New Approaches in Porous Silicon Based Optical Immunosensors

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Submitted on the 25th of March 2014
in fulfilment for the degree of Doctor of Philosophy (PhD)
Accepted on the 28th of August 2014

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Abstract

The rapid and reliable detection of diseases and pathogens is essential to modern healthcare systems. Development of new and more efficient sensing techniques is continuously being undertaken to meet this requirement with a large focus on immuno- and biosensing devices. The work conducted in this thesis seeks to address these needs via the development of new detection mechanisms and receptor immobilisation techniques for porous silicon (pSi) based optical immunosensors.

Initial investigations focused on the fabrication of an appropriate pSi optical sensor platform via changes in the electrochemical etching parameters. Higher current density applied during the etching cycle resulted in increased pore size, porosity and etching rate of n-type pSi. Monolayer pSi containing higher levels of porosity were demonstrated to be more sensitive to changes in refractive index through interferometric reflectance spectroscopy (IRS). Optimisation of these parameters yielded a sensitive and flexible sensor platform.

Development of a new absorbance based pSi optical biosensor was then undertaken. Detection of a human IgG analyte was achieved via a cascade of immunological reactions at the pore walls to form a sandwich assay. The detection strategy involved an alkaline phosphatase (AP) labelled secondary antibody and precipitation of the enzyme substrate 5-bromo-4-chloro-3-indoyl phosphate (BCIP)/nitro blue tetrazolium (NBT) within the porous matrix. The intense colour change and strong absorbance of the biocatalysed BCIP/NBT compounds at 600 nm provided a measureable response on the intensity of the reflected optical profile of the porous layer. This approach yielded a limit of detection of 2.14 ng/mL, well within the working range required for analysis of clinical samples.

Following development of the new pSi sensor, a special protein based IgG affinity coating was investigated as a new method of receptor immobilisation on optical sensors. Adaptation of a previously reported genetically modified bacterial surface layer (S-layer) protein from *Lysinibacillus sphaericus* containing twin IgG binding domains ($SbpA_{31\text{-}1064}$/ZZ) provided a route to the formation of a self-

assembling protein layer capable of immobilising receptor IgG molecules with defined orientation. In vitro self-assembly of purified recombinant $rSbpA_{31-1068}$/ZZ fusion protein was demonstrated by the formation of crystalline protein layers on various surface chemistries. IgG binding capacity was shown on $rSbpA_{31-1068}$/ZZ coated ELISA microtiter plates via the immobilisation of IgG capture antibodies and detection of human IgG and human Interleukin-6 analytes. Integration of this coating into the previously developed pSi biosensor yielded a general improvement in sensor performance compared to covalent attachment of capture antibodies indicating that this new approach resulted in less receptor inhibition and greater numbers of viable binding sites.

Finally, the development of a new pSi optical interferometric biosensor based on metallic deposition was investigated. Chemical reduction of silver and deposition within gold treated pSi was found to result in a significant decrease to the EOT of the material due to a refractive index change. This refractive index 'contrast' enhancement was demonstrated on both gold nanoparticle decorated pSi and gold plated pSi and optimised to provide maximum signal change. An enzyme mediated silver deposition system was then developed using alkaline phosphatase and a synthesised enzyme substrate, hydroquinone diphosphate. Enzyme mediated silver deposition on gold plated pSi was demonstrated and optimised. Finally, adaptation of this system to a pSi optical immunosensor was demonstrated via the detection of human IgG.

The new organic and metallic enhancement immunosensors developed in this thesis demonstrate strong sensor platforms and with further investigation may be viable as future diagnostic techniques. In addition, the S-layer affinity coating has vast potential for use in a variety of immunosensors and a swath of other applications including patterned microarrays, biomimetics and drug delivery.

Declaration

‘I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text. I also certify that the entirety of the experimental work represented herein was conducted solely by the author unless otherwise stated.’

Andrew Oliver Jane

Acknowledgements

I would like to express my very great appreciation to Dr Roman Dronov who initially worked on this project as a colleague and eventually became my supervisor and friend. His support, both academic and financial, was essential in finishing this work. I would also like to thank Professor Nico Voelcker who encouraged me to undertake a PhD and was instrumental in the project's conception and direction. His willingness to take an active role and generously give his time has been very much appreciated and is recognized by all of his students. To my other supervisors, Dr Alastair Hodges and Professor Joe Shapter go my eternal thanks for their expansive knowledge and advice on the practicalities of biosensing; and especially to Joe for taking me under his wing when circumstances left me without a supervisor.

My great friend Chris Williams has shared this journey with me from the beginning of undergraduate studies and I would like to offer heartfelt thanks for keeping my spirits up and understanding the many trials and tribulations associated with a PhD when many others wouldn't. To the members of the cockpit, Marty and Steve, I thank you for your camaraderie and support. The office may be long gone but our friendship remains.

I am particularly grateful for the assistance given by Dr Endre Szili who taught me everything I know about porous silicon biosensing and provided financial support when things became difficult. To Dr Anthony Quinn and Lastek, you are the best employer anyone could ask for. Your ongoing flexibility and active support of my studies has allowed me the time and space needed to finish research work and write this thesis.

I wish to acknowledge my parents for their loving support and encouraging my education. They made me who I am today and instilled a passion for knowledge and logic, without their guidance I would never have made it so far. To my son Noah, your arrival into this world was a bright ray of light. I thank you for the happiness and laughter that you bring to my life.

Finally, and most importantly, I would like to thank my beautiful, loving and supportive wife, Megan. She has my deepest gratitude for her encouragement during the rough times and for single handedly running the household and a business so I could concentrate on my studies. You are my rock and have my undying thanks.

