Development of High Voltage Pulse Inducement Method for Biological Cell

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Abstract— **Electroporation (EP) system is a process of controlling cell functions by using electromagnetic fields (EMF) to create pores through a cellular membrane that causes cell lysis and apoptosis. In this paper we present an experimental setup for fundamental studies on cell EP. An adjustable high voltage pulse generator (3kV/10μs – 600μs pulse length) system were connected to the EP chamber which subsequently allow real time observation of membrane permeability changes and cellular physiology.**

In order to initiate higher cell viability rate, high transfection efficiency, lower sample contamination and smaller Joule heating the modification of EP chamber need to be implemented. . Following that, HeLa cell culture has been projected as cell that will be used in this study. Finally, some suggestions are proposed for the future studies.

Keywords-electroporation; high voltage pulse; real time monitoring;electromagnetic field

I. INTRODUCTION

This research involves in-vitro technique to evaluate specific cellular level interactions with exposed EMF under controlled environments outside living organism. This fascination of controlling cell functions by using EMF has led to the discovery of EP and have been a topic of great interest in physiological and morphological changes [1-3].

The detail work to understand such behavior through simulation effort has been conducted and reported [4]. From previous simulation effort together with current experimental work we can foresee that main advantage of this studies is some of the exposure conditions can be easily and precisely controlled (e.g., changing exposure duration, background temperature, or exposure field intensity) as a means of determining dose-response relationships, and the effect of applying different threshold levels [5-8].

In order to obtain a deeper insight on the quantitative interaction mechanisms between electric field and biological cells, an experimental setup confines the observation of a cell during exposure to electric field and provide the analysis of changes in cellular mechanism.

II. CHARACTERISTICS OF THE PROPOSED EXPERIMENTAL SETUP

The general characteristic of the experimental setups for EP is to create pores that can be temporary (reversible EP) or permanent (irreversible EP) [9, 10]. These characteristics conducted to insert biological samples into cells (reversible EP) and also electroporated irreversibly to release their intercellular contents for further biological investigations.

Previously EP technique e.g. flow cytometry, require the exposure of large suspension volumes and commercially available EP cuvettes with electrode gaps of 1, 2, and 4 mm have the volume of 100μl, 200μl and 400μl. However the cuvette based EP cannot be observed in real time, at least several seconds after the procedures of taking the sample out of the pulse generator and placing it under a microscope.

In this paper we developed an experimental setup presents several advantages such as real time monitoring of cells poration, single cell analysis and the effects of high voltage pulse electrical fields to cell plasma membrane.

Fig. 1 shows the main components of the experimental setup. A Nikon inverted research microscope (Ti series) offers improved system speed, increased flexibility and efficient multi-mode microscopy as part of a fully integrated microscope system that is ideal for high end research and live cell imaging.

Furthermore to controlled environment of the cell, chamlide TC is connected through a microscope stage and accepts both various chamlide magnetic chambers and commercial culture dishes. Chamlide TC includes special glass covers for disposable culture wares to maintain humidity and provide excellent transmission for imaging. The external subsystem is controller (CU-109) which controls temperatures of the incubator main body, incubator cover, humidifier, and lens warmer, as well as adjust the flow rate of the mixed CO^2 gas by using the flow meter (less than 100 ml/min).

In order to expose biological cells to high voltage pulse electric field, two main subsystem needed: (i) imaging

chamber system (chamlide EC) dedicated to the applications field stimulation which supplied with a pair of platinum electrodes, and (ii) high voltage pulse system (ECM® 830) which allows delivering 10µs – 600µs duration pulses with adjustable amplitude up to 3kV.

Once the EP systems are determined, it allows the penetration of genes or other therapeutic molecules inside the cell, hence visualization of their effects can be monitored.

III. RESULTS AND DISCUSSION

Once the overall setup is identified, there are several tasks that need to be seen and among them are the EP chamber and the type of cell. To ensure successful EP process the design and construction of controlled EP chamber is very important. For initial study the HeLa cells were selected as the material used to be exposed to the electric field excitation in EP chamber. This selection is due to the fact that HeLa cells is one of the cells that available from the Animal Cell Cultures Laboratory (*Faculty of Bioscience and Bioengineering UTM*).

A. Electroporation (EP) Chamber

The EP modified chamber used consists of two platinum electrodes with a distance 0.5mm. In order to achieve the high electric field intensity of 3kV, the cells need to be placed between electrodes.

When compared with conventional EP devices, these EP chamber devices have several advantages such as it can controls the temperatures, pH and humidifier of the incubator as shown in fig. 2. According to these advantages of EP devices it can improves the higher cell viability rate, high transfection efficiency, lower sample contamination, and smaller Joule heating effect. As a result with the modification made to this chamber it will allow a further biological investigation. In related research studies that investigated the effects of different parameters such as field strength, ionic concentration, pulse strength and duration on the cell permeabilization can be achieved.

