Pulse electric field effect on cancer cell properties: Reversible electroporation study

Nur Adilah Abd Rahman¹, Muhammad Mahadi Abdul Jamil¹, Mohamad Nazib Adon¹ and Tengku Nadzlin Tengku Ibrahim²

¹Biomedical Modelling and Simulation Research Group, Faculty of Electrical and Electronics Engineering, Universiti Tun Hussein Onn Malaysia, Batu Pahat, Johor.
²Center for diploma studies, Universiti Tun Hussein Onn Malaysia, Hab Pendidikan Tinggi Pagoh, KM1, Jalan Panchor, Pagoh, Muar, Johor.
*drmuhammadmahadi@gmail.com

Abstract. Controlling cell function by using pulses of electrical fields have been used widely in chemotherapy application. The pulse electric field induced will create pores through the cell membrane and causes other substance around it to be absorbed into the cell. This method has led to a variety of medical applications, particularly in cell studies. In this preliminary, a range voltage of the pulse electric field used will be demonstrated. Theoretically, pulse electric field does give variety of effect on cancer cell. Which can either be a reversible electroporation or irreversible electroporation. The effect can either heal or kill the cells induced. Thus, will lead towards either cell cancer treatment factors plus the presence of electric field as the stimulator for aggressive adsorption of the anti-cancer agent into the cells or as enhancer towards wound healing applications. The outcome of this techniques will contribute towards understanding the cancer cell treatment factors which may lead towards a new method for cancer cell treatment or wound healing applications. This study will focus on applying pulse electric field on Breast cancer cell to investigate the effect of electroporation process. In addition, this study will discuss the effectiveness of low and high pulse electric field parameter, to look at anti-proliferation effect on the cancer cell towards cancer treatment applications.

1. Introduction

Electroporation or electro-permeabilization is a phenomenon where the lipid bilayer and proteins on cell membrane were momentarily disrupted and permeable to ions and macromolecules when it induced to short but high voltage field pulses [1–3]. There are two types of electroporation, which is reversible (temporal pores) and irreversible electroporation (permanent pores). Temporal open pores means that the cell survive for few microseconds after removing the electric field [8, 9]. While permanently open pores, can cause cell die due to lysis. Square wave electric field has shown to be effective in electroporation method to the cells [16]. It was introduced due to better control of electric field parameter due to the concept of capacitor discharge where the power switch is capable of fast switching and the voltage supply constantly charges the capacitor. The square wave pulse is produced by a partial discharge of a large capacitor, which requires the interruption of high currents against high voltages. Typically, voltage, pulse length, and number of pulses are all directly set on the instrument. Square wave pulses have well defined electric field amplitude, are both effective and also relatively mild to cells to yield higher viability. The membrane potential is the voltage between the external and internal surrounding of living cells. The typical value of this membrane potential is in the range of -30 to -90mV with respect to the exterior of the membrane [17].
In the cell membrane, there are protein channels, pores, and pumps present. The proteins are dependent on the transmembrane potential cause the closing and the opening of these channels. When an electric field induces, many protein channels that sensitive to voltage will open due to the lipid molecules change in orient resulted in creating hydrophilic pores [5]. The pores size could be varied dependent on time range from hundreds of microseconds to milliseconds and will stand through the duration of pulses, shorter pulse durations are thought to create much smaller pores that will allow ions but not large molecules to pass through [12]. But if those parameters exceed the optimal values it can lead to permanent pores (irreversible electroporation) occur on the cell membrane which can cause the cell to die because of lysis [10]. Electroporation is a simple technique that considered to be a non-invasive and nonchemical method [14]. Where this technique does not change the function of the target cell and the biological arrangement itself. The factors that effects the efficiency of this technique are electric field parameters, the chemical composition of media and cell characteristic. Among these factors, the most influential is electric field parameters which consist of amplitude, duration and number of the pulse. The optimal electric pulse parameters were selected based on the selected application [15]. There is an application that requires reversible such as foreign molecule insertion and some other application require irreversible electroporation such as tissue ablation. As for foreign molecule insertion, the parameter selection depends on the type of the molecule. As for small molecule, a repetitive of short pulses with a time duration in the range of micro to millisecond is sufficient. While for large molecule, longer pulses in the range of few millisecond or combination of higher voltage, short pulse duration and low voltage, the longer pulse duration is used. An opening pores, allowed transporting or introducing of therapeutic materials from small to large molecules [6]. Which can be applied in many medical applications. Current applications of this technique include transfer of genes and drug delivery into cells [11], food processing or bacterial decontamination in food technology, micro biotechnology and cancer treatment. It’s depends on the parameter applied used. As elaborated, Electroporation method are still in research and investigations on various field and study on electroporation method were performed, focusing on the parameter used for various applications as shown on table 1.

