Optimization of Pulse Duration Parameter for Hela Cells Growth Rate

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***Abstract*— To introduce a cell or tissue with a gene or DNA, electroporation parameter plays the most important role. Research on electroporation parameters is still in its early stages. Different researches have used different parameters while performing their research. Electroporation is a mechanism of temporarily disrupting the bilayer membrane, in such a way that a hydrophilic pore is established, allowing a pathway into the cell for molecules such as DNA, which then heals up, once again protecting the cell from the outside. There are many theories as to how this can occur, but the simplest view is that a short pulse creates an increase in the trans-membrane potential which if it exceeds a certain threshold (dependent on size and shape of cells) can lead to a thinning of the bilayer, either due to a local dislocation in the membrane. In this study, cervical cancer cells (HeLa cells) was used to expose to single pulse electric field. Thus, for the purpose of this study, the field strength of 1kV/cm was selected and the pulse duration was varied (30µs, 70µs, 100µs, 200µs, 300µs and 600µs) to obtain higher proliferation for a growth rate of HeLa cells. From this study, it is determined that, HeLa cell exposed to 1kV/cm with a pulse duration of 100µs and single pulse revealed the highest and fastest percentage confluence when compared to growth rate of HeLa cell exposed for 30µs, 70µs, 200µs, 300µs and 600µs pulse duration.**

***Index Terms*— Growth Rate; Hela Cells; Microsecond Pulsed Electric Field (Mspef).**

I. INTRODUCTION

Most of the unipolar pulse generator that has been used in bioelectric experiments produce microsecond to millisecond pulses. The most common pulse shape is a square pulse which is the most efficient at producing pores. Typically, electric field strengths of 1kV/cm and 100µsec pulses are used for drug delivery [1-2] and low field and longer pulses, such as 200V/cm, 20-50msec are used for gene therapy [3-6] In addition, the electric field and pulse duration (between 10^{-5} and 10^{-7}) are used for bacterial decontamination. The electric field employed under these parameters can facilitate transfer plasmid DNA into the bacterial cell. However, application of higher fields can result in bacteria death as it is possible to open permanent pores. Figure 1 shows the typical parameter range for different biological applications. The electroporation system designed here is targeted at drug delivery and gene therapy. It is therefore desirable to be able to control a range of electric fields between 10^3 and about 10^4 V/cm and pulse durations varying from 10^{-5} to 10^{-3} sec.

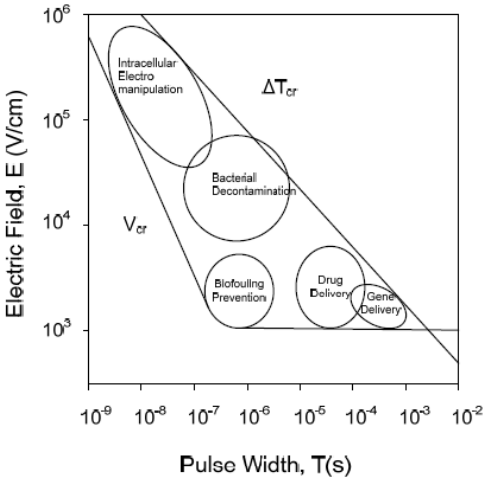

Figure 1: Range of electric field and pulse width for biological applications [7].

To illustrate electroporation, the lipid bilayer of a cell membrane with a directly applied electric field pulse (Figure 2). The lipid bilayer consists of two layers of elongated molecules that are hydrophilic at one end and hydrophobic at the other. In an aqueous environment, these molecules form a bilayer with the hydrophilic heads pointing outwards. Such a structure is very good at isolating the contents of the cell from the outside thus, providing a barrier to entry. If the pulse electric field exceeds a certain threshold, it can lead to a thinning of the bilayer, either due to a local dislocation in the membrane, or local field intensity maximum usually near the electrode. This can allow a hydrophobic pore to be created (Figure 2d) which looks like a tear or discontinuity in the bilayer. The layer will then try to reorganize itself locally as the hydrophilic tails and will be repelled by the proximity of the aqueous medium. That is now able to get into the membrane and the hydrophilic heads will rearrange themselves to face this medium. This results in a hydrophilic pore as shown in Figure 2 Electric fields pulses can induce pore formation, if the electric field pulse is appropriately selected (typically 300-400mV for <1ms across the membrane). Too much field can result in cell rupture and the formation of a permanent pore.

