Original Article

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The Effects of Microseconds Electroporation on Pore Size, Viability and Mitochondrial Membrane Potential of Cervical Cancer Cells

Mikrosaniye Elektroporasyonun Rahim Ağzı Kanseri Hücrelerinin Gözenek Boyutu, Canlılığı ve Mitokondri Membran Potansiyeli Üzerine Etkileri

Güney GÜRSOY[1], Meriç Arda ESMEKAYA[2], Zehra ÇİÇEK[3]

[1]Kırşehir Ahi Evran University Faculty of Medicine, Department of Biophysics, Kırşehir, Türkiye
[2]Gazi University Faculty of Medicine, Department of Biophysics, Ankara, Türkiye
[3]University of Health Sciences Türkiye, Gülhane Faculty of Medicine, Department of Physiology, Ankara, Türkiye

ABSTRACT

Objective: Electroporation (EP) is a method in which the membrane permeability is increased by applying electrical pulses. The determination of modifications that occur in cells subsequent to EP with varying pulse parameters holds significant importance in establishing the foundations of EP theory. Therefore, we sought clarification regarding the phenomenon of pore formation on the membrane of the electroporated human cervical cancer cell line (HeLa) cells.

Methods: The pores created on the cell membrane due to EP was observed using a scanning electron microscope. The change in the viability and mitochondrial membrane potential (ΔΨm) of cells was determined by WST-8 and JC-1 assays.

Results: The surface of the electroporated cell membrane exhibited a relatively uniform pore population. The viability of HeLa cells was significantly reduced with increasing electric field intensities. A slight decrease in ΔΨm was observed between the control and the 0.8 and 1.6 kV/cm EP groups, but ΔΨm was higher in the 2.4 and 3.2 kV/cm EP groups compared to the control group.

Conclusion: In conclusion, our study showed that the application of EP to the cervical cancer cell line resulted in the formation of pores of varying sizes on the membrane. While cell viability decreased with increasing electric field amplitude, no significant

ÖZ

Amaç: Elektroporasyon (EP), elektrik darbeleri uygulanarak membran geçirgenliğinin artırıldığı bir yöntemdir. EP sonrası hücrelerde değişen nabız parametreleriyle meydana gelen değişikliklerin belirlenmesi, EP teorisinin temellerinin oluşturulmasında büyük önem taşımaktadır. Bu nedenle, EP'ye tabi tutulan insan rahim ağzı kanseri hücre dizisi (HeLa) hücrelerinin zarı üzerinde gözenek oluşumu olgusuna ilişkin açıklama aradık.

Yöntemler: EP'ye bağlı olarak hücre zarında oluşan gözenekler taramalı elektron mikroskobu kullanılarak gözlemlendi. Hücrelerin canlılığı ve mitokondriyal membran potansiyelindeki (ΔΨm) değişiklik, WST-8 ve JC-1 analizleri ile belirlendi.

Bulgular: EP'ye tabi tutulmuş hücre zarının yüzeyi nispeten tekdüze bir gözenek popülasyonu sergiledi. HeLa hücrelerinin canlılığı, artan elektrik alan yoğunluklarıyla önemli ölçüde azaldı. Kontrol grubu ile 0,8 ve 1,6 kV/cm EP grupları arasında ΔΨm'de hafif bir azalma gözlendi ancak 2,4 ve 3,2 kV/cm EP gruplarında ΔΨm kontrol grubuna göre daha yüksekti.

Sonuç: Sonuç olarak çalışmamız, EP'nin rahim ağzı kanseri hücre hattına uygulanmasının, membran üzerinde değişen boyutlarda gözeneklerin oluşmasına neden olduğunu gösterdi. Elektrik alan genliğinin artmasıyla hücre canlılığı azalırken, EP tedavisi ve kontrol grupları arasında ΔΨm'de anlamlı bir değişiklik gözlenmedi. EP'ye

Address for Correspondence: Güney Gürsoy, Kırşehir Ahi Evran University Faculty of Medicine, Department of Biophysics, Kırşehir, Türkiye
E-mail: gursoy.guney@gmail.com **ORCID ID:** orcid.org/0000-0003-1849-9068

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ABSTRACT

change was observed in ΔΨm between EP treatment and control groups. It should be noted that further research is needed to determine the pore distributions in electroporated cells and the resulting changes at different electric field amplitudes.

Keywords: Electroporation, cervical cancer, pore size, cell viability, mitochondrial membrane potential

ÖZ

tabi tutulmuş hücrelerdeki gözenek dağılımlarını ve farklı elektrik alanı genliklerinde ortaya çıkan değişiklikleri belirlemek için daha fazla araştırmaya ihtiyaç olduğu unutulmamalıdır.

