Gold Nanoparticles Enhanced Electrochemical Impedance Sensor (EIS) for Human Papillomavirus (HPV) 16 Detection E6 region

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Abstract. The persistent infection by high risk HPV is a necessary but not sufficient cause of this cancer which develops over a long period through precursor lesions which can be detected by electrochemical impedance sensor. The HPV driven molecular mechanisms underlying the development of cervical lesions have provided a number of potential biomarkers for both diagnostic and prognostic use in the clinical management of women with HPV related cervical disease and these biomarkers can also be used to increase the positive predictive value of current methods. The most influential methods for the detection and identification of HPV using gold nanoparticle (GNP) included electrochemical impedance sensor will visit their sensitivity, selectivity and characteristic detection on synthetic target which are complement of the DNA, non-complement of the DNA and mismatch of the DNA. In difference concentration of synthetic target, which stage can get the exactly value to determine the HPV in strain 16 was evaluated in this research studies.

1. Introduction
The HPV infection is the most common risk factor and causative agent of cervical cancer [1]. It is widely used in HPV DNA testing at cervical cancer screening programs to improve the detection of high-grade lesions by using electrochemical impedance sensor for recognition of the HPV DNA hybridization. Single-stranded 24-mer DNA was immobilized at its 5'end onto gold electrode surfaces and specific detection or synthetic target which are mismatch, complement, non-complement in DNA using a small molecule-modified gold electrode [2-6].

Human Papillomavirus (HPV) is the most common sexually transmitted virus. HPV is a type of virus which can produce abnormal growth of the tissue and other cell changes. Certain types of HPV infections can cause cervical cancer for a long time. HPV may also be important to certain other cancers such as anal, vaginal, vulvar, penile and oropharyngeal [7].
The direct sexual contact from the skin and mucous membranes of infected persons to the skin and mucous membranes of their partners can also easily spread more than 40 types HPV. Vaginal, anal and oral sex can be transmitted. For non-genital warts, other types of HPV are responsible. Wart is an increasing growth in the skin surface or in another organ that is not transmitted sexually.

The electrochemical impedance spectroscopy (EIS) determines the dielectric properties of the DNA probe. This is measured cervical cancer interaction with the dipole moment of a particular sample usually stated by selective and sensitivity of DNA probe. This is regarded as an experimental technique that describes electrochemical systems. This method measured system impedance over a series of frequencies. Most of the time, the data analysis gathered through electrochemical impedance spectroscopy is graphically conveyed. Electrochemical impedance spectroscopy is also known as dielectric spectroscopy.

Electrochemical DNA can be used by specific devices that are electrochemical impedance sensors (EIS) for label-free detection and labelling. The current between a metal electrode and a sample of the analyte can be recognized and measured (target) can be identified (Azizah et al, 2016). Due to the flexibility, simplicity, high affectability, availability with mass assembly, the possibility of innovative and versatile micro-manufactured products, Electrochemical bio-sensors have gained impressive consideration in recognition for DNA hybridism, making the great opportunity for care diagnosis DNA [8].

For optical biosensing utilizing GNP, the optical properties provide a wide range of opportunities, all of which ultimately arise from the collective oscillations of conduction band electrons. The sensing capacity of this new chip has been evaluated by impedance measurements with gold nanoparticles (GNP). GNP coated with mercaptoundecanoic acid was also used to greatly improve chemical affinity for the silicone substrate as a biomolecular model. Gold was not used for sensing these kinds of molecules with most biological molecules. The low affinity of gold adherence to silicone dioxide is, however, much more difficult and expensive to manufacture. Gold electrodes are far more corrosion resistant and more biocompatible.

