DEVELOPMENT OF SCREEN-PRINTED IMPEDANCE BIOSENSOR FOR CHARACTERIZATION OF CANCER CELLS

BY

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ABSTRACT

Electric Cell-Substrate Impedance Sensing (ECIS) is currently gaining widespread application in biomedical field such as cell monitoring. The ability to monitor growth and death of mammalian cells has made this technique more and more popular in drug discovery. Currently chemotherapy treatment subject patients to trial and error therapy, resulting in unoptimized dosage and unnecessary pain for patients. Optimal treatment effectiveness can only be accomplished when the chemotherapy dosage is individualized to each patient. In this study, a silver/silver chloride screen-printed impedance biosensor on glass was developed to characterize A549 lung cancer cell growth in the presence of collagen I, Bovine as substrate coating. The sensor comprise of Interdigital Electrodes Structures (IDEs) with variable spacing. The sensor's optimum spacing between electrodes has been simulated using COMSOL Multiphysics. The sensor was fabricated and incorporated with a culture well made of polydimethylsiloxane (PDMS). A549 cells were cultured in the chambers and impedance measurements were taken at 12 hours intervals for 120 hours. Cell Index (CI) were calculated from the impedance measurement and plotted against time together with the growth profile of the cells using conventional method. A549 cells were also treated with chemotherapy drug; Paclitaxel and its response were monitored over 5 days. Experimental results using biosensor show a similar trend with conventional growth profile of cells in T-flask. Significant reduction in Cell Index (CI) was observed after exposure to Paclitaxel indicating the reliability of the impedance biosensor. Lower spacing between the electrodes has been found to be more sensitive, producing higher Cell Index during cell growth. This work indicates that silver/silver chloride screen printed sensor offers a low cost solution for personalized sensors as it provides a rapid, real time, continuous, reliable method for chemotherapy drug screening.

خلاصة البحث

يكتسب استشعار المقاومة الكهربائية للخلية-الركيزة (ECIS) مؤخراً انتشاراً واسعاً لتطبيقاته في مجال الطب الحيوي مثل رصد الخلية. فالقدرة على رصد نمو وموت خلايا الثدييات تجعل هذه التقنية أكثر شعبية في اكتشاف العقاقير. لأن أسلوب العلاج الكيميائي في الوقت الحاضريُخضع المرضى إلى أخطاء في التشخيص والعلاج، مما يتسبب في إعطاء المريض جرعة غير مناسبة وآلام إضافية كان من الممكن تجنبها. وفعالية العلاج الأمثل لا يمكن تحقيقها بإفراد جرعة العلاج الكيمياوي لكل مريض. في هذه الدراسة، تم تطوير شاشة الفضة/ كلوريد الفضة المطبوعة لجهاز مقاومة الاستشعار البيولوجي على الزجاج لتوصيف نمو خلية سرطان الرئة A549 في ظل وجود مادة الكولاجين البقري الأول بوصفها ركيزة الطلاء. تتألف أجهزة الاستشعار من هياكل أقطاب الأفوات (IDEs) على مسافات متغيرة. تمت محاكاة التباعد الأمثل لأقطاب أجهزة الاستشعار باستخدام برنامج COMSOL Multiphysics. صنعت أجهزة الاستشعار وأدمجت ببيئة مصنعة جيداً من الـpolydimethylsiloxane (PDMS). كانت خلايا A549 مستزرعة في غرف وأخذت قياسات المقاومة في فترات 12 ساعة لمدة 120 ساعة. تم حساب المؤشر الخليوي (CI)من قياس المقاومة ورسمت مقابل الوقت بالإضافة إلى بيانات نموالخلايا باستخدام الطريقة التقليدية. كما تم معالجة خلايا A549 بعقاقير العلاج الكيمياوي Paclitaxel وتم رصد استجابتها خلال 5 أيام. النتائج التجريبية باستخدام جهاز الاستشعار البيولوجي أظهرت اتجاهاً مماثلا مع البيانات التقليدية لنمو الخلايا في القارورة-T. وقد تم ملاحظة انخفاض كبير في المؤشر الخليوي (CI) بعد التعرض لعقار Paclitaxel مما يشير إلى إمكانية الاعتماد على جهاز استشعار المقاومة البيولوجي. وتم العثور على تباعد أقل بين الأقطاب لتكون أكثر حساسية، ولتنتج مؤشر خليوي أعلى خلال نمو الخلايا. يشير هذا العمل إلى أن شاشة الفضة/ كلوريد الفضة المطبوعة لجهاز مقاومة الاستشعار البيولوجي تقدم حلاً منخفض التكلفة لأجهزة الاستشعار الشخصية، حيث أنها توفر طريقة سريعة ومستمرة وموثوقة في الوقت الحقيقي لفحص عقاقير العلاج الكيمياوي.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science in Electronics Engineering.

