URINARY HUMAN PAPILLOMAVIRUS DNA DETECTION USING PIEZOELECTRIC BIOSENSOR

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ABSTRACT

Cervical cancer is a disease that remains a concern for women worldwide. Despite the implementation of standard Pap test and the HPV test, the screening coverage is still low due to its invasive nature as both involve the collection of cervical samples. The HPV screening test itself is expensive, extensive and label-dependent. In this pilot study, piezoelectric biosensor was used for HPV DNA detection in urine due to its non-invasive approach, simplicity, low instrumentation costs, and label-free detection. Urine samples were collected from 21 women with abnormal Pap Test results and 19 women with normal Pap Test results. HPV HR piezoelectric biosensor was developed for the detection of 3 high-risk HPV DNA strains (16, 18, and 33) and HPV 16 piezoelectric biosensor is for the detection of only HPV 16 DNA. Probe optimisation and calibration experiments were carried out. Amplified urinary DNA samples were analysed using the biosensors. Results showed that the optimum probe concentration for both biosensors was 1.0 μM. The biosensor was able to detect the presence of complementary target DNAs with high specificity. For HPV HR piezoelectric biosensor, the sensitivity was 97.99 Hz μM^{-1}, the instrument detection limit was 16.36 Hz and the concentration detection limit was 0.10344 μM. Meanwhile, for HPV 16 DNA piezoelectric biosensor, the sensitivity was 99.19 Hz μM^{-1}, the instrument detection limit was 15.14 Hz and the concentration detection limit was 0.088 μM. The clinical sensitivity and specificity for both types of piezoelectric biosensor were both 100%. These preliminary results allow for the possibility of implementing the piezoelectric biosensor for the detection of urinary HPV DNA as a potential alternative screening method.

ABSTRAK

Kanser serviks adalah penyakit yang boleh dicegah, yang masih merupakan masalah bagi wanita di Malaysia dan di seluruh dunia. Ia disebabkan oleh jangkitan berterusan *Human Papillomavirus (HPV)* di kawasan serviks. Walaupun ujian Pap biasa dan ujian HPV telah diperkenalkan, liputan saringan masih rendah kerana kedua-dua ujian ini invasif dan memalukan. Ini disebabkan oleh pengumpulan sampel dilakukan di bahagian serviks. Ujian saringan HPV adalah mahal, rumit dan memerlukan label. Dalam kajian pilot ini, biosensor piezoelektrik digunakan untuk mengesan HPV DNA dalam air kencing kerana pendekatannya tidak invasif, mudah, kos peralatan yang rendah, boleh dipantau secara masa nyata, tidak memerlukan enzim dan label. 21 orang wanita yang mempunyai hasil ujian Pap yang tidak normal dan 19 wanita yang mempunyai hasil ujian Pap yang normal, telah menyertai kajian perintis ini. HPV HR biosensor piezoelektrik telah dibangunkan untuk mengesan 3 jenis HPV DNA berisiko tinggi (16, 18, 33) dan HPV 16 biosensor piezoelektrik adalah untuk mengesan hanya HPV 16 DNA. Pengoptimuman dan eksperimen penentukuran telah dijalankan bagi kedua-dua biosensor. Sampel DNA air kencing daripada peserta telah dianalisa oleh kedua-dua biosensor. Hasil kajian menunjukkan bahawa kepekatan prob yang optimum untuk kedua-dua biosensor adalah 1.0 μM. Biosensor ini boleh mengesan kehadiran DNA dengan kadar pemilihan dan pengkhususan yang tinggi. Untuk HPV HR biosensor piezoelektrik, sensitivitinya adalah 97.99 Hz μM^{-1}, had pengesanan untuk alat ialah 16.36 Hz dan had pengesanan untuk kepekatan ialah 0.10344 μM. Sementara itu, bagi HPV HR biosensor piezoelektrik, sensitivitinya adalah 99.19 Hz uM^{-1}, had pengesanan untuk alat ialah 15.14 Hz dan had pengesanan untuk kepekatan ialah 0.088 μM. Nilai sensitiviti dan pengkhususan secara klinikal untuk kedua-dua jenis biosensor apabila dibandingkan dengan kaedah Ujian Pap sebagai rujukan, kedua-duanya adalah 100%. Hasil kajian awal ini membolehkan kaedah saringan untuk kanser serviks boleh dilakukan secara tidak invasif dengan mengesan HPV DNA di dalam sampel air kencing dengan menggunakan biosensor piezoelektrik.

