REDUCED GRAPHENE OXIDE-MULTI WALLED CARBON NANOTUBES HYBRID MATERIAL AS ELECTRODE FOR DNA BIOSENSOR

by

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LIST OF ABBREVIATIONS

a.u.	Arbitrary unit
AFM	Atomic-force microscopy
Al	Aluminum
AutoCAD	Automated computer-aided design
BOE	Buffer oxide etch
CH_3COOH	Acetic acid
CNT	Carbon Nanotubes
DIW	Deionized Water
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded DNA
EDC	1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide
EDX	Energy-dispersive X-ray spectroscopy
FESEM	Field Emission Scanning Electron Microscope
FET	Field effect transistor
FTIR	Fourier transform infrared spectroscopy
GO	Graphene oxide
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
HCl	Hydrochloric acid
HNO_3	Nitric acid
HPM	High power microscope
IDEs	Interdigitated electrodes
IV	Current voltage characteristic

$K_2S_2O_8$	Potassium persulfate
$KMnO_4$	Potassium permanganate
LPM	Low power microscope
MWCNT	Multi Walled Carbon Nanotubes
NaOH	Sodium hydroxide
NH_4OH	Ammonium hydroxide
NHS	N-hydroxysuccinimide
P_2O_5	Phosphorus pentoxide
PBS	Phosphate buffer solution
PH	A measure of the acidity or alkalinity of a solution
POC	Point of care
PR	Photoresist
PTFE	Polytetrafluoroethylene
PVD	Physical vapor deposition
RC1	Radio Corporation of America 1
RC2	Radio Corporation of America 2
rGO	Reduced graphene oxide
rGO-MWCNTs	Reduced graphene oxide-Multi Walled Carbon Nanotubes
RPM	Revolutions per minute
SEM	Scanning electron microscope
Si	Silicon
SiO_2	Silicon dioxide
ssDNA	Single-stranded DNA
SWCNT	Single Wall Carbon Nanotubes

LIST OF SYMBOLS

%	Percentages
°C	Degree Celsius
µL	Micro liter
µm	Micro meter
µM	Micromole
C	Concentration
cm	Centimeter
g	Gram
I	Current
L	Liter
mA	Miliampere
mg	Milligram
min	Minute
mm	Millimeter
mL	Milliliter
mV	Millivolt
mole	Molar mass
nm	Nano meter
Pa	Atmosphere standard pressure
rpm	Ramp per meter
s	Second
T	Temperature
Torr	Gas pressure measurement unit
V	Voltage
v/v	Percentage of volume per volume
Ω	Ohm
Θ	Theta
W	Watt

Graphene Dikurangkan Oksida -Multi Walled Carbon Nanotube Hybrid Bahan Elektrod Untuk Dna Biosensor