### Table 1. Previous study on electroporation technique with different parameter used

| References | Cell Type       | Electrical Parameter | Pulse Width | Pulse Number | Purpose                                      |
|------------|----------------|----------------------|-------------|--------------|----------------------------------------------|
| [19]       | Endothelial cells | 1000 V/cm            | 12.8 ms     | 1            | Endothelial cell seeding of prosthetic surfaces |
| [20]       | Liver (live rat)  | 50V                  | 50 ms       | 8            | Determine the optimum conditions for local gene therapy |
| [11]       | Jurkat cells     | ~100kV/cm (100V/cell diameter) | 60ns        | 1            | Monitor change in membrane potential in response to nsPEF |
| [12]       | HeLa             | 500 – 3kV/cm         | 30µs        | 1            | Wound Healing application                     |
| [21]       | HeLa             | 2kV/cm               | 30µs 600µs  | 1            | Wound healing applications and treatment applications |
| [22]       | MCF7             | 1008 V and 1280 V    | 20 µs       | 1            | Combination therapy for clinical applications |
| [18]       | HT29             | 600V/cm              | 500µs       | 1            | Wound healing application                     |
| [23]       | hippocampal neurons | 3 kV/cm (range: 1.9–4 kV/cm) | 200ns      | 1            | VGSC activation AP induction                  |
| [24]       | HeLa             | 2kV/cm               | 30µs        | 1            | Wound Applications Healing                   |
2. Literature Review

2.1. Breast cancer cell
Breast cancer is one of the most common cancers develop among female in Malaysia with the highest percentage of patient who died because of it with 52% death among Malaysian people [25]. Breast cancer cell developed from breast tissue with the sign symptom of change in breast shape, an existence of lump in the breast, fluid coming from the nipple, dimpling of skin or red scaly patch of skin. The risk factor is female, obesity, lack of physical activities, drinking alcohol, hormone replacement therapy during monopose, ionizing radiation, and early age at first menstruation, having child late or not at all, older age and family history. It commonly develop in cells from lining of milk ducts and the lobules that supply the ducts with milk. The provided treatment is surgery, radiation therapy, chemotherapy, hormonal therapy and targeted therapy. Breast cancer cell is a metastatic disease, it can spread beyond the original organ such as bone, liver, lung and brain.

2.2. Electroporation (EP)
Electroporation is a method that usually used to apply electric field onto the cells. This phenomenon causes the cell to permeable to ions and macromolecules when inducing with short high voltage pulses [26]. This is due to the voltage breakdown happen on the cell membrane which causes the lipid bilayer to be folded, this phenomenon causes an open pathway on the cell membrane which called an opening pore on the cell membrane. The opening pore on the cell membrane can be used for many applications such as introduction protein, large and small molecule and also cell fusion. A temporary open pore is called reversible electroporation. But if the voltage inducing is too high it can cause cell destruction, which called irreversible electroporation. The study presented in this paper focus on the reversible electroporation

2.2.1. Reversible Electroporation (RE)
Reversible electroporation is a temporal open pore in membranes and cell survival after the removal of the electric field. Reversible electroporation is primarily used for delivery of molecules into the cell. RE is often used to introduce substances into cells, such as dyes, drugs, protein and nucleic acids [27, 28]. Creating small pores in biological and artificial membranes can be done by applying an electric pulse of sufficient amplitude and duration. In the case of reversible, low-amplitude and short-duration pulses is sufficient to produce the pore on the cell membrane and the pores will close within milliseconds to minutes. Which safer in the inducement of the electrical field. This type of electroporation was chosen to see the cell response of breast cancer cell with the reversible range of electroporation method.

3. Material and Methods

3.1. Breast cancer cell (MCF7) culture
Breast cancer cell line is an immortal cell line that mostly used for scientific research. It is the oldest and most frequently used human cell line. The main advantage of this cell is as long as the basic cell survival conditions are met, it can be divided into an unlimited number of times. Therefore, breast cancer cell was used as the primary cell type. These breast cancer cell samples were acquired from the animal laboratory cell cultures (Kulliyyah of Allied Health Sciences, IIUM).

3.2. Experimental setup for electroporation method
The equipment used for electroporation system are consist of High Voltage Pulse Generator (ECM 830) and Nikon inverted microscope TS100 that connected with Dino Camera and Dino capture 2.0 Software. The cell were suspended inside the 4 mm gap size of cuvette and attach to the safety stand that connected to ECM 830. While the parameter that will be set into the pulse generator was 100 – 1000 V/cm (with 100V/cm interval) of electric field intensity, 30 μs of pulse duration and a single number of pulse.
4. Preliminary Result

