



**GOLD NANOSTRUCTURES IN MEDIATING HIGH-  
PERFORMANCE MEDICAL DIAGNOSIS OF HUMAN  
BLOOD DISEASE BIOMARKERS**

by

**ISWARY LETCHUMANAN  
1841812752**

A thesis submitted in fulfillment of the requirements for the degree of  
Doctor of Philosophy

**INSTITUTE OF NANO ELECTRONIC  
ENGINEERING  
UNIVERSITI MALAYSIA PERLIS**

2020

## ACKNOWLEDGMENT

First, I would like to thank my parents Letchumanan Perumal and Rajaswary Packirysamy, for giving birth to me at the first place and supporting me spiritually throughout my life.

Secondly, I would like to express my sincere gratitude to my supervisor Assoc. Prof. Dr. Subash Chandra Bose Gopinath for the continuous support of my Ph.D study and research, for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better supervisor and mentor for my Ph.D study. Thank you so much for forcing me to look at research and my work in different ways and for opening my mind. Your support was essential to my success here.

Besides my supervisor, I would like to thank my co-supervisor Assoc. Prof. Ir. Dr. Mohd Khairuddin Md Arshad for his encouragement, insightful comments and hard question which incited me to widen my research from various perspectives.

My sincere thanks also go to Dr. Asanka Rajapaksha for enlightening me the first glance of research. He continually and convincingly conveyed a spirit of adventure in regard to research. Without his guidance and persistent help this dissertation would not have been possible.

Last but not the least, I would like to thank my fellow labmates in Institute of Nanoelectronic Engineering (INEE) Nur Dalila Rizuan, Santhraleka, Nur Atiqah Ahmad, Indragandhi Subramaniam and Conlathan Ibau for the stimulating discussions, for the sleepless nights we were working together before deadlines, and for all the fun we have had in the last one years. To them I say “we meet to part, but more important we part to meet.”

## TABLE OF CONTENTS

	<b>PAGE</b>
<b>DECLARATION OF THESIS</b>	<b>ii</b>
<b>TABLE OF CONTENTS</b>	<b>iv</b>
<b>LIST OF TABLES</b>	<b>x</b>
<b>LIST OF FIGURES</b>	<b>xi</b>
<b>LIST OF ABBREVIATIONS</b>	<b>xx</b>
<b>LIST OF SYMBOLS</b>	<b>xxii</b>
<b>ABSTRAK</b>	<b>xxiii</b>
<b>ABSTRACT</b>	<b>xxiv</b>
<b>CHAPTER 1 : INTRODUCTION</b>	<b>1</b>
1.1 Research Background	1
1.2 Problem Statement	4
1.3 Objectives	5
1.4 Research Scope	6
<b>CHAPTER 2 : LITERATURE REVIEW</b>	<b>8</b>
2.1 Human Blood Diseases	8
2.1.1 Cardiovascular disease	9
2.1.2 Cancer	13
2.1.3 Haemophilia	15
2.2 Nanotechnology for High-Performance Sensing	18
2.2.1 Gold as an outstanding sensing element	19
2.2.1.1 Synthesization of gold nanomaterial	20

2.2.1.2	Configuration of Gold nanoparticles	24
2.1.1.3	Types of gold nanoparticles/nanostructures	27
2.2.2	Overview of ZnO nanomaterial	38
2.3	Biosensor in Health Care Diagnostics	38
2.3.1	Interdigitated electrode	47
2.3.1.1	Impedance biosensor	50
2.3.1.2	Capacitance biosensor	52
2.3.2	Field-Effect Transistor based biosensor	53
<b>CHAPTER 3 : METHODOLOGY</b>		<b>60</b>
3.1	Device fabrication	61
3.1.1	Materials	61
3.1.2	Device fabrication	62
3.1.2.1	Cleaning of the wafer	62
3.1.2.2	Thermal oxidation on the silicon substrate	63
3.1.2.3	Pattern designing on chrome mask	63
3.1.2.4	Aluminium/Gold deposition	64
3.1.2.5	Gold etchant preparation	64
3.1.2.6	In-depth photolithography	64
3.1.3	Gold-nanorod Enhances Dielectric Voltammetry Detection of C-reactive protein: A Predictive Strategy for Cardiac Failure	67
3.1.3.1	Surface functionalization on nanogapped	67
3.1.3.2	Scanning Electron Microscopy (SEM)	67
3.1.3.3	Atomic Force Microscopy (AFM)	67
3.1.3.4	3D Surface (Nano) Profilometer	68