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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In the name of Allah, the most gracious, the most merciful

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LIST OF SYMBOLS

A	Area of plates (m)
C	Capacitance (F)
d	Distance between plates (m)
E	Electric Field (V m^{-1})
ε/ ε_0	Permittivity of Free Space (8.85418782 × 10^{-12} m^{-3} kg^{-1} s^4 A^2)
ε_r	Relative Permittivity of material
I	Electric Current (A)
M_1/M_2	Cell concentration inside Flask (cells ml^{-1})
R	Resistance (Ω)
R_{cell}	Resistance of cells (Ω)
R_b	Resistance of media without cells (Ω)
V	Electric Potential (v)
V_1/V_2	Volume (l)
X/X_c	Reactance (Ω)
ω	Angular Frequency
Z	Impedance (Ω)
Θ	Phase (°)
σ	Conductivity of material (S m^{-1})
∇	Gradient of Electric Field

LIST OF ABBREVIATIONS

3D	Three Dimensional
A549	Non-Small Lung Cancer Cell Lines
AC	Alternating Current
Ag	Silver
AgCl	Silver Chloride
ATCC	American Type Culture Collection
BAW	Bulk Acoustic Wave
CI	Cell Index
CMOS	Complementary Metal–Oxide–Semiconductor
CO_2	Carbon Dioxide
DMEM	Dulbecco Modified Eagle Medium
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
ECIS	Electrical Cell-Substrate Impedance Sensing
ECM	Extracellular Matrix
EIS	Electrochemical Impedance Spectroscopy
FBS	Fetal Bovine Serum
GOx	Glucose Oxidase Enzymes
IC	Integrated Circuit
IC_{50}	50% Inhibitory Concentration
IDEs	Interdigital Electrodes
IUPAC	International Union of Pure and Applied Chemistry
OSCC	Oral Squamous Cell Carcinoma
QCM	Quartz Crystal Microbalance
PBS	Phosphate-Buffered Saline
PDMS	Polydimethylsiloxane
RT-CES	Real Time Cell Electronic Sensing
SAW	Surface Acoustic Wave
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
UV	Ultra Violet

CHAPTER ONE

INTRODUCTION

1.1 INTRODUCTION

Previously, the conversion of a biological response into an electrical signal was a challenging task due to the difficulty in providing an appropriate electronic device that can detect specific biological conditions. Nowadays, growing numbers of biosensors have made it possible to develop point-of-care sensing devices, but it is still bounded with the issue of miniature and lack of cost-effective devices (Grieshaber, MacKenzie, Voeroes, & Reimhult, 2008; Q. Liu et al., 2009). Therefore, there have been demands to produce safe, reliable, inexpensive, and easy to use devices for biological event related studies. Many biological branches can indeed benefit from the development of biosensors; one of them specifically, is cell studies.

In vitro cell culture has become a very common method to study cell response and growth. Current conventional methods for cellular analysis include fluorescent imaging, chemiluminescence, radioactive detection, and even animal experiments that sometimes may cause loss of important biological information or even worse, destruction of the cells (Arias, Perry, & Yang, 2010; Q. Liu et al., 2009). In cancer cell studies, there is still a necessity to develop fast, simple and continuous techniques to understand the interactions of cells, drugs and toxins since the current conventional methods do not provide continuous monitoring of samples (Arias et al., 2010). Having a label-free and non-invasive study of cells will be a great advantage to provide real-time and continuous monitoring of cell activities. As a summation, a cost effective,

reliable cell-based method will greatly improve the development process in studying cancer cells, such as their interactions with drugs, drug discovery and drug screening.

1.2 **BIOSENSORS**

A biosensor is an analytical device which has the ability to monitor and convert biological responses into measurable and processable signals such as electrical signals (Grieshaber et al., 2008). The International Union of Pure and Applied Chemistry (IUPAC) defines a biosensor as a self-contained integrated device capable of providing at least semi-quantitative analytical information by using direct spatial contact of biological recognition element with the transducer elements (Koyun, Ahlatcolu, Koca, & Kara, 2012). The first biosensor was introduced in 1962 by Clark and Lyons, who improved their own electrochemical sensors by adding enzyme transducers coated on electrode surfaces. Since then, researchers have started to innovate on this concept thus leading towards the development of a variety of biosensors.

