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Cell electrotransfection and viability dependence on electrotransfection and viability dependence on electoporation medium

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Electroporation is a physical method that uses electric fields to increase membrane permeability. The method is widely used for intracellular drug and gene delivery. In this study we aimed to investigate the importance of the medium for efficiency of cell electrotransfection and viability following the application of electric field pulses.

The experiments were performed using 2 different cell lines: Chinese hamster ovary (CHO) and Chinese hamster lung fibroblast (DC-3F) and 2 different electroporation media: SMEM (medium conductivity equal to 1.3 S/m) and laboratory-made low-conductivity (0.1 S/m) electroporation (EP) medium. Cells suspended in these media were supplemented with plasmid (10 μg/ml) encoding luciferase and then were treated with 1, 5, or 10 high-voltage (1200 V/cm, 100 μs, at 1 Hz) pulses. Transfection efficiency was determined by luciferase activity 24 h after cell treatment, while cell viability was determined by clonogenic assay.

Results showed significant differences between cell lines and used electroporation media. CHO transfection was higher when electroporation was performed in low conductivity EP medium. Low transfection efficiency in SMEM medium resulted from low viability. In contrast, transfection efficiency of DC-3F cells was higher in SMEM. Possible mechanisms governing these differences are discussed.

Key words: gene electrotransfer, electroporation medium, low-conductivity medium, SMEM, CHO, DC-3F

INTRODUCTION

External electrical fields at properly chosen parameters can reversibly affect cell membrane permeability to various exogenous molecules (Kotnik et al., 2015). First reported in 1972 this phenomenon was called electroporation (Neumann, Rosenheck, 1972). The leading electroporation theory claims that hydrophilic electropores in the membrane is a reason for facilitated exogenous molecule transfer to cells (Weaver, Chizmadzhev, 1996). Hydrophilic electropores occur

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when transmembrane potential induced by an external electrical field surpasses transmembrane voltage threshold (Tsong, 1991). Reversible electropore formation enables electrotransfer of small exogenous molecules (Kramar et al., 2007; Weaver, Chizmadzhev, 1996). Electrotransfer is also observed when molecules (i. e. plasmid DNA) are larger than the estimated size of induced electropores. Therefore, DNA electrotransfer processes are more complex (Rols, Teissie, 1998). It is known that during electric field application due to electrophoresis DNA migrates toward the positively charged electrode and forms complexes with the electroporated cell membrane at the membrane surface facing the negatively charged electrode (Golzio et al., 2002). It is presumed that some of DNA/electroporated membrane complexes enter the cell via endocytosis (Pavlin et al., 2012).

Electrotransfection mechanism is not yet fully understood (Rosazza et al., 2012). For example, there is no data of external medium impact on the electrotransfection efficiency. So far various electroporation media are being used for electrotransfection. The most frequently used electroporation mediums *in vitro* are SMEM and laboratory-made medium having lower conductivity (Kotnik et al., 2001; Krinos et al., 1999; Pavlin et al., 2005; Phillips-Jones, 1990).

In this study we aimed to investigate the importance of the medium for efficiency of cell electrotransfection and viability following the application of electric field pulses. The experiments were performed on 2 frequently used cell lines, namely DC-3F and CHO. Two most often used electroporation media were chosen.

MATERIALS AND METHODS

Cell cultures

Chinese hamster ovary (CHO, radius 9.7 μm) and Chinese hamster lung fibroblast (DC–3F, radius 5.1 μm) cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Sigma, D5546) supplemented with 10% Foetal Bovine Serum (FBS) (Sigma, F7524), 1% L–glutamine (Sigma, G7513), and 1% penicillinstreptomycin solution (Sigma, P0781). Cells were passed every 2–3 days and always a day before the experiment.

Used plasmid and electroporation mediums

Luciferase coding plasmid (LUC) containing synthetic *Photinus pyralis* luciferase gene under control of SV40 promoter (Promega, E6681) was used. Final plasmid concentration was 10 μg/ml. Two different electroporation media were used: Spinner Modification Essential Eagle Medium (SMEM) (Sigma, M8167) and laboratory-made sucrose-based electroporation medium (EP). The composition of laboratory-made EP medium (pH 7.2) is shown in Table 1. Measured SMEM and EP medium conductivities were 1.3 and 0.1 S/m, respectively.

