Correlation between Electrical Field Strength and Pulse Width Analysis on Cell Viability

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Abstract. Electric field pulses can make living cell membrane electroporated either reversibly or irreversibly. This study determined the effects of electric field pulses on HeLa cancer cell viability. First set of experiment - Pulses electric fields (control, 200V/cm, 400V/cm, 600V/cm, 800V/cm, and 1000V/cm) with fixed pulse width (200µs) were performed on HeLa cells. Second set of experiment - Pulses width of (control,100µs, 500µs,1ms,5ms,10ms) with constant electric field strength of (600V/cm) were performed on HeLa cells. Number of pulses fixed to 1 for both sets of experiments. The quantitative determinations of cell viability measurement were performed by the aid of haemocytometer and trypan blue staining. The data shows that HeLa cell viability decreases as the electric field strength and pulse width. This is because electric pulses alter the cell's transmembrane potential, causing disruption of the lipid bilayer, and increase the cell membrane permeability. Efficient electroporation outcome only will be gained by fine tuning the appropriate electric field parameter according to the application needed.

1. Introduction
Electroporation, a microbiology technique being favourably applied in several areas like medicine, biotechnology, biology, food processing and such due to the versatility [1]. It works with any cell, with fewer steps, where it becomes a uniquely beneficial method. In medicine field, electroporation is used for electrochemotherapy. It is a new technological approach treatment; where cytotoxicity of anti-cancer drugs is increased by means of electroporation to the tumorous cells [2]. While in biotechnology, electroporation system used for sterilizing water. Electric field is effective in killing bacteria and pathogens without the chemicals use. Electroporation creates impermanent pores in microorganism cell membranes which decrease dosages of chlorine in water disinfection [3-4]. Apart from that, electroporation technique plays a major role in food processing. It has shown a promising outcome in food preservation, where pulsed electric fields used for microbial inactivation with least effect on food quality [5]. No matter what application it is, for a successful outcome, pulse parameter is the important key in electroporation-based application. Meaning that, efficient electroporation is achieved only after careful fine-tuning on the parameters which includes pulse amplitude, pulse duration, and number of pulse. However, each application differs in the setting of pulse parameter according to the cell size, cell type, orientation of cells and so on [6]. However, in the present review an attempt has made to investigate the effect of electric field strength on HeLa cancer cell by measuring cell viability percentage.
2. Methodology

2.1. Cell culture

In this study, HeLa cells were used as an experiment sample. HeLa cells were cultured in RPMI1640 medium supplemented with 10% of fetal bovine serum (FBS) and 1% of antibiotic (penicillin and streptomycin) from Gibco USA. Cells were kept in incubator at 37°C containing 5% of CO2 in order to grow and maintain their optimal temperature and humidity. HeLa cells will undergo sub-culture process every 3 to 5 days after which it reaches confluency of 80-90%. For sub-culture process, old medium will be aspirated and discarded from culture flask. Then cells were washed thrice with 2ml of phosphate buffered saline (PBS) to remove the serum of media, so that trypsin will be able to detach the cell from flask surface. Cells were digested with 2ml of trypsin and incubated for about 5 to 10 minutes. Double volume of complete growth medium added into flask to stop the effect of the trypsin and then centrifuged at 1000 rpm for 5 minutes. Supernatant will be removed and cell pellet will be re-suspended in complete growth of medium for a uniform.

2.2. Electroporation

ECM 830 square wave electroporation system is appropriate for drug, protein, gene delivery. ECM 830 generators devised for in vitro and in vivo applications. The ECM 830 comes with combination of a wide array of BTX specialty electrodes and accessories to intensify molecular and drug delivery experiments. ECM 830 electroporator were used to generate electric pulses needed to electroporate the cells. This device comes with a wide range of voltages from 5 to 3,000 V and pulse durations from 10μsec to 10sec. It also available with digital display of actual voltage and pulse length delivered.

After detaching process, eight hundred microliter (800 μl) of cells suspension, then poured into a 4mm electrode gap cuvette (BTX Harvard Apparatus). A single pulse electric field with an intensity of 200V/cm, 400V/cm, 600V/cm, 800V/cm, 1000V/cm with a constant pulse duration of 200μs and another set of experiment with constant field strength of 600V/cm with a varied pulse duration of 100μs, 500μs, 1ms, 5ms, 10ms were utilized in electroporating the cells. Then, cell suspension from each cuvette, were then seeded into wells of six-well culture flasks containing 2ml of complete growth medium. Similarly, 800μl cells from the same initial flask without electroporation were also seeded in another well containing 2ml of medium. Latterly cells will be incubated at 37°C and 5% of CO2. Viability of cell was observed after 48 hours of incubation.

2.3. Measurement of cell viability

Cell viability measured after 48 hours of incubation with the aid of haemocytometer. Haemocytometer is a device where we used to determine the concentration of cells in a given sample. It is a special type of microscope slide consisting of two chambers, which is divided into nine (1.0mm x 1.0mm) large squares which are separated from one another by triple lines.