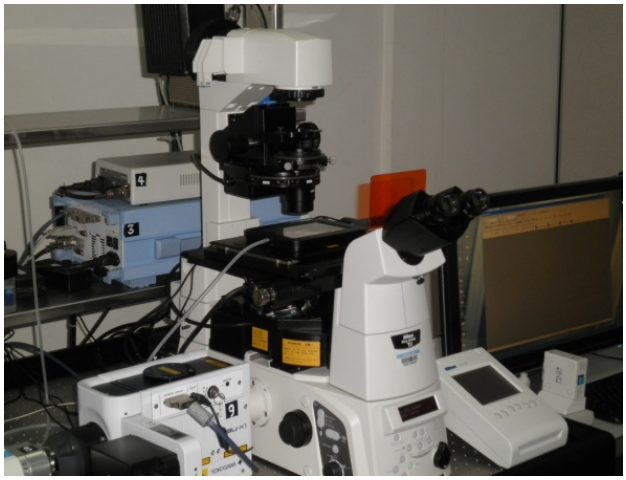

Figure 1. Figure Picture of Nikon Inverted Microscope (Ti series)

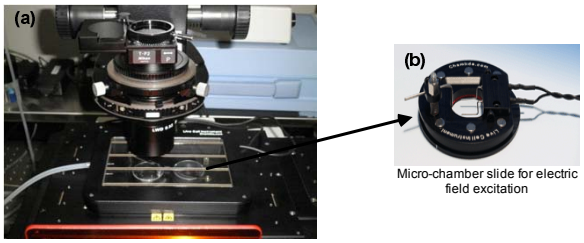

Figure 2. Picture of (a) Chamlide TC stage, (b) microchamber slide (Chamlide EC).

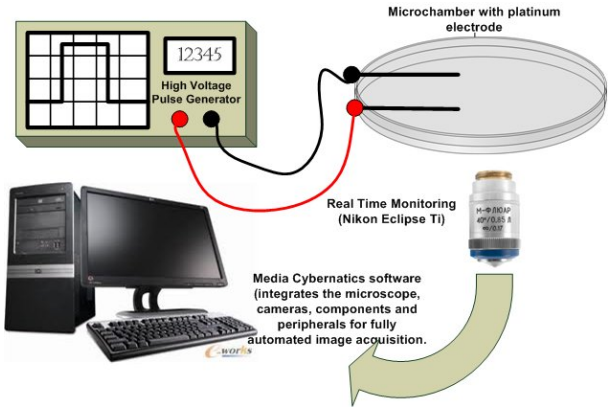

Figure 3. Diagramatic representation of a fully integrated system for EP process.

B. Cells

Most researches on EP are conducted using in-vitro techniques. Most of these techniques using cells in the sample, in conjunction with this study can be developed by studying the lysis of cells, transfection, and electrofusion.

In this study we decided to use HeLa cells [11-14] (Human cervical cancer cells). HeLa cells cultured in Dulbecco's Modified Eagle Medium (Gibco) with 10% fetal bovine serum until 90% confluency (fig.4), are first harvested by incubation with trypsin (Invitrogen) for 3 min (fig. 5) and suspended in RPMI 1640 medium (Sigma) with 10% fetal bovine serum to neutralize the trypsin. The suspension is then centrifuged and resuspended in Dulbecco's phosphate buffered saline (PBS, Sigma).

These preliminary results demonstrate feasibility to use our EP chamber applying electric field near the cell membrane that is usually refers as EP or electropermeabilization.

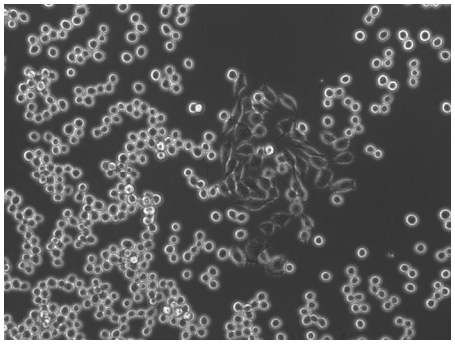

Figure 5. Harvested by incubation with Trypsin

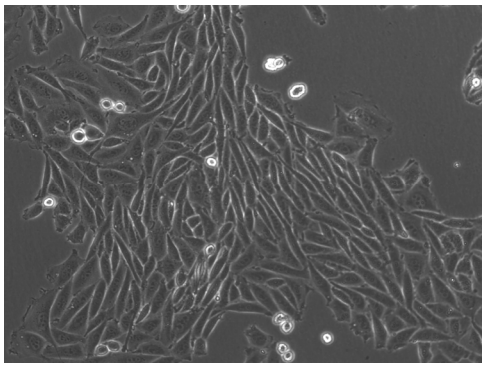

Figure 4. HeLa cells (90% confluence)

IV. CONCLUSION

From this study a preliminary work were has been completed by selecting HeLa cells, thus the cells were cultured successfully. This study constitutes a major step towards the possibility to use EP chamber slide for further investigations on intracellular components of living cells when induced with high voltage pulse generator.

A method permitting to control pH, temperature, and humidity on EP chamber, its key feature to improve the higher cell viability rate, high transfection efficiency, lower sample contamination and smaller Joule heating effect.

Furthermore, this experimental setup demonstrated on this study may enable the application of high voltage pulse on living cells for real time visualization.

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