Assessing cancellation effect using numerical modeling of pore formation

Ana-Maria Sandu[a], Damijan Miklavčič[b], Maria Scuderi[b], Janja Dermol-Černe[b]

[a] *Doctoral School of Electrical Engineering, University ″Politehnica″of Bucharest, 313 Splaiul Independenței, 060042, Bucharest, Romania*

[b] *University of Ljubljana, Faculty of Electrical Engineering, Tržaška cesta 25, 1000, Ljubljana, Slovenia*

E-mail: janja.dermol-cerne@fe.uni-lj.si

Abstract *Electroporation is a phenomenon when cell membrane permeability increases due to exposure to the pulsed electric field. Recently, it has been suggested that instead of long monopolar pulses, short high-frequency bipolar pulses (HF-EP) can be applied to reduce pain and muscle contractions. However, higher amplitudes are required for HF-EP, and the cancellation effect could be responsible for this. This phenomenon occurs when bursts of HF-EP pulses are applied, and the effect of the first pulse is canceled by the second pulse. In our study, we aim at explaining the cancellation effect. We modeled pore formation during one HF-EP burst with the inter-phase and inter-pulse delays of either 1 µs or 10 ms in low and high-conductivity buffers and validated the model with the experimental results. To calculate the uptake of molecules we used the area under the curve (AUC) considering the temporal evolution of the number of pores. We compared our results with the percentage of permeabilized cells obtained experimentally. Our numerical results for a high-conductivity buffer corresponded to the experimental ones and we could say that the cancellation effect could be partially explained by pore formation.*

Keywords—Numerical modeling, Cancellation effect, Short-bipolar pulses, Pore formation, Area Under the Curve, Electroporation

I. INTRODUCTION

Electroporation is a phenomenon in which cell membrane, due to exposure to high pulsed electric fields becomes permeable to ions and molecules that otherwise cannot cross the cell membrane [1]. Depending on pulse parameters: electric field strength, pulse duration, pulse polarity and shape, number of pulses, inter-phase delay, and pulse repetition frequency, electroporation can be either transient (reversible electroporation) or permanent (irreversible electroporation). Electroporation is used in medicine [2], biotechnology [3], food [4], and biomass processing [5]. In medicine, it is used in electrochemotherapy (ECT) for delivering chemotherapeutic drugs [6], in gene therapy for delivering DNA in cells and tissues [7], or for tissue ablation using irreversible electroporation (IRE) [8]. In ECT, usually we delivered eight 100 µs long pulses with a repetition frequency of 1 Hz or 5 kHz. The IRE treatments usually consist of several tens of 70-100 µs long monopolar pulses delivered at 1 Hz repetition frequency or paced by patients' ECG directly to the target tissue.

Such high-voltage cause electrical stimulation of excitable cells in the body, leading to muscle contractions and acute pain. During the treatment, therefore it is necessary to administer neuromuscular blocking agents, anesthesia and to synchronize the pulses with the heart activity [9], [10]. In irreversible electroporation treatments, it was suggested that these drawbacks could be reduced by delivering bursts of high-frequency short bipolar pulses, i.e., the high-frequency electroporation (HF-EP) [11]. Later, it was also shown that HF-EP pulses can be successfully used to introduce dyes [12], [13], and chemotherapeutics into the cells [14], and were recently even applied in gene electrotransfer [15]. However, to obtain comparable results of HF-EP pulses and standard longer pulses, a higher amplitude of electric field must be delivered for HF-EP than for longer monopolar pulses. One explanation for the need to apply a higher electric field, besides the usual shape of the strength-duration curve, could be the cancellation effect present in HF-EP, where the effect of the first pulse is reduced by the second pulse of the opposite polarity [13], [16]. This cancellation effect was first observed for nanosecond pulses, but the range was later extended to one or more bipolar pulses with the duration up to 10 µs for the positive or the negative pulse, and the inter-phase delay between the positive and the negative pulse up to 10 ms [13]. The reason why the cancellation effect occurs is still not completely understood , however, in literature, the suggested mechanisms are assisted membrane discharge; reversed electrophoretic ion transport, and two-step oxidation of membrane phospholipids (formation of reactive oxygen species, followed by membrane oxidation) [16].

