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DEVELOPMENT OF PLANAR ELECTRODES FOR REAL-TIME ELECTROPORATION

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Abstract. In this paper the concept of planar electrodes for real-time electroporation on a microscope stage is presented and the structure is analyzed using finite element method (FEM) analysis. A multiparametric investigation of the chip topology is performed in COMSOL Multiphysics environment to define the configuration of electrodes, electric field distribution and other electroporation parameters to ensure a homogeneous cell exposure. Based on the simulation results, an optimal electrode configuration, which is suitable for the investigation of the permeabilization thresholds during electroporation, is proposed.

Keywords: electroporation, electropermeabilization, electric field, planar electrodes, COMSOL model, simulation.

Introduction

The application of high-voltage pulses to generate an electric field across a biological membrane, which causes reorientation of lipids and formation of pores in the cell membrane is termed electroporation or electro-permeabilization (Denzi et al., 2015). The electroporation increases the permeability of the cell membranes by dielectric breakdown allowing temporary access to the cell interior (reversible electroporation), thus increasing molecular transport (Bennett, Sapay, & Tieleman, 2014) – an effect that is used for gene delivery into cells. The cell membrane is then resealed once the application of pulses is stopped (Sundararajan, 2009).

For optimal effectiveness of the electroporation application, the physical parameters of the applied electric pulses must be carefully chosen by setting the most appropriate electric field strength, pulse amplitude, duration, waveform and number of pulses (Flisar, Puc, Kotnik, & Miklavcic, 2003). Depending on these parameters, the extent of the effect and the effect itself can be reversible (temporary) or non-reversible, where reversibility/irreversibility is related to cell survival/death (Rebersek & Miklavcic, 2011; Schoenbach, Beebe, & Buescher, 2001). Subsequently, a variety of the electroporation protocols have been developed and reported for various biotechnological applications (Pucihar, Krmelj, Reberšek, Napotnik, & Miklavčič, 2011). For example, for reversible electroporation (delivery of drug or other molecules into cells) pulses in the range of 1 kV/cm (hundreds of microseconds to milliseconds) are usually used (Pucihar et al., 2011). For larger molecules or non-reversible electroporation, the intensity of the electric field may be increased from several kV/cm to tens of kV/cm (Xiao et al., 2013). However, efficiency of the electroporation depends on the affected cell type, tissue, or buffer the parameters, which create a motivation for investigation of the cell permeabilization process in detail.

The most common way of investigating electroporation in *vitro* is to study the cell uptake of dyes: either fluorescent molecules (fluorophores) or color stains (such as trypan blue) (Batista Napotnik & Miklavčič, 2018). The cells are put into a cuvette with electrodes and after pulsing procedures the release or loading of the dye is observed (V. Novickij, Tabasnikov, Smith, Grainys, & A. Novickij, 2015). The most common electroporation systems use a standard cuvette with metal electrodes. The usual distance between electrodes vary from 1 mm to 4 mm. However, this method does not allow to research and monitor the dyes dynamic in real-time, due to the required cell handling from the cuvette to the microscope slide (Novickij et al., 2015). As result, the observation of the dye release dynamics is not possible during pulsing procedures.

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In order to overcome the drawbacks of the standard cuvette electroporators, the microfluidics-based electroporation technique is rapidly developing (Geng & Lu, 2013). Even though this technique is advanced with its unique characteristics of miniaturization and integration, it is still difficult to study fast, nanoscale pore formation dynamics using real-time fluorescent microscopy. In order to perform such experiments, the electrode structures, which are capable of delivering uniformly distributed electric field to the biological cells, is required.

