L6 Influence of the orientation of the electric field on the formation of the DNA membrane complex

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Duration of the experiment: 90 min
Max. number of participants: 4
Location: Cell Culture Laboratory
Level: Advanced

PREREQUISITES

Participants should be familiar with Laboratory safety (S1) and Electroporation hardware safety (S2). The basic knowledge of handling with cells is required for this laboratory practice.

The aim of this laboratory practice is to demonstrate how different orientations of the electric field influence the formation of the DNA membrane complex.

THEORETICAL BACKGROUND

Gene electrotransfer is a non-viral method used to transfer genes into living cells by means of high-voltage electric pulses [1]. An exposure of a cell to an adequate amplitude and duration of electric pulses leads to transient increase of cell membrane permeability for molecules which are otherwise deprived of membrane transport mechanisms. This allows various nonpermeant molecules, including DNA, to be transported across the membrane and enter the cell. Although mechanisms of the process are not yet fully elucidated, it was shown that several steps are crucial for gene electrotransfer: interaction of plasmid DNA (pDNA) with the cell membrane, translocation of pDNA across the membrane, migration of pDNA towards the nucleus, transfer of pDNA across the nuclear envelope and gene expression [2-4].
Many parameters (such as electric pulse protocol) or DNA size [5] can influence the first step (interaction of DNA with the cell membrane) and by that gene electrotransfer efficiency. Therefore, different electric pulse protocols are used in order to achieve maximum gene transfection [6], one of them is changing the electric field orientation during the pulse delivery [7, 8]. Since DNA is a negatively charged molecule and it is dragged towards the cell with the electrophoretic force in the opposite direction of the electric field, changing electric field orientation increases the membrane area competent for DNA entry into the cell.

EXPERIMENT

We will focus on the interaction of DNA with the cell membrane by using TOTO-1 dye. For the experiment, we will use Chinese hamster ovary cells (CHO-K1) and plasmid DNA (pEGFP-N_1) that codes for GFP (green fluorescent protein). To generate and deliver electric pulses a βtech electroporator (Electro cell B10, Betatech, France) and electrodes with 4 mm inter-electrode distance will be used. Pulses will be monitored on oscilloscope (LeCroy 9310C).

Pulse protocols (see also Figure 1):

a) SP (single polarity): the direction of electric field is the same for all pulses
b) BP (both polarities): the direction of the electric field is changed between the pulses

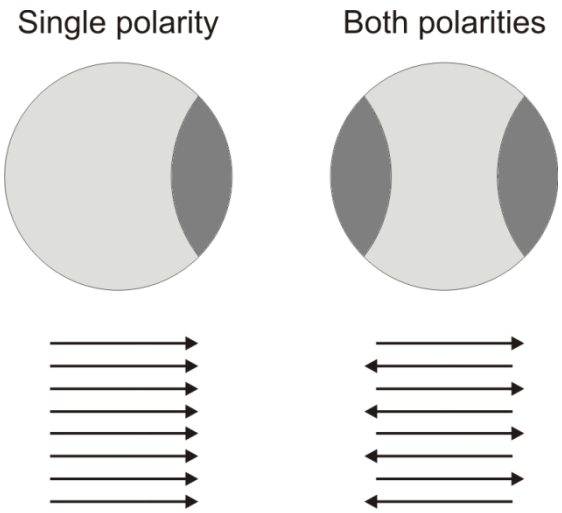

Figure 1: Two different pulse protocols will be used: single polarity (SP) and both polarities (BP).

Protocol:

Interaction of DNA with the cell membrane: CHO cells will be grown in Lab-Tek chambers as a monolayer culture in Ham's tissue culture medium for mammalian cells with 10% fetal bovine serum (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) at 37° C. Cells will be plated 1 h before the experiment in concentration 1 x 10^5 cells per chamber.

To visualize DNA interaction with cell membrane TOTO-1 nucleic acid stain (Molecular Probes-Invitrogen, Carlsbad, California, USA) will be used. The plasmid pEGFP-N1 will be labelled on ice with 2.3 x 10^{-4} M TOTO-1 DNA intercalating dye 1 h before the experiment. Plasmid concentration will be 1 µg/µl, which yields an average base pair to dye ratio of 5.

Just before the experiment remove culture medium and rinse the cells with 1 ml of electroporation buffer (10 mM phosphate buffer K_2HPO_4/KH_2PO_4, 1 mM $MgCl_2$, 250 mM sucrose; pH = 7.4). Afterwards add 500 µl of electroporation buffer containing 5 µg of labelled plasmid DNA. Then apply a train of eight pulses with amplitude of 350 V, duration of 1 ms and repetition frequency 1 Hz using single polarity or both polarities (see Pulse protocols).

Immediately after exposure of cells to electric pulses rinse the cells three times with 1 ml of electroporation buffer. Add again 500 µl of electroporation buffer and observe the interaction of DNA with the cell membrane with fluorescent microscopy using TOTO filter with excitation at 514 nm.

REFERENCES:

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EXPECTED RESULTS

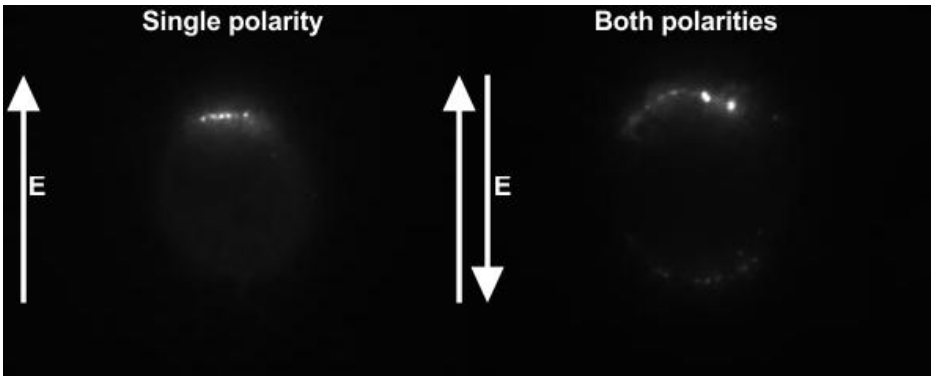

Figure 2: DNA membrane complex on one side of the membrane when the electric field is applied in one direction – single polarity (left image) or in both directions – both polarities (right image).

NOTES & RESULTS