In our study we aim at explaining the cancellation effect which could be responsible for the HF-EP pulses being less efficient than longer monopolar pulses using numerical modeling. It is known that the cancellation effect occurs when bursts of high-frequency short-bipolar pulses are applied close to each other, where the second pulse cancels the effect of the first pulse. Therefore, we decided to upgrade the assisted discharge theory with the model of pore formation during one HF-EP burst with different inter-phase and inter-pulse delays and voltages and validate with the experimental results, from [13] and [14].

II. METHODS

A. Numerical modeling

We constructed a 2-D axial symmetric model of a single cell in the extracellular medium using the finite element (FEM) environment COMSOL Multiphysics v5.6.

The cell with radius *R* was located inside a rectangular enclosure with the dimensions 200 µm x 100 µm where we set the boundary conditions, $V_{terminal} = burst$ for the top, ground for the bottom, axial symmetry on the left wall, and electrical insulation on the right wall. We simulated two electroporation buffers,low- and high-conductivity (Table 1) which were used in experiments [13]. The time-dependent problem for the geometry was solved in application mode *Electric Currents* of the *AC/DC* module *(Time-Dependent Study)* by solving the Laplace equation:

$$-\nabla(\sigma_s \nabla V) - \nabla \frac{\partial(\varepsilon_s \nabla V)}{\partial t} = 0 \qquad (1)$$

where σ_s and ε_s represent the conductivity and dielectric permittivity of each subdomain. The cell membrane was modeled with a boundary condition *Distributed Impedance* [17] defined by the expression:

$$n \cdot J = \frac{\sigma_m}{d_m}(V_i - V_e) + \frac{\varepsilon_m}{d_m}\left(\frac{\partial V_i}{\partial t} - \frac{\partial V_e}{\partial t}\right) \qquad (2)$$

where $\boldsymbol{n}$ is a unit vector normal to the boundary surface, J is the electric current density, V_i is the intracellular electric potential, V_e is the extracellular electric potential, σ_m, ε_m and d_m are the membrane conductivity, membrane dielectric permittivity, and membrane thickness, respectively. To calculate pore formation we coupled the differential pore formation equation in COMSOL with the *Weak Form Boundary PDE* application mode as suggested in [17]:

$$\frac{dN}{dt} = \alpha e^{\left(\frac{ITV}{V_{ep}}\right)^2}\left(1 - \frac{N}{N_0} e^{-q\left(\frac{ITV}{V_{ep}}\right)^2}\right) \qquad (3)$$

where N is the pore density, N_0 the initial pore density, when ITV is 0 V, and α, q, and V_{ep} are the constants of the electroporation process. The ITV was defined as the difference between intracellular electric potential V_i and extracellular electric potential V_e. Inducement of pores leads to an increase in the membrane conductivity that can be described by the expression [17]:

$$\sigma_{ep} = N \frac{2\pi r_p^2 \sigma_p d_m}{\pi r_p + 2 d_m} \qquad (4)$$

where r_p and σ_p are the radius and pore conductivity of a single pore.

Table 1 Parameters of the model, their symbols, and values

Parameter	Symbol	Value
Cell radius	R	10 µm [13]
Cell membrane thickness	d_m	5 nm
Extracellular conductivity	σ_e	0.176 S/m * [13] 1.912 S/m ** [13]
Extracellular relative permittivity	ε_e	80
Intracellular conductivity	σ_i	0.3 S/m [13]
Intracellular relative permittivity	ε_i	70
Membrane conductivity	σ_m	3×10^{-7} S/m [13]
Membrane relative permittivity	ε_m	4.5
Pore conductivity	σ_p	$\frac{\sigma_e - \sigma_i}{\ln(\frac{\sigma_e}{\sigma_i})}$
Pore radius	r_p	0.76 nm
Electroporation constant	q	2.46
Electroporation parameter	α	10^9 m^{-2}s^{-1}
Characteristic voltage of electroporation	V_{ep}	0.258 V
Equilibrium pore density	N_0	1.5×10^9 m^{-2}

Values were taken from [17] except if noted otherwise. *Low-conductivity electroporation buffer; **High-conductivity electroporation buffer

B. Area Under the Curve

The area under the curve (AUC) is a metric applied in pharmacokinetic analysis to describe and quantify aspects of the plasma concentration-time profile of an administered drug. It is calculated as an integral of drug concentration over time. Many medical applications use the AUC to quantify the effect in time, e.g., to assess the pain intensity post analgesic drug administration [18]. In our study, we used the AUC to calculate the uptake of molecules considering the temporal evolution of the number of pores. We considered the AUC as the metric for the molecular uptake. The AUC was implemented in MATLAB using a trapezoidal method for the integration of the number of pores as a function of time.

