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Short communication

Effect of Mg ions on efficiency of gene electrotransfer and on cell electropermeabilization

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ABSTRACT

Gene electrotransfer is a promising nonviral method that enables DNA to be transferred into living cells with electric pulses. However, there are many parameters that determine gene electrotransfer efficiency. One of the steps involved in gene electrotransfer is interaction of DNA with the cell membrane. Divalent cations in the electroporative media can influence the anchoring of DNA to the cell membrane and by that gene electrotransfer efficiency. Here we report the effect of different concentrations of Mg^{2+} on electropermeabilization for small molecule (propidium iodide), gene electrotransfer and viability of the cells. We also used TOTO-1 dye to visualize DNA-cell membrane interaction for different [Mg]. For this purpose, we used different electroporative media with increasing [Mg]. Our study shows that higher [Mg] lead to higher electropermeabilization for propidium iodide and higher viability, while causing lower gene electrotransfer efficiency. Because we observed higher TOTO-1 labeled DNA at cell surface when using higher [Mg], we suggest that Mg^{2+} ions can bind DNA at cell surface at such strength that cannot pass into the cell during application of electric pulses, which can lead to lower gene transfection. There may also be other mechanisms involved, since there are many steps of gene electrotransfer on which Mg^{2+} ions can have an effect on. Our results also imply that membrane permeability changes are not sufficient for an efficient gene electrotransfer.

1. Introduction

Exposing cells to short intense electric pulses increases permeability of cell membrane. It has been shown on lipid membrane model that electric field hastily reorients lipid heads in the membrane and that the number of local spots with fluid conformation increases [1]. The phenomenon is shown as electroporation or electropermeabilization. It has been used for many years to introduce small molecules that otherwise cannot pass the cell membrane into cells [2–4]. In 1982 Neumann with colleagues first achieved successful transfection of a foreign gene into eukaryotic cells with electric pulses [5,6]. Even though today gene electrotransfer is widely used to transfect all types of cells and represents a safer alternative to viral vectors, the processes underlying transfer of genetic material through cell membrane and into the cell are still not completely understood [7,8].

Different mechanisms of electropermeabilization for small molecules have been reported [9–14]. In addition, gene electrotransfer mechanisms are also available in the literature which suggests that several steps are involved in gene electrotransfer [10,13,15–21]: (a) formation of a complex between DNA and cell membrane, (b) translocation of DNA across the permeabilized membrane, (c) transfer of DNA from cytoplasm into nucleus and (d) gene expression.

The main obstacle in gene electrotransfer of mammalian cells is its low efficiency, which depends not only on permeability changes of cell membrane but also on the way DNA interacts with the membrane and migrates towards the nucleus. Although several studies showed that different parameters (e.g. cell type, temperature, parameters of electric pulses, ions in electroporative media) have influence on the efficiency of uptake of small molecules as well as on gene electrotransfer [10,22–39], it was also suggested that one of the key parameters which affect the process of DNA interaction with the cell membrane is the concentration of ions in media [15,17,40]. It was suggested that especially divalent cations (such as Ca^{2+} or Mg^{2+}) may have important impact on forming a complex between DNA and the cell membrane during application of electrical pulses, which can lead to the improvement of gene electrotransfer [17,29,40]. Namely, since DNA is negatively charged polyelectrolyte, divalent cations can bridge the DNA with negatively charged cell membrane during application of electric pulses. This hypothesis was supported by the study of anionic unilamellar vesicles, where DNA adsorption to vesicle membrane was greatly enhanced with increasing concentration of divalent cations such as Ca^{2+} ions [41,42].

Up to now only few researchers have experimentally investigated the effect of different ions on gene electrotransfer efficiency in vitro or in vivo [5,6,17,28,29,32,40], however their results are contradictory.

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In one of the in vitro studies researches observed increased gene electrotransfer efficiency for increased [Mg] from 1 to 8 mM [17]. However, this is not in agreement with Neumann et al. [5,6] study. This can be a consequence of small DNA concentration used by Xie and Tsong [17], which resulted in a relatively low percentage of transfection (max. 9%) and therefore higher [Mg] could have a positive effect at such low plasmid concentration.

In in vivo studies the influence of a wide range of ionic composition in electroporative media on gene electrotransfer was also analyzed. It was demonstrated that gene expression decreased for higher concentrations of ions in used media [28]. Further some researchers showed that higher concentration of divalent cations limits plasmid DNA entrance into the cell during electroporation [32]. They proposed that higher concentration of divalent cations alters stability and physical properties of DNA molecules. However, in another in vivo study it was shown, that gene electrotransfer efficiency in mice is improved by increasing the concentrations of ions in initial injected media [29].