3.1.3.5	Interaction between anti-CRP antibody and CRP	68
3.1.3.6	Interaction between anti-CRP-antibody and gold-conjugated CRP	68
3.1.3.7	Detection of CRP in human serum – Specificity analysis	69
3.1.3.8	Enzyme-linked immunosorbent assay (ELISA)	70
3.1.4	Gold Nano-urchin Integrated Label-free Amperometric Aptasensing Human Blood Clotting Factor IX: A Prognosticative Approach for “Royal Disease”	71
3.1.4.1	Chemical modification on interdigitated electrode	71
3.1.4.2	Surface morphological analysis	71
3.1.4.3	Biotin-streptavidin conjugation system	72
3.1.4.4	GNUs integrated biotin-streptavidin system and interactive analysis of anti-FIX aptamer and FIX	72
3.1.4.5	Specific FIX detection in human serum	73
3.1.4.6	Enzyme-linked apta-sorbent assay (ELASA)	73
3.1.5	Divalent ion-induced Aggregation of Gold Nanoparticles for Voltammetry Immunosensing: Comparison of Transducer Signals in an Assay for the Squamous Cell Carcinoma Antigen.	75
3.1.5.1	Sensing surface functionalization	75
3.1.5.2	Surface characterization analysis	75
3.1.5.3	Enzyme-linked Immunosorbent Assay (ELISA)	76
3.1.5.4	Assembly and Disassembly of GNPs	76
3.1.5.5	Binding event between anti-SCC and SCC-Ag	77
3.1.5.6	Binding event between CaCl <sub>2</sub> -induced aggregated GNPs attached anti-SCC and SCC-Ag	77
3.1.5.7	Analytical high-performance	78

<b>CHAPTER 4 :</b>	<b>RESULTS AND DISCUSSION</b>	<b>79</b>
4.1	Device Characterization	79
4.1.1	Surface morphological analysis	79
4.1.2	Physical Characterization	80
4.1.3	Voltammetry measurement on the bare dielectric	84
4.1.4	Voltammetry measurements for pH scouting	88
4.2	Gold-nanorod Enhances Dielectric Voltammetry Detection of C-reactive protein: A Predictive Strategy for Cardiac Failure	94
4.2.1	Surface morphology analysis – Nanoscale imaging	95
4.2.1.1	Sensing surface profiling	95
4.2.1.2	Gold nanorod imaging	97
4.2.2	Validation of molecular interactions	98
4.2.2.1	UV-Vis Spectrophotometry	98
4.2.2.2	Enzyme-linked Immunosorbent Assay (ELISA)	99
4.2.2.3	Voltammetry analysis on the interaction of anti-CRP antibody and CRP antigen and sensitivity determination	101
4.2.2.4	Voltammetry analysis on the interaction of anti-CRP antibody and gold-nanorod conjugated CRP antigen and sensitivity determination	104
4.2.2.5	Specificity and stability analyses	107
4.3	Gold Nano-urchin Integrated Label-free Amperometric Aptasensing Human Blood Clotting Factor IX: A Prognosticative Approach for “Royal Disease”	111
4.3.1	Surface characterization	112
4.3.1.1	IDE surface	112
4.3.1.2	Gold-nanourchins (GNUs)	116
4.3.2	Validation of molecular properties	118
4.3.2.1	Spectral analysis	118

