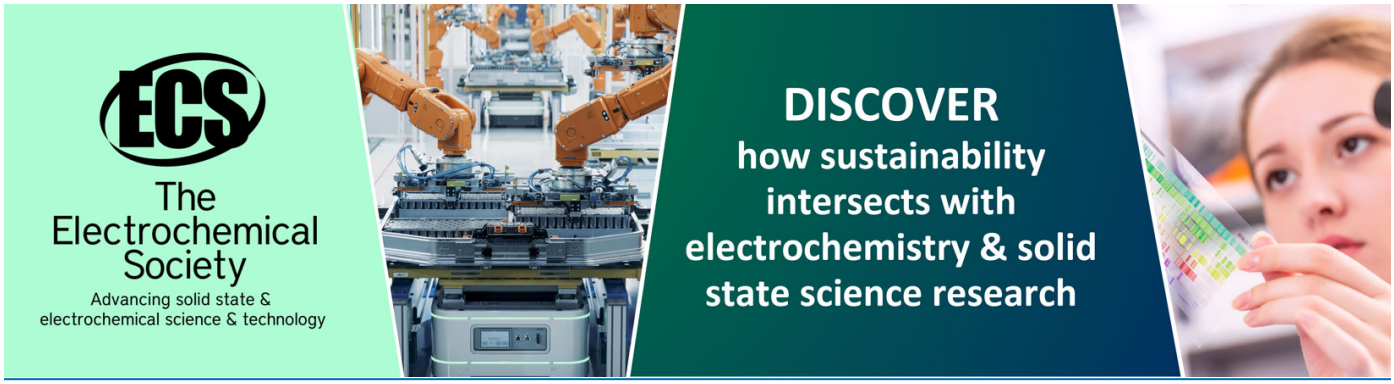

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Fabrication and Characterizations of Poly-Si Nanowire Biosensor using Conventional Photolithography Technique for Detection of Dengue Virus DNA Type 2 (DENV-2)

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Abstract. Nowadays, nanotechnology has become a vast expanding application which can be used all across the science field such as chemistry, biology, physic, material science and engineering. In this paper, a poly-Si nanowire biosensor was fabricated by using the conventional photolithography technique. In addition, this technique is used to define the initial poly-Si with the dimension of 1 µm. After the conventional photolithography process, the photoresist undergone the development using resist developer and etched with reactive ion etching (RIE). Meanwhile, for the electrical part, it was observable that there was an increase in current when the nanowire has been hybridized with Dengue DNA type-2 (DENV-2) ranging from 10 fM – 10 µM. The morphology of the poly-Si nanowire was characterized by optical microscopy whilst electrically characterized by measuring the two-terminal current-voltage (I-V) characteristic.

1. Introduction

Over the last several decades, the World Health Organisation (WHO) has estimated about 2.5 billion of people which is two-fifth of the world population were at risk of dengue virus [1]. Many treatments and diagnosis have been implemented in order to overcome this tropical disease. One of the stated treatment was by using the polymerase chain reaction (PCR)-based fluorescent microarrays for the detection of the dengue virus. However, this technique suffers from producing an exact profile gene [2]. To resolve this problem, polysilicon (poly-Si) nanowire biosensor has been introduced where this biosensor exhibits a more subtle, label-free, and electrical tool making it one of the most applicable devices for researchers in the past years [3]. An abundant of methods can be applied to fabricate the biosensor, but the most common was by using the 'top-down' method. The reason for using this technique was because of the combination of conventional photolithography and size reduction strategy which leads to a simpler yet economical solution. Thus, the e-beam lithography can also be utilized to fabricate the poly-Si nanowire device with such precision and in a control manner [4-5].