Hence, we report here a study of the influence of different [Mg] in electroporative media on electropermeabilization for propidium iodide (PI), cell viability and gene electrotransfer. The main objective of our study was to understand the role of Mg^{2+} ions on different steps of gene electrotransfer by analyzing separately effects of Mg^{2+} ions on electropermeabilization for PI, viability and transfection.

2. Materials and methods

2.1. Cell culture

Chinese hamster ovary cells (CHO-K1) were grown in Ham's tissue culture medium for mammalian cells with 10% fetal bovine serum (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) at 37 °C in a humidified 5% CO_2 atmosphere in the incubator (Kambič, Slovenia).

Electroporation was performed on 24 hour old cell culture in different electroporative media.

2.2. Plasmid DNA

Plasmid pEGFP-N_1 (Clontech Laboratories Inc., Mountain View, CA, USA) encoding green fluorescent protein (GFP) was amplified in DH5α strain of Escherichia coli and isolated with HiSpeed Plasmid Maxi Kit (Qiagen, Hilden, Germany). Plasmid DNA concentration was spectrophotometrically determined at 260 nm and confirmed by gel electrophoresis.

2.3. Electropermeabilization for propidium iodide

To evaluate electropermeabilization for propidium iodide of CHO cells in different electroporative media with different concentration of Mg^{2+} ([Mg] = 1, 4, 10 and 50 mM), propidium iodide (PI) was used. PI is a small molecule which enters a cell, if the membrane of the cell is permeabilized [43]. All electroporative media were isoosmolar (10 mM phosphate buffer, NaH_2PO_4/Na_2HPO_4, pH = 7.4), where media with [Mg] = 1 mM represents standard electroporative media.

Cell suspension was prepared by 0.25% trypsin/EDTA solution (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany), centrifuged for 5 min at 1000 rpm (180×*g*) at 4 °C (Sigma, Germany) and resuspended in different electroporative media to a cell density of $\rho = 2.5 \times 10^6$ cells/ml. Because of easier survey of the results we used only electroporative media with [Mg] = 1 mM, 4 mM, 10 mM and 50 mM. For electroporation, cuvettes with built in aluminium electrodes were used (Eppendorf, Hamburg, Germany).

The volume of the cells placed in cuvette was 200 µl (for each electric pulse parameter). Immediately before electric pulse application, 2 µl of 0.15 mM PI was added to the media. Final concentration of PI in a sample was 10 µg/ml.

Samples were then exposed to electric pulses to deliver PI into the cells using Cliniporator™ (IGEA s.r.l., Carpi, Modena, Italy) pulse generator. A train of four rectangular pulses with duration of 200 µs and repetition frequency 1 Hz was applied. The applied voltages were 240 V, 400 V and 560 V which resulted in 0.6 kV/cm, 1.0 kV/cm and 1.4 kV/cm *E*, respectively. Applied electric field is defined by

$$E = U(\text{appl}) / d, \tag{1}$$

where *U*(appl) denotes applied voltage and *d* electrode distance (*d* = 4 mm). To achieve saturation of fluorescence of PI in cells we also exposed cells to *E* = 1.8 kV/cm. No electric pulses were applied to cells in control.

After pulses were applied, cells were incubated for 3 min at room temperature (22 °C) and then centrifuged for 5 min at 1000 rpm (180×*g*) at 4 °C to remove extracellular PI that did not enter the cells. 200 µl of fresh media was added and the uptake of PI was evaluated with spectrofluorometer (Tecan infinite M200, Tecan Austria GmbH) at 617 nm. The permeabilization (uptake of PI) is defined as:

$$\text{Permeabilization } (\%) = \frac{F_{(\text{PI,E})} - F_{(\text{PI,E}=0)}}{F_{(\text{PI,max})} - F_{(\text{PI,E}=0)}} \cdot 100, \tag{2}$$

where $F_{(\text{PI, E})}$ denotes fluorescence intensity of cells subjected to electric pulses, $F_{(\text{PI, E}=0)}$ fluorescence intensity of cells at *E* = 0, i.e. cells in control, and $F_{(\text{PI, max})}$ fluorescence intensity of cells at *E* = 1.8 kV/cm, i.e. where saturation fluorescence is achieved.

