LABEL-FREE GOLD INTERDIGITATED MICROELECTRODES IMMUNOSENSOR FOR PROSTATE CANCER BIOMARKER

by

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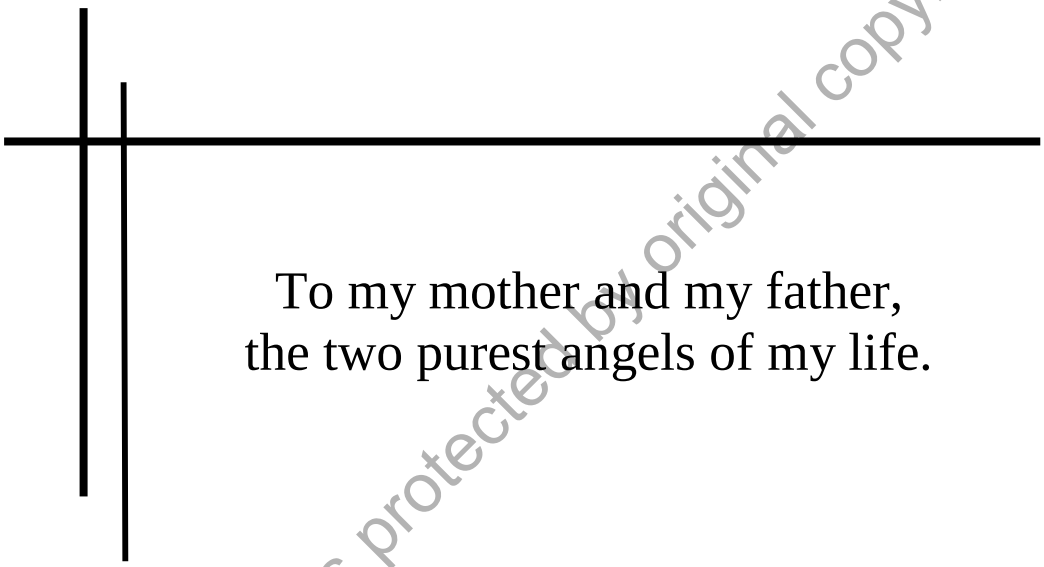

To my mother and my father,
the two purest angels of my life.

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TABLE OF CONTENTS

LIST OF TABLES

PAGE

LIST OF FIGURES

LIST OF ABBREVIATIONS

-COOH	Carboxylic
$-NH_3^+$	Amine
-OH	Hydroxyl
-SH	Thiol
16-MUA	16-mercaptoundecanoic acid
AC	Alternating current
AFM	Atomic force microscopy
Anti-PSA	Prostate-specific antigen monoclonal antibody
APTES	3-aminopropyltriethoxysilane
Au	Gold
AuNPs	Gold nanoparticles
BE	Binding energy
C-C	Alkyl carbon
CE	Counter electrode
CMOS	Complementary metal-oxide semiconductor
CV	Cyclic voltammetry
DC	Direct current
DIW	De-ionized water
DNA	Deoxyribonucleic acid
DPV	Differential pulse voltammetry
DRE	Digital rectal examination
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDS	Energy dispersive spectroscopy
EIS	Electrochemical Impedance Spectroscopy
eV	Electron-volts
Fc	Ferrocene
f-EIS	Faradaic-mode Electrochemical Impedance Spectroscopy
$[Fe(CN)_6]^{4-/3-}$	Ferrocyanide/ferricyanide redox
GA	Glutaraldehyde
GCE	Glassy carbon electrode
hK2	Human kallikrein 2
HPM	High power microscope

