



**ELECTRICAL LABEL-FREE SENSING OF  
CARDIAC TROPONIN BIOMARKER:  
FET-BASED INTEGRATION WITH  
SUBSTRATE-GATE COUPLING**

by

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## TABLE OF CONTENTS

	<b>PAGE</b>
<b>DECLARATION OF THESIS</b>	<b>i</b>
<b>ACKNOWLEDGMENT</b>	<b>ii</b>
<b>TABLE OF CONTENTS</b>	<b>v</b>
<b>LIST OF FIGURES</b>	<b>ix</b>
<b>LIST OF TABLES</b>	<b>xiv</b>
<b>LIST OF ABBREVIATIONS</b>	<b>xv</b>
<b>LIST OF SYMBOLS</b>	<b>xix</b>
<b>ABSTRAK</b>	<b>xxi</b>
<b>ABSTRACT</b>	<b>xxii</b>
<b>CHAPTER 1 INTRODUCTION</b>	<b>1</b>
1.1 Background	1
1.2 Research Problem Statement	3
1.3 Research Objectives	5
1.4 Research Scopes	6
1.5 Thesis Organization	8
<b>CHAPTER 2 LITERATURE REVIEW</b>	<b>10</b>
2.1 Introduction	10
2.2 Acute Myocardial Infarction	10
2.2.1 Conventional Method for Diagnosis of AMI	11
2.2.2 Cardiac Biomarkers as Alternative Method for Diagnosis of AMI	13
2.2.2.1 Cardiac Troponin	13

2.2.2.2	Conventional Assay for the Cardiac Troponin Detection	16
2.3	Biosensors for Cardiac Troponin Detection	19
2.3.1	Biosensors	19
2.3.1.1	Bio-receptor	20
2.3.1.2	Transducer	22
2.3.2	Label-based Biosensor	23
2.3.2.1	Chemiluminescence immunoassays	23
2.3.2.2	Fluorescence immunoassays	26
2.3.2.3	Colorimetric detection	27
2.3.3	Label-free Biosensor	29
2.3.3.1	Surface Plasmon Resonance	30
2.3.3.2	Electrical Biosensor	34
2.4	Field-Effect Transistor-based Biosensor	45
2.4.1	History	45
2.4.2	Principle of Operation	46
2.4.3	Immobilization Techniques of Bio-receptor	48
2.4.4	Integration with Additional Gate	49
2.4.5	FET-based Biosensors for Detection of Cardiac Troponin Biomarker	50
2.4.5.1	Silicon Nanowires	53
2.4.5.2	Conducting Polymers	58
2.4.5.3	Tin Oxide Nanobelt	61
2.4.5.4	Carbon-based Materials	62
2.5	Nanostructured Metal-oxide Materials for Biomolecules Detection	64
2.5.1	Zinc Oxide	66

2.5.2	Synthesis of Zinc Oxide via Sol-gel and Spin-Coating Techniques.	66
2.5.3	Surface Funtionalization of Zinc Oxide	70
2.6	Chapter Summary	70
<b>CHAPTER 3 METHODOLOGY</b>		<b>72</b>
3.1	Introduction	72
3.2	Selectivity Verification of MAb-cTnI as Bio-receptor via ELISA	74
3.3	Device Modelling of FET-based Biosensor	75
3.4	Synthesis of ZnO-NPs Seed Solution	77
3.5	Fabrication of ZnO-FET Biosensor	78
3.6	Surface Functionalization and Immobilization of ZnO-FET Biosensor	81
3.7	Characterizations	83
3.7.1	ZnO-NPs Thin film Characterization	83
3.7.2	Surface Functionalization and Immobilization Characterization	83
3.7.3	Electrical Characterization of ZnO-FET Biosensor	84
<b>CHAPTER 4 RESULTS AND DISCUSSIONS</b>		<b>86</b>
4.1	Introduction	86
4.2	Selectivity Verification of MAb-cTnI as Bio-receptor	87
4.3	Fabricated Device Structure	90
4.4	ZnO-NPs Thin Film Characterization	93
4.4.1	Surface Morphology	93
4.4.2	Structural and Crystalline Phase	96
4.4.3	Electrical Characteristics	97
4.5	Surface Functionalization and Immobilization Characterization	99
4.5.1	Fourier-transform Infrared	99