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LIST OF ABBREVIATIONS

HPV	-Human Papillomavirus
DNA	-Deoxyribonucleic acid
Pap	-Papanicolaou
LSIL	-Low-grade Squamous Intraepithelial Lesion
HSIL	-High-grade Squamous Intraepithelial Lesion
QCM	-Quartz Crystal Microbalance
PCR	-Polymerase Chain Reaction
NaOH	-Sodium Hydroxide
NaCl	-Sodium Chloride
DC	-Direct Current

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Cervical cancer or carcinoma is the second most common malignancy in females in the world affecting about 500,000 people every year [1]. It represents the third most common cause of female mortality worldwide [1][2]. In Malaysia, cervical cancer is the third most common malignancy in females and is the fourth common cause of female mortality [3]. Over the years, the worldwide incidence and mortality of this disease have reduced due to the implementation of Pap test. However, about 274,000 women still die from this disease every year [4] [5]. In Peninsular Malaysia, 847 cases of cervical cancer were recorded in 2006, although the report varied by racial groups, whereby the Indian women had the highest score, followed by Chinese and Malay. The incidence increased with age after 30 years and peaked at the age of 65 to 69 [6].

Cervical cancer is caused by persistent infection of high-risk strains of Human Papillomavirus (HPV) in the cervix, causing cytological changes, resulting in the development of malignant lesions [7]. The process involves the integration of the viral DNA into the human host cell genome [7]. It has been proven that early detection or screening of precancerous lesions, managed to prevent cervical cancer disease [1]. In addition to the screening, HPV vaccinations have also been

implemented as a primary prevention strategy for the infection and other related diseases [1].

1.2 Problem Statement

Cervical cancer screening tests are important to detect pre-cancerous changes in the cervical squamous cells, so that, cervical cancer can be prevented from developing by early medical or surgical intervention. Although these tests have managed to reduce the cervical cancer incidence worldwide, they still have limitations that can be potentially improved in the future.

The standard cervical cancer screening is usually done through Pap test by cytological procedure. However, this test causes variability in the result interpretation as it depends on visual evaluation by different experts. The molecular-based screening tests use PCR and hybrid capture techniques to detect the presence of HPV DNA in samples. Nevertheless, the PCR-method is complicated as it involves labelling approach using enzymes, fluorophores, and radioactivity. Moreover, this test is associated with cross-reaction and cross-hybridisation which results in less accurate results. Apart from that, both cytology and molecular-based screening tests are invasive which causes discomfort for patients, as these tests require pelvic examination using a speculum to collect samples from the cervical area. In addition to these, all the tests require extensive procedures and expensive instrumentations.

In order to overcome the above limitations, in this research, the use of simpler, cheaper, quantitative and label-free technique using piezoelectric biosensor was used to detect the presence of HPV DNA in urine, which involves a non-invasive and pain-free sampling method. Probe optimisation experiment for the biosensor was necessary to know the optimum probe concentration. Calibration

experiment was also required to confirm that the biosensor is an acceptable instrument for HPV DNA detection. Lastly, the biosensor had to be tested whether it could detect HPV DNA in the real clinical urine samples and the result should be compared to the detection by PCR.

1.3 Study Objectives

This study aims to investigate the appropriate probe concentration for immobilisation reaction on the Quartz Crystal Microbalance (QCM) gold electrode of the piezoelectric biosensor. It also aims to calibrate the HPV DNA biosensor using different synthetic target DNA concentrations which was never done by any studies before. Furthermore, this study aims to investigate the ability of the biosensor to detect HPV DNA in real urine samples from patients.

1.4 Study Scope

In this study, two types of piezoelectric biosensors were tested for the HPV DNA detection. Type 1 (HPV HR) is for screening positive samples of 3 high-risk HPV strains namely HPV 16, 18, and 33, using a degenerate probe. Meanwhile, Type 2 (HPV 16) is for the detection of only HPV 16 DNA. The HPV HR biosensor allows fast detection or screening of the virus presence, without identifying the particular strain involved. Ideally, only if the samples are identified positive from the first screening, they need to undergo subsequent analysis using the HPV 16 biosensor for genotyping of specifically HPV 16 strain, which is the main culprit of cervical cancer. These two steps aim to reduce cost and analysis time.

Urine samples were collected from female subjects who have been sexually exposed and have had positive Pap Test results which include atypical squamous

cells, low-grade squamous intraepithelial lesion (LSIL) or high-grade squamous intraepithelial lesion (HSIL).

To achieve the objectives, series of experiments were conducted which involve probe optimisation, biosensor calibration study, determination of the limit of detection, reusability of the biosensor, and the ability of the biosensor to detect HPV DNA in clinical urine sample.

1.5 Significance of Study

This study is important as it calibrated the HPV DNA piezoelectric biosensor accordingly, so that it could be a valid instrument to be used in the real clinical settings using real clinical urine samples. The optimum concentration of biotinylated probe as well as the optimum concentration of target DNA was also determined in this study.

The outcome of this research project will improve the number of females attending for cervical cancer screening test as it introduces an alternative non-invasive and simpler screening test. This will eventually improve the diagnostic and prognostic procedure of detecting cervical cancer at early stage so that early effective intervention for the disease can be made. Furthermore, this study will improve the worldwide and the national healthcare service for patients. In addition, this study will help to increase the number of national publication, thus contributing to the national medical research.

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