The influence of Mg^{2+} ions on gene electrotransfer efficiency L1

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Duration of the experiment: day 1: 60 min; day 2: 60 min
Max. number of participants: 4
Location: Cell Culture Laboratory 3
Level: Basic

PREREQUISITES

Participants should be familiar with the Safety rules and Rules for sterile work in cell culture laboratory. No other specific knowledge 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 (Figure 1).

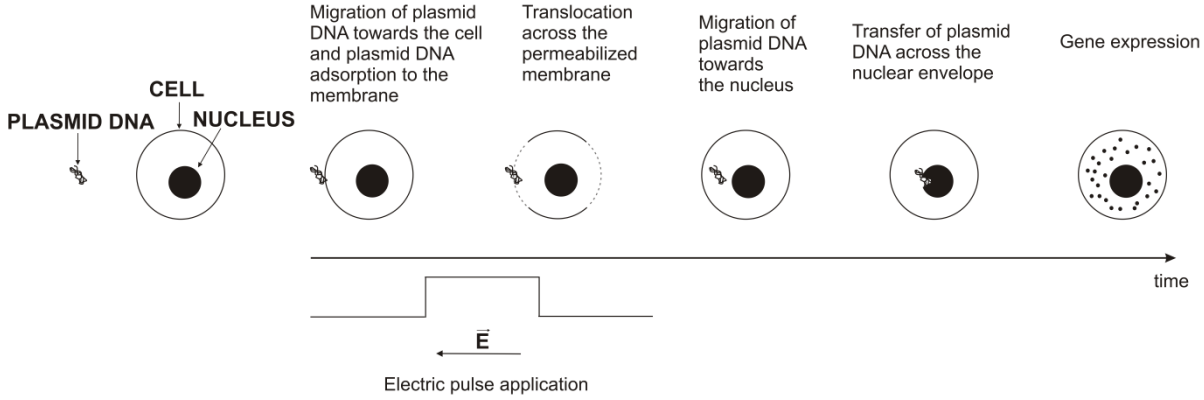

Figure 1. Steps involved in gene electrotransfer.

Many parameters have been described, which can influence the efficiency of gene electrotransfer. A few published reports have shown that the concentration of Mg^{2+} ions in electroporation media have important impact on forming a complex between DNA and the cell membrane during application of electrical pulses. Namely, DNA is negatively charged polyelectrolyte and Mg^{2+} ions can bridge the DNA with negatively charged cell membrane. But it was shown that Mg^{2+} ions at higher concentrations may bind DNA to the cell membrane strong enough to prevent translocation of DNA into the cell during electroporation and gene electrotransfer efficiency is decreased.

The aim of this laboratory practice is to demonstrate how different Mg^{2+} concentrations in electroporation media affect the efficiency of gene electrotransfer and cell viability.

EXPERIMENT

We will transfect Chinese hamster ovary cells (CHO-K1) with plasmid DNA (pEGFP-N_1) that codes for GFP (green fluorescent protein) using two different electroporation media (see Protocol section). To generate electric pulses Jouan GHT 1287 electroporator (Jouan, St. Herblain, France) will be used. Pulses will be monitored on osciloscope (LeCroy 9310C).

We will determine gene electrotransfer efficiency and cell viability for both electroporation media.

