Impedimetric-based Biosensor for Cardiac Troponin I Detection: Sensing Strategy aided by Hybrid rGO and Gold Interdigitated Electrode

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

1D	1 Dimensional
2-ABA	2-aminobenzyl Amine
2D	2-Dimensional
3D	3-Dimensional
4-ATP	4-aminothiophenol
AFM	Atomic Force Microscope
Ag	Argentum
AMI	Acute Myocardial Infarction
APTES	(3-Aminopropyl) triethoxysilane
Au	Aurum
Au-IDE	Gold Interdigitated Electrode
Au-SPE	Gold Screen Printed Electrode
BA	Blocking Agent
CK-MB	Creatine Kinase-MB
CNT	Carbon Nanotube
CO_2	Carbon Dioxide
C-RP	C-reactive Protein
cTnI	Cardiac Troponin I
cTnT	Cardiac Troponin T
CuO	Copper Oxide
CV	Cyclic Voltammetry
CVDs	Cardiovascular Diseases
CVD	Chemical Vapor Deposition
DIW	Deionized Water
DNA	Deoxyribonucleic Acid
DPV	Differential Pulse Voltammetry
EDC	1-ethyl-3-dimethylaminopropyl Carbodiimide
EDX	Energy Dispersive X-ray
EIS	Electrochemical Impedance Spectroscopy
FET	Field-Effect Transistor
FTIR	Fourier-Transform Infrared Spectroscopy
GCE	Glassy Carbon Electrode
GMWCNT	Graphene Multi Walled Carbon Nanotube
GO	Graphene Oxide

GQD	Graphene Quantum Dot
HPM	High Power Microscope
IDE	Interdigitated electrode
ITO	Indium Tin Oxide
LOB	Limit of Blank
LOD	Limit of Detection
M1	Method-1
M2	Method-2
M3	Method-3
Mb	Myoglobin
MnO_2	Manganese (II) Oxide
NCDs	Noncommunicable Diseases
NHS	N-hydroxysuccinimide
NiO	Nikel Oxide
NMP	N-Methyl-2-Pyrrolidone
PAMAM	Polyamidoamine
PBE	Paper-based Electrode
PBS	Phosphate Buffer Saline
Pd	Palladium
PDDA	Poly-diallyldimethylammonium Chloride
PEI	Polyethyleneimine
Pr-GO	Porous Graphene oxide
Pt	Platinum
rGO	reduced Graphene Oxide
RSD	Relative Standard Deviation
S-1	Strategy-1
S-2	Strategy-2
S-3	Strategy-3
S-4	Strategy-4
SEM	Scanning Electron Microscope
SnO_2	Tin (II) oxide
SPE	Screen-printed electrode
STM	Scanning Tunneling Microscope
TEM	Transmission Electron Microscope
TiO_2	Titanium Oxide
UV-Vis	Ultraviolet – Visible

WO3-rGO	Tungsten Trioxide reduced Graphene Oxide
XPS	X-ray Photoelectron Spectroscopy
XRD	X-ray Diffraction
ZnO	Zinc Oxide

LIST OF SYMBOLS

µL	Microliter
µM	Micrometer
Å	Angstrom
AC	Alternating Current
C	Capacitance
˚C	Celsius
C_{dl}	Double Layer Capacitance
eV	Electronvolt
G	Conductometric
Hz	Hertz
I	Current
J	Imaginary Part
mM	Milimolar
nM	Nanometer
R	Resistance
R_{ct}	Charge Transfer Resistance
R_s	Solution Resistance
V	Voltage
Z	Impedance
Z’	Real-part Impedance
Z”	Imaginary-part Impedance
σ_{lowest}	Standard Deviation of Lowest Concentration
σ_{probe}	Standard Deviation of Probe

Biopenderia berasaskan Permeteran Galangan untuk Pengesanan Troponin Jantung I: Strategik Penderiaan dibantu oleh rGO Hibrid dan Elektrod Berselang-seli Emas