Anahtar Sözcükler: Elektroporasyon, rahim ağzı kanseri, gözenek boyutu, hücre canlılığı, mitokondri membran potansiyeli

Introduction

The membrane permeability is risen by the application of electrical field pulses of appropriate amplitude and parameters. This method is referred to as "electropermeabilization" or "electroporation" (EP). With EP application, particles that cannot cross the membrane under normal cell conditions are allowed to pass through the membrane. Intense and short-term electrical pulses result in an rise in the transmembrane potential (TMP) on the cell membrane (1-5). When the TMP reaches a critical value, the formation of aqueous pores will allow molecular transitions through the membrane. Despite the fact that a precise mechanism at the molecular level cannot be fully expressed, molecular flow has been demonstrated in the membrane regions where the highest TMP is observed (6-8). The effectiveness of EP depends on the applied electrical pulse parameters (duration, intensity pulse shape and pulse number). Based on the impact of these parameters, EP can be reversible or irreversible (9-11). Reversible EP has many applications in the fields of medicine and biotechnology including electrogenotherapy and electrochemotherapy (ECT) (5,12). Irreversible EP is utilized for tumor ablation (due to its non-thermal effect) and sterilization purposes (11-13).

The amount of molecules penetrating into the cell by EP is related to the size of the pores formed on the membrane. Hence, it is very important to determine the size of pores created on cell membrane owing to application of short-term and high-intensity electrical pulses to enhance the efficacy of EP. There are several theoretical and experimental studies on the pore formation kinetics and reclosing in the literature. Different pore sizes and resealing times have been reported depending on different cell lines and EP parameters in these studies. Furthermore, there are a limited number of studies that demonstrate the presence of electropores (14-19).

The cells frequently adapt to physiological requirements in order to maintain their vitality and internal balance. Cell injury is defined as the adverse effects of internal or external stimuli that are severe enough to disrupt cell homeostasis or where the cell is unable to adjust without being damaged. As a consequence of cell injury, the cell may or may not be repaired and the cell death may occur. The primary causes of cell damage include damage to membranes, DNA, proteins, and mitochondria, as well as an upraised amount of intracellular calcium and reactive oxygen species (ROS) (20-22).

The application of EP may result in lipid peroxidation and damages to proteins embedded within the cell membrane. Therefore, the formation of pores due to EP is a cell injury (23-25). The main cause of cell death after EP remains unclear, as it overlaps with many types of cell damage and cell death. The excessive entry of Ca^{2+} into the cell may result in cell death due to EP. Ca^{2+} regulates many cellular mechanisms including cell proliferation, cell death and cell cycle. The pulsed electric fields (PEFs) may also trigger oxidative stress and initiate the production of ROS. Furthermore, EP administration may cause damage to mitochondria, which are known to play an important role in apoptosis. The causes of cell death after EP are closely related to each other. For this reason, it is quite complex to determine which death stimulus is the effect or result of the pathological condition that occurs after EP (26-30). Mitochondria have an important role in the intrinsic death pathways. It may be beneficial to understand the cellular mechanisms of action of EP by determining the change in mitochondrial membrane potential (ΔΨm) due to the application of PEF, EP may cause a loss of ΔΨm.

Chemotherapy is a prevalent treatment option for cervical cancer. The administration of substantial doses of chemotherapeutic agents utilized in treatment can nevertheless result in significant side effects. Research has shown that ECT is an influential treatment method for certain types of cancer (12,31) providing low doses and a significant decrease in side effects. Nevertheless, the lack of a study examining the formation of pores on electroporated human cervical cancer cell line (HeLa) cell membrane, suggests a deficiency in this field. We aimed to investigate the effects of EP application on the cell viability and ΔΨm in HeLa cells and to determine the size of electropores created on the surface of the HeLa cell membrane after EP application.

Methods

Cell Culture and Electroporation Protocol

The human cervical cancer cell line HeLa was used in our study. The cells were incubated with culture medium at 5% CO_2 and 37 °C [cell culture medium; (Dulbecco's Modified Eagle's medium (DMEM), Capricorn DMEM-HA) containing 10% fetal bovine serum (Capricorn FBS-11A) and 1% penicillin/streptomycin antibiotic (PSA, HyClone)]. 10^5 cells at 100 µL were incorporated into EP cuvettes with a gap of 4 mm and subsequently placed in the BTX pulse generator chamber. Then, increasing electric field amplitudes of 0.8 and 3.2 kV/cm at 100 µs pulse width, eight square waves and 1 Hz repetition frequency were applied to each EP cuvette. After each application, the cells were kept in the room environment for approximately 10

minutes. Then, ten thousand cells per 100 µL were seeded into 96-well plates and incubated for 24 hours. Control cells were placed in the EP chamber in the bathtub but no EP treatment was made.