Furthermore, to the best of our knowledge, poly-Si nanowire biosensor inquires great biosensing mechanism due to its ultrasensitive and excellent electrical characteristic. The thing that separates this biosensor and other sensors is the presence of biological/organic identification which enables them to detect significant biological molecule in the medium [6]. The functional group of amine which is from the 3-aminopropyltriethoxysilane (APTES) was used as the surface modification for the detection of

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dengue virus. Whenever the modification activates the surface, the effectiveness of oligonucleotides is modified with functional group which allows covalent attachment with the reactive group on the surface [7]. Nevertheless, for the detection of the dengue DNA to be a success, the amine group APTES requires another linker which is the glutaraldehyde (GA). It is popularly known as a cross linker, that is a dialdehyde with high affinity for the free primary amine groups of amino acids [8]. A biosensor is an analytical piece of device that in the presence of a compatible enzyme in the bio recognition layer which then provides an electro active substance for the detection of the transducer by converting it into measuring signal [9]. In this paper, the morphology and dimension of the device were carried out by using an atomic force microscope (AFM) and high-power microscope (HPM). Meanwhile, for the electrical part, a KEITHLEY 6487 pico-ammeter/voltage source was used to measure the current-voltage (I-V) of the poly-Si nanowire after being hybridized with Dengue DNA type-2 (DENV-2) ranging from 10 fM – 10 μM.

2. Experimental

2.1. Material

The material being used in this experiment was Si-SiO_2-Si_3N_4-poly-Si as the substrate layer for the fabrication of poly-Si nanowire. The functional group amine which is 3-aminopropyltriethoxysilane (APTES) and glutaraldehyde (GA) were used as surface modification. For DNA immobilization and hybridization part, a 10-μM DNA probe solution and 27-mer complementary DNA targets in the range from 10 fM – 10 μM concentrations has been make use of in this experiment.

2.2. Device fabrication

In this work, the fabrication of the poly-Si nanowire started off with the cleaning process using RCA1 and RCA2 to eliminate any organic or inorganic compound on the substrate layer. Then, conventional photolithography process was carried out on top of the cleaned surface to produce the nanowire. Furthermore, the surface of poly-Si was then coated with positive photoresist via spin-coater for about 3000 rpm for 40 seconds which in result produce a thickness of 1.8 μm. To improve adhesion to the layer, the coated photoresist was then soft-baked 110°C for 120 seconds on a hot-plate. Afterwards, the coated photoresist was exposed with ultraviolet light (UV) with wavelength of 365 nm for 10 seconds for pattern transfer and followed by the development process.

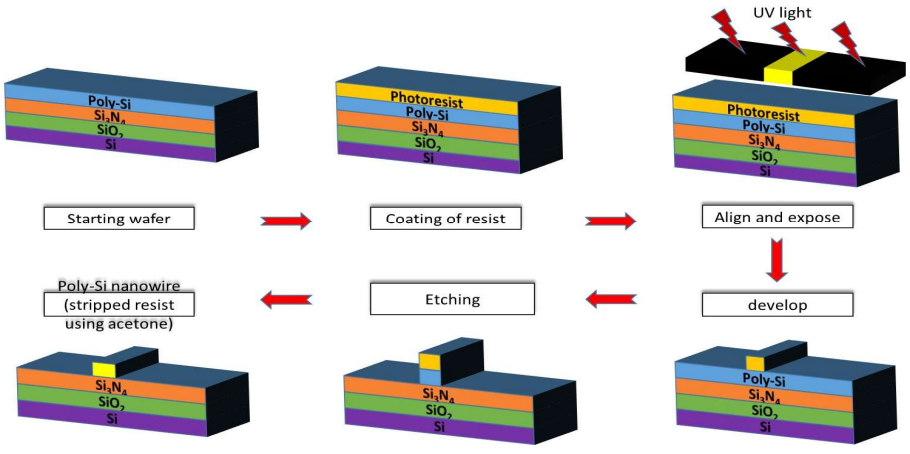

Figure 1. Step-by-step fabrication process of poly-Si nanowire using conventional photolithography.

