Novel Planar Interdigital Sensors for the Detection of Bacterial Endotoxins

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Electronics Engineering at Massey University, Palmerston North, New Zealand

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2012

To my parents

Abdul Rahman Daud

Mariah Bt Deraman

To my wife

Nurhanina Mohamad Akib

And to my children

Nurafifah Afrina

Muhammad Afiq

Muhammad Ammar

Muhammad Asyraf

Muhammad Aiman

Abstract

Food poisoning caused by endotoxins or Lipopolysaccharide (LPS) are associated with Gram-negative bacteria. Foodborne pathogens, *Escherichia coli* (*E. coli*) and *Salmonella* are examples of Gram-negative bacteria which could cause large food poisoning outbreaks. New types of planar interdigital sensors have been fabricated with different coating materials to assess their response to endotoxins. A carboxyl-funtional polymer, APTES (3-Aminopropyltriethoxysilane) and Thionine were chosen for coating the novel interdigital sensors. All coated sensors were immobilized with PmB (Polymyxin B) which has specific binding properties to LPS. The sensors were tested with different concentrations of LPS O111:B4, ranging from 0.1 μg/ml to 1000 μg/ml. Analyses of sensors' performance were based on the Impedance Spectroscopy method. The impedance spectra were modelled using a Constant Phase-Element (CPE) equivalent circuit, and a Principal Component Analysis (PCA) was used for data classification. Sensors coated with APTES have shown better selectivity towards LPS detection. The experiments were repeated by coating APTES and immobilizing PmB to a newly improved design of silicon based interdigital sensor. Scanning electron microscope (SEM) and atomic force microscope (AFM) images were taken to analyse the APTES coating surface and PmB immobilization. The images of non-coated sensors and coated silicon sensors were studied and the thickness of a single layer coating was estimated (≈268 nm). Analyses of results with LPS O111:B4 showed that these silicon sensors have higher sensitivity and selectivity to the target biomolecule LPS. The complex non-linear least squares (CNLS) fitting method was used to fit the measured impedance spectra based on chosen equivalent circuit model. PCA results were clustered, showing the parameters were related and have identified process which related to the diffusion, charge transfer and adsorption of molecules on sensors' surface. It was also found that these sensors can detect the standard endotoxin as low as 0.01 EU/ml which is equivalent to 1 pg/ml. Selectivity, stability and sensitivity of different thickness of coated sensors were analysed. It was observed that the optimum thickness layer is 3-layers of coating which is equivalent to 800 nm. Analyses of results with food samples have shown the developed novel interdigital sensors can detect the presence of endotoxin in contaminated food samples.

Research Outputs

The research outputs which have been published are listed below. The research outputs are in conjunction with the author's Ph.D candidacy:

Book Chapters

1. Mohd Syaifudin Bin Abdul Rahman, Subhas C. Mukhopadhyay, Pak-Lam Yu, Michael J. Haji-Sheikh and Cheng-Hsin Chuang. *"Sensors for Food Inspections"*. Chapter 4 in Modern Sensors, Transducers and Sensor Networks (Book Series: *Advances in Sensors: Reviews, Vol. 1*). International Frequency Sensor Association (IFSA) Publishing. 15 May 2012. ISBN: 978-84-615-9012-4.

2. A. R. Mohd Syaifudin, Subhas Chandra Mukhopadhyay, Pak-Lam Yu, Michael J. Haji-Sheikh, and Cheng-Hsin Chuang. *"Impedance Spectroscopy of Novel Interdigital Sensors for Endotoxin Detection"*. Taylor and Francis Group- CRC Press: Lecture Notes on Impedance Spectroscopy - Measurement, Modelling and Applications - Kanoun (ed). Volume 2. Copyright 2012, CRC Press, Taylor & Francis Group, London. Pp. 103- 112. ISBN 978-0-415-69838-2.

Journals

1. Mohd Syaifudin Abdul Rahman, Subhas Chandra Mukhopadhyay, Pak-Lam Yu, Javier Goicoechea, Ignacio R. Matias, Chinthaka P. Gooneratne, and Jűrgen Kosel. *"Bacterial Endotoxin in Food: New Planar Interdigital Sensors based Approach". Elsevier:* Journal of Food Engineering 114 (2013). Pp. 346–360, DOI: 10.1016/j.jfoodeng.2012.08.026. 14th September 2012.

2. A. R. Mohd Syaifudin, Subhas Chandra Mukhopadhyay, Pak-Lam Yu, Michael J. Haji-Sheikh, Cheng-Hsin Chuang, John D. Vanderford, and Yao-Wei Huang. *"Measurements and Performance Evaluation of Novel Interdigital Sensors for Different Chemicals Related to Food Poisoning"*. IEEE Sensors Journal, Vol. 11, No. 11, Pp. 2957-2965. DOI: 10.1109/JSEN.2011.2154327. November 2011.

