Extending the uses of lipid-membrane coated electrodes: Next generation of lipid membrane biosensors and smart implantable cell-electrode devices

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

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Certificate of Original Authorship

I, Amani Alghalayini, declare that this thesis is submitted in fulfillment of the requirements for the award of a Doctor of Philosophy, in the School of Life Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. This document has not been submitted for qualifications at any other academic institution.

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Amani Alghalayini

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Dedication

To the one whom I discovered my life with,

To my soulmate,

To my paradise on earth,

To Tariq

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Publications

Refereed journal publications

- **Alghalayini, A.**, A. Garcia, T. Berry, and C.G. Cranfield, *The Use of Tethered Bilayer Lipid Membranes to Identify the Mechanisms of Antimicrobial Peptide Interactions with Lipid Bilayers.* Antibiotics, 2019. **8**(1): p. 12.

Conference Presentations

- **Conference oral presentations**

  - **Amani Alghalayini**, Lele Jiang, Xi Gu, Guan Yeoh, Charles G. Cranfield, Victoria Timchenko, Bruce Cornell, Stella M. Valenzuela. Heat transfer characteristics of nanoparticles measured with tethered bilayer lipid membranes. 10th International Nanomedicine Conference. 2019 Pier One Sydney Harbour Hotel, Sydney, Australia

  - **Amani Alghalayini**, Lele Jiang, Xi Gu, Guan Yeoh, Charles G. Cranfield, Victoria Timchenko, Bruce Cornell, Stella M. Valenzuela**.** Study of heat transfer from laser-irradiated gold nanoparticles using tethered bilayer lipid membranes. Asian biophysics association symposium and annual meeting 2018 of the Australian society for biophysics. RMIT University, Melbourne, VIC, Australia

  - **Amani Alghalayini**, Lele Jiang, Xi Gu, Guan Yeoh, Charles G. Cranfield, Victoria Timchenko, Bruce Cornell, Stella M. Valenzuela. Real-time monitoring of heat transfer between gold nanoparticles and tethered bilayer lipid membranes. ICEAN 2018 the International Conference on Emerging Advanced Nanomaterials. Newcastle Exhibition & Conference Centre, Newcastle, NSW, Australia

  - **Alghalayini, Amani**; Parviz, Maryam; Cornell, Bruce A.; Cranfield, Charles G.; Valenzuela, Stella M. Cell-electrode interfaces and optimal architecture for cell detection. IDEAL-ARC Hub Annual Forum 2018. University of South Australia, Cancer Research Institute, Adelaide, Australia.

- **Conference poster presentations**

  - **Alghalayini, Amani**; Parviz, Maryam; Cornell, Bruce A.; Cranfield, Charles G.; Valenzuela, Stella M. Optimizing cell-electrode interface mimicking the natural environment. International Conference on Nanoscience and Nanotechnology ICONN 2018 the University of Wollongong, Wollongong, Australia

  - **Alghalayini, Amani**; Parviz, Maryam; Cornell, Bruce A.; Cranfield, Charles G.; Valenzuela, Stella M. Ion conduction recording of neuronal cell activity utilizing tethered lipid membrane. The 2017 annual meeting of the Australian Society for Biophysics. UTS, Sydney, Australia.

  - **Alghalayini, Amani**; Parviz, Maryam; Cornell, Bruce A.; Cranfield, Charles G.; Valenzuela, Stella M. Development of a cell-based sensor using functionalized tethered lipid bilayer membrane. The New Horizon, 2017. University of Sydney, Sydney, Australia

  - **Alghalayini, Amani**; Parviz, Maryam; Cornell, Bruce A.; Cranfield, Charles G.; Valenzuela, Stella M. Improving implantable technologies by optimizing specificity and efficiency between electrode-cell interfaces. The New Horizon 2016, Sydney, Australia.

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Appendix

List of Abbreviations

Å	Angström (10-10 m)
AC	Alternative current
AFM	Atomic Force Microscopy
Ala	Alanine amino acid
Alpha-IMS	Alpha-Institute for Microelectronics Stuttgart
AM199	Zwitterioninc lipids comprised 70% C20 diphytanyl-ether-glycero-phosphatidylcholine and 30% C20 diphytanyldiglyceride ether lipids
AM215	mobile lipids mixture of 3:7 molar ratio of GDPE: DPEPC
Arg	Arginine amino acid
Asn	Asparagine amino acid
Asp	Aspartate amino acid
BLM	Black lipid membrane
B-PE	1-oleoyl-2-(12-biotinyl (aminododecanoyl))-sn-glycero-3-phosphoethanolamine
BSA	Bovine serum albumin
C_m	Membrane capacitance
cRGDfK(B-PEG)	Cyclo [Arg-Gly-Asp-D-Phe-Lys (Biotin-PEG-PEG)]
CHO-K1	Chinese hamster ovary cells
Con-A	Concanavalin A
Cys	Cysteine amino acid
D_2O	Deuterated water
d31-POPC	Deuterated 1-palmitoyl-(d31)-2-oleoyl-sn-glycero-3-phosphatidylcholine
DAPI	4′,6-diamidino-2-phenylindole
DC	Direct current
DLP	Half-membrane is spanning
DMEM	Dulbecco's modified eagle's medium
DOTAP	1, 2-dioleoyl-3-trimethylammonium-propane (chloride salt)
DPBS	Dulbecco’s phosphate-buffered saline
DPEPC	diphytanyl ether phosphatidylcholine
DP-NGPE	1,2-dipalmitoyl-sn-glycero-3phosphoethanolamine-N-(glutaryl) (sodium salt)
ECIS	Electrical cell-substrate impedance sensors
ECM	Extracellular matrix
E-coli	Escherichia coli
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
EDTA	Ethylenediaminetetraacetic acid
EIS	Electrochemical impedance spectroscopy
f	Frequency