4.3.2.2	Enzyme-linked apta-sorbent assay (ELASA)	119
4.3.2.3	Current vs Voltage (I-V) measurements for surface modifications	121
4.3.2.4	Electrical analysis for streptavidin-biotin conjugation	122
4.3.3	Molecular interaction analysis	123
4.3.3.1	Analysis of FIX and anti-FIX aptamer with integration of streptavidin-biotin	123
4.3.3.2	Analysis of FIX and anti-FIX aptamer with GNUs complexed streptavidin-biotin	127
4.3.3.3	Analytical high-performance	129
4.3.3.4	Analysis of FIX and anti-FIX aptamer with integration of streptavidin-biotin using nanogapped	132
4.4	Divalent ion-induced Aggregation of Gold Nanoparticles for Voltammetry Immunosensing: Comparison of Transducer Signals in an Assay for the Squamous Cell Carcinoma Antigen	134
4.4.1	Choice of material	134
4.4.2	Surface morphology analysis	135
4.4.3	Investigates Disassembly and CaCl <sub>2</sub> -induced Assembly of GNPs	136
4.4.4	Molecular interaction analysis	139
4.4.4.1	Enzyme-linked Immunosorbent Assay (ELISA)	139
4.4.4.2	Interactive study by voltammetry between anti-SCC-Ag and SCC-Ag	141
4.4.4.3	Interactive study by voltammetry between SCC-Ag and GNPs conjugated anti-SCC-Ag	143
4.4.4.4	Evaluation of interaction between SCC-Ag and CaCl <sub>2</sub> -induced assembly of GNPs conjugated anti-SCC-Ag by voltammetry	145
4.4.4.5	Analytical high-performance analysis	147
<b>CHAPTER 5 : CONCLUSION</b>		<b>155</b>

**REFERENCES**

**158**

**APPENDIX**

**175**

©This item is protected by original copyright

## LIST OF TABLES

	<b>PAGE</b>
Table 1: Comparison work among all the detection methods for blood disease biomarkers with different strategies.	59
Table 2: Specification data;The height, area size and pitch of the fabricated sensor was visibly noted on the table.	81
Table 3: Comparison of different sensing strategies for CRP detection.	110
Table 4: Comparison between different sensing methods for FIX detection	129
Table 5: Comparison among the currently available SCC detections by different sensing systems	153

©This item is protected by original copyright

## LIST OF FIGURES

	<b>PAGE</b>
Figure 2.1: Atherosclerosis is the plaque formation at the wall of the coronary artery which can trigger the high formation of CRP molecules.	8
Figure 2.2: Amino acid sequence for CRP which contain approximately 206 amino acids available in Genbank with the Accession number AAA52075.	10
Figure 2.3: Crystal structure of CRP in pentameric orientation. Each subunit cotains monomer and intramolecular disulphide bond. This structure available in Protein Data Bank with the PDB ID:1GNH. ( <a href="https://www.rcsb.org/3d-view/1GNH/1">https://www.rcsb.org/3d-view/1GNH/1</a> )	10
Figure 2.4: (a) CRP molecule is packed with five anti-CRP (bioreceptor protein) due to the existence of five subunit for each CRP molecule; (b) Electrical measurements were performed after each surface modification and the semi-circle diameter was monitored.	11
Figure 2.5: Amino acid sequence for FIX, available in Genbank with accession number AAA98726.	16
Figure 2.6: Crystal structure of FIX. This structure available in Protein Data Bank with the PDB ID: 5F84	17
Figure 2.7: Bleeding disease, a so-called ‘Haemophilia B’ or ‘Christmas disease’ caused due to deficiency of FIX and highly connected to most of the proteins.	18
Figure 2.8: Brief gold reduction method was illustrated clearly	25
Figure 2.9: EDC and NHS interaction mechanism.	27