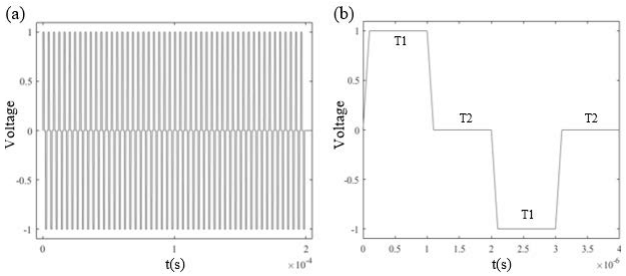

Fig. 1. Numerical modeling of short bipolar pulses. (a) One burst consisted of 50 bipolar pulses. (b) Each bipolar pulse consisted of a positive and a negative pulse, both of length 1 µs (T1) and the inter-phase delay and inter-pulse delay of 1 µs (T2). During our simulation, T2 was either 1 µs or 10 ms. The rise time and fall time for each pulse were 100 ns.

III. RESULTS

We calculated the cancellation effect using numerical modeling of pore formation after applying one burst consisting of 50 bipolar pulses with 1 µs duration (T1) and two different inter-phase delays (T2), 1 µs and 10 ms (Fig. 1), and compared with the experimental results from [13].

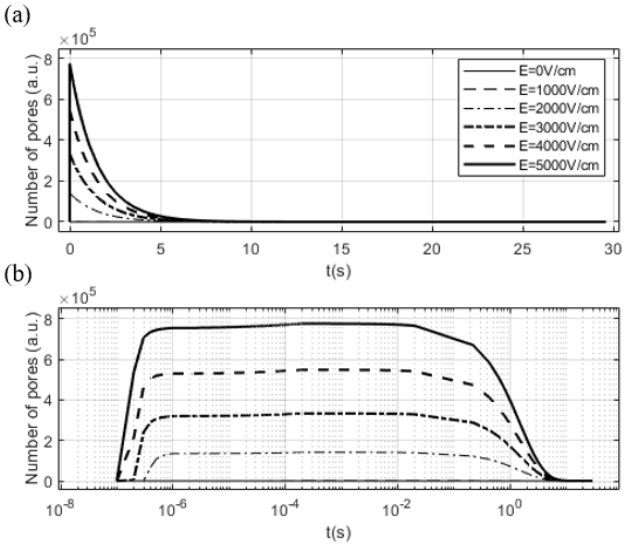

Fig. 2. Number of pores as a function of time for different values of the applied electric field in low conductivity buffer for one burst of 50 bipolar pulses, each pulse with the duration of 1µs and inter-phase delay of 1 µs. (a) In linear scale we can observe the dynamics of pore resealing; (b) In a logarithmic scale we can observe the initial steps in pore formation.

In Fig. 2 we show the increase in number of pores by increasing the magnitude of the applied electric field, within the range of 0 to 5000 V/cm, for a low-conductivity buffer. The number of pores was calculated by integrating the pore density obtained with Eq. (3). A higher electric field caused a higher number of pores.

Fig. 3 shows the pore formation for two inter-phase delays 1 µs and 10 ms, in a low-conductivity buffer at 1 kV/cm applied electric field. We choose both representations, linear and logarithmic scale, to observe better the increasing of pore number. From Fig. 3(b), it can be observed that for 10 ms inter-phase delay the number of pores is gradually increasing in comparison with 1 µs where the increase is very steep. Fig. 4 shows the AUC values calculated for 1 µs and 10 ms inter-phase delay, as function as the applied electric field in low and high-conductivity buffers.

