

Carbon-Based Nanobiohybrid Thin Film for Amperometric Glucose Sensing

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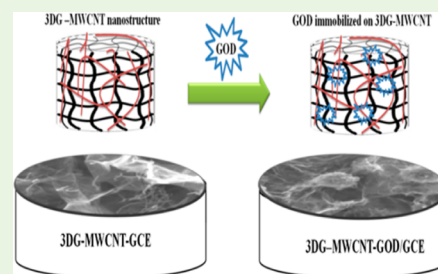
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ABSTRACT: This pioneering study involved the fabrication of a new class of nanohybrid-based electrochemical glucose biosensor. First, three-dimensional (3D) graphene was fabricated as a platform of multiwalled carbon nanotube (MWCNT). Then, it was used to immobilize glucose oxidase (GOD) on nanohybrid thin film via the entrapment technique. The modified glucose biosensor indicated excellent biocatalytic activity toward the glucose measurement with a sensitivity of up to 49.58 $\mu\text{A mM}^{-1} \text{cm}^{-2}$ and a wide linear dynamic range up to 16 mM. The fabricated biosensor shows an excellent stability of 87.8%, with its current diminishing after 3 months. This facile and simple electrochemical method for glucose monitoring using a modified glassy carbon electrode (GCE) by 3DG-MWCNT-GOD could open new avenues in producing of a inexpensive and sensitive glucose nanobiosensors.

KEYWORDS: glucose detection, nanobiohybrid, biomedical application, carbon nanomaterials, electrocatalysis, enzymatic electrodes



1. INTRODUCTION

Recently, carbon-based nanomaterials have been used for adsorption function because of properties such as low density, chemical compatibility, structural diversity, and suitability for large-scale production.^{1,2} 1D carbon allotropes have been used extensively for last few decades in nanoelectronics and bioelectronics due to its outstanding characteristics.^{3,4} Some of their characteristics include significant electrochemical properties, large surface-to-volume ratio, extraordinary mechanical strength, and higher electrical conductivity, which makes them suitable for electrochemical electrodes for the purpose of energy storage and energy conversion and sensing.^{5,6} Graphene is a recent discovery. Although it is structurally 2D, it shares some characteristics with those of CNTs. It reports a unique surface area and mobility, with unparalleled charge carrier.⁷ Thus, graphene is fast substituting CNTs in applications such as electrochemical electrodes, nanoelectronics, and electronic sensors. Several attempts have been made to synergize 2D graphene and 1D CNTs.⁸ Electrochemical electrodes, which are composed of graphene and CNTs, exhibit random networks that are usually planar, in the shape of the carbon materials.⁹ The accumulation between the individual components and random electrical contacts in the structure are responsible for its active surface area and conductivity.¹⁰ The high surface area of electrodes is fundamentally confined by their 2D planar geometry. The compatible electrochemical electrode reports remarkable performance compared to common 2D electrodes used in supercapacitors and biofuel cells.¹¹

This paper focused on modifying glassy carbon electrode using nanobiohybrids of 3DG-MWCNT biocomposite and glucose oxidase (GOD), and its use to detect glucose. Graphite

was used as a starting material for synthesis of the 3DG, and it was subsequently hybridized with MWCNT and GOD. These processes resulted in high linear dynamic range and advisable glucose sensitivity. The high activity and excellent stability of the fabricated nanobiohybrid is indeed excellent for glucose detection.

2. EXPERIMENTAL SECTION

2.1. Apparatus. The potentiostat/galvanostat in a cell of 50 mL that is controlled by NOVA software was used to measure all the electrochemical potentials via a conventional system of three electrodes.¹² The modified GCE that is 3 mm in diameter is the working electrode, the counter electrode was constructed with a platinum rod, whereas the reference electrode was Ag/AgCl_{3 M KCl}. Field-emission scanning electron microscope (FESEM) images were collected using Carl Zeiss SUPRA 35VP, whereas high-resolution transmission electron microscopy (HRTEM) (Hitachi, Japan) images were used to study the morphology of the samples.

2.2. Materials. 0.1 M PBS (phosphate buffered saline) that was used as supporting electrolyte for conducting the electrochemical studies was prepared via the combination of KCl, Na₂HPO₄, and NaH₂PO₄. Graphite powder, GOD, H₃PO₄, NaH₂PO₄, Na₂HPO₄, KMnO₄, and KCl were obtained from Sigma-Aldrich.

