Electrochemical Immunosensor for Detection of *Mycobacterium tuberculosis*

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Abstract: A rapid and sensitive sandwich electrochemical immunosensor was developed based on the fabrication of the graphene/polyaniline (GP/PANI) nanocomposite onto screen-printed gold electrode (SPGE) for detection of *Mycobacterium tuberculosis* (*M. tuberculosis*) antigen. The chemical bonding and morphology of GP/PANI-modified SPGE were studied by Raman spectroscopy and field enhance scanning electron microscopy-energy dispersive X-ray spectroscopy (FESEM-EDX), respectively. From both studies, it clearly showed that GP/PANI was successfully coated onto SPGE through drop cast technique. Cyclic voltammetry (CV) was used to study the electrochemical properties of the modified electrode. The effective surface area for GP/PANI-modified SPGE was enhanced when compared with bare SPGE. Differential pulse voltammetry (DPV) was used to detect the *M. tuberculosis* antigen. This proposed electrochemical immunosensor is sensitive, low sample volume, rapid, and disposable, which is suitable for tuberculosis detection in real samples.

Keywords**:** Tuberculosis, electrochemical sensors, screen-printed gold electrode (SPGE), graphene, polyaniline.

INTRODUCTION

Tuberculosis (TB) is a contagious disease that caused by *Mycobacterium tuberculosis (M. tuberculosis)* and causes high mortality and morbidity compared to other disease (WHO, 2016). An early diagnosis of TB is very important for reducing the number of fatal cases and allow fast recovery. The current diagnostic methods such as sputum smear microscopy and culture methods are required skilled personnel and more than 2 weeks to confirm the result. To overcome this problem, many diagnostic methods have been developed such as fluorescence microscopy and mycobacterium growth indicator tube (MGIT) but still require more than 10 days and expensive (Kim *et al*., 2013). Therefore, electrochemical immunosensor based on GP/PANI-modified SPGE was used in this study for detection of TB due to its sensitivity and able to reduce time consuming.

MATERIALS AND METHODS

Graphene/polyaniline (GP/PANI) nanocomposite was synthesized in a solution of poly(methyl vinyl ether alt-maleic acid) (PMVEA) by oxidative polymerization using ammonium persulfate (APS) as the oxidant known as self-assembled method (Mohamad *et al*., 2017). After 6 hours reaction, the black-green precipitate was observed that indicates the formation of the nanocomposite. The prepared nanocomposite was then used for fabrication of the immunosensor by drop casting it onto SPGE, then baked in oven at 70 °C after 24 hours dried at room temperature. After that, the modified electrode

was immersed in EDC/NHS solution for 30 minutes, followed by incubation of capture antibody (CapAb) for 2 hours at 37 °C, classified as CapAb/GP/PANI-SPGE. Then, the CapAb/GP/PANI-SPGE was then immersed in bovine serum albumin (BSA) which act as blocking buffer to avoid non-specific binding for 1 hour at room temperature. Then, the *M. tuberculosis* antigen was drop casted onto prepared electrode and incubated for 1 hour at 37 °C. Finally, the Fe/Au MNPs conjugated with primary antibody (Ab) was then drop casted onto it and incubated for 40 minutes at 37 °C, then ready for measurement using differential pulse voltammetry (DPV) technique.

RESULTS AND DISCUSSION

The GP/PANI nanocomposite was successfully synthesized with average size of 85 nm. Then, the prepared GP/PANI nanocomposite was used for modification of SPGE. The surface area of GP/PANI-modified SPGE provide 5 times greater than bare SPGE which were calculated using Randles-Sevcik equation. There are several parameters that have been studied in order to get the optimum condition for *M. tuberculosis* detection including significant of treatment the electrode before being used, amount of GP/PANI coated onto SPGE, scan rate, and time incubation of EDC/NHS as a cross linker. Finally, the different concentration of *M. tuberculosis* antigen were studied to calculate the limit of detection for this developed method.

CONCLUSIONS

In this study, we developed a simple, non-invasive and rapid electrochemical technique for *M. tuberculosis* detection based on GP/PANI-modified SPGE. As we modified the electrode using GP/PANI nanocomposite, the surface area of the working electrode was increased 5 times compared to bare SPGE. By using this developed method, *M. tuberculosis* antigen could be detected within 1 day.

ACKNOWLEDGMENT: The authors would like to thank the NanoMalaysia Berhad for their financial support and Hospital Universiti Sains Malaysia (HUSM), Kubang Kerian, Kelantan for their cooperation to provide the real samples.

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