

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

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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).

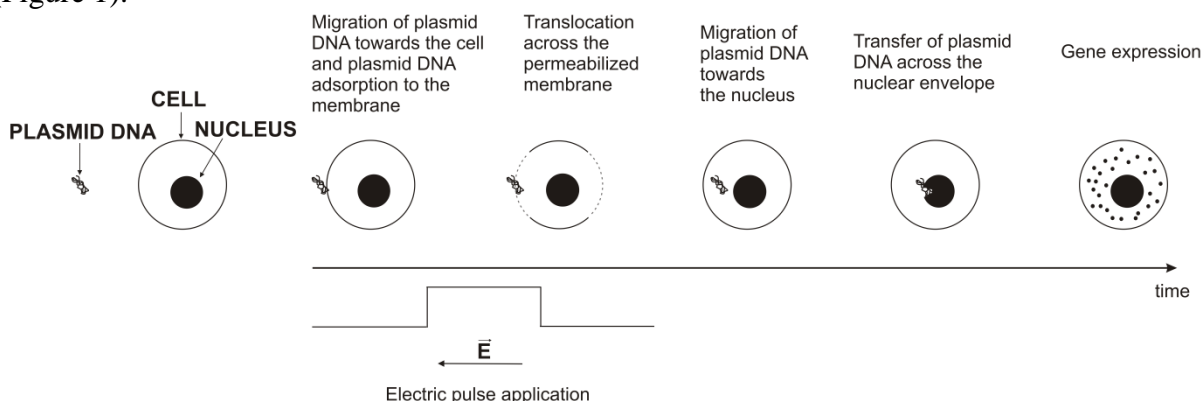


Figure 1. Steps involved in gene electrotransfer.

Many parameters have been described to 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 strongly enough to prevent translocation of DNA into the cell during electroporation thus 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-N₁) 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 oscilloscope (LeCroy 9310C).

