GOLD NANOPARTICLES ENHANCED DNA BIOSENSOR BASED ON SILICA INTERDIGITATED ELECTRODES FOR DETECTION OF HUMAN PAPILLOMAVIRUS

by

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LIST OF ABBREVIATIONS

AC	Alternating current
AFM	Atomic force microscope
APTES	3-Aminopropyl triethoxysilane
GNPs	Gold nanoparticles
BLAST	Basic Local Alignment Search Tool
CD	Critical dimension
ddH_2O	Deionized distilled water
DIW	Distilled water
DC	Direct current
DNA	Deoxyribonucleic acid
dsDNA	Double stranded DNA
ES	Electrochemical sensor
EDX	Energy dispersive X-ray
ELISA	Enzyme Linked Immunosorbent Assay
HPM	High power microscope
I-V	Current-voltage
LPM	Low power microscope
LOD	Limit of Detection
MW	Molecular weight
PBS	Phosphate buffer saline
PR	Photoresist
PCR	Polymerase Chain Reaction
CMOS	Complementary metal-oxide semiconductor
LOC	Lab-on-chip
EBL	Electron beam lithography
ICP-RIE	Inductively coupled plasma-reactive ion etching
LOD	Limit of detection
pH	Power of hydrogen
POC	Point-of-care
CD	Critical dimension
MOH	Malaysia Ministry of Health

NH2	Amine
SEM	Scanning electron microscope
Si	Silicon
SiH_4	Silane
S	Source
SAM	Self-assemble monolayer
SiOH	Silanol
SMU	Source measurement unit
SOI	Silicon-on-insulator
SiO_2	Silicon dioxide
ssDNA	Single stranded DNA
T	Temperature
T	time
Tm	Melting temperature
UV	Ultraviolet

LIST OF SYMBOLS

%	Percentages
nm	Nanometer
µL	Microliter
mL	Milliliter
L	Liter
g	Gram
mg	Milligram
s	Second
min	Minutes
h	Hours
M	Molar
mM	Milli Molar
Mol	Molar mass
I	Current
V	Volt
rpm	Revolutions per minute
G	Gravitational force
°C	Degree Celsius
t	Thickness

Nanopartikel Emas untuk Peningkatan Biosensor DNA berasaskan Silika Elektrod Interdigitasi untuk Pengesanan Virus Papilloma