4.5.2	X-ray Photoelectron Spectroscopy	103
4.6	Susbrtate-gate Effect on Electrical Characteristic of ZnO-FET Biosensor	106
4.7	Detection of Cardiac Troponin I via ZnO-FET biosensor	111
4.7.1	Device Modelling Simulation	112
4.7.2	Electrical Characterization	114
4.7.3	Sensitivity	115
4.7.4	Limit of Detection	118
4.7.5	Selectivity	120
4.7.6	Reproducibility	121
<b>CHAPTER 5 CONCLUSION</b>		<b>124</b>
5.1	Introduction	124
5.2	Conclusion	124
5.3	Future Works	130
<b>REFERENCES</b>		<b>133</b>
<b>APPENDIX A</b>		<b>150</b>
<b>APPENDIX B</b>		<b>151</b>
<b>LIST OF PUBLICATIONS</b>		<b>152</b>
<b>LIST OF AWARDS</b>		<b>163</b>

## LIST OF FIGURES

NO.		PAGE
2.1	ST segment in schematic representation of ECG. (a) normal patient ECG (Becker, 2006); (b) patient with NSTEMI ECG (Daga et al., 2011).	12
2.2	Tn complex build from cTnI, cTnT and cTnI in cardiac muscle (Shave et al., 2010).	14
2.3	Release time of several cardiac biomarkers after AMI (Wright et al., 2011).	14
2.4	Protein sequence of human cTnI protein (UniProtKB, 2007).	15
2.5	Heart-related problem based on cTn concentration level (Agewall et al., 2011).	16
2.6	ELISA used to detect antigen in a given sample. Different concentrations of the Tn can be titrated. Appropriate primary Ab to be used for binding. For detection, enzyme-conjugated anti-rabbit/mouse need to be used as a secondary Ab. Substrate is used for the colour development and measured at an appropriate wavelength (Tian, Xie, Fu, Pei, & Zhao, 2012).	17
2.7	The representation of biosensor (Grieshaber et al., 2008).	20
2.8	Biomolecule interaction of Ab (bio-receptor) binds with respective analyte (target). (a) Before and (b) direct format, and (c) Indirect format (Monošik et al., 2012).	21
2.9	Various classes of transducers utilized in biosensors (Monošik et al., 2012).	22
2.10	Mitsubishi PATHFASTs principle of operations (Kurihara et al., 2008).	25
2.11	Schematic diagram of the sandwich immunoassay using antigen/Ab binding (System 1) and avidin/biotin affinity binding (System 2) on the FMGC. The avidin/biotin couple was used to enhance the signal (S. Y. Song et al., 2011).	27
2.12	Schematic diagram of the silver enhancement colorimetric experimental procedure for detection of cTnI using PDMS–AuNPs composite films biosensor (Wu et al., 2010).	28

2.13	Basic setup for SPR. Changes in the refractive index are shown with changes in the angles. Changes in the optical waves are indicated (Homola et al., 1999).	31
2.14	Ultra-sensitivity and high selectivity electrical biosensor showing the advantages i.e. enables integration on a chip, allows multiplexed detection of several biomarkers at once, and real sample monitoring in real time (Chua, Chee, Agarwal, Wong, & Zhang, 2009).	35
2.15	(i) Measurement setup. (ii) Structure of the biosensor and placement of the Ab-antigen complex. (iii) Biosensor response to cTnI. (a) Capacitance versus frequency characteristics: after biosensor fabrication, (b) after anti-cTnI immobilization, and (c) after dropped cTnI solution (de Vasconcelos et al., 2009).	37
2.16	Comparison between (a) bulk silicon PMOS FET (Neamen, 2010) and (b) SOI FET-based biosensor (S. Liu & Guo, 2012) in term of device architecture and electrical characteristic.	47
2.17	(a) Fabrication of SiNW based FET devices. (i) Thermal oxidation to thin the device layer of the SOI wafer down to ~50 nm. (ii) Thin film of silicon dioxide (SiO <sub>2</sub> ) deposited by PECVD. (iii) SiO <sub>2</sub> patterned by lithography. (iv) Wet etching using the TMAH solution to produce SiNWs. (v) Deposition of metal contact for source, and drain, followed by rapid thermal annealing to obtain ohmic contact. (vi) Fabrication of SiO <sub>2</sub> /SiN <sub>x</sub> passivation layer by PECVD, lithography and RIE processes, followed by covalent modification of MAb-cTnI (Kong et al., 2012). (b) Schematic diagram of the chemical process for surface functionalization of SiNW devices. The hydroxyl-terminated SiO <sub>2</sub> surface of the nanowire binds to the silanol groups of APTES. GA converts the amine-terminated surface to an aldehyde-terminated surface that is able to bind with the N-terminus of the MAb-cTnI. Antigen-Ab interactions cause cTnI to bind specifically to the MAb-cTnI probes on the surface, causing changes in SiNW conductance (Chua et al., 2009).	55
2.18	The cross-section view of the single PANI nanowire-based biosensor (I. Lee et al., 2012).	60
2.19	Schematic diagram of the cTnI sensing scheme depicting the detailed assembly procedure of Abs on the nanobelt surface and subsequent detection of the antigen (Cheng et al., 2011).	61
2.20	The sol-gel technique overview. Synthesis examples of (a) films from a colloidal sol, and (b) powder from a colloidal sol transformed into a gel (Lamia Znaidi, 2010).	67

