

Table of Contents

S. No.	Description	Page No.
a)	List of Figures	xi
b)	List of Tables	xvi
c)	List of Abbreviations	xvii
d)	List of Symbols	xx
e)	Preface	xxiii
1.0	Chapter 1: Introduction	1
1.1	Biosensor	1
1.2	Cell-based biosensor	3
1.3	Classification of cell-based biosensor	5
1.3.1	Electrochemical biosensor for CBB	5
1.3.1.1	Amperometric biosensor	6
1.3.1.2	Potentiometric biosensor	7
1.3.1.3	Impedimetric biosensor	11
1.3.2	Optical biosensor	15
1.3.2.1	Surface plasma resonance (SPR)	16
1.3.2.2	Resonant waveguide grating (RWG)	17
1.3.2.3	Luminescence-based optical chemical sensing (OCS)	18
1.3.3	Piezoelectric biosensor	19
1.4	Strategies for the design and characterization of CBB	20
1.4.1	Design for fabricating the CBB	21
1.4.1.1	Choice of sensing material	21
1.4.1.2	Structure for fabricating the CBB	22
1.4.1.3	Thin film deposition techniques for fabricating the CBB	23
1.4.2	Classification techniques for the fabricated CBB	24
1.4.3	Fundamental behavior of the electric field	25
1.5	Literature review	28
1.5.1	Review on ECIS systems for CBB	29
1.5.2	Review on thin film based electronic devices for CBB	30
1.5.3	Major observation from the literature survey	31

1.6	Research objectives	32
1.7	Scope of the thesis	33
2.0	Chapter 2: Low-cost Electric Cell–Substrate Impedance Sensing System	35
2.1	Outline	35
2.2	Materials and methods	36
2.2.1	Material	36
2.2.2	Microelectrode design and fabrication	37
2.2.3	Cell culture procedure	37
2.2.4	Voltage controlled constant current source (VCCS) unit construction and testing	38
2.2.5	Equivalent electrical circuit Model for the cell-electrode interface	41
2.2.6	Complete Electric Cell-substrate Impedance Sensing (ECIS) system	41
2.3	Results and discussion	46
2.4	Conclusion	59
3.0	Chapter 3: Aluminium Oxide Thin-Film based Biosensing Device	60
3.1	Outline	60
3.2	Materials and methods	61
3.2.1	Materials	61
3.2.2	Fabrication of MIM biosensor	62
3.2.3	Cell culture procedure	63
3.3	Results and discussion	63
3.4	Conclusion	70
4.0	Chapter 4: Zinc oxide coated Metal-Semiconductor-Metal based Biosensing Device	71
4.1	Outline	71
4.2	Materials and methods	72
4.2.1	Materials	72
4.2.2	Sol-gel synthesis of ZnO	72
4.2.3	Sensor fabricated process	72
4.2.4	Cell culture procedure	74
4.3	Results and discussion	75

4.3.1	Thin film characterization of spin coated ZnO device	75
4.3.2	Surface modification of ZnO thin film	80
4.3.2.1	Fourier Transform Infrared (FT-IR) spectroscopic study	82
4.3.2.2	Cell viability and proliferation	84
4.3.3	Electrical characterisation of fabricated cell MSM device	85
4.3.4	Optical and electrical characterisation of fabricated gelatin-GA-APTES-cell MSM device	87
4.3.4.1	Case 1-1000 cells/well concentration	87
4.3.4.2	Case 2-5000 cells/well concentration	90
4.4	Conclusion	92
5.0	Chapter 5: Extended Large Area Heterojunction Biosensing Device	94
5.1	Outline	94
5.2	Experimental details	96
5.2.1	Sensor fabrication procedure	96
5.2.2	Primary cortical neuron cell culture procedure	97
5.2.3	Surface modification	98
5.2.4	Fluorescent staining of nuclei and actin cytoskeleton of neuronal cells	99
5.3	Results and discussion	99
5.3.1	Electrical characterisation of fabricated extended larger area heterojunction device	100
5.3.2	Optical and electrical characterisation of fabricated cell culture attached extended larger area heterojunction device	102
5.4	Conclusion	106
6.0	Chapter 6: Summary and future scope	107
6.1	Summary	107
6.2	Future scope	110
	References	111
	Author's Relevant Publications	140