2.4. Cell viability

Cell viability was evaluated with crystal violet dye elution method (CVDE) as previously described [44]. After exposing cells to electric pulses with plasmid DNA in concentration 40 µg/ml, the cells were plated in multiwells at a cell density of $\rho = 1 \times 10^5$ cells/ml and grown for 24 h in cell culture medium at 37 °C in a humidified 5% CO_2 atmosphere in the incubator. Because of easier survey of the results we used only electroporative media with [Mg] = 1, 4, 10 and 50 mM.

After 24 h samples were stained with 0.1% crystal violet (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) solution in sodium phosphate media (10 mM phosphate buffer, NaH_2PO_4/Na_2HPO_4, pH = 7.4) for 30 min at room temperature (22 °C). After incubation, crystal violet was removed and cells were washed with sodium phosphate media and lysed with 10% acetic acid. The same staining protocol with crystal violet was performed also in wells without cells (background wells). CVDE is a simple assay that evaluates cell density by staining DNA. After elimination of the excess dye, the absorbance at 540 nm was measured with spectrofluorometer (Tecan infinite M200, Tecan Austria GmbH), which is proportional to the amount of viable cells in the well.

The experimental fraction of cells stained with crystal violet (CV) is defined as:

$$f(\text{CV}) = \frac{A_{(\text{CV,E})} - A_{(\text{CV,bg})}}{A_{(\text{CV,E}=0)} - A_{(\text{CV,bg})}}, \tag{3}$$

where $A_{(\text{CV, E})}$ denotes absorbance of cells subjected to electric pulses, $A_{(\text{CV, bg})}$ background absorbance and $A_{(\text{CV, E}=0)}$ absorbance of cells at *E* = 0, i.e. that of non-pulsed cells.

The fraction of stained cells equals cell viability, therefore *f*(CV) = *f*(viability) and:

$$\text{Viability } (\%) = f(\text{CV}) \cdot 100. \tag{4}$$

2.5. Gene electrotransfer

Electroporation was performed on CHO cells that were in the exponential growth phase. Cell suspension was prepared in the same

way as for electropermeabilization for PI using different electroporative media.

Plasmid DNA was added to cell suspension in concentration 40 μg/ml. After 2–3 min incubation of DNA with CHO cells at room temperature (22 °C), samples were exposed to square wave electric pulses using Cliniporator™ pulse generator. For gene electrotransfer the same electric pulse protocol was used as for electropermeabilization for PI. After exposing cells to electric pulses, fetal calf serum (FCS-Sigma, USA) was added (25% of sample volume) to preserve cell viability. Cells were then incubated for 5 min at 37 °C to allow cell membrane resealing and then grown for 24 h in cell culture medium at 37 °C in a humidified 5% CO_2 atmosphere in the incubator. After 24 h cells were trypsinized, centrifuged for 5 min at 1000 rpm (180×*g*) at 4 °C and resuspended in phosphate buffered saline (PBS) to a cell density of $\rho = 1\times10^6$ cells/ml. The samples were analyzed with Coulter EPICS Altra flow cytometer (Beckman Coulter Electronics) equipped with a laser emitting at 509 nm. 9000 events were recorded. As a representative case, histograms obtained from the flow cytometer and scatter plots are displayed in Fig. 1. Gene electrotransfer efficiency was determined by measuring fraction of cells expressing green fluorescent protein:

$$f(GFP) = N_{GFP} / N, \tag{5}$$

where $N_{(GFP)}$ denotes number of viable fluorescent cells and N number of all viable cells in a given sample. The fraction of cells expressing green fluorescent protein equals fraction of transfected cells for the given conditions, $f(\text{GFP}) = f(\text{transfection})$, therefore

$$\text{Transfection } (\%) = f(GFP)\cdot 100. \tag{6}$$

All experiments were repeated three times on different days. Results from different repetitions of experiments were pooled together and are presented as mean and standard deviation of the mean.

2.6. Visualization of DNA-cell membrane interaction for different [Mg]

To visualize DNA interaction with cell membrane TOTO-1 (Molecular Probes — Invitrogen, Carlsbad, California, USA) nucleic acid stain was used which was already shown to enable direct visualization of DNA with the cell membrane [45]. The plasmid pEGFP-N_1 was labeled with 2.3×10^{-4} M TOTO-1 DNA intercalating dye for 60 min on ice. Plasmid concentration was 1 μg/μl, which yields an average base pair to dye ratio of 5.

