Design and Development of Parallel Biosensing System for Personalized Chemotherapy Treatment

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1 RESEARCH PROBLEM

Chemotherapy administration can sometimes inflict negative side effects to the patient. The regimen or cocktails of the drugs introduced into a patient's body has always needed careful consideration. Currently, the combinations are determined by strength of the regimen based on empirical technique; which is the observation of the response exhibited by the patients. However, an individual's drug absorption rate is influenced by many factors such as age, gender, metabolism, disease state, organ function, drug-to-drug interactions, genetics, and obesity. Consequently, different patients can have different body response towards the chemotherapy. Clinical studies have proved that optimal treatment effectiveness can be achieved only when the chemotherapy treatment is individualized for each patient (Zhang et al., 2013).

Current chemosensitivity assay such as MTT Assay, ATP assay and molecular probes are tedious, time consuming, labor intensive and expensive (Kiilerich-Pedersen and Rozlosnik, 2012; Lazcka et al., 2007). The tedious nature of these types of assays prohibit individualized testing for patients before chemotherapy. Therefore, there is a need for low-cost point-of-care biosensors which can predict the patient's response towards different chemotherapy regimens.

2 OUTLINE OF OBJECTIVES

Non-destructive monitoring of cell behaviors have gained wide attention over the past decade. The concept of Electrical Cell-Substrate Impedance Sensing (ECIS) was pioneered by Giaever and Keese in 1984 and evolved to be the most stable and effective technique of measuring cultured cells on microelectrodes with real-time impedance monitoring (Cui et al., 2017; Hong et al., 2011).

In this research, the ECIS concept will be applied to monitor adhesion, proliferation and death of cancer cells in vitro due to exposure to chemotherapy drugs. Chemosensitivity analysis will be performed using the developed impedance biosensing system by correlating the response of cell samples towards several chemotherapy regimens. A comparison will be made between tests conducted using biosensors and the actual chemotherapy treatment prescribed to patients.

3 STATE OF THE ART

Rapid and effective treatment of cancer is crucial to improve patients' quality of life and chance of survival. Currently, cancer cell growth, apoptosis and response to chemotherapeutic treatment involve colorimetric assays, which require complex laboratory equipment and extensive cell and drug preparation. Measurements and cell preparation are made at each endpoint, making the process labour-intensive and high cost. As such personalized studies on the efficacy of chemotherapeutical drugs on patients are rarely done due to its high cost and tedious process. Better disease-free survival rates have been reported using neoadjuvant therapy where treatment is given before surgery and is followed by systemic chemotherapy (Ancona et al., 2001; Lowy et al., 1999). In recent years, numerous efforts have been made to develop better chemotherapeutic regimens, resulting in improved outcomes and prolonged survival (Sjoquist et al., 2011). To facilitate this, there is great demand for rapid and real-time techniques for studying cancerous cells especially in terms of their reactions to drug and toxins (Bramwell et al., 1997).

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Biosensors can be used to test the response of tumours to different chemotherapy agents, similar to how microorganisms are tested against different antibiotics in vitro before the actual drugs are employed in the patients. This enables an accurate prediction as to which chemotherapy agent or combination of agents that are likely to be successful against the tumours without subjecting patients to trial-and-error therapy as tumour behaviours are unpredictable and differs from each individual patient. This may also minimize the side effects of the drugs as patients will not suffer unnecessarily from ineffective treatment. Biosensors may also be used to predict the aggressiveness of the tumour by measuring the rate of tumour growth. This information will be helpful to clinicians in deciding which patients require more aggressive treatment to prevent disease progression during treatment.

ECIS is the first impedance-based technique for studies of quantifying cell behaviours. In ECIS, a small gold electrode (250μm) is immersed in culture medium at the bottom of the tissue culture wells. Electrode surface is pre-coated with certain proteins to enhance cell adhesion with the electrode. Two electrodes, working and counter electrodes, exist in the system. A relatively small circular gold electrode behaves as a working electrode as compared to the larger counter electrode at the bottom. In ECIS biosensor systems, an AC signal with 1V amplitude is applied through a 1MΩ series resistor at 4 kHz frequency. The voltage across the electrodes is measured using an amplifier (Luong et al., 2001). Various cellular study using ECIS has been reported such as in monitoring growth, proliferation and differentiation of cells, cell migration and cytotoxicity (Cui et al., 2017; Anh-Nguyen et al., 2016; Sun et al., 2013; Mansor and Nordin, 2018)

4 METHODOLOGY

There are several aspects that need to be considered when developing a biosensing system to predict the outcome of chemotherapy. ECIS is the gold standard for monitoring cellular interaction towards drugs exposure using impedance biosensing technique. This technique, however, requires expensive equipment and electrodes to be implemented in high throughput testing (HTT). By leveraging the cheap mass fabrication costs of printed circuit board (PCB) in the electronics industry, we propose as an alternative Lab-on-Chip the Lab-on-PCB as a single use, disposable biosensor. Optimization of electrode configuration will be done analytically and experimentally to find the best design. A portable and wireless impedance data acquisition system will also be developed by embedding commercialized AD5933 Integrated Circuit (IC) impedance converter IC with microcontroller. Finally, chemosensitivity analysis will be performed using the developed impedance biosensing system by correlating the response of cell samples toward several chemotherapy regimen tested using biosensor and the actual chemotherapy outcome of the patients.