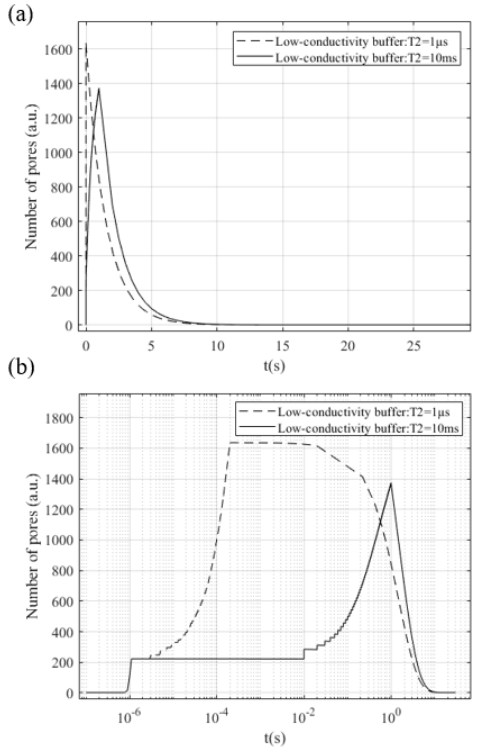

Fig. 3. Number of pores as a function of time for two different inter-phase delays (T2), either 1 µs or 10 ms, in low-conductivity buffer at 1 kV/cm; (a) In linear scale, we can focus on the pore resealing; (b) In a logarithmic scale, we can focus on initial pore formation during the burst application.

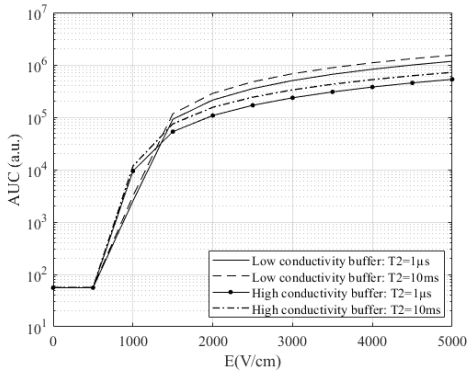

Fig. 4. Area under the curve as a function of the applied electric field in low and high-conductivity buffers for one burst of 50 bipolar pulses. Pulse duration (T1) was 1 µs, and the inter-phase delay (T2) was either 1 µs or 10 ms. In the linear scale, the AUC values increase linearly.

Fig. 5 shows a comparison of experimental and simulated data. Experimental data is median fluorescence of propidium iodide (PI), obtained from [14] and simulated data is the pore formation. From this comparison, it can be seen that both curves are dependent on the magnitude of the electric field, i.e., they have a similar trend of increasing.

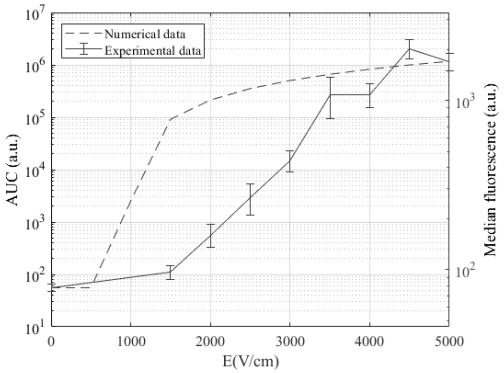

Fig. 5. Comparison of measured and simulated molecular uptake based on fluorescence of propidium iodide and pore formation. The measured values are shown as a mean ± standard deviation and were obtained from [14].

IV. DISCUSSION

In our study, we aimed at explaining the cancellation effect by modeling high-frequency short bipolar pulses and evaluating pore formation. We performed calculations for two conductivity buffers that were previously used in experiments [13]. From numerical results, we observed that the pore formation depends on the magnitude and duration of the applied electric field (Fig. 2). With electric fields of up to 1500 V/cm, we observed that the pore formation increases step-by-step and follows roughly the shape of the applied burst (Fig. 3). For values higher than 1500 V/cm, this increase is steeper as the membrane is immediately permeabilized and more conductive and the following pulses do not contribute much. In Fig. 3, we compared pore formation for both values of inter-phase delay. We observed that for 1 µs inter-phase delay, we obtain more pores but for a shorter time than for 10 ms inter-phase delay, where we obtain less pores but for a longer time. The importance of the duration when pores are open is corroborated by Fig. 4 where for both buffers, the AUC value is higher for 10 ms delay than for 1 µs delay for all electric fields and both buffers.

