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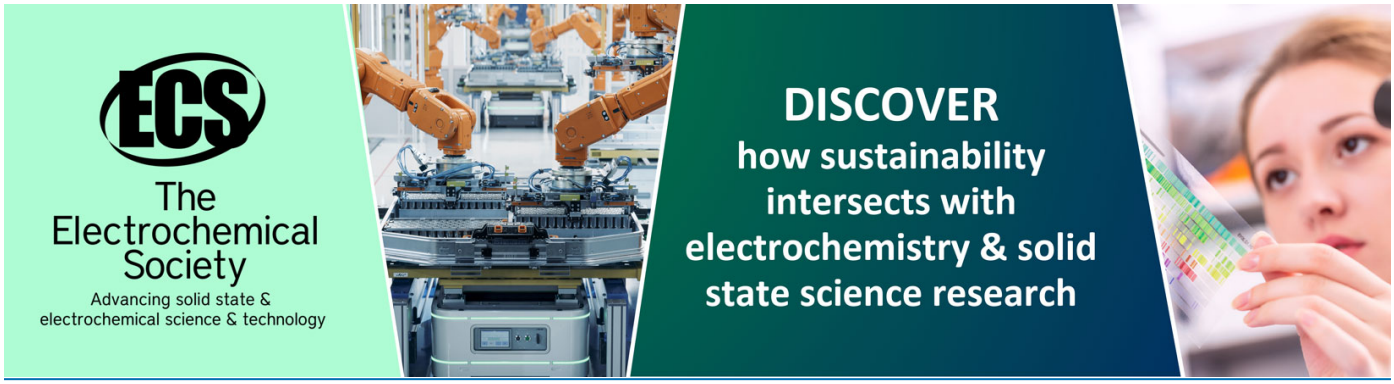

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Electrochemical DNA Biosensor based on 30 nM Gold Nanoparticle Modified Electrode by Electro Less Deposition for Human *Papillomavirus* (HPV) 18 E6 Region

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Abstract. The aim of this work was to develop a novel, simple, inexpensive, sensitive an electrochemical DNA biosensor based on interdigitated electrodes (IDEs) integrated gold nanoparticle modified electrode by electro less deposition for HPV 18. The biosensor was designed with a 30 mer E6 region of HPV 18 DNA modified probe. The E6 region has been used for their clinical importance properties and suitable as recognition biomarker region. Three different target types were tested which complementary target, non-complementary target and mismatch target. All target were analyzed for detection of HPV 18 in early stages by using Dielectric Analyzer (DA), Alpha-A High- performance Frequency Analyzer, Novocontrol Technologies, Handsagen, Germany associated with the software package Windeta. Complementary target gives a positive result in HPV detection, while non-complementary and mismatch target give negative results. IDE device with 5 nm gap sizes has demonstrated a high performance towards the detection of HPV18 ssDNA target by modified with 30 nm gold nanoparticle. The electrochemical biosensor showed better performance compared to agarose gel electrophoresis assay. This technology can be used as a new and attractive sensor development for detection of virus infection in human bodies.

1. Introduction

Human *Papillomavirus* (HPV) also known as HPV is the most popular virus lead to cervical cancer [1–4]. HPV 18 was classified as high risk (HR), and responsible for 70% of cervical cancer in the worldwide population[5]. HPV was very dangerous where it is able to infect the epithelial cells of our skin, genital mucosa and oral. Generally, HPV can be easily be passed to a person even there have no symptoms. It can be passed during vaginal or anal sex[6]. Usually, it is hard to detect and there is no any symptom at the beginning infected by the virus until it is developed after years. A symptom such genital wart appears with vary of size with cauliflower sized at the genital area causing a health problem where the next step will be cancer. This can be detected by healthcare by looking at the genital area.

E6 region was constantly expressed in the high risk of HPV and responsible for malignancy transformation of cervical cancer [7]. E6 region represent the ideal biomarker region for the development of biosensor [8], which potentially eliminate pre-existing lesions and tumors by generate immunity of cellular mechanism against HPV infected cells. Cancer takes a longer time to develop for a person who has HPV; people with a weak immune system cannot survive. HPV is not only can be cervical cancer but also can be other cancers[9]. There are many types of HPV strain which affect human body at high and a low risk, hundred and fifty to be exact. From all of them, fifteen strains was identified as dangerous strains to infected person for cervical malignancy.