ABSTRAK

Penyakit kardiovaskular (CVD) adalah penyebab nombor satu kematian antara penyakit tidak berjangkit di peringkat global. CVD merujuk kepada penyakit-penyakit yang berkaitan dengan aliran darah yang tidak normal di jantung dan sering dikaitkan dengan keadaan 'serangan jantung' atau dalam istilah klinikal sebagai infarksi miokardium akut (AMI). Biopenanda Troponin jantung I (cTnI) diterima secara meluas sebagai "*piawaian emas"* untuk pengesanan AMI kerana pengkhususannya yang amat tinggi. Oleh itu, keperluan penderia mudah-alih dan peka dengan pengesanan pantas adalah sangat diperlukan untuk mendiagnosis AMI bagi rawatan cepat. Di sini, imunopenderia cTnI berasaskan galangan tanpa-label telah difabrikasi dan prestasi analitik telah dinilai dan dilaporkan dalam tesis ini. Grafena kekurangan oksida (rGO) dan saling berkait elektrod berpadu emas (Au-IDE) digunakan untuk membangunkan imunopenderia. Kaji-selidik ini terbahagi kepada dua bahagian utama, pertama adalah untuk mendapatkan pengendapan rGO yang seragam melalui teknik jatuh-tuangan titisan-tunggal dan yang kedua adalah untuk memfabrikasikan imunopenderia cTnI yang dibantu oleh rGO. Keputusan pengendapan mendedahkan kaedah-3 yang melibatkan teknik pasca-sonikasi menghasilkan nanokepingan rGO yang besar dan memasang-diri melalui jatuh-tuangan titisan-tunggal tanpa menggunakan pelarut kimia. Tambahan pula, peranti Au-IDE yang diubahsuai dengan rGO menunjukkan mobiliti elektron yang sangat baik di mana kekonduksian elektrik meningkat kira-kira 1000 kali ganda berbanding dengan peranti kosong. Oleh itu, ampaian rGO yang disediakan telah digunakan untuk memfabrikasi imunopenderia. Empat strategik pengubahsuaian permukaan yang berbeza pada bioelektrod masing-masing dibantu oleh ampaian rGO telah dikaji-selidik. Spektroskopi galangan elektrokimia (EIS) telah dijalankan untuk mencirikan imunopenderia dengan penyapuan frekuensi dari 0.1 - 500 kHz pada voltan AC kecil (25 mV). Hasil kajian menunjukkan bahawa satu daripada empat imunopenderia yang dibangunkan melalui strategik-4 menunjukkan prestasi analitik yang amat baik untuk pengesanan antigen cTnI dalam pepaku penimbal dan serum manusia. Fungsionalisasi permukaan yang berpotensi ini dibantu oleh kumpulan berangkap satah dasar rGO dan telah dipostulat berdasarkan analisa XPS dan FTIR. Imunopenderia yang difabrikasi menunjukkan julat lelurus yang luas (10 ag/mL - 100 ng/mL) dalam pengesanan antigen cTnI dalam serum manusia dengan pekali regresi linear (R^2) sebanyak 0.9716 dan analit terendah yang dikesan adalah 10 ag/mL, di mana ia menunjukkan sifat yang sangat selektif dan peka. Imunopenderia ini juga menunjukkan pengesanan pantas yang hanya memerlukan 5 minit untuk kuar dan sasaran mengikat dan stabil selama sembilan hari. Bioelektrod ini boleh dihasilkan semula. Oleh itu, strategik pengubahsuaian permukaan pengesanan elektrod seperti ini dapat diekstrapolasi lebih jauh untuk aplikasi selanjutnya dalam diagnosis berbagai penyakit yang dibantu oleh biopenanda.

Impedimetric-based Biosensor for Cardiac Troponin I Detection: Sensing Strategy aided by Hybrid rGO and Gold Interdigitated Electrode