Electroporation

High-voltage (HV) electric pulses (1200 V/cm, 100 μs duration, repeated at 1 Hz) were used with an electroporator constructed in Kaunas University of Technology and Vytautas Magnus University. 50 μl of cell containing 9×10^4 cells and plasmid (10 μg/ml) was placed between stainless plate electrodes, separated by 2 mm and subjected to electric pulses. After that, electroporation cells were

Table 1. Components of laboratory-made electroporation medium (EP)

	Na_2HPO_4	NaH_2PO_4	$MgCl_2$	Sucrose
Electroporation medium (0.1 S/m)	5.59 mM	3.00 mM	1.73 mM	242.19 mM

put in 35 mm petri dishes and incubated for 10 minutes. Thereafter, petri dishes were supplemented by 2 ml of DMEM medium. From 2050 µl of cell suspension, 223 µl was placed in a 96-well plate and incubated for 24 hours for transfection measurements.

Measurement of luciferase expression

After 24-hour incubation, growth medium was removed and 100 µl of solution from the luciferase assay system (Promega, E6120) was added to cell suspension. After 5 min, luminescence was measured using a microplate reader (GENios Pro, Tecan).

Cell viability measurements

Cell viability measurements were performed using clonogenic assay. After electroporation, 10 min incubation, 2 ml of DMEM growth media was in cell suspension. From this suspension 100 µl was put into another 35 mm petri dish with 1.9 ml DMEM growth medium. From that plate 178 µl was put into another 35 mm petri dish with 1.8 ml DMEM growth medium. This plate was put into an incubator and held for 6 days, then fixed with 70% ethanol and stained with crystal violet staining solution. Grown colonies were counted and normalized with control.

Statistics

All experiments were repeated at least 6 times. Error bars represent the standard error of the mean. Statistical significance between experimental groups was calculated using Student's t-test.

RESULTS

CHO cell transfection with LUC plasmid in both electroporation media is shown in Fig. 1A. As seen, CHO electrotransfection is more efficient with a higher number of electric pulses. Higher transfection efficiency is obtained using laboratory-made EP medium. The difference in transfection efficiency gets greater when more electric pulses are used. After 10 HV pulse combination in laboratory-made EP medium electrotransfection was more than 3 times higher than that in SMEM medium.

DC-3F cell electrotransfection with LUC plasmid in both media is shown in Fig. 1B. DC-3F electrotransfection is more efficient with a higher number of electric pulses. There are no significant electrotransfection differences in EP and SMEM media after 1 HV pulse. However, after 10 HV pulses transfection efficiency in SMEM is more than 4 times higher than that in EP medium.

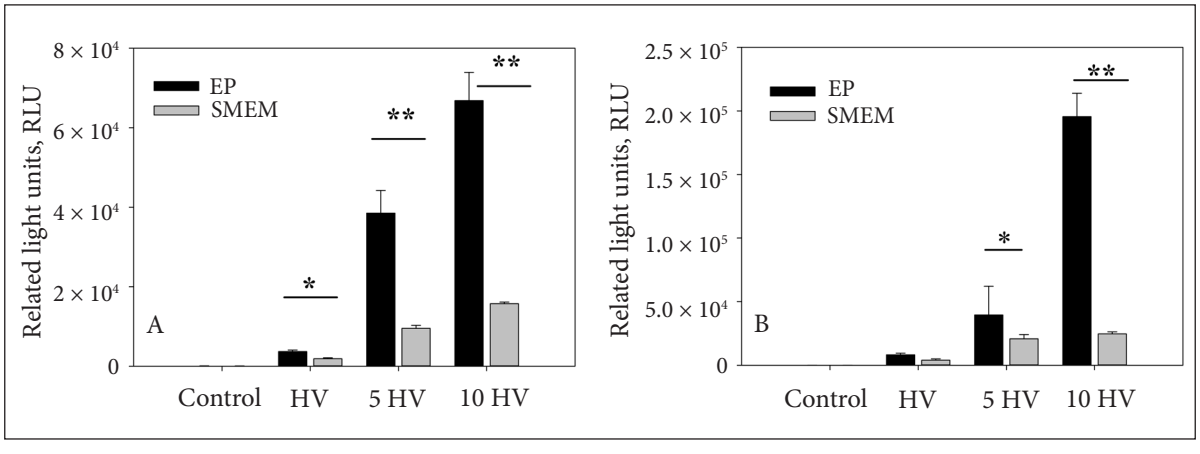

Fig. 1. Luciferase activity in CHO (A) and DC-3F (B) cells after cell treatment with 1, 5, and 10 HV electric pulses of 1200 V/cm pulse strength, 100 µs pulse duration repeated at 1 Hz in dependence of medium used: EP medium (dark bars) and SMEM medium (light bars). Cell treatment with electric pulse was performed following addition of luciferase coding plasmid (10 µg/ml). Here: ** $p < 0.01$ and *** $p < 0.001$

