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Evaluation of DMSO effects on cell electrical parameters using dielectrophoresis

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Abstract

Mouse fibroblasts were exposed to buffers containing various concentrations of DMSO and dielectrophoretic spectra were acquired. Based on these spectra, electric parameters of the cell (cytosol conductivity, membrane conductivity and membrane permittivity) were computed. A decrease of all electrical parameters was observed with the increase of the DMSO concentration. Results were analyzed in terms of DMSO perturbation of the structured water layers adjacent to the membrane and of lipid packing.

1. Introduction

Cell membrane permeabilization is an important process used in various applications in pharmacology, cosmetics, biotechnology, and clinical medicine. It can be accomplished either by physical (e.g., electric pulses, ultrasounds, laser radiation) or by chemical (e.g., detergents, aprotic solvents, ionophores, antibiotics) agents [1]–[3].

Since permeabilization is supposed to affect the electric properties of the plasma membrane and cell cytosol, dielectrophoresis (DEP) appears to be a method of choice to investigate this process. DEP is the phenomenon by which a dielectric body exposed to a non-homogenous alternating electric field, experiences a force along the electric field amplitude gradient [4].

We previously used DEP to analyze the changes caused in cells by their exposure to permeabilizing electric fields [5], [6]. These short, intense electric pulses cause a transient or permanent cell membrane permeabilization, process known as electroporation [1]. In the present study we explore the modulation of the cell permeabilization not by a physical agent, but by a chemical one, dimethyl sulfoxide (DMSO).

DMSO is a molecule used in various biological and biotechnological applications (e.g., as drug delivery enhancer [7], or as cell fusogenic agent [8]) mainly due to its effect on the lipid packing of the cell membrane. DMSO is also used as a cryoprotectant [9] and as an aprotic solvent [10]. It was shown that DMSO increases the permeability of the lipid membrane [11], [12].

DEP spectra were acquired on hamster lung fibroblasts (DC3F) suspended in buffers with different concentrations of DMSO. Based on these spectra, electric parameters of cells (cytosol conductivity, membrane conductivity and membrane permittivity) were computed.

2. Materials and Methods

The 3DEP dielectrophoresis analysis system from DEPtech - LABtech (UK) was used to acquire the DEP spectra. This device is a multichannel function generator which delivers specific AC frequencies on different wells of a 20 wells chip (DEPwell 805). After an equilibration time, images are acquired on each well, and, from the analysis of the cell density distribution, the relative DEP force is computed for each applied frequency.

The following were used for cell growth, passage, and detachment: DMEM culture medium (Dulbecco's Modified Eagle's Medium, Sigma-Aldrich, D5796), fetal bovine serum (Sigma-Aldrich, F7524), L-glutamine (ATCC, 30-2003) and Trypsin-EDTA (Sigma-Aldrich, T4174).

Cells were grown in 75 cm^2 flasks containing DMEM medium (Dulbecco's Modified Eagle's Medium, Sigma-Aldrich, D5796), supplemented with 10% fetal bovine serum (Sigma-Aldrich, F7524) and 1% L-glutamine (ATCC, 30-2003). The cells were harvested by Trypsin-EDTA (Sigma-Aldrich, T4174). The cell suspension was centrifuged at 300 x g for 5 minutes at 24 °C. The supernatant was discarded, and the pellet was washed twice

with 300 mM sucrose solution. The pellet was resuspended in one of the buffers of different DMSO concentrations (5, 20, 30 % v/v in 250 mM Sucrose, 8 mM Na_2HPO_4, 2 mM KH_2PO_4, 1 mM $MgCl_2$, pH 7.4, 286 mOsm/kg). The final conductivity was adjusted to exactly 0.01 S/m using the 300 mM Sucrose solution. The DEP chip was loaded with 100 µL cell suspension.

After 10 min of incubation in DMSO, the acquisition of the spectra was done (frequency range 10 kHz – 40 MHz, during 60 s). Each experiment was repeated ~ 12 times.

Medium conductivity after 10 min of incubation and cell diameters were measured for further use in electric parameters computation.

DEP spectra were acquired using the 3DEP 1.5.1.68 software (DEPtech – LABtech, UK).

3. Data processing

Data processing was realized with the use of a DEP spectra analysis program developed by our own laboratory (DEP Plotter 1.1) presuming the single shell model for describing the DEP spectrum of a cell.

The electric parameters of the cells (cytosol conductivity, membrane conductivity and membrane permittivity) were obtained by fitting the DEP spectra based on formulas described below.
DEP force (F_{DEP}) is given by equation (1):

$$F_{DEP} = 2\pi r^3 \varepsilon_e Re(CM) \nabla E^2 \qquad (1)$$

where r is the cell radius, ε_e the external medium absolute permittivity, E the electric field amplitude, and $Re(CM)$ the real part of the Clausius Mossotti factor which describes the DEP force dependence on the field frequency and on the geometrical and electric parameters of the cell. In terms of complex electric parameters, for a dielectric homogenous spherical particle representing a cell, CM is given by [4]:

$$CM(\omega) = \frac{\hat{\varepsilon}_p - \hat{\varepsilon}_e}{\hat{\varepsilon}_p + 2\hat{\varepsilon}_e} \qquad (2)$$

where ω is the field angular frequency ($\omega = 2\pi f$, f is AC field frequency), $\hat{\varepsilon}_p$ the complex permittivity of the particle, and $\hat{\varepsilon}_e$ the external medium complex permittivity. Considering the single shell model, the equivalent complex permittivity of that particle is given by [4]:

