Voltammetric DNA Biosensor for Human Papillomavirus (HPV) Strain 18 Detection

M. A. Mhd Akhir3, N. A. Parmin1*, Uda Hashim1,2, Subash C.B. Gopinath1,3, Zulida Rejali4, Amilia Afzan4, M.N.A Uda3, M.N.Afnan Uda3 and V.C. Hong1

1Institute of Nano Electronic Engineering, Universiti Malaysia Perlis, 01000 Kangar, Perlis, Malaysia. 2School of Microelectronic Engineering, Universiti Malaysia Perlis, 01000 Kangar, Perlis, Malaysia. 3School of Bioprocess Engineering, Universiti Malaysia Perlis, 02600 Arau, Perlis, Malaysia. 4Department of Obstetrics and Gynaecology, Faculty of Medicine & Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

E-mail: azizahparmin@unimap.edu.my

Abstract. This research was developed to focus on the study of the voltammetric DNA biosensor for the detection of HPV strain 18. In this research, electrical DNA biosensor was expected to detect HPV strain 18 more efficiently by using electrical characterization. In this project, device inspection was conducted to make sure the functional of the gold interdigitated electrode (IDE) by using Scanning Electron Microscope (SEM). 3-Aminopropyl Triethoxysilane (APTES) solution was used for the process of surface modification to form the amine group on the surface of the device to facilitate the attachment of the DNA probe. In this project, synthetic DNA sample and DNA from the saliva of several Biosystems Engineering students were used as the target DNA. The current-voltage (I-V) electrical characterization was conducted to detect the presence of HPV strain 18 in both DNA samples. As the results, perfect alignment between the electrodes on the IDE was detected under SEM. Surface modification of the biosensor successfully conducted which is the covalent bond between APTES and DNA probe increase the electrical. Synthetic DNA shows the presence of HPV strain 18 while there was no HPV strain 18 detected in the DNA from saliva samples.

1. Introduction

Human Papillomavirus (HPV) became a virus that can lead to cervical cancer by infecting the skin or infecting the mucous membrane of the patient. HPV belongs to the family of Papillomaviridae [1,2]. This virus can be transmitted through sexual activities. There are more than 100 types of HPV that can cause warts and cervical cancer [3]. HPV can be classified as two types which are low-risk and high-risk. The low-risk types usually cause non genital warts and genital warts. HPV-6 and HPV-11 are the common viruses that cause genital warts and these strains are non-cancerous to human. On the other hand, the high-risk HPV consist of 7 types of strains (18,16,45,31,33,52,58) [3]. The common strain contribute to the cervical cancer is HPV strain 18 which approximately 70% of cervical cancer is from this strain [4-6].

HPV cannot be cured if the human cells were already infected and forming cancerous cells since there are no vaccines produced to cure the infection [7]. Two types of vaccines were produced by scientists to protect from the infection of HPV for women which are Gardasil and Cervarix [8,9].
Gardasil works effectively in protecting women from HPV strain 18 by blocking the movement of the virus to mucous membrane and eradicate the DNA of the HPV [10]. Cervarix also preventing the infection of this strain but still in approval process by Food and Drug Administration (FDA). Even though both of these vaccines can prevent the infection of HPV strain 18, woman still needs to take screening test regularly. Not only HPV strain 18 can cause cervical cancer but other type of strains such as HPV strain 31, strain 33, strain 45, strain 52 and strain 58 can also cause cervical cancer even though they are not the main contributor to cervical cancer [11]. For a person who infected by the non-cancerous low risk HPV which causing warts on the skin, treatment such as electro cautery and surgical removal can be used to remove the warts but the person still carrying the strain inside their body. The strain usually will perish after a year’s time depending on the person’s immune system.

Conventional method such as Pap test was the most popular screening test in detecting HPV [12]. Samples from cervical cell were collected and observed under the microscope by the doctor or science officer to detect any abnormal cells which referring to cancer cell caused by HPV. Even though this method is popular to detect cervical cancer, this method is only can detect the cell which already infected by HPV and does lack in sensitivity which can cause false positive results. The less sensitive and time-consuming of conventional method lead to the invention of biosensors [4-6].

