IMMOBILIZATION OF HEPATITIS B ANTIBODY ONTO THE REDUCED GRAPHENE OXIDE

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Abstract - Hepatitis B (Hep B) is a viral infection caused by hepatitis B virus (HBV) that infects the human liver. HBV is blood-borne and transfusion-transmitted human pathogen that has a large impact on blood safety and public health worldwide. Approximately 5% of the Malaysian population is infected by HBV. The treatment to this disease is possible if early detection is done before irreversible liver damage occurs. The presence of Hepatitis B virus surface antigen (HBsAg) is an evidence of the presence of HBV infections. Presently, the conventional methods used for detection shows weakness in terms of time and efficiency. In this research, we aim to study on the feasibility of reduced graphene oxide (rGO) as the base material for immune biosensor for the detection of HBsAg. The graphene oxide was prepared via Modified Hummer's Method due to its ability to produce graphene in large quantity. The fabrication of immunosensor was conducted by immobilizing antibody onto the graphene. Nafion-graphene nanosheet was grown and later treated with Thionine solution. Following that, a solution of primary antibodies against Hep B surface antigen (anti-HBsAg IgG) was incubated on the biosensor. After the immobilization, the antigen was dropped onto the biosensor, followed by incubation of secondary antibodies conjugated to Horseradish Peroxidase (HRP). Upon addition of Tetramethylbenzidine (TMB), the colour of the solution changes to blue.

Keywords - Biosensor, Graphene, Hepatitis B Surface Antigen

INTRODUCTION

Hep B is a disease caused by HBV that attacks the liver. A HBV infection can cause both acute and chronic diseases (Ding *et al.*, 2010) which is a major health problem occurring in all parts of the world (Yap, 1994). The World Health Organisation (WHO) mentioned that approximately 600, 000 people die each year due to the acute or chronic consequences of Hep B. In Malaysia, 5% of its population is affected by Hep B (Lim, 2013). Hence, the best treatment for Hep B is to eliminate the virus before the occurrence of irreversible liver damage. For this to be successful, accurate detection of HBV infections in the early stages is crucial. The conventional methods of detecting HBV such as ELISA and PCR is time consuming (Liu, Qi, & Xiong, 1999), hence, the necessity for low cost, rapid, high selectivity and sensitivity bio-diagnosis markers present at ultra-low level during early stages of infections. Therefore, the research on graphene based biosensor for the purpose of early detection of HBV is crucial in terms of cost and effectiveness.

Graphene is much favored for the usage as a biosensor due to its unique physicochemical properties such as having high surface area and extraordinary electron transfer ability (Geim & Novoselov, 2007) which is useful for the purpose of a biosensor. Previous researches proven that further reduction of graphene oxide improved the properties exhibit by graphene. The presence of a large number of oxygen-containing functional groups and structural defects (Park, *et al.*, 2000) is the reason behind rGO exhibiting enhanced chemical activity compared to graphene.

AIM: To study on the feasibility of rGO as the base material for immuno-biosensor for the detection of HBsAg.

MATERIALS

Nafion 117 solution and thionine acetate salt were obtained from Sigma Aldrich. The Hepatitis B virus surface antigen (HBsAg) was purchased from MP Biomedicals Asia Pacific Pte. Ltd. The Hepatitis B antibodies (anti-HBsAg IgG)

and tetramethylbenzidine (TMB) were purchased from Gene Tex.