3.1	Overall methodology in development of ZnO-FET biosensor integrated with substrate-gate coupling for detection of cTnI target biomarker.	73
3.2	The process of selectivity verification of MAb-cTnI as bio-receptor via conventional indirect ELISA.	75
3.3	Device modelling simulation process flow.	75
3.4	Illustration of designed ZnO-FET biosensor simulated in Silvaco ATLAS software.	76
3.5	Synthesis of ZnO-NP seed solution.	78
3.6	Fabrication process of ZnO-FET biosensor integrated with substrate-gate coupling.	79
3.7	Overall stages of the surface functionalization and immobilization on the ZnO-NPs thin film.	82
3.8	Electrical label-free detection of cTnI biomarker by ZnO-FET biosensor integrated with substrate-gate coupling. (a) The 3-dimension (3D) representation of the biosensor. (b) The surface functionalization of ZnO-NPs thin film with APTES and GA; followed by surface immobilization process with MAb-cTnI for capturing cTnI biomarker. (c) The fabricated biosensor, comparable in size with Malaysia's 5 cent.	85
4.1	Visual inspection on the microtiter plate for selectivity verification of MAb-cTnI as bio-receptor by using conventional ELISA.	87
4.2	Optical density versus sample concentration graph of cTnI (target) and cTnT biomarkers (negative control) from ELISA reader.	89
4.3	Fabricated ZnO-FET biosensor integrated with substrate-gate coupling. (a) Fabricated device; (b) SEM images of (i) source, (ii) channel, (iii) drain, and (iv) substrate gate regions; and (c) EDX spectra located at (i) Si, (ii) channel, (iii) SiO <sub>2</sub> , and (iv) terminal pads.	92
4.4	AFM characterization of ZnO-NPs thin films with different number of deposited layers via sol-gel and spin-coating techniques. 2D (a) and 3D (b) views for (i) 1, (ii) 3, and (iii) 5 deposited layers.	94
4.5	FESEM images of ZnO-NPs thin films with different number of deposited layers via sol-gel and spin-coating techniques. (a) 1, (b) 3, and (c) 5 deposited layers.	95