4.1. Cell Culture Method

These are the preliminary result for this study. Where the Procedure of splitting cell were done. In order to do the splitting procedure, Cancer cells must be 80 – 90% of confluence in 25 cm² flask as shown in figure 2. In order to split the cells, first cancer cells were washed once with 5 ml of PBS and aspirated the PBS. The 2 ml of trypsin was added enough to cover the surface of the 25 cm² flask, in order to detach the cancer cells from the surface of the 25 cm² flask. The flask was sealed and incubated for 30 minutes. After the cells fully detached, the flask examined under a high-resolution microscope, a fully trypsinized cell appeared rounded and no longer attached to the surface of flask as shown in Figure 2. After the cells were trypsinized, 2 ml of RPMI media was added to neutralize the trypsin inside the flask. After that, 0.5 – 1 ml of harvested cells were taken using pipette and seeded into a new 25 cm² flask that contained 5 ml of new media RPMI + 10% FBS + 1% Pen/Strep. The flask were sealed and incubated until they reached 80 – 90% confluency or 5 – 6 days for the next splitting process.
Figure 3 shows how cancer cell grew in 36 hours. In order to maintain a controlled cells culture method, reagents protocol and sterile work area are important factor that influenced the growth rate of cancer cells. The same splitting technique was applied in every experimental in order to get the consistency of morphology and proliferation rates of cancer cells when exposed to high pulse electric field. These cells will be tested with the series of low electric field range which is 100 – 1000 V/cm and pulse duration of 10, 30 and 100 µs with single number of pulse. In order to look at the pulse electric field effect on the cell properties which is anti-proliferation, cell viability and cell length towards cancer treatment application.

4.2 Pulse Electric Effect on cell length

![Figure 4. Low range pulse electric field effect on cell length](image)

The preliminary result for this study as shown on the graph in figure 4. It show the cell length after induce with several low ranges of pulse electric field compared with the untreated cells. As shown above, it shows that cell well expands when induced with 100 and 200 V/cm with 81.34±1.36 mm and 80.47±1.15 mm and cell are shorter on cell length when inducing with 400 V/cm with a mean of 32.09±0.60 mm of cell length. This result showed an effect of pulsed electric field on the cancer cell. Which the result is shown and expansion of cell length after 48 hours incubated. The result showed a reversible electroporation effect where the cell survives after the pulse electric field inducement and continue to grow and shown a variance of cell length. The importance of investigating the cell length is due to the greater of cell length could contribute on the enhancement of cell to covering or fulfilling’s the wound area for the faster wound healing process [8].

5. Conclusion

In conclusion, Electroporation method is a convenient and non-invasive technique that does not require any chemical method in applying to the cells. It is a phenomenon where the cell permeable to any surrounding molecule due to an opening pore that causes by the voltage breakdown on the cell membrane. These happened due to the electric field effect causes the lipid molecule to change orientation and resulted in creating hydrophilic pores. The pulse electric field inducement influence the cell morphological changes dependent on what range used for the inducement. As explained before there is 2 type electroporation, reversible (temporary) and irreversible (permanently) electroporation which can be used either killing or healing the cell. This study will be more focused on the reversible electroporation which is temporal pores occur on cell membrane and cell survive for few microseconds after removing the electric field. The reasons for choosing reversible electroporation is due to the temporary open pore and the cells survive after the inducement. Thus, it may not harm the normal cell surround it and can be used to enhance the adsorption of the anti-cancer agent into the cancer cells without harming the other normal cell. The aim is to test the low range of pulse electric field which is starting from 100 – 1000 V/cm (with 100 V/cm interval) on breast cancer cells, monitor the cell responds and find the best parameter of pulse electric field inducement, which may enhance the anti-proliferative of the cancer cell. The effects of this range of pulse electric field will be monitored by examining proliferative properties and cell viability of the cells. Thus, the optimum
parameter of pulse electric field identified may have great implication for future biomedical applications such as cancer treatment or wound healing applications.

Acknowledgments
Authors wishing to acknowledge the Ministry of Education Malaysia for financial support of this work through Geran Penyelidikan Pascasiswazah (GPPS), VOT U949, Universiti Tun Hussein Onn Malaysia.