The most critical part of the development process was to control the time and the resist profile. The resist undergoes a series of trimming process by using a resist developer to achieve the smallest size possible which was rinsed with DIW afterward. Subsequently, the poly-Si layer was etched via the inductive couple plasma reactive ion etching (ICP-RIE). Thus, the remaining resist was completely stripped by acetone and rinsed with DIW. A more detailed presentation of the fabrication process is shown in figure 1. The process continued with the deposition of nickel/Gold (Ni/Au) thin films on the sample as adhesion and contact pad via thermal evaporator. The prepared structure was then optically characterized using HPM and AFM.

2.3. Surface modification

In this experiment, for the device to identify a specific type of target molecule, functionalization of poly-Si nanowire surface with bio-receptor is utterly important. Primarily, with the presence of native oxide on the poly-Si nanowire surface was immersed in 2% APTES (v/v) in a mixture of 95% ethanol and 5% water for 2 hours at room temperature to obtain a surface exposed amine-terminated groups (NH_2). During this time, covalent bonding was formed between the oxygen atom of the hydroxyl-terminated groups and the silicon atom in the molecule of APTES. Afterwards, the sample was cleaned using ethanol to remove any excess APTES and dried on a hot plate at 120°C for 10 minutes. Next, the functionalized APTES was immersed in 2.5% glutaraldehyde (with PBS solution) and kept in the solution for 1 hour at room temperature. The process continues with PBS cleaning and rinsed with DI water for 5 minutes to remove excess GA. The GA acts a linker to ensure the bonding between amine-terminated groups and aldehyde groups (COH) on the surface.

2.4. DNA immobilization

For DNA immobilization, 10-μM DNA probe solution (diluted with PBS solution at pH 7.4) was injected to the sensing area of the poly-Si nanowire which was then followed by incubation process at room temperature for 4 hours. Any unbound probe was washed away with PBS solution. This is due to the fact that aldehyde group can be used as DNA immobilization in which a 27-mer amine-terminated probe was linked to the aldehyde-terminated groups.

2.5. DNA hybridization

Following the immobilization step, 27-mer complementary DNA targets in the range from 10 fM – 10 μM concentrations was dropped on the sensing area (nanowire) in order to hybridize the immobilized DNA. Subsequently, the sample was incubated at room temperature overnight in order to keep it hybridized. Later, the sample was washed away with PBS solution to remove excess DNA target. The sample was stored at 4°C when it is not in used. The mentioned step-by-step of surface modification until DNA hybridization is detailed out in Fig 2. Electrical characterization was carried out to ensure successful fabrication and functionalization of poly-Si nanowire by measuring the I-V characteristics, specificity, and sensitivity of the device. The drain voltage was swept from 0 to 1 V with the source grounded to test the fabricated Silicon nanowire, amine-terminated APTES, DNA immobilization, and hybridization through the use of Keithley 6487 picoammeter/voltage source.