4.1 Design of Electrodes

The extensive research efforts in lab-on-a-chip (LoC) in biomedical field have shown the advantages and feasibility of the devices in real-life application. However, despite the said advantages, LoC has less commercialization potential due to expensive setup for mass-manufacturing (Moschou and Tserepi, 2017). Recently technology of lab-on-PCB (lab-on-printed circuit board) has re-emerged as a potential alternative to LoC in biomedical field. PCB is widely used in the electronics industry, thus established manufacturing companies for fabrication process can easily be found. The biosensor proposed is a thin film nickel-gold finishing plated on copper electrodes through electroplating. Previous study found that the gold-plated PCB was biocompatible with human K562 cells (Mazzuferi et al., 2010).

Optimization of electrodes will be done to determine the highest electric field generated on the electrode surface. This proposed design will also be compared with conventional Interdigitated Electrodes (IDEs) configuration to come out with the best design. Width (w), spacing (s) and length (l) will be varied and simulated using COMSOL Multiphysics to find the optimum design. The design of the IDEs is optimized to maximize sensitivity towards changes in the cells using ECIS technique. the IDEs geometry will be optimized such that both the cut-off frequency of the interfacial impedance and the solution resistance are minimized. This allows the highest electric field to be generated by the IDEs.

4.2 Design of Data Acquisition System

Although many researches have been conducted using the impedance monitoring technique, most work rely on impedance spectroscopy measurement using traditional impedance measurement instruments such as HP 4284A precision LCR meter (Zou et al., 2007), HP 4194A Impedance/Gain-Phase Analyzer (Webster et al., 2009), Agilent 4294A Impedance Analyzer (Price et al., 2009). However,

these traditional instruments are mostly bulky, expensive and are difficult to be used in a portable environment.

Intensive research has been growing to construct a miniaturized module to replace these instrumentations for point of care setting. The first commercially available impedance network analyzer implemented as a single integrated circuit (IC) was designed and introduced by Analog Device Inc that combines a frequency generator with a 12-bit, 1MSPS (sampling per second), analog-to-digital converter (ADC). In this research, AD5933 will be embedded with microcontroller unit (MCU) to make a portable wireless data acquisition system for impedance monitoring of the cells.

4.3 Biological Test

4.3.1 A549 Cell Lines Test

The first biological study using A549 lung cell lines will be conducted to test the performance and biocompatibility of the sensor. This is to come out with the optimal electrode configuration and to ensure the sensors are non-toxic and suitable for monitoring cellular activities in vitro.

Electrode surface will be coated with extracellular matrix (ECM) coating such as collagen type I to promote cellular adhesion on to the surface of the electrodes.

4.3.2 Chemosensitivity Test

Once the design is fixed, the chemosensitivity test will be performed using primary lung cancer cells. Samples will be taken from biopsy of the patients and will be cultured in the lab until it reached suitable passage for biosensor testing. Targeted samples are between 10-30 different samples, as an essential standard for pilot study of clinical device.

Chemosensitivity response of conventional chemotherapy drugs used in treating lung cancer will be tested against the cultured cells on the sensor, to predict the response of each drugs on the cells. Correlation between the sensors' results with the actual chemotherapy response of the patients will be made to analyse the significance of the results predicted by the sensors.

5 EXPECTED OUTCOME

The expected outcome of this project would be a chemosensitivity device that is able to predict the response of chemosensitivity based on in vitro cell culture using the ECIS techniques. The general system architecture is shown in Figure 1 below.

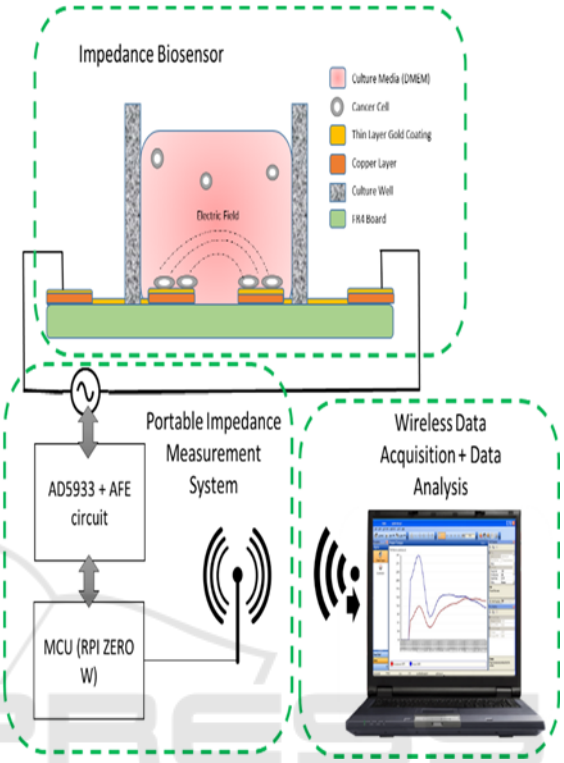

Figure 1: Overall system architecture for personalized chemotherapy response.