2.3. Preparation of 3DG-MWCNT Nanohybrid As a Biocatalyst Support. The primary step for preparing 3D graphene is to synthesize graphene oxide (GO) by exfoliating graphite to form graphite oxide, as per.¹³ To prepare a homogeneous solution, 1 mL of GO was aqueously dispersed in 40 mL of deionized water and the mixture was stirred for 30 min. Then, 0.1 mg of MWCNT and 10 μL

Received: May 24, 2017

Accepted: July 26, 2017

of hydrazine were added into it solution, and its pH was adjusted to 9. It was then sealed inside a 50 mL autoclave for 24 h at 80 °C. The synthesized self-assembled 3D graphene was removed and stored for 48 h inside a freeze-dryer.

2.4. Fabrication of Modified Glassy Carbon Electrode with 3DG-MWCN-GOD. Ten milligrams of synthesized 3DG-MWCNT was placed in a test tube that contains 5 mL of ethanol and it was sonicated for 30 min to obtain a homogeneously dispersed solution. The subsequent step is to add an appropriate amount of GOD into the solution. Then, 5 μ L of the nanobiohybrid solution drop-casted to the top surface of glassy carbon electrode, then air-dried at ambient temperature for 15 min.

3. RESULTS AND DISCUSSION

3.1. Raman Studies. Figure 1 illustrates the Raman spectra of prepared 3D graphene, as well as GO. The spectra for all of

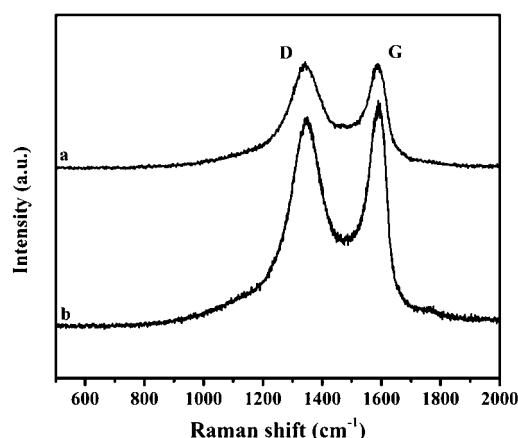


Figure 1. RAMAN spectra result: (a) graphene oxide (GO) and (b) 3D graphene.

the samples exhibit two vibrations at ~ 1348 and 1577 cm^{-1} , which are associated with D-band and G-band for reduced graphene oxide (RGO). D-band is a breaking point of k -point for the A_{1g} symmetry, which is generated by the disruption of the graphitic lattice that is symmetrical and hexagonal in shape.¹⁴ The G-Band is attributed to the phonon of E_{2g} for the symmetric stretching in the case of sp^2 (C–C) bond.¹⁵ The ratio between the intensities of the D and G bands (I_D/I_G) is a measure of the disorder, as expressed by the sp^2/sp^3 carbon ratio.¹⁶ Compared with graphene oxide (1.09), the 3D graphene (0.92) has a slightly higher I_D/I_G value, which is attributed to an increase in size of sp^2 domains upon the crystallization process.¹⁷

3.2. Surface Morphology of 3DG-MWCNT-GOD Nanobiohybrid. Figure 2A shows the FESEM image of 3DG and its highly porous structure. The FESEM image of hybridized 3DG-MWCNT is presented in Figure 2B. Figure 2C shows the hybridized 3DG-MWCNT, while Figure 2D displays the high-resolution transmission electron microscopy (HRTEM) image of the hybridized 3DG-MWCNT. In fact, the porous structure of 3DG and wire-like nature of MWCNT enfolded the GOD prevents the immobilized enzyme from denaturation and leaching while facilitating electron transfer from enzyme-to-electrode and improving the enzyme stability.¹⁸ The surface characterization of 3DG-MWCNT-GOD has been investigated using FESEM and HRTEM analyses.

3.3. Electrochemical Performances of Modified Glassy Carbon Electrode with Nanobiohybrid. As per Figure 3, cyclic voltammograms (CV) was carried out (at pH 7) for