4.6	XRD analysis ZnO-NPs thin films with different number of deposited layers via sol-gel and spin coating techniques. (a) 1, (b) 3, and (c) 5 deposited layers.	96
4.7	Electrical characterization of ZnO-NPs thin films with different number of deposited layers via sol-gel and spin-coating techniques. (a) Current-voltage characteristics and (b) Resistance histogram of the deposited thin film layers.	98
4.8	Surface functionalization characterization of ZnO-NPs thin film. (a) FTIR spectra adsorption of ZnO-NPs thin film deposited with (b) APTES, (c) GA, and (d) MAb-cTnI to functionalize the surface for capturing cTnI biomarker.	102
4.9	High resolution XP spectra of functionalization (with APTES and GA) and immobilization (with MAb-cTnI) of ZnO-NPs thin film. (a) Wide scan as well as normalized (b) N 1s, and (b) C 1s.	104
4.10	Comparison in term of $I_D$ against $V_D$ characteristic between (a) fabricated and (b) simulated device when different $V_{SG}$ bias applied on the ZnO-FET biosensor with substrate-gate coupling.	107
4.11	Cutline location of the simulated 2D FET structure. (a) Electron concentration at BOX/Si substrate interface, underneath the source, gate, and drain region. (b) Hole concentration near the surface of the source, gate and drain region for different $V_{SG}$ bias on the ZnO-FET biosensor with substrate-gate coupling.	109
4.12	Shifting of $V_T$ and $I_D$ in the (a) $I_D$ against $V_D$ characteristics due to (b) formation of hole conduction layer at the channel during (i) without, (ii) negatively, and (iii) positively bias of $V_{SG}$ on the ZnO-FET biosensor with substrate-gate coupling.	110
4.13	Effect of extra charges introduced at the surface of the ZnO-NPs thin film as channel of the ZnO-FET biosensor with substrate-gate coupling. (a) Hole concentration along the cutline of the simulated 2D FET structure near the surface of the source, gate and drain region. (b) $I_D$ against $V_D$ characteristic of the biosensor with introduction of (i) negative and (ii) positive $Q_F$ .	113
4.14	$I_D$ against $V_D$ characteristic of the ZnO-FET biosensor ( $V_D$ sweep from 0 to 1 V with $V_{SG}$ bias of $-2$ V) at different cTnI target biomarker concentrations (1 ng/ml to 10 $\mu$ g/ml).	115
4.15	$I_D$ response curve of the ZnO-FET biosensor with substrate-gate coupling ( $V_D$ bias of 1 V with $V_{SG}$ bias of $-2$ V) at different cTnI target biomarker concentrations.	116

4.16	Sensitivity comparison of ZnO-FET biosensor integrated with substrate-gate coupling with previous FET-based biosensor.	117
4.17	The relative change in $I_D$ calibration curve of the ZnO-FET biosensor with substrate-gate coupling ( $V_D$ bias of 1 V with $V_{SG}$ bias of $-2$ V), for LOD determination.	119
4.18	The relative change of $I_D$ curve of the ZnO-FET biosensor with substrate-gate coupling ( $V_D$ bias of 1 V with $V_{SG}$ bias of $-2$ V) for $10 \mu\text{g/ml}$ cTnT (negative control protein) and $10 \mu\text{g/ml}$ of cTnI target biomarker.	121
4.19	Reproducibility of ZnO-FET biosensor for the $10 \mu\text{g/ml}$ cTnI target biomarker.	122
5.1	Progression of cTn biomarkers devices detection from the past, present, and future perspective, a transition from benchtop towards translational research.	132

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## LIST OF TABLES

NO.		PAGE
2.1	Comparison of conventional assays, label-based, and label-free biosensors for cTnI and cTnT biomarker with details on their specifications (Fathil et al., 2015, 2016).	42
2.2	FET-based biosensors for cTnI/cTnT biomarker with details on their specifications (Fathil et al., 2016).	51
4.1	Optical density for various concentrations of cTnI and cTnT biomarker bind with MAb-cTnI from ELISA reader.	89
4.2	ZnO-NPs thin film surface morphology characterization using stylus profilometer and AFM.	94
4.3	Changes in $V_T$ and $I_D$ of fabricated ZnO-FET biosensor when biased with various $V_{SG}$ .	111
5.1	Comparison of available FET-based biosensors for cTnI biomarker detection with their specifications (Fathil et al., 2016).	128

## LIST OF ABBREVIATIONS

2D	2-dimension
3D	3-dimension
Ab	Antibody
AFM	Atomic force Microscope
ALD	Atomic layer deposition
ALP	Alkaline phosphatase
AMI	Acute myocardial Infarction
APTES	3-aminopropyltriethoxysilane
ASIC	Application-specific integrated circuit
AuNP	Gold nanoparticles
BNP	B-type natriuretic peptide
BOE	Buffered oxide etch
BOX	Buried oxide
BSA	Bovine serum albumin
CCD	Charge-coupled device
CK-MB	Creatine kinase-MB
CK-MM	Creatine kinase-MM
CL	Chemiluminescence
CLEIA	Chemiluminescence enzyme immunoassay
CLIA	Chemiluminescence immunoassay
CMOS	Complementary metal-oxide semiconductor
CNF	Carbon nanofiber