Significant differences are seen when comparing CHO and DC-3F electrotransfection results. After CHO treatment in EP medium (0.1 S/m) transfection efficiency was always higher. In comparison to SMEM medium, cell treatment with 1, 5, and 10 HV pulses in EP medium resulted in 1.95, 4, and 4.25 times greater transfection efficiency, respectively. Similar results were observed using DC-3F cells. Electrotransfection in SMEM medium resulted in 2.1, 1.9, and 7.9 times greater transfection efficiency compared to EP 0.1 S/m medium, when 1, 5, and 10 HV pulses were used.

CHO and DC-3F transfection using the same electroporation media are shown in Fig. 2. It is seen a higher CHO transfection efficiency in laboratory-made EP medium compared to DC-3F. CHO had the same transfection efficiency with 1 HV pulse, however 1.85 and 2.71 times higher transfection efficiency compared to DC-3F after 5 and 10 HV pulses, respectively.

Figure 2A shows opposite transfection efficiency results with DC-3F when SMEM was used. Indeed, DC-3F cells resulted in a higher transfection efficiency when compared to CHO cells. After 1, 5, and 10 HV DC-3F cell transfection compared to CHO cells was, respectively, 4.3, 4.12, and 12.43 times higher.

The highest CHO transfection efficiencies, namely 66835 ± 7097 and 15724 ± 403 RLU were obtained in EP 0.1 S/m and SMEM, respectively, using 10 HV pulses. The highest DC-3F transfection efficiencies, namely 24630 ± 1641 and 195551 ± 18330 RLU were obtained in EP 0.1 S/m and SMEM medium, respectively.

In order to evaluate cell viability influence on electrotransfection efficiency, a cell colony test was performed (Fig. 3). Results show that after 1, 5, and 10 HV pulses the viability of CHO using EP and SMEM media was 99.46 ± 9.84, 50.43 ± 10.6, 33.87 ± 11.12 and 81.33 ± 10.54, 22.58 ± 2.62, 6.38 ± 0.76%, respectively. Using the same 1, 5, and 10 HV pulses the viability of DC-3F in EP and SMEM media was 99.89 ± 2.09, 45.81 ± 3.93, 22.58 ± 2.616 and 95.91 ± 2.57, 85.77 ± 1.31, 82.68 ± 4.50%, respectively.

As seen in Fig. 3, CHO and DC-3F viability is similar after electroporation in EP medium using various pulse combinations. Contrary to that, viability of DC-3F cells was affected to a much lesser extent when using SMEM medium. Viability difference between CHO and DC-3F in SMEM medium after cell treatment with 1, 5, and 10 HV pulses was 15, 63, and 76%, respectively.