The planar electrode structures proved to be advantageous for this type electroporation experiments (Geng & Lu, 2013; Novickij et al., 2017), but various issues, such as the spatial non-uniformity of the electric field distribution, complexity of electrode fabrication, biological compatibility issues, and the voltage breakdown occurrence between the electrodes has to be solved (Novickij et al., 2017). Due to this, the variety of available planar electrodes for electroporation is currently limited due to the complexity, price and the electrodes fabrication challenges. As result, the need to research the various electrodes configurations and their parameters (such as thickness, electrode gap, finger width) influence on the generated electric field intensity and homogeneity still exists.

In this paper, the finite element method simulations are applied for development of the planar electrodes for real-time electroporation. The generated electric field intensity and homogeneity dependency on the electrodes configuration is evaluated and the optimal configuration is proposed.

1. Planar electrodes for electroporation

In order to investigate an electric field distribution and make preparation works for the electrodes prototype development, the planar electrodes computer model was developed using COMSOL Multiphysics software (COMSOL Inc., USA).

Firstly, the requirements for the electrodes were defined, which are enforced by the biological objects. The gap between the electrodes must be big enough to ensure sufficient field of view to accommodate multiple cells (for statistical analysis of the observed effect). The typical mammalian cell size in the range of 8–10 μm and the permeabilization threshold is in the range of 0.3–1 kV/cm (Krassowska & Filev, 2007; Novickij et al., 2016), therefore it was defined that the minimum effective gap should be at least 80 μm. On the other hand, to have more flexibility during the experiment, the gap between the electrodes should to be wider than the effective gap. For this reason, the gap between electrodes is selected to vary from 150 μm to 500 μm, while the generated electric field should be at least 1 kV/cm.

Secondly, the biocompatibility of the electrodes was a concern. Cells are sensitive to oxidation and the pH changes in the electrode/medium interface (Li et al., 2015), thus only biocompatible and highly conductive metals should be used. As a result, the choice was limited to gold and platinum, however, taking into account future availability of infrastructure and the specifics of fabrication process, platinum has been selected.

Lastly, electroporation is highly dependent on the parameters of the electric field (i.e. direction, pulse form, duration etc.), therefore the structure should support bipolar pulses and delivery of pulses with different angles, which will allow a more flexible study the spatial distribution of pores in the cell membranes by real-time fluorescent dye diffusion. As a result, at least several (more than 2) electrodes must be present in the structure, while we limited the structure to 4.

For the Multiphysics environment simulations, the Electric Currents interface of the COMSOL Multiphysics software has been selected. The selected interface provides a solution of the current conservation equation which is based on Ohm's law using the scalar electric potential as the dependent variable. For the results resolution fine element resolution, the free tetrahedral mesh was selected with a minimum and maximum finite element size of 1.65 μm and 38.5 μm, respectively. The specific size of each element is influenced by the geometry of the electrodes. The simplified electrodes mesh structures are represented in Figure 1.

As it can be seen from Figure 1, the structure consists of 4 symmetrical electrodes, which form 2 pairs (vertical, horizontal). The goal is to estimate the influence of electrode geometry (gap) on the electric field homogeneity both in vertical and horizontal planes, which will ensure equal exposure of cells to pulsed electric field.

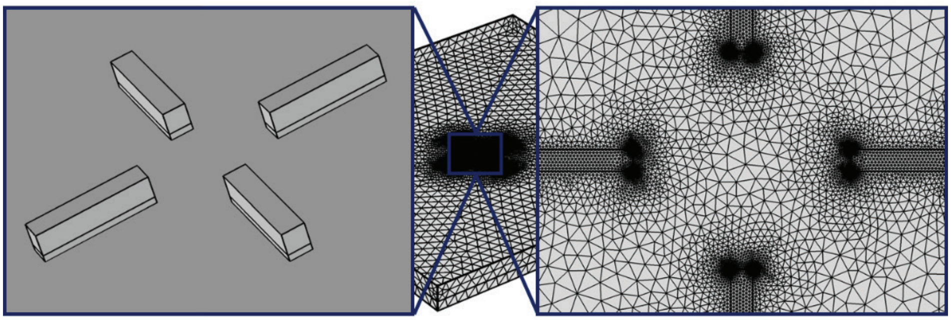

Figure 1. 3D simplified electrodes (left) and free tetrahedral mesh (right) structures of planar electrodes for electroporation

2. COMSOL model

Four platinum electrodes with titanium layer on the top were positioned on the 1.1 mm width (and the same depth) 0.03 mm height silica glass plate. On the top of the silica glass plate, the electrode structures are surrounded by 0.05 mm height water container. Both represent the typical medium in which the electrodes are used.