Figure 2.10: Graphical abstract explained the overall detection method.	36
Figure 2.11: Basic component of biosensor manufactured for medical diagnostic purposes.	39
Figure 2.12: The dipole moment on the IDE surface was shown clearly when the strong binding event between antigen and antibody/aptamer.	41
Figure 2.13: Surface modification on silica based substrate for the biomolecular binding such as CDI, GOPTS and Glu.	44
Figure 2.14: Schematic of selective MPA protein capture compared to a needle and syringe and proposed a variation of MPA geometry to increase protein capture.	45
Figure 2.15: General setup for amperometric based biosensor using IDE. This is a two probe system, where it will be connected to the anode and cathode parts of the IDE. Electrical measurements will be transmitted to the picoammeter connected to the software which able to display the results.	47
Figure 2.16: Graphical illustration clearly explained the FET biosensor for cancer biomarker detection using miRNA	56
Figure 3.1: Detailed fabrication step for the development of a dielectric sensor.	66
Figure 3.2: Brief illustration on fabrication flow for both gold and aluminium dielectric. All the steps are same except the electrode material. The final design of dielectric electrode was photographed and presented.	66
Figure 3.3: Schematic diagram explained the overall flow of the detection process.	69

Figure 4.1: Autocad mediated design (a). The proposed dielectric sensor was printed on chrome mask; The fabricated dielectric structure for sensing purpose (b).	80
Figure 4.2: The electrode edges were smooth and sharp as can see in the 3D view image.	81
Figure 4.3: Surface roughness (a) and waviness (b) based on the black line shown in 2D image.	82
Figure 4.4: 2D view (c); The gap between the electrodes were fabricated according to the design dimensions.	82
Figure 4.5: The EDX data was apparently displayed that the presence of aluminium inside the dielectric with SEM images.	83
Figure 4.6: The EDX data was apparently displayed that the presence of gold inside the dielectric with SEM images.	83
Figure 4.7: Voltammetry analysis for bare devices. Aluminium dielectric (a), Equivalent circuit (Figure inset); Gold dielectric (b)	84
Figure 4.8: Stability analysis to justify the performance of the dielectric electrode. Aluminium dielectric (a); Gold dielectric (b).	86
Figure 4.9: pH scouting. Aluminium dielectric (a); Gold dielectric (b). pH ranges from 1-12 were tested.	89
Figure 4.10: Mechanism on the sensing surface. Mobility of the ions between (cathode and anode) electrodes (a). Mode of transduction between electrodes (b).	93
Figure 4.11: Surface characterization of the dielectric gapped sensing surface imaging. Scanning Electron Microscopy observation.	95
Figure 4.12: The edge of the nanogapped electrode through High Power Microscopy.	96

Figure 4.13: The 3D structure of electrode surface via 3D Surface (Nano) Profilometer	96
Figure 4.14: Surface topography of the electrode via Atomic Force Microscopy	97
Figure 4.15: Morphological characterization for gold nanorod. Atomic Force Microscopy (a) and Scanning Electron Microscopy (b).	98
Figure 4.16: UV-Vis measurements. Only proteins (a); Proteins and GNR (b); only GNR (c). Anti-CRP-antibody (i); CRP (ii); CRP-GNR complex (iii).	99
Figure 4.17: Enzyme Linked-Immunosorbent Assay. The color development with the substrate is shown by a photograph.	101
Figure 4.18: Reproducibility analysis. Three different parallel measurements with bare devices are shown.	102
Figure 4.19: Surface analysis after the antigen-antibody binding event at the dielectric gapped. 3D Surface (Nano) Profilometer (a); and Atomic Force Microscopy (b).	103
Figure 4.20: The current to voltage (I-V) measurements prior to surface modifications (a); Different concentrations of CRP without GNR conjugates (b).	105
Figure 4.21: Amperometric measurements were performed using 0 V to 2 V at 0.01 V step voltage was used through the analysis. Biomolecular interaction and high-performance analysis with; linear regression analysis for the interaction of CRP and anti-CRP antibody. Limit of detection was calculated based on $3\sigma$ calculation.	106