Scanning Electron Microscope and the Distribution of Pore Size

The pores created on the plasma membrane of HeLa cells at an electric field strength of 1.6 kV/cm were observed by scanning electron microscope (SEM). The electric field strength of 1.6 kV/cm was found to be the optimal electric field strength with minimal cell death and maximum membrane permeability for HeLa ECT (32). SEM is a highly effective technique for obtaining both quantitative and qualitative data regarding porous structures. It is commonly used for the determination of the average size of pores and their distributions. In order to prevent the closure of pores, cells were placed on ice for a duration of 10 minutes subsequent to the application of EP prior to undergoing SEM observations (14,15). Cells were washed with PBS solution three times and fixed with 2.5% glutaraldehyde (Capricorn PBS-1A) After that, the samples were rinsed with PBS and the glutaraldehyde was removed. This was followed by the dehydration of samples with increasing concentrations of ethanol from 30% to 100%. After dehydration, the samples were dried and subsequently photographed using a high resolution SEM at various positions with a suitable magnification (x100.000-x200.000). The measurements of the size of pores created by electrical pulses were determined from SEM images using ImageJ, which is unable to provide an accurate analysis of scientific images. We analyzed 10 SEM micrographs and measured pore size at randomly selected locations in each SEM micrograph. The size distribution of pores created by electrical pulses was determined by measuring the radius of 250 pores on the entire cell membrane.

The Measurement of Cell Viability and ΔΨm

Cell viability was measured 24 hours after EP treatment using the WST-8 assay. 10 µL of cell proliferation reagent WST-8 (ABP Biosciences) was added to each well, followed by a three-hour incubation. After a period of waiting, the absorbance values at the appropriate wavelength (450 nm) were determined using an ELISA reader. The cell viability results were presented as a percentage relative to the control cells. ΔΨm of the cells was measured JC-1 (ABP Biosciences) assay 24 hours after EP. After incubation, the cell culture medium was aspirated, 200 µL of PBS and 2 µL of JC-1 stock solution was added to each well (Capricorn PBS-1A). After half an hour of incubation in the incubator, the well was washed twice with PBS and the same amount of PBS was added each well. The ratio of red to green was determined by measuring the green and red fluorescence values with a spectrofluorometric plate reader.

Statistical Analysis

All experiments were performed in triplicate and data are presented as mean standard deviation. Control comparisons were made using One-Way ANOVA test and Tukey test was used as post-hoc. $P<0.01$ was considered statistically significant. GraphPad Prism 9 program was used for statistical analysis.

Results

The surface of the electroporated membrane was observed by means of SEM. High intensity µs EP induced nanopores on HeLa cell membrane (Figure 1). The surface of the plasma membrane of the electroporated HeLa cells showed relatively homogeneous pore populations. On the non-electroporated control HeLa cells, no pore-like structures were observed. The surface of the non-electroporated cell membrane exhibited a rougher surface than

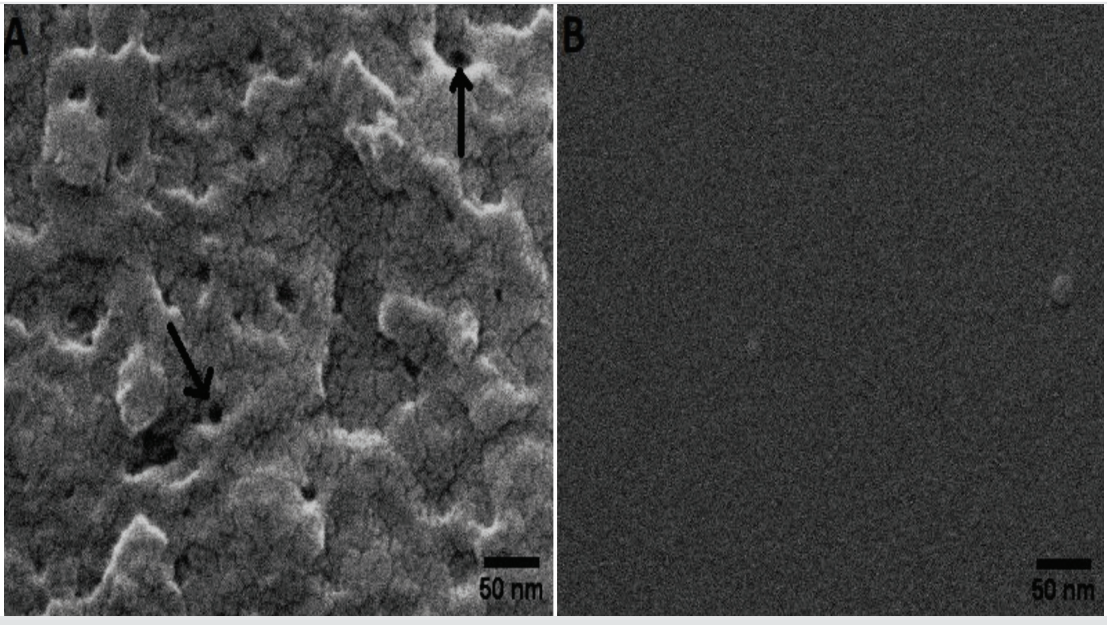

Figure 1. SEM images of A) electroporated and B) non-electroporated HeLa cell membrane, the black arrows indicate pores
SEM: Scanning electron microscope, HeLa: Human cervical cancer cell line

that of the electroporated cell membrane. The mean radius of electropores created on the electroporated HeLa cell membrane was 9.23 nm (±2.8 nm) (Figure 2). The mean pore size observed in this study was large enough to enable the passage of many chemotherapeutic agents through the cell membrane.