This study focused on analysis of gold nanoparticles enhanced electrochemical impedance sensor (EIS) for Human Papillomavirus (HPV) 16 Detection E6 region. The detection of HPV 16 E6 region 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. DNA Extraction for Saliva Samples

The saliva was collected in 60 microcentrifuge tubes called with difference type of person and store. The Proteinase K and Lysis Buffer L were added into the Eppendorf tube contains saliva and the vortex were used. Function added Proteinase K and Lysis Buffer L are to degraded of DNA strand. Incubated the Eppendorf tube then, mixed thoroughly the Binding Buffer was added and store the Eppendorf tube on ice. Binding Buffer mainly like glue that help the pellet attach into the wall of Eppendorf. Next, the Eppendorf tube was spin for 3 minutes at 1350 rpm to make sure supernatant and pellet was separated. The clear supernatant was transferred into new tube. The lysate was applied up onto spin column and centrifuge. Sometime, the lysate volume is not passing through, spin again with higher speed up. Buffer SK was applied to column and centrifuge. The balance for solution was throwing out and continues with the Wash Solution A to column and centrifuge again. Residue of liquid in column with throw out that has only for DNA-stranded. The column was place into a fresh 1.7 mL Elution tube provided with the kit. Then, the Elution Buffer B solution was added and incubated at room temperature. Then, it was centrifuged for maximum spin. The purified genomic double-stranded of DNA has been stored at -20 °C in freezer for longer term storage.
2.2. Denaturation of genomic double stranded DNA
The lysate of DNA has been released in the freezer from several days. Then, the lysate in the Eppendorf tube has been dislodged with thaw method. Then, the Double-stranded of DNA was heated up at 94 °C on thermo-shaker size (20 × 0.5 + 12 × 1.5) mL TS-100 Biosan Model within 5 minutes to denatured and got single-stranded of DNA also it was transfer to the fresh Eppendorf tube. After that it was placed on ice bag within 10 minute to stop reaction from denatured in DNA stranded.

2.3. DNA Characterization
The dsDNA and ssDNA was measured by used nano-drop established by DeNovix as in figure 3.5. Nano-drop was a simple and easy to measure characterize of DNA stranded.

2.4. The Electrical Characterization
The devices with completed IDE were also inspected using the dielectric analyzer for electrical characterization. The software package WinDeta was associated Dielectric analyzer (DA), Alpha-A High-Performance Frequency Analyzer, Novocontrol Technologies, Handsagen, Germany were used in this characterization. The electrical measurement set-up is depicted, where DA was connected to the probe station, vacuum stage and source measurement unit of probes. During the measurement set-up, fabricated nanogap device was placed in the middle of vacuum stage that is connected to the probe station and the two source measurement units’ probes individually aligned to IDE.

2.5. Surface Modification of IDE
The IDE was prepared with difference gap which are 130 μm gap and 5 μm gap. The IDE was measured on device started from bare of IDE. Firstly, the IDE was cleaned by flew the air from the blower. Then, the IDE was measured within 30 minutes used EIS and continue by wash on IDE. After that, GNP was dropped on IDE and incubated in room temperature then started measurement. Next, the step followed up repeated with MUA and APTES by layers after dry for each.

2.6. Synthetic Functionalization of IDE
This procedure must continue after have layered APTES bound on the sensing area of IDE. The DNA was used for the detection using nanogap device working as a biosensor. The detection procedure starts with the immobilization of single-stranded of DNA probe-16, the 24-mer single-stranded DNA (ssDNA) probe-16 was premodified by amine linker.

2.7. Hybridization with Synthetic Target
The DNA target was applied on the probe bound sensing area surface between nanogap electrodes and incubated at room temperature. These treatments allowed the target sequence of complement, non-complement and mismatch to hybridize with ssDNA probe one by one to form a double-stranded DNA (dsDNA) on the surface between the nanogap electrodes. The electrical characterization was carried out to measure the dsDNA duplex formation using EIS device.