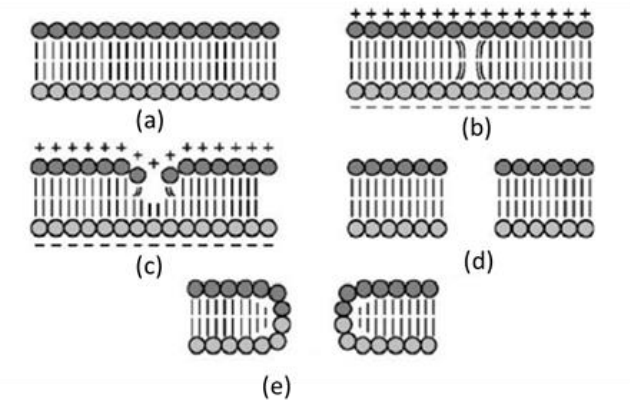

Figure 2: Formation of pores (a) Initial cell membrane condition, (b) a cell exposed to pulse electric field. This results in non-uniform molecular structure (c) Carving of cell membrane (d) Short-term hydrophobic pore on cell (e) Restructuring cell membrane [8].

The electric field pulse applied results in a rapid polarization change that can deform mechanically unconstrained cell membranes leading to local thinning as described above. At critical field strength, a rapid localized rearrangement of the lipid morphology is caused as described above. A temporary hydrophobic pore is rearranged at the pore edge. Eventually, the lipid heads fold over to create a hydrophilic interface during the transition to a conductive state. The pores are formed in the membrane and reseal after a short period time depending on applied electric field and bilayer edge energy. Several researchers have demonstrated that electroporation can be successfully applied to different types of cells such as mammalian cells, yeast, bacteria, plant cells [8-10], cancer cells [11-17] and blood cells [18-19]. Different applications of electroporation require different EP parameters for different cells type. An intense external electric field could damage the cell membrane and lead to cell lysis. Therefore, examining of EP parameter for different applications is very significant and extended experimental knowledge with theoretical models is needed.

II. MATERIALS AND METHODS

A. *Cell line and Cell Culture Protocol*

The HeLa cells are cultured in an incubator with 5% humidified CO_2. Medium RPMI-1640 is used for cells with 10% fetal bovine serum (FBS), while 1% of penicillin-streptomycin is also used. The temperature of the incubator is set to 37˚C for cells culture. To perform cells subculture the confluence of HeLa cells must reach 80-90%. Figure 3 demonstrates the microscopic view of HeLa cells with 80-90% confluence.

After this process, HeLa cells were washed once with 5ml PBS and the PBS were removed from the flask. Once PBS is removed, the flask was introduced with 2ml of trypsin for the surface of 25cm^2 flask and left for 10 minutes in the incubator. After the process of incubation, HeLa cells were examined under a high-resolution microscope. In Figure 4 the view of trypsinized cells, which are circular in shape and detached from the flask surface.

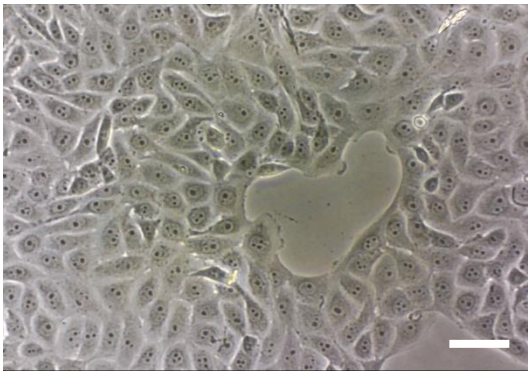

Figure 3: HeLa cells at 80 – 90% confluence (scale bar = 50µm)

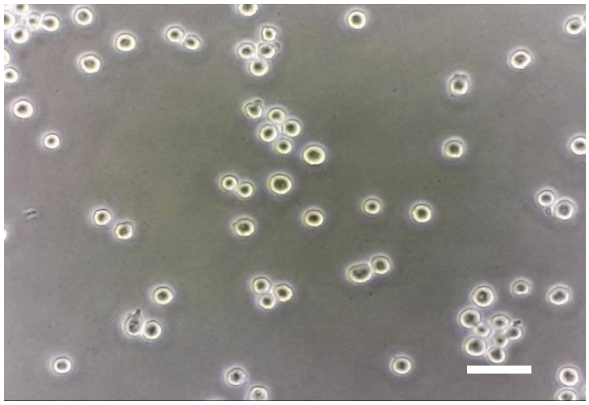

Figure 4: HeLa cells trypsinization image (scale bar = 50µm)

In this research, HeLa cell concentration after detaching is calculated by hemocytometer was 1.6×10^6 cell/ml.

