Study of Effect of Microsecond Pulsed Electric Fields on Threshold Area of HeLa Cells

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Abstract— **Microsecond pulse electric field (μsPEF) application development substantially affected the development of research process including controlling cell functions by using pulses of electrical fields to create pores through a cellular membrane causes cell lysis and apoptosis commonly known as electroporation. Here we demonstrate the influence of the μsPEF on the threshold area (TA) of human cervical cancer cells (HeLa) membrane. The electric field for μsPEF is 3kV/cm while the pulse interval is 100ms. The pulse length and the number of pulses were fixed at 10μs and 5, respectively. While the cultured skin cells are placed in 9 mm-gap EP electrode chamber for allowing 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 effect the modification of EP chamber need to be done which can be controlled by pH scale, temperature and humidity. The experiment using high pulse electrical field with simply repetitive pulses shows the threshold area of cell membrane was decreasing gradually to 44.59μm, and is settled within hundreds of second. We found that the threshold area of cells membrane was affected when exposed to high voltage pulse electric field. The dependence of the threshold area on the HeLa cell membrane might be associated with the electrical impedance of the plasma membrane that begins to fluctuate after the application of a certain level of μsPEF.**

Keywords- microsecond pulsed electric field (μsPEF); electroporation; HeLa cell

I. INTRODUCTION

Biological effects of microsecond pulsed electric fields (μsPEF) have been intensively investigated over the last decade. C.chen et al. have been studying the biological effect of μsPEF and found that the transient increase in the permeability of cell membranes is used to introduce DNA or other molecules into cells. This phenomenon is potentially, the basis for many in vivo applications such as electro chemotherapy and gene therapy. However, it still lacks a comprehensive theoretical basis.

This research involves in-vitro technique to evaluate specific cellular level interactions with exposed μsPEF under controlled environments outside of living organism. This fascination of controlling cell functions by using μsPEF has led to the discovery of EP and has been a topic of great interest in physiological and morphological changes [1-3]. The detail work to understand such behavior through simulation has been conducted and reported [4].

Based on previous simulation combined with current experimental work, we can observe that the main advantage of this study 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 mean of determining the dose-response relationships and the effect of applying different threshold levels [5-8].

In order to delve deeper into the quantitative interaction mechanisms between electric field and biological cells, an experimental setup that confines cell observation during exposure to electric field has been reported [9]. Here, we are reporting the effect of threshold area on the transient increase in the permeability of cell membranes for cultured HeLa cells. Besides that, we are also monitoring this effect in real time using Nikon inverted research microscope (Ti series). There is 3kV/cm amplitude voltage applied with pulsing sequences of 10μs long pulses. These are simply repetitive pulses produced by a commercially available pulsed power generator [10]. Due to the fact that the cell membrane permeability depends on the applied pulse number, the experiment was performed on 5 pulse number for the amplitude voltage.

II. MATERIALS AND METHODS

A. Cell lines and Culture conditions

In this study we decided to use HeLa cells. HeLa cells cultured in Dulbecco's Modified Eagle Medium (Gibco) with 10% fetal bovine serum until 90% confluence, are first

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harvested by incubation with trypsin (Invitrogen) for 3 min and suspended in RPMI 1640 medium (Sigma) with 10% fetal bovine serum to neutralize the trypsin. They were settled in a CO_2 incubator until used.

B. µspef Exposure System

The commercial electroporation ECM 830 made by BTX Harvard Apparatus can provide two modes of operation. First, a high voltage mode ranging from 30V to 3kV and pulse length of 10µs to 600µs (1µs resolution). The second mode is a low voltage which gives an output voltage of 5V to 500V and pulse length of 10ms to 999ms (1ms resolution).

C. Electric Field (EF) Stimulation Chamber

The EF stimulation chamber system was designed and built thus allowing for observation of the HeLa cells changes following µsPEF exposure applications. These chambers consist of two platinum electrodes with a distance of 9mm as shown in Fig. 1. In order to achieve the high electric field intensity of 3kV/cm, the cells were needed to be placed between the electrodes. When compared with conventional EP stimulation chamber devices, this EP chamber device has several advantages such as that it can control the temperatures, pH and humidity of the incubator as shown in Fig. 2. Henceforth, this particular EF device can provide a higher cell viability rate, high transfection efficiency, lower sample contamination, and smaller Joule heating effect. As a result, the modification made to this chamber will allow for further biological investigations.

