Investigation on Suitability of Pulse Duration Parameter for HeLa Cells Line Proliferation Properties towards Anti-Cancer Application

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Abstract. In order to bring up a cells or tissue with a gene or DNA, Electroporation parameter such as pulse duration, field strength and number of pulse plays the most important aspect. Thus, the aim of this paper is to optimize the pulse duration parameter for HeLa cells line. This is very important because nowadays, research on EP factors is still in limited mode. Moreover, suitable and applicable pulse duration parameter would ensure successful treatment with least possible side effect. In this paper, HeLa cells or known as cervical cancer cells was used to induce into a single pulse electric field. Therefore, to achieve the aim of this study, field strength of 500V/cm was preferred and the pulse duration was varied in the range of 100 µs, 500 µs, 1 ms, 2 ms and 5 ms to obtain lowest proliferation rate and inhibit growth of HeLa cells. The result obtained shows that HeLa cell induced to 500 V/cm with pulse duration of 100 µs, induced the lowest proliferation rate (45%) as compared to initial reading density (control) after 48 hours of culture. Thus, further research based on this work may pave the way for an anti-cancer application through the study of the effect of pulse duration parameter on HeLa cells.

1. Introduction

Electroporation (EP) is a process of the biophysical effect on cells induced by an external electrical field [1]. It is a molecular biology technique in order to create pores through a cell membrane, increase rapidly the permeability of the cell membrane, allowing chemicals, drugs, or DNA to be introduced into the cell [2]. In modern molecular medicine and biotechnology field, tissue electroporation is performed by using special electrodes placed in the target area of the body.
Moreover, in an experimental setup, EP is done by insertion of a biological sample between two electrodes, by that produce an electric field between the electrodes either in-vitro or in-vivo EP [5]. Cell EP by in-vitro is used mainly for transfection by DNA introduction, but many other interventions are possible, including microbial killing. On the other side, in-vivo electroporation of tissues enhances molecular transport through tissues and into their constitutive cells whilst ex-vivo electroporation provides manipulation of cells that are reintroduced into the body [5].

By applying electrical pulses across cells can have a variety of outcomes; from no effect to Reversible EP or to Irreversible EP. Reversible EP will allow transient molecular transport through the pores and after a few minutes, cellular membrane will reseal and cell functions are restored. In contrast, Irreversible EP offers an ability to destroy unwanted tissue in the body and could be practiced as in the absence of chemo drug to destroy the cancerous cells. There are many benefits of EP technique, such as, this technique does not change and modify the biological structure and function of the target cells, and it is safer, high ability and efficiency and less immunologic [6-11].

Furthermore, the efficient and capability of EP is achieved by controlling the pulse duration, one of the important parameter in EP technique which have the biggest clout on the outcome of the experiment [1-5]. In addition, pulse duration need to be controlled for a particular cell type, cell orientation, cell size and cell density. Hence, in this study, the efficiency of EP was determined by measuring the percentage of proliferation rate of electroporated HeLa cells.

2. Materials and Methods

2.1 Cell Culture

In this research, HeLa cells line or known as cervical cancer cells were used in this experiment. The HeLa cells line were cultured in an incubator with 5% CO2. Then, the HeLa cells line were cultured in the medium RPMI-1640 enhanced with 10% Fetal Bovine Serum (FBS) and 1% antibiotic Penicillin-streptomycin from Gibco, USA. The humidified incubator is set to 37°C for cell growth. To perform cells subculture, the confluence of HeLa cells must reach 80 – 90 % confluent and then the HeLa cells were sub-cultured every 3 to 5 days shown in Figure 1.

- HeLa cell line were cultured as a monolayer in RPM11640 (plus L-glutamine)
- RPM11640 was enhanced with 10% Fetal Bovine Serum with 1% antibiotic (Penicillin and Streptomycin)
After HeLa cells reached 80 – 90 % confluent, the old medium was first aspirated and discarded. Then, 3ml of Phosphate Buffer Saline (PBS) was added for washing the HeLa cells and later the PBS was removed from the old culture flask [12-13]. Once the PBS is removed, 2ml of Trypsin Express solution was introduced into the 25cm² old culture flask and left for 10 – 15 minutes in the incubator for cell detachment [12-13]. This is because Trypsin Express solution works very well in a warm condition. After incubation process, the HeLa cells were monitored under a high resolution microscope to make sure the cells are circular in shape and fully detached from the culture flask surface after trypsinization process in Figure 2.

**Figure 1.** Flow chart of the cell culture process.

(a) The cells before detachment process
After trypsinization, HeLa cells appeared rounded and fully detached from the flask surface. Figure 2. Comparison between the HeLa cells conditions.

2.2 Electroporation (Ep)

Figure 3 shows an ECM830 device from BTX Harvard Apparatus that was used for electroporating the HeLa cells line in suspension. Moreover, ECM830 has two modes of operation, where the first mode is a low voltage mode between pulse length 10ms until 999ms (1ms resolution), whereas high voltage mode ranging from 10µs to 600µs (1µs resolution). In addition, EP process involves several parameters and the parameters to measure the effectiveness of EP effect are the pulse duration, electric field strength and the number of pulse.