From our numerical results, we could see that both inter-phase delays have the same trend in the low and high-conductivity buffers. Fig. 4 showed that the longer inter-phase delay increases the uptake which could explain the cancellation effect. At the same time, looking at the experimental data, we observed that the uptake in [13] has a different trend for low and high conductivity electroporation buffers, which indicates that this cancellation effect phenomenon is more complicated than modeled. For high-conductivity buffer, we obtained similar results as is shown in the experiments, and we could explain the cancellation effect with pore formation. Meanwhile, this phenomenon cannot be seen in permeabilization experiments with the low-conductivity buffer, meaning that there is a difference between low and high-conductivity buffer, probably related to the biology or chemistry of electroporation which is not included in the model. Sucrose, which is present in high

concentration in the low-conductivity buffer is added to buffers to keep the osmolality in the physiological range and prevent the cells from collapse while keeping the buffer's electric conductivity low. In [19] we observed that cells response were different in low-conductivity buffer with a high concentration of sucrose than without.

We compared our results with the experimental ones from [13] where the percentage of permeabilized cells was showed. The percentage of permeabilized cells increased in a sigmoid-like fashion and after a certain applied electric field, the maximum number of permeabilized cells was achieved. However, from our results (Fig. 4) we observed that by increasing the electric field the uptake kept increasing. On a linear scale, the increase of the AUC value is also linear – with higher electric fields more dye enters the cells, although experimentally we already reached 100% permeabilization. Since in [13] the experimental results shown only the percentage of permeabilized cells, we compared our data with the median fluorescence from [14]. In Fig. 5 we observed that the median fluorescence and the AUC value have a similar trend, even though in experiments, cells are already 100% electroporated at 3500 V/cm.

There are some limitations of our model and assumptions that we made. First, we modeled only one burst of 50 bipolar pulses, instead of eight, that was used in the experimental data, to reduce computation time and resources. Other pulse parameters were kept the same as in the already published study [13]. Second, our model does not include the pore expanding or shrinking, only formation of pores of fixed size. Third, we did not calculate the transport mechanisms (electrophoresis and diffusion). We assumed that the number of pores is correlated to the transport, i.e., by increasing the number of pores, the transport increases, which we approximated by calculating the Area Under the Curve, (AUC) value. The adequacy of using the number of formed pores as an indicator of the transport across the membrane was shown in [20] where the modeled number of formed pores was proportional to experimentally determined intracellular calcium concentration.

V. Conclusion

Cancellation effect in high-conductivity buffer could be explained by pore formation as with 10 ms delay, a higher AUC value was obtained than with 1 µs delay. However, in the low-conductivity buffer, the experimental results cannot be explained by pore formation, the phenomenon is more complex than the model used in this study.

Acknowledgment

This research was conducted during an Erasmus+ program between Doctoral School of Electrical Engineering, University ″Politehnica″ of Bucharest and Laboratory of Biocybernetics, Faculty of Electrical Engineering, University of Ljubljana. The authors acknowledge the financial support from the Slovenian Research Agency (ARRS).

References

[1] T. Kotnik, L. Rems, M. Tarek, and D. Miklavcic, "Membrane Electroporation and Electropermeabilization: Mechanisms and Models," *Annu. Rev. Biophys.*, vol. 48May 2019

[2] B. Geboers *et al.*, "High-voltage electrical pulses in oncology: Irreversible electroporation, electrochemotherapy, gene electrotransfer, electrofusion, and electroimmunotherapy," *Radiology*, vol. 295, no. 2, May 2020

[3] T. Kotnik, W. Frey, M. Sack, S. Haberl Meglič, M. Peterka, and D. Miklavčič, "Electroporation-based applications in biotechnology," *Trends Biotechnol.*, vol. 33, no. 8 Aug. 2015

[4] S. Mahnič-Kalamiza, E. Vorobiev, and D. Miklavčič, "Electroporation in Food Processing and Biorefinery," *J. Membr. Biol.*, vol. 247, no. 12, Nov. 2014