List of figures

Figure No.	Description	Page No.
1.1	Illustration of biosensor.	1
1.2	The biosensor concept proposed by L.C Clark.	2
1.3	Cell-based biosensing device and zoomed images showing the influence of various stimuli namely chemical, biological, and physical on the cells to cause cellular functions and the changes is being converted into a measurable electric signal by the transducer element. The image is adapted from [21] with some modifications.	4
1.4	Electrodes of electrochemical biosensor (a) two-electrode system, and (b) three-electrode system.	5
1.5	Types of potentiometric sensors used (a) ISFET, (b) EIS capacitive sensor, and (c) LAPS devices.	8
1.6	EnFET's structure with its principle of operation.	9
1.7	Bio-impedance technique based on the type; (a) active, and (b) passive type bio-impedance. (re-used with slight modifications with permission from Springer Nature copyright [43]).	12
1.8	ECIS system having a pair of co-planar electrode, signal generator, current limiting resistor, and lock-in amplifier.	14
1.9	Adherent cells growing on an electrode system with its corresponding change in impedance value due to progression of cellular functions; where (1) when no cells is inoculated; an increase in the impedance value is due to (2) cells starts to adhere on the surface, (3) cellular proliferation, (4) cell-cell interaction on the surface; and decrease in the impedance value due to (5) loss of tight junction or adhesion or cell membrane integrity.	14
1.10	Surface plasmon resonance (SPR) in Kretschmann configuration for CBB.	17
1.11	Resonant waveguide grating (RWG) for CBB.	18

1.12	Luminescence-based OCS for CBB.	19
1.13	Quartz crystal microbalance (QCM) for CBB.	20
1.14	Types of co-planar electrode arrangement used; (a) Interdigitated electrode (IDE), and (b)-(e) dissimilar & similar-sized electrodes.	22
1.15	Sol-gel spin coating process	24
1.16	(a) Thermal, and (b) Electron beam vacuum deposition method.	24
1.17	(a) A schematic diagram of an inverted fluorescence microscope, and (b) Pictograph of the semiconductor parameter analyser (SPA).	25
1.18	Biological cells in electric field, while (a) cell placed in an electric field., (b) cell membrane is represented by as a capacitor due to its hydrophobic nature, (c) adherent cells grown on a conducting surface (metal).	27
2.1	(a) Microelectrode design pattern used; (b) Customized cell-culture chamber developed.	37
2.2	Calibration graph using different R_1 values.	40
2.3	(a) Actual ECIS system, and (b) Constructed experimental (wireless) setup used with 2.5 kHz exciting frequency.	42
2.4	Schematic pictorial representation of the developed device.	43
2.5	Flowchart for calculating the impedance; (a) Transmitting end, and (b) Receiving end.	45
2.6	Morphological changes of C2C12 cells at various time point. (a) 3 h, (b) 6 h, (c) 18 h, (d) 32 h, (e) 48 h, and (f) 72 h; Scale bar: 20 μm .	46
2.7	Change in cell proliferation with respect to average magnitude of impedance over the calibrated area. (a) Frequency vs. Magnitude (b) Frequency vs. Phase (c) Nyquist plot shows the change in semi-circle diameter due to the change in transfer of electrons between the metal-electrode interface resistances.	48

2.8	Rate of proliferation with respect to impedance (a) Normalized impedance vs. frequency plot, and (b) Time point vs. change in impedance and average cell count per image.	49
2.9	Morphology of the C2C12 cells present near the electrode (a) 6 h, (b) 18 h, (c) 36 h, (d) 48 h, and (e) 72 h; Scale bar: 20 μm .	51
2.10	Equivalent circuit model of biological cells (a) Fricke-Morse model, (c) modified Fricke-Morse model, (d) Cole-Cole model, and (f) modified Cole-Cole model. While (b & e) are simplification step.	52
2.11	Shows the experimental data and its fitting.	55
3.1	Fabrication process of EBE deposition Al_2O_3 thin film based biosensing device.	63
3.2	Morphology of C2C12 cells at various time intervals: (a) 8 h, (b) 24 h, (c) 48 h, and (d) 72 h; Scale bar: 50 μm .	64
3.3	Changes in the characteristic electrical properties of the Al_2O_3 thin film due to dynamic behaviour of myoblast cells in culture: (a) capacitance vs. frequency plot, (b) magnitude of impedance plot, (c) phase of impedance plot, (d) Nyquist plot and (e) Average cell count at different time point.	66
3.4	Equivalent electrical circuit model to represent the surface-electrode-cell interface: (a) fabricated device component, (b) tissue component, (c) fabricated device with tissue component and (d) cell-device.	68
4.1	ZnO sol-gel preparation method and its spin coating process.	73
4.2	Transparent ZnO thin film coated MSM based biosensor fabrication process.	74
4.3	(a) XRD pattern of spin coated ZnO thin film, (b) Williamson-Hall (W-H) plot used for calculating average strain (ϵ) and crystallite size.	76
4.4	(a) AFM micrograph, and (b) HR-SEM image of spin coated	77