CNT	Carbon nanotube
CP	Conducting polymer
CRP	C-reactive protein
cTn	Cardiac troponin
cTnC	Cardiac troponin C
cTnI	Cardiac troponin I
cTnT	Cardiac troponin T
CV	Co-efficient of variation
CVD	Chemical vapour deposition
DNA	Deoxyribonucleic acid
ECB	ELISA coating buffer
ECG	Electrocardiography
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDX	Energy dispersive X-ray
ELISA	Enzyme-linked immunosorbent Assay
EnFET	Enzyme field-effect transistor
EOC	ELISA-on-chip
FESEM	Field-emission scanning electron microscope
FET	Field-effect transistor
FI	Fluorescence immunoassay
FMGC	Fluoro-microbead guiding chip
f-RG	Functionalized reinforcing bar graphene
FTIR	Fourier-transform infrared
GA	Glutaraldehyde

GCE	Glassy carbon electrode
GO	Graphene oxide
HRP	Horseradish peroxidase
IDE	Interdigitated electrode
ISFET	Ion-sensitive field effect transistor
IUPAC	International Union of Pure and Applied Chemistry
LOD	Limit of detection
MAb	Monoclonal antibody
MEA	Monoethanolamine
MHDA	Mercaptohexadecanoic acid
MPA	3-mercaptopropionic acid
MWCNT	Multi-walled carbon nanotube
Myo	Myoglobin
NEA	Nanoelectrode array
NHS	N-hydroxysuccinimide
NMO	Nanostructured metal-oxide
NP	Nanoparticle
NSTEMI	Non-ST segment elevation myocardial infarction
OEG	Oligo (ethylene glycol)
PANI	Polyaniline
PBS	Phosphate buffer saline
PDMS	Poly(dimethylsiloxane)
PECVD	Plasma-enhanced chemical vapour deposition
PEDOT	Poly(3,4-ethylenedioxythiophene)

PEI	Polyethyleneimine
POCT	Point-of-care testing
PPY	Polypyrrole
PyBuNHS	1-pyrenebutyric acid N-hydroxysuccinimide ester
PyMe	1-pyrenemethyl
PyMe-NH <sub>2</sub>	1-pyrenemethyl hydrochloride
ReBar	Reinforcing bar
RNA	Ribonucleic acid
SAM	Self-assembled monolayer
SDS	Sodium dodecyl sulphate
SEM	Scanning electron microscope
SiNW	Silicon nanowire
SP	Screen-printed
SPE	Screen-printed electrode
SOI	Silicon-on-insulator
SPA	Semiconductor parametric analyzer
SPR	Surface plasmon resonance
SWCNT	Single-walled carbon nanotube
TMAH	Tetramethylammonium hydroxide
Tn	Troponin
UniProtKB	Universal Protein Resources Knowledgebase
XP	X-ray photoelectron
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction

## LIST OF SYMBOLS

Al	Aluminium
Al <sub>2</sub> O <sub>3</sub>	Alumina
Au	Gold
c	Y-intercept
C	Carbon
CH <sub>4</sub>	Methane
HCl	Hydrochloric acid
HfO <sub>2</sub>	Hafnium (IV) oxide
HNO <sub>3</sub>	Nitric acid
I	Current
I <sub>D</sub>	Drain current
I <sub>D0</sub>	Immobilization drain current
IgG	Immunoglobulin G
L	Length
m	Slope
μ	Mean
N	Nitrogen
Ni	Nickel
O	Oxygen
ρ	Resistivity
pI	Isoelectric point
Pt	Platinum

$Q_F$	Interface charge density
$R$	Electrical resistance
$R_a$	Average roughness
$R_{et}$	Electron transfer resistance
$\Delta R_{et}$	Difference in electron transfer resistance
RMS	Root mean square
RSD	Relative standard deviation
Si	Silicon
$SiO_2$	Silicon dioxide
$\sigma$	Standard deviation
$SnO_2$	Tin oxide
$t$	Thickness
$V$	Voltage
$V_D$	Drain voltage
$V_{SG}$	Substrate-gate voltage
$V_T$	Threshold voltage
$W$	Width
ZnO	Zinc oxide

