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 incented 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

LIST OF TABLES

PAGE

LIST OF FIGURES

PAGE

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-1ºAb	primary capture antibody
AQ-2ºAb	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_1	Adamantine-modified primary antibodies
CM-β-CD	Carboxymethyl-β-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

LIST OF SYMBOLS

μM	Micromolar
M	Molar
h	Hour
fM	Femtomolar
pM	Picomolar
mM	Millimolar
ºC	Degree Celcius
μm	micrometer
A	Ampere
ψm	Metal potential
ΔV_{th}	Threshold voltage

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 ~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 permukaanya 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 elektrikal 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