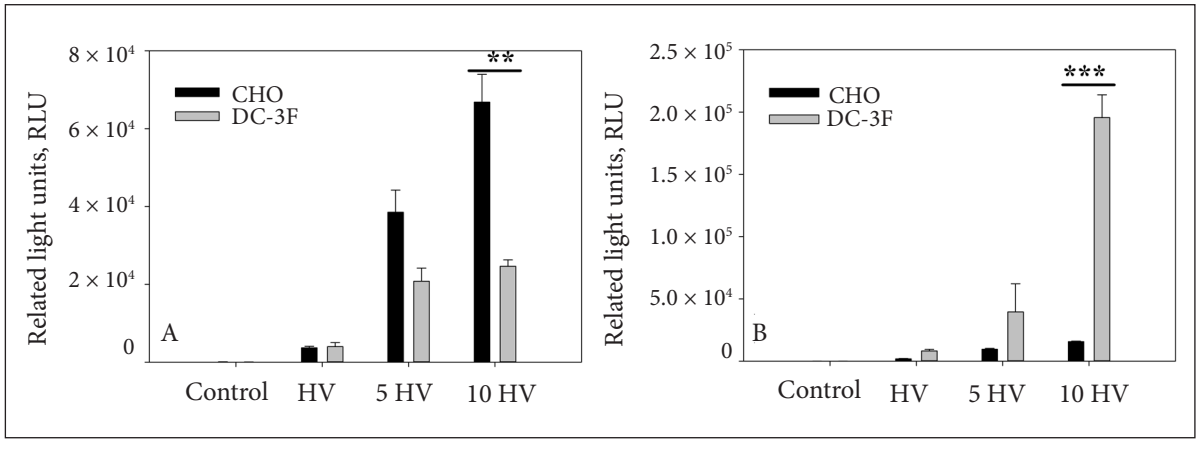

Fig. 2. Cell transfection efficiency in EP (A) and SMEM (B) medium after cell treatment with 1, 5, and 10 HV electric pulses of 1200 V/cm pulse strength, 100 μs pulse duration repeated at 1 Hz in dependence of cell line used: CHO cells (dark bars) and DC-3F cell (light bars). Cell treatment with electric pulse was performed following addition of luciferase coding plasmid (10 μg/ml). Here: ** $p < 0.01$ and *** $p < 0.001$

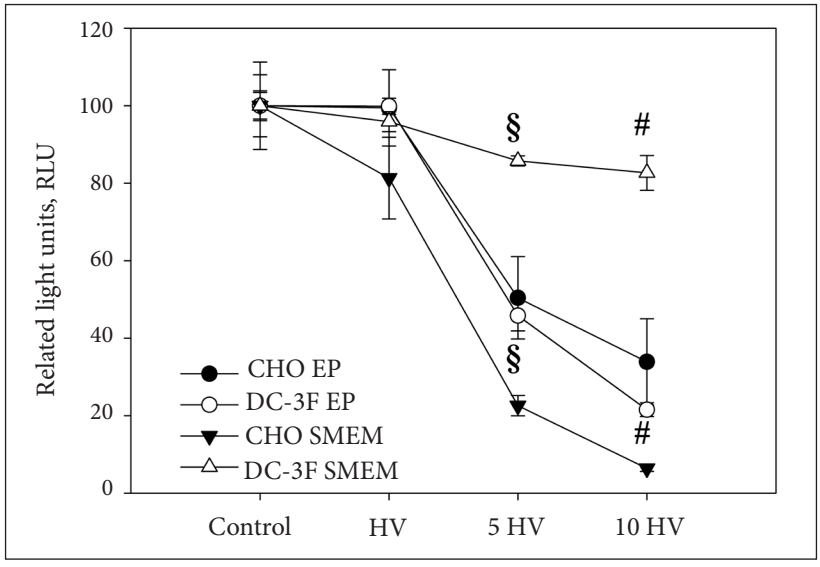

Fig. 3. CHO and DC-3F cell viability after cell treatment with 1, 5, and 10 HV electric pulses of 1200 V/cm pulse strength, 100 μs pulse duration repeated at 1 Hz in EP and SMEM media. § represents t-test significance ($p < 0.01$) between CHO and DC-3F using 5 HV pulse combination. # represents t-test significance ($p < 0.01$) between CHO and DC-3F using 10 HV pulse combination

DISCUSSION

The electroporation phenomenon is dependent on the magnitude of induced transmembrane potential $\Delta\psi_i$, which can be calculated using a simplified equation (Marszalek et al., 1990):

$$\Delta\psi_i = f \times r \times E \times \cos\theta, \quad (1)$$

where f is cell shape factor (for spherical cell f is 1.5), r is cell radius, E is external electric field, θ is the angle between the site on the cell membrane where $\Delta\psi_i$ is measured and the direction of the external field.

As indicated above, the mean radius of CHO and DC-3F cells is different. According to equation the induced transmembrane potential was 1.746 V for CHO and 0.918 V for DC-3F cells. At the used electric field strength, the membrane area affected by electric pulses was larger in larger CHO cells. Therefore, after cell electroporation plasmid can interact with a larger membrane area. This can explain why CHO cell transfection efficiency, in comparison to DC-3F, after 5 and 10 HV pulses was by 2.7 times higher when using EP medium. On the other hand, in comparison to CHO cells, electrotransfection efficiency in DC-3F was higher in SMEM medium.

