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It is certified that the work contained in the thesis titled "***Fabrication and Characterization of Thin Film Nanomaterials based Biosensing Devices for Assessing Dynamic Behaviour of Mammalian Cells***" by "***Mr. Uvanesh K.***" has been carried out under our supervision and that this work has not been submitted elsewhere for a degree.

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I, ***Uvanesh K***, certify that the work embodied in this Ph.D. thesis is my own bonafide work and carried out by me under the supervision of ***Prof. Neeraj Sharma*** and ***Dr. Sanjeev Kumar Mahto*** from "***21st July 2015***" to "***30th September 2020***" at **School of Biomedical Engineering, Indian Institute of Technology (BHU), Varanasi.** The matter embodied in this thesis has not been submitted for the award of any other degree/diploma. I declare that I have faithfully acknowledged and given credit to the research workers wherever their works have been cited in my work in this thesis. I further declare that, I have not willfully copied any other's work, paragraphs, text, data, results, etc. reported in the journals, books, magazines, reports, dissertations, theses, etc., or available at websites and have not included them in this Ph.D. thesis and have not cited as my own work.

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Date : 29/09/2020

Place : Varanasi **(Uvanesh K.)**

Dedicated
To
My Family

Table of Contents

List of figures

List of Tables

LIST OF ABBREVIATIONS

Abbreviation	Details
CBA	Cell-based assay
CBB	Cell-based biosensor
FETs	Field-effect transistors
MOSFET	Metal oxide semiconductor field-effect transistor
MISFET	Metal-insulator-semiconductor field-effect transistor
ECIS	Electric cell-substrate impedance sensing
CO_2	Carbon dioxide
OP-AMP IC	Operational amplifier integrated circuit
DMEM	Dulbecco's Modified Eagle's Medium high glucose
PBS	Phosphate buffered saline
FBS	Fetal bovine serum
CMOS	Complementary metal-oxide-semiconductor
RF	Radio frequency
VCCS	Voltage controlled constant current source
EEC	Equivalent electrical circuit model
DAQ	Data Acquisition
DSO	Digital storage oscilloscope
CPE	Constant phase element
MIM	Metal-insulator-metal
AFM	Atomic force microscopy
SEM	Scanning electron microscopy
EDX or EDS	Energy dispersive X-ray spectroscopy
XRD	X-ray diffraction
FTIR	Fourier transform infrared spectroscopy
UV-*Vis*	Ultraviolet-visible
MSM	Metal-semiconductor-metal

MOS	Metal-oxide-semiconductor
SPA	Semiconductor parameter analyser
SMU	Source and measuring unit
MIS	Metal-insulator-semiconductor
ZnO	Zinc oxide
Au	Gold
Ag	Silver
Al	Aluminum
ITO	Indium-doped tin oxide
Si	Silicon
Al_2O_3	Aluminum Oxide
HCl	Hydrochloric acid
EBE	Electron beam evaporation unit
PDMS	Polydimethylsiloxane
APTES	(3-Aminopropyl)-triethoxysilane
GA	Glutaraldehyde solution
MEA	Monoethanolamine
PVDF	Polyvinylidene difluoride
MTT assay	3-(4, 5 dimethylthiazol-2)-2, 5-diphenyltetrazolium bromide
DMSO	Dimethyl sulfoxide
DAPI	4,6-diamidino-2-phenylindole
BSA	Bovine serum albumin
PLL	Poly-L-lysine
FWHM	Full width half maxima
IDE	Interdigital electrode
rms	Root mean square
sccm	Standard cubic centimeters per minute
KCl	Potassium chloride

Na^+	Sodium ion
K^+	Potassium ion
Ca^{2+}	Calcium ion
Cl^-	Chloride ion
H^+	Hydrogen ion
O_2	Oxygen
H_2O	Water
Cd^{2+}	Cadmium ion
Cu^{2+}	Cupric ion
Hg^{2+}	Mercuric cation
Pb^{2+}	Lead ion
WE	Working electrode
RE	Reference electrode
pH	potential of hydrogen
EMG	Electromyography
EOG	Electrooculography
ECG	Electrocardiogram
EEG	Electroencephalogram
IS	Impedance spectroscopy
ECL	Electrochemiluminescence
RI	Refractive index
DIC	Digital interference contrast microscopy
SPR	Surface plasmon resonance
RWG	Resonant waveguide grating
OCS	Optical chemical sensing
RA	Resonance angle
QCM	Quartz crystal microbalance
SAW	surface acoustic wave

LIST OF SYMBOLS

Symbol	Details
λ	Wavelength
θ	Diffraction angle
T	Transmittance
A	Absorbance
I-V	Current-voltage
C-V	Capacitance-voltage
W	Channel width
L	Channel length
e	Charge of electron
C	Cell membrane
X_C, Q	Reactance
R_1, R, R_f	Resistance
Z	Total impedance
C_{dl}	Double layered capacitance
R_{dl}	Double layered resistance
α, n	Dimensionless exponent parameter
R^2	Correlation coefficient
Z_{re}	True or real impedance
R_0	Lower frequency component resistance
R_∞	Higher frequency component resistance
R_i	Intracellular resistance
R_e	Extracellular resistance
M	Mole

h	Hour
min	Minute
s	Second
ºC	degree Celsius
RT	Room Temperature
mm	Millimeter
nm	Nanometer
rpm	Revolutions per minute
ε	strain
W	watt
V	volt
g	Gram
µg	Microgram
µL	Microliter
mL	Milliliter
Ω	Ohm
kΩ	Kilo Ohm
MΩ	Mega Ohm
Hz	Hertz
kHz	Kilohertz
MHz	Megahertz
ϕ	Phase
R_m	Membrane resistance
C_m	Membrane capacitance
µ	Micro