ABSTRAK

Tesis ini membentangkan rencaman saput nipis graphene oksida terturun-karbon nanotiub berbilang dinding (rGO-MWCNTs) sebagai elektrod saput penderiaan untuk mengesan penetapan dan penghibridan asid deoksiribonukleik (DNA). Projek ini terdiri daripada tiga bahagian iaitu penyediaan dan pencirian rencaman saput nipis rGO-MWCNTs, pembikinan peranti, dan diikuti oleh penetapan dan penghibridan DNA. Dalam bahagian pertama, tesis ini menerangkan penyediaan graphene oksida (GO) daripada serbuk grafit melalui kaedah Hummer yang diperbaiki. Manakala, MWCNTs telah diubahsuai melalui proses pengoksidaan asid nitrik. Proses pengurangan kimia telah dilaksanakan menggunakan pelarut hidrazin sebagai agen pengurangan untuk memperoleh rGO. Seterusnya, bahan hibrid rGO-MWCNTs ini telah disediakan dengan mencampurkan rGO-MWCNTs melalui proses ultrabunyi normal. Bahan-bahan seperti MWCNTs, GO, dan rGO-MWCNTs telah disembur secara mekanikal ke atas permukaan silikon dioksida (SiO_2) saluran peranti dengan teknik sembur. Larutan kitosan telah dicampur bersama bahan-bahan ini dan disembur ke atas permukaan peranti untuk meningkatkan kelikatan dan menguatkan rekatan bahan-bahan tersebut terhadap permukaan SiO_2 dengan menukarkan ciri permukaan daripada hindar air kepada hidrofilik. Morfologi dan topologi bahan-bahan tersebut telah dicerapkan menggunakan mikroskop elektron imbasan pancaran medan (FESEM). Ikatan kimia bahan-bahan tersebut telah diperiksa menggunakan spektroskopi infra-merah jelamaan Fourier (FTIR). Struktur fasa bahan-bahan itu diselidiki melalui belauan sinar-X (XRD). Yang kedua, rekabentuk, pembikinan, dan penilaian peranti tersebut telah diterangkan secara terperinci. Secara khususnya, proses pembikinan peranti terdiri daripada beberapa proses seperti, 1) pengoksidaan untuk penumbuhan lapisan SiO_2, 2) pengendapan wap fizikal (PVD) yang telah digunakan untuk endapan lapisan aluminium di atas substratum silikon untuk membentuk punca dan saliran, 3) merekabentuk, mencetak, dan menggunakan topeng dalam proses pemindahan corak dan 4) proses fotolitografi yang telah dilaksanakan untuk menghasilkan saluran peranti tersebut. Kendalian elektrod itu adalah berdasarkan penjerapan cas permukaan pada antara muka bahan saput. Akhirnya, dalam seksyen penetapan dan penghibridan DNA, di mana novelti penyelidikan ini telah diperkenalkan, penderia biologi ini telah memperlihatkan kepekaan yang tinggi terhadap sasaran DNA pelengkap dengan julat lelurus daripada 500 pM ke 100 pM. Selain itu, penderia biologi ini telah mempamerkan kememilihan, kebolehkeluaran semula, dan kestabilan jangka panjang yang baik untuk pengesanan DNA. Peranti ini telah menunjukkan kemampuan yang mencukupi untuk membezakan antara sasaran DNA pelengkap dan DNA yang berbeza jujukan seperti DNA bukan pelengkap dan DNA tak padanan tunggal. Proses penghibridan DNA bukan pelengkap mempunyai tindak balas terkecil (39 μA) disebabkan DNA terpintal dua tidak terbentuk secara efektif. Manakala, the DNA tak padanan tunggal telah menunjukkan tindak balas yang lebih rendah (55 μA) berbanding DNA pelengkap (65 μA) disebabkan oleh tapak tak padanan tunggal. Kejituan peranti telah diselidik dan didapati pada kadar 11.28%. Memandangkan penderia biologi ini memberikan tindak balas yang amat baik dan memperlihatkan

kemampuan pengesanan yang cemerlang, ianya sangat disarankan untuk pengesan penanda biologi tertentu dan protein-protein sasaran yang lain.

Reduced Graphene Oxide-Multi Walled Carbon Nanotubes Hybrid Material as Electrode for Electrical Sensing

