L8 Influence of the orientation of the electric field on gene electrotransfer efficiency and cell viability

Saša Haberl Meglič, Matej Reberšek
University of Ljubljana, Faculty of Electrical Engineering

Duration of the experiment: 60 min
Max. number of participants: Unlimited
Location: Online ONLY
Level: Advanced

PREREQUISITES

No specific knowledge is required for this laboratory practice.

The aim of this laboratory practice is to demonstrate how different orientations of the electric field influence the efficiency of gene electrotransfer and cell viability.

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 non permeant 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

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 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 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) OBP (orthogonal both polarities): the direction of the electric field is changed between the pulses

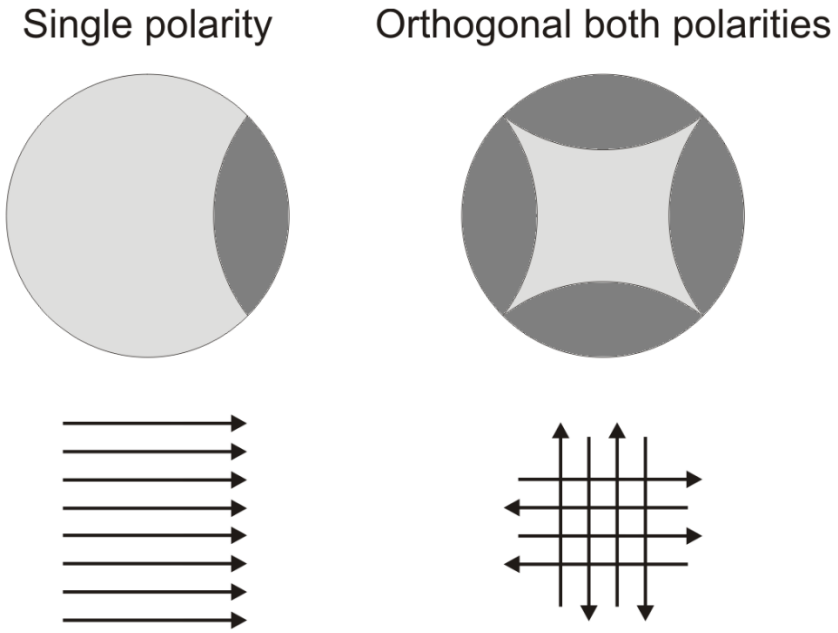

Figure 1: Two different pulse protocols will be used: single polarity (SP) and orthogonal both polarities (OBP)

Protocol 1/2 (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 37° 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 225 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 37° C and then add 1 ml of culture medium.

Protocol 2/2 (Determining gene electrotransfer efficiency and cell viability): After 24 h incubation at 37° 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.

REFERENCES:

[1] Neumann E., Schaefer-Ridder M., Wang Y., Hofschneider P.H. Gene transfer into mouse lyoma cells by electroporation in high electric fields. *EMBO J,* 1:841-845, 1982.

[2] Golzio M., Teissié J., Rols M.P. Direct visualization at the single-cell level of electrically mediated gene delivery. *PNAS,* 99:1292-1297, 2002.

[3] 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.

[4] Rosazza C., Haberl Meglič S., Zumbusch A., Rols M.P., Miklavčič D. Gene electrotransfer: a mechanistic perspective. *Curr Gene Ther,* 16:98-129, 2016.

[5] Sachdev S., Moreira S. F., Keehnen Y., Rems L.Kreutzer M. T., Boukany P. E. DNA-membrane complex formation during electroporation is DNA size-dependent. *BBA*, 1862: 183089, 2020.

[6] Urbanskas E., Jakstys B., Venckus J., Malakauskaite P., Satkauskiene I., Morkvenaite-Vilkonciene I., Satkauskas S. Interplay between electric field strength and number of short-duration pulses for efficient gene electrotransfer. *Pharm*, 17: 825, 2024.

[7] 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.

[8] 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.

[9] 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.

EXPECTED RESULTS

Single polarity: A lower efficiency of gene electrotransfer is expected, but with a higher viability of the cells.

Orthogonal both polarities: A higher efficiency of gene electrotransfer is expected, but with a lower viability of the cells.

NOTES & RESULTS

Table 1: Gene electrotransfer efficiency and cell viability 24 h after application of different orientations of electric field (single polarity and orthogonal both polarities).

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