## **Pengesanan Elektrik tanpa Label untuk Penanda Biologi Troponin Jantung: Berasaskan Transistor Kesan Medan Disepadukan Gandingan Get-substratum**

### **ABSTRAK**

Infaksi myokardium akut (AMI) merupakan punca utama kematian di seluruh dunia walaupun dengan adanya kemajuan terapi. Oleh itu, kaedah diagnosis awal menggunakan biopenanda-biopenanda jantung adalah diperlukan supaya tindakan yang tepat dapat dilaksanakan. Troponin jantung I (cTnI) merupakan salah satu biopenanda jantung untuk diagnosis awal AMI dan dianggap sebagai “piawai emas” untuk menentukan kecederaan otot jantung. Pengesanan cTnI melalui biopenderia berasaskan elektrikal membolehkan pengesanan tanpa label dengan menukarkan pengikatan biomolekul kepada isyarat elektrikal yang ketara melalui sebuah pemindaharuh semikonduktor. Biopenderia ini memanfaatkan keberaliran untuk menentukan kewujudan biomolekul. Salah sebuah biopenderia berasaskan elektrikal ini yang dikenali sebagai biopenderia berasaskan transistor kesan medan (FET) telah menarik banyak perhatian kerana memiliki konsep pemindaharuan cas; di mana ia membolehkan diagnosis segera biopenanda jantung dengan kadar sensitiviti yang tinggi secara khusus pada kepekatan rendah di peringkat awal. Dalam kajian ini, biopenderia berasaskan FET-zink oksida (ZnO) digandingkan dengan get-substratum telah direka bentuk dan difabrikasi untuk pengesanan cTnI. Saput nipis ZnO sebagai bahan separa-pengalir jenis-n dan juga merupakan pemindaharuh serasi dengan biologi telah diendapkan menggunakan teknik-teknik gel-sol dan penyalutan putar di antara terminal punca dan salir jenis-p, yang terletak di atas substratum silikon-atas-penebat (SOI) untuk menghasilkan simpang *p-n-p*, sebuah peranti yang berupaya untuk aplikasi pengesanan biologi. Morfologi permukaan salut nipis ini telah dicirikan melalui mikroskop daya atom (AFM) dan mikroskop elektron imbasan pancaran medan (FESEM). Saput nipis ini memperlihatkan fasa wurzit heksagon seperti yang telah dipaparkan oleh analisa belauan sinar-X (XRD) adalah bersesuaian dengan interaksi biomolekul. Permukaan saput nipis ZnO ini telah ditetapkan dengan antibodi monoklonal cTnI (MAb-cTnI) melalui kaedah pengikatan kovalen untuk mengesan biopenanda cTnI. Proses ini telah dibuktikan melalui infra-merah jelmaan fourier (FTIR) dan spektroskopi fotoelektron sinar-X (XPS). Struktur peranti ini telah diselakukan di dalam perisian penyelaku 2-dimensi Silvaco ATLAS bertujuan untuk menghuraikan ciri elektrikal peranti tersebut, secara khususnya kepekatan elektron di dalam terusan dan permukaan oksida tertanam/substratum. Peranti ini mempamerkan strategi baru melalui pencirian elektrikal, apabila digandingkan dengan get-substratum yang mempertingkatkan pembentukan lapisan pengaliran lubang pada saluran yang terletak di antara kawasan saluran dan punca. Akhirnya, biopenderia ini menunjukkan peningkatan pada perubahan nisbi aras arus saluran yang ketara dalam julat lurus daripada 6.2 ke 16.5% dengan peningkatan kepekatan biopenanda cTnI yang bercas positif daripada 1 ng/ml ke 10 µg/ml. Sensitiviti pengesanan peranti ini adalah pada  $2.51 \% \cdot (\text{g/ml})^{-1}$  dengan had pengesanan (LOD) serendah 3.24 pg/ml.