The application of detecting biological gene using electrical methodologies are the future steps of small, portable and high sensitivity biomarker detection. There are three basic components of the biosensor which is bio receptor, transducer, and detector [13-17]. Voltammetric biosensors measure current as the potential and ramped at a given rate. The current response usually showed a peak that was proportional to the concentration of target analyte [6,18].

This study focused on analysis of electrical DNA biosensor for Human Papillomavirus (HPV) Strain 18 detection in synthetic and saliva samples. The detection of HPV 18 has become more accurate since the biosensor has been introduced as the biosensor researchers utilize the HPV strain to study the bio recognition element and the transducer.

2. Experimental

2.1 Material and samples collection
3-Aminopropyltriethoxysilane (APTES) solution was purchased from Sigma Aldrich (USA) and QIAamp DNA mini kit was purchased from Qiagen Company. 15 samples of saliva were obtained from third year students of Biosystems Engineering in UniMAP and the synthetic DNA with modified COOH group obtained from Institute of Nanotechnology UniMAP. Gold IDE were purchased from Dropsen.

2.2 Device Inspection
The first stage was observing the morphology of the gold IDE before the process of surface modification. SEM was used in the inspection. This process was done slowly and steady to identify any defects between the gaps of the gold electrodes because this process was determine the functional group of the DNA biosensor. Devices with defects such as scratches were not being used in this research.

2.3 DNA Extraction and Measurement of Absorbance, Concentration and Purity
The saliva samples were undergone DNA extraction in order to obtain the pure DNA for the I-V characterization. The kit used in this process was QIAamp DNA Mini Kit [19] with 10 materials included in this kit as shown in Table 1. There were 5 steps in the DNA extraction which are lysate preparation, binding column, column wash, DNA elution and DNA storage.

2.4 I-V Characterization for Synthetic DNA and DNA from Saliva Samples
The characterization by using current-voltage (I-V) was conducted by using synthetic DNA and DNA from saliva samples.
2.5 Surface Modification
After the inspection of the device was done, the next step was the surface modification of the device. 1μl of 24% APTES concentration was dropped on the active area of the device and dried in the dry cabinet for 10 minutes. This process formed an amine functional group on the surface of glass substrate. Then, I-V electrical characterize was measured with the APTES solution on the surface of the device. The curve of I-V electrical characterize before surface modification with APTES also measured. The curves from both I-V electrical characterize graphs were recorded as constant variables.

2.6 Surface Functionalization
The process of immobilization was done in this stage. The 1.0μl of the 10.0μM probe was dropped on the surface of the dried APTES solution. The device was dried again in the dry cabinet for 10 minutes. The curve of I-V electrical characterization was measured after the device is fully dried.

2.7 Characterization for Different Concentration of Synthetic DNA
The whole process for I-V characterization was repeated and using different type of concentration of synthetic DNA which is nano molar, micro molar, pico molar and femto molar.

2.8 Hybridization Using Single Stranded DNA from Saliva Samples
After the surface functionalization, the device proceeded to the hybridization process. 1.0 μl of the 1.0μM of DNA samples from the DNA extraction method was dropped on the IDE that contain immobilize DNA probe and incubated for 10 minutes. The I-V electrical characterization was measured after the device is fully dried. The curve of the I-V graph was observed and compared with the graph from surface functionalization to determine whether there is hybridization occurred between DNA probes with the single stranded DNA sample.

3 Results and Discussions
3.1 Device Characterization
The gold interdigitated electrode (IDE) was inspected under a Scanning Electron Microscope (SEM) and showed the alignment between the two comb-shaped gold electrode as shown in figure 1. From the inspection, the comb-shaped gold electrode was in good condition and showed no defects. Thus, the device was used for the detection of Human Papillomavirus (HPV) strain 18. Generally, a perfect alignment of the comb-shaped electrodes giving an accurate reading of ampere since there is no contamination or defect between the electrodes [20].