ABSTRAK

Kes peningkatan kanser pangkal rahim yang disebabkan oleh Virus Papilloma (HPV) adalah penyebab masalah utama bagi kesihatan wanita di seluruh dunia. Di Malaysia, lebih daripada 5,000 penghidap kanser pangkal rahim atau dikenali sebagai barah pangkal rahim meninggal dunia berpunca daripada kelewatan mengesan sel kanser yang merebak ke peringkat akhir pada tahun 2015. Persatuan Kebangsaan Kanser Malaysia (NCSM) melaporkan lebih daripada 11,000 wanita telah disahkan menghidap kanser pangkal rahim setiap tahun terutamanya wanita muda berusia lewat 30-an. Kaedah pengesanan pantas dan pencegahan diperlukan untuk menyelesaikan masalah kesihatan dan yang berkaitan dengan virus patogenik ini. Kaedah pengesanan sedia ada memerlukan penyediaan sampel spesimen yang banyak dan prosedur ujian yang mengambil masa yang sangat lama. Oleh itu, kajian penyelidikan ini memberi tumpuan kepada pembangunan kaedah pengesanan pantas yang mampu mengesan virus ini dengan kepekaan tinggi. HPV 16 digunakan sebagai rujukan DNA untuk pembangunan kaedah pantas ini. IDE telah dibangunkan untuk mengenal pasti saiz sensor mini tetapi mempunyai kepekaan yang tinggi bersifat biosensor untuk kegunaan kejuruteraan bioperubatan bagi mengesan asid deoksiribonukleik (DNA) HPV penyebab kanser pangkal rahim dengan litografi konvensional (CL) untuk fabrikasi peranti. Biosensor elektrik berdasarkan partikel nano emas (GNP) telah dibina sebelum penambahan 3-aminopropiltriethoxysilana (APTES). Pengoptimuman IDE untuk mengesan DNA HPV telah diubahsuai dengan menggunakan APTES melibatkan pengubahsuaian penderia DNA HPV dengan kumpulan karboksil (-COOH) pada penghujung 5’ pemegunan oleh ikatan kovalen melalui amina (-NH2) pada APTES di atas permukaan IDE berasaskan GNP. Analisis struktur permukaan dengan mikroskopi elektron pengimbasan (SEM) digunakan untuk mencirikan perubahan pada permukaan IDE. Analisa spektroskopi inframerah transformasian Fourier (FTIR) digunakan untuk menilai prosedur ikatan kimia. Prinsip pengesanan berfungsi dengan mengesan perubahan dalam arus elektrik IDE yang untuk pemegunan penderia DNA dan penghibridan dengan DNA sasaran. Didapati bahawa sensor menunjukkan selektiviti HPV target DNA dalam fasa linear dengan kepekatan dari 1 pM hingga 1 μM. Dengan analisis ini, kita boleh mencapai had pengesanan (LOD) dianggarkan 1 pM dan setanding dengan sensor sedia ada. Di samping itu, peranti biosensor yang terkini dapat membezakan antara sasaran daripada sumber sintetik, ketidakpadanan pada 1 mer dan urutan DNA yang tidak melengkapi. Kaedah komersil HCII Hybrid yang berasaskan ELISA untuk mengesan 13 jenis HPV yang berisiko tinggi termasuk HPV 16 dan 18 telah digunakan sebagai satu teknik pengesahan untuk IDE biosensor berasaskan GNP dalam sampel sebenar. Kelebihan dalam memendekkan masa pengesanan tanpa aplikasi penglabelan amat berguna dalam mengenal pasti. DNA HPV yang menyebabkan kanser pangkal rahim. Sistem biosensor elektrik ini akan dapat digunakan dalam pembangunan peranti untuk analisis di lokasi pada masa akan datang.

Gold Nanoparticles Enhanced DNA Biosensor based on Silica Interdigitated Electrodes for Detection of Human Papillomavirus

ABSTRACT

The increment in cervical cancer cases caused by the genital Human *Papillomavirus* (HPV) is a major worldwide problem for the women healthcare. In Malaysia, more than 5,000 cervical cancer patients, die from the delay in detecting cancer cells that are spreading to the final stage in 2015. The National Cancer Society of Malaysia (NCSM) reports that more than 11,000 women have been diagnosed with cervical cancer every year, especially young women in the late 30s. Rapid detection methods for the prevention and identification are required to solve the health and safety problems related to this pathogenic virus. Current detection methods require extensive specimen sample preparation and prolonged assay procedures. Thus, this research has focused on developing rapid detection methods, which are capable of sensing these viruses at a higher sensitivity. HPV 16 was used as the standard reference strain for the development of rapid methods. Nanoscaled interdigitated electrodes (IDEs) has been developed for the identification and miniaturizing the size of sensor but have higher performance for the biomedical engineering usage by detecting deoxyribonucleic acid (DNA) of HPV caused cervical cancer. With the conventional lithography (CL) for device fabrication, an electrical biosensor based on gold nanoparticle (GNP) IDE was constructed before the addition of 3-aminopropyltriethoxysilane (APTES). The optimized IDE was then employed for the detection of HPV DNA by the introduced two-steps mechanism after the surface modification by APTES. APTES is linking the modified HPV DNA probe with carboxyl group (-COOH) immobilization by covalent binding via amine (-NH2) coupling APTES on the sensing surface based IDE, and DNA hybridization. Surface structure analysis with scanning electron microscopy (SEM) was used to characterize the changes in the surface appearance. Fourier transform infrared (FTIR) spectroscopy analysis was used to assess the attachment procedures. The detection principle works by detecting the changes in the electrical current of IDE, which bridges the source and drain terminal to sense the immobilization of HPV DNA probe and hybridization with target DNA. It was found that the sensor showed the selectivity for HPV DNA target in a linear range with the concentrations ranges from 1 pM to 1 µM. With this analysis, the sensitivity limit of detection (LOD) was approximately 1 pM and it is comparable with the currently available sensors. In addition, the developed biosensor device was able to discriminate among complementary synthetic target, single mismatch, and non-complementary DNA sequences. A commercial, HCII Hybrid capture based Enzyme-Linked Immunosorbent Assay (ELISA) method for 13 types of high-risk HPV including HPV 16 and 18 was used as a validation technique for confirming the effectiveness of GNP based IDE electrical biosensor in real samples. The advantage of this sensor is fast detection without labeling application and is useful in identifying the strength of HPV DNA probe binding to HPV target. This electrical biosensor system will be useful for the development of devices with on-site analysis.