The viability of HeLa cells for four different electric field strengths having repetition frequency of 1 Hz, an eight square waves and pulse duration of 100 μs parameters which are used in classical ECT treatment was given in Figure 3. As shown in figure, a slight reduce in cell viability was observed at the electric field intensity of 0.8 kV/cm, even so, it was not statistically significant ($p>0.05$). The change in cell viability at 1.6 kV/cm of electric field strength was statistically significant when compared to the control group. We found that the cell viability was 27.09% at 3.2 kV/cm, which we selected as the highest field strength ($p<0.0001$). Figure 4 depicts the morphological modifications of the cells in response to four distinct field strengths applied under an inverted microscope. The live cells had transparent, light-colored, and clearly defined membranes. At high electric field strength levels (2.4 and 3.2 kV/cm), the cell shape was distorted. The electric field amplitude of 1.6 kV/cm was the critical amplitude for cell viability. The viability of cells was significantly reduced above this electric field amplitude due to EP application.

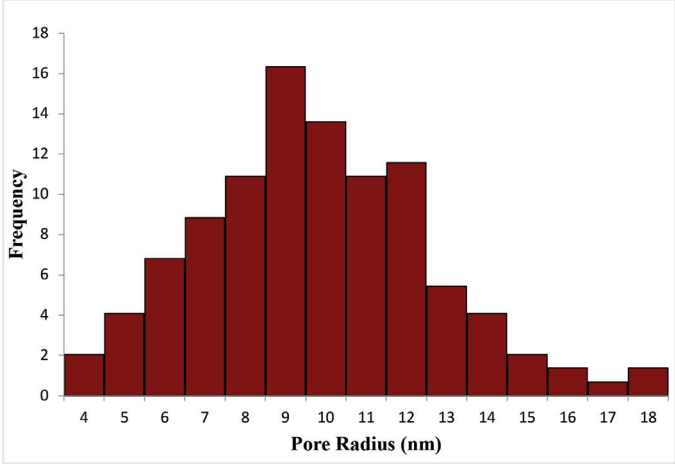

Figure 2. Pore size distribution histogram of electropores created on HeLa cell membrane

HeLa: Human cervical cancer cell line

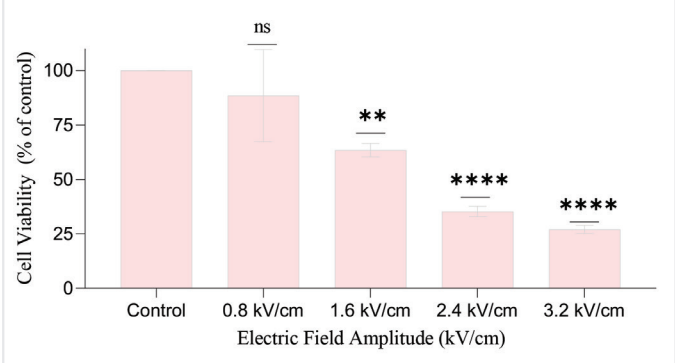

Figure 3. Cell viability in cells at increasing electric field amplitudes (0-3.2 kV/cm)

The change in ΔΨm, which occurs at these intensities by changing only the electric field intensities from the pulse parameters, was depicted in Figure 5. The slight depolarization tendency was determined in ΔΨm in EP applications with an electric field amplitude between 0.8 and 1.6 kV/cm ($p>0.05$). When we further increased the electric field amplitude, the change in ΔΨm tended to increase, but this increase was not statistically significant compared to the control group ($p>0.05$). At low electric field intensities, a slight depolarization tendency was observed in the ΔΨm. However, fluctuating changes occurred at high electric field amplitudes.

Discussion

EP treatment has the ability to induce various types of cell death, inclusive of apoptosis and necrosis. This circumstance is closely associated with the cell type application conditions and EP parameters employed in the study (23,33,34). In this study, we examined the impact of electrical pulses having amplitudes of 0.8, 1.6, 2.4, and 3.2 kV/cm, a pulse width 100 μs, an eight square waves and a repetition frequency of 1 Hz on the viability and ΔΨm of HeLa cells. The EP pulse parameters; 100 μs pulse width, eight square waves and 1 Hz repetition frequency are commonly employed in ECT applications. The viability of HeLa cells decreased with the increase in the applied electric field amplitude. The cell viability was found to be 88.5% and 63.49%, respectively, at electric field intensities of 0.8 and 1.6 kV/cm. These field intensities were among the most efficient values for ECT applications. The application of EP had a greater damaging effect on HeLa cells at higher electric field amplitudes. The cell viability was significantly decreased to 35.35% (compared to control cells) at a field strength of 2.4 kV/cm.