![Figure 1. Gold electrode under (a) x500 magnification (b) under x200 magnification](image)

3.2 Measurement of Concentration, Absorbance and Purity of DNA Samples
The fifteen samples of saliva from students of Biosystems Engineering undergo DNA extraction to obtain pure DNA samples using the QIAamp DNA Mini Kit. Then, the samples undergo measurement to obtain the value of concentration, absorbance, and purity by using DS-11 Spectrophotometer. The result was shown in table 1. From table 1, there were only 6 samples of single-stranded DNA with the...
purity between ranges of 1.8 to 2.0 and the rest were above 2.0 and below 1.8. This could be caused by the contamination of the samples during the process of DNA extraction.

Table 1. Measurement of absorbance, concentration and purity for double stranded (ds) DNA samples

| Samples DNA | Concentration of dsDNA (ng/μl) | $A_{260}$ | $A_{260/230}$ | $A_{260/280}$ |
|-------------|---------------------------------|----------|---------------|---------------|
| 1           | 3.940                           | 0.0788   | 0.03          | 2.94          |
| 2           | 9.689                           | 0.1938   | 0.04          | 2.05          |
| 3           | 11.335                          | 0.2267   | 0.06          | 4.73          |
| 4           | 3.889                           | 0.0780   | 0.11          | 2.40          |
| 5           | 5.863                           | 0.1173   | 0.16          | 2.01          |
| 6           | 5.290                           | 0.1058   | 0.17          | 2.21          |
| 7           | 5.872                           | 0.1174   | 0.16          | 2.43          |
| 8           | 6.027                           | 0.1205   | 0.11          | 2.28          |
| 9           | 3.326                           | 0.0665   | 0.07          | 2.25          |
| 10          | 6.237                           | 0.1247   | 0.02          | 1.99          |
| 11          | 11.381                          | 0.2276   | 0.02          | 2.07          |
| 12          | 5.51                            | 0.1030   | 0.17          | 1.88          |
| 13          | 5.436                           | 0.1086   | 0.17          | 2.38          |
| 14          | 5.171                           | 0.1036   | 0.04          | 2.11          |
| 15          | 9.591                           | 0.1918   | 0.01          | 3.15          |

DNA sample with a purity between ranges of 1.8 to 2.0 has the highest purity of DNA with a low amount of impurities which could lead to high output signal during the I-V characterization. Therefore, the process of DNA extraction should be done more carefully and avoid any contamination of the samples. The tips of the micropipette should be clean and avoid touching the tip while dropping the samples into the tubes. Impurities such as excess carbohydrate from the original sample could alter the reading of a sensor which resulting inaccurate result [21]. Table 2 showed the measurement of absorbance, concentration and purity for single stranded (ss) DNA samples after denaturation method by heating at 94°C for 1 minute to denature the hydrogen bonding. The concentration of ssDNA became half of the concentration of dsDNA due to the heating process.
Table 2. Measurement of absorbance, concentration and purity for single stranded (ss) DNA samples

| Samples DNA | Concentration of ssDNA (ng/μl) | $A_{260}$ | $A_{260}/230$ | $A_{260}/280$ |
|-------------|--------------------------------|----------|--------------|--------------|
| 1.          | 2.111                          | 0.0640   | 0.020        | 1.904        |
| 2.          | 6.721                          | 0.2037   | 0.038        | 1.952        |
| 3.          | 7.915                          | 0.2398   | 0.059        | 4.840        |
| 4.          | 3.036                          | 0.092    | 0.128        | 2.213        |
| 5.          | 4.114                          | 0.1247   | 0.163        | 2.476        |
| 6.          | 3.168                          | 0.0960   | 0.143        | 1.935        |
| 7.          | 3.390                          | 0.1021   | 0.136        | 1.895        |
| 8.          | 3.323                          | 0.1007   | 0.090        | 2.311        |
| 9.          | 1.643                          | 0.0498   | 0.053        | 4.889        |
| 10.         | 3.852                          | 0.1107   | 0.017        | 1.891        |
| 11.         | 5.180                          | 0.1570   | 0.014        | 2.504        |
| 12.         | 2.888                          | 0.0875   | 0.150        | 1.421        |
| 13.         | 2.975                          | 0.0901   | 0.139        | 1.842        |
| 14.         | 3.295                          | 0.0999   | 0.038        | 2.279        |
| 15.         | 5.365                          | 0.1626   | 0.006        | 3.515        |

