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Changes in living cell morphology induced by electroporation

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ABSTRACT

Electroporation is a modern technique with many applications in cancer therapy and food industry. With high voltage electric pulses we can obtain reversible or irreversible electroporation of the living cell membrane. In our study we have investigated the cell volume changes in non-porated and porated cells, finding that a significant cells swelling occurs at poration voltages higher than 0.75 V/cm as compared to non-porated cells and cells porated with electrical pulses of 0.75 V/cm.

Keywords: *electroporation, cell swelling, permeabilization.*

1. INTRODUCTION

Exposing the living cell to an external electric field causes an accumulation of charges on both sides of the plasma membrane and consequently the formation of an induced transmembrane voltage (ITV). If ITV reaches a certain value (~0.2– 1 V), membrane permeability increases, allowing molecules for which the plasma membrane is poorly permeable under physiological conditions, to enter or exit the cell [1]. Electroporation mainly results in transient increase in membrane permeability when these membranes – which are mainly composed of lipid bilayers – are exposed to very short and intense electric fields. The physical mechanism responsible for increased permeability is thought to be the formation of nano-scale defects termed pores (thus electroporation) in the lipid structure [2]. When the electric field is not too strong and the exposure is not too long, the pores reseal in seconds to minutes after exposure and the cells restore their normal activity [1]. Our goal was to study how the volumes of electroporated and nonporated cells change in an increasing electric field. Three different types of cells 1/ nonporated, 2/ porated at E=0,75kV/cm and porated at E = 2.3 kV/cm, were investigated.

Two properties of a lipid bilayer would render it susceptible to influence by an applied electric field: the charges or the electric dipoles of the lipid molecule and the small but nevertheless finite permeability of the bilayer to ions. The former would cause lipid molecules to reorient under an intense electric field, thus creating hydrophilic pores and impairing the bilayer to serve as a barrier against ions [3].

Swelling was reported to be a general behavior of cells after electropermeabilization [4] and is thought to be an important factor for the post-poration evolution of the cell [5].

In our present work we propose a strategy to study the cellular swelling process after poration, using a *combined technique comprising optical tweezers and image analysis*. The study was conducted using different poration voltages.

2. EXPERIMENTAL SECTION

2.1. Cells.

B16F10 cells were grown for 48 h in DMEM (w 4.5 g/L Glucose, w 1% L-Glutamine, w Phenol Red, Sigma Aldrich, EU), supplemented with antibiotics (Penicillin-Streptomycin-Neomycin, Sigma Aldrich, EU) and 10% Fetal Bovine Serum (Sigma Aldrich, EU). Cells were detached using Trypsin (0.5 g/L Porcine Trypsin – 0.2 g/L EDTA-4Na in 0.09%NaCl, Sigma Aldrich, EU). Mannitol solution (300 mM in MilliQ water, pH 7-7.4, between 10 and 15 µS/cm) was used to wash the cells 3 times by centrifugation (200g, for 3 minutes at room temperature). The supernatant was each time carefully removed to reduce the electric conductivity of the suspension as much as possible and finally resuspended in Mannitol at a concentration of approximately 5 x 10^5 cells/ml. As a rule, the final conductivity was below 15 µS/cm.

2.2. Electroporation.

50 µl of cellular suspension were placed in an electroporation cuvette (with 2 mm gap between the Aluminum electrodes). The cells were electroporated with 8 unipolar rectangular pulses (100µs pulse duration, 0.75 and 2.3 kV pulse amplitude) delivered using Electro Cell B10 from β Tech Generator (Toulouse, France).

2.3. Image acquisition and processing.

After poration, a drop of cellular suspension was placed on a microscope slide. One single cell was trapped using the optical tweezers and images were recorded within 100 seconds after poration using the AxioCam camera MRm (Carl Zeiss, Germany) and Axio Vision Rel.4.8 software, as follows: an initial image immediately after cell trapping and a final image at 100 seconds after pulse application. Cells volumes were computed using ImageJ 1.46r (NIH, Bethesda, Md, USA). Using a macro, we fitted the shape of the cell to an ellipse and finally got the volume of each cell at the two moments described above.

3. RESULTS SECTION

It is known from literature that with the increasing pulse voltage, the area of porated membrane is increasing. Our results show that nonporated cells as well as cells porated at 0.75 kV/cm do not show a measurable increase in volume, while cells porated at 2.30 kV/cm swell by approximately 8 % (vol.) (Table 1). The Kruskal-Wallis test for nonparametric independent samples applied to porated cells as compared to controls (nonporated cells) gave for E= 0.75 kV/cm, $p = 0.14$ (no significant difference) and for E= 2.30 V/cm, $p = 4.5 \times 10^{-4}$ (strongly significant difference between sample and control) as shown in Fig. 1. A possible explanation of these results could be that at 0.75 kV/cm the poration process may be reversible while at 2.30 kV/cm it is, most probably, irreversible.

Figure 1. The comparative representation of relative volume variation of nonporated cells and cells exposed to electric pulses of E=0,75kV/cm and E = 2.3 kV/cm

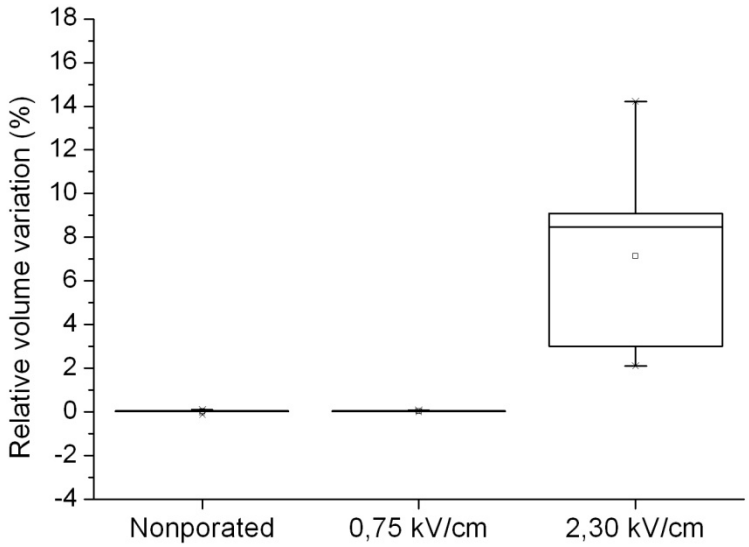

Table 1. The relative variation of the cellular volume (ΔV/V) for nonporated and porated cells at two different voltage values

ΔV/V (%)		
Nonporated	**Ep 0.75 (kV/cm)**	**Ep 2.3(kV/cm)**
0.062	0.004	8.472
0.057	-0.007	2.099
0.067	0.080	14.226
0.038	-0.003	9.023
0.029	-0.010	9.092
0.047	0.042	3.971
0.011	-0.004	3.011
0.005	-0.010	
-0.139	-0.004	
0.025	0.042	
-0.009		
0.096		

4. CONCLUSIONS

The details of the cellular changes occurring under electroporation process are important since electroporation is a modern technique with many applications in cancer therapy and food industry. It is progressively improving based on results of research regarding the details of the molecular and cellular mechanisms of the electroporation process. We show in our work that irreversible swelling of the cells occurs at pulse voltages higher than 0.75 kV/cm. Further experiments will refine these results, in order to detect the threshold pulse voltage at which a significant swelling occurs. This is varying, of course, with the cell type and pulse characteristics, other than voltage; these parameters will be taken into account in a future study.

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