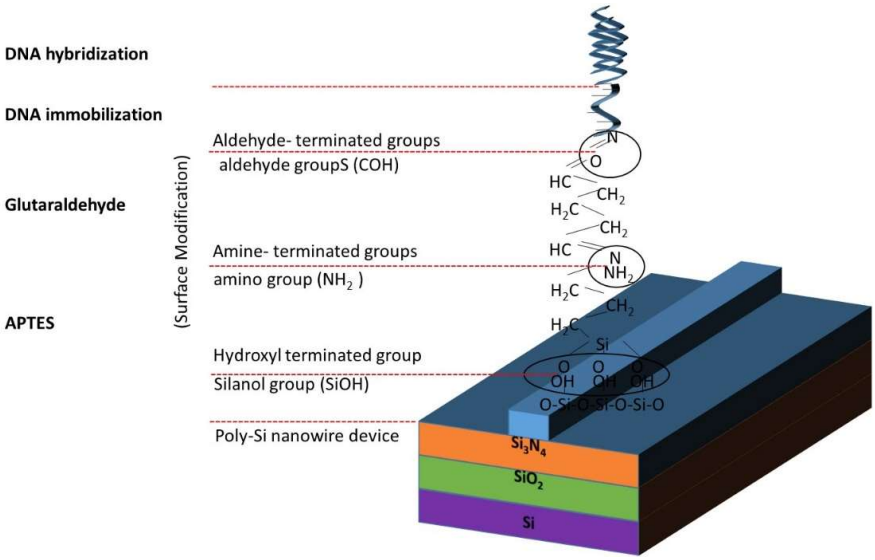

Figure 2. Surface modification from the functional group amine.

3. Results and Discussions

3.1. Physical characterization of poly-Si nanowire

As shown in figure 3, the normal developed resist was taken under the HPM for the fabricated poly-Si nanowire after conventional photolithography process with the length of 496.94 μm. Other than a normal developed resist, there are several problems that may occur if the development process was not controlled properly. The type of resist problems that may arise during the development process are such as underdevelopment, incomplete development, and overdevelopment [10-11]. Optical inspection was carried out once the device was completely fabricated. In this work, the surface morphology image was performed by using the AFM. Figure 4 and 5 show the 3D image and morphology profile of the poly-Si nanowire, respectively. Both figures clearly present a successful fabrication after the poly-Si layer managed to lose it width by 100 – 150 nm after the photoresist was developed using a resist developer and etched via reactive ion etching (RIE).

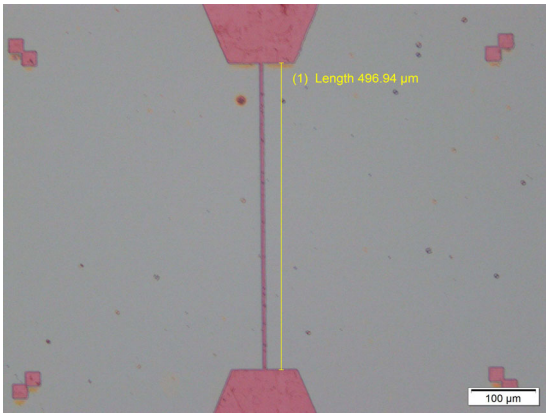

Figure 3. The overview image of poly-Si nanowire after conventional photolithography.

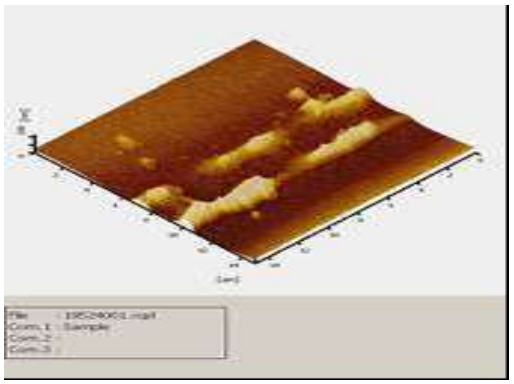

Figure 4. 3D AFM image of poly-Si nanowire.

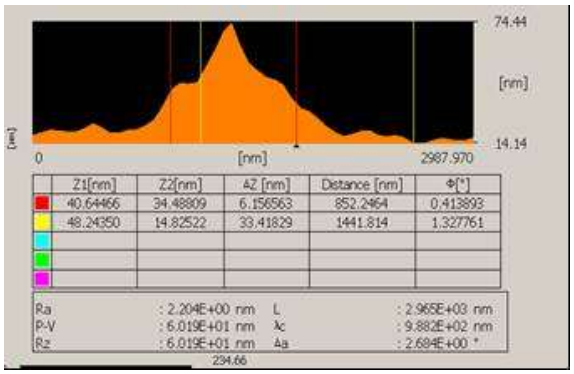

Figure 5. Surface morphology of poly-Si nanowire.