CHAPTER 1 : INTRODUCTION

1.1 Background

Genital Human *Papillomavirus* (HPV) strains are among the most common virus responsible for cervical cancer (Crosbie, Einstein, Franceschi, & Kitchener, 2013) and can be categorized as a potential microbe in sexually transmitted infection (Cosette Marie Wheeler, 2013). Based on the World Health Organization (WHO), over 290 million women had been infected by cervical cancer by the end of 2013 (World Health Organisation, 2013; World Health Organization, 2016). HPV was first discovered in 1984 by Harald zur Hausen (Lehtinen, 2013; Mammas, Sourvinos, & Spandidos, 2014). He found and ascribed the cervical disease to HPV 16 and 18 (Długońska, 2009). HPV gets its name due to the strain that causes warts, known as *Papillomas* (Dürst, Gissmann, Ikenberg, & zur Hausen, 1983). Hundred and fifty types of HPV strains were identified and classified as high and low risks (Elfström et al., 2015). From 150 strains, fifteen were recognized as a high risk for cervical malignancy (Ault, 2007; Cronin et al., 2016; Seiki et al., 2015). Depend on the problem of cervical cancer malignant, disadvantage due to the survival rate of patient and deficiency of early detection rapidness commonly have a meaningful medical effect in the developing country, such as Malaysia, Vietnam, Singapore, and the Philippines (Akinyemiju, 2012; Rao, Escobar-Hoyos, & Shroyer, 2016). Cervical cancer death rate in Chile, Santiago is four times bigger than the developed countries (Ferreccio et al., 2013).

At present, most of the current conventional methods of detection for HPV that caused cervical cancer is time-consuming and labor-intensive. Clinical techniques for the recognition of cervical cancer include the following fundamental methods; pap smear (Hyacinth, Adekeye, Ibeh, & Osoba, 2012), HPV genotyping (Grozdanov et al., 2014; Meijer, Snijders, & Castle, 2006), cytology screening (Dijkstra et al., 2014) and serological confirmation (Van Doorslaer, Reimers, Studentsov, Einstein, & Burk, 2010). All HPV screening tests right now are being used depending on the identification of viral nucleic acids based on the fact that HPV can't be cultured (Brink, Snijders, & Meijer, 2007; Yuce et al., 2012).

Currently, the growth of a biosensor for HPV virus diagnosis make a promise a fast, sensitive and simple to perform (Wang et al., 2011; Zari et al., 2009). Biosensor comes with the incomparable competency for actual-time and on-spot analysis. On the spot detection of HPV virus is significant since it helps medical doctor especially Obstetrics and Gynecologist (O&G) to diagnose cervical cancer in faster ways. Cervical cancer can be treated if the associated HPV infection detected at an earlier stage before developing a precancerous or cancerous change in the cervix (Leinonen et al., 2012).

Biosensors show a modern and interesting technology with abilities for convenient and fast detection. Biosensors could be defined as an analytic devices, capable to capture the biomolecules as a responsive surface in tight contact with a transducer and turn the binding of the analyte to the capturing molecule into a measurable signal (Hilmiye, 2013; Perumal & Hashim, 2014; Piorek, Andreou, Moskovits, & Meinhart, 2014; Ronkainen, Halsall, & Heineman, 2015; Scarano,

Mariani, & Minunni, 2015; Thakur & Ragavan, 2013; D. Zhang & Liu, 2016; G. Zhou et al., 2014).