2.8. Characterization with Selectivity and Sensitivity Test
A novelty of a biosensor is the capability and feasibility of the device to specifically select the complementary target DNA and detect at the lowest limit. The selectivity test was done by observing the electrical characterization of the hybridized complementary, non-complementary and mismatch target of DNA with probe DNA on the sensing area surface of IDE between nanogap electrodes. Meanwhile, the detection limit of the nanogap device was done by measuring different concentrations, from 10 nM to 1 fM of complementary saliva target of DNA.
3. Results and Discussions

3.1. DNA Extraction of Saliva Samples
The process of DNA extraction, divided into three main steps, breaks open the cell wall has several are lysis, destroys membranes and precipitates DNA within cells from the supernatant-pellet solution. In this experiment, the saliva sample was mashed manually which was used Proteinase K solution and Lysis Buffer L solution. At this point, DNA was ready for molecular biological procedures when combining Proteinase K with Lysis Buffer. This process was successfully disrupted where the DNA, along with protein molecules and other cellular miscellanea, was released from the cells into the solution.

The stranded DNA molecules were made of a double-helical polymer composed of a sugar-phosphate backbone with a perpendicular nitrogenic base. The cell contents mixed into a solution and the DNA was separated from the other molecules called as precipitation process. A transparent white solution began that was form at the top called supernatant that contained of the DNA and the thick substance as pellet that attaches to the wall of Eppendorf tube.

3.2. DNA Characterization
The DNA concentration was assessed with absorbance by optical density with 60 specimen of saliva tabulated as shown on table 1 with different categories which are double-stranded and single-stranded of DNA. The table 1 shown that only get 12 specimens for the purity of DNA which are specimens number 1, 2, 14, 20, 23, 36, 40, 42, 43, 45, 46, 56 and 60. The nucleic acid absorb ultraviolet (UV) light due to the heterocyclic rings of nucleotides are the sugar-phosphate backbone does not contribute to absorption. The wavelength of maximum absorption for both DNA is 260 nm with a characteristic value for each base. The 260 nm absorbance was used to calculate nucleic acid concentration. A\textsubscript{260} = 1 has dsDNA at length of 1 cm and a concentration of 50 μg/ml. The absorbance value is also dependent on the amount of secondary structure in the stranded DNA due to hypochromicity. For reliable spectrophotometric DNA quantification A\textsubscript{260} readings must lie between 0.1 and 1.0.

A\textsubscript{260}/A\textsubscript{280} ratio was calculated to determine the purity of DNA was measured protein contamination. The pure DNA preparations have an A\textsubscript{260}/A\textsubscript{280} ratio of greater than or equal to 1.8. The A\textsubscript{260}/A\textsubscript{280} values are often lower than the corresponding A\textsubscript{260}/A\textsubscript{230} values for the pure saliva sample. Around 230 nm of organic compounds or chaotropic salts are strongly absorbed which can be seen in the purified DNA. A ratio of 230 nm to 260 nm was helped evaluated the level of salt carry over in the purified DNA. The lower the ratio was present the greater the amount of salt. The A\textsubscript{260}/A\textsubscript{230} ratio was greater than 1.5 that ideally closer to 1.8.

3.3. Electrical Measurement Test On the Interdigitated Electrodes
The Interdigitated electrodes (IDE) played important roles as a transducer or sensor and consist of twin electrodes which was arranged in a comb-like structure with the band gaps between two electrodes that responsible to any changes [9-18]. The IDE based electrical DNA biosensor was used to detect a synthetic 24-mer oligonucleotide with the sequence corresponding to HPV type 16 DNA by measured electrical signal response from electrochemical impedance sensor.

The results obtained from this characterization shows that nano-gap device with 5 nm was more stable compare to the bigger size gap 130 nm (figure 1). These can be concluded that 5nm nano-gap device is significant for analysis especially for biomolecules detection.
3.4. Electrical Measurement Test on the Synthetic Target

The electrical characterization of the device was made to study the method for spatially arranged DNA immobilization on 10 nm gold nanoparticle (GNP) was deposited on Interdigitated Electron (IDE). The key advantages of nanogap biosensor using both characterizations are a homogeneous interrogation electric field and the opportunity for surface-bound molecules to occupy a significant portion of the detection volume, thereby increasing sensitivity. The gold nanoparticle (GNP) are covalent bound to the surface via silane chemistry which is practical interest as precursor to elemental of silicon was monitored by scanning transmission electron microscope (SEM).