3. A. R. Mohd Syaifudin, S. C. Mukhopadhyay and P. L. Yu. *"Modelling and Fabrication of Optimum Structure of Novel Interdigital Sensors for Food Inspection"* Wiley: International Journal of Numerical Modelling: Electronic Networks, Devices and Fields. Vol. 24, Issue 6. Pp. 1-18. DOI: 10.1002/jnm.813. ISSN: 1099-1204. 1st February 2011.

4. Mohd. Syaifudin Abdul Rahman, Subhas C. Mukhopadhyay and Pak-Lam Yu. *"Novel Sensors for Food Inspections"*, Sensors & Transducers Journal**.** Vol. 114, Issue 3, March 2010, Pp. 1- 40. ISSN: 1726- 5479.

Proceedings and Conference Papers

1. A.R. Mohd Syaifudin, Asif I. Zia, S. C. Mukhopadhyay, P.L. Yu, Chinthaka P. Gooneratne and Jűrgen Kosel. *"Improved Detection Limits of Bacterial Endotoxins Using New Type of Planar Interdigital Sensors"*. Proceeding of IEEE Sensors 2012 Conference, Taipei, Taiwan. Pp. 2034-2037. 28th–31st October, 2012. IEEE Catalog Number: CFP12SEN-USB. ISBN: 978-1-4577-1765-9.

2. A.R. Mohd Syaifudin, S. C. Mukhopadhyay, P.L. Yu, Cheng-Hsin Chuang, Hsun-Pei Wu, Jürgen Kosel, and Chinthaka P. Gooneratne. *"Characterizations and Performance Evaluations of Thin Film Interdigital Sensors for Gram-Negative Bacteria Detection"*. Proceeding of 5th International Conference on Sensing Technology (ICST 2011), Palmerston North, New Zealand. Pp. 186 – 191. 28th Nov – 1st Dec 2011. IEEE Catalog Number: CFP1118E-CDR. ISBN: 978-1-4577-0167-2.

3. Asif. I. Zia, A.R. Mohd Syaifudin, S.C. Mukhopdhyay, I. H. Al-Bahadly and P.L. Yu, C.P. Gooneratne and Jürgen Kosel. *"Development of Electrochemical Impedance Spectroscopy Based Sensing System for DEHP Detection"*. Proceeding of 5th International Conference on Sensing Technology (ICST 2011), Palmerston North, New Zealand. Pp. 703 – 711. 28th Nov – 1st Dec 2011. IEEE Catalog Number: CFP1118E-CDR. ISBN: 978-1-4577-0167-2.

4. A.R. Mohd Syaifudin, S. C. Mukhopadhyay, P.L. Yu, Ignacio R. Matias, J. Goicoechea, Jürgen Kosel, and Chinthaka P. Gooneratne. *"Analyses of Performance of Novel Sensors with Different Coatings for Detection of Lipopolysaccharide"*. Proceeding of IEEE Sensors 2011 Conference, Limerick, Ireland. Pp. 588 – 591. October 28-31, 2011. IEEE Catalog Number: CFP11SEN-CDR. ISBN: 978-1-4244-9288-6. DOI: 10.1109/ICSENS.2011.6127023.

5. A.R. Mohd Syaifudin, S. C. Mukhopadhyay, P.L. Yu, Michael J. Haji-Sheikh, Cheng-Hsin Chuang and Hsun-Pei Wu. *"Detection of Natural Bio-Toxins Using an Improved Design Interdigital Sensors"*. Proceeding of IEEE Sensors 2011 Conference, Limerick, Ireland. Pp. 1028 - 1031. October 28-31, 2011. IEEE Catalog Number: CFP11SEN-CDR. ISBN: 978-1-4244-9288-6. DOI: 10.1109/ICSENS.2011.6127038.

6. A.R. Mohd Syaifudin, S. C. Mukhopadhyay, P.L. Yu, Michael J. Haji-Sheikh and Cheng-Hsin Chuang. *"Novel Interdigital Sensors: Analysis, Measurement and Evaluations"*. Proceeding of the IEEE: International Instrumentation and Measurement Technology Conference, (I^{2}MTC). Binjiang, Hangzhou, China. Pp 358-362. May 10-12, 2011. ISBN: 978-1-4244-7934-4.