References
[1] Andreas Blicher 2011 Electrical aspects of lipid membranes (University of Copenhagen)
[2] Abd Rahman N A, Buhari M H and Jamil M A 2015 An Overview: Investigation Of Electroporation Technique On Cell Properties Cultured On Micropatterned Surface J. Teknol. 6 61–5
[3] Abd Rahman N A 2017 Fundamental study of pulse electric field effects on HeLa cell cultured over extracellular matrix protein micropatterned surface (Universiti Tun Hussein Onn Malaysia)
[4] Cooper G M 2000 Cell Membranes The Cell: A Molecular Approach (Sunderland: Sinauer Associates)
[5] Tarek M 2005 Membrane electroporation: a molecular dynamics simulation. Biophys. J. 88 4045–53
[6] Casciola M and Tarek M 2016 A molecular insight into the electro-transfer of small molecules through electropores driven by electric fields Biochim. Biophys. Acta. - Biomembr. 1858 2278–89
[7] Buchmann L, Frey W, Gusbeth C, Ravaynia P S and Mathys A 2019 Effect of nanosecond pulsed electric field treatment on cell proliferation of microalgae Bioresour. Technol. 271 402–8
[8] Subhra Santra T, Wang P-C and Gang Tseng F 2013 Electroporation Based Drug Delivery and Its Applications
[9] Jiang C, Davalos R V. and Bischof J C 2015 A review of basic to clinical studies of irreversible electroporation therapy IEEE Trans. Biomed. Eng. 62 4–20.
[10] Tarek M 2005 Membrane Electroporation: A Molecular Dynamics Simulation Biophys. J. 88 4045–53.
[11] Frey W, White J A, Price R O, Blackmore P F, Joshi R P, Nuccitelli R, Beebe S J, Schoenbach K H and Kolb J F 2006 Plasma membrane voltage changes during nanosecond pulsed electric field exposure Biophys. J. 90 3608–15
[12] Adon M N 2015 Pulse Electric Field Exposure Effect on Morphological (Universiti Tun Hussein Onn Malaysia)
[13] Warindi, Hadi S P, Berahim H, Suharyanto, Raz-prag D, et. al. 2017 Effect of cell electroporation on the conductivity of a cell suspension Biophys. J. 1828 14012
[14] Meacham J M, Durvasula K, Levent Degertekin F and Fedorov A G 2014 Physical Methods for Intracellular Delivery: Practical Aspects from Laboratory Use to Industrial-Scale Processing HHS Public Access J Lab Autom 19 1–18
[15] Sadiq A A, Zaltum M A M, Mamman H B, Adon M N, Othman N B, Dalimin M N and Jamil M M A 2015 An overview: Investigation of electroporation and sonoporation techniques Proc. - 2015 2nd Int. Conf. Biomed. Eng. ICoBE 2015
[16] Jordan D W, Gilgenbach R M, Uhler M D, Gates L H and Lau Y Y 2004 Effect of pulsed, high-power radiofrequency radiation on electroporation of mammalian cells IEEE Trans. Plasma Sci. 32 1573–8
[17] Hanna H, Denzi A, Liberti M, André F M and Mir L M 2017 Electropenetrabilization of Inner and Outer Cell Membranes with Microsecond Pulsed Electric Fields: Quantitative Study with Calcium Ions Sci. Rep. 7
[18] Buhari M H 2017 Optimization of low amplitude pulse electric field exposure on colon cancer cell for wound healing application (Universiti Tun Hussein Onn Malaysia)
[19] Kotnis R A, Thompson M M, Eady S L, Budd J S, James R F L and Bell P R F 1995 Attachment, replication and thrombogenicity of genetically modified endothelial cells Eur. J. Vasc. Endovasc. Surg. 9 335–40
[20] Suzuki T, Shin B C, Fujikura K, Matsuzaki T and Takata K 1998 Direct gene transfer into rat liver cells by in vivo electroporation FEBS Lett. 425 436–40
[21] Milad Zaltum M A, Adon M N, Hamdan S, Dalimin M N and Abdul Jamil M M 2015 Investigation a critical selection of pulse duration effect on growth rate of HeLa cells 2015 Int. Conf. BioSignal Anal. Process. Syst. ICBAPS 2015 33–6
[22] Sree V G, Muthuraman C and Sundararajan R 2015 Anti-proliferation control of breast cancer cells using electric pulses Proc. IEEE Int. Conf. Prop. Appl. Dielectr. Mater. 2015-Octob 160–3
[23] Pakhomov A G, Semenov I, Casciola M and Xiao S 2017 Neuronal excitation and permeabilization by 200-ns pulsed electric field: An optical membrane potential study with FluoVolt dye Biochim. Biophys. Acta - Biomembr. 1859 1273–81
[24] Rahman N A A and Jamil M M A 2017 Investigation of pulsed electric field on cancer cell cultured on patterned surface Proc. - 6th IEEE Int. Conf. Control Syst. Comput. Eng. ICCSCE 2016 25–7
[25] Yong Chee Meng 2018 The latest treatments for advanced ovarian cancer Star2 Health
[26] Neumann E, Sowers A E and Jordan C A 1989 Electroporation and Electrofusion in Cell Biology (Boston, MA: Springer US)
[27] Garcia P A, Rossmeisl J H and Davalos R V. 2011 Electrical conductivity changes during irreversible electroporation treatment of brain cancer 2011 Annual International Conference of the IEEE Engineering in Medicine and Biology Society (IEEE) pp 739–42
[28] Sundararajan R, Salameh T, Camarillo I and Campana L 2011 Effective use of electrical pulses on cancer cells to control proliferation 2011 - 14th International Symposium on Electrets (IEEE) pp 231–2.