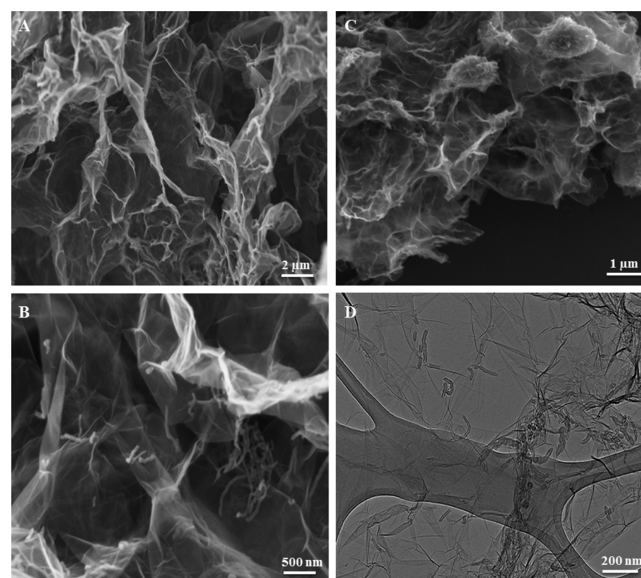


Figure 2. (A) FESEM of 3DG, (B) FESEM of hybridized 3DG-MWCNT, (C) FESEM of successfully immobilized GOD on hybridized 3DG-MWCNT, (D) HRTEM image of hybridized 3DG-MWCNT.

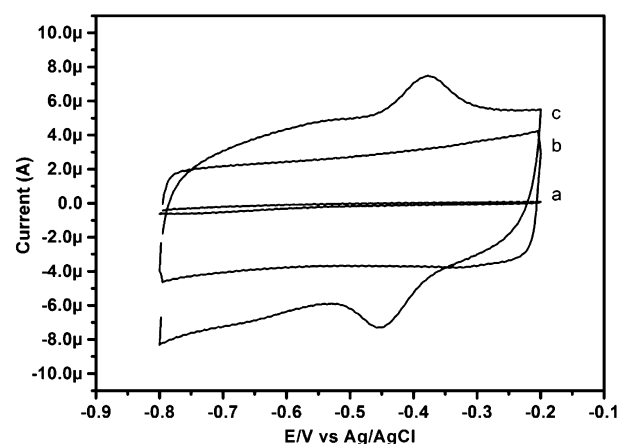


Figure 3. Cyclic voltammetry results at pH 7, (a) bare GCE, (b) 3DG-GCE, (c) 3DG-MWCNT-GOD-GCE.

distinguishing 3DG-MWCNT-GOD-GCE performance with that of bare GCE. The electrochemical peaks attained from the 3DG-MWCNT-GOD-GCE are quite a few times more than GCE. The GOD immobilization at 3DG-MWCNT-GCE confirms by CV analyses, by pair of precise redox peaks at -0.43 V and -0.39 V . Thus, fabricated 3DG-MWCNT-GOD nanobiohybrid is able to transfer electron to the surface of electrode. Consequently, the greater 3DG-MWCNT performance for electrochemical measurement has been confirmed.¹⁹ There is no significant decrease of redox peak current and shift in the peak potential of GOD (FAD/FADH_2) in the CV, which suggests that GOD is highly stable at the 3DG-MWCNT-GOD hybrid biocomposite. Figure 4 indicates the schematic immobilization of the GOD.

Figure 5 shows the linearly cathodic/anodic peak of current from the 3DG-MWCNT-GOD-GCE scaling with the scan rate's square root. It can be seen that the reaction is a surface-controlled quasi-reversible electrochemical route, and that the GOD even at higher scan rates retains its bioactivity.²⁰ Scan

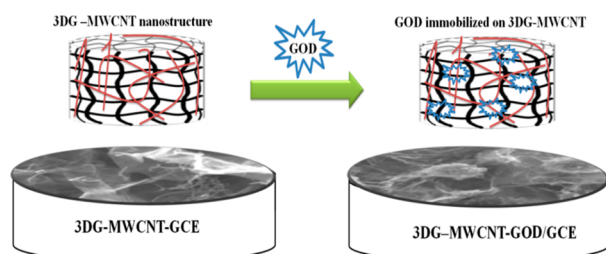


Figure 4. Schematic illustration of 3DG-MWCNT-GOD-GCE.

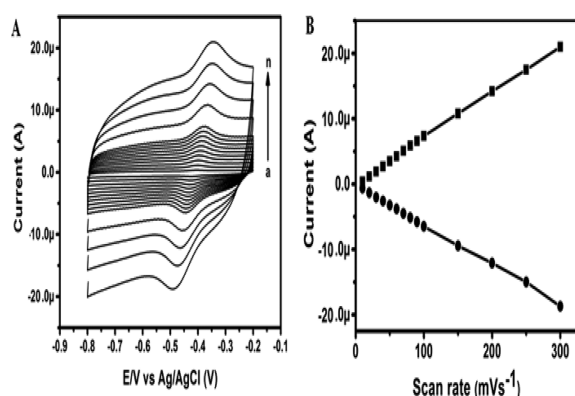
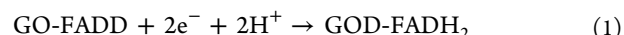


Figure 5. (A) The cyclic voltammetry for 3DG-MWCNT-GOD-GCE at different scan rates in N_2 -saturated solution (pH 7). The scan rates from a to n are 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, and 300 $mV s^{-1}$. (B) Linearly of I_{pa} (■) and I_{pc} (●) on the scan rate (10 to 300 $mV s^{-1}$).