Figure 4.22: Linear regression analysis for the interaction of CRP-GNR complex and anti-CRP antibody. Limit of detection was calculated based on $3\sigma$ calculation.	106
Figure 4.23: Specificity analysis; Specific binding of CRP antibody against different proteins available in human serum was analyzed. Immobilized antibody (i); Factor IX (ii); Serum albumin (iii); C-reactive protein(iv); Human serum (v); Human serum spiked C-reactive protein (vi).	108
Figure 4.24: Stability of the sensor. Bare device (i); APTES (ii); Glutaraldehyde (iii); Anti-CRP antibody (iv); Ethanolamine (v).	109
Figure 4.25: Surface morphological examination on the electrode bandgaps sensing zone. Scanning Electron Microscopy (SEM) observation.	113
Figure 4.26: Prior to ZnO deposition; EDX profile of sensing zone after ZnO deposition.	113
Figure 4.27: Presence of zinc was confirmed by X-ray diffraction analysis; and the close view of sensing zone using Field-emission Scanning Electron Microscopy.	114
Figure 4.28: The morphology between the electrodes bandgap via Atomic Force Microscopy	114
Figure 4.29: Clear cut image of sensor by 3D Surface (Nano) Profilometer.	115
Figure 4.30: Physical characterization after the attachment of linker (CDI). Scanning Electron Microscopy (a); Atomic Force Microscopy (b); 3D Surface (Nano) Profilometer	116
Figure 4.31: Surface topography analysis on gold nano-urchin by Scanning Electron Microscopy	117

Figure 4.32: Transmission Electron Microscopy (b) and figure inset present the enlarged view.	117
Figure 4.33: Uniform size distribution of GNU via Atomic Force Microscopy.	118
Figure 4.34: UV-Vis measurements for gold-nanoparticle (i) and gold nanourchin (ii)	120
Figure 4.35: Enzyme-linked apta-sorbent assay. The colour changes after the substrate addition were shown by a photograph (b). The colour changes occurred at highest concentration. First well starting from 3 pM, 6 pM, 12 pM, 25 pM, 50 pM, 100 pM, 125 pM, 1 nM then non-specific protein which is (CRP) followed by only coating buffer, both act as the control.	120
Figure 4.36: Surface characterization after the attachment of CDI. I-V measurements was carried out to justify the surface modification.	121
Figure 4.37: The current versus voltage analysis using 0 V to 2 V at 0.1 V step voltage. Streptavidin-biotin conjugation assay using a different concentrations of biotin without GNUs.	125
Figure 4.38: The current versus voltage analysis using 0 V to 2 V at 0.1 V step voltage. Streptavidin-biotin conjugation assay using a different concentrations of biotin with GNUs.	125
Figure 4.39: Interactive analysis for FIX and anti-FIX aptamer with streptavidin-biotin strategy without GNUs.	126
Figure 4.40: Interactive analysis for FIX and anti-FIX aptamer with streptavidin-biotin strategy with GNUs.	126
Figure 4.41: Analytical high-performance analysis. Linear regression curve for the interaction of FIX and anti-FIX aptamer. Limit of detection was determined based on $3\sigma$ calculation.	128

- Figure 4.42: Analytical high-performance analysis. Linear regression curve for the interaction of GNUs integrated FIX and anti-FIX aptamer. Limit of detection was determined based on  $3\sigma$  calculation. 128
- Figure 4.43: Specificity analysis (c); Immobilized aptamer (i); Serum albumin (ii); C-reactive protein (iii); Human serum (iv); Human serum spiked 3 pM of Factor IX (v); Human serum spiked 6 pM of Factor IX (vi). The capability of FIX to bind with different proteins available in human serum was monitored. 131
- Figure 4.44: Stability of the sensor (d). Bare device (i) Reproducibility analysis was presented by figure inset where three different measurements by the bare device; CDI (ii); Streptavidin (iii); Ethanolamine (iii); Biotin (iv); anti-FIX aptamer (v). 131
- Figure 4.45: The current flow for each concentrations tested on nanogapped sensor. 133
- Figure 4.46: Morphological inspection for dispersed and agglomerated GNPs on the active surface area of the sensor. 3D Surface Nano-profilometry observation with (a) dispersion and (b) agglomeration; (c) Scanning Electron Microscopy observation with dispersion and (d) agglomeration; Atomic Force Microscopy observation with (e) dispersion and (f) agglomeration. 135
- Figure 4.47: UV-Vis measurements for different concentrations of  $\text{CaCl}_2$  with GNPs; Figure inset shows the optical density reading at 520 nm for all the  $\text{CaCl}_2$  tested. 137
- Figure 4.48: Voltammetry analysis for all  $\text{CaCl}_2$  concentration with GNPs. Figure inset (i) shows the color transition from red to purple. Figure inset (ii) 3D Surface (Nano) Profilometer imaging after the attachment of agglomerated GNPs on the sensor surface. 138