In general, a biosensor consists of three main parts; biological samples, transducers, and an electronic system. Samples are the targeted analytes or biological elements to be studied while the electronic system is the equipment for signal processing and display. A transducer can be divided into two parts, which are the bioreceptor—a biomolecule to identify and bind samples together, and the electrical interface, where signals are produced from the biological events occurring surrounding it (Grieshaber et al., 2008).

1.3 **CANCER**

By definition, cancer is a group of diseases where cells are having an abnormal and uncontrolled growth in the human body. The condition continuously deteriorates when the cancer starts to spread to other organs, hence disrupting the organ's normal functions, upon which—if late action is taken—could result in the death of the patient

(Ferlay et al., 2015). Cancer cases and mortality rates increase annually, making it one of the leading causes of death around the world. Recent statistics showed that an estimated 8.2 million deaths were caused by cancer (Ferlay et al., 2015). Different regions are susceptible to different types of cancer with various numbers of cases, but the most common cases include breast cancer, lung cancer, prostate cancer and colorectal cancer. In 2012, Southeast Asia had been reported to have the highest percentage of deaths (19.4%) from lung cancer among other types of cancers in that region with the rate of nearly 95 deaths per 100,000 people (Ferlay et al., 2015). Lung cancer is considered a life-threatening disease because it has rapid growth and spread rates before displaying any apparent symptoms for detection. Most patients diagnosed with lung cancer were already in their middle or late stages of the cancer. A549 lung cancer cells are an example of non-small lung cancer cells that were taken from a cancer patient's body.

Cancer diagnosis and treatment is an area of concern in healthcare institutions and organizations. Large expenditures are allocated for cancer medical research in order to develop new technologies such as equipment and devices to prevent, detect, treat, and cure as well as drug discovery. Although well-known as a malignant disease, cancer can still be treated and cured with early detection and proper treatments. Most of these treatments require infusion of medicines or drugs on patients; i.e., chemotherapy, targeted therapy and photodynamic therapy.

1.4 **PROBLEM STATEMENT AND SIGNIFICANCE**

Chemotherapy administration can sometimes inflict negative side effects to the patient. The dose of the drugs introduced into a patient's body has always been a matter of concern. Currently, the dose of drugs administered for cancer patients is determined

by observing a particular calculation method—the body surface area (BSA). This formula only requires an individual's weight and height. However, an individual's drug absorption rate is also influenced by many other factors such as age, gender, metabolism, disease state, organ function, drug-to-drug interactions, genetics, and obesity. As a consequence, some patients may be under-dosed while others may be overdosed. This will result in unnecessary exposures to excess chemotherapy on patients (Rosenberger, English, Bose, Sani, & Moy, 2014; B. Zhang, Wang, Wang, & Li, 2013). Clinical studies proved that optimal treatment effectiveness can be achieved only when the chemotherapy dosage is individualized for each patient (B. Zhang et al., 2013). Therefore, in order to implement this concept, a reliable, highly sensitive, non-invasive, label-free, cheap and point-to-care sensor must be developed so that a patient's drug interaction can be monitored to determine respective optimal doses for chemotherapy.

1.5 **RESEARCH OBJECTIVES**

This dissertation is focused on the development of a screen-printed impedance biosensor that can be used for monitoring cancer cell behaviour. The main objectives of this research are:

I. To design and simulate a screen-printed impedance biosensor.
II. To fabricate a screen-printed impedance biosensor.
III. To test and evaluate the performance of the biosensor with A549 lung carcinoma cells under the influence of Taxol (Chemotherapy drug).

1.6 **RESEARCH METHODOLOGY**

The research methodologies taken in this dissertation will be discussed sequentially in this section. The first part is the literature review of related topics regarding impedance

bio-sensing in cancer cell studies. The next step is proposing the biosensor structure and design. Simulations using COMSOL Multiphysics will be done to test the proposed design. Afterwards, the design will be fabricated using screen printing techniques. The curing conditions (drying time and heating temperature) of the sensor will be optimized to obtain good adhesion between the printed ink and the substrate. Subsequently, the biosensors will be tested with A549 cells cultured on the electrodes of the sensors. The results will be compared with the conventional method performed prior to the biosensor tests. Cytotoxicity tests will be conducted to further assess the compatibility of the biosensors with drugs. The results will later be analyzed and discussed.