METHODS

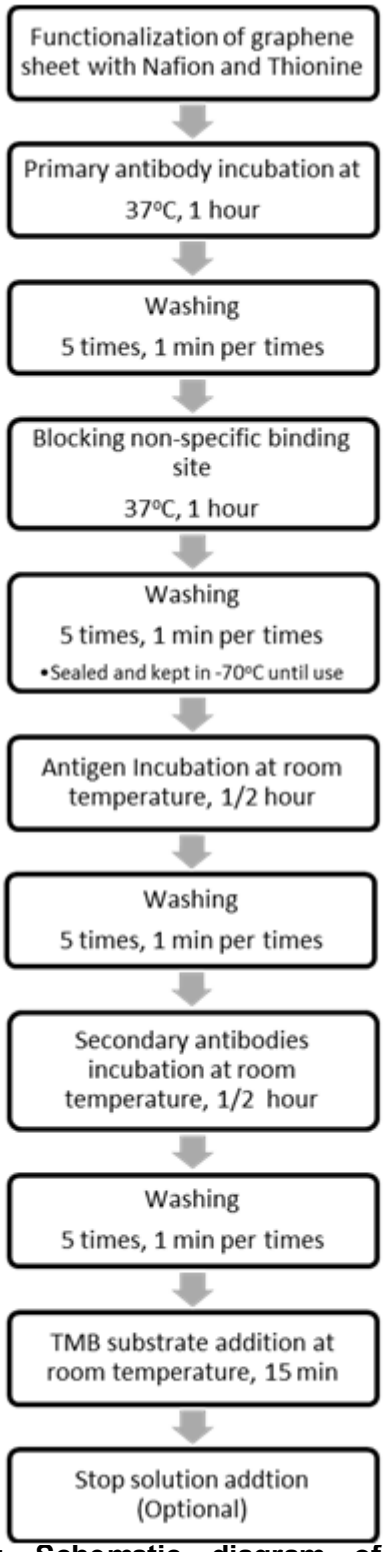

Fig 1: Schematic diagram of the fabrication of rGO biosensor for the detection of HBsAg.

The rGO was prepared by using Modified Hummer's Method (Jae *et al.*, 2010; Su *et al.*, 2009). The prepared rGO was kept in vacuum desiccator until used. Following this is the fabrication of the biosensor. The rGO was used to grow the rGO-Nafion nanosheet by ultrasonication in 0.25% Nafion-water for 30-50 minutes to obtain a homogenous, well distributed suspension of rGO-Nafion composite. The prepared rGO-Nafion solutions were stored until time of use.

Subsequently, 100 μL of rGO-Nafion was dropped onto a treated surface and allowed to dry to fabricate the biosensor. When the rGO-Nafion layer dried, it was dropped with 3mM Thionine solution and soaked for 10 minutes. This is followed by the incubation of primary antibody (100 μL) at 37°C for an hour. Then washed with 1X PBS (250 μL/well) 5 times with an approximate of 1 minute per wash. Block non-specific binding with 1% BSA/PBS by adding 200 μL/well and incubated for an hour at 37°C. The fabricated biosensor was sealed and frozen at -70°C until use.

Fig. 1 shows the methodology developed for the detection of HBsAg using the rGO biosensor. The antigen was introduced onto the sensor and incubated for half an hour at room temperature and followed by washing with 1X PBS 5 times with an approximate of 1 minute per wash. The secondary antibody (conjugated with HRP) was dropped on the sensor and incubated for half an hour at room temperature and followed by washing with 1X PBS 5 times. Then, 100 μL/well of TMB was added. To stop the reaction of TMB, a stop solution (1M H_2SO_4) was added.

RESULTS

The binding of HBsAg with antibody was confirmed with the introduction of TMB substrate at room temperature for 15 minutes. As shown in Fig. 2, TMB substrates detected HRP activity, thus yielding a blue colour solution. After the addition of H_2SO_4, the colour changes from blue to yellow.

