Analysis of electric field orientations on gene electrotransfer – efficiency and visualization at the membrane level L8

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Duration of the experiment: day 1: 90 min; day 2: 60 min
Max. number of participants: 4
Location: Cell Culture Laboratory 1
Level: Advanced

PREREQUISITES

Participants should be familiar with the Safety rules and Rules for sterile work in cell culture laboratory. The basic knowledge of handling with cells is required for this laboratory practice.

THEORETICAL BACKGROUND

Gene electrotransfer is a non-viral method used to transfer genes into living cells by means of high-voltage electric pulses. 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.

Many parameters (such as electric pulse protocol) 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, one of them is changing the electric field orientation during the pulse delivery. 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.

The aim of this laboratory practice is to demonstrate how different pulse polarity affects formation of DNA – membrane complex, the efficiency of gene electrotransfer and cell viability.

EXPERIMENT

The experiment will be conducted in two parts. In the first part (a) we will focus on the interaction of DNA with the cell membrane by using TOTO-1 dye and in the second part (b) we will determine the efficiency of gene electrotransfer and cell viability. For both parts 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 high-voltage prototype generator and electrodes with four cylindrical rods, which were developed at a Laboratory of Biocybernetics will be used. Pulses will be monitored on osciloscope (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
c) OBP (orthogonal both polarities): the direction of the electric field is changed between the pulses

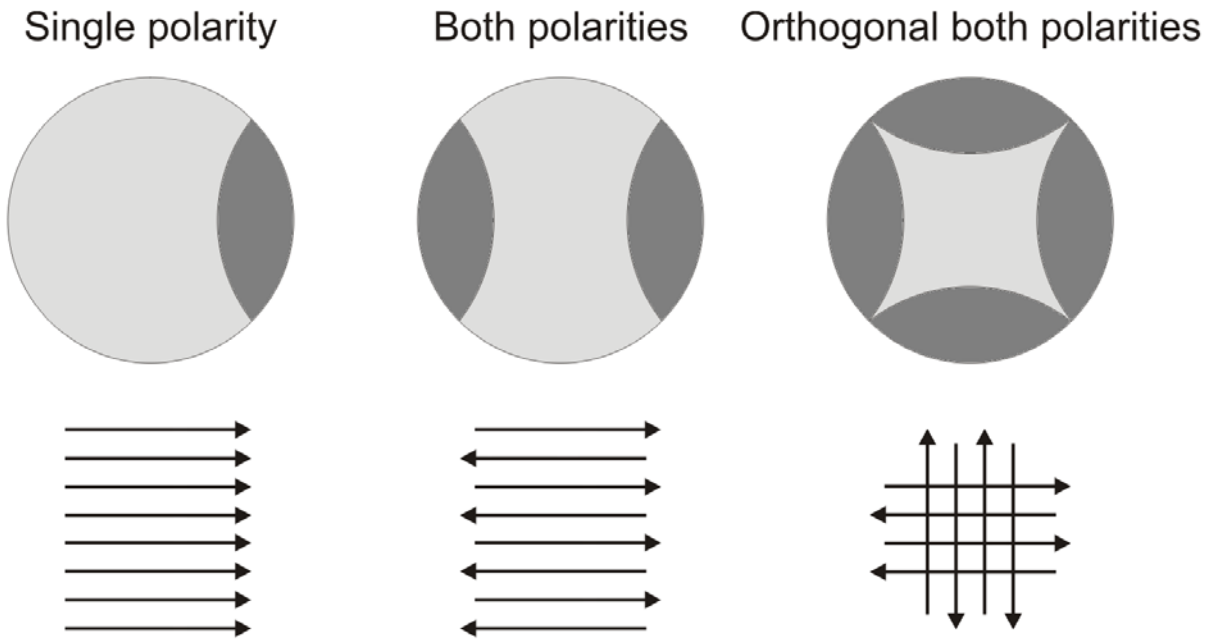

Figure 1: Three different pulse protocols will be used: single polarity (SP), both polarities (BP) and orthogonal both polarities (OBP)

Protocol 1/2:

a. 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 37º 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 (Zeiss 200, Axiovert, Germany) using 100x oil immersion objective using TOTO filter with excitation at 514 nm.

b. Gene electrotransfer with different pulse parameters: CHO cells will be grown in multiwells as a monolayer culture in Ham's tissue culture medium for mammalian cells with 10% fetal bovine serum at 37º C. Cells will be plated 24h before the experiment in concentration 5 x 10^5 cells per well.

Just before the experiment remove culture medium and replace it with 150 µl of electroporation buffer containing plasmid DNA with concentration 10 µg/ml. Incubate cells with plasmid for 2-3 minutes at room temperature. Then apply a train of eight pulses with amplitude of 350 V, duration of 1 ms and repetition frequency 1 Hz using single polarity and orthogonal both polarities (see Pulse protocols) to deliver plasmid DNA into the cells.
Cells in the control are not exposed to electric pulses.
Immediately after exposure of cells to electric pulses add 37 µl of fetal calf serum (FCS-Sigma, USA). Incubate treated cells for 5 minutes at 37º C and then add 1 ml of culture medium.

Protocol 2/2 (Determining gene electrotransfer efficiency and cell viability): After 24 h incubation at 37º C determine the difference in gene electrotransfer efficiency and cell viability for both pulse protocols by fluorescent microscopy (Leica, Wetzlar, Germany) at 20x magnification using GFP filter with excitation at 488 nm.

You will determine gene electrotransfer efficiency from the ratio between the number of green fluorescent cells (successfully transfected) and the total number of cells counted under the phase contrast. You will obtain cell survival from phase contrast images as the ratio between the number of viable cells in the treated sample and the number of viable cells in the control sample.

FURTHER READING:

Faurie C., Reberšek M., Golzio M., Kandušer M., Escoffre J. M., Pavlin M., Teissie J., Miklavčič D., Rols M. P. Electro-mediated gene transfer and expression are controlled by the life-time of DNA/membrane complex formation. *J Gene Med* 12: 117-125, 2010
Golzio M., Teissié J., Rols M. P. Direct visualization at the single-cell level of electrically mediated gene delivery. *PNAS* 99: 1292-1297, 2002
Reberšek M., Faurie C., Kandušer M., Čorović S., Teissié J., Rols M.P., Miklavčič D. Electroporator with automatic change of electric field direction improves gene electrotransfer *in vitro*. *Biomed Eng Online* 6: 25, 2007
Reberšek M., Kandušer M., Miklavčič D. Pipette tip with integrated electrodes for gene electrotransfer of cells in suspension: a feasibility study in CHO cells. *Radiol Oncol* 45: 204-208, 2011
Video Article:
Pavlin M., Haberl S., Reberšek M., Miklavčič D., Kandušer M. Changing the direction and orientation of electric field during electric pulses application improves plasmid gene transfer in vitro. *J Vis Exp*, 55: 1-3, 2011

NOTES & RESULTS

Pulse parameters	Gene electrotransfer efficiency [%]	Cell viability [%]
Single polarity		
Orthogonal both polarities		