

Feasibility of Parylene Coating for Planar Electroporation Copper Electrodes

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This paper is focused on the feasibility study of parylene as a biocompatible coating for planar electroporation microelectrodes. The planar parallel and the circular interdigitated electrodes are applied in the analysis. The electrodes feature 100 μm width with a 300 μm gap between anode and cathode. The parylene coating thickness was varied in the 250 nm – 2 μm range. The resultant electric field distribution evaluation has been performed using the finite element method. The electrodes have been applied in electroporation experiments with *Saprolegnia parasitica*. For reference the additional experiments using conventional electroporation cuvette (1 mm gap) have been performed. It has been determined that the parylene coating with hydrophobic properties has limited applicability for the passivation of the planar electroporation electrodes.

Keywords: microelectrodes, pulsed electric field, biological cells.

1. INTRODUCTION

Electroporation is a biological cell treatment method based on the application of the pulsed electric field to induce reversible and non-reversible changes in the cell membrane permeability [1–4]. Conventional electroporation includes a pulsed power generator for high voltage pulse formation and a cuvette for biological cells with integrated electrodes, where a high intensity and defined duration electric field is exerted [5, 6]. In this case the design of the electrodes usually consists of two parallel metal plates (anode and cathode) in direct contact with the cell medium, where a uniform electric field is generated [7]. Such configuration is typical for many electroporation setups [8–11]. Depending on the physical or biological treatment parameters such as pulse duration, amplitude, polarity, repetitive frequency, waveform or cell geometry [12–15] the electroporation effect can be reversible and irreversible [1, 16–18]. Both outcomes are desirable in biotechnology and have found vast applications in cancer treatment (electrochemotherapy), DNA transfer (genetic engineering), food processing (sterilization) and many others [15, 19–21].

Since the electroporation phenomenon is popular biomedical technique, the biophysics of the process is studied in detail. The research for possibility to observe the electroporation process in real-time on a microscope stage has resulted in the increased interest in microfluidic systems [22–24]. Different planar configurations of the electrodes have been proposed, where planar parallel strip structures and interdigitated electrode configurations are common [25–27]. However, various problems arise, 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 [28]. As a result planar electroporation in the present form is limited due to the complexity, price and the electrodes fabrication challenges [29].

At the same time parylene is frequently used as a substrate for microfluidic devices and has proven to have good biomedical compatibility when used as a passivation layer for metals even for *in vivo* applications [30–32]. Nevertheless, the number of works focusing on the development of planar microelectrodes for electroporation and featuring passivation of the electrode surface with parylene is low.

The main reasons can be based on the problems of parylene adhesion to the surface of microfluidic devices and the negative influence of parylene coating on the distribution of the electric field [33, 34]. On the other hand introduction of parylene layer to planar electroporation could potentially enable application of non-biocompatible materials (such as copper) [35], thus simplify the fabrication process and reduce the total cost of the device [36].

In this work we present the experimental feasibility study of the parylene as a biocompatible coating [37] for planar electroporation. The influence of parylene layer thickness on the electric field distribution and the respective parameters of electroporation are determined.

2. MATERIALS AND METHODS

2.1. Electrodes

Planar parallel strip and circular interdigitated microelectrode [38] configurations are applied in the study. The microelectrodes have been fabricated using laser

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etching (LPKF, USA) on a FR-4 glass epoxy substrate based copper board (LPKF, USA). The copper thickness was 18 μm . The gap between electrode fingers was 300 μm . The surface of the chips has been covered with parylene in the parylene deposition tool (SCS 2010 Labcoter, USA). As parylene is known to have conformal coating properties, coating thickness was in a range of 250 nm to 2 μm , which was a reasonable trade-off between dielectric effect on electric field (refer to Fig. 2) and aspect ratio of polymer to coated copper features. The coating thickness has been measured using spectroscopic reflectometer (Nanometrics NanoSpec 3000, USA) on the reference samples for each batch. The structure of the electrodes is shown in Fig. 1.