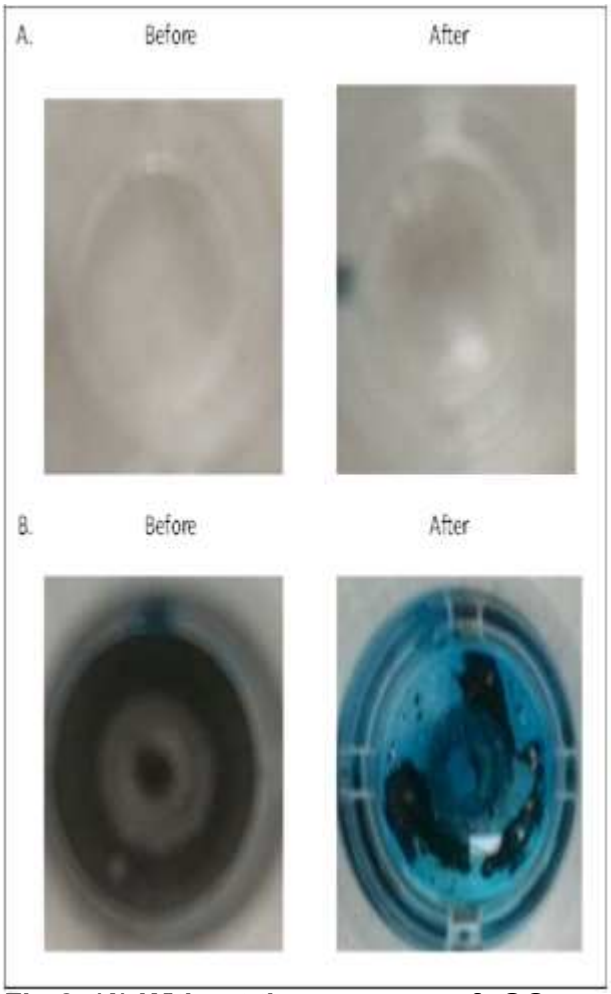

Fig 2: (A) Without the presence of rGO-Nafion (B) Presence of rGO-Nafion. Both undergo the complete immobilization process.

To summarize, the developed immobilization process requires 1 hour and 25 minutes to proof that the Hep B antigen was detected. Hence, the aim of this research was reach as it is feasible to use rGO as the base material for immuno-biosensor for the detection of HbsAg.

DISCUSSION

Summary of results:

1. The development of a rGO based immunoassay for the detection of HBsAg is feasible as shown in the results obtained. The colour changed obtained from visual inspection has proven the feasibility of rGO as a based material for biosensor fabrication. This is probably due to the unique characteristics (Jung *et al.*, 2010) of having high surface area and also the presence of boundless active sites which are used for the binding of antibody which captures the antigen.
2. It also shows that without the presence of rGO, anti-HBsAg IgG is unable bind as there is no base material present. Hence, with the presence of rGO as the base material the anti-HBsAg IgG is able to bind to the rGO and later followed by the HBsAg. TMB is a colourless substance which only changes colour in the presence of HRP contained in the secondary antibody.

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REFERENCES

Ding, C., Li, H., Hu, K., & Lin, J.-M. (2010). Electrochemical immunoassay of hepatitis B surface antigen by the amplification of gold particles based on the nanoporous gold electrode. *Talanta 80*, 1385-1391.

Geim, A. K., & Novoselov, K. S. (2007). The rise of graphene. *Nat. Mater. 6*, 183.

Jae, H., Doo, S., Fei, L., Kang, B., & Tae , S. (2010). A graphene oxide based immuno-biosensor for pathogen detection. *Angew. Chem. Int. Ed.* , 5708-5711.

Jung, J., Cheon, D., Liu, F., & Seo, T. (2010). Graphene oxide based immuno-biosensor for pathogen detection. *Angewandte Chemie International Edition*, 5708–5711.

Lim, Y. (2013, May 30). Malaysians encouraged to screen for hepatitis. Petaling Jaya, Selangor, Malaysia.

Liu, X., Qi, Z., & Xiong, S. (1999). Laboratory diagnostics of viral hepatitis. *Second Ed. People Health Press*, 3-76.

Park, J., Cho, E., Lee, D., Park, J., Le, Y., Choi, E. (2000). Receptor-mediated endocytosis of hepatitis B virus presi protein in EBV-transformed B-cell line. *J. Microbiol. Biotech 10*, 844-850.

Su, H., Yuan, R., Chai, Y., Zhuo, Y., Hong, C., Liu, Z. (2009). Multilayer structured amperometric immunosensor built by self-assembly of a redox multi-wall carbon nanotube composite. *Electrochimica Acta, 54*, 36-45.

Yap, S. F. (1994). Chronic hepatitis B infection in Malaysians. *Malaysian Jounal of Pathology*, 3-6.