ABSTRACT

This thesis presents a novel thin film of reduced graphene oxide-multiwalled carbon nanotubes (rGO-MWCNTs) composites as a sensing film electrode for Deoxyribonucleic acid (DNA) immobilization and hybridization detection. This project consisted of three parts, which are the rGO-MWCNTs composite thin film preparation and characterization, the device fabrication processes description, and followed by the DNA immobilization and hybridization. In the first part, the thesis describes the graphene oxide preparation from graphite powder using improved Hummers' method. Whereas, the multiwalled carbon nanotubes (MWCNTs) was functionalized through nitric acid oxidation process. Chemical reduction process was used to obtain the reduced graphene oxide using hydrazine as reduced agent. The MWCNTs, GO, and rGO-MWCNTs materials were mechanically sprayed on the silicon dioxide (SiO_2) surface of the device channel using spray technique. Chitosan solution was mixed with the materials and sprayed on the device surface in order to increase the viscosity of the materials and strengthen their adhesion with the silicon dioxide surface by changing the surface characteristic from hydrophobic to hydrophilic. The morphology of the rGO-MWCNTs composite thin films were observed by field emission scanning electron microscope. The bonding of the rGO-MWCNTs were examined using Fourier transform infrared spectroscopy. The phase structure of the materials were confirmed via X-ray powder diffraction. Secondly, the design, fabrication and evaluation of the device were descripted in details. In addition, the device fabrication processes contained of oxidation process for silicon dioxide layer growing, physical vapor deposition process which was used to deposit an aluminum layer on the silicon substrate to form the source and drain, mask designed, printed, and utilized in the pattern transfer process, and photolithography process which was carried out to create the channel of the device. The operation of the electrode is based on the surface charge adsorption of the film material interface. Finally, in the DNA immobilization and hybridization where the novelty of the research introduced, the biosensor demonstrated high sensitivity to the complementary DNA target with a linear range from 500 pM to 100 pM. Furthermore, the biosensor demonstrated good selectivity, reproducibility, and long-term stability for DNA detection. The device has shown sufficient capability to distinguish between targets complementary DNA and different DNA sequences, such as non-complementary and single-mismatched DNA. The hybridization process of the non-complementary DNA has the smallest response (39 µA) due to the double standard DNA was not effectively formed. Whereas, the single-mismatched DNA has shown less response (55 µA) comparing with the complementary DNA (65 µA) due to the single mismatched base. The device accuracy was investigated and found to be 11.28 %. Since, the biosensor responded very well and demonstrated excellent detection capabilities, it is highly recommended to be used in detecting specific biomarkers and other targeted proteins.

CHAPTER 1

INTRODUCTION

1.1. Overview

Point-of-care (POC) monitoring and diagnosis technology is defined as the medical diagnoses testing that brings the diagnosis test immediately and conveniently to the patient place (V. Kumar, Shorie, Ganguli, & Sabherwal, 2015). Due to the increasing demand for POC testing devices, the scientists are investigating the establishment and development of cheap, rapid and small biosensors. This process is growing rapidly every year. The biosensor is defined as a sensing device consisted of combination of a specific biological sensitive material such as (DNA, enzyme, antibody, antigen and organic molecules) integrated with a transducer (Lien et al., 2010). The specific biological material can only recognize and react with a specific analyte; this biochemical reaction can be converted into a measurable signal, usually in the form of electrical, optical, or acoustic form. This signal can be further amplified, processed and analysed. Furthermore, DNA biosensor is one of the sensors that has grown dramatically since the invention of the DNA by Watson and Crick in 1953 (Watson & Crick, 1953).

The biosensor technology was introduced by Clark and Lyons in 1962 (LC & C, 1962), which was an enzyme-based glucose sensor. On the other hand, the concept of the DNA biosensor is depending on the single stranded (ssDNA) molecules of known sequences immobilization on a modified surface of the biosensor device and the complementary DNA target detection through hybridization reaction process. In fact, there are two types of DNA detection methods which are the labelling detection and label-free detection method. The labelling detection is conventional method including the fluorescence, enzymatic-labels or radioactive. The labelling process should be applied to either the DNA probe or the target DNA (Feng, Zhang, Ren, & Qu, 2014). However, the label free detection process do not required any labelling agents to be attached to the DNA and it be performed without any biomolecule modification (Lien et al., 2010). The currently reported label-free detection method are the DNA biosensor, optical biosensors, electrical biosensors, electrochemical biosensors and microgravimeteric sensors. There is various application for the DNA detection such as clinical research, medical diagnoses, criminal investigations and etc. (Teles & Fonseca, 2008).

The capacitor-based biosensors are one of the electrical charge biosensors such as aluminium electrode biosensor, carbon-based material biosensor and screen-printed electrode biosensors. The capacitor-based biosensors have the ability to measure the target biomolecules reaction with the DNA probe by a simple preparation processes. In particular, the biomaterials produced from the binding of the target analytes with the bio-receptors on the surface of the electrode initiate the difference in the distribution of charges, or changed the dielectric properties, or it cause the conductance changes of these sensors (Fathil et al., 2016). These electrical charges usually amplified, recorded and further investigated.