Figure 3. ECM830 device

2.3 Measurement Of Cell Proliferation

After 48 hours in culture mode, HeLa cells were detached and counted by using Hemocytometer method. Figure 4 shows the Hemocytometer method that was conducted to monitor the cell’s proliferation growth rate. It is a device used to count the number of cells using Trypan Blue solution.
Based on the method, after 48 hours the cell densities were compared with the initial plating density (control) to observe the effect of EP on HeLa cells. Furthermore, experiments were performed in triplicate for consistency, and the mean of HeLa cells proliferation’s rate was calculated for analysis purpose.

**Figure 4. Hemocytometer method to count cells number**

3. **Experimental results**

In this experiment, HeLa cells were exposed with different pulse duration parameter to monitor cells growth proliferation rate. EP technique involves several important parameters and the most
effective EP result support by the length of the field applied known as pulse duration. The pulse duration parameter is controlled by the commercial EP device.

After harvesting the cells with Trypsin Express solution and neutralizing with an equal volume of growth medium, 0.8 ml of cells suspension was then poured into a 4mm electrode gap cuvette. An electric field with an intensity of 500V/cm was used in electroporating the cells and different pulse duration ranging between 100µs-5ms was utilized.

Subsequently, the cells suspension after exposed with EP technique were then seeded into a 6-wells plates containing 1.2ml of complete growth medium and were incubated at 37℃ and 5% of CO2. Similarly, for control, same method was repeated from same in initial flask but without exposed with EP technique. All the flasks are cultured under the same condition and were monitored for 48 hours.

Moreover, after 48 hours in culture condition, the cells was detached and counted using Hemocytometer method for each treatment to study the EP effect on HeLa cell's proliferation rate. The results are shown in Table 1, and quantitatively revealed the dependence of cell’s proliferation rate on pulse duration parameter.

Furthermore, Figure 5 show images of HeLa cells exposed by various pulse duration of 100µs, 500 µs, 1ms, 2ms, and 5ms with pulse amplitude (electric field) of 500V/cm after 48 hours in cultured as compared to initial plating number of cells. It was found that the cell’s proliferation rate started to reduce by 40% at 100µs for 500V/cm as compared to initial number of seeding cells (control group) after 48 hours in culture conditions.

**Table 1. HeLa Cells Proliferation Rate for Different Pulse Duration Parameter after 48 Hours**

| Different pulse duration (S) | Proliferation rate of HeLa cells after 48 Hours (%) |
|-----------------------------|-----------------------------------------------|
| CONTROL                     | 55.00                                          |
| 100µs                       | 10.00                                          |
| 500µs                       | 22.50                                          |
| 1ms                         | 22.50                                          |
| 2ms                         | 12.50                                          |
| 5ms                         | 12.50                                          |

(a) Pulse duration parameter: control  
(b) Pulse duration parameter: 100µs
In addition, Figure 6 shows that the lowest proliferation rate of HeLa cells was achieved at a pulse duration of 100µs at 500V/cm. Besides, this short and fast pulse duration inhibit the proliferation rate of HeLa cells and it revealed the 100µs at 500V/cm is 45% lower in cell’s proliferation rate than the control group. The results of the experiment showed that HeLa cells proliferation is considerably affected when treated with short pulse duration. At this stage, qualitative observation towards HeLa cells at this pulse duration showed a change in color of media from red to orange due to the electrolysis process at short pulse duration. Moreover the color changes occurred because of pH of media from neutral to acidic. Therefore, this changes in pH of the media might contributed to the higher number of cell death at shortest pulse duration.

**Figure 5.** HeLa Cells Growth Rate images after 48 hours.
Figure 6. HeLa cells proliferation rate for different pulse duration parameter after 48 hours.

The result demonstrated that short pulse duration works the best to inhibit the HeLa cells proliferation rate and suitable for anti-cancer cell’s application. This is because shortest pulse duration could result to electrolysis of the media and reduce the proliferation rate of HeLa cells. The experiments were performed in triplicate for consistency, and the mean or average proliferation factor was calculated.

4. Conclusion

In this study, the fundamental ideas and methods of EP were highlighted and the suitability of parameter of EP method is performed with shortest pulse duration to inhibit the HeLa cells proliferation rate. To achieve this objective, the culture and sub-culture technique of HeLa cells is done practically. Experimental data shows that HeLa cells exposed to 100µs with 500V/cm in single pulse shows the best anti-proliferation compared to other parameters. Later, the study will be continued by combining both EP method and porcupine bezoar (PB) extract that has the potential in becoming chemopreventive agents and requires further study and analysis. Finally, the outcome of this experiment might open the door in finding an alternative method for anti-cancer treatment.

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