ABSTRACT

Cardiovascular diseases (CVD) are the number one cause of death among the noncommunicable diseases globally. CVD are referred to diseases that associated with the abnormalities of blood flow in heart and often related with 'heart attack' condition or in clinical term as an acute myocardial infarction (AMI). Cardiac Troponin I (cTnI) biomarker is widely accepted as *gold standard* for AMI recognition due to its high specificity. Hence, the need for portable and highly sensitive sensor with fast detection is utmost required to diagnose AMI for fast treatments. Herein, a label-free impedimetric-based cTnI immunosensors were fabricated and their analytical performances were assessed and reported in this thesis. Reduced graphene oxide (rGO) and gold interdigitated electrode (Au-IDE) were employed to develop the immunosensors. The research work is divided into two main sections; first is to obtain a uniform deposition of rGO through single-droplet drop-casting technique and second is to fabricate the cTnI immunosensor aided by rGO. The deposition results revealed the method-3 involving post-sonication technique produces a large and self-assembled of rGO nanoflakes through single-droplet drop-casting without the use of any chemical solvent. Furthermore, the rGO modified Au-IDE devices show an excellent electron mobility, whereby the electrical conductivity was enhanced approximately ~1000-fold compared to the bare devices. Thus, the as-prepared rGO suspension was used to fabricate the immunosensors. Four different surface modification strategies on respective bioelectrodes mediated by the as-prepared rGO suspension were investigated. Electrochemical impedance spectroscopy (EIS) was performed to characterize the immunosensors by sweeping frequencies from 0.1 - 500 kHz at a small AC voltage (25 mV). Results revealed that one of the four immunosensors developed through strategy-4 shows an excellent analytical performance for the cTnI antigen detection in spiked buffer and human serum. A potential surface functionalization mediated by rGO basal plane functional groups was postulated based on XPS and FTIR analyses. The as-fabricated immunosensor showed a wide linear range (10 ag/mL – 100 ng/mL) of cTnI antigen detection in human serum with a linear regression coefficient (R^2) of 0.9716 and the lowest analytes detected was 10 ag/mL, whereby it shows highly selective and sensitive trait. The immunosensor also showed a fast-detection that only required 5 minutes for probe and targets binding and stable for nine days. The bioelectrodes were highly reproducible. Hence, such an electrode sensing surface modifications strategy can be further extrapolated for later applications in various biomarker mediated disease diagnoses.

CHAPTER 1 : INTRODUCTION

1.1 Background Study

Home is a living place for human mankind and biosensors becoming a part of human life which revolutionizing disease diagnosis methods. A biosensor is a diagnostic device predominantly used in healthcare sectors especially for disease recognitions. It offers numerous benefits such as portability, rapid detection, inexpensive, easy to use and eliminates the need for highly trained personnel. Technically, a 'biosensor' is an integrated device that contains two major components namely a biological recognition element (bioreceptor/probe) and a transducer (electrode) where both components are engaged in a specific way to provide a specific analytical information (Ribeiro & Cordeiro, 2020; Sabu & Henna, 2019; Souto, 2017). Figure 1.1 (a) shows the generic components of a biosensor that mainly comprises of bioreceptor and transducer. Biosensors can be classified based on bioreceptors (e.g. DNA biosensors, enzymatic biosensors, immunosensors, aptamer biosensors) or transducers (e.g. optical biosensors, electrochemical biosensors, piezoelectric biosensors). Besides this classification, biosensors also can be categorized as label-based or label-free detection as shown in Figure 1.1 (b). Label-based detection requires "labelling" or "tagging" procedures to bioreceptors in order to detect specific targets (Cohen & Walt, 2019; Sang, Wang, & Feng, 2016). Several examples of label-based detection are fluorescent, chemiluminescent and nanoparticle-labelling. Label-based assay involves more complex procedures, time consuming and requires special instrumentations, thus very expensive (X. Luo, Davis, & Davis, 2013; Syahir & Usui, 2015; Y. Zhou, Fang, & Ramasamy, 2019). Furthermore, some synthetic challenges during labelling procedures may exhibit interference with the

bioreceptor binding site and it could alter an intrinsic nature of the bioreceptor. Meanwhile, a label-free detection has overcome the above-mentioned limitations and it provides a great deal of information on biological interaction event. An electrochemical biosensor is an example of label-free detection. Recently, research on label-free detection by using electrochemical biosensor grows tremendously because it offers numerous benefits such as simple fabrication procedure, real-time diagnosis, high sensitivity, high selectivity and cost effective (Luka & Ahmadi, 2015; Ranjan, Parihar, & Jain, 2020; Roointan, Ahmad Mir, & Ibrahim Wani, 2019). The electrical measurements modes are based on voltammetry, amperometry, conductometric or impedimetric transductions, which depending upon the transducer's structure.