Zhou et al. (35) studied the effects of EP pulse amplitude (0-1000 V) with a pulse duration of 100 s and a repetition frequency of 1 Hz on HeLa cells. They found that 400 V was the threshold pulse amplitude level for reversible EP, and cell viability was decreased with an increase in pulse amplitude. Miller et al. (36) demonstrated that besides the thermal effects of an IRE application, whole-cell ablation could occur in different electrical parameters. Another study investigated the reversible and irreversible EP parameters of HepG2 cells. This study revealed that the IRE electric field amplitude was approximately 4 kV/cm for almost all types of cell death. Furthermore, it was found that the maximum electric field amplitude for reversible EP was 1 kV/cm (37). Saczko and colleagues conducted an ECT study on OvBH-1 and SKOV-3 cells, and reported that EP was safe up to 2 kV/cm (38).

In this study, we examined the change in ΔΨm resulting from EP application for four distinct electric field amplitudes. A slight decrease in ΔΨm was observed between the control and the 0.8 and 1.6 kV/cm EP groups, but ΔΨm was higher in the 2.4 and 3.2 kV/cm EP groups compared to the control group. Beebe et al. (26) reported an enhancement in cell

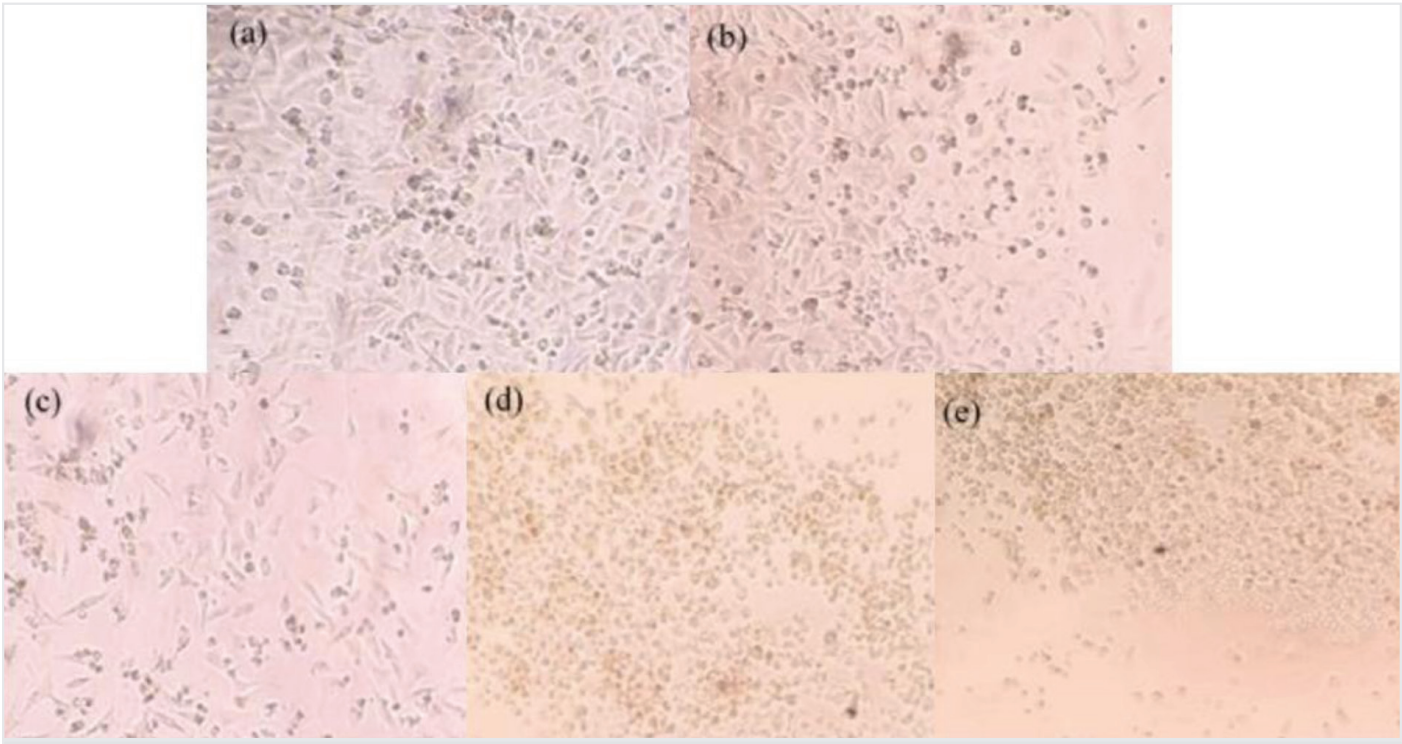

Figure 4. Invert microscope images of EP applications at x100 magnification in HeLa cell line, (a) control, (b) 0.8 kV/cm, (c) 1.6 kV/cm, (d) 2.4 kV/cm, (e) 3.2 kV/cm. It has seen that the number of HeLa cells decreases and cell morphologies are distrupted as the electric field value increases