Figure 4.49: Enzyme-linked-Immunosorbent Assay. The color development with the substrate is shown by a photograph. Diagrammatic representation is shown by the figure inset. The bar graph represents the absorbance value for each concentration.	140
Figure 4.50: Voltammetry current analysis prior to surface modifications. Figure inset displays the performance of the sensor; (i) Bare device; (ii) APTES; (iii) EDC and NHS; (iv) Anti-SCC antibody; (v) Ethanolamine. Error bars indicate the averaged values by the replicates (n=3).	142
Figure 4.51: Interactive analysis between antigen and antibody without the integration of gold nanoparticles.	144
Figure 4.52: Interactive analysis between antigen and antibody with the integration of dispersed state GNPs.	145
Figure 4.53: Interactive analysis between antigen and antibody with the integration of agglomerated state GNPs.	146
Figure 4.54: Comparison of current analysis. For all three studies to determine the better strategy for the efficient detection of SCC-Ag to obtain accurate and quick results.	147
Figure 4.55: Linear regression plot with averaged values from triplicates (n=3) for the interaction of SCC-Ag and anti-SCC-Ag with the standard deviations are ranging from $\pm 0.5$ to $2.5 \times 10^{-5}$ A.	148
Figure 4.56: Linear regression plot with averaged values from triplicates (n=3) for the interaction of anti-SCC-Ag-GNP complex and SCC-Ag with the standard deviations are ranging from $\pm 1.0$ to $3.0 \times 10^{-5}$ A.	148
Figure 4.57: The current dependence square root of scan rate	149
Figure 4.58: Linear regression plot with averaged values from triplicates (n=3) for the interaction of anti-SCC-Ag-GNP (agglomerated)	

complex and SCC-Ag with the standard deviations are ranging from  $\pm 0.2$  to  $2.0 \times 10^{-5}$  A.

151

Figure 4.59: Specificity analysis ; (i) Immobilized antibody ; (ii) Serum albumin ; (iii) Factor IX ;(iv) C-reactive protein; (v) Human serum spiked 10 fM of SCC-Ag ;(vi) Human serum spiked 100 fM of SCC-Ag ; (vii) Human serum. The current to voltage (I-V) measurements were performed from 0 to 2 V.

152

©This item is protected by original copyright

## LIST OF ABBREVIATIONS

ELISA	Enzyme-linked Immunosorbent Assay
SPR	Surface Plasmon Resonance
IDE	Interdigitated Electrode
ZnO	Zinc Oxide
GN	Gold nanoparticle
SEM	Scanning Electron Microscopy
AFM	Atomic Force Microscopy
HPM	High Power Microscope
AuNPs	Gold nanoparticles
NP	Nanoparticle
NF	Nanoflower
SELEX	Systemic Evolution of Ligands
CDI	N,N'-Carbonyldiimidazole
GOPTS	3-glycidoxypropyltrimethoxysilane
Glu	Glutaraldehyde
APTES	3-Aminopropyltriethoxysilane
FET	Field Effect Transistor
MPA	Microprojection Array
FIX	Factor IX
CRP	C-Reactive Protein
CVD	Cardiovascular Disease
mCRP	Monomer CRP
Anti-1°Ab	primary capture antibody
AQ-2°Ab	anthraquinone-labeled signaling secondary antibody
EDC	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
NHS	N-Hydroxysuccinimide
OD	Optical density
I-V	Current versus voltage
RD6	Resist developer
Si	Silicon
PVD	Thermal evaporator