The Table 1 shows the summary main parameters of the developed COMSOL model of the planar electrodes for electroporation.

Table 1. Summary of simulation parameters

Parameter	Value
Electrode width	10 μm
Electrode length	50 μm
Electrode thickness of titanium layer	0.01 μm
Electrode thickness of platinum layer	0.1 μm
Distance between electrodes	150–500 μm
Applied voltage	30 V
Water electrical conductivity	0.1 S/m
Water relative permittivity	78
Platinum electrical conductivity	8.9 × 106 S/m
Platinum relative permittivity	2.6
Titanium electrical conductivity	2.6 × 106 S/m
Titanium relative permittivity	100
Silica glass electrical conductivity	1 × 10–14 S/m
Silica glass relative permittivity	2.09

As it can be seen the electrode gap were changed during simulation from 150 μm to 500 μm in order to determine the optimal value. Since the electrodes represent identical pairs, the simulation results only of one pair are further presented in the study.

3. Results

The example of electric field distribution between two planar electrodes pairs in YZ and XY planes is presented in Figure 2. The distance between electrodes is set to 350 μm and the applied voltage is 30 V.

If the gap between electrodes is set to 150 μm, the electric field in the middle is 1.1 kV/cm, which is applicable for reversible electroporation procedures of the biological cells and tissues (Pucihar et al., 2011). The influence of electrode gap on the electric field amplitude in the middle of the chip is summarized in Figure 3.

As it can be seen in Figure 3 the field strength decreases (from 1.1 kV/cm to 0.40 kV/cm) with increase of electrode gap from 150 μm to 450 μm, respectively. However, the homogeneity of the exposure is a concern, since the cells should be treated equally.

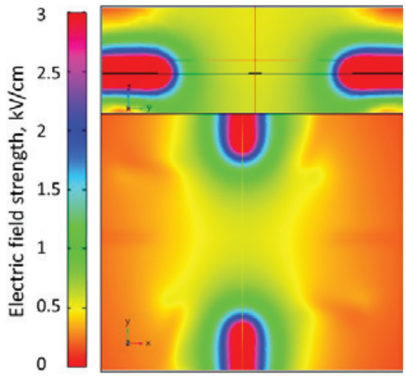

Figure 2. Electric field distribution of planar electrode pair in YZ plane (top) and XY plane (bottom) when electrode gap is equal to 350 μm