7. A.R. Mohd Syaifudin, P.L. Yu, S. C. Mukhopadhyay, Michael J. Haji-Sheikh and J. Vanderford. *"A performance evaluation of new developed novel planar interdigital sensors".* Proceeding of the International Instrumentation and Measurement Technology Conference, (I^2MTC). Hilton. Austin, Texas. United States. Pp 731-736 May 3-6, 2010. DOI: 10.1109/IMTC.2010.5488245.

8. A.R. Mohd Syaifudin, C.P. Gooneratne, S. C. Mukhopadhyay, P.L. Yu, C-H. Chuang, Y-W. Huang, Michael J. Haji-Sheikh and J. Vanderford. "*A comparative study of three different designs of novel planar interdigital sensors for food inspections.*" Proceeding of the 4th International Conference on Sensing Technology (ICST 2010). *Lecce, Italy*. Pp 560-564. June 3-5, 2010.

9. A.R. Mohd Syaifudin, M. A. Yunus, K. P. Jayasundera and S. C. Mukhopadhyay. *"A Novel Planar Interdigital Sensor for Environmental Monitoring".* Proceeding of the IEEE SENSORS 2009 Conference. Christchurch Convention Centre, Christchurch, New Zealand. Pp 105-110, 25-28 October 2009. DOI: 10.1109/ICSENS.2009.5398227.

10. A.R. Mohd Syaifudin, K. P. Jayasundera , and S. C. Mukhopadhyay. *"A novel planar interdigital sensor based sensing and instrumentation for detection of dangerous contaminated chemical in seafood".* Proceeding of the IEEE: International Instrumentation and Measurement Technology Conference (I^2MTC), Singapore. Pp 701-706, 5-7 May 2009. DOI: 10.1109/IMTC.2009.5168540.

11. A. R. Mohd Syaifudin, S. C. Mukhopadhyay and P. L. Yu. *"Electromagnetic Field Computation using COMSOL Multiphysics to Evaluate the Performance of Novel Interdigital Sensors".* Proceeding of the IEEE Applied Electromagnetic Conference. Hyatt Regency Kolkata, India. 4 pages. 14-16 Dec 2009. DOI: 10.1109/AEMC.2009.5430661.

Seminars

1. A.R. Mohd Syaifudin, S. C. Mukhopadhyay and P. L. Yu. *"Bacterial Endotoxin: Analyses using New Type of Planar Interdigital Sensors".* Electronics, Information and Communication System (EICS) Seminar, 2nd August 2012. Massey University, New Zealand.

2. A.R. Mohd Syaifudin, S. C. Mukhopadhyay and P. L. Yu. *"Characterization and Measurement of Novel Interdigital Sensors using Impedance Spectroscopy".* 2011 IEEE (NZ) Sensors, Measurement and Instrumentation Workshop. AUT, Auckland. 10th – 11th March 2011.

3. A. R. Mohd Syaifudin, S. C. Mukhopadhyay, and P.L. Yu. *"Novel Planar Interdigital Sensors for Detection of Dangerous Toxins*". IEEE Instrumentations

and Measurement Society New Zealand Chapter Workshop, 1st – 2nd September 2010. Victoria University, Wellington. New Zealand.

4. A. R. Mohd Syaifudin, S. C. Mukhopadhyay and P.L. Yu. *"Novel Interdigital Sensors Analysis, Measurement and Performance Evaluations*". IEEE Postgraduates Seminar, IEEE New Zealand Central. 31st August 2010. Massey University, Palmerston North. New Zealand.

5. A. R. Mohd Syaifudin. *"Novel Sensors for Endotoxins Detection"*. Electronics, Information and Communication System (EICS) Seminar, 31st May 2010. Massey University, New Zealand.

6. A. R. Mohd Syaifudin. *"Novel Sensors for Food Inspections"*. PhD confirmation seminar. 17th February 2010. Massey University, Palmerston North. New Zealand.

7. A. R. Mohd Syaifudin. *"A Smart Sensor System for Raw Seafood Inspection"*. IEEE Post Graduate Presentations. 4th September 2009. Victoria University. Wellington. New Zealand.

Awards

1. Listed in 10 best articles award in 2010 from International Frequency Sensor Association (IFSA) and Sensor & Transducers Journal's Editorial Board for the article titled: *"Novel Sensors for Food Inspections"*, Sensors & Transducers Journal**.** Vol. 114, Issue 3, March 2010, Pp. 1- 40. ISSN: 1726- 5479.