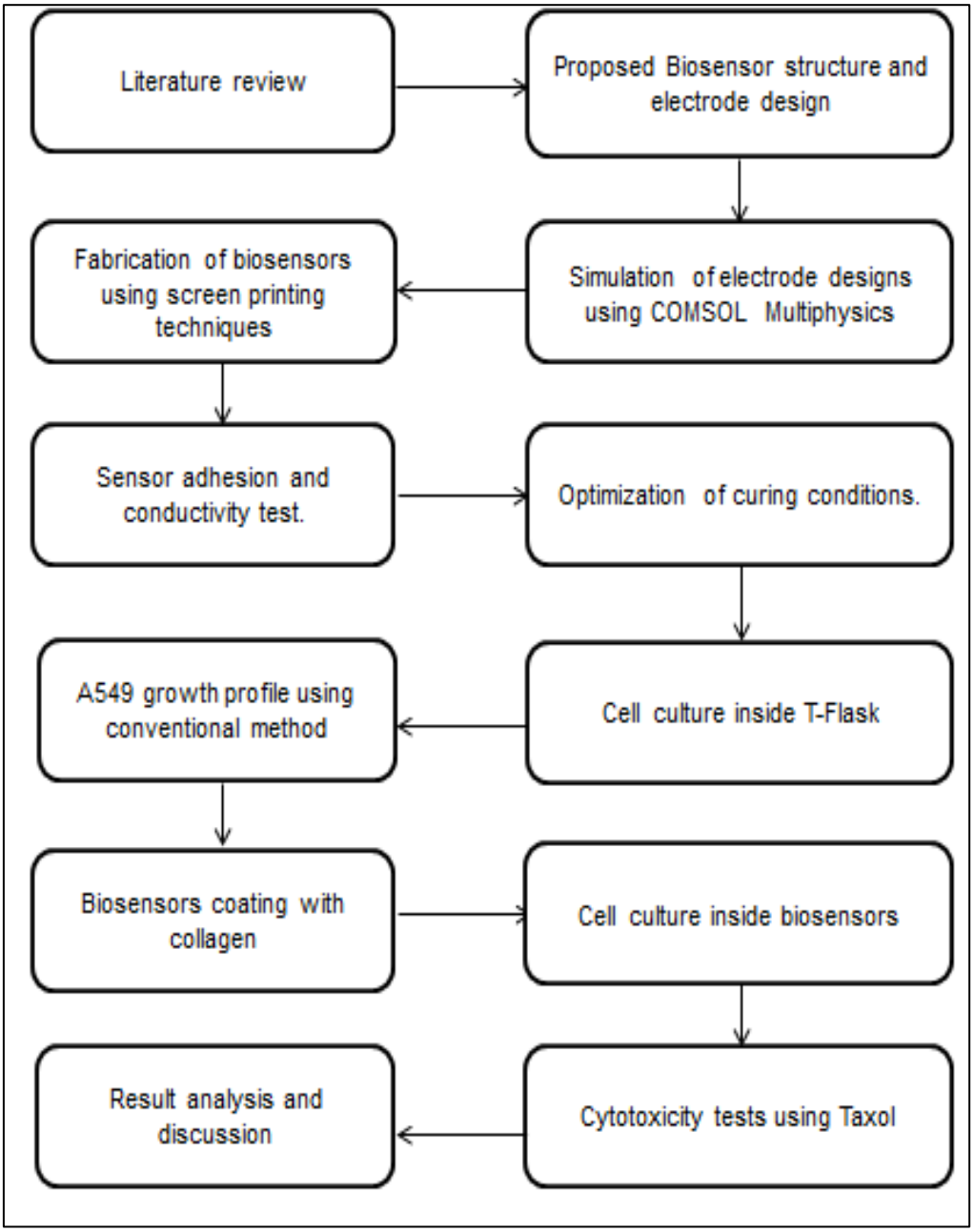
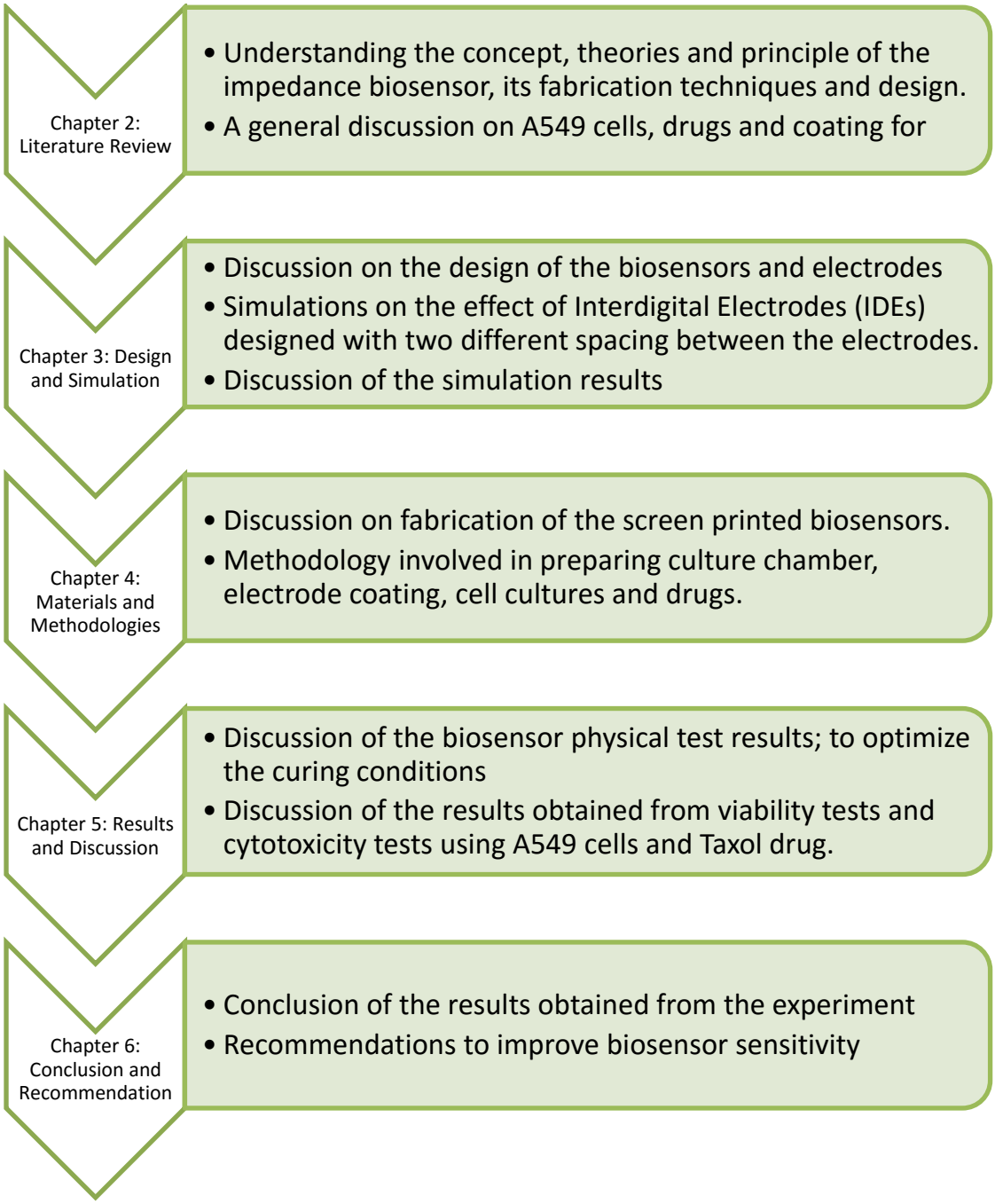