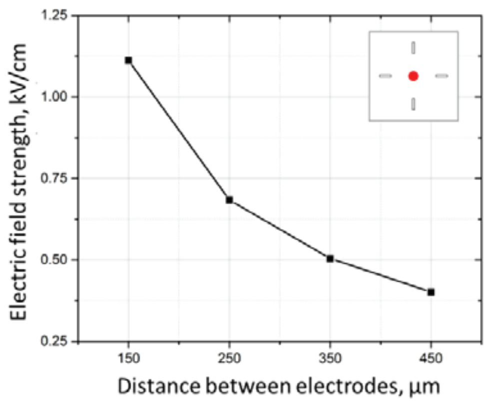

Figure 3. Electric field strength dependence on the distance between electrodes

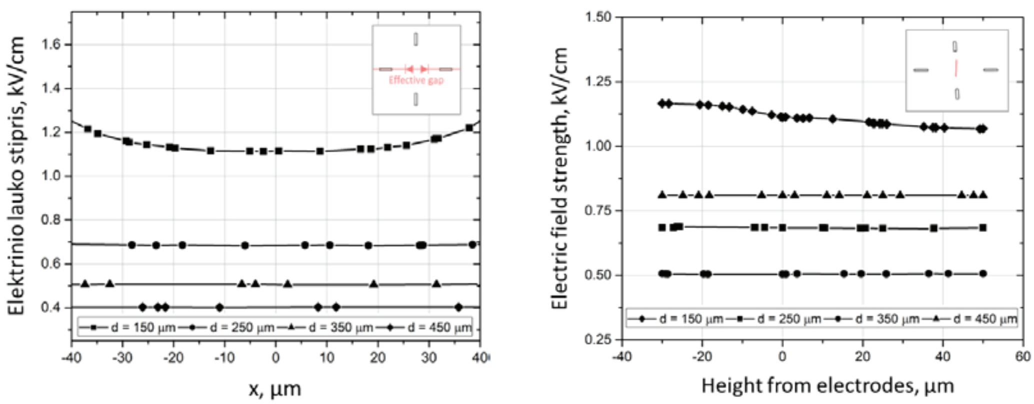

Figure 4. Electric field strength distribution in x-plane (top) and z-plane (bottom) dependence on the distance between electrodes

Therefore, the distribution of electric field values in the effective gap (80 μm) is presented in Figure 4. As it can be seen in Figure 4 (top) the electric field is homogeneous (80 μm gap) when the 250–450 μm gap between electrodes are used. As a trade-off, the electric field amplitude is reduced. However, the fabrication process allows application of voltage higher than 30 V, thus the drop of amplitude can be compensated by increase of voltage.

Another important parameter is the electric field homogeneity in the vertical plane (z-plane). The electric field strength distribution (z-plane) dependence on the distance between electrodes is summarized in Figure 4 (bottom). Similar to x-plane distribution in the z-plane the 250–450 μm gap electrodes offer the best homogeneity. Heights up to 60 μm from the electrodes where investigated, which is sufficient since the cells are small (8–10 μm).

Based on the results, it was concluded that the 250–350 μm gap electrodes have the most potential since they offer a tradeoff between the applied voltage (less than 100 V) and acquired electric field parameters (more than 1 kV/cm).

Conclusions

In this paper, planar electrodes for real-time electroporation were proposed and a COMSOL model was developed. Proper distance between the electrodes must be chosen in order to achieve homogeneous distribution of electric field. It was determined that the 250–350 μm gap electrodes are the most suitable for real-time planar electroporation and will be fabricated. The results of experiments will be covered in future works.

References

Batista Napotnik, T., & Miklavčič, D. (2018). In vitro electroporation detection methods – an overview. *Bioelectrochemistry, 120*, 166-182. https://doi.org/10.1016/j.bioelechem.2017.12.005

Bennett, W. F. D., Sapay, N., & Tieleman, D. P. (2014). Atomistic simulations of pore formation and closure in lipid bilayers. *Biophysical Journal, 106*(1), 210-219. https://doi.org/10.1016/j.bpj.2013.11.4486

Denzi, A., Merla, C., Palego, C., Paffi, A., Ning, Y., Multari, C. R., … & Liberti, M. (2015). Assessment of cytoplasm conductivity by nanosecond pulsed electric fields. *IEEE Transactions on Biomedical Engineering, 62*(6), 1595-1603. https://doi.org/10.1109/TBME.2015.2399250

Flisar, K., Puc, M., Kotnik, T., & Miklavcic, D. (2003). Cell membrane electropermeabilization with arbitrary pulse waveforms. *IEEE Engineering in Medicine and Biology Magazine, 22*(1), 77-81. https://doi.org/10.1109/MEMB.2003.1191453

Geng, T., & Lu, C. (2013). Microfluidic electroporation for cellular analysis and delivery. *Lab Chip, 13*(19), 3803-3821. https://doi.org/10.1039/C3LC50566A