I	current
$	United states dollar (USD)
A	Ampere
μA	Microampere
F	farads
$\vec{E}$	Electric field
I_D	drain current
I_{DS}	Source-drain current
V_G	Gate potential
$V_{p\text{-}p}$	Peak - peak voltage
%	Percentage
Å	Angstrom
wt.	weight

PREFACE

Biosensor is an analytical device that consists of a biological component to detect an analyte, coupled with a physical transducer to produce a measureable signal. Biosensors have been widely explored and extensively utilized as a platform for applications in pharmaceutical, environment, food and medical field. Amongst the various classes of biosensors developed so far, cell-based biosensors express promising potential in comprehending fundamental biological processes with reference to a whole cell or a group of cells. The direct use of living cells offers exceptional opportunities for bio-detection and drug discovery. The biochemical effect of the direct spatial contact of living cells is converted into quantitative electrical signals by sensors or transducers, thus bridging the gap between electronics and biology. Cell-based biosensors are endowed with certain advantages in comparison to molecule-based approaches for example, noninvasive long-term recordings, label-free detection, reduced response time, etc. In order to achieve the objectives, the thesis has been divided into following chapters.

Chapter-1 introduces the basics about biosensor and cell-based biosensor; Types of cell-based biosensor currently being used. Different types of cell-based biosensor based on its transduction principle; Strategies for the Design and Characterization of CBB and fundamental behaviour of cells in electric field. Literature review and the observation; Research objective and scope of the present study.

Chapter-2 describes the investigation of functional behaviour of myoblast (C2C12) cells using a co-planar silver metal electrode system integrated to a low-cost ECIS system. Typically, the silver metal was thermally coated on the conversional glass substrate to construct metal-insulator-metal (MIM) structure *via* shadow mask method, to achieve an active layer's dimensions of 1.5 mm wide and 4 mm length. At the same time, to develop

a ECIS system, we used the electronic components available at the laboratory to construct a low-cost impedance measuring circuitry and calibrated the developed circuit, against the standard commercial resistor. Further, the MIM device was integrated to the developed impedance measuring circuitry to construct a low-cost ECIS system. To increase the cell adhesion process, the MIM device was coated with 2% gelatin protein and the gelatin-coated MIM device was used as a platform to study the phenotypic change of adherent mammalian cells under the influence of applied electric field. Further, the fabricated biosensor was electrically and optically characterised to validate the outcome.

Chapter 3 focuses on fabrication and characterization of metal-oxide thin film nanomaterials based biosensing devices for assessing dynamic behaviour of adherent mammalian cells. Typically, the Chapter 3 investigates the effect induced by cellular functional behaviour on the characteristic electrical properties of the e-beam deposited aluminium oxide (Al_2O_3) thin film nanomaterial-based metal-insulator-metal (MIM) device. A patterned indium tin oxide (ITO) coated glass substrate having a co-planar electrode dimension of 12×5 mm^2 at each side with a gap of 1 ± 0.2 mm (at center) was used as a transparent electrode system. To fabricate the MIM device, Al_2O_3 thin film of ~100 nm thickness was deposited on ITO electrode system and used as the active sensing interface. To increase the cell adhesion process, the MIM device was coated with 2% gelatin protein and the gelatin-coated MIM device was used as a platform to study the phenotypic change of adherent mammalian cells under the influence of applied electric field. Further, the fabricated biosensor was electrically and optically characterised to validate the outcome.

Chapter 4 investigates the effect induced by cellular functional behaviour on the characteristic electrical properties of the sol-gel synthesized spin coated zinc oxide (ZnO) thin film nanomaterial-based metal-semiconductor-metal (MSM) device. A patterned

indium tin oxide (ITO) coated glass substrate having a co-planar electrode dimension of 12×5 mm^2 at each side with a gap of 1 ± 0.2 mm (at center) was used as a transparent electrode system. To fabricate the MSM device, ZnO thin film of ~100 nm thickness was deposited on ITO electrode system and used as the active sensing interface. To increase the cell adhesion process, the MSM device was coated with 2% gelatin protein and the gelatin-coated MIM device was used as a platform to study the phenotypic change of adherent mammalian cells under the influence of applied electric field. Further, the fabricated biosensor was electrically and optically characterised to validate the outcome.

Chapter 5 investigates the effect induced by cellular functional behaviour on the characteristic electrical properties of the sol-gel synthesized spin coated zinc oxide (ZnO) thin film nanomaterial-based larger area heterojunction device. To fabricate the larger area heterojunction device, ZnO thin film of ~200 nm thickness was deposited p-type Si substrate, and to create ohmic contact, the aluminium metal electrode was used on both p-Si and n-ZnO. While, ZnO was used as the active sensing interface. To increase the cell adhesion process, the fabricated heterojunction device was functionalized with poly-L-lysine and the poly-L-lysine functionalized ZnO thin film based extended larger area heterojunction device was further characterized for assessing the cell-induced electrical characteristic property change.

Finally, **Chapter 6** summarizes the major objectives and concludes the major findings of the present thesis. This chapter also outlines some future scope of works related to this thesis.