The most current technique that has been used for identification of HPV DNA was the electrophoresis method by using gel electrophoresis method [10]. The disadvantages of this method are time consuming and required well-trained personnel. Nowadays, the technology gives us much advantageous and effectiveness on detection of HPV strain by the growth of a biosensor in detection the virus of HPV at early stages [1–4]. A biosensor has become the most important roles in HPV virus identification and detection. Therefore, a rapid and sensitive step in detection of HPV especially on HPV 16, HPV 18, HPV 31 and HPV 33 was proposed by integrating the simple DNA extraction with the gene-based biosensor [4,6,11–14]. Recently, the biosensor is used to detect by analyzing the concentration of the biological analyte [3]. A modern biosensor consists of 3 major components which are the component to recognize the analyte and produce a signal, a signal transducer and a reader device.

DNA biosensor was required to detect a specific DNA sequence from E6 region of HPV18. The efficiency of DNA biosensor test kit has dependency by the way of the attachment of probe to the support and the sequence of the capture probes [7,15]. To develop the best biosensor, many procedures should be considered and it should be quick, covalent, and be able to reproduce. The procedures such surface modification, surface functionalization, immobilization and lastly hybridization were characterized by dielectric analyser. Thus, this research mainly focuses for development of electrochemical DNA biosensor by using 30 nm gold nanoparticles in order to enhanced signal for the detection of HPV 18.

2. Experimental

2.1 Material and samples collection

3-Aminopropyltriethoxysilane (APTES) solution was obtained from Sigma Aldrich (https://www.sigmaaldrich.com/) and QIAamp DNA mini kit was purchased from Qiagen Company. APTES were prepared using 12 APTES and 38 of deionized water to functionalize the biosensor. Mercapto- hexadecanoic acid (MHDA) was used as the cross-linking and stabilizing agents for linking COOH and NH_2. MHDA was exposed to the IDE sensor to eliminate non-specific binding.

2.2 Gold Nanoparticles Inspection

The first stage was observing the morphology of the gold IDE before the process of surface modification. Gold nanoparticles (AuNP) were used to modify the surface of biosensor and to enhance the signal on interdigitated electrode IDE.

2.3 Surface Modification

The electrical measurement was conducted by using dielectric analyzer. It begins with the bare test of 5μm IDE, the data was recorded. Then, continue the IDE was washed with 70 % of ethanol and then washed again with deionized water to remove the unwanted particle on IDE Biosensor. By using the blower, The IDE has been blow dried then continues with electrical measurement. The IDE is denoted as silicon oxide (SiO_2). Additionally, 1 μL of 30 nm of AuNPs were added on the surface of IDE and it incubated for 15 minutes to let it dried on the surface. It continued with electrical measurement by using picoammeter measurement. After that, 1 μL of 2-Mercaptoethanol has been drop onto the

modified IDE to block the non-specific bonding on the top of IDE. After 15 minutes, the electrical measurement was quantified. The characterization by using current-voltage (I-V) was conducted.

2.4 Surface functionalization

The silicon oxide (SiO_2) surface was undergo the process of functionalization with an (3-Aminopropyl) Triethoxysilane (APTES) which has been homogenized with the ratio of 12 µl of APTES and 36 µl of deionized water. Silanization uses the silanol group (SiH_3) which existed in APTES was chemically bind to the hydroxyl-rich SiO_2. Moreover, the amino group (NH_2) in APTES act as a glue layer for the AuNPs where AuNPs would be linked to probe DNA. For the surface functionalization, 1 µL of APTES solution was deposited on the IDE surface and has been incubate for 15 minutes. Then, the surface has washed 3 times and has been blow dried. After that, the IDE was continued with electrical measurement.

2.5 Probe Immobilization

One µL Probe DNA of HPV 18 was deposited onto the IDE with AuNPs modified for immobilization and has been incubated at room temperature for 15 minutes. Afterwards, the IDE was washed with deionized water in order to remove any un-bonded DNA probe material and dried at room temperature. The probe modified devices represent as Si/SiO2/AuNPs/APTES/DNA, and was ready for electrical measurements.

2.6 Synthetic DNA Target Hybridization

1 µL Target DNA was deposited onto IDE surface layered with the DNA probe for hybridization detection. Then, the reaction has been incubated for 15 minutes. After that, it was ready for measurement using Electrochemical Characterization by Electrochemical Impedance Spectroscopy (EIS). Characterization by comparison with selectivity test included complementary, non-complementary and mismatch.

2.7 IDE Characterization

The IDE modified with of the AuNPs has been characterized using a scanning electron microscope (SEM) to get the image and the exact sizing of gold nanoparticle on IDE. Other than that, the prove HPV 18 also deposited onto IDE then undergo the process of characterization using SEM. The DNA immobilization and hybridization has been tested using a dielectric analyzer (Alpha-A High-Performance Frequency Analyzer, Novocontrol Technologies, and Germany).