B. *HeLa cells exposed to Pulse Electric Field*

Electroporation process involves several parameters. The most important parameters for effective electroporation are the electric field strength, length of the field applied (pulse duration) and number of the pulse. These factors are controlled by the commercial electroporation ECM 830 made by BTX Harvard Apparatus having two modes of operation. In this study, 0.25ml of the HeLa cells suspension is put in a 4mm cuvette. The cuvette is then placed in the electroporator chamber, for the purpose of this study, the field strength of 1kV/cm was selected and the pulse duration was varied to six pulses duration (30µs, 70µs, 100µs, 200µs, 300µs and 600µs) to obtain higher proliferation for a growth rate of HeLa cells. After being exposed to the electric field, the cell is inserted in a six-well plate with 1.75ml of complete medium for further investigation.

C. *Imaging system*

Electroporation process involves several parameters. The most important parameters for effective electroporation are the electric field strength, length of the field applied (pulse duration) and number of pulse. These factors are controlled by the commercial electroporation ECM 830 made by BTX Harvard Apparatus having two modes of operation.

III. RESULTS

Pulse electric field parameter is the main key for a rapid growth rate of cells. Thus, HeLa Cells are exposed to one

pulse, at a constant field strength of 1kV/cm, along with various pulse durations (30µs, 70µs, 100µs, 200µs, 300µs and 600µs). Experimental data shows that there is pulse duration effect on HeLa cell growth rate. The result data in Table 1 shows the average percentage confluence rate of HeLa cell line over time (6, 18, 24 and 36 hours respectively) for each pulse duration parameter. Figure 5 also gives a graphical representation of data in Table 1.

Table 1
HeLa cell growth rate over time

Time (Hour)	Pulse Duration 30µs	Pulse Duration 70µs	Pulse Duration 100µs	Pulse Duration 200µs	Pulse Duration 300µs	Pulse Duration 600µs
6	10	10	10	9	7	5
18	20	25	30	20	20	5
24	40	45	60	40	30	6
36	70	80	90	80	70	6

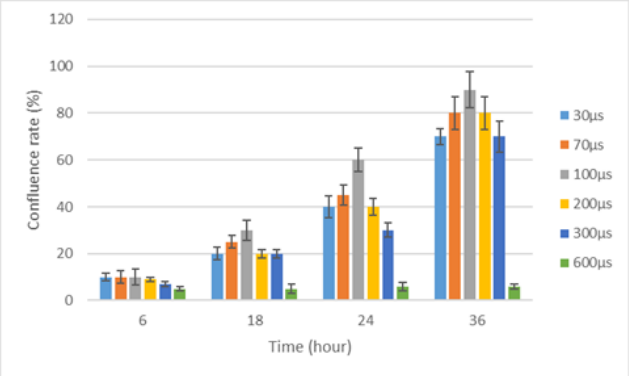

Figure 5: Percentage of HeLa Cells growth rate, the values are SEM. of triplicates (P<0.001)

The analysis of Figure 5 shows that the fastest confluence rate of HeLa cell growth was achieved at a pulse duration equal to100µs which took 36 hours to reach 90% confluence. While with a pulse duration of 70µs and 200µs, the confluence rate of HeLa cell growth took 60 hours to reach 90% confluence. Furthermore, pulse duration of 30µs and 300µs, the confluence rate of HeLa cell growth took 72 hours to reach 90% confluence. On the other hand, the HeLa cell growth rate at 600µs pulse duration achieved only 6% confluence after 36 hours. This means that 600µs pulse duration suppressed the growth rate of HeLa cells.

IV. DISCUSSION

From this work it is determined that, HeLa cell exposed to 1kV/cm pulse with a duration of 100µs and single pulse revealed the highest and fastest percentage confluence when compared to growth rate of HeLa cells exposed for 30µs, 70µs, 200µs, 300µs and 600µs pulse duration. However, this research requires further investigation to identify the critical process of growth rate which might lead us to innovate a technique for wound healing process. In order to measure the proliferation rate of HeLa cells under the influence of electric field, the field amplitude and varied the pulse duration is fixed between 30µs to 600µs. The influence of six selected duration from the given range was analyzed. From the result obtained, 600µs pulse duration induced death of HeLa cell to a great level. Hence, this duration can be used in tissue ablation of cancer cells [20]. On the other hand, 100µs stimulate the proliferation of HeLa cells. Hence 1kV/cm at 100µs could use in the proliferative stage of wound healing to facilitate cell growth.

V. CONCLUSION

In this study, optimization of PEF parameter is performed. In order to achieve this objective, the subculture of HeLa cells is done. Accordingly, HeLa cells are exposed to one pulse, at a constant field strength of 1kV/cm, along with various pulse durations (30µs, 70µs, 100µs, 200µs, 300µs and 600µs). Experimental data shows that HeLa cells exposed to 1kV/cm, 100µs pulse duration and single pulse shows the best growth rate compared to other parameters. Finally, the current finding does give us a dimension to explore further down the cellular level which may contribute towards wound healing and treatment applications.