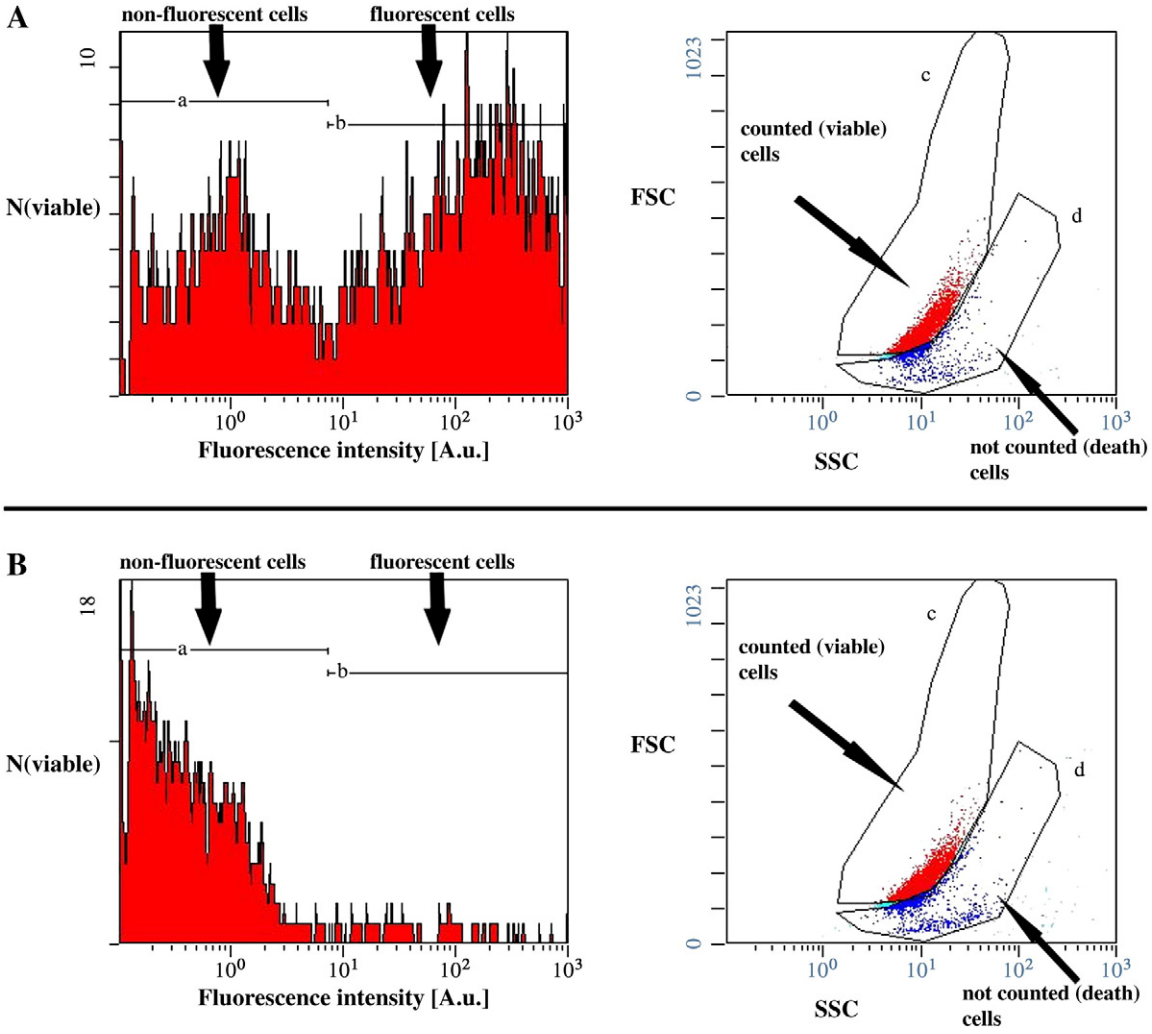

Fig. 1. Representative histograms for GFP fluorescence (left) and scatter plots (right) obtained from the flow cytometer for (A) [Mg] = 0.5 mM and (B) [Mg] = 50 mM. Fraction of cells expressing GFP was calculated as a number of viable fluorescent cells divided by number of all viable cells (see Eqs. (5) and (6)). Histograms for GFP fluorescence (left) shows in region (a) counted viable non-fluorescent cells and in region (b) counted viable fluorescent cells. Scatter plots (right) shows in region (c) counted (viable) cells and in region (d) not counted (death) cells. 4×200 μs pulses (E = 1.4 kV/cm) with repetition frequency 1 Hz were used at room temperature (T = 22 °C).