FBS	Fetal bovine serum
G418	Geneticin
gA5XB	biotinylated gramicidin
gAYYSSBn	tethered gramicidin
GDPE	glycerodiphytanylether
Gln	Glutamine amino acid
Glu	Glutamic acid amino acid
Gly	Glycine amino acid
G_m	Membrane conduction
GNPs	gold nanoparticles
GNUs	gold nano urchins
GRGDS	Glycine-arginine–glycine–aspartic acid-serotonin (GLY-ARG-GLY-ASP-SER)
Hz	Hertz
I	Current
ICS	Ion-channel switch
IKVAV	Ile-Lys-Val-Ala-Val
K	Kilo
laser	light amplified stimulated emission of radiation
LB	lysogeny broth
Leu	Leucine amino acid
Lys	Lysine amino acid
M	Molar
mg	Milligram
ml	milli-Litre
MLP	Mobile lipid phase
mM	milli-Molar
MSLa	Biotinylated tethered membrane-spanning lipid
MTSDDL	Met-Thr-Ser-Asp-Asp-Leu (Methionine-Threonine-Serine-Aspartate-Aspartate-Leucine)
mV	Millivolt
MΩ	Megaohm
nF	NanoFarad
NHS	N-hydroxysuccinimide
nm	Nanometer
nM	Nanomolar
NNEN	Asn-Asn-Glu-Asn (Asparagine Asparagine Glutamate Asparagine)
NR	Neutron Reflectivity
^{o}C	Degree Celsius
OEG	Oligo ethylene glycol
OmpF	Outer membrane protein
PBS	Phosphate buffered saline
PC	Phosphatidylcholine

PE	Phosphotidylethanolamine
PEG	Polyethylene glycol
PFA	paraformaldehyde
PHSRN	Pro-His-Ser-Arg-Asn
PLL	Poly-L-lysine
POEGA	Poly(oligoethylene glycol) acrylate
POPC	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine
POPE	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylethanolamine
PS	Phosphatidylserine
Q	Momentum transfer
QCM	Quartz Crystal Microbalance
R	Resistance
RGD	Arginine–glycine–aspartic acid (Arg-Gly-Asp)
Rm	Resistance
RYD	Arginine–tyrosine –aspartic acid (Arg-Tyr-Asp)
SAMs	Self-assembled monolayers
SAMs	self-assembled monolayers
Ser	Serine amino acid
SFM	serum-free medium
SH-SY5Y	Neuroblastoma cell line
SiO_2	Silicon dioxide
SLB	Supported lipid bilayer
SLD	Scattering Length Density
SPR	Surface Plasmon Resonance
t	Time
T10	10 percent of tethering molecules
T100	fully tether molecules percentage
TAT	GRKKRRQRRRPQ
tBLM	Tethered bilayer membrane
TEG	Triethylene glycol
Triton-X100	Octylphenyl-nonaoxyethylene
Trp	Tryptophan amino acid
Tyr	Tyrosine amino acid
UV	Ultraviolet
V	Voltage
Val	Valine amino acid
YIGSR	Tyr–Ile–Gly–Ser–Arg
Z	Impedance
|Z|	The magnitude of the impedance
ΔA	Percentage surface area expansion
θ	Phase
λ	Wavelength
µg	Microgram

µl	micro-Litre
µM	Micromolar
µS	Microsiemens
σ	Roughness
2D	Two-dimensional
3D	Three-dimension

Abstract

The ability to combine both a functional sensing and signalling membrane-electrode interface system is crucial for developing new technologies that can directly connect the living biosphere with electrical devices. However, there is a considerable distinction between both the chemical and biomechanical properties of live cell membranes versus synthetic electrical prostheses, thus there remain significant challenges that must be overcome in order to establish stable and functionally predictable interactions between these different components. The sparsely tethered bilayer lipid membrane possesses the necessary skeleton onto which novel chemistries can be added in order to succeed in the first iteration of correctly integrating electronic coupling with biological tissue.

This dissertation presents an investigation into controlling the ionic and the electronic interface and then detecting ion fluxes arising from nearby biologically active cells at the nanometer scale, by using the detectable electrical signals derived from interfacing of membranes with a gold electrode. In it, the feasibility of implementing tBLMs as either an interface between biological systems and electrical devices or for continual sensing in real-time or for diagnostic purposes is investigated.

Commencing is a comprehensive review of variant artificial lipid membrane models and the impedance spectroscopy approach (Chapter 1). A demonstration of the intimate nanoscale contacts of cells with the surface of the electrode is presented in Chapter 2. The aim of this study was to examine the feasibility of applying tBLMs in bio-implantable devices to offer specific transmission of electrical signals to individual target neurons to improve signal fidelity. This was to be achieved by reducing leakage pathways, thereby minimizing electrophoretic ion currents being lost into the surrounding interstitial

medium. Chapter 3 describes how, instead of using the lipid membrane-covered electrodes to signal to cells, the electrode might be used to as a nano-biosensor for cell detection. Various approaches to increase sensitivity were explored to enhance this capability. The necessity for detection at the nanometer scale is explored in Chapter 4, recording in real-time the laser-generated heat pulses arising from laser-illuminated gold nanoparticles. Detection of these heat pulses required attachment of the gold nanoparticles to the membrane surface, while non-specific binding of gold nanoparticles failed to elicit a measurable response.

Conclusions and perspectives are presented in Chapter 5, sum up of the significant achievements presented in this dissertation, which has focused on extending our understanding of cell membrane interactions and exploring the feasibility of using these across a range of applications.