PBS	Phosphate Buffer Saline
UV	Ultra-violet
16-MDA	16-mercaptohexadecanoic acid
PoC	Point-of-Care
HRP	Horse Radish Peroxidase
FESEM	Field-emission Scanning Electron Microscopy
ELASA	Enzyme-linked apta-sorbent assay
GNR	Gold nano-rod
FTIR	Fourier-transform Infrared Spectroscopy
GNU	Gold nano-urchin
ADA-Ab <sub>1</sub>	Adamantine-modified primary antibodies
CM- $\beta$ -CD	Carboxymethyl- $\beta$ -cyclodextrin
ISFET	Ion sensitive field effect transistor
EIS	Electrochemical impedance spectroscopy
MOS	Metal oxide-semiconductor
EDL	Electrical double layer
MOSFET	Metal oxide-semiconductor field effect transistor

©This item is protected by original copyright

## LIST OF SYMBOLS

$\mu\text{M}$	Micromolar
M	Molar
h	Hour
fM	Femtomolar
pM	Picomolar
mM	Millimolar
$^{\circ}\text{C}$	Degree Celcius
$\mu\text{m}$	micrometer
A	Ampere
$\psi\text{m}$	Metal potential
$\Delta V_{\text{th}}$	Threshold voltage

©This item is protected by original copyright

# STRUKTUR NANO EMAS DALAM MEMEDIASI DIAGNOSIS PERUBATAN BERPRESTASI TINGGI DALAM BIOMARKER PENYAKIT DARAH MANUSIA

## ABSTRAK

Penyelidikan semasa telah dijalankan dengan menggunakan tiga jenis protein penyakit darah manusia yang berbeza iaitu C-Reaktif Protein (CRP), protein untuk sistem pembekuan darah dan juga protein untuk kanser kulit. Dua jenis elektrod berlainan telah digunakan dalam penyelidikan ini. Elektrod dengan jurang  $\sim 100$  nm telah direka dan diubahsuai untuk menyelidik aktiviti biopengesanan sasaran iaitu CRP. Manakala, protein untuk sistem pembekuan darah dan juga protein untuk kanser kulit telah dikaji dengan menggunakan elektrod interdigitated (IDE) yang mempunyai struktur seperti jari dengan permukaannya meliputi zink oksida. Nanorod emas dengan panjang 119 nm dan lebar 25 nm telah digunakan untuk meningkatkan status biopengesanan sasaran iaitu CRP. Nano-urchin emas (GNUs) dengan garis pusat 60 nm disatukan dengan strategi 'streptavidin-biotinylated aptamer' untuk menguji kemampuan mengesan protein sistem pembekuan darah. Tambahan pula, struktur nano emas dengan diameter 30 nm telah diaplikasikan dalam kes pengujian protein kanser kulit. Dalam kes ini tiga jenis kajian telah dilakukan iaitu dengan adanya struktur nano emas, tanpa struktur nano emas dan dengan struktur nano emas terkumpul. Pengujian fizikal telah dilakukan dengan menggunakan mikroskop daya atom, mikroskop elektron imbasan, 3D nano-profilometri, mikroskop berkuasa tinggi and UV-Vis spektroskopi. Pengujian dari segi elektrik telah dijalankan untuk menganalisis perbezaan prestasi dengan adanya struktur nano emas, tanpa struktur nano emas dan dengan struktur nano emas terkumpul untuk menangkap sasaran protein kanser kulit. Teknik amperometrik dengan voltan linear 0 hingga 2 V pada voltan 0.1 V voltan telah digunakan untuk mengkaji kebolehan interaksi dikalangan protein penyakit darah manusia. Pengesanan ini menyerlah ke tahap pico- hingga julat femtomolar. Oleh itu, novel yang diketengahkan dalam penyelidikan ini adalah penggunaan nanostruktur emas yang berbeza dan juga strategi yang digunakan untuk meningkatkan sistem pengesanan. Oleh itu, prestasi tinggi sensing ini boleh mengurangkan had pengesanan pico-kepada julat femto-molar dan memegang prestasi cemerlang dalam aplikasi biopengesanan.

