Zeta potential for cell surface – nanoenvironment interaction assessment

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Introduction

An extensive literature has described the efficiency of cell electro-permeabilization (electroporation) based on electric pulse parameters and chemical composition of the media used. In particular, for the effect of media conductivity and composition, it has shown that decreasing media conductivity, electroporation efficiency was increased. Media conductivity had also a significant effect on cell survival. Moreover, gene electrotransfer and cell electrofusion are media composition dependent [1].

So far, a widely used step in most electroporation protocols is the transfer of cells from the cell culture media to the standard electroporation buffer or to another buffer with a different composition. Therefore, it is crucial to assess firstly the cell surface-media interaction (interface) to understand the subsequent phenomena due to the application of pulsed electric fields.

Here we attempt to evaluate the influence of different buffers, used for cell electroporation, on cells with measuring Zeta potential. Our results confirm our initial assumption about the influence of changing cell culture media to another media (electroporation buffer) on the cell surface-nanoenvironment interaction: the Zeta potential values in different buffers were different from the one measured in culture media. We conclude the interaction of the cell surface-cell-surrounding nanoenvironment is important for understanding the subsequent effects of pulsed electric fields.

Materials and Methods

Cell culture and sample preparation

Human cancer bone osteosarcoma epithelial cells (U2OS) were obtained from American Type Culture Collection, human non-transformed retinal pigment epithelium cells (RPE1; immortalized cells stably expressing telomerase reverse transcriptase hTERT) were obtained from Dr. M. Bonhivers (Université Bordeaux, Bordeaux, France). Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and antibiotics (100 U/ml penicillin, and 0.1 mg/ml streptomycin). Cells were cultured at 37°C in a humidified and 5% CO_2 atmosphere until confluence in 25 cm^2 flasks. Typically, cells were passaged by trypsinization (0.025% Trypsin, Sigma). Cell pellet was collected by centrifugation (250×g, 7 min, 4 ºC) and re-suspended in corresponding buffers. Each sample contained the same density of cells 1×10^5 cells/ml.

Buffers and their properties

Three buffers were considered in addition to the cell culture media. The buffers were prepared with chemicals purchased from Sigma. Additionally, to their chemical composition, the buffers were further characterized by measuring their conductivity, pH, viscosity, dielectric permittivity and refractive index. All measurements were carried out at 37 ºC. Table 1 summarizes physicochemical properties of buffers and used culture medium.

Zeta potential

The Zeta (ξ) potential is the electrostatic potential that exists at the shear plane of a particle and it is related to both surface charge and the local environment of the particle. ZetaPALS from Brookhaven Instruments Corporation (USA) was used for the determination of the Zeta potential of our samples in different buffers at 37 ºC. The Zeta potential was calculated based on the Smoluchowski equation of the electrophoretic mobility. Each value was obtained as the average of three subsequent runs of the instrument, each with at least 25 measurements. The measurements were carried out three times.

Results and discussion

Buffers physicochemical properties

The properties of the buffers are shown in Table 1. The viscosity (η) was determined with the μRheology mode of the ZetaPALS instrument and using a polystyrene research particles probe of 0.970 μm diameter (Microparticles GmbH, Germany). The results show that low conductivity buffers have a higher viscosity (LCB1=0.83 mPa.s and LCB2= 0.825 mPa.s, probably due to high sucrose content) compared to the cell culture media and high conductivity buffer (DMEM=0.706 mPa.s and HCB1=0.742 mPa.s).

Refractive index was measured with a refractometer (DSRλ, SCHMIDT + HAENSCH GmbH & Co. Germany). The results show a slight difference between low conductivity buffers and high conductivity buffers and cell culture media.

Dielectric permittivity was extracted from capacitance measurements of the buffers with an Impedance analyzer (4192 A-HP, 5 Hz-13 MHz) at the frequency 10 MHz. The results show that the dielectric permittivity of the low

Table 1: Buffers and their physicochemical properties at 37 ºC

	DMEM	HCB1	LCB1	LCB2
Composition	Full DMEM	10 mM KH_2PO_4/K_2HPO_4, 1 mM $MgCl_2$, 150 mM NaCl	10 mM HEPES, 250 mM Sucrose, 0.7 mM $MgCl_2$, 0.3 mM $CaCl_2$	10 mM KH_2PO_4/K_2HPO_4, 1 mM $MgCl_2$, 250 mM Sucrose
pH	7.25	7.20	7.20	7.70
Conductivity [mS/cm]	14.425	15.6	0.351	0.989
Refractive index	1.3333	1.3316	1.3418	1.3415
Viscosity [mPa.s]	0.706	0.742	0.830	0.825
Dielectricc permitivity	72.7	72.7	61.9	72.6

conductivity buffer (LCB1) is lower ε= 61.9 compared to other buffers and cell culture media which they are around ε= 72.