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REFERENCES

[1] Jaroszeski MJ, Gilbert R, Nicolau C, Heller R. In vivo gene delivery by electroporation. *Advanced drug delivery reviews*, 1999, 35(1):131-7.
[2] Prausnitz MR. Microneedles for transdermal drug delivery. *Advanced drug delivery reviews*, 2004, 56(5):581-7.
[3] Imai E, Isaka Y. Gene electrotransfer: Potential for gene therapy of renal diseases. *Kidney international*, 2002, 61:S37-41.
[4] Kobayashi S, Dono K, Tanaka T, Takahara S, Isaka Y, Imai E, Nagano H, Tomoaki K, Umeshita K, Nakamori S, Sakon M. Gene transfer into the liver by plasmid injection into the portal vein combined with electroporation. *The journal of gene medicine*. 2003 Mar 1;5(3):201-8.
[5] Cemazar M, Jarm T, Sersa G. *Cancer electrogene therapy* with interleukin-12. Current gene therapy. 2010 Aug 1;10(4):300-11.
[6] C Heller L, Heller R. Electroporation gene therapy preclinical and clinical trials for melanoma. *Current gene therapy*. 2010 Aug 1;10(4):312-7.
[7] He H, Chang DC, Lee YK. Using a micro electroporation chip to determine the optimal physical parameters in the uptake of biomolecules in HeLa cells. *Bioelectrochemistry*. 2007 May 31;70(2):363-8.
[8] Wall K, MacGregor SJ, Anderson JG, Mackersie JW. Evidence of nuclear membrane damage in yeast cells treated with pulsed electric fields. *In Power Modulator Symposium*, 2004, pp. 623-626. IEEE.
[9] Katsuki S, Moreira K, Dobbs F, Joshi RP, Schoenbach KH. Bacterial decontamination with nanosecond pulsed electric fields. *In Power Modulator Symposium*, 2002, pp. 648-651. IEEE.
[10] Wu TF, Tseng SY, Su DS, Chen YM, Chen YK. Applications of soft-switching full-bridge converter and rotational electric field to transdermal drug delivery. *In Applied Power Electronics Conference and Exposition*, 2004, Vol. 2, pp. 919-925. IEEE.
[11] Baker RJ, Johnson BP. Stacking power MOSFETs for use in high speed instrumentation. *Review of scientific instruments*. 1992 Dec;63(12):5799-801.
[12] Adon MN. *Pulse electric field exposure effect on morphological properties of hela cells* (Doctoral dissertation, Universiti Tun Hussein Onn Malaysia).
[13] Li C, Yao C, Sun C, Guo F, Zhou W, Xiong Z. Dependence on electric field intensities of cell biological effects induced by microsecond pulsed electric fields. *IEEE Transactions on Dielectrics and Electrical Insulation*. 2011 Dec;18(6).
[14] Adon MN, Dalimin MN, Jamil MM, Kassim NM. Development of high voltage pulse inducement method for biological cell. *In Biomedical Engineering (ICoBE)*, 2012, pp. 501-503. IEEE.
[15] Zaltum MA, Adon MN, Hamdan S, Jamil MM. Feasibility study on MCF-7 cell membrane swelling properties induced by microsecond

pulsed electric field. *In Biomedical Engineering (ICoBE)*, 2015, pp. 1-3. IEEE.

[16] Mamman HB, Jamil MM. Investigation of electroporation effect on HT29 cell lines adhesion properties. *In Biomedical Engineering (ICoBE)*, 2015, pp. 1-4. IEEE.

[17] Pehlivanova VN, Tsoneva IH, Tzoneva RD. Multiple effects of electroporation on the adhesive behaviour of breast cancer cells and fibroblasts. *Cancer cell international*. 2012 Mar 22;12(1):9.

[18] Mouneimne Y, Barhoumi R, Myers T, Slogoff S, Nicolau C. Stable rightward shifts of the oxyhemoglobin dissocation curve induced by encapsulation of inositol hexaphosphate in red blood cells using electroporation. *FEBS letters*. 1990 Nov 26;275(1-2):117-20.

[19] Gehl J. Electroporation: theory and methods, perspectives for drug delivery, gene therapy and research. *Acta Physiologica*. 2003 Apr 1;177(4):437-47.

[20] Lin G, Yang R, Banie L, Wang G, Ning H, Li LC, Lue TF, Lin CS. Effects of transplantation of adipose tissue-derived stem cells on prostate tumor. The Prostate. 2010 Jul 1;70(10):1066-73.