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Abbreviations

SEAT	School of Engineering and Advanced Technology
LPS	Lipopolysaccharide
PmB	Polymyxin B
E. coli	*Escherichia coli*
VTEC	Verotoxin-producing *Escherichia coli*
STEC	Shiga Toxin-producing *Escherichia coli*
DNA	Deoxyribonucleic Acid
PCR	Polymerase Chain Reaction
ELISA	Enzyme Linked Immunosorbent Assays
LAL	Limulus Amoebocyte Lysate
FDA	United States Food and Drug Administration
EU	Endotoxin Unit
LOD	Limit of Detection
AMP	Antimicrobial Peptides
SPR	Surface Plasmon Resonance
FTIR	Fourier Transform Infrared Spectroscopy
ISE	Ion-Selective Electrodes
ISFET	Ion-Sensitive Field Effect Transistors
EIS	Electrochemical Impedance Spectroscopy
IS	Impedance Spectroscopy
AC	Alternating Current
QCM	Quartz Crystal Microbalance
SAW	Surface Acoustic Wave
NMR	Nuclear Magnetic Resonance
SNR	Signal to Noise Ratio
IDTs	Interdigital Transducers
IDAM	Interdigitated Array Microelectrode
IDES	Interdigitated Electrode Structures
EC	Electrolyte Conductivity
MUT	Material Under Test
DUT	Device Under Test

dH_2O	Distilled water
APTES	3-aminopropyltrietoxysilane
NaCl	Sodium Chloride
LPS O111:B4	Lipopolysaccharides from *Escherichia coli* O111:B4
CNLS	Complex Non-linear Least Square
PCA	Principal Component Analysis
PC	Principal Component
SAS	Statistical Analysis Software
SEM	Scanning Electron Microscope
AFM	Atomic Force Microscope
Sensor 1_11	Sensor with eleven negative electrodes in between two positive electrodes. The configuration is 1_11_1
Sensor 1_5	Sensor with five negative electrodes in between positive electrodes. The configuration is 1_5_1_5_1
Sensor 1_3	Sensor with three negative electrodes in between positive electrodes. The configuration is 1_3_1_3_1_3_1
Din_10mil	Conventional interdigital sensor design of pitch length 10 mil
Din_20mil	Conventional interdigital sensor design of pitch length 20 mil
Din_40mil	Conventional interdigital sensor design of pitch length 40 mil
FR4	Fiberglass reinforced epoxy laminates for PCB fabrication
Alumina-Al_2O_3	Aluminium Oxide
PCB	Printed Circuit Board
PR	Photo Resist
MEMS	Micro-electromechanical Systems
Si	Silicon
SiO_2	Silicon Dioxide
TMAH	Tetra-Methyl Ammonium Hydroxide
Cr	Chromium
Au	Gold
Si_3N_4	Silicon Nitride
PECVD	Plasma enhanced chemical vapour deposition
RIE	Reactive ion etching
LCR 821	Measuring instrument for impedance (inductance, capacitance and

	resistance)
RS232	Serial communication port
HFORCE	Carries the signal current source. Connected to the +ve side of the DUT
LFORCE	Accepts the signal current return. Connected to the –ve side of the DUT.
HSENSE	Together with Lsense, monitors the Potential,V. Connected to the +ve side of the DUT.
LSENSE	Together with Hsense, monitors the Potensial, V. Connected to the –ve side of the DUT
GND	Electrical ground
CPE	Constant Phase-Element
PDEs	Partial differential equations
R	Resistance
C	Capacitance
Q	Charge
V	Voltage
I	Current
f	Frequency
Z	Impedance
θ	Phase Angle, theta
W_e	The electrostatic energy density
D	The electric displacement
E	The electric field intensity
ε_0	The permittivity of vacuum which sets to be 8.854×10^{-2} F/m
ε_r	The relative permittivity
ε	The permittivity
ω	The angular frequency
UV	Ultra-violet
Nyquist	Data presentation in complex impedance plot
Bode	Data presentation as a function of frequency
MilliQ	Ultra-pure water which undergo the proses filtration and deionisation that has been characterised in terms of resistivity (typically 18.2 MΩ·cm)
pH	Potential of hydrogen
H^+	Hydrogen ions

OH^-	Hydroxide ions
Coating A	Electrospun membrane of Carboxylic functional polymer
Coating B	Spin coated Carboxylic functional polymer
Coating C	Dip-Coated silica coating funcitonalized with APTES
Coating D	Dip coated silica with Thionine
$Na_2B_40_7$*$10H_20$	Sodium Tetraborate
H_3BO_3	Boric Acid
$Na_2HPO_4.2\ H_2O$	Disodium Hydrogen Phosphate
KH_2PO_4	Potassium Dihydrogen Phosphate
TEOS	Tetraethylorthosilicate
EtOH	Ehanol
HCl	Hydrochloric Acid
C_{dl}	Double-layer capacitance
R_s	Solution resistance
R_{ct}	Charge/electron transfer resistance
Z_W	Warburg impedance
σ_w	Warburg coefficient
C_{ad}	Adsoption capacitance