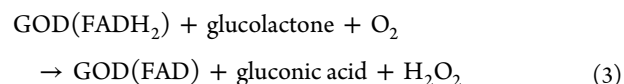
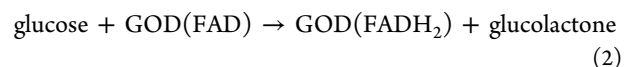
rates on fabricated 3DG-MWCNT-GOD-GC electrode, in N_2 saturated solution, differs from 0.01–0.1 V (pH of 7). As per Figure 5, scan rate is directly correlated to the current peak. Correspondingly, the potential peak was lifted negatively with increasing the scan rates from 10 to 300 $mV s^{-1}$ within −0.8 to −0.2 V.²¹

3.4. Electrocatalytic Activity of 3DG-MWCNT-GOD-GCE for Glucose Monitoring. Figure 6A shows the highly sensitive amperometric response obtained from the modified GCE by 3DG-MWCNT-GOD. The designated PBS concentration was 0.1 M, while applied potential was −0.45 V. The current resulted in a consecutive addition of glucose. As the amount of injected glucose increases, the current of the

amperometric response remains linear, at a range up to 16 mM, proving the high affinity between 3DG-MWCNT-GOD-GCE and glucose. The result confirms that fabricated biosensor is suitable for detection of blood glucose concentration of (4–6 mM) (Figure 6B). However, the detection mechanism of the biosensor remains similar to other subclasses of glucose biosensor, but the immobilization process confirms that the microenvironment changes enzyme and affect the inherent properties of the sensor. The improved detection mechanism could result in the devices' improved affinity to glucose. The results indicate that the redox reaction of GOD 3DG-MWCNT-GOD-GCE belongs to two protons ($2H^+$) and two electrons ($2e^-$) process, as shown in the equation:²⁴



This process is electrocatalysis of glucose by GOD in the presence of oxygen saturated PBS. The possible reaction mechanism at modified electrode can be clarified by following equations:



For confirming the anti-interference property of fabricated nanobiosensors, the influence of interference compounds were studied (Figure 7). The addition of 0.1 mM of dopamine, uric acid, ascorbic acid, fructose, and urea did not increase the current values, although, a significant current change was seen due to adding of 0.1 mM of glucose. The fabricated sensor was rinsed with water and stored, there is almost no reduction of biocatalytic current in glucose after storing the biosensor for a month at 4 °C. Table 1 compares the limit of detection (LOD), dynamic range (LDR) and sensitivity of different graphene-based modified electrodes for glucose detection. The electron transfer mechanism of the GOD in glucose biosensor remains stable and constant after 100 cycles of potential scanning.

3.5. Reproducibility and Stability of Modified Glassy Carbon Electrode with 3DG-MWCNT-GOD. The 3DG-MWCNT-GOD-GC reproducibility and storage stability were checked and verified. Relative standard deviation (RSD) of glucose biosensor for detection of 1 mM glucose was 1.9–3.8%

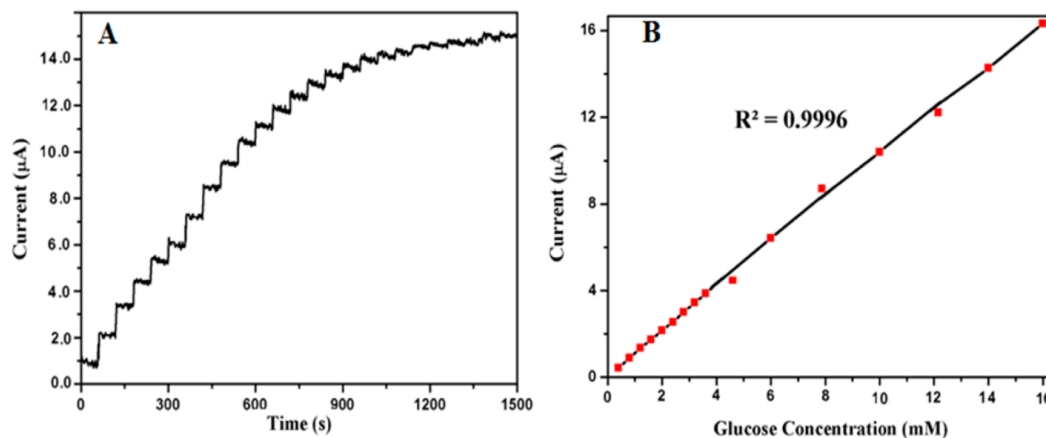


Figure 6. (A) Chronoamperometric result to glucose successive injections in air saturated 0.1 M PBS for 3DG-MWCNT-GOD-GCE at −0.45 V. (B) Calibration curve for modified electrode at different concentrations of glucose.