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

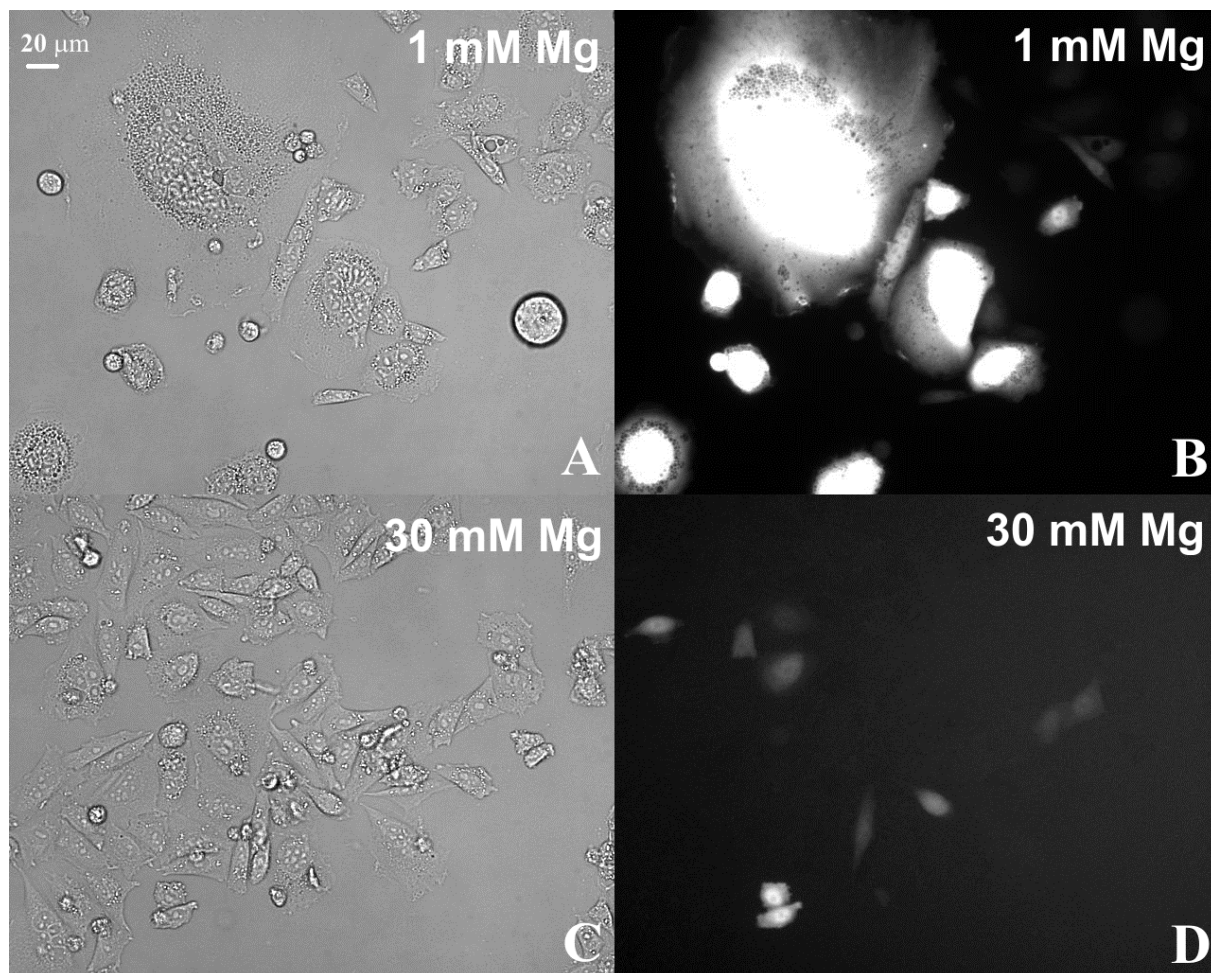


Figure 2. Gene electrotransfer of plated CHO cell 24 h after pulse application in 1 mM Mg or 30 mM Mg media. 8 x 1 ms (stainless steel wire electrodes with inter-electrode distance $d = 2$ mm; applied voltage $U = 140$ V resulting in electric field strength $E = 0.7$ kV/cm) pulses were applied with repetition frequency of 1 Hz to deliver pEGFP-N₁ (concentration of DNA in electroporation media was 10 μ g/ml) into the cells. Phase contrast images of treated cells for (A) 1 mM Mg and (C) 30 mM Mg media and fluorescence images of treated cells for (B) 1 mM Mg and (D) 30 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 37° C. Cells will be plated 24 h before the experiment in concentration 5×10^4 cells per well.

Just before the experiment remove culture media and replace it with 150 μ l of electroporation media containing plasmid DNA with concentration 10 μ g/ml. Use 1 mM or 30 mM electroporation media. Sucrose molarity has also been changed in order to attain the molarity of the media:

- a) **1 mM Mg media** (10 mM phosphate buffer Na_2HPO_4/NaH_2PO_4 , 1 mM $MgCl_2$, 250 mM sucrose; pH = 7.2)
- b) **30 mM Mg media** (10 mM phosphate buffer Na_2HPO_4/NaH_2PO_4 , 30 mM $MgCl_2$, 160 mM sucrose; pH = 7.2)

Incubate cells with plasmid DNA for 2-3 minutes at room temperature. Then apply a train of eight rectangular pulses with duration of 1 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 37.5 μ l of fetal calf serum (FCS-Sigma, USA). Incubate treated cells for 5 minutes at 37°C and then add 1 ml of culture media.

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 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 viable 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

Haberl S., Kandušer M., Flisar K., Bregar V.B., Miklavčič D., Escoffre J.M., Rols M.P., Pavlin M. Effect of different parameters used for in vitro gene electrotransfer on gene expression efficiency, cell viability and visualization of plasmid DNA at the membrane level. *J Gene Med* 15: 169-181, 2013

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

NOTES & RESULTS

Electroporation media	Treated viable cells	Viable cells in control	Green fluorescent cells	Gene electrotransfer efficiency [%]	Viability [%]
1 mM Mg media					
30 mM Mg media					

NOTES & RESULTS