3. Results and Discussions

3.1 Optimization of IDE Biosensor with Complementary, Non-Complementary and Mismatch

Electrochemical hybridization biosensor can be used in specific DNA detection. EIS simplified the procedures of several assays, facilitated the specific detection of nucleic acid sequence [4,14]. DNA hybridization based on the ability of bioreceptor in single stranded form recognize it complementary DNA sequences, which forming DNA duplex. A hybridization of complementary DNA with the probe was analyzed. Figure 1 representing graphs after hybridization with the complementary target.From the graphs, the capacitance response was showed a positive result with modified of IDE / wash / GNP / APTES and immobilized with HPV 18 probe. The capacitance peak was increased above the probe HPV line. Based on the graph in figure 2, the graph showed negative result because the capacitance was decreased below the probe DNA line.

Figure 3 showed the graph after hybridization with the mismatch target. Based on the graph, it showed that the mismatch target line is below than the probe line which means it was the negative result. To make sure that the reading of the target is lower than the probe value, the frequency at 0.086 Hz was taken and plotted to generate the bar chart.

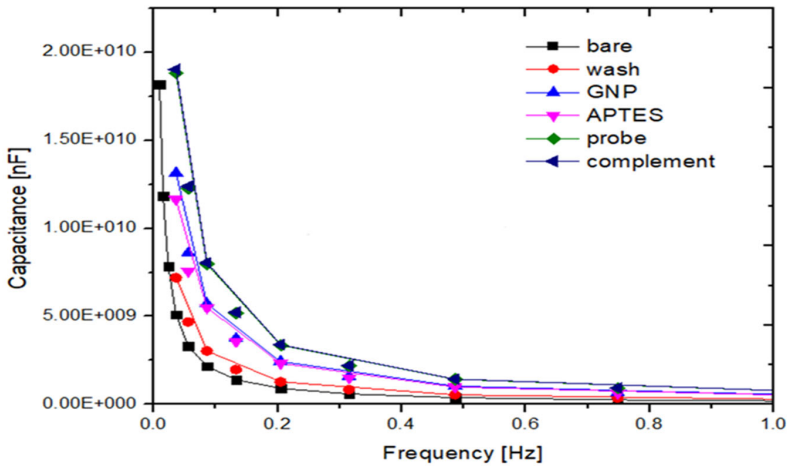

Figure 1. Graph of capacitance versus frequency of complementary target.

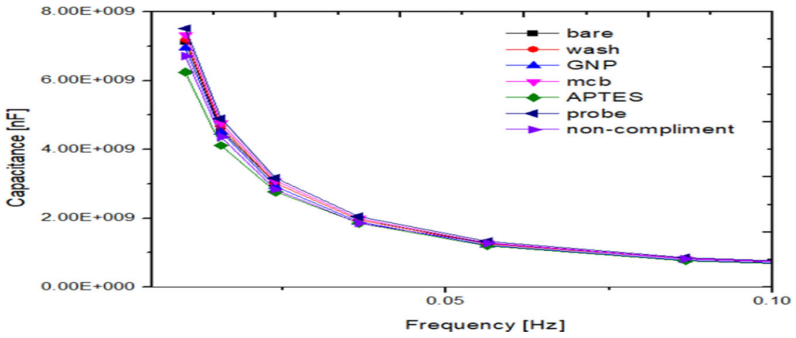

Figure 2. Graph of capacitance versus frequency of non-complementary target.