# **GOLD NANOSTRUCTURES IN MEDIATING HIGH-PERFORMANCE MEDICAL DIAGNOSIS OF HUMAN BLOOD DISEASE BIOMARKERS**

## **ABSTRACT**

The current research was carried out using three (3) different human blood disease biomarkers which were C-reactive protein (CRP), blood clotting factor IX (FIX) and squamous cell carcinoma antigen (SCC antigen). Two different types of dual probing system electrode were utilized in this research. A nano gapped electrode with the gap of ~100 nm was designed and modified to capture the target, CRP. Meanwhile, factor IX and SCC antigens were diagnosed by using an interdigitated electrode (IDE), which had finger like structure with the zinc oxide surface. In order to increase the amount of antigen to be captured a gold nanorod (GNR) of a 119 nm in length and 25 nm in width was integrated in CRP detection system. In addition, gold nano-urchins (GNUs) with 60 nm in diameter was integrated into a streptavidin-biotinylated aptamer strategy in Factor IX diagnosis technique. Whereby, dispersed and agglomerated state of gold nanoparticles with 30 nm was used in SCC antigen detection scheme. The physical characterization for the sensing surface and gold nanostructures was properly carried out atomic force microscopy, scanning electron microscopy, 3D nano-profilometry, high-power microscopy and UV-Vis spectroscopy. A comparative analysis in the existence and non-existence of gold nanostructures utilization was performed using electrical characterization. The amperometric measurement by a linear sweep voltage of 0 to 2 V at 0.01 V step voltage was implemented to study the sensitivity and specificity of the blood biomarker interaction. Current research using three different biomarkers which responsible for three different blood disease reveals a lower limit of detection as compared to real concentration of specific biomarkers in human serum. The obtained detection limit as low as pico- to femtomolar range was due to the conjugation of gold nanostructures with antibody (Probe) and the strategy used like streptavidin-biotinylated aptamer for Factor IX detection. Hence, the highlighted novelty of the research is the utilization of different gold nanostructures and also the strategy applied to enhance the detection system. Hence, gold mediated high-performance sensing able to lower the limit of detection down to pico- to femto-molar ranges and hold an outstanding performance in biosensing application.

## CHAPTER 1 : INTRODUCTION

### 1.1 Research Background

Blood is the essential living tissue in an individual body which encompasses of solid and liquid portions. The liquid part is made of water, salts and protein, called plasma and more than half the blood is plasma (Jankowska et al., 2020). Red blood cells, white blood cells and platelets are in the solid part of blood. Blood disease and disorders affect one or more parts of the blood and dysfunctioning the blood. Mainly, the disorders are generated due to the gene malfunctions. Whereby, there are other causes involved, such as lack of nutrients in diet and side effects of the medicine (Parnetti et al., 2019).

There are few human blood disorders for an instance bleeding disorder as haemophilia, blood cancer (Jankowska et al., 2020), eosinophilic disorders (Wu et al., 2019) known as hematologic diseases. Blood studies with health and disease are defined as 'hematology', includes red blood cells, white blood cells, platelets, blood vessels, bone marrow, lymph nodes, spleen, bleeding and coagulation proteins (hemostasis and thrombosis). At present era, healthcare system is facing new challenges, which need a magnificent technology development to decline the mortality rate (Subash C.B. Gopinath et al., 2020). It is due to the fatal diseases with the rate increment by the human blood diseases that can be observed apparently. Robust development in the medical diagnosis of human blood disorder is going on primarily to cut-down the death rate. Therefore, emphasizing on primary and rapid diagnosis of blood disorders is as vital as the discovery of diagnostic and prognostic biomarkers to predict the risk factors. Whereby, molecules