3.2. Electrical characterization of poly-Si nanowire

As soon as the device was fabricated, the testing and measuring process was conducted. Electrical characterization was carried out in order to verify the effectiveness of the biosensor by investigating the I-V characteristics of the poly-Si nanowire using a KEITHLEY 6487 picoammeter/voltage source. The drain voltage was swept from 0 – 1 V between the two metal contacts of the poly-Si nanowire with the source being grounded to test the fabricated device. The device was measured for the surface modification using functional group of amine and aldehyde from APTES and GA, followed by DNA probe immobilization and DNA hybridization ranging from 10 fM – 10 μM. Figure 6 shows an I-V graph being plotted with the result obtained due to the different concentration of surface functionalization.

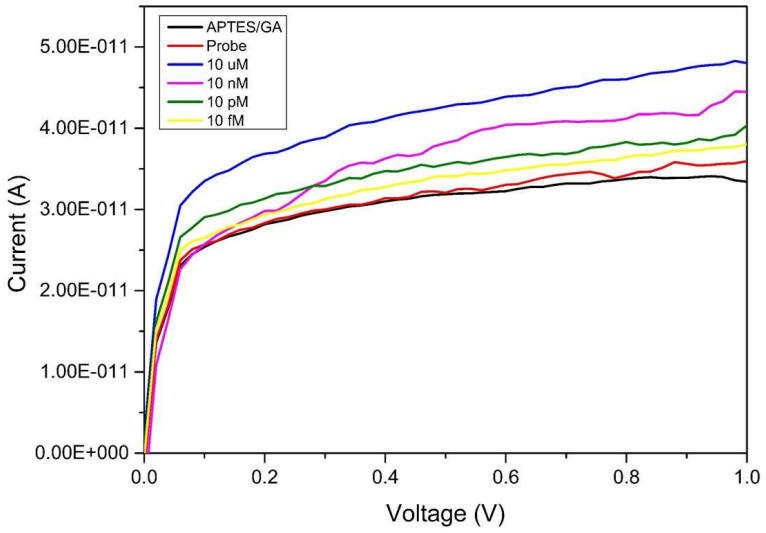

Figure 6. The current- voltage (I-V) for APTES/GA, DNA probe immobilization and target DNA hybridization of DENV-2.

Based on the I-V characteristic, the fabricated poly-Si nanowire biosensor had shown good electrical characterization. Initially, the device was tested with surface modification APTES which shows the presence of current due to the APTES providing an amine-terminated group (exposed-NH_2) into the picture when the oxygen atom of the hydroxyl-terminated group that is naturally present on the poly-Si nanowire surface forms a covalent bond with the molecule of APTES. On the other hand, glutaraldehyde (GA) functions as a linker for the amine-terminated group and the aldehyde group as a purpose of ensuring chemical bonding. Subsequently, the device was immobilized with DNA probe resulting a slight increase in current. This is because 10-μM DNA solution which has been diluted with buffer solution at pH 7.4 being injected to the surface of poly-Si nanowire biosensor and therefore affects the change in value of the result. Lastly, the device was hybridized with target DNA ranging from 10 fM – 10 μM. The current increases as the concentration of the target DNA increases on account of the accumulation of negative charge carriers which differs from each of the target DNA that leads to an increase in the current being measured, hence decreasing the resistance [12].

4. Conclusion

In this research, a simple and low-cost method to fabricate poly-Si nanowire biosensor was a success via conventional photolithography. As a result, the poly-Si layer loses it width by 100 – 150 nm. After the photoresist was developed thoroughly using a resist developer and etched with RIE afterwards. Subsequently, for the electrical part, it is visible that there was an increase in conductance when the device was being test with surface modification APTES, DNA probe and target DNA ranging from 10 fM – 10 μM. The variation in conductivity of the poly-Si nanowire was affected by the charge contain in the surface functionalization. However, the sensitivity of detection can further be improved when a smaller dimension of poly-Si nanowire is fabricated due to an increase of surface to volume ratio.

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