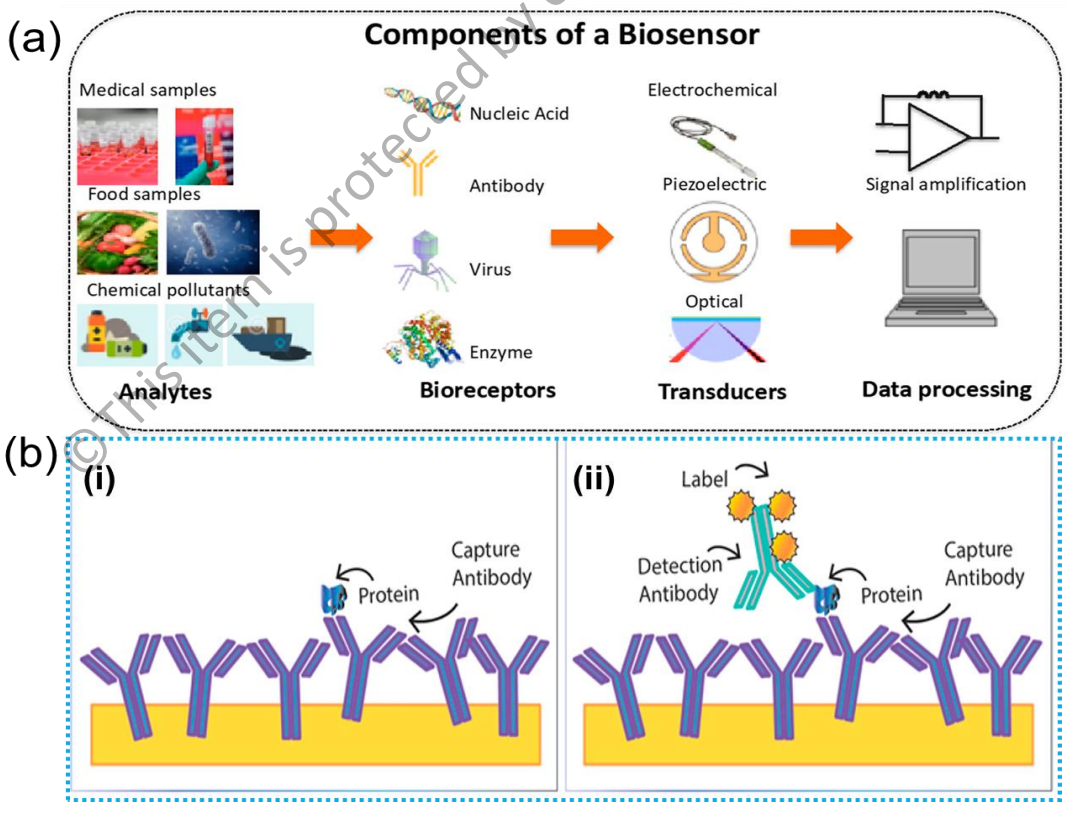

Figure 1.1 (a) Schematic illustration of classification and components in biosensor (Y. Zhou et al., 2019). (b) comparison between (i) a label-free and (ii) a label-based immunosensor (Cohen & Walt, 2019).

In mean time, the advent of nanotechnology has led to application of various types of nanomaterials (carbon-based nanomaterials, metal nanoparticles) in biosensors development. This is due to their high surface area to volume ratio which enhances bioreceptors immobilization area, thus escalates biosensors analytical performance tremendously (eg. limit of detection, sensitivity and selectivity) (W. Jiang, Rutherford, Vuong, & Liu, 2017; Shrivas et al., 2020). In this context, graphene-based nanomaterials have attracted a strong scientific and technological interest owing to its unique optical, electronic, mechanical, electrochemical and thermal properties (K.S. Novoselov, 2004). The number of research articles that published containing the word "graphene" is significantly increased year by years and the major portion of the research are devoted to biomedical fields, ranging from biosensors, drug delivery, stem cell, tissue engineering and imaging therapy (Mao et al., 2013; Scognamiglio & Arduini, 2019). Graphene was first discovered by A. K Geim and Novoselov through a technique known as mechanical exfoliation or scotch tape cleavage technique (J. W. Jiang, 2015), (Novoselov & Geim, 2004a). Since then, several nanomaterials pertaining to the graphene are known and obtained in the efforts to synthesize the pristine graphene. The nanomaterials can be identified as graphene-based nanomaterials or in other words as 'graphene derivatives' which include graphene oxide (GO) and reduced graphene oxide (rGO) (Kochmann, Hirsch, & Wolfbeis, 2012).