There are three main parts in the system. The first part is the impedance biosensor, which will be fabricated using gold-plated PCB. The electrodes will be applied with low alternating voltage at 10kHz frequency for monitoring the cellular behaviour based on cellular adhesion on the electrodes.

The second part is the portable impedance measurement system, which mainly consist of AD5933 impedance analyser IC which will be controlled by MCU together with the Analog Front End (AFE) as the signal conditioning circuit for controlling current exposure to the cells on the electrodes.

The last part is the wireless data acquisition system, which is also controlled by the MCU connected with the network. All raw data measured by the system will be transferred and stored in a cloud server and can be remotely access from other location.

The system will be embedded in a small device packaging to make it suitable to be placed inside the incubator during testing. Cellular behaviour of the cells can continuously be monitored for several days

to show the response of chemotherapy drugs on the cancer cells.

6 STAGE OF THE RESEARCH

This research has arrived at the stage of development of data acquisition system and sensor validation. Electrode design was analytically optimized to obtain the best configuration for the sensor. Simulation using COMSOL was done such as shown in Figure 2. Optimization of electrode configuration was done in term of finding lower cut-off frequency and minimizing bulk resistance by varying width of electrodes (W), spacing between electrodes (S) and total number of electrodes (N). After simulation, the design was sent out for fabrication using gold coated PCB technique. Prototype sensor is shown in Figure 3.

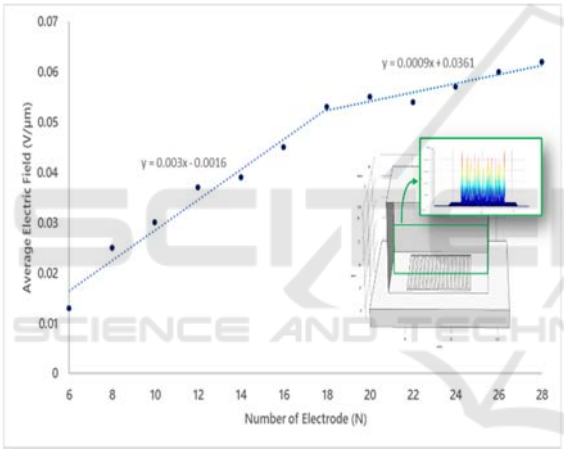

Figure 2: COMSOL Simulation of optimizing the parameter for the electrode configuration. Simulation suggested N=18 is the optimal number of electrodes for having saturated average electric field on electrode's surface.

As of the current stage, sensors are tested with A549 lung cancer cell lines to optimize cell seeding, coating concentration and to check for toxicity of the biomaterial. Next step involves development of wireless data acquisition system and establishment of primary lung cancer cell culture protocol before chemosensitivity testing using biosensor. Figure 4 shows the flows of expected outcome of the research.

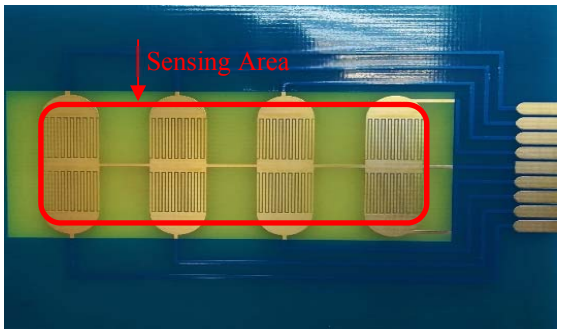

Figure 3: Fabricated sensor with gold coated surface finishing PCB.

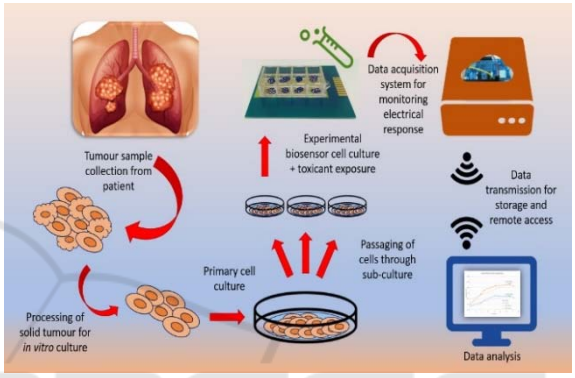

Figure 4: Flows of expected outcome of the research.

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