It is known that highly electroporated cells do not survive (Tsong, 1991). This might give the explanation why survival of CHO cells and consequently luciferase activity were low. Indeed, as it is seen from Fig. 3, viability of CHO cells decreases when using SMEM medium. Viability of DC-3F cells, contrary to CHO cells, was higher in SMEM medium. This might be an explanation of 12.4 time higher transfection efficiency in DC-3F cells than in CHO cells.

Viability can explain the changes in transfection efficiency tendencies between CHO and DC-3F. Figure 2A and Fig. 3 show that CHO viability in SMEM decreased from 81.33 ± 10.54% after 1 HV to 6.38 ± 0.76% after 10 HV; nevertheless, luciferase activity increased 8.3 times from 1887 ± 237 to 15724 ± 403 RLU. Higher transfection might be due to increase in DNA electrophoretic movement when 10 HV pulses were used (Cepurniene et al., 2010; Pavlin et al., 2010) or a longer persistence of induced electropores in the membrane (Weaver, Chizmadzhev, 1996).

CONCLUSIONS

The obtaied results show the importance of electroporation medium and cell line choice for electrotransfection performance. Here, we show that CHO cell transfection is up to 2.7 times more efficient when using laboratory-made (0.1 S/m) medium compared to SMEM (1.3 S/m). DC-3F showed up to 7.9 times higher transfection efficiency when using SMEM compared to laboratory-made medium. CHO and DC-3F viability was similar when low-conductivity medium was used for electroporation. However, SMEM medium improved DC-3F and decreased CHO viability. Our data suggest that CHO electrotransfection decrease using SMEM medium is due to decrease in cell viability.

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References

1. Cepurniene K, Ruzgys P, Treinys R, Satkauskiene I, Satkauskas S. Influence of plasmid concentration on DNA electrotransfer in vitro using high-voltage and low-voltage pulses. J Membr Biol. 2012; 236(1): 81–5.
2. Golzio M, Teissie J, Rols MP. Direct visualization at the single-cell level of electrically mediated gene delivery. Proc Natl Acad Sci US A. 2002; 99(3): 1292–7.
3. Kotnik T, Frey W, Sack M, Haberl Meglič S, Peterka M, Miklavčič D. Electroporation-based applications in biotechnology. Trends in Biotechnology. 2015; 33(8): 480–8.
4. Kotnik T, Mir LM, Flisar K, Puc M, Miklavcic D. Cell membrane electropermeabilization by symmetrical bipolar rectangular pulses. Part I. Increased efficiency of permeabilization. Bioelectrochemistry. 2001; 54(1): 83–90.
5. Kramar P, Miklavcic D, Lebar AM. Determination of the lipid bilayer breakdown voltage by means of linear rising signal. Bioelectrochemistry. 2007; 70(1): 23–7.
6. Krinos C, High AS, Rodgers FG. Role of the 25 kDa major outer membrane protein of Legionella pneumophila in attachment to U-937 cells and its potential as a virulence factor for chick embryos. J Appl Microbiol. 1999; 86(2): 237–44.
7. Marszalek P, Liu DS, Tsong TY. Schwan equation and transmembrane potential induced by alternating electric field. Biophys J. 1990; 58(4): 1053–8.
8. Neumann E, Rosenheck K. Permeability changes induced by electric impulses in vesicular membranes. J Membr Biol. 1972; 10(3): 279–90.
9. Pavlin M, Flisar K, Kanduser M. The role of electrophoresis in gene electrotransfer. J Membr Biol. 2010; 236(1): 75–9.
10. Pavlin M, Kanduser M, Rebersek M, Pucihar G, Hart FX, Magjarevic R, Miklavcic D. Effect of cell electroporation on the conductivity of a cell suspension. Biophys J. 2005; 88(6): 4378–90.
11. Pavlin M, Pucihar G, Kanduser M. The role of electrically stimulated endocytosis in gene electrotransfer. Bioelectrochemistry. 2012; 83: 38–45.
12. Phillips-Jones MK. Plasmid transformation of Clostridium perfringens by electroporation methods. FEMS Microbiol Lett. 1990; 66(1–3): 221–6.
13. Rols MP, Teissie J. Electropermeabilization of mammalian cells to macromolecules: control by pulse duration. Biophys J. 1998; 75(3): 1415–23.
14. Rosazza C, Phez E, Escoffre JM, Cezanne L, Zumbusch A, Rols MP. Cholesterol implications in plasmid DNA electrotransfer: Evidence for the involvement of endocytotic pathways. Int J Pharm. 2012; 423(1): 134–43.
15. Tsong