Pristine graphene is defined as a single 2D planar/a flat monolayer of sp^2-bonded carbon atoms that are tightly arranged in honeycombs lattice structure (A. K. Geim, 2007). Graphene having a strong ambipolar electric field effect, a very high crystal quality structure and exhibit massless Dirac fermions characteristics because of the valence band and the conduction band touch the Brillouin zone corners (Mao et al., 2013).

Meanwhile, GO is a precursor material in the effort to synthesis the pristine graphene and it is widely used in micro- and nano-environment especially in biosensors and bioelectronics fields. This is due to a large number of oxygen functional groups in GO (Paredes, Marti, Tasco, & Martı, 2008; Stankovich, Dikin, & Piner, 2007). GO is electrically insulating material because the sp^2 bonding networks are disrupted with oxygen-containing functional groups. The electrical conductivity of GO can be recovered by restoring the π-network through the reduction methods, yielding rGO. In general, rGO is a flat monolayer composed of carbon and oxygen atoms whereby some oxygenated groups have been removed. Hence, the rGO shows an improvement in electrical conductivity and the remaining oxygen groups are still useful to develop biosensors (Gilje, Han, Wang, Wang, & Kaner, 2007). Moreover, rGO also resemble pristine graphene in terms of electrical, thermal, mechanical and optical properties. It exhibits a large surface area to volume ratio with good electrical conductivity that act as "electron wire" between bioreceptor and electrode surface (G. Wei, 2015).

Based on the above literature surveys, clearly a biosensor with label-based detection has its own limitations and the development of label-free biosensor is much preferred. Besides that, an integration of nanomaterials with biosensor hold a great promise for high analytical performances. The nanomaterials can improve biosensor's physicochemical properties such as electro-active surface area and electron transfer rate. The success of the best performs biosensor depend on the right selection of bioreceptors and electrode surface modifications technique (Choudhary, Kumar, & Singh, 2013). Hence, in this thesis, a label-free impedimetric-based biosensor using rGO and gold interdigitated electrode (Au-IDE) is presented to demonstrate the detection of cardiac troponin I (cTnI) biomarker.

1.2 Problem Statements

Cardiovascular diseases (CVD) are the leading cause of death among the noncommunicable diseases (NCDs) globally. According to the latest report based on NCDs country profiles by World Health Organization, the CVD were responsible for 17.9 million death accounting for 44% of total 41 million NCDs death world-widely on 2016 (World Health Organisation, 2018). A CVD is referred to a collection of diseases that associated with abnormalities of blood flow in arteries and is often related to 'heart attack' or in clinical term as an acute myocardial infarction (AMI). It was reported, 85% from 17.9 million of CVD deaths globally was due to heart attack. The current AMI diagnoses methods are based on the analysis from electrocardiogram, echocardiogram, cardiac computer tomographic or coronary angiogram. These methods required hospitalization, medical experts, costly equipment and time-consuming as well as expensive. Hence, an early stage detection plays a vital role in preventing this fatal disease to save human life. With respect to that, cardiac troponin I (cTnI) is considered as *gold standard* biomarker for AMI recognition because it is released from myocardial cells upon cardiac injury and has a very high specificity (Singal & Srivastava, 2014; Tuteja & Bhalla, 2014; Q. Wu, Li, Sun, & Wang, 2017). Thus, a label-free based biosensor with high sensitivity and selectivity that offers fast detection is indeed essential for early treatments. Hence, an integration of rGO with transducer will facilitates to materialise such a high-perform biosensor. However, the development of such biosensor using rGO possess new challenges in the electrode surface modifications. As mentioned earlier, rGO contains plenty amount of oxygen functional groups at the basal plane and at the edge of the nanosheet. The oxygenated groups such as carboxyl and carbonyl are located at the edges, whereas hydroxyl and epoxy are located at the basal plane of the