D. Integrated devices of real time imaging system

The EP stimulation chamber was examined at 100X magnification using Nikon inverted research microscope (Ti series). Differential interference contrast (DIC) is the selected analyzer because this technique can be used to enhance the contrast in unstained and transparent sample. Furthermore, to control the cellular environment, chamlide TC is connected through a microscope stage which accepts both different variations of chamlide magnetic chambers and commercial culture dishes. Chamlide TC includes special glass covers for disposable culture wares to maintain the humidity and provide excellent transmission for imaging. The external subsystem controller (CU-109),provides control to the temperatures of the incubator main body, incubator cover, humidifier, and lens warmer, as well as adjusting the flow rate of the mixed CO_2 gas by using the flow meter (less than 100 ml/min).

III. RESULT AND DISCUSSION

Experimental data show that there is an electric field effect on the HeLa cells. Fig. 3 indicates the time course of the threshold area activity of HeLa cells membrane exposed to the induced a high voltage of 3kV/cm with a pulse length of 10µs and a pulse number of 5.

From the diagram, it can be seen that the threshold area for the HeLa cells membrane decreased gradually to 44.59µm prior to increasing again after a hundreds seconds.

This was due to the µsPEF which is known to cause temporal membrane defects such as electroporation. It was also experimentally shown that the electrical impedance of the plasma membrane began to fluctuate after the application of a certain level of µsPEF before settling within several hundreds of second. This delay is likely dependent on the energy of the pulses provided.

Figure 1. Picture of 9mm – gap EP electrode chamber for µsPEF excitation.

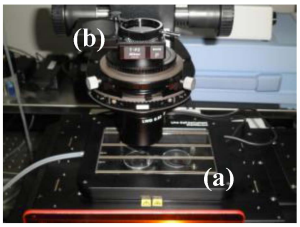

Figure 2. Picture of (a) Chamlide TC stage, (b) Nikon Inverted Microscope.

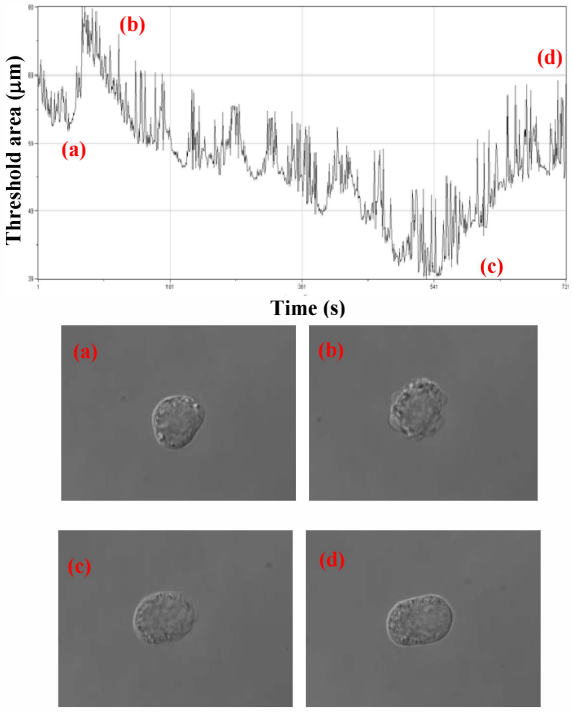

Figure 3. Threshold area response of HeLa cells membrane (a) TA = 75.25µm before µsPEF (b) TA = 77.71µm during µsPEF (c) TA=44.59µm after 541s µsPEF and (d) TA=75.78µm after 721s µsPEF. Field strength and the number of pulses were fixed at 3 kV/cm and 5 pulses, respectively. Pulse length rate was set at 10µs.

IV. CONCLUSION

Overall, we have proven that the threshold area of membrane changes is caused by the μsPEF. This is due to cell lysis of the cell membrane which causes the permeability and conductivity to vary when subjected to μsPEF. This change occurs when the high voltage amplitude is set up to 3kV with a pulse length 10μs and the number of pulse 5. All of the data were recorded with real time observation using the Ti series Nikon microscope. Due to the more easily controlled high voltage amplitude, the optimized high voltage pulse is preferred when the μsPEF is repetitively applied to biological targets.

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