HRP	Horseradish peroxidase
HSA	Human serum albumin
Hz	Hertz
ICTS	Immunochromatography test strip
IDμE	Interdigitated microelectrode
IPA	Isopropanol
LOD	Limit of detection
LOQ	Limit of quantification
LSV	Linear sweep voltammetry
MAb1	Monoclonal antibody 1
MAb2	Monoclonal antibody 2
MSCC	Metastatic spinal cord compression
MWCNTs	Multi-walled carbon nanotubes
nf-EIS	Non-Faradaic mode Electrochemical Impedance Spectroscopy
NHS	N-Hydroxysuccinimide
OCH_3	Methoxy
O=C-N	Amide
OCP	Open circuit potential
PAMAM	Polyamidoamine
PAP	Prostatic acid phosphatase
PBS	Phosphate buffered saline
PCa	Prostate cancer
PEB	Post exposure bake
PtNPs	Platinum nanoparticles
PoC	Point-of-care
PSA	Prostate-specific antigen
QD	Quantum dot
RCA 1	Radio Corporation of America 1
RCA 2	Radio Corporation of America 2
RE	Reference electrode
rGO	Reduced graphene oxide
RNA	Ribonucleic acid
R.S.D	Relative standard deviation

RT	Room temperature
SAM	Self-assembled monolayer
SB-PBS	Starting Block phosphate buffered saline
scAb	Single-chain antibody
SEM	Scanning electron microscopy
Si	Silicon
SiH_4	Silane
SiO_2	Silicon dioxide
Si-OH	Silanol
SPE	Screen-printed electrode
SWV	Square wave voltammetry
TH	Thionine
Ti	Titanium
tPSA	Total prostate-specific antigen
PTMs	Post-translational modifications
TRUS	Transrectal ultrasound
UV	Ultraviolet
WE	Working electrode
XPS	X-ray photoelectron microscopy

LIST OF SYMBOLS

σ	Standard deviation
$\sum_{i=1}^{N} x_i$	Sum of all numbers of data set
ω	Angular frequency
φ	Phase angle
b	Sensor's sensitivity computed from the slope of calibration curve
C	Capacitance
c	Y-intercept
C_{CP}	Capacitance change of capture probe
C_{DL}	Double layer capacitance
C_{INS}	Capacitance change of insulating SAM layer
ΔC	Change in capacitance value
f	Frequency
F	Faraday Constant
i	Quantity of electron charges passed
I_0	Amplitude of applied current
j	Imaginary number equal to $\sqrt{(-1)}$
n	Rate of reaction and amount of electrons
Q	Constant phase element
R^2	Correlation coefficient
R_a	Mean surface roughness
R_{ct}	Charge transfer resistance
R_{max}	Maximum roughness
R_s	Resistance of electrolyte solution
V(t)	Applied voltage expressed in a function of time
V_0	Amplitude of applied voltage
Y_0	Magnitude of admittance at angular frequency = 1
Z'	Real part of impedance
Z"	Imaginary part of impedance
|Z|	Absolute impedance
ΔZ	Change in impedance value

Z_Q	Constant phase element
Z_W	Warburg impedance

Peranti Pengesan Tindakbalas Imun Berdasarkan Interdigit Mikro-Elektrod Emas Bebas Label Untuk Penanda-Bio Barah Prostat