Recently, DNA biosensor technology which depend on the specific binding of DNA probe to DNA target has drawn much concern of scientist for virus identification due to the great benefit of detecting various microbes with a high level of sensitivity, selectivity, reusability, rapidity, simplicity, and real-time analysis (Wang et al., 2017). The DNA biosensor has the competency to find out the complementary DNA fragment with a low detection limit making it convenient for a practical point-of-care (POC) stand with a low target number in clinical applications without using Polymerase Chain Reaction (PCR) for amplification (L. Wang et al., 2017). Beyond that, the latter progress in up-to-date materials combined with nanotechnology has gained a lot of interest in the development of biosensor technology such as gold nanoparticles (GNPs) in IDE (Perumal, Hashim, Gopinath, Haarindraprasad, Poopalan, et al., 2015).

This thesis represents the development of sensitive, rapid and real-time detection method for HPV based on DNA biosensing, which uses Interdigitated Electrodes (IDE) based electrical biosensor. The application of GNPs as a nanosignal enhancer in IDE biosensor approaches was conducted to increase the detection sensitivity. The IDE based DNA biosensor system was then used to determine HPV in real clinical samples including swab from cervix tissues. The analyzed result from DNA biosensor using IDE was compared with established methods such as commercial ELISA kit, liquid-based cytology (LBC), and HPV DNA genotyping based PCR.

1.2 Problem Statements

There are three problem statements are identified in this research study, including the application of detecting HPV that cause cervical cancer. The problem statements are as follows:

i. The common diagnosis kit for cervical cancer detection based on conventional methods and not specified to specific DNA sequences. Cervical cancer was positioned number two after breast cancer, among the most common cancers affected women, particularly in Malaysia (Zaridah, 2014). Among all the cancer deaths, cervical cancer has been reported to be positioned at fifth (Torre et al., 2015). Currently, there is no particular treatment or an effectual vaccine has ready to be developed for curing the disease. Co-testing of cytology and HPV screening was suggested by the American Society for Colposcopy and Clinical Pathology in cervical cancer detection with married women around 30 and 65 years and recommended for regular testing at 5 years interval for prevention (Preventive & Task, 2011; Saslow et al., 2012). Thus, the reliable diagnostic method for clinical diagnosis is required to determine cervical cancer precisely and rapidly afterward treat HPV infection at an early phase. Besides that, the specific DNA sequence that related to HPV strains must be identified to choose the right method of treatment.

ii. The conventional methods for the detection of HPV that cause cervical cancer and other HPV strains have been detected based on conventional polymerase chain reaction (PCR) requires long assay times. PCR has emerged as a nucleic

acid diagnostic tool in 1983 started from the work of Kary Mullis (Dorado, Besnard, Unver, & Hernández, 2015; Kary B. Mullis, Henry A. Erlich, David H. Gelfand, Glenn Horn, 1990; K. B. Mullis, 1985). The PCR may increase the chance of cross-contamination and time consuming due to the preparation of gel staining. Furthermore, PCR requires a large and expensive instrument for thermal cycling steps. Enzyme-Linked Immunosorbent Assay (ELISA) and screen-printed electrode (SPE) for the detection of cervical cancer cannot be reusable. Thus, there is a strong demand for a development of sensitive, label-free, fast response, and portable sensing device as a replacement for the time consuming, complexity, and label-based assays.

iii. The advancement of nanomaterial enhancer in nanotechnology has begun up the chances in an electrical measurement system for the biomolecule. The modification in the biological or chemical reaction usually creates the alteration in the electrical characteristics of the biomolecule. Thus, electrical detection based biosensor can be simpler, fast detection, and come out with a transportable version. IDE has become a suitable device for application in miniaturizing biosensor device (Hashim, Lakshmipriya, Hashim, & Gopinath, 2017; Sh. Nadzirah, Azizah, Hashim, Gopinath, & Kashif, 2015; Perumal, Hashim, Gopinath, Haarindraprasad, Poopalan, et al., 2015). IDE also has been establishing as a potential device for label-free and very sensitive identification for biomolecules, such as DNA, viruses, and proteins. As bio sensing elements, IDE has been studied as a detection device, which showed a fast response, label-free method, and has the ability to detect target analyte in very low concentrations. An easy utilization of DNA biomarkers in cervical tumor

screening is the immediate identification of the markers by biochemical characterization in cervical samples (Wentzensen & von Knebel Doeberitz, 2007a). DNA has used a biomarker probe due to the simple characteristic and denaturation process (Board et al., 2008; Riccelli et al., 2001). These preferences are important for the expansion of IDE bases DNA electronics for early identification of cervical cancer through DNA hybridization method and strong demand for the development of reusable sensing devices to replace for the one time label-based assays.

1.3 Research Objectives

The intention of this research is to develop a portable, sensitive, selective, rapid, and label-free DNA detection on Nano chip-based Interdigitated Electrodes (IDE) for early detection of Human *Papillomavirus* (HPV) that cause cervical cancer by quantitative measurements. In this research study, IDE acts as a transducer for a low bio-cytotoxicity, and a low-concentration of HPV DNA using *in vitro* clinical samples. This project was accomplished with the following specific objectives:

i. To design the specific DNA sequences related to HPV genes on the modified IDE.

ii. To develop and characterize the integrated GNPs with IDE based biosensor that acts as a transducer to convert qualitative bio reaction signal to quantitative electrical measurement.

iii. To examine and validate the performance of the functionalized GNPs based IDE device for DNA of HPV detection based on electrical measurement of DNA hybridization using high-performance analysis.

1.4 Research Scopes

The first research scope is initiated by reviewing the current progression in early detection strategies for cervical cancer related to molecular biology, immunoassay, biosensor, nanotechnology, fabrications of Interdigitated Electrodes (IDE) and its application. The conventional methodologies used for the detection of HPV like HPV genotyping actually robust and reproducible but at the same time involve complex protocols that take longer times to complete the assay. The proper diagnosis of HPV has been improvised since researchers in biosensor are utilizing HPV virus strains as a model to study the interaction between transducer and bio receptor element. The uses of IDE as a transducer for the electrical biosensor to detect HPV DNA have been discussed in the first research scope. The pattern of IDE constitutes twin electrodes, which are organized in a comb-like frame to form gaps between the two electrodes. The design was transferred to transparent sheets forming the photomask. An insulating layer, which is SiO_2 introduced using a wet oxidation on a cleaned silicon wafer to avoid the leakage of the electron. The IDEs were fabricated on a silicon wafer by using a conventional photolithography method. IDE acts as a transducer for a low bio-cytotoxicity and a low concentration of HPV DNA by using a synthetic target.

In the second scope, molecular techniques applied for HPV detection have been discussed. Molecular diagnosis techniques were taking over an essential method in

clinical detection of cervical cancer involve visual and cytological identification for accurate differentiation of HPV strains that categorized as high, intermediate and low oncogenic risks. DNA analysis based on the complementarity principle of DNA strands can identify the target amplification of a fragment by Polymerase Chain Reaction (PCR) and signal amplification based oligonucleotide hybridization assay. PCR mainly used by a research center and specific health services due to expensive equipment, time-consuming, and complex protocols that required a specialized operation.

The third scope has involved the development of IDE biosensor for early detection of HPV cause cervical cancer through the deposition of GNPs on the top of IDE. The GNPs was chosen due to their characteristics, which are biocompatible, stable structure, cost-effective, high surface-to-volume ratio and the efficient electron transportation. Different sizes of GNP (10, 15, 30, and 80 nm) were studied for the best deposition on top of IDE in term of usage in the biosensor. 3-Aminopropyl triethoxysilane ($C_9H_{23}NO_3Si$, Formula Weight: 221.37 g/mol), as known as APTES was utilized for immobilization of carboxylic end modified 5' single strain HPV DNA probes. The characteristic of the immobilization process was identified through the vibrancy of the components in FTIR. The measurement of hybridization process is carried out by using current-voltage (I–V) characterization (KEITHLEY, 6487) to define the alteration in current flow for the GNP based IDE active area before and after DNA hybridization.

Finally, a real-time of current-voltage measurement was performed upon the immobilization and hybridization processes. The changes in current-voltage during immobilization and hybridization processes were studied. The stability, reproducibility,