In this project, an aluminium electrode biosensor was fabricated for DNA detection through rGO-MWCNTs hybrid film and demonstrated the electrical sensing mechanism due to its advantages which is high signal-to-noise ratio, excellent compatibility, low cost and miniaturization (Heller, Mãnnik, Lemay, & Dekker, 2009). The gate surface of the electrode coated by rGO-MWCNTs hybrid thin film. The MWCNTs assembled with rGO as a hybrid through non-covalent π-π stacking to form rGO-MWCNTs hybrid material. The rGO-MWCNTs has exceptional properties such as, high chemical stability, high sensitivity, high electrical conductivity and high surface area, thus these properties are making the rGO-MWCNTs suitable to be used in the DNA biosensor. The device mechanism was made by immobilizing well-known ssDNA onto the surface of the transducer, which is the hybrid film of rGO-MWCNTs. The device transducer has the ability to convert the specific recognition process of two single strands DNA through the hybridization process into measurable signal.

1.2. Problem statement

DNA biosensors are highly significant and valued tools in a number of areas related to the human health such as genetic analysis, forensic applications, diagnosis of infection diseases and clinical purpose (Teles & Fonseca, 2008). There are a number of DNA detection methods that are available such as colorimetry (Thavanathan, Huang, & Thong, 2014), fluorescence (Yue, Jiang, Xu, Ma, & Bai, 2015), electrochemistry (Mani, Devadas, & Chen, 2013), electrochemiluminescence (Kochmann, Hirsch, & Wolfbeis, 2012), quartz crystal microbalance (Lien et al., 2010) and surface Plasmon resonance spectroscopy (J. Liu, Liu, Barrow, & Yang, 2015). However, these techniques required high technology instruments and specialized laboratory setups, which are complicated, expensive and time consuming. On the other hand, the aluminum electrode biosensor as capacitor-based biosensor is cost effective, easy to fabricate and can provide good result as point-of-care diagnoses. However, the capacitor-based biosensor required ultra-sensitive material to be deposited on the device gate surface to electrochemically react with the biomolecule in order to perform the DNA probe immobilization and hybridization.

CNTs is promising and considered material to be used as ultra-high sensitive material. The CNTs has reported with high electrical conductivity, excellent electrochemical reaction and stability and good mechanical strength (B. Kim et al., 2012). Furthermore, the DNA biosensors that used CNTs as thin film are usually required some organic solvents to suspend the CNTs, which is insoluble. However, it has been found that the CNTs tend to agglomerate in the organic dispersion because it is highly insoluble (Y. Wang et al., 2011). On the contrary, the addition of the organic solvents is affected the electrical properties of the biosensor and it has some limitations such as the organic solvents are not able to form strong and excellent interaction with biological systems (Tran & Mulchandani, 2015).

Therefore, it would be highly recommended to suspend the CNTs in an aqueous solution, without the need for such surfactants or organic solvents. The graphene oxide has been reported to be better dispersant of CNTs to form hybrid material GO-MWCNTs (Mani, Devadas, et al., 2013) and the rGO-MWCNTs exhibit high electrical conductivity, good surface biocompatibility, large surface area and excellent catalytic properties comparing with the GO (V. Kumar et al., 2015). Therefore, the novelty of this research is to use the rGO-MWCNTs hybrid material as ultra-sensitive thin film for the DNA biosensor.

1.3. Research objective

1.3.1. General research objective

The aim of this research is to fabricate an electrical biosensor and investigate the feasibility of using rGO-MWCNTs hybrid thin film as sensing film for the DNA detection.

1.3.2. Specific Research Objectives

The research accomplished with the following specific objectives:

i. To prepare and characterize the functionalized MWCNTs, GO, and rGO-MWCNTs hybrid and use rGO-MWCNTs thin film as sensing film for DNA detection.
ii. To design and fabricate aluminum electrode biosensor based on rGO-MWCNTs hybrid thin film as sensing film for DNA detection application.
iii. To determine the sensitivity, selectivity, stability, reusability and the electrical properties of the rGO-MWCNTs based biosensor.

1.4. Research scope

This research is embarked based on the following scopes; the first scope of this research project is to prepare the rGO-MWCNTs hybrid thin film as a sensing film, which is deposited on the gate of the device. There are four main processes applied in order to obtain the rGO-MWCNTs hybrid composite material, which are the MWCNTs