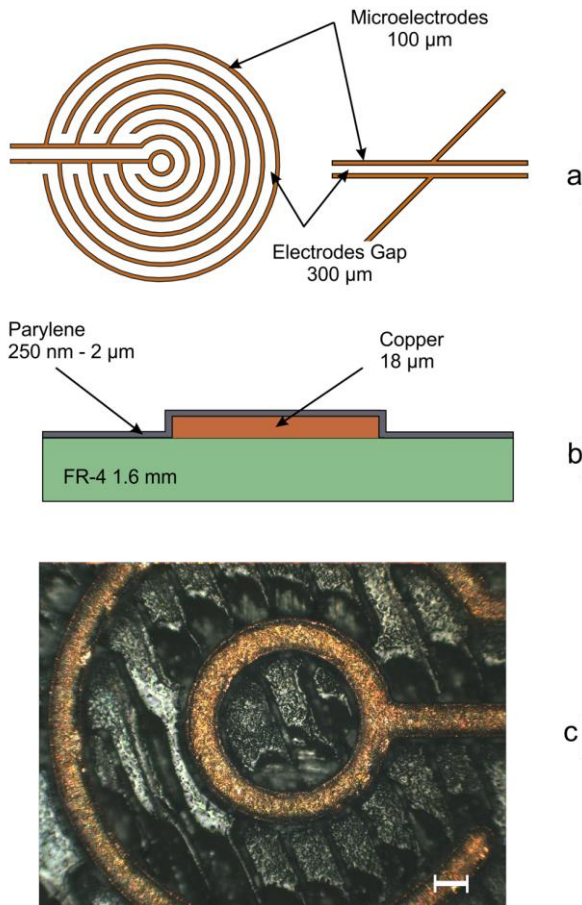


Fig. 1. a – configuration of the circular interdigitated and parallel strip planar microelectrodes; b – fabricated layer structure of one of the fingers of the microelectrodes; c – microphotograph of the laser etched electrode fingers, where the scale mark represents 100 μm

As it can be seen in Fig. 1 the application of interdigitated electrode configuration allows increasing the number of separate experimental instances proportionally to the number of equivalent pairs of anodes and cathodes. In order to evaluate the resultant electric field distribution between planar fingers and estimate the influence of parylene layer the finite element method (FEM) analysis has been applied (COMSOL Multiphysics, Sweden).

2.2. FEM analysis

The model of the microelectrodes has been constructed in the 2D plane. The parameters that were used are

summarized in Table 1. In the simulation a 100 V voltage has been applied to the electrode contacts in order to generate high intensity electric field. The results of the FEM simulation for the parallel strip microelectrodes are shown in Fig. 2 a, b. As it can be seen in Fig. 2 a parallel strip electrode configuration allows generating a spatially non-uniform electric field.

Table 1. FEM model parameters

Parameter	Value
Electrode width, μm	100
Electrode gap, μm	300
FR-4 relative permittivity	4.2
Buffer relative permittivity	71
Parylene relative permittivity	2.65
Applied voltage, V	100

There are reports for possibilities of application of non-uniform electric fields in electroporation, however spatial uniformity is desirable. In Fig. 2 a the > 90 % homogeneity region is marked with a dot rectangle. In order to estimate the effect of parylene on the electric field distribution the parylene layer has been introduced to the simulation as shown in Fig. 1 b. The magnitude of the electric field in the gap between electrodes has been estimated at a fixed height ($z = 5 \mu\text{m}$). The results for different thicknesses of the parylene coating are presented in Fig. 2 b.