EP: Electroporation, HeLa: Human cervical cancer cell line

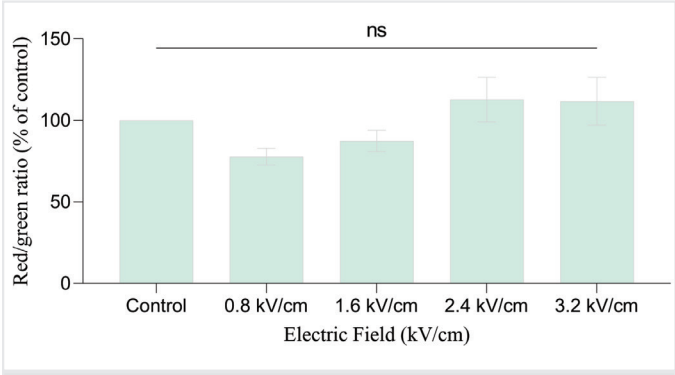

Figure 5. Mitochondrial membrane potential change at increasing electric field amplitudes in HeLa cells (0-3.2 kV/cm)

HeLa: Human cervical cancer cell line

viability and distruption of ΔΨm following the application of a nanosecond PEF (nsPEF) for varying exposure periods in N1-S1 hepatocellular carcinoma cell line. They observed that the primary trigger for cell death was the loss of ΔΨm resulting from the application of EP. Another study performed in human T lymphocyte cells reported decreased ΔΨm and increased mitochondrial membrane permeability following to application of nsPEF (39). In their study on the SMMC-7721 cell line, Mi et al. (30) found a gradual decrease in ΔΨm depending on the voltage amplitude and pulse duration. They found that higher field strengths and longer exposure periods were more effective. The study performed on HepG2 cell line demonstrated that the application of prolonged electrical pulses with an electric field amplitude of 10 kV/cm, a pulse duration of 500 ns, and a repetition frequency of 1 Hz induced apoptosis (40). The study also reported an increase in intracellular Ca^{2+} level and loss of ΔΨm. Gibot et al. (41) applied EP in combination with Ca^{2+} to human dermal fibroblasts and HCT-116 line and showed that the combined application of EP in combination with Ca^{2+} induced cell death without induction of genotoxicity. They concluded that the cytotoxicity resulted from the application of EP in combination with Ca^{2+} was associated with a dramatic decrease in ΔΨm and ATP depletion (41). Until now, it has been demonstrated that a reduction in ΔΨm leads to apoptotic cell death. However, recent scientific studies have demonstrated that an increase in m may be responsible for apoptosis (42-44). Furthermore, excess levels of ROS, ATP and Ca^{2+} may affect mitochondrial activity. All of these factors make up a complex network capable of influencing each other. This intricate sequence renders the explanation of mitochondrial dysfunction arduous (29).

We observed a pore size distribution ranging from 4 to 18 nm, and the most common pore size was around 9 nm in this study. There are a limited number of studies that have examined the impact of EP on pore size. This is the reason we conducted this study. An electrical pulse EP of 40 kV/m for a duration of one microsecond caused the formation of electropores with an

average radius of 22 nm (15). The study conducted by Chang and Reese (14) revealed the existence of a non-homogeneous pore distribution on the membrane of human red blood cells. Some other authors also reported different average pore sizes depending on pulse parameters such as amplitude and duration of electrical pulses used in the studies (15,45-50).

Study Limitations

In this study, the distribution of pore sizes formed in the membrane by EP application in HeLa cells was examined. In addition, in our study, changes in cell viability and mitochondria membrane potential were demonstrated with EP application. Examining pore formation is a very difficult and complex process. Therefore, the limitation of the study was that the pore formation was examined at a single electric field value. This study lays an important foundation for examining membrane pore formation in cervical cancer cells by EP. We think that examining cellular changes in different EP parameters will allow more comprehensive findings to be obtained.

Conclusion

In summary, it was demonstrated that the application of EP to the cervical cancer cell line resulted in the formation of pores of varying sizes on the membrane. It was observed that increasing the electric field amplitude led to a significant decrease in cell viability after a critical value, but no significant change in $\Delta\Psi m$ was observed between EP treatment and control groups.

Ethics

Ethics Committee Approval: Ethics committee approval is not required.

Informed Consent: Informed consent is not required.