CHO-K1 cells were plated as a monolayer culture in Labtech chamber for 1 h in cell culture medium at 37 °C in a humidified 5% CO_2 atmosphere in the incubator at cell density of $\rho = 1 \times 10^5$ cells/ml. After 1 h culture medium was removed and replaced with electroporative media with different concentration of Mg^{2+} ([Mg] = 1 mM and 50 mM). Immediately before electric pulse application, labeled plasmid was added to cells in concentration 10 μg/ml. Samples were then exposed to electric pulses using Jouan GHT 1287 electroporator (Jouan, St. Herblain, France). An oscilloscope Wave surfer™ 422 (Le croy, Chestnut Ridge, New York, USA) monitored pulse shape. A train of eight pulses with duration of 5 ms and repetition frequency 1 Hz was applied ($E = 0.7$ kV/cm). The distance between a pair of two wire stainless steel parallel electrodes was $d = 2$ mm.

To monitor the interaction of DNA with the cell membrane fluorescent microscopy (Zeiss 200, Axiovert, ZR Germany) was used with 100× oil immersion objective. The images (see Fig. 3) were recorded using imaging system (MetaMorph imaging system, Visitron, ZR Germany).

3. Results

Main objective of our study was to determine the role of Mg^{2+} ions on DNA adsorption on cell surface by means of electric pulses, to analyze separately different effect of these ions on electropermeabilization for PI, viability and gene electrotransfer and to determine whether higher [Mg] improves gene electrotransfer efficiency.

The effect of [Mg] was followed by determining the uptake of PI (permeabilization), the fraction of viable cells (viability) and fraction of cells expressing green fluorescent protein (transfection). The electroporation protocol was the same in all experiments: applied voltages were 240 V, 400 V and 560 V which resulted in electric field strengths 0.6 kV/cm, 1 kV/cm and 1.4 kV/cm; four pulses with pulse duration 200 μs and repetition frequency 1 Hz were used.

Fig. 2A shows the percentage of permeabilization for PI, which represent the uptake of PI for different electric field strength (E) as a function of different Mg^{2+} concentrations in electroporative media. Increasing electric field strength results in an increase in electropermeabilization efficiency for PI irrespective of the electroporative media used. Increasing the [Mg] resulted in increasing electropermeabilization efficiency for PI.

We also tested the viability of the cells in different media. The increase of the electric field strength was followed by the decrease of the number of viable cells in all media (Fig. 2B). The highest viability was observed in media with [Mg] = 1 mM at $E = 0.6$ kV/cm, where approximately 83% of cells survived. At higher electric field strengths above the electroporation threshold (1.0 kV/cm and 1.4 kV/cm) we however obtained higher viability with higher [Mg]. Our results therefore showed that increasing [Mg] in electroporative media results in better viability of electroporated cells.

In Table 1 percentage of transfection, which represent the gene electrotransfer efficiency is shown with different [Mg] in electroporative media for different E. In general, gene electrotransfer efficiency increased with increasing E. At [Mg] = 0.5 mM we consistently observed the highest gene electrotransfer at each field. Maximum gene expression was observed at highest E (1.4 kV/cm) where approximately more than 50% of cells were expressing GFP gene similarly as in our previous study [34]. Further increase in [Mg] (from 1 to 50 mM) resulted in a decrease of gene expression at each field, contrary to what was observed for electropermeabilization for PI. The percentage of transfection in electroporative media with [Mg] = 50 mM was the lowest, where even at $E = 1.4$ kV/cm only 13% of transfected cells were obtained. Altogether the gene electrotransfer efficiency in electroporative media with [Mg] = 50 mM dropped to less than a half in comparison with gene electrotransfer efficiency in electroporative media with only [Mg] = 0.5 mM.

