The Dependence of Molecular Transmembrane Electrotransfer Efficiency on Medium Conductivity and Osmotic Pressure

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Electroporation is a process when after application of external electric field the permeability of cell plasma membrane increases due to formation of aqueous electropores [1]. This increase of permeability can be used for transmembrane transportation of different molecules that cannot pass the plasma membrane at normal conditions. Molecules that can be introduced into the cell in this way range from small charged species to macromolecules such as DNA. Because of versatility of the method it was adopted in clinics for drug and gene electrotransfer to treat malignant tissues [2, 3] and also for extraction of bioactive compounds from plants or food stocks [4]. In many preclinical trials molecules to be delivered in to target tissues are prepared in various solutions with different conductivities. Moreover the target tissues also have different conductivities. Therefore it is essential to know the effect of external media conductivity on the intensification of molecular transmembrane electrotransfer. The experiments whose results are shown below were performed in order to investigate the effect of external media conductivity on intracellular electrotransfer efficiency and kinetics and of ethidium bromide. Ethidium bromide was used because the quantum efficiency of this dye increases upon binding to nucleic acids inside the cell. Also, ethidium bromide was reported as a plausible dye to visualize electroporation [5].

Experiments were carried out on Chinese Hamster Ovary (CHO) cells that were grown in Dulbecco's Modified Eagle Medium (DMEM) in 37°C and 5% CO₂. The cells were passaged one to two hours prior experiment. This was enough for cells to attach on microscope coverslip, yet spherical shape was still maintained. At the start of experiment, coverslip with cells was placed on copper electrodes (constructed on microscope slide) with 2 mm gap. The medium was supplemented with 600 μM ethidium bromide. The electrodes then were placed under fluorescent microscope (Motic AE31) and subjected to high voltage electric pulse (640 V/cm or 1200 V/cm pulse strength, 100 μs pulse duration). The ethidium bromide fluorescence was then observed for 120 seconds, taking fluorescent images once per second using camera (Moticam Pro 282B). Pictures then were processed with open-source image processing program ImageJ to calculate Correlated Total Cell Fluorescence (CTCF) which is a relative measure of the cell fluorescence associated with ethidium bromide binding intracellular nucleic acids; it is measured in relative units (r. u.). CTCF was calculated as integrated fluorescence density of the cell area after background subtraction.
Three different sets of experiments were conducted using electroporation media of different conductivities (0.1 S/m, 0.5 S/m or 0.9 S/m in isoosmotic pressure) or different osmotic pressures (170 mOsm, 270 mOsm or 370 mOsm when maintaining constant conductivity of 0.1 S/m). Third set of experiments were made with different electric field amplitudes. Standard 1200 V/cm electric field was chosen as it is most common in electrotransfer experiments. Also electric field of 640 V/cm was selected to attain transmembrane potential in the range close to electroporation threshold voltage. Transmembrane potential threshold for electroporation to start was calculated from the adapted electroporation model where average radius of CHO cells were counted as 8.45 μm [6]. Each experimental point was repeated three times and at least three independent experiments were performed for each condition.

Gained results show statistically significant (p < 0.05, two-tailed Student's t-test) ethidium bromide electrodelivery when comparing 0.1 S/m and 0.9 S/m or 0.5 S/m conductivity electroporation media. Significance started from 9th second after electric pulse. There was also statistically significant difference between cells electroporated in 0.5 S/m and 0.9 S/m conductivity media that started 35 seconds from the electrical pulse application (Fig. 1). It is also seen that the efficiency of electrotransfer, measured calculating CTCF, decreases when the conductivity of external medium increases.

![Fig. 1. CTCF dependence on conductivity of electroporation media. Every fifth data point is shown to improve clarity of the figure](image)

Other set of experiments were conducted using 0.1 S/m media having hypoosmotic (170 mOsm), isoosmotic (270 mOsm) or hyperosmotic (370 mOsm) pressures. There were no statistically significant differences observed between cells electroporated in hyperosmotic and isoosmotic electroporation media. However, there was a statistically significant difference observed between cells electroporated in isoosmotic and hypoosmotic electroporation media. Difference was observed from 2nd second after the delivery of electrical impulse and lasted until 78th second after that time all the observed differences were not statistically significant (Fig. 2).
The experiments conducted using different electric pulse strengths showed statistically significant differences between cells electroporated in 0.1 S/m medium and using electrical pulse of different voltages that started at 6 seconds from the delivery of the pulse onwards. However, no statistically significant differences were observed when comparing cells electroporated using different voltages in 0.9 S/m conductivity medium. In addition to that, no statistically significant differences were observed between 0.9 S/m and 0.1 S/m conductivity media when the strength of the pulse was lowered to threshold value [5] of electroporation for CHO cells (Fig 3.) That serves to prove that the effect of external media conductivity is dependent on the strength of electric field and no significant difference is observed when the electric pulses are lowered to critical values required for electroporation and the electrotransfer efficiency is limited by the electrical component.

Fig 2. The dependence of CTCF on osmotic pressure of electroporation media. Error bars represent standard error of mean (SEM); every fifth data point is shown to improve the clarity of the figure.

Fig 3. The dependence of electrotransfer efficiency dependent on the voltage of electric impulse. Every fifth point is shown to improve the clarity of the figure.
In conclusions, we have shown statistically significant inversely proportional ratio in between external media conductivity and small molecule electrotransfer efficiency.

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**References**


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The electrotransfer efficiency was evaluated for different external medium conductivities, osmotic pressures and electric pulse voltages. It was found that increase in conductivity or decrease in electric pulse strength decreases electrotransfer efficiency. Decrease in osmotic pressure tends to decrease electrotransfer efficiency.