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Footnotes

Authorship Contributions

Design: G.G., M.A.E., Data Collection or Processing: G.G., M.A.E., Z.Ç., Analysis or Interpretation: G.G., M.A.E., Z.Ç., Literature Search: G.G., M.A.E., Z.Ç., Writing: G.G., M.A.E.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Weaver JC. Electroporation theory. Concepts and mechanisms. Methods Mol Biol. 1995;55:3-28.
2. Gehl J. Electroporation: theory and methods, perspectives for drug delivery, gene therapy and research. Acta Physiol Scand. 2003;177:437-47.
3. Tryfona T, Bustard MT. Enhancement of biomolecule transport by electroporation: a review of theory and practical application to transformation of Corynebacterium glutamicum. Biotechnol Bioeng. 2006;93:413-23.
4. Chang DC. Electroporation and Electrofusion. 2006.
5. Jaroszeski MJ, Dang V, Pottinger C, Hickey J, Gilbert R, Heller R. Toxicity of anticancer agents mediated by electroporation in vitro. Anticancer Drugs. 2000;11:201-8.
6. Gimsa J, Marszalek P, Loewe U, Tsong TY. Electroporation in rotating electric fields. Bioelectrochemistry and Bioenergetics. 1992;29:81-9.
7. Gehl J, Skovsgaard T, Mir LM. Enhancement of cytotoxicity by electropermeabilization: an improved method for screening drugs. Anticancer Drugs. 1998;9:319-25.
8. Zheng QA, Chang DC. High-efficiency gene transfection by in situ electroporation of cultured cells. Biochim Biophys Acta. 1991;1088:104-10.
9. Yarmush ML, Golberg A, Serša G, Kotnik T, Miklavčič D. Electroporation-based technologies for medicine: principles, applications, and challenges. Annu Rev Biomed Eng. 2014;16:295-320.
10. Kanduser M, Miklavcic D. Electroporation in Biological Cell and Tissue: An Overview. New York, NY: Springer; 2009.
11. Teissié J, Eynard N, Gabriel B, Rols MP. Electropermeabilization of cell membranes. Adv Drug Deliv Rev. 1999;35:3-19.
12. Heller R, Gilbert R, Jaroszeski MJ. Clinical applications of electrochemotherapy. Adv Drug Deliv Rev. 1999;35:119-29.
13. Chen X, Ren Z, Zhu T, Zhang X, Peng Z, Xie H, et al. Electric ablation with irreversible electroporation (IRE) in vital hepatic structures and follow-up investigation. Sci Rep. 2015;5:16233.
14. Chang DC, Reese TS. Changes in membrane structure induced by electroporation as revealed by rapid-freezing electron microscopy. Biophys J. 1990;58:1-12.
15. Krassowska W, Filev PD. Modeling electroporation in a single cell. Biophys J. 2007;92:404-17.
16. Saulis G. Cell electroporation: estimation of the number of pores and their sizes. Biomed Sci Instrum. 1999;35:291-6.
17. Rols MP, Teissié J. Electropermeabilization of mammalian cells to macromolecules: control by pulse duration. Biophys J. 1998;75:1415-23.
18. Sowers AE, Lieber MR. Electropore diameters, lifetimes, numbers, and locations in individual erythrocyte ghosts. FEBS Lett. 1986;205:179-84.
19. Chiapperino MA, Bia P, Lamacchia CM, Mescia L. Electroporation modelling of irregular nucleated cells including pore radius dynamics. Electronics. 2019;8:1477.
20. Kumar V, Abbas AK, Aster JC, Deyrup AT. Robbins basic pathology. 10th ed. Philadelphia, Pennsylvania: Elsevier; 2018:935.
21. Zhivotovsky B, Orrenius S. Calcium and cell death mechanisms: a perspective from the cell death community. Cell Calcium. 2011;50:211-21.
22. Lemasters JJ. Chapter 1 - Molecular Mechanisms of Cell Death, in Molecular Pathology. 2nd. In: Coleman WB, Tsongalis GJ, editors. Academic Press; 2018.p.1-24.