	ZnO thin film.	
4.5	(a) Absorbance spectra, and (b) Tauc's plot of spin coated ZnO thin film.	79
4.6	EDX pattern of spin coated ZnO thin film with elemental composition table.	79
4.7	Schematic illustration of gelatin functionalization on spin coated ZnO thin film.	81
4.8	FT-IR spectra of pristine gelatin, surface unmodified ZnO thin film and surface modified ZnO thin film.	83
4.9	Cell viability and proliferation of C2C12 cells using MTT assay.	85
4.10	(a) $I-V$ characteristics of fabricated and simulated MSM device (b) Band diagram for the device structure.	86
4.11	Morphological changes of C2C12 cells at various time: (a) 6 h, (b) 24 h, (c) 48 h, (d) 72 h, (e) 96 h, and (f) 120 h; Scale bar: 20 μm .	88
4.12	Electrical properties of the ZnO thin film with respect to change in cell proliferation (1000 cells seeded): (a) $I-V$ characteristics plot, (b) Capacitance vs. frequency plot, (c) Magnitude of impedance plot, (d) Phase of impedance plot, (e) Nyquist plot, and (f) Time vs. change in impedance and average cell count plot.	89
4.13	Morphological changes of C2C12 cells at various time: (a) 24 h, (b) 48 h, (c) 72 h, (d) 96 h, (e) 120 h, (f) 144 h, (g) 168 h, and (h) 210 h ; Scale bar: 20 μm .	91
4.14	Electrical properties of the ZnO thin film with respect to change in cell proliferation (5000 cells seeded): (a) $I-V$ characteristics plot, (b) Capacitance vs. frequency plot, (c) Magnitude of impedance plot, (d) Phase of impedance plot, (e) Nyquist plot, and (f) Time vs. change in impedance and average cell count plot.	92
5.1	An extended larger area heterojunction device with cell	98

	culture well fabrication process.	
5.2	(a) Energy band diagram for the Si/ZnO heterojunction structure, (b) C-V characteristics of the fabricated Si/ZnO heterojunction, and (c) $I-V$ characteristics of fabricated and simulated Si/ZnO heterojunction.	101
5.3	Change in electrical (resistance, conductive) properties of the spin coated ZnO thin film with respect to change in various cell functionality. (a) $I-V$ characteristics plot; (b) Magnitude of impedance plot; (c) Phase of impedance plot; and (d) Nyquist plot.	103
5.4	Fluorescent image shows morphological changes of cortical neuronal cells (a-c) Day 3, and (d-f) Day 5 ; Scale bar: 20 μm .	105
5.5	SEM image shows morphological changes of cortical neuronal cells (a) Day 3, and (b) Day 5.	105
5.6	Micrograph shows morphological changes of cortical neuronal cells at various time point (a) Day 1, (b) Day 3, and, (c) Day 5.	106

List of Tables

Table No.	Description	Page No.
2.1	Cost comparison of the device in USD	44
2.2	Analytical values through an equivalent electrical circuit model of adherent cell-electrolyte-electrode system (Fitting data derived from best of five developed microelectrode system).	57
3.1	Calculated EEC model parameters.	70
4.1	Calculated XRD parameters.	76
4.2	Calculated AFM parameters.	78
4.3	Parameters used for the simulation of ITO/ZnO/ITO device.	86
5.1	Parameters used for the simulation of Al/Si/ZnO/Al heterojunction.	101