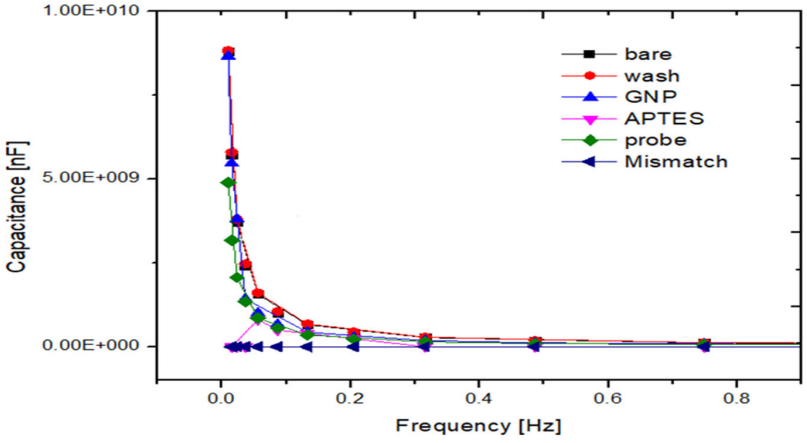

Figure 3. Graph of capacitance versus frequency of mismatch target

Figure 4 showed the combination graph of complementary, non-complementary, and mismatch of target data where from the graph obtained, the peak of complementary had increased above the graph of the probe which indicated as a positive result whereby, non-complementary and mismatch target has decreased below the probe. To confirm that the complementary reading was higher than the data of probe, the frequency at 0.133 Hz has been taken and generates the bar chart. Figure 5 showed the bar chart which indicated that the complementary reading was higher than the probe. This means that complementary has a positive result to HPV 18 meanwhile, non-complementary and mismatch ware negative result of HPV 18.

The previous study had stated that upon the perfect match binding of ssDNA probe and complement ssDNA target allows the electron to passes between the electrodes gives a huge increment in the electrical signal, compared to the bonding with ssDNA non-complementary and ssDNA mismatch. In the previous study, mismatch bonding between ssDNA probe and ssDNA MM has decreased the stability of hydrogen bond, thus decreased the measurement signal of the mismatched sequence. This means that the device has high specificity towards complementary ssDNA, ssDNA mismatch, and ssDNA non-complement. Moreover, It is also proves that the potential of developed IDE device as a biosensor for HPV 18 detection.

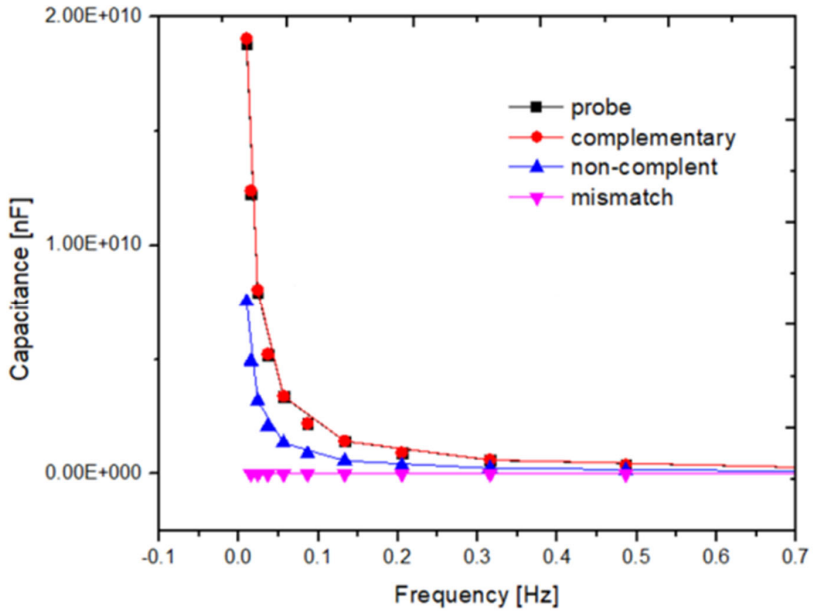

Figure 4. Graph of capacitance versus frequency of mismatch target

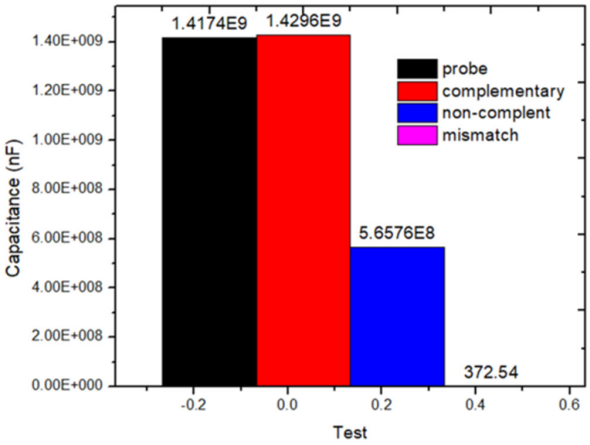

Figure 5. Graph of frequency reading at 0.133 Hz

4. Conclusions

In conclusion with these above results, it was clear that the IDE device with 5 nm gap size has demonstrated a high performance towards the detection of HPV18 ssDNA target by modified with 30 nm gold nanoparticle. By comparing to another biosensor, this IDE can detect up 1 pM concentration of target complementary DNA. Other than that, this IDE also capable to distinguish its DNA target to others non-complementary and single mismatches as shown in the graphs.

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