ABSTRAK

Barah prostat ialah sejenis barah yang merebak secara senyap dan tidak bergejala di dalam kelenjar prostat. Ia merupakan barah kedua tertinggi yang sering didiagnosis di seluruh dunia dengan catatan 28% kematian dalam kalangan pesakit. Walaupun dengan pengenalan proses saringan barah pada beberapa puluh tahun yang lalu, teknik saringan tersebut tidak mampu dicapai oleh kalangan pesakit yang tinggal di kawasan pedalaman kerana sifat proses saringan yang kompleks dan tidak mudah-alih. Pembangunan platfom bio-pengesan bebas label dan mudah-alih menggunakan penanda-bio berpiawaian emas seperti antigen spesifik prostat telah dilancarkan untuk menyelesaikan masalah ini, seperti mana juga dengan pembangunan peranti pengesan tindakbalas imun berdasarkan interdigit mikro-elektrod emas yang dilakukan dalam hasil kajian ini. Dalam kajian ini, peranti interdigit mikro-elektrod dipilih berdasarkan daya pengesannya yang unggul, mudah-alih dan daya pembuatan massa yang bagus. Peranti pengesan ini difabrikasi di dalam makmal menggunakan teknik fotolitografi berpiawai yang menghasilkan mikro-elektrod bersaiz 10 µm dan jurang interdigit bersaiz 10 µm, diintegrasi dengan elektrod kaunter emas dan elektrod rujukan samaran emas pada substrat silikon dioksida. Untuk menjadikan peranti pengesan-bio, dua skema modifikasi pada pemukaan transduser dilakukan. Skema 1 melibatkan modifikasi lapisan mono terkumpul sendiri menggunakan proses ‘amino-silanisasi’ daripada bahan kimia 3-aminopropyltriethoxysilane dan imobilisasi antibodi monoklonal yang specifik kepada antigen spesifik prostat di atas permukaan silikon dioksida, manakala Skema 2 menggunakan tindakbalas ‘thiolasi’ oleh asid 16-merkaptoundekanoik dan imobilisasi antibodinya di atas mikro-elektrod emas. Kejayaan proses modifikasi ini disahkan menggunakan teknik mikroskopi tenaga atom, pengukuran sudut hubungan air, spektroskopi fotoelektron bertenaga x-ray dan voltammetri kitaran. Pengukuran kuantitatif terhadap peristiwa ikatan antigen spesifik prostat di atas transduser dilakukan menggunakan teknik Spektroskopi Impedans Elektrokimia dalam mod Faradaic (kehadiran spesies redoks) dan mod bukan-Faradaic (tanpa spesies redoks). Pengukuran mod Faradaic dilakukan dengan mengukur perubahan rintangan pemindahan caj di dalam sistem elektrokimia apabila peristiwa ikatan sasaran berlaku, manakala dalam mod bukan-Faradaic, pengesanan dilakukan dengan mengukur perubahan kapasitans dua lapisan. Pengesanan sasaran bio dalam mod Faradaic menghasilkan julat pengesanan linear 5000 ng/ml ke 0.5 ng/ml dengan had pengesanan pada 0.377 ng/ml dengan menggunakan pengesanan Skema 1. Sementara itu, dengan menggunakan Skema 2, pengesanan sasaran mod Faradaic menghasilkan julat pengesanan linear 100 ng/ml ke 0.01 ng/ml dan had pengesanan pada 0.01 ng/ml. Dalam mod bukan-Faradaic, julat pengesanan linear yang dihasilkan ialah daripada 5000 ng/ml ke 0.5 ng/ml dan had pengesanan pada 0.5 ng/ml. Pengamatan terhadap tahap kebolehulangan, kekhususan dan kestabilan alat pengesan

sasaran bio tersebut menunjukkan prestasi yang unggul untuk terus dibangunkan untuk direalisasikan sebagai platfom peranti titik penjagaan yang berkesan.

Label-Free Gold Interdigitated Microelectrodes Immunosensor for Prostate Cancer Biomarker

ABSTRACT

Prostate cancer is a slowly proliferating and non-symptomatic form of malignancy in the prostate gland. It is the second most commonly diagnosed type of cancer worldwide, with 28% deaths among diagnosed cases. Although with an introduction of cancer screening in the last few decades had saved countless lives, this technique could not reach out to the patients who live in rural areas due to its inherent complexities and non-portability. Recent developments in label-free and portable biosensing platforms using the gold-standard prostate-specific antigen are catered towards solving this current diagnoses loophole. Similarly, this research work develops a label-free gold interdigitated microelectrodes immunosensor which target to solve these limitations in the current cancer diagnostic technologies. In this work, an interdigitated microelectrodes architecture is chosen due to its superior detection sensitivity, portability and mass manufacturability. The device is fabricated in-house using an established Complementary Metal-Oxide Semiconductor fabrication process, with a gold microelectrode size of 10 µm and interdigitated gap size of 10 µm, integrated with a gold counter electrode and gold pseudo-reference electrode on a silicon dioxide substrate. For biosensing device development, two transducer surface modification schemes are demonstrated. Scheme 1 involves a self-assembled monolayer modification using amino-silanization of 3-aminopropyltriethoxysilane and immobilization of monoclonal antibody specific to prostate-specific antigen on the silicon dioxide substrate, meanwhile Scheme 2 involves modification of self-assembled monolayer using thiolation chemistry of 16-mercaptoundecanoic acid and immobilization of the monoclonal antibody on gold microelectrode surface. Successful sensor's modification steps are validated using atomic force microscopy, water contact angle measurement, X-ray photoelectron microscopy, and cyclic voltammetry characterization techniques. The binding event of prostate-specific antigen target on device transducer is quantitatively measured using a highly-sensitive Electrochemical Impedance Spectroscopy technique in both Faradaic (in presence of redox species) and non-Faradaic (in absence of redox species) modes of measurement. The Faradaic measurement is performed by measuring the changes in charge transfer resistance in the electrochemical double layer upon target binding, meanwhile in non-Faradaic mode, the detection is made by measuring the changes in the double layer capacitance of the electrochemical system. Using the detection Scheme 1, the bio-detection of prostate-specific antigen in Faradaic mode reveals a linear detection range of 5000 ng/ml to 0.5 ng/ml, with a limit of detection at 0.377 ng/ml. On the other hand, in Scheme 2, Faradaic measurement reveals a linear detection range of 100 ng/ml to 0.01 ng/ml and limit of detection of 0.01 ng/ml. In non-Faradaic mode, linear detection range of 5000 ng/ml to 0.5 ng/ml and limit of detection of 0.5 ng/ml are reported. The sensors' reproducibility, specificity and stability studies reveal highly promising sensing performances that warrant for future development into a point-of-care biosensing platform.