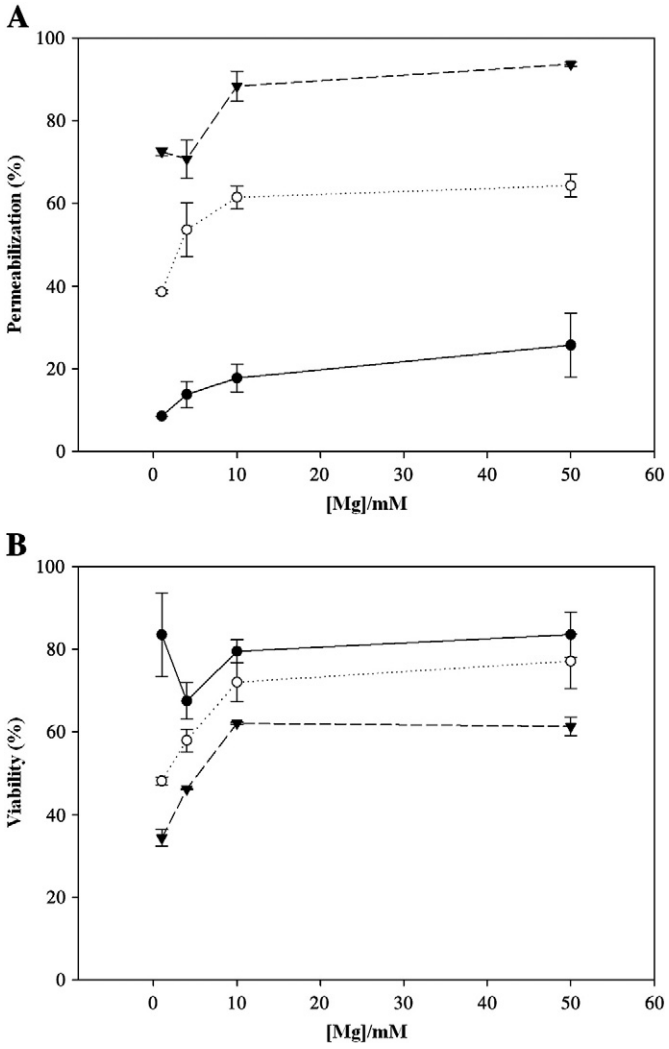

Fig. 2. Effect of Mg^{2+} ions on (A) cell electropermeabilization for PI and on (B) cell viability. (A) the percentage of permeabilization (uptake of PI — see Eq. (2)) and (B) the percentage of viability (see Eqs. (3) and (4)) as a function of different [Mg] in electroporative media (1, 4, 10 and 50 mM) for different electric field strengths: (●) 0.6 kV/cm; (○) 1.0 kV/cm and (▼) 1.4 kV/cm is shown; 4 × 200 μs pulses with repetition frequency 1 Hz were used at room temperature ($T = 22$ °C). Cell density $\rho = 2.5 \times 10^6$ cells/ml. Values represent means ± standard deviation.

To understand if the decrease in gene electrotransfer efficiency when using higher [Mg] is a consequence of intense binding of DNA at cell membrane, visualization of DNA interaction with membrane was performed by using TOTO-1 labeled plasmid DNA. The labeled plasmid was added to cells and when electric pulses were applied, accumulation of the labeled DNA at the cell membrane was observed,

Table 1
Effect of Mg^{2+} ions on gene electrotransfer. The percentage of transfected cells (%) — cells expressing GFP (see Eqs. (5) and (6)) in electroporative media with different [Mg] is presented for different electric field strengths. Other conditions are as in the caption of Fig. 2. Values represent means ± standard deviation.

Transfection (%)	0.6 kV/cm	1.0 kV/cm	1.4 kV/cm
0.5 mM [Mg]	7.1(±0.3)	41.1(±0.3)	53.3(±0.9)
1 mM [Mg]	5.7(±0.9)	37.2(±4.0)	50.7(±5.8)
2 mM [Mg]	5.3(±1.0)	31.1(±3.5)	46.7(±4.7)
4 mM [Mg]	4.2(±0.6)	28.2(±2.7)	40.6(±4.4)
6 mM [Mg]	3.5(±0.4)	23.8(±0.6)	34.9(±0.9)
8 mM [Mg]	3.0(±0.3)	20.0(±0.3)	30.8(±1.2)
10 mM [Mg]	3.5(±0.2)	20.7(±1.2)	32.7(±1.1)
50 mM [Mg]	1.5(±0.1)	8.3(±0.6)	13.2(±1.0)

as was already shown before [45–47]. No fluorescence caused by spontaneous adsorption of DNA to the plasma membrane was detected when no pulses were applied (control sample - data not shown). When using [Mg] = 1 mM, localized fluorescence spots were observed only on the membrane side facing the cathode (see Fig. 3B). Increase in concentration of Mg^{2+} ions to 50 mM resulted in an increase of fluorescence intensity of accumulated DNA at whole cell membrane surface (Fig. 3D). We repeated the experiment three times and this effect was always observed.

4. Discussion and conclusions

Transfer of DNA into the cells by membrane electroporation is an established method for gene delivery both in vitro and in vivo. The main problem in gene electrotransfer of mammalian cells in vivo is however it's relatively low efficiency. In some of the reports researchers emphasize an importance of cations on gene electrotransfer efficiency and suggest that divalent cations enhance adsorption of negatively charged DNA onto negatively charged cell membrane (bridging effect) which could improve gene electrotransfer efficiency [17,40].

