UNIVERSITI PUTRA MALAYSIA

FABRICATION OF CHITOSAN-INTEGRATED SINGLE-MODE TAPERED OPTICAL FIBER DENV II E PROTEIN SENSOR

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By

NADIA BINTI MOHD AMIN @ MOHD NASIR

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Chair : Muhammad Hafiz bin Abu Bakar, PhD
Faculty : Engineering

Globally, diseases infected by dengue virus (DENV) prevails among major public health problem, especially in tropical and sub-tropical areas. 500 000 people are estimated infected with severe dengue require hospitalization every year and 2.5% is estimated case fatality. Quantitative assessment by enzyme-linked immunosorbent assay (ELISA) is known to be used by laboratories to produce better clinical monitoring but it needs complex laboratories infrastructure as well as expertise to operate it. For the past decades, tapered single mode fiber has shown versatility and enticing sensitivity towards changes in its surrounding refractive index, making it suitable for sensing applications. In 2018, a research developing tapered optical fiber sensor targeting dengue virus envelope (E) protein originates from DENV II which is among 4 distinct DENV serotypes has been published. DENV II E protein is the interested determinant since it is located at the outermost of dengue virus structure, hence detecting the protein signifies the presence of the virus itself. As a result, high sensitivity and specificity within rapid detection 15 minutes is achieved. This biosensor is enhanced further by utilizing inorganic material which is graphene to facilitate greater surface area for sensing enhancement. However, graphene is known to have mild toxicity and its effect to DENV II E protein is yet to be determined.

This study looks into the use of organic nanomaterial namely chitosan for enhancement of tapered fiber sensing response. A layer of chitosan was introduced to single mode tapered fiber functionalized for the detection of DENV II E protein. Tapered optical fiber was fabricated and functionalized using Sodium Hydroxide (NaOH), 3-(Aminopropyl) triethoxysilane (APTES), and Glutaraldehyde. Chitosan immersion time up to 60 minutes was then tested

yielding working immersion time of 20 (CHIT20), 30 (CHIT30), 35 (CHIT35), 40 (CHIT40) and 45 minutes (CHIT45). Subsequently, the experiment proceeded with the immobilization of antibody. The immersion time for antibody was optimized for CHIT20, CHIT30, CHIT35, CHIT40 and CHIT45 at 25, 30, 33, 35 and 38 minutes, respectively.

After that, different concentration of DENV II E protein solution ranging from 0.0nM to 1.0nM with increment of 0.2nM were introduced. Prior to that, optimum incubation time of DENV II E protein for CHIT20, CHIT30, CHIT35, CHIT40 and CHIT45 was observed at 30, 35, 38, 40 and 43 minutes respectively. The spectral shift with the introduction of DENV II E protein was then recorded and analyzed. This set of experiment was conducted in triplicates. Consistent red shift of spectra at increasing concentration is observed for CHIT20. It obeys the linear relationship between concentration and refractive index which altered the effective refractive index and caused the red shift. For CHIT30, CHIT35, CHIT40 and CHIT45, consistent red shift of spectra was also noted. Increment of the sensitivity value is observed as CHIT20, CHIT30 and CHIT35 recorded 6.28 nm/nM, 10.68 nm/nM and 14.19 nm/nM, respectively. However, the sensitivity decreased for CHIT40 and CHIT45 with corresponding value of 12.24 nm/nM and 11.34 nm/nM. From these values, it is noted that the best sensitivity obtained for the sensor is at CHIT35 with 14.19 nm/nM. The work proceeded with the investigation on limit of detection (LOD) and the sensor was tested with different concentration ranging from 0.1pM to 0.1μM. The Langmuir curve plotted from the findings denoted LOD of 1pM. In conclusion, this study highlights the feasibility of using organic nanomaterial which has better biocompatibility and environmental friendly for the enhancement of DENV II E protein detection.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

FABRIKASI PENDERIA PROTEIN E DENV II GENTIAN OPTIK TIRUS SATU MOD BERSEPADUKAN CHITOSAN

Oleh

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Di seluruh dunia, penyakit yang dijangkiti oleh virus denggi (DENV) mengungguli masalah kesihatan awam, terutamanya di kawasan tropika dan sub-tropika. Dianggarkan 500 000 manusia dijangkiti denggi yang kritikal sehingga dimasukkan ke hospital dan 2.5% dianggarkan kes yang membawa kematian. Penilaian kuantitatif dari enzyme-linked immunosorbent assay (ELISA) diketahui untuk digunakan di makmal untuk mendapatkan pengawasan klinikal yang lebih baik tetapi ia memerlukan infrastruktur makmal yang kompleks dan juga kepakaran dalam pengendalian. Beberapa tahun yang lampau, gentian optik tirus telah menunjukkan kebolehan dan kepekaan terhadap perubahan indeks biasan didalam medium menyebabkan kesesuaian untuk diterapkan dalam mekanisma sistem deria. Pada tahun 2018, penyelidikan dalam membangunkan alat penderia gentian optik tirus mensasarkan protein virus denggi envelope (E), berasal daripada DENV II yang mana dalam kalangan 4 serotaip DENV yang berbeza telah diterbitkan. Disebabkan protein DENV II E terletak paling luar pada struktur virus denggi, maka mengesan protein itu menunjukkan kehadiran virus itu sendiri. Kesan daripada itu, sensitiviti yang tinggi dan khusus dalam 15 minit pengesanan pantas telah diperolehi. Penderia bio ini diperbaiki lebih lagi dengan penggunaan bahan bukan organik iaitu grafin untuk menyediakan kawasan permukaan yang lebih besar untuk penambahan penderiaan. Walaubagaimanapun, grafin diketahui untuk mengadungi toksik yang tidak kuat dan kesannya kepada protein DENV II E belum ditentukan lagi.

Kajian ini memperkenalkan penggunaan nanomaterial organik yang bernama Chitosan untuk penambah pada gerak balas penderiaan di gentian tirus. Satu lapisan Chitosan telah dikenalkan kepada satu mod gentian tirus yang telah difungsikan untuk pengesanan protein DENV II E. Gentian optik tirus telah dibuat dan difungsikan menggunakan Natrium Hidroksida (NaOH), 3-(Aminopropyl) triethoxysilane (APTES), dan Glutaraldehyde. Masa rendam

sehingga 60 minit telah diuji menghasilkan masa rendam berfungsi 20 (CHIT20), 30 (CHIT30), 40 (CHIT40) dan 45 minit (CHIT45). Selepas itu, eksperimen diteruskan dengan pelumpuhan antibodi. Masa rendam untuk pelumpuhan antibodi telah dioptimumkan untuk CHIT20, CHIT30, CHIT40 dan CHIT45 dan masa optimum yang dihasilkan untuk setiap keadaan ialah 25, 30, 35 dan 38 minit.

Selepas itu, larutan protein DENV II E dengan kepekatan berbeza antara 0.0nM sehingga 1.0nM dengan kenaikan 0.2nM telah diperkenalkan. Sebelum itu, masa rendam optimum untuk protein DENV II E telah dilihat pada 30, 35, 40 dan 43 minit bagi CHIT20, CHIT30, CHIT40 dan CHIT45. Pengalihan spektrum disebabkan oleh protein DENV II E telah direkod dan dihuraikan. Eksperimen ini dijalalankan sebanyak tiga kali. Pengalihan ke kanan yang berterusan pada kepekatan meningkat telah dilihat untuk CHIT20, CHIT30, CHIT40 dan CHIT45. Kenaikan dalam nilai kepekaan menunjukkan sebagaimana CHIT20, CHIT30 and CHIT35 memperoleh 6.28 nm/nM, 10.68 nm/nM and 14.19 nm/nM. Walaubagaimanapun, kepekaan menurun untuk CHIT40 dan CHIT45 dengan nilai masing-masing 12.24 nm/nM and 11.34 nm/nM. Dari nilai-nilai ini, kepekaan terbaik yang diperoleh ialah 14.19 nm/nM. Kerja ini diteruskan lagi dengan menyiasat had pengesanan dan penderia diuji dengan kepekatan berbeza dalam julat 0.1pM to 0.1μM. Lengkungan Langmuir telah diplot dan LOD yang diperolehi ialah 1pM. Ia mematuhi hubungan lelurus diantara kepekatan dan indeks biasan dan menyebabkan pengalihan ke kanan. Kesimpulannya, kajian ini menonjolkan penggunaan nanomaterial yang boleh dilaksana yang mana mempunyai keserasian bio yang lebih baik dan mesra alam untuk penambah penderiaan protein DENV II E.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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LIST OF ABBREVIATIONS

AFM	Atomic force microscope
APTES	3- (Aminopropyl) triethoxysilane
Au	Gold
AuNPs	Gold nanoparticles
C	Capsid
cDNA	Complementary DNA
DENV	Dengue virus
DF	Dengue fever
DHF	Dengue haemorrhagic fever
DNA	Deoxyribonucleic acid
DSS	Dengue shock syndrome
E	Envelope
EDX	Energy-dispersive X-Ray
ELISA	Enzyme linked immunosorbent assay
FESEM	Field emission scanning electron microscope
FSR	Free spectral range
GA	Glutaric acid
GO	Graphene oxide
LOD	Limit of detection
LP_{01}	Fundamental mode
LSPR	Localized surface plasmon resonance
M	Membrane
NaOH	Sodium hydroxide
nM	Nanomolar

NS	Non-structural
OH	Hydroxyl
OSA	Optical spectrum analyzer
PAMAM	Polyamidoamine
PBS	Phosphate buffer solution
PNA	Peptide nucleic acid
PSiNs	Porous silica nanospheres
SPR	Surface plasmon resonance
WHO	World health organization

CHAPTER 1

INTRODUCTION

1.1 Overview

Dengue virus (DENV) infection is the most common viral infection transmitted by mosquito [1]. Four distinct serotypes have been acknowledged which are DENV-1, DENV-2, DENV-3 and DENV-4 [2]. Infection by any of these serotypes can cause classical Dengue fever and the more acute Dengue haemorrhagic fever (DHF) and Dengue shock syndrome (DSS). Up to date, there are no licensed vaccines that have been published for Dengue. Consequently, containment of the infection relies on proper clinical management that should begin during febrile phase which is first three days of infections [3]. Hence, early detection is crucial to ensure the survival of Dengue patients.

Current trend to diagnose this disease for early detection is by using antigenic determinants as a target in sensing systems. DENV is composed of 10 distinct kinds of proteins with its 11 000 nucleotide-based genome [4]. Three of the proteins are structural proteins for the capsid (C), membrane (prM) and envelope (E) glycoprotein, whereas another 7 as non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5) [5]. From the variety types of proteins, invention of detection kits targeting specific protein has been reported [6]. For example, detection kits targeting NS1 proteins are already obtainable in the market [6][7]. Nevertheless, these kits are based on qualitative assessment and only for rapid detection.

Conversely, quantitative assessment produces a better clinical monitoring where clinical laboratories typically employ the enzyme-linked immunosorbent assay (ELISA) method, or the IgM/IgG antibody capture ELISA (MAC-ELISA) [7]. The principle of operation is based on absorbance changes on the interaction of ligand-targeted antibody bound on the sensing solid phase. Although this technique produces quantitative output, the procedure is complex and time consuming which also requires expensive laboratories infrastructure and highly trained personnel [7]. Another quantitative method available is real-time polymerase chain reaction (RT-PCR) [8]. It can specifically detect DENV at very low concentration and possesses high sensitivity. However, it may show false positives when there is a presence of even very small impurities [8]. In light of this, the more common quantitative assessment opted for infection monitoring is platelet count [9]. Nonetheless, monitoring via platelet is not specific to DENV only as other viral infections could also affect platelet count [10].

Hence, the alternative is to pursue a simpler and cheaper quantitative detection method such as the implementation of tapered optical fiber which is known to be small in size, flexible and highly sensitive [11][12]. Recent studies have been published on the use of tapered optical fiber sensors to detect NS1 [13] as well as DENV II E protein [14]. DENV II E protein is of interest since it is located at the outermost of DENV structure, hence it will interact with host cell first compared to other proteins. The detection of the E protein itself allows diagnosis of DENV infection at its onset, thus allowing rapid clinical response.

The transducer in [14] was further enhanced through integration of nanomaterial in the sensing layer [15]. By introducing graphene oxide (GO) onto the single mode tapered optical fiber surface, the effective sensing area was expanded which led to a substantial increase in sensitivity and emphasized the immense potential of nanomaterial integration in the sensing layer of tapered optical fiber transducer. This is proven as the sensitivity obtained was increased from 5.02 nm/nM [14] to 12.77 nm/nM [15].

1.2 Problem Statement

Despite numerous DENV sensing methods available nowadays, there are drawbacks that impede their deployment as comprehensive, accessible and user-friendly diagnostic tools. For example, although ELISA and RT-PCR are sensitive and quantitative, they are highly complex and costly in terms of time and chemical reageants. Whereas, for rapid detection strip test, the results are solely qualitative and it targets NS1 which is not a primary determinant. Hence from the drawbacks mentioned, the emergence of outstanding alternatives such as the tapered fiber sensor are highly desired. Enhancement of the sensor through nanomaterial integration has further solidified the feasibility of taper sensor to address issues pertaining to current DENV detection schemes. Nevertheless, inorganic nanomaterial that has been explored such as graphene oxide could still become an issue in terms of its decomposition as well as toxicity. Additionally, its effect to the protein sensing layer and organic sample remains undetermined.

1.3 Aim and Objectives

This research aims to explore the use of organic nanomaterial, chitosan, for enhancement of functionalized single-mode tapered fiber DENV II E protein sensor. The following objectives of this research are:

I. To deposit chitosan on functionalized single-mode tapered fiber
II. To fabricate a chitosan-integrated single-mode tapered optical fiber DENV II E protein sensor
III. To compare the performance between chitosan-integrated and non chitosan-integrated single mode tapered optical fiber DENV II E protein sensor

1.4 Scope of Work

The scope of work in this research is summarized in Figure 1.1. Generally, this work was focused on dengue diagnostics specifically using optical-based sensor. The optical transducer of choice was tapered single mode fiber due to its fabrication simplicity and high sensitivity accorded by evanescent wave excited along the narrowed region. In addition, the spectral-shift based detection provides accurate and power-independent sensing output. The DENV determinant detected by the single mode fiber transducer was Dengue II E protein. E protein was chosen because it is located at the outermost structure of the virus, hence detecting the E protein signifies the presence of the virus itself while serotype II was selected as it is the most prevalent type in Malaysia. The sensor in this work was integrated with nanomaterial with the aim of enhancing the response by creating more binding sites during antibody immobilization. Chitosan, an organic nanomaterial was chosen for its nontoxicity, biodegradability and biocompatibility. DENV II E protein concentration within nanoMolar (nM) range was chosen for the investigation to make it comparable to other similar prior studies.

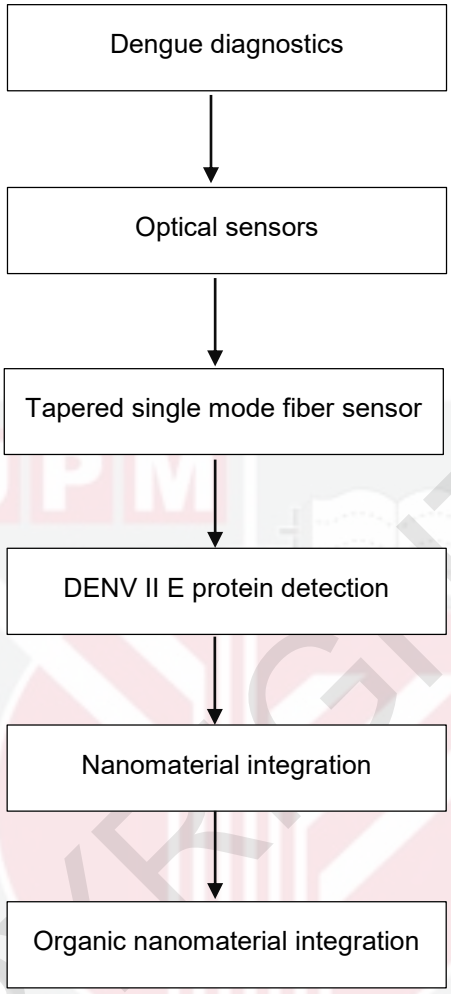

Figure 1.1: Scope of Work

1.5 Organization of Thesis

The organization of this thesis is outlined as following:

Chapter 1 consists of the introduction and overview of the research area. It provides a brief explanation on dengue viruses and infection. Current dengue detection method and their challenges are highlighted along with the aim and the objectives that are formed to address those issues. The scope of work and thesis organization are also included in this chapter.

Chapter 2 introduces the classical methods and modern trends to diagnose Dengue. This includes thorough discussion and reviews on established dengue detection methods. Emerging technologies such as tapered optical fiber sensor are also presented in this chapter.

Chapter 3 merges the methodology used in this work as well as results obtained from the whole process. The steps taken starting from the fabrication of single mode tapered optical fiber until the complete detection of DENV II protein are detailed. Analysis of the results as well as comparison to prior works are also included.

Last but not least, chapter 4 concludes the research work. All the important findings corresponding to the set objectives are highlighted and recommendations for future work are also stated.

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