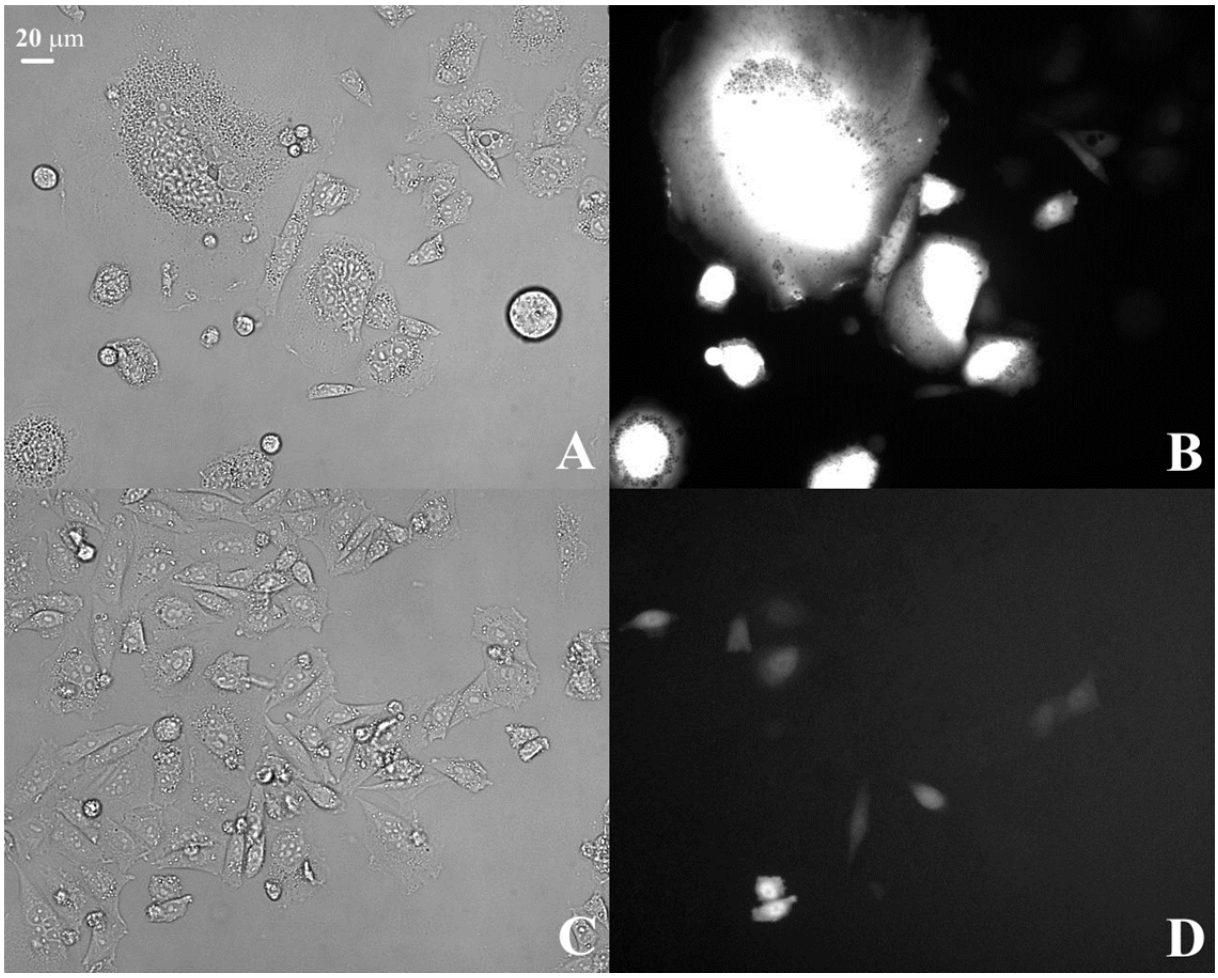

Figure 2. Gene electrotransfer of plated CHO cell 24 h after pulse application in 1 mM Mg or 50 mM Mg media. 8 x 5 ms (stainless steel wire electrodes with inter-electrode distance d = 2 mm; applied voltage U = 140 V resulting in electric field strength E = 0.7 kV/cm) pulses were applied with repetition frequency of 1 Hz to deliver pEGFP-N_1 (concentration of DNA in electroporation media was 10 μg/ml) into the cells. Phase contrast images of treated cells for (A) 1 mM Mg and (C) 50 mM Mg media and fluorescence images of treated cells for (B) 1 mM Mg and (D) 50 mM Mg media are presented. To visualize transfection 20x objective magnification was used.

Protocol 1/2 (Gene electrotransfer with different electroporation media): 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 (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) at 37º C. Cells will be plated 24 h before the experiment in concentration 5 x 10^5 cells per well.
Just before the experiment remove culture media and replace it with 150 µl of electroporation media containing plasmid DNA with concentration 10 µg/ml. Use 1 mM or 50 mM electroporation media:

a) 1 mM Mg media (10 mM phosphate buffer Na_2HPO_4/NaH_2PO_4, **1 mM $MgCl_2$**, 250 mM sucrose; pH = 7.4)
b) 50 mM Mg media (10 mM phosphate buffer Na_2HPO_4/NaH_2PO_4, **50 mM $MgCl_2$**, 100 mM sucrose; pH = 7.4)

Incubate cells with plasmid DNA for 2-3 min at room temperature. Then apply a train of eight rectangular pulses with duration of 5 ms, U = 140 V resulting in electric field strength E = 0.7 kV/cm and repetition frequency 1 Hz to deliver plasmid DNA into the cells. Use stainless steel wire electrodes with inter-electrode distance d = 2 mm. 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 min at 37ºC and then add 1 ml of culture media.

Protocol 2/2 (Determining gene electrotransfer efficiency and cell viability): After 24 h incubation at 37ºC determine the difference in gene electrotransfer efficiency and cell viability for both electroporation media 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:

Haberl S., Pavlin M., Miklavčič D. Effect of Mg ions on efficiency of gene electrotransfer and on cell electropermeabilization. *Bioelectrochemistry* 79: 265-271, 2010.
Kandušer M., Miklavčič D., Pavlin M. Mechanisms involved in gene electrotransfer using high- and low-voltage pulses-An in vitro study. *Bioelectrochemistry.* 74: 265-271, 2009.
Wong T.K., Neumann E. Electric field mediated gene transfer, *Biochem Biophys Res Commun* 107: 584-587, 1982.

NOTES & RESULTS

Electroporation media	Gene electrotransfer efficiency [%]	Cell viability [%]
1 mM Mg media		
50 mM Mg media		

NOTES & RESULTS