Krassowska, W., & Filev, P. D. (2007). Modeling electroporation in a single cell. *Biophysical Journal, 92*(2), 404-417. https://doi.org/10.1529/biophysj.106.094235

Li, Y., Wu, M., Zhao, D., Wei, Z., Zhong, W., Wang, X., … & Li, Z. (2015). Electroporation on microchips: the harmful effects of pH changes and scaling down. *Scientific Reports, 5*, 17 817. https://doi.org/10.1038/srep17817

Novickij, V., Girkontaite, I., Grainys, A., Zinkevičiene, A., Lastauskiene, E., Švediene, J., … & Novickij, J. (2016). Measurement of transient permeability of Sp2/0 myeloma cells: flow cytometric study. *Measurement Science Review, 16*(6), 300-304. https://doi.org/10.1515/msr-2016-0038

Novickij, V., Tabasnikov, A., Smith, S., Grainys, A., & Novickij, J. (2015). Analysis of planar circular interdigitated electrodes for electroporation. *IETE Technical Review (Institution of Electronics and Telecommunication Engineers, India), 32*(3), 196-203. https://doi.org/10.1080/02564602.2014.1000982

Novickij, V., Tabašnikov, A., Smith, S., Grainys, A., Novickij, J., Tolvaišienė, S., & Markovskaja, S. (2017). Feasibility of parylene coating for planar electroporation copper electrodes. *Medziagotyra, 23*(2), 93-97. https://doi.org/10.5755/j01.ms.23.2.14953

Pucihar, G., Krmelj, J., Reberšek, M., Napotnik, T. B., & Miklavčič, D. (2011). Equivalent pulse parameters for electroporation. *IEEE Transactions on Biomedical Engineering, 58*(11), 3279-3288. https://doi.org/10.1109/TBME.2011.2167232

Rebersek, M., & Miklavcic, D. (2011). Advantages and disadvantages of different concepts of electroporation pulse generation. *ATKAFF, 52*(1), 11-19. https://doi.org/10.1080/00051144.2011.11828399

Schoenbach, K. H., Beebe, S. J., & Buescher, E. S. (2001). Intracellular effect of ultrashort electrical pulses. *Bioelectromagnetics, 22*(6), 440-448. https://doi.org/10.1002/bem.71

Sundararajan, R. (2009). Nanosecond electroporation: another look. *Molecular Biotechnology, 41*(1), 69-82. https://doi.org/10.1007/s12033-008-9107-y

Xiao, D., Yao, C., Liu, H., Li, C., Cheng, J., Guo, F., & Tang, L. (2013). Irreversible electroporation and apoptosis in human liver cancer cells induced by nanosecond electric pulses. *Bioelectromagnetics, 34*(7), 512-520. https://doi.org/10.1002/bem.21796

PLANARIŲJŲ ELEKTRODŲ MODELIS REALIOJO LAIKO ELEKTROPORACIJOS TYRIMAMS

P. Butkus

Santrauka

Šiame straipsnyje pristatomas planariųjų elektrodų, skirtų realiojo laiko elektroporacijos tyrimams, ant mikroskopo objektinio staliuko baigtinių elementų modelis. Pasiūlytas daugiaparametrio elektrodų lusto topologijos tyrimas atliekamas „COMSOL Multiphysics“ aplinkoje, siekiant nustatyti tinkamą elektrodų konfigūraciją, elektrinio lauko pasiskirstymą ir kitus elektroporacijos parametrus, kuriems esant būtų užtikrintas tolygus ląstelių poveikis. Remiantis modeliavimo rezultatais, siūloma optimali elektrodų konfigūracija, kuri užtikrina pakankamą permeabilizacijos slenkstinę įtampą elektroporacijos tyrimų metu.

Reikšminiai žodžiai: elektroporacija, permeabilizacija, elektrinis laukas, planarieji elektrodai, COMSOL modelis, modeliavimas.