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List of Abbreviations

Abbreviation	Full Name
Ab	Antibody
ABS	Antigen Binding Site
ADH	Alcohol dehydrogenase
AFM	Atomic Force Microscopy
AP	Alkaline Phosphatase
APTES	3-aminopropyl triethoxysilane
Atg	Antigen
ATR	Attenuated total reflectance
AuNP	Gold nanoparticle
BCIP	5-bromo-4-chloro-3-indoyl phosphate
BCIP-indigowhite	5,5-dibromo-4,4-dichloro-indigowhite
BSA	Bovine serum albumin
CCD	Charge-Coupled Device
CtC	Centre-to-centre distance
CV	Coefficient of variation
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
E. Coli	Escherichia Coli
EDAX	Energy Dispersive X-ray Analysis
ED	Electroless Deposition
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
ELISA	Enzyme-Linked Immunosorbent Assay
EOT	Effective Optical Thickness
etc	et cetera
EtOH	Ethanol
FFT	Fast Fourier Transform
FTIR	Fourier Transformed Infrared Spectroscopy

GnHCl	Guanidine hydrochloride
HAc	Acetic acid
HF	Hydrofluoric acid
HQ	Hydroquinone
HQDP	Hydroquinone diphosphate
HRP	Horseradish peroxidase
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-6	Interleukin-6
IPTES	3-isocyanatopropyl triethoxysilane
IPTG	Isopropyl β-D-1-thiogalactopyranoside
IR	Infrared
IRS	Interferometric Reflectance Spectroscopy
L. sphaericus	Lysinibacillus sphaericus
LB	Lysogeny Broth
LOD	Limit of Detection
Method 1	Solution based immobilisation of AuNP's on pSi
Method 2	Drying based immobilisation of AuNP's on pSi
milliQ	Ultrapure water, resistivity 18.2 MΩ.cm
MS	Mass spectroscopy
MUA	11-Mecaptoundecanoic acid
mλ	Spectral order of the Fabry-Pérot fringe (m) times wavelength of the incident light striking the surface at an incident angle of 0˚ (λ)
Na(HQDP)	Sodium Salt of hydroquinone diphosphate
NB	Nutrient Broth
NBT	Nitro Blue Tetrazolium
nd	Average refractive index of a porous silicon layer (n) times porous silicon layer thickness (d)
NHS	N-Hydroxysuccinimide
NMR	Nuclear Magnetic Resonance spectroscopy
n-type	Phosphorous doped silicon

OD	Optical Density
OPD	O-Phenylenediamine dihydrochloride
Ozone Silicon	Ozone oxidised silicon, treated with mild thermal oxidation
PBS	Phosphate Buffer Saline
PBS-T	PBS-Tween® 20
PDMS	Polydimethylsiloxane
PEG	Polyethylene Glycol
PEG Silane	n-(triethoxysilylpropyl)-o-polyethylene oxide urethane
PFCS	Pentafluorophenyl dimethylchlorosilane
pSi	Porous silicon
PSS	Polystyrene sulfonate
Pty Ltd	Proprietary Limited
p-type	Boron doped silicon
QCM	Quartz Crystal Microbalance
RIA	Radio-immunoassay
RMS	Root mean squared
S/N	Signal to noise ratio
SA	Silver acetate
SbpA	S-Layer protein from *L. sphaericus*
SbpA-ZZ	Recombinant S-layer fusion protein, $rSbpA_{31-1068}/ZZ$
SCWP	Secondary Cell Wall Polymer
SDS-PAGE	Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis
SEM	Scanning Electron Microscopy
S-layer	Surface layer protein
SPR	Surface Plasmon Resonance
Sulfo-NHS	N-Hydroxysulfosuccinimide
TDFCS	Tridecafluoro-1,1,2,2-tetrahydrooctyl-dimethylchlorosilane
TIR	Total Internal Reflectance
TIRF	Total Internal Reflectance Fluorescence
TM	Trademark
TMB	3,3',5,5'-Tetramethylbenzidine

Tris	Trizma base
Tris-HCl	Trizma HCl
Tris-T	Tris buffer-Tween® 20
™	Trade Mark
UV-Vis	Ultraviolet-Visible
Z	Synthetic IgG binding domain
α	Anti
λ	Wavelength
®	Registered

List of Units

Unit Abbreviation	Full Name
%	Percentage
~	Approximately
<	Less than
>	Greater than
≤	Less than or equal to
≥	Greater than or equal to
°	Degrees
°C	Degrees Celsius
^{1}H	Proton
A	Ampere
au	Arbitrary units
avg	Average
C	Coulomb
cm	Centimetre
cm^{-1}	Reciprocal centimetres (wavenumbers)
d	Porous layer thickness
eV	Electronvolt
g	Grams
hr	Hour
J	Coupling constant (Hz).
kDa	Kilo Daltons
kV	Kilovolt
M	Molar
m/z	Mass to charge ratio
mA	Milliamps
min	Minute
mL	Millilitre
mM	Millimolar

mmol	Millimoles
n	Refractive index
ng	Nanogram
nm	Nanometre
ø	Diameter
pH	Potential of hydrogen
pKa	Acid dissociation constant
S	Singlet
sec	Seconds
v/v	Volume per volume
W	Watts
w/v	Weight per volume
ε	Molar extinction coefficient
µg	Microgram
µL	Microlitre
µm	Micrometre
µM	Micromolar
Ω	Ohms

Peer Reviewed Publications

Szili, E.J., Jane, A., Low, S.P., Sweetman, M., Macardle, P., Kumar, S., Smart, R.St.C., Voelcker, N.H., 'Interferometric porous silicon transducers using an enzymatically amplified optical signal', Sensors and Actuators B, 160 (2011), 341-348.

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