Zeta potential

Experimental results of Zeta potential measurements of cells in media (control) and in different buffers with different physicochemical properties are shown in fig. 1. The zeta potential characterizes the electrical double layer potential on the cell surface; its value should be dependent on the biochemical composition of the plasma if the solvent composition is constant as was measured in culture media for different cell type [2]. The zeta potential of U2OS was -8.94±1.97 mV, whereas the zeta potential for RPE1 cells was -11.36±1.14 mV in DMEM culture media. It is well established that cells of different tissue origin differ in their plasma membrane lipid, protein, and carbohydrate composition. The negative values of zeta potential of cell membrane at physiological conditions are dependent on charged lipid concentration and on bound proteins [3]. The results of Zeta potential of the cells after transfer to different buffers are very interesting.

The fig.1 of U2OS cells (upper figure) shows the value of Zeta potential measurements in high conductivity buffer slightly decreased from -8.94±1.97 mV in culture media to -12.23±1.78 mV. Similarly was observed with RPE1 cells when transferred to high conductivity buffer (lower figure), where the Zeta potential value slightly changed from -11.36±1.14 mV in culture media to -12.70±2.22 mV. On the same figure, when U2OS cells were transferred to low conductivity buffers, the Zeta potential values dropped significantly from -8.94±1.97 mV in DMEM to -24.08±3.15 mV and -29.69±3.63 mV in LCB1 and LCB2 respectively. Whereas, for RPE1 cells when transferred to low conductivity buffers, the Zeta potential values were not significantly changed. The value of Zeta potential in culture media dropped from -11.36±1.14 mV to -17.40±4.00 mV and -18.31±4.94 mV for LCB1 and LCB2 respectively.

Based on these results of Zeta potential measurements, we demonstrate that the physicochemical properties of buffers and the cell type are tightly dependent via their interaction. We show that Zeta potential is a useful parameter for cell surface-buffer interaction assessment, which takes in account buffer physicochemical properties and cell surface properties (cell type).

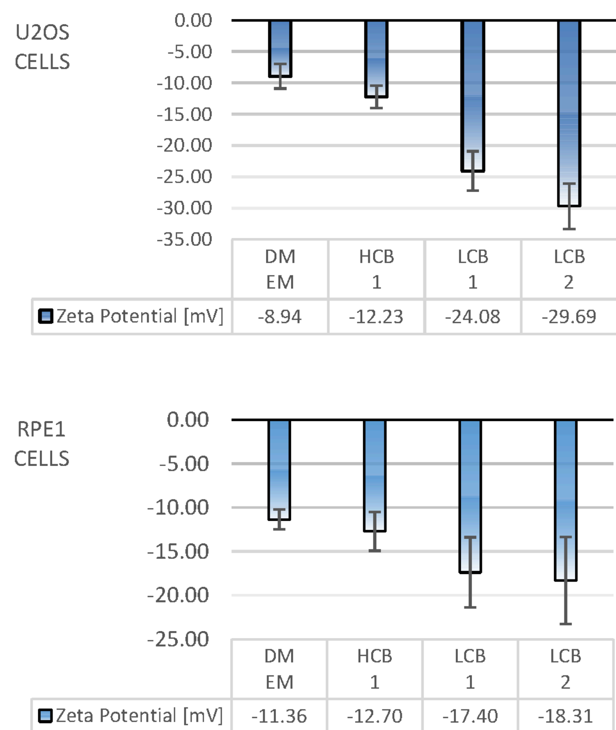
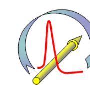


Figure 1: Zeta potential measurements of cells in media and in different buffers, U2OS cells (upper) and RPE1 cells (lower). Error bars standard deviation for N=9.

The importance of cell surface-buffer interaction as a key step for pulsed electric field effects assessment can be supported by the works done recently, such as the work of Dermol et al [4], where they tested different buffers similar to ours at 37 ºC for cell sensitization by microsecond pulsed electric field. They found that cell electro-sensitization is present only in cell culture media, therefore is buffer dependent phenomena. Moreover, in the work of Dutta et al [5], they exposed cells to nanosecond pulses, and they demonstrated that nanosecond pulsed electric field (nsPEF) could significantly change the cell membrane surface charge densities. Therefore, we believe that our work shed additional light on the existing works explaining



the subsequent effects of pulsed electric fields with a particular interest to the synergistic effect of the cell membrane - surrounding nanoenvironment interaction.

Conclusions

The distribution of electric charges near the cell membranes is the key for many fundamental phenomena associated with the interaction of cells with external electromagnetic fields. Herein, it is shown that cell surface-surrounding nanoenvironment interaction has a prodigious influence on the final electric charge distribution, thus the subsequent phenomena caused by the pulsed electric field. It is also shown that Zeta potential characterizes this interaction taking in account the physicochemical properties of buffers and cell type simultaneously.

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