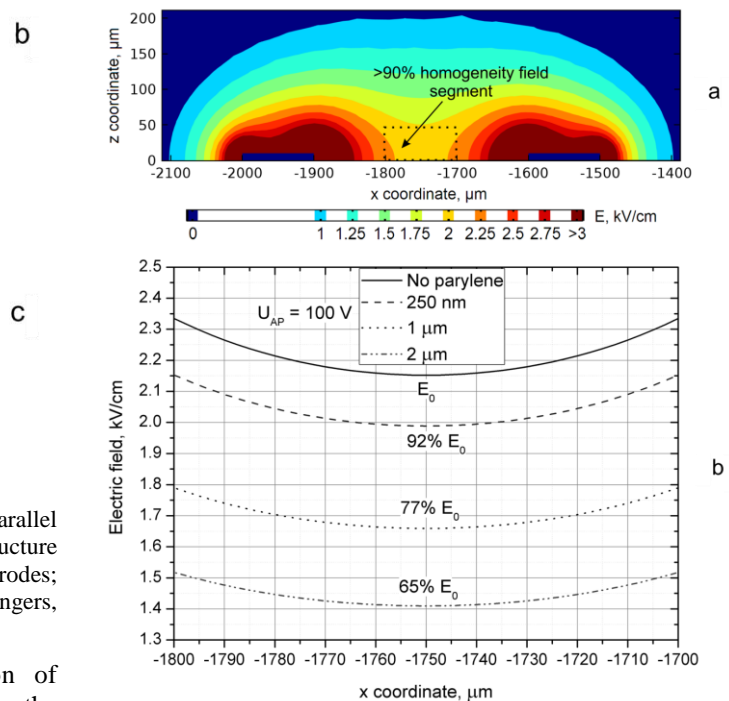


Fig. 2. a – FEM analysis of the XZ plane electric field distribution in the parallel strip electrode structure; b – influence of parylene layer on the distribution of the electric field in the > 90 % homogeneity region between electrode fingers, $z = 5 \mu\text{m}$

The FEM simulation results indicate that the magnitude of the electric field is reduced up to 65 % E_0 (E_0 – electric field amplitude generated without parylene layer) for the 2 μm parylene coating. The same tendency is observed when the interdigitated electrodes are applied, thus the

results are not shown.

2.3. Pulse delivery setup

The square wave electrical pulse generator developed in High Magnetic Field Institute (Vilnius, Lithuania) has been applied in the study [11]. The setup is capable of delivering repetitive 1 μ s – 10 ms 0 – 1 kV square wave and bipolar pulses. The maximum current is internally limited to 100 A. The electrodes have been connected directly as a load of the generator. The additional experiments using conventional electroporation cuvette have been performed (BTX, Cuvette plus™, Nr. 610, San Diego, USA) to provide reference measurements.

The waveform of the pulse is shown in Fig. 3.

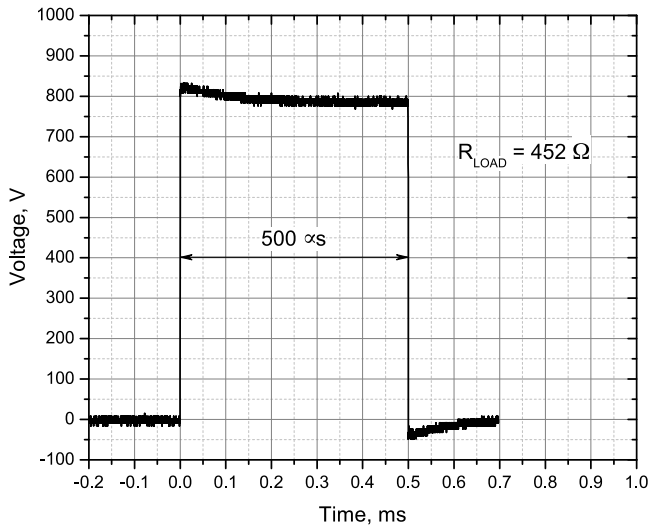


Fig. 3. The waveform of the electrical pulse

During experiments the charging voltage has been varied in the 0.1 – 0.8 kV range. Based on the FEM simulation results the charging voltage has been adjusted for each applicator depending on the parylene layer thickness and electrodes type. The 1 – 8 kV/cm 20 pulses bursts (1 Hz) have been applied in the experiments.

2.4. Biological cells

The freshwater mold *Saprolegnia parasitica* has been used in the experiments. The specimens have been isolated from the water samples collected in a freshwater pool (Vilnius, Verkiiai park). The colony has been incubated for several days at room temperature using the hemp seed as bait. Later the fragments of hyphae and the reproductive spores were cut from colony and a suspension using distilled water as a base has been formed. For the conventional electroporation the samples of 80 μ l have been used for each instance, while the 5 μ l samples were used for the planar electroporation.

3. EXPERIMENT

The experiments using the 250 nm and 2 μ m parylene coating electrodes and the conventional BTX cuvette have been performed. The electroporation effect has been evaluated by the morphological analysis of the cell membrane integrity. The number of the cells with the compromised cell membrane integrity was estimated as a

percentage to the total number of the cells that were analysed in the experimental instance during the light microscopy. The summary of the results and the microphotograph of the *S. parasitica* with the compromised membrane integrity are shown in Fig. 4.

