UNIVERSITI TEKNOLOGI MARA

DEVELOPMENT OF IMPEDIMETRIC IMMUNOSENSOR BASED ON MODIFIED PT DISK MICROELECTRODE FOR DETECTION OF AMYLOID BETA IN BRAIN TISSUE LYSATE

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ABSTRACT

Alzheimer's disease (AD) is a fatal neurodegenerative disease and clinically characterized by progressive deterioration of memory and decline of cognitive function. Since the only reliable diagnosis for the pathology is histochemical post-mortem examination, therefore, there is a continuing demand for fast, reliable, and sensitive detection technique. Here, a miniaturized biosensing platform based on monoclonal amyloid-beta antibodies (mAβ_{ab}) that were immobilized on a disc-shaped platinum/iridium (Pt/Ir) microelectrode surface coupled with an impedimetric signal transducer has been developed for a label-free and sensitive detection of amyloid-beta peptide fragment 1−40 (Aβ40), a reliable biomarker for diagnosis of AD. The Pt/Ir microelectrode was electropolymerized with poly (ortho-phenylenediamine), a conducting free amine-containing aromatic polymer; followed by crosslinking with glutaraldehyde (GA) for subsequent coupling of mAβ_{ab} on the microelectrode surface. This modification strategy efficiently improved the impedimetric detection performance of Aβ40 in terms of charge transfer resistance and normalized impedance magnitude percentage change (~40 % increase) as compared to passive adsorption-based immobilization method. The sensitivity of the micro-immunosensing assay was 1056 pg/mL/ (kΩ•cm^2) and the limit of detection of 4.81 pg/mL with a dynamic range of 1−10^5 pg/mL (R^2=0.9932) were obtained. The repeatability of the assay via relative standard deviation was 12.8 %, demonstrating its reliability and accuracy. In respect of sensor durability and stability, the immobilized mAβ_{ab} on the microelectrode surface was capable of maintaining 80 % of its binding activity to Aβ40 after leaving in PBS (pH 7.4) solution for 48 h at ambient temperature. To validate the real applicability, the developed assay was tested in brain tissue lysates prepared from AD-induced rats. The results were presented which includes the quantification data of Aβ40 presence in the samples. As a conclusion, this study demonstrates the potential application of the Pt-PPD-GA-mAβ_{ab}-Aβ40 immunosensing assay for diagnosing the AD‘s biomarker. The method used in this work would offer a useful means for quantifying Aβ in a biological matrix, and be valuable in the design of new types of electrochemical biosensors for the detection of other disease-related biomarkers.

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CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND OF STUDY

Alzheimer's disease (AD) is the most common type of dementia (Pahnke et al., 2009; Mosconi et al., 2007) where it able to damage the brain, resulting in impaired memory, problem-solving, language, thinking and behaviour. This disease is normally common among the elderly at the age of 65 years. Above this age, a person's risk of developing AD doubles approximately every five years. It is expected that the percentage of this disease worldwide will increase sharply from the year 2050 as the life of expectancy increases (Prince et al., 2015). Moreover, according to Alzheimer's disease Foundation Malaysia (2016), it is predicted that currently there are about 50,000 people in Malaysia with this disease and it is also will continue to increase from 6.3 % in 2000 to 12 % by the year 2030. Whilst the number of people reaching old age is increasing, it is become a concern to health care providers and government. Due to this reason, AD is believed to have a huge impact on socio-economic crisis with the total estimated worldwide cost of dementia is US $818 billion, and it will become a trillion dollar disease by 2018 (Prince et al., 2015).

The histopathological hallmark of AD is the presence of proteins build up in the brain deposits to form structures called 'plaques' and 'tangles'. Researchers have claimed that, a key component of these 'plaques' is known to be as amyloid β-peptide (Aβ) protein which is generated from the amyloid precursor protein (APP) by sequential action of β-secretase (BACE1 and BACE2) and γ-secretase (Liu & Zhou., 2015). This Aβ protein begins as a solitary molecule but tends to aggregates and initially builds up into clusters that are still soluble and can travel freely in the brain. Due to this, Aβ can bind strongly to a receptor on nerve cells which are interconnected through synapses. Theoretically, these signals are transmitted across the synapse in the form of chemicals that are known as neurotransmitters. Neurotransmitters are passed from one neuron cell, across the synapse (connection) and to the receiving neuron cell, which collects the neurotransmitter with a receptor. The receiving cell can then send