Since Mg^{2+} ion is one of the most common divalent cation used in standard electroporative media, the aim of our study was to evaluate the effects of various Mg^{2+} concentrations on cell electropermeabilization for PI, viability and gene electrotransfer in order to better understand mechanism of gene electrotransfer (specially the formation of a complex between DNA and cell membrane during electric pulses), and furthermore to determine whether higher [Mg] can improve gene electrotransfer.

Our results show that higher [Mg] in electroporative media increase electropermeabilization for PI as shown in Fig. 2A. This is in agreement with other studies which showed that changing ion concentration in the media can have an effect on reorganization of membrane lipid bilayer, which can result in a higher uptake of small molecules such as PI [48].

To determine the influence of [Mg] on gene electrotransfer efficiency, we performed gene electrotransfer with plasmid DNA encoding GFP in different media. In general transfection efficiency decreased with increasing [Mg] for all electric filed strengths (see Table 1). We also tested gene electrotransfer efficiency in media with even lower concentrations of Mg^{2+} ([Mg] = 0.5 mM) with the same pulse parameters as for other media. The percentage of cells expressing GFP gene was higher than in standard media with [Mg] = 1 mM.

In order to directly analyze the effect of Mg^{2+} concentrations on the formation of a complex between DNA and cell membrane, we performed visualization of DNA interaction with the cell membrane using TOTO-1 nucleic acid stain (Fig. 3). As was already shown by others [45–47], plasmid interacted with the membrane by forming localized spots. Although gene electrotransfer efficiency decreased for higher [Mg], it appears that quantity of accumulated DNA at the cell membrane increased with increasing [Mg]. It was already proven that Mg^{2+} ions facilitate the absorption of DNA at the cell surface and are therefore necessary for successful gene electrotransfer [6]. But maybe at some point Mg^{2+} at higher concentrations bind DNA at the cell membrane at such intensity, that DNA cannot cross the membrane during electroporation.

However, since formation of a complex between DNA and cell membrane is only one step necessary for successful gene electrotransfer, the decrease in transfection efficiency for increasing [Mg] could be also a consequence of other factors. Namely, divalent cations such as Mg^{2+} ions interact with plasmid DNA and can alter the stability and topology of DNA molecule and therefore can induce structural transition of a DNA into a compact form [49]. It was shown by some researchers that ions strongly affect biologically significant behavior of DNA in the cell, such as transcriptional initiation and elongation [46], which can consequently result in decreased gene expression. It was also shown that for some conditions divalent ions