[5] A. Golberg *et al.*, "Energy-efficient biomass processing with pulsed electric fields for bioeconomy and sustainable development," *Biotechnol. Biofuels*, vol. 9, no. 1, 2016

[6] N. Esmaeili and M. Friebe, "Electrochemotherapy: A Review of Current Status, Alternative IGP Approaches, and Future Perspectives," *J. Healthc. Eng.*, , 2019

[7] L. Lambricht, A. Lopes, S. Kos, G. Sersa, V. Préat, and G. Vandermeulen, "Clinical potential of electroporation for gene therapy and DNA vaccine delivery," *Expert Opin. Drug Deliv.*, vol. 13, no. 2, Feb. 2016

[8] K. N. Aycock and R. V. Davalos, "Irreversible Electroporation: Background, Theory, and Review of Recent Developments in Clinical Oncology," *Bioelectricity*, vol. 1, no. 4, Dec. 2019

[9] A. Deodhar *et al.*, "Irreversible Electroporation Near the Heart: Ventricular Arrhythmias Can Be Prevented With ECG Synchronization," *AJR. Am. J. Roentgenol.*, vol. 196, no. 3, p. W330, Mar. 2011

[10] B. Mali, T. Jarm, M. Snoj, G. Sersa, and D. Miklavcic, "Antitumor effectiveness of electrochemotherapy: A systematic review and meta-analysis," *European Journal of Surgical Oncology*, vol. 39, no. 1. Eur J Surg Oncol, Jan. 2013

[11] C. B. Arena *et al.*, "High-frequency irreversible electroporation (H-FIRE) for non-thermal ablation without muscle contraction," *Biomed. Eng. Online*, vol. 10, Nov. 2011

[12] D. C. Sweeney, M. Reberšek, J. Dermol, L. Rems, D. Miklavčič, and R. V. Davalos, "Quantification of cell membrane permeability induced by monopolar and high-frequency bipolar bursts of electrical pulses," *Biochim. Biophys. Acta - Biomembr.*, vol. 1858, no. 11, Nov. 2016

[13] T. Polajžer, J. Dermol-Černe, M. Reberšek, R. O'Connor, and D. Miklavčič, "Cancellation effect is present in high-frequency reversible and irreversible electroporation," *Bioelectrochemistry*, vol. 132, Apr. 2020

[14] M. Scuderi, M. Rebersek, D. Miklavcic, and J. Dermol-Cerne, "The Use of High-frequency Short Bipolar Pulses in Cisplatin Electrochemotherapy in Vitro," *Radiol. Oncol.*, vol. 53, no. 2, 2019

[15] T. Potočnik, D. Miklavčič, and A. Maček Lebar, "Gene transfer by electroporation with high frequency bipolar pulses in vitro," *Bioelectrochemistry*, vol. 140, Aug. 2021

[16] A. G. Pakhomov *et al.*, "Cancellation of cellular responses to nanoelectroporation by reversing the stimulus polarity," *Cell. Mol. Life Sci.*, vol. 71, no. 22,, Nov. 2014

[17] L. Rems, M. Ušaj, M. Kandušer, M. Reberšek, D. Miklavčič, and & Gorazd Pucihar, "Cell electrofusion using nanosecond electric pulses" Scientific Reports, 2013

[18] J. C. Cappelleri, A. G. Bushmakin, G. Zlateva, and A. Sadosky, "Pain responder analysis: Use of area under the curve to enhance interpretation of clinical trial results," *Pain Pract.*, vol. 9, no. 5, Sep. 2009

[19] J. Dermol, O. N. Pakhomova, A. G. Pakhomov, and D. Miklavčič, "Cell Electrosensitization Exists Only in Certain Electroporation Buffers," *PLoS One*, vol. 11, no. 7, Jul. 2016

[20] J. Dermol-Černe, T. B. Napotnik, M. Reberšek, and D. Miklavčič, "Short microsecond pulses achieve homogeneous electroporation of elongated biological cells irrespective of their orientation in electric field," *Sci. Reports 2020 101*, vol. 10, no. 1, Jun. 2020