$$\hat{\varepsilon}_p = \frac{\left(\frac{r}{r-d}\right)^3 + 2\left(\frac{\hat{\varepsilon}_i - \hat{\varepsilon}_m}{\hat{\varepsilon}_i + 2\hat{\varepsilon}_m}\right)}{\left(\frac{r}{r-d}\right)^3 - \left(\frac{\hat{\varepsilon}_i - \hat{\varepsilon}_m}{\hat{\varepsilon}_i + 2\hat{\varepsilon}_m}\right)} \qquad (3)$$

where r is the cell radius, d the membrane thickness and $\hat{\varepsilon}_i$ and $\hat{\varepsilon}_m$ the cytosol and the membrane complex permittivities, respectively. The complex permittivity is calculated by the equation below:

$$\hat{\varepsilon} = \varepsilon - j\frac{\sigma}{\omega} \qquad (4)$$

where σ is the electrical conductivity, ω the field angular frequency and ε the absolute permittivity.

Statistics (ANOVA One way with a given level of 0.05 and Tukey test, given that there is a Gaussian distribution of data) was done in FluorEssence™ 2.1 (developed by Horiba under OriginPro™).

4. Results and Discussion

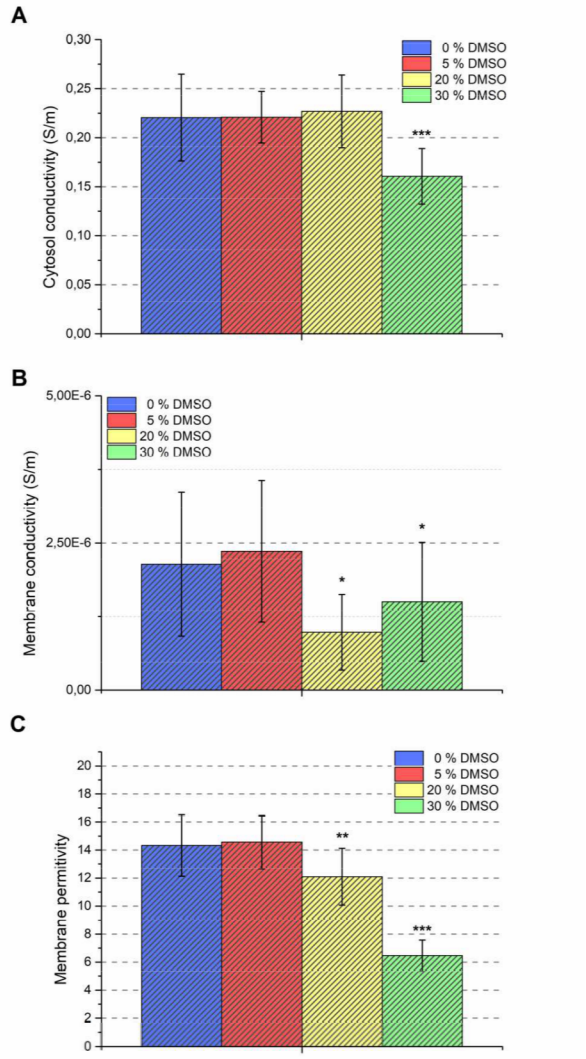

Figure 1. Electric parameters of the cells: (A) cytosol conductivity, (B) membrane conductivity and (C) membrane permittivity of DC3F cells suspended in 0.01 S/m sucrose based buffers with different concentrations of DMSO (0, 5, 20 and 30 % v/v). *, ** and *** signifies P < 0.05, 0.01 and 0.001, with respect to the control.

As can be seen in Figure 1, the concentration of 5 % v/v DMSO does not modify any of the computed electrical parameters of the cells. The 20 % v/v DMSO concentration lowers both membrane electrical parameters, conductivity and permittivity, and this effect is statistically different when compared to the control. At 30 % v/v DMSO there is a statistically significant decrease of all three electrical parameters. Even more, in the case of the membrane permittivity this statistical difference is also present between the 20 and 30 % v/v DMSO concentrations.

The behavior of the membrane electric parameters in the presence of DMSO is different: while the membrane electrical conductivity has a drop between 5 and 20 % v/v DMSO, the permittivity evolves gradually with the increase of the DMSO concentration.

In previous work [12] molecular dynamics (MD) simulations on DOPC lipid bilayers showed the existence of three regimes of action of DMSO, depending on its concentration in the bilayer: membrane loosening, pore formation and bilayer collapse. Based on those MD results, the decrease of the cytosol conductivity seen at 30 % v/v DMSO concentration may be attributed to the permeabilizing effect of DMSO, which allows the leakage of ions out of the cell.

The membrane electrical parameters, as observed by DEP measurements, are determined not only by the intrinsic electric properties of the lipid bilayer but also by the existence of adjacent structured water layers [13]. Considering that DMSO conductivity is lower than the one of water (3×10^{-6} vs. 5×10^{-6} S/m), it is reasonable to conclude that the presence of DMSO decreases the observed membrane conductivity. The same rationale applies for membrane permitivity modifications induced by DMSO, since DMSO permitivity is ~ 45, while the one of bulk water is 78.

5. Conclusions

Our study showed that DEP is an appropriate method to determine modifications of cell electrical properties induced by a chemical agent able to destabilize the membrane, in our case, the DMSO. It was possible to evidence the role of DMSO in the perturbation of the structured water layers adjacent to the membrane.

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