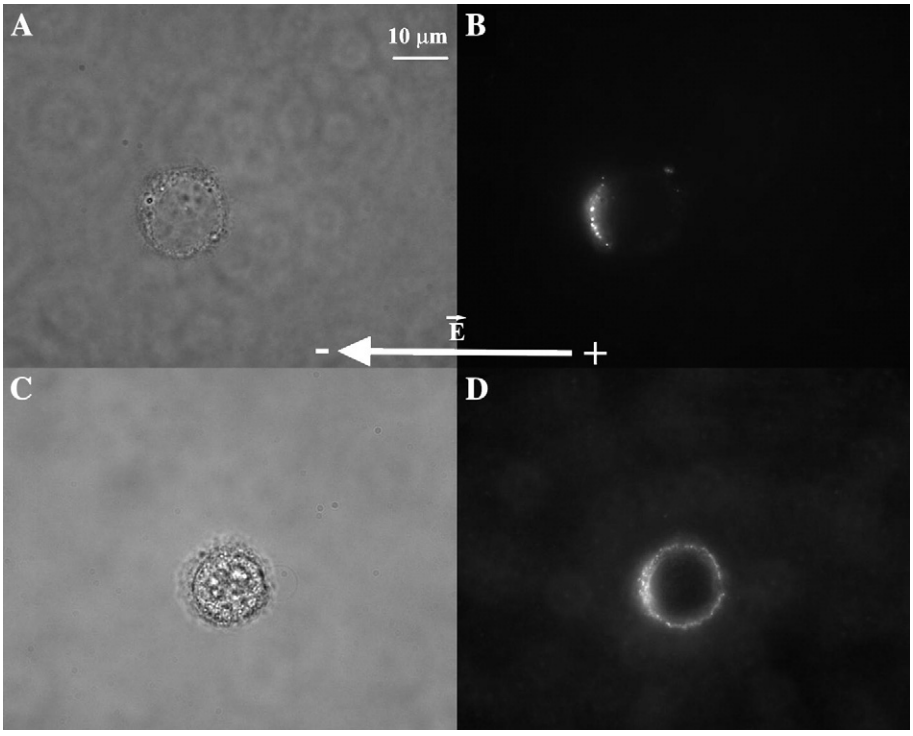

Fig. 3. Fluorescence microscopy observation of DNA-membrane interaction when using different [Mg]. Plated cells were incubated in presence of TOTO-1 labeled DNA (pEGFP-N_1) and different [Mg] in electroporative media (1 mM and 50 mM). 8×5 ms (E = 0.7 kV/cm) pulses were applied with repetition frequency of 1 Hz to observe DNA–membrane interaction (concentration of labeled DNA in electroporative media was 10 μg/ml). (A) phase contrast image of treated cells in [Mg] = 1 mM; (B) fluorescence image of treated cells in [Mg] = 1 mM; (C) phase contrast image of treated cells in [Mg] = 50 mM; (D) fluorescence image of treated cells in [Mg] = 50 mM. The white arrow in the middle indicates the direction of the applied electric field.

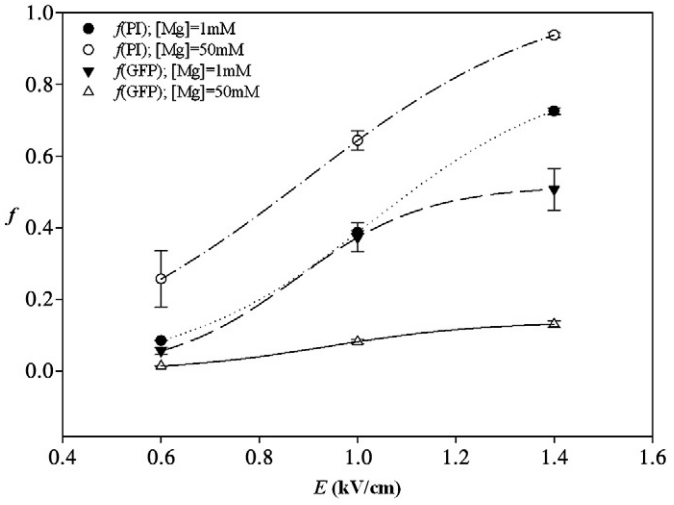

Fig. 4. Correlation between electropermeabilization for PI and gene electrotransfer at [Mg] 1 mM and 50 mM. Uptake of PI - f(PI) = Permeabilization/100 (see Eq. (2)) and fraction of cells expressing green fluorescent protein - f(GFP) (see Eq. (5)) as a function of different electric filed strength used is presented: (●) f(PI) in [Mg] = 1 mM; (○) f(PI) in [Mg] = 50 mM; (▼) f(GFP) in [Mg] = 1 mM; (△) f(GFP) in [Mg] = 50 mM. Values represent means ± standard deviation.

(mostly Mg^{2+}) increase activity of DNAse enzyme, which as a result can decrease the gene electrotransfer efficiency [50], in electroporative media with high concentration of added Mg^{2+} ions.

Our analyses of correlation between electropermeabilization for PI and gene electrotransfer in combination with Mg^{2+} ions led us to the conclusion that although electroporation is crucial for transfection, gene electrotransfer efficacy is not strictly correlated to permeability changes in cell membrane. This is also in agreement with other authors, where they also obtained that gene electrotransfer is a more complex process than classical electroporation [25] and that several steps are involved. From Fig. 4 it is clearly apparent, that generally at a given E, f(GFP) i.e. transfection for DNA is fraction-wise different from f(PI) i.e. permeabilization/100 for PI and that permeabilization of cells for PI is not the same as permeabilization for DNA.

In order to separate different effects of Mg^{2+} ions on gene electrotransfer efficiency, we tested whether Mg^{2+} inhibitory effect on gene electrotransfer is a result of lower cell viability. We obtained, that increasing [Mg] in electroporative media results in higher viability of electroporated cells, which can be explained that addition of Mg^{2+} ions improves the recovery of electrolyte homeostasis as has been already shown in vivo [51].

In conclusion, we have shown that cell electropermeabilization for PI, gene electrotransfer efficiency and cell viability depend on the concentration of Mg ions in electroporative media. While electropermeabilization for PI and viability increases with higher [Mg], gene electrotransfer decreases. Because of the opposite effect of Mg^{2+} ions on cell electropermeabilization for PI and on gene electrotransfer efficiency, we can conclude that membrane permeabilization is necessary but not sufficient for an efficient gene transfer as already shown by other study [25]. We suggest that Mg^{2+} ions at higher concentrations bind DNA to the cell membrane strong enough to prevent translocation of DNA into the cell during electroporation. However, there may be other mechanisms involved, since there are many steps of gene electrotransfer on which Mg^{2+} ions can have an effect on.

Acknowledgements

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