23. Batista Napotnik T, Polajžer T, Miklavčič D. Cell death due to electroporation - A review. Bioelectrochemistry. 2021;141:107871.
24. Chen W, Zhongsheng Z, Lee RC. Supramembrane potential-induced electroconformational changes in sodium channel proteins: a potential mechanism involved in electric injury. Burns. 2006;32:52-9.
25. Maccarrone M, Bladergroen MR, Rosato N, Finazzi Agrò AF. Role of lipid peroxidation in electroporation-induced cell permeability. Biochem Biophys Res Commun. 1995;209:417-25.
26. Beebe SJ, Chen YJ, Sain NM, Schoenbach KH, Xiao S. Transient features in nanosecond pulsed electric fields differentially modulate mitochondria and viability. PLoS One. 2012;7:e51349.
27. Batista Napotnik T, Wu YH, Gundersen MA, Miklavčič D, Vernier PT. Nanosecond electric pulses cause mitochondrial membrane permeabilization in Jurkat cells. Bioelectromagnetics. 2012;33:257-64.
28. Brini M, Carafoli E. Calcium signalling: a historical account, recent developments and future perspectives. Cell Mol Life Sci. 2000;57:354-70.
29. Brookes PS, Yoon Y, Robotham JL, Anders MW, Sheu SS. Calcium, ATP, and ROS: a mitochondrial love-hate triangle. Am J Physiol Cell Physiol. 2004;287:817-33.
30. Mi Y, Sun C, Yao C, Li C, Mo D, Tang L, al. Effects of steep pulsed electric fields (SPEF) on mitochondrial transmembrane potential of human liver cancer cell. Annu Int Conf IEEE Eng Med Biol Soc. 2007;2007:5815-8.
31. Jaroszeski MJ, Heller R, Gilbert R. Electrochemotherapy, Electrogenetherapy, and Transdermal Drug Delivery. 2000.
32. Gursoy G, Esmekaya MA, Cicek Z. Treatment of cervical cancer by electrochemotherapy with bleomycin, cisplatin, and calcium: an in vitro experimental study. Med Oncol. 2022;40:52.
33. Faroja M, Ahmed M, Appelbaum L, Ben-David E, Moussa M, Sosna J, Nissenbaum I, Goldberg SN. Irreversible electroporation ablation: is all the damage nonthermal? Radiology. 2013;266:462-70.
34. Kim HB, Sung CK, Baik KY, Moon KW, Kim HS, Yi JH, et al. Changes of apoptosis in tumor tissues with time after irreversible electroporation. Biochem Biophys Res Commun. 2013;435:651-6.
35. Zhou W, Xiong Z, Liu Y, Yao C, Li C. Low voltage irreversible electroporation induced apoptosis in HeLa cells. J Cancer Res Ther. 2012;8:80-5.
36. Miller L, Leor J, Rubinsky B. Cancer cells ablation with irreversible electroporation. Technol Cancer Res Treat. 2005;4:699-705.
37. Lindelauf KHK, Baragona M, Baumann M, Maessen RTH, Ritter A. Pulse Parameters and Thresholds for (ir)Reversible Electroporation on Hepatocellular Carcinoma Cells *in Vitro*. Technol Cancer Res Treat. 2023;22:15330338221136694.
38. Saczko J, Kamińska I, Kotulska M, Bar J, Choromańska A, Rembiałkowska N, et al. Combination of therapy with 5-fluorouracil and cisplatin with electroporation in human ovarian carcinoma model in vitro. Biomed Pharmacother. 2014;68:573-80.
39. Beebe SJ, Fox PM, Rec LJ, Willis EL, Schoenbach KH. Nanosecond, high-intensity pulsed electric fields induce apoptosis in human cells. FASEB J. 2003;17:1493-5.
40. Xiao D, Yao C, Liu H, Li C, Cheng J, Guo F, et al. Irreversible electroporation and apoptosis in human liver cancer cells induced by nanosecond electric pulses. Bioelectromagnetics. 2013;34:512-20.
41. Gibot L, Montigny A, Baaziz H, Fourquaux I, Audebert M, Rols MP. Calcium Delivery by electroporation induces in vitro cell death through mitochondrial dysfunction without DNA damages. Cancers (Basel). 2020;12:425.
42. Kadenbach B, Arnold S, Lee I, Hüttemann M. The possible role of cytochrome c oxidase in stress-induced apoptosis and degenerative diseases. Biochim Biophys Acta. 2004;1655:400-8.
43. Kadenbach B, Ramzan R, Moosdorf R, Vogt S. The role of mitochondrial membrane potential in ischemic heart failure. Mitochondrion. 2011;11:700-6.
44. Iijima T, Mishima T, Tohyama M, Akagawa K, Iwao Y. Mitochondrial membrane potential and intracellular ATP content after transient experimental ischemia in the cultured hippocampal neuron. Neurochem Int. 2003;43:263-9.
45. López-Alonso B, Hernáez A, Sarnago H, Naval A, Güemes A, Junquera C, et al. Histopathological and ultrastructural changes after electroporation in pig liver using parallel-plate electrodes and high-performance generator. Sci Rep. 2019;9:2647.
46. Kaushik A, Nikkhah-Moshaie R, Sinha R, Bhardwaj V, Atluri V, Jayant RD, et al. Investigation of ac-magnetic field stimulated nanoelectroporation of magneto-electric nano-drug-carrier inside CNS cells. Sci Rep. 2017;7:45663.
47. Shil P, Bidaye S, Vidyasagar PB. Analysing the effects of surface distribution of pores in cell electroporation for a cell membrane containing cholesterol. J Phys D Appl Phys. 2008;7:551.
48. Neumann E, Kakorin S, Toensing K. Fundamentals of electroporative delivery of drugs and genes. Bioelectrochem Bioenerg. 1999;48:3-16.
49. Saulis G. Kinetics of pore disappearance in a cell after electroporation. Biomed Sci Instrum. 1999;35:409-14.
50. Saulis G, Saulė R. Size of the pores created by an electric pulse: Microsecond vs millisecond pulses. Biochimica Biophys Acta. 2012;1818:3032-9.