After cell detachment, 100 μL of cell suspension will be mixed gently with 100 μL of trypan blue. Next by using a pipette, 100 μL of Trypan Blue-treated cell suspension slowly filled on both chambers underneath the coverslip, allowing the cell suspension to be drawn out by capillary action. Next the device will be placed on the microscope stage and the cell suspension is counted. In order to get the
cell viability, add together the live and dead cell to obtain a total cell count. Then, divide the live cell count by the total cell count to calculate the percentage viability. 100µs, 500µs, 1ms, 5ms, 10ms were utilized in electroporating the cells. Then, cell suspension from each cuvette, were then seeded into wells of six-well culture flasks containing 2ml of complete growth medium. Similarly, 800μl cells from the same initial flask without electroporation were also seeded in another well containing 2ml of medium. Latterly cells will be incubated at 37°C and 5% of CO₂. Viability of cell was observed after 48 hours of incubation.

3. Experimental result

3.1. HeLa cell viability

Electroporation is a technique which is used extensively to enhance the permeability of living cell. The proficiency of electroporation depends on the electric field parameters. Cell membrane permeability increases when electric field applied and subsequently allows molecules, drugs, ions to be introduced into cell for an effectual treatment. Percentage of cell viability was measured. Viability measurements used to estimate the life or dead of cancerous cells. The results obtained in this study, as shown in Table 1 and Figure 2 quantitatively revealed the dependence of cell viability on electrical parameters. Control, 200V/cm, 400V/cm, 600 V/cm, 800V/cm, 1000V/cm electric field strength with a constant pulse width of 200µs showed 100%, 98.2%, 96.5%, 96%,93.3%,89.2% of cell viability respectively. Based on the result obtained, cell survival decreases with an increasing of field strength from 200V/cm to 1000V/cm with fixed pulse width of 200µs and single pulse.

| Electroporation parameter | Cell Viability (%) |
|---------------------------|--------------------|
| Control, 0V/cm            | 100                |
| 200V/cm, 200µs            | 98.2               |
| 400V/cm, 200µs            | 96.5               |
| 600V/cm, 200µs            | 96                 |
| 800V/cm, 200µs            | 93.3               |
| 1000V/cm, 200µs           | 89.2               |

Figure 2. HeLa cell viability percentage with different field strength and fixed pulse width
Another set of experiment carried out, where pulse duration varied (control, 100µs, and 500µs, 1ms, 5ms, and 10ms) and field strength fixed at 600V/cm with single pulse. Result obtained as in Table 2 and Figure 2 showed 95.3%, 97.2%, 95.4%, 86.1%, 75.2%, and 53.3% of cell viability percentage. Cell viability found to be diminishing as the pulse width increases.

**Table 2.** Hela cell viability percentage with varied pulse width and fixed electric field strength (600V/cm).

| Electroporation parameter | Cell Viability (%) |
|----------------------------|--------------------|
| Control, 0V/cm             | 100                |
| 100µs, 600V/cm             | 97.2               |
In a way to discover the effects of electroporation with different field strength and pulse duration on tumour cell, viability of cell was measured. Experiment results showed that cell survival is significantly affected when treated with pulse electric field. Both set of experiments demonstrates a decreasing number of percentages in HeLa cell viability. Higher the voltage and pulse width, lower the cell viability becomes. Electric pulses alter the cell’s transmembrane potential results in membrane permeation. This happens when induced transmembrane potential exceeds the breakdown potential lipid bilayer. Next pores are formed in membranes and maintained by the electrical pulse field. Cell membrane pores created when the energy stored in the membrane capacitor exceed the energy required to keep the membrane impaired against pore expansion. Electric field parameter with high percentage viability stimulates the cells in absorbing more nutrients from the media through the formation of pores. While the higher field strength may lead to cell apoptosis. In these phenomena, mitochondria the power house of biological cells play a potent role. An early signal for the programmed cell death is a decrease of mitochondrial transmembrane potential, which leads to opening of pores in the inner mitochondrial transmembrane. Decrease in mitochondrial transmembrane potential causes the release of an enzyme called cytochrome c from mitochondria into cytosol. This enzyme will activate pro-apoptotic caspases [7]. Qualitative observation of the media 1640 at higher pulse amplitude, showed a change of colour from red to orange. This could be the change of pH of the medium from neutral to acidic due to high number of cell death.

4. Conclusion

In this paper attention was given to the electric field parameter on cell viability. The result of this paper demonstrates that cell viability slowly decreases when the electrical field strength and pulse duration increases. Thus, the result showing that cell viability is influenced by electrical field parameters (field strength and pulse width). Efficient electroporation outcome only will be gained by fine tuning the appropriate electric field parameter according to the application needed. The result of this study can be used for further investigation in order to identify the optimal electroporation condition according to the application needed.

Figure 4. HeLa cell viability percentage with different pulse duration and fixed electric field strength of 600V/cm
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