CHAPTER 1 : INTRODUCTION

1.1 Motivation

Since the first sighting on prostate cancer (PCa) by (Adams, 1853), it took the whole 88 years to discover an androgen-ablation therapy by Charles Huggins in 1940s, the first effective treatment to combat this disease. Furthermore, it was not more than only three decades ago since the prostate-specific antigen (PSA) screening was introduced had revolutionized our approach to tackle this endemic fatality. Albeit the long-time taken, these medical discoveries are nothing from the sort of miracles, except from the hard works among the scientists that led to these scientific breakthroughs that had saved countless lives.

There are many interesting effects due to discovery of PSA. For a start, the increase in the number of PCa prognosis from PSA screening is apparent, which translate to greater possibility of lives being saved. This life-saving fact holds true as reported shows the PSA screening has increased the success rate of PCa detection to ~70-80%, that is 50% increased before the use of PSA screening (Catalona, Smith, Ratliff, & Basler, 1993). It was also reported that the combination of PSA screening with digital rectal examination (DRE) test have led to a greater sensitivity in detecting the organ-confined PCa compared to DRE or transrectal ultrasound (TRUS) alone (Brawer et al., 1992; Crawford et al., 1996; Mettlin et al., 1993). Albeit these successes, the current PSA screening method still relies on the time-consuming and complicated laboratory-based tests that prolong the diagnosis time. Other than that, technique such as biopsy poses the risks of internal bleedings and infections, furthermore, not to

mention the psychological effects that lead to embarrassment among the patients. These loopholes have led to hesitation among men to undergo such procedures, resulting in complications to detect PCa in its early stage. Therefore, the principle motivation of this research is to eliminate these diagnostic loopholes by transitioning the existing methods into a label-free, portable, and highly sensitive immunosensing platform for early detection of PCa. The term 'immunosensing' describes the application of a sensing platform in this research work that incorporates the highly-specific immuno-response of the immobilized monoclonal antibody (mAb) towards the PSA target antigen.

1.2 Research background

An accurate diagnosis in earlier cancer stage is crucial to yield better disease monitoring and successful treatment for PCa. Many conventional PSA detection methods have been developed, such as, enzyme-linked immunosorbent assay (ELISA) (Matsumoto et al., 1999, 2000), fluorescence immunoassay (Choi et al., 2013), bioluminescent immunoassay (Ito et al., 2007), chemiluminescence immunoassay (Jolly, Damborsky, et al., 2016), and time-resolved fluorescence assay (Härmä et al., 2003; Siivola et al., 2000). Albeit their good sensitivity, these methods are complicated, require labels or tags, laborious, non-portable, time-consuming, and expensive. Another interesting method, the immunochromatography test strip (ICTS) (Li et al., 2014) offers the speed, portability, simplicity and point-of-care (PoC) testing, however it requires high antigen loading to produce translatable output. Furthermore, most of the reported ICTS outputs are of qualitative nature at most, and rarely semi-quantitative.