The measurement for ssDNA detection was taken 30 min after the hybridization process. The relative capacitance changed at frequency 25 KHz shows a different reading between complementary and non-complement ssDNA. The relative capacitance of complementary ssDNA increase with the concentration increment, meanwhile the non-complementary results show little to no change when concentration increase.

The IDE 1 shown as IDE with 130nm gap while IDE 2 have 5nm of electron gap. The graph in figure 2 showed that the measurement for the complement of ssDNA was observed got a lower value on the IDE 2 than IDE 1. It is because IDE 2 got a small gap which is 5nm and more sensitivity because the range of frequency on IDE 1 is between 0 Hz - 2000 Hz while IDE 2 between 100 Hz - 1200 Hz. The pattern of the IDE-1 graph was almost same to the IDE-2 graph but have differentiation on the complement target. The complement target was matched on the probe 16. IDE 1 got the narrow than IDE 2 because at that phase DNA was combining together and produced double-stranded DNA.

Based on figure 3, IDE 2 is closely measurement than IDE 1 but at IDE 1 got half curve from the IDE 2. It is because resistor was not parallel to capacitance and below 1.0V also cannot obtain the shape of graph. Besides, focus on the sample line positioning type which are mismatch target and probe-16 is different with complement target. The position of probe-16 is higher than mismatch target. The arrangement of ssDNA does not match with probe-16.

Based on figure 4, the two different graphs for non-complement showed that IDE 2 smoothly was arranged than IDE 1, this has caused certain thing that was disturbed with lighting and cell phone. The light and cell phone was disturbed the electron during the experiment.
Figure 2. Graph of the comparison between IDE 1 and IDE 2 for complementary target hybridization.

Figure 3. Graph of the comparison synthetic target between IDE 1 and IDE 2 for mismatch of the DNA target.

Figure 4. Graph of the comparison of synthetic target between IDE 1 and IDE 2 for non-complement of DNA target.
3.5. The Sensitivity and Selectivity Test for IDE biosensor

The detection of a specific DNA was authenticated that capability by the sensor, the DNA-biosensor must be able to distinguish among the target of single-stranded DNA complementary with different concentrations. A serial dilution of DI water has been used to dilute the different concentrations of HPV DNA in 1 μM, 1 nM, 1 pM, 1 fM. The 1.0 μL specimens from each concentration have been collected for testing in figure 5. Figure 5 showed a frequency with different HPV analyte DNA concentrations measured with the aid of the EIS device for GNP 10 nm deposited biosensor.

In this research study, a sensitive and fast DNA detection technique based on the single-stranded oligonucleotide test was demonstrated on interdigitated electron (IDE). The development of an electrical DNA biosensor for the detection of the most representative high-risk HPV strain which is HPV 16. The current method, the recognition of the presence of HPV was performed with good sensitivity and specificity within the turnaround time of 30 minutes by used IDE biosensor.

![Graph of the capacitance over frequency detection with the complementary DNA target at different concentrations in serial dilution.](image)

4. Conclusions

The capacitance and impedance of nano-gap with electrodes gap dimensions; 130 nm and 5 nm were measured using the dielectric analyzer at a frequency range from 0.1 Hz to 1.0 MHz with bare (no samples) and DI-water. Higher capacitance and impedance were achieved from 5 nm nano-gap device compared to 130 nm sized gaps. The capacitance result shows that the value was increased with the dimension of gap decreased. The nano-gap device performance was first optimized by conducted a sensitivity test using a same electrical system. The IDE sensor was successfully applied as a label-free biosensor for HPV detection.

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