Figure 1.1: Flow chart of research methodologies for the study

1.7 **DISSERTATION ORGANIZATION**



CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION TO BIOSENSORS

2.1.1 Classification of Biosensors

Technological advancements in this era have been improving human life; each day better than the day before. Many researchers from various fields are now working on new inventions and pushing innovation of current technologies to the next level. Biotechnology, biochemistry and biomedicine are some of the fields that have experienced extensive growth in research over the past few decades. For instance, a medium for translating biological data into an easier processable electrical signal is required in order to improve accuracy and efficiency of monitoring biological responses. Hence, biosensors were introduced in the form of transducers that have the ability to convert a particular biological response into an electrical signal without interfering with the related biological processes.

In 1909, Sorenson presented his findings of the pH concept and standardized pH scale based on hydrogen ion concentration. This acid/base chemistry discovery was the beginning of biosensor development. A few other researchers then examined the relation of electrodes with the pH of human blood and tissues (Shruthi, Amitha, & Mathew, 2014). In 1962, the first biosensor was designated by C. Clark, which was called the glucose oxidase biosensor. Glucose oxidase enzymes (GOx) were immobilized on the oxygen electrode surface so that glucose concentration can be quantified from the samples (Nambiar & Yeow, 2011; Sassolas, Blum, & Leca-Bouvier, 2012). He explained that an electrochemical sensor can be amended and made