## Electrical Label-Free Sensing of Cardiac Troponin Biomarker: FET-based Integration with Substrate-gate Coupling

### ABSTRACT

Acute myocardial infarction (AMI) is a leading cause of death worldwide despite the existence of therapy's advances. Therefore, an early diagnosis method by using cardiac biomarkers is essential to enable correct countermeasures. Cardiac Troponin I (cTnI) is one of the cardiac biomarkers for early diagnosis of AMI and considered as 'gold standard' for cardiac muscle injury determination. The detection of cTnI through an electrical-based biosensor allows label-free detection by converting biomolecular binding event into a significant electrical signal via a semiconductor transducer. It utilizes conductivity to specify the existence of biomolecules. One of the electrical-based biosensors known as field-effect transistor (FET)-based biosensor has drawn much attention for owning the concept of charge transduction; thus, allows early, high sensitivity, high selectivity, and rapid diagnosis of the specific cardiac biomarker at low concentrations. In this work, the zinc oxide (ZnO)-FET biosensor coupled with substrate-gate has been designed and fabricated for the detection of cTnI biomarker. ZnO thin film, as n-type biocompatible semiconductor material, and also as transducer was deposited via sol-gel and spin coating techniques between p-type source and drain terminal on SOI substrate, forming a *p-n-p* junction, a device capable of bio-sensing application. The surface morphology of the thin film was characterized by using atomic force microscopy (AFM) and field emission scanning electron microscopy (FESEM). The thin film, which demonstrated hexagonal wurtzite phase as shown by X-ray diffraction (XRD) analysis appropriate for biomolecules interaction. The surface of the ZnO thin film was immobilized with cTnI monoclonal antibody (MAb-cTnI) as biological receptor via covalent binding technique for capturing cTnI biomarker. The process was validated by Fourier transform-infrared (FTIR) and X-ray photoelectron spectroscopy (XPS). The device structure was simulated in Silvaco Atlas 2D-simulator, to elucidate its electrical characteristic, by means of hole and electron concentration in the channel and buried oxide/substrate interface, respectively. The device demonstrated a new strategy via electrical characterization with the introduction of substrate-gate coupling that enhanced the formation of hole conduction layer at the channel between drain and source region. Finally, the biosensor shown significant increment in relative changes of drain current level in a linear range of 6.2 to 16.5 % with the increase of positively charge cTnI biomarker concentrations from 1 ng/ml to 10  $\mu$ g/ml. The device sensitivity of the detection is at  $2.51 \text{ \%} \cdot (\text{g/ml})^{-1}$  with limit of detection (LOD) down to 3.24 pg/ml.

# CHAPTER 1

## INTRODUCTION

### 1.1 Background

Biosensors are frequently defined as integrated diagnostic devices comprising of three elements, which are bio-receptor, transducer, and a signal processing unit (Conroy, Hearty, Leonard, & O’Kennedy, 2009). Generally, a suitable transducer surface of biosensor is immobilized with a biological receptor material (i.e. antibody (Ab), deoxyribonucleic acid (DNA), or ribonucleic acid (RNA)). It produces a measurable signal upon bio-receptor interaction with the specific biomolecules (Goode, Rushworth, & Millner, 2015; Qureshi, Gurbuz, & Niazi, 2012). The generated signals is in the mode of either electrochemical (Gomes-Filho, Dias, Silva, Silva, & Dutra, 2013; Horak, Dincer, Qelibari, Bakirci, & Urban, 2015), optical (H.-Z. He et al., 2013; C.-H. Leung et al., 2013; K.-H. Leung et al., 2015; Lu et al., 2014), mass change (piezoelectric/acoustic wave) (Joonhyung Lee et al., 2013), or magnetic (J. Liu, Zhang, Wang, Zheng, & Sun, 2014). The development of biosensors for diversity of biomolecules detection has cover many field, including medicine (J. Wang, 2006), food testing (Huet et al., 2010), environmental (Weller, Schuetz, Winklmaier, & Niessner, 1999), and process control monitoring (Venugopal, 2002). The developed biosensors that come with several advantages (i.e. portable, inexpensive tools for the rapid detection of pathogens, proteins and other biomolecules) are intended to provide as an alternative method to the conventional bioanalytical approaches (Fathil et al., 2016). Commonly, conventional bioanalytical