3.3 I-V Characterization of Synthetic DNA

The process of I-V characterization for surface modification, device functionalization and device hybridization were conducted by using synthetic target which is positive of HPV strain 18. The purpose of this process is to proof the functional of the gold IDE as a biosensor that can detect the presence of HPV strain 18 in the synthetic DNA and to differentiate the result with the real samples of saliva. As the result, HPV strain 18 was detected in the synthetic DNA due to the hybridization of the DNA probe and synthetic DNA. Generally, reading for hybridization higher than the reading before the hybridization since there is a chemical process occurred between two complementary DNA or RNA which lowering down the reading of the sensor [22]. The results were shown in the Figure 2. Each test was repeated three times to obtain accuracy of the sensor in detecting the presence of HPV strain 18. The three readings from each test were close to each other indicating that the sensor is high in precision in detecting the samples.

From figure 2, the bare IDE with no attachment gave the current reading to be $2.1245 \times 10^{-7}$ at 0.6V and peak to $3.1719 \times 10^{-7}$ at 0.7V followed with attachment of APTES which gave the reading to be $2.2825 \times 10^{-7}$ at 0.6V and peak to $3.3299 \times 10^{-7}$ at 0.7V. After the process of device functionalization using probe, the current reading increased from $2.4348 \times 10^{-7}$ at 0.6V to $3.4907 \times 10^{-7}$ at 0.7V which was the highest reading. After the process of device hybridization using synthetic DNA, the reading was lower than device functionalization which is $2.3199 \times 10^{-7}$ at 0.6V to $3.3734 \times 10^{-7}$ at 0.7V due to the net changes between the complementary nucleotides proving that the hybridization of DNA occurred between DNA probes with synthetic DNA.
Characterization for DNA from Saliva Sample

The six samples of single-stranded DNA from saliva undergone I-V characterization to detect the output signal for surface modification, device functionalization and device hybridization using Keithley 6487. The purpose of using the sample of saliva of the students is to make as a negative result of HPV strain 18. An additional purpose of using saliva sample is to investigate whether the students were infected by the HPV strain 18 or not. As the result, 6 of them were free from HPV strain 18 infection since the I-V characterization shows no hybridization between DNA probe and their DNA samples. No hybridization occurred between non-complementary DNA and RNA. Each test was repeated three times to obtain accuracy of the sensor in detecting the presence of HPV strain 18. The three readings from each test were close to each other indicating that the sensor is high in precision in detecting the samples.

From figure 3, the bare IDE with no attachment gave the current reading to be $5.5264 \times 10^{-6}$ at 0.6V and peak to $6.7106 \times 10^{-6}$ at 0.7V followed with attachment of APTES which gave the reading to be $5.7576 \times 10^{-6}$ at 0.6V and peak to $6.8408 \times 10^{-6}$ at 0.7V. After the process of device functionalization using probe, the current reading increased from $6.0019 \times 10^{-6}$ at 0.6V to $7.0318 \times 10^{-6}$ at 0.7V. After the process of device hybridization using DNA1, the reading is $6.1522 \times 10^{-6}$ at 0.6V to $7.1508 \times 10^{-6}$ at 0.7V which is the highest current reading due to no hybridization occurred between probe DNA and DNA1.

Conclusions

The analysis for the operational of the DNA biosensor was successfully conducted in this project. Saliva samples show no presence of HPV strain 18 while synthetic DNA shows the presence of HPV strain 18 through hybridization process between DNA probe and DNA target. Based on the I-V
characterization graphs, we can conclude that all of the saliva samples produce the same pattern of the graph which the reading for the bare IDE was the lowest while the DNA reading is the highest. The readings for DNA samples were higher than the probe indicated that there was no hybridization of DNA sample and DNA probe. As a conclusion, this electrical DNA biosensor was capable to be used for the detection of HPV strain 18.

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