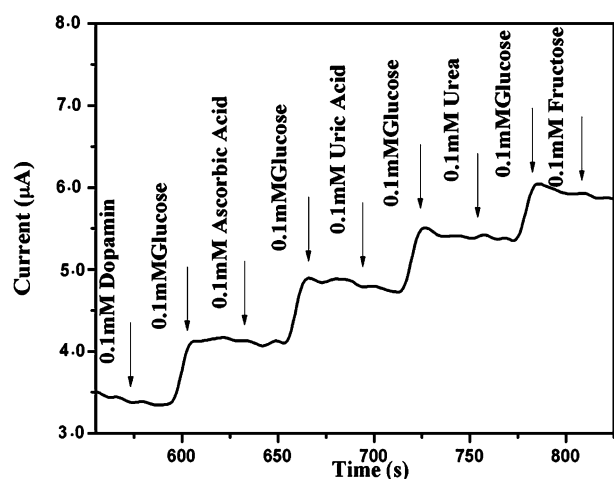


Figure 7. Chronoamperometric result with additions of different interfering species for modified electrode.

Table 1. Limit of Detection (LOD), Linear Dynamic Range (LDR), and Sensitivity of Different Graphene-Based Modified Electrodes for Glucose Detection

modified electrode	limit of detection (LOD) (mM)	linear dynamic range (LDR) (mM)	sensitivity ($\mu\text{A}/\text{mM cm}^2$)	ref
GOD/Cu _{nano} /Gr-nafion/GCE	0.005	Up to 12	34	22
RGO/HAp/GOD-modified GCE	0.003	0.1–11.5	16.9	23
reduce graphene sheet/GOD	0.1	1–10	1.08	24
ERGO-AuPdNPs	0.0069	Up to 3.5	266.6	25
graphene/PANI/Au NPs/GOD	0.001	0.2–11.2	20.32	26
graphene/Au NPs/GOD	0.004	0.02–2.26	3.8	27
C-PPy/graphene/GOD	0.000001	0.01–1		28
graphene/ZnO/GOD	0.01	1–10	0.1	29
glucose oxidase/Pt/functional graphene sheets/chitosan	0.0006	up to 5		30
GCE/PDA-RGO/GOD/PtNPs	0.10	0.2–1.0	33	31
3DG-MWCNT-GOD-GCE	0.0001	Up to 16	49.58	present Study

for five successive additions. The fabricated biosensor was stored in a dry place at intervals of 1 week, and its performance was analyzed. It retained $\sim 87.8\%$ of its original sensitivity after 3 months.

3.6. Analytical Recovery and Glucose Monitoring in Human Blood Serum. The performance of the 3DG-MWCNT-GOD-GCE to glucose detection was examined using human blood serum attained from hospital patients. Prior to its characterization, the obtained blood sample was allowed to clot, and then centrifuged for 15 min to remove the clot. The resulted serum was stored at -20°C . Ten milliliters addition of PBS (pH 7) was used to solve 2 mL of the serum, followed by measuring the blood glucose concentration. According to the results, the blood glucose concentration was 4.8 mM, which agrees with the referenced values of the typical colorimetric method of the hospital (4.90 mM). The fabricated

3DG-MWCNT-GOD-GCE glucose biosensor's analytical recovery for glucose solution was 96 ± 2 , confirming its excellent accuracy of the 3DG-MWCNT-GOD modified electrode.

CONCLUSION

The nanobiohybrid thin film, by using 3DG-MWCNT-GOD-GCE was presented for glucose detection. The fabricated biosensor revealed excellent sensitivity with an LDR of up to 16 mM. The glucose biosensor reports a high response to glucose due to its large specific surface area and the fast electron transfer of entrapped GOD in 3D-MWCNT nanohybrid. Moreover, the modified GCE with 3DG-MWCNT-GOD shows a long-term stability and excellent reproducibility. The glucose sensor was tested using the real sample, with the spiked samples a good recovery was reported. The results declare that the 3DG-MWCNT-GOD nanohybrid biosensor is suitable for home care and clinical applications due to rapid determination of glucose, as well as alcohol, lactate, cholesterol, acetylcholine, xanthine, and hypoxanthine.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank University Malaya for financially supporting the project with a research grant (RP038B-1SHTM).

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