As it can be seen in Fig. 4 the electroporation can induce irreversible damage to the cell membrane (6 – 8 kV/cm, 20 pulses, 1 Hz). However during experiments we were not able to detect any statistically significant changes in the morphology of the cells when the planar electrodes with parylene coating were used. On the opposite the conventional electroporation procedure resulted in up to 20 % of the cells being damaged.

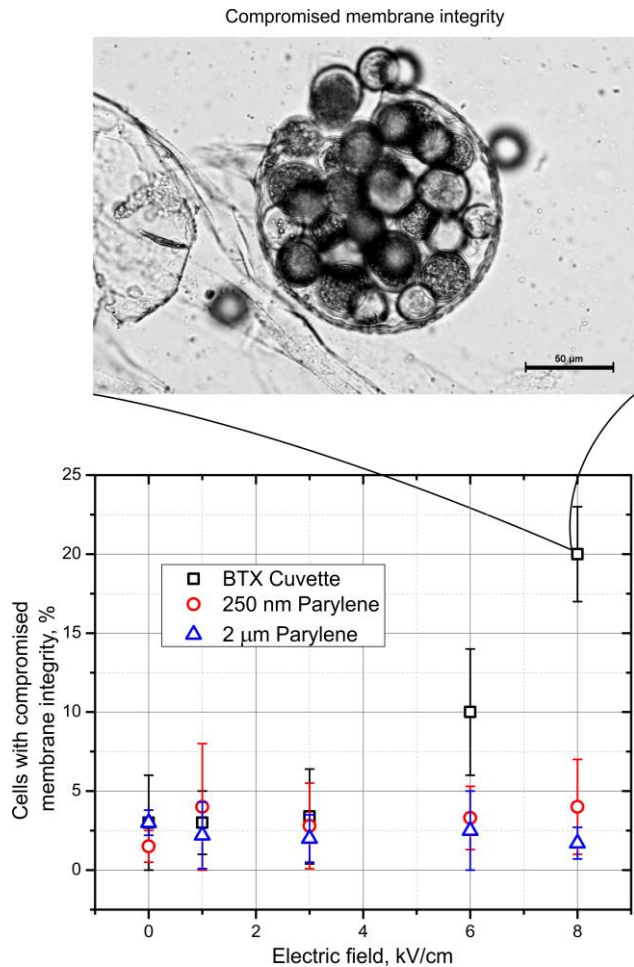


Fig. 4. The microphotograph of the *S. parasitica* with compromised membrane integrity after electroporation and the fraction of the damaged cells compared to the total number of cells; the scale mark represents 50 μ m

It has been concluded that the result was influenced by the hydrophobic properties of the parylene coating, resulting in a higher cells spatial $z \gg 5 \mu$ m position under the coverslip, thus the induced transmembrane potential was not high enough for the permeabilization phenomenon to occur.

4. DISCUSSION AND CONCLUSION

The planar electroporation electrodes have been applied in the study and the feasibility of the parylene coating has been analysed. It has been shown that the parylene coating results in the reduction of the peak

electric field (up to 65 % E_0 in the 2 μm coating case). The experimental data indicated that the hydrophobic properties of the parylene that was deposited according to the described methodology negatively influence the electroporation efficacy. We were not able to acquire any statistically significant morphological changes in the *S. parasitica*, while the reference experiments using the conventional electroporation cuvette at the same conditions were successful. It has been concluded that the induced transmembrane potential was not high enough for the permeabilization phenomenon to occur due to the higher cells spatial $z \gg 5 \mu\text{m}$ position under the coverslip.

The next step and the object of the future works for a potential solution to this issue would be the treatment of the parylene coating by the oxygen plasma, thus removing a proportion of the coating thickness and rendering the surface of parylene coating to hydrophilic. This would require accordingly the deposition of a higher thickness of the parylene layer. Such configuration could potentially enable application of the non-biocompatible materials (such as copper) for electroporation experiments, thus simplify the fabrication process and reduce the total cost of the device, compared to the platinum or gold electrodes.

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