UNIVERSITI TEKNOLOGI MARA

SURFACE ENGINEERING OF NANOPOROUS ANODIC ALUMINIUM OXIDE FOR BIOSENSOR APPLICATION IN THE DETECTION OF DENGUE VIRUS TYPE II

ABDUL HADI BIN MAHMUD

Thesis submitted in fulfilment
of the requirements for the degree of
Master of Science
(Applied Chemistry)

Faculty of Applied Sciences

November 2017

ABSTRACT

In this work, nanoporous anodic aluminium oxide (AAO) membrane-based optical biosensor was constructed for targeting the DNA Dengue virus type II. The AAO membrane was fabricated by electrochemical anodization of aluminium foil applying different voltages from 40 – 100 V. To increase the sensitivity of the biosensor device, amino (1-propyl-4,5-dihydro-1*H*-1,2,3-triazol-4-yl)-triethoxysilane (click compound) was synthesized and introduced on to the AAO by silanization approach followed by 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide (EDC) mediated immobilization of amino (NH_2)-modified probe ssDNA. Fluorescent-tagged (Texas red) complementary target DNA was used for DNA hybridization process on modified AAO. Characterization of the AAO membrane's morphology was observed by means of Field Emission Scanning Electron Microscope (FE-SEM). To confirm the silanization of click compound on AAO surfaces, Fourier transforms infrared spectroscopy (FTIR) analysis and X-Ray photoelectron spectroscopy (XPS) analysis was conducted. The detection of DNA hybridization process on AAO surfaces was determined by the presence of fluorescent signal using confocal microscopy. FE-SEM characterization revealed that prolong anodization time improved the pore morphology of anodized aluminium oxide in the case of 40 V. However, the pore wall started to collapse when the voltage is applied at 100 V. FTIR spectra of modified AAO surface showed emerging peaks at 2960 , 2928 and 2884 cm^{-1} that correspond to the C-H stretching of an aromatic methyl group from silane compound. XPS survey scan showed traces of element carbon, nitrogen, oxygen, and silicon on the silanized AAO surface, which showed the success of surface modification technique. Confocal microscopy images confirmed the fluorescent-tagged complementary target DNA was successfully hybridized on the modified AAO surface. Results obtained showed that the developed optical biosensor is capable of specific detection of DNA Dengue virus type II with detection limits recorded as low as five nMol. The presented results suggest that nanoporous AAO-based DNA biosensor show promise for a sensitive and specific diagnostic device in detecting DNA Dengue's virus type II.

ACKNOWLEDGEMENT

Firstly, I wish to say Alhamdullilah and thank God, for giving me the opportunity to embark on my Master and for completing this long and challenging journey successfully. My gratitude and thanks go to my supervisor Dr. Abdul Mutalib Md Jani, and co-supervisor, Dr. Mohd Tajudin Mohd Ali, without their support, patience and ideas in assisting me; this project will not be completed.

I also would like to express my gratitude to all members of PCMaG (Physic Chemistry Materials Research Group) of UiTM Arau especially Ass Prof. Dr. Nafisah Othman and Madam Hanani Yazid whom have helped me in completing my research. Also Special thanks to my colleagues and friends for helping me with this project.

Finally, this thesis dedicated to my very dear father and mother for the vision and determination to educate me, and for all the support that has assisted me to complete my study. This piece of victory devoted to both of you, Alhamdulillah.

TABLE OF CONTENTS

CHAPTER ONE
INTRODUCTION

1.1 Background of Study

Dengue virus is the prevalent parvovirus in Southeast Asia, Africa and Central America. This Dengue virus belongs to the Flaviviridae family comprising of four unique serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) and consists of an antigenic complex in the genus Flavivirus. These four dengue viruses are recognized in over 100 countries and territories, and the worldwide annual infection rate is estimated to be between 50 and 100 million per year (Iyer et al., 2014) spread by mosquito *Aedes aegypti* bites (Deng & Toh, 2013). Clinical symptoms demonstrated from dengue fever patient include myalgia, arthralgia, maculopapular rash, petechiae, bruising and thrombocytopenia. However, these symptoms will manifest long after the infection of dengue virus. Therefore, more efficient diagnostic tools need to be developed in order for proper and early treatment.

In tropical countries, numerous infectious diseases coexist, and as a result, possibilities of co infections are common. In this circumstance, infections with human immunodeficiency virus and dengue virus (DENV) are serious public health problems in tropical and subtropical regions and represent major public health problems in several countries, including Malaysia. DENV belongs to the genus Flavivirus, family Flaviviridae, a small group of enveloped viruses with a positive-sense RNA virus that co-circulates in endemic regions as four closely-related distinct serotypes that are identified as DENV-1, DENV-2, DENV-3 and DENV-4 (Guzman & Harris, 2015). In various regions of Malaysia, dengue epidemics occurred for decades, contributing to the high rates of morbidity and mortality a year after year. Data obtained from the Malaysia's dengue website (idengue.remotesensing.gov.my) stated that in 2016 from 3 January to 9 March was reported 29571 cases of dengue with 62 deaths in Malaysia. The symptom of dengue virus infection varies from an acute febrile illness usually benign to a severe form of hemodynamic disorders, severe hemorrhagic manifestations, cavity effusions and shock (Torrentes-carvalho et al., 2016).