DNA Biosensor Using Gold-Nanoparticles Interdigitated Electrodes for Detecting Human *Papillomavirus* in Cervical Cancer

by

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LIST OF ABBREVIATIONS

AFM	Atomic Force Microscopy
ATR	Attenuated Total Reflection
AUNPS	Gold-nanoparticles
APTES	3-Aminopropyl) triethoxysilane
BLAST	Basic Local Alignment Search Tool
BSA	Bovine Serum Antigen
CC	Cervical Cancer
CEA	Carcino-embryonic antigen
CV	Cyclic Voltammetry
DNA	Deoxyribonucleic Acid
DPV	Different Pulse Voltammetry
EDX	Energy Dispersive Xray
EIS	Electrochemical Impedance Spectroscopy
ELISA	Enzyme-Linked Immunosorbent Assay
ERGO	Electrodepositing reduced graphene oxide
FESEM	Field Emission Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
GC	Guanine-Cytosine
GO	Graphene Oxide
HCII	Hybrid Capture II
HPV	Human Papillomavirus
IDE	Interdigitated Electrodes
ITO	Indium Tin Oxide
IV	Current-Voltage
LOD	Limit of Detection
MEMS	Micro-Electrochemical Systems
MWCNT	Multi-walled Carbon Nanotubes
PANI	Polyaniline
POC	Point of care
PCR	Polymerase Chain Reaction
PSA	Prostate-Specific Antigen
RNA	Ribonucleic Acid
RT	Room Temperature

SAM	Self-Assemble Monolayer
SAW	Surface Acoustic Wave
SEM	Scanning Electron Microscopy
SERS	Surface-enhanced Raman scattering
SPCE	Screen Printed Carbon Electrode
TEM	Transmission Electron Microscopy
UV-VIS	Ultra-Visible Spectroscopy
WHO	World Health Organization

LIST OF SYMBOLS

a	Atto
Δ	Current changes
°	Degree
=	Equal
µ	Micro
n	Nano
%	Percentage
Θ	Theta

Biosensor DNA menggunakan Elektrod Interdigit Nanozarah Emas untuk pengesanan Virus Papilloma Manusia berbagai bagi Kanser Serviks

ABSTRAK

Di Malaysia, kira-kira 1,740 kes kanser serviks baru didiagnosis setiap tahun, di mana 991 daripadanya meninggal dunia setiap tahun disebabkan oleh kelewatan mengesan sel-sel kanser yang merebak secara tidak sedar di dalam badan. Oleh itu, kaedah pengesanan pantas dan mudah untuk pencegahan dan pengenalpastian diperlukan bagi menyelesaikan morbiditi dan kematian yang berkaitan dengan virus patogenik. Penyelidikan ini menumpukan pada pembangunan kaedah pengesanan berbilang yang mudah dan pantas untuk mengesan virus pada kepekatan yang lebih rendah, dengan HPV 16, 18, dan 58 sebagai strain rujukan untuk strategi pengesanan yang cepat. Pertama, morfologi permukaan menggunakan Pengimbas Mikroskopi Elektron (SEM) dan 3D Profiler menjelaskan lebar pengukuran elektrod jari dan jurang untuk 50 µm dan 1.5 µm, masing-masing. Seterusnya, sifat fisiokimia koloid Nanozarah Emas (AuNPs) telah dikaji menggunakan Mikroskopi Elektron Penghantaran (TEM) (D: ±9.5 nm) dan Ultra Kelihatan (UV-Vis) (521 nm). IDE berskala nano telah dibangunkan untuk mengenal pasti dan mengecilkan saiz penderia. Tambahan pula, IDE berfungsi dengan AuNP koloid yang dibina dengan APTES sebagai penghubung. IDE yang dioptimumkan kemudiannya digunakan untuk mengesan HPV ssDNA menggunakan mekanisme dua langkah yang diperkenalkan selepas pengubahsuaian permukaan oleh APTES. APTES memautkan kuar ssDNA rekaan HPV yang diubah suai dengan kumpulan karboksil (-COOH) melalui imobilisasi menerusi pengikatan kovalen melalui amina (-NH2). Akibatnya, setiap probe ssDNA HPV telah digunakan untuk mengesan sasaran ssDNA tertentu semasa hibridisasi. Prinsip pengesanan berfungsi dengan mengesan perubahan dalam arus elektrik IDE yang menjambatani sumber dan terminal longkang untuk merasakan imobilisasi kuar ssDNA HPV dan hibridisasi dengan ssDNA sasaran. Semasa proses hibridisasi, arus elekrik meningkat secara drastic daripada negatif ke positif menghasilkan arus elektrik yang tinggi. Selain itu, didapati bahawa sensor menunjukkan kepekaan untuk sasaran HPV ssDNA dalam julat linear dengan kepekatan antara 1 aM hingga 100 µM. Pekali regresi untuk HPV 16, 18 dan 58 ialah 0.99857, 0.98928 and 0.99583 secara tepat. Manakala, ujian sensitiviti untuk HPV 16,18,58 telah diukur pada 0.00302 AM^{-1}, 0.074 AM^{-1} and 0.28089 AM^{-1}. Dengan analisis ini, sensitiviti Had Pengesanan (LOD) adalah kira-kira 0.01 aM, 0.002 aM dan 0.0025 aM untuk HPV 16, 18 dan 58. Selain itu, virus daripada HPV 18 dan 58 telah dapat dikesan dengan peritus keberkesanan sebanyak 90.42% dan 91.92% juga disahkan dengan tindak balas polimer berantai (PCR) secara signifikan. Kemudian, kebolehgunaan semula biosensor untuk HPV 16, HPV 18, dan HPV 58 adalah sehingga dua minggu selepas ia dibangunkan. Oleh itu, kajian ini menjelaskan biosensor elektrod mikro-IDE pertama berdasarkan NP yang didepositkan Au untuk pengesanan awal kanser serviks.

DNA Biosensor Using Gold-Nanoparticles Interdigitated Electrode for Detecting Human Papillomavirus in Cervical Cancer

ABSTRACT

In Malaysia, about 1,740 new cases of cervical cancer are diagnosed every year and 991 of them die annually because the cancer cells that are spreading unconsciously in the body are not detected in time. Thus, rapid and simple detection methods for prevention and identification are required to solve the morbidity and mortality related to the pathogenic virus. This research has focused on developing a simple, rapid, and novel multi-detection method to detect HPV 16, 18, and 58 at significantly lower concentrations, using these strains as reference to establish effective rapid detection strategies. Firstly, the surface morphology is analyzed using scanning electron microscopy (SEM) and 3D profiler to determine the width and spacing of the finger electrode to 50 µm and 1.5 µm, respectively. Subsequently, the physiochemical properties of colloidal gold Nanoparticles (AuNPs) were investigated using transmission electron microscopy (TEM) (D: ±9.5 nm) and ultraviolet (UV-Vis) (521 nm). Nanoscale IDEs were developed to determine and reduce the sensor size. In addition, IDEs were functionalized with colloidal AuNPs and constructed with APTES as linkers. The optimized IDEs were then used to detect HPV ssDNA by the presented two-step mechanism after surface modification by APTES. APTES links the modified HPV-designed ssDNA probe with the carboxyl group (-COOH) through immobilization by covalent binding via amine (-NH_2). Consequently, each HPV ssDNA probe was used to detect its specific ssDNA target during hybridization. The detection principle works by detecting changes in the electrical current of the IDE, bridging the source and drain junction to detect immobilization of the HPV sDNA probe and hybridization with the target sDNA. During the hybridization process, the measured current of the target was higher than its probe as drastic changes in charges generated a spike in the current profile. It was also found that the sensor showed sensitivity for HPV ssDNA target in a linear range with concentrations from 1 aM to 100 µM. The regression coefficient for HPV 16, 18 and 58 was 0.99857, 0.98928 and 0.99583 respectively, and the sensitivity test for HPV 16, 18 and 58 measured at 0.00302 AM^{-1}, 0.074 AM^{-1} and 0.28089 AM^{-1} respectively. With this analysis, the limit of detection (LOD) was approximately 0.1 aM, 0.02 aM, and 0.025 aM for HPV 16, 18, and 58 respectively. Next, HPV 18 and 58 were detected in the real sample with an accuracy of 90.42% and 91.92% also validated with PCR significantly. The reusability of the biosensors for HPV 16, HPV 18, and HPV 58 is up to two weeks after their development. Therefore, the first IDEs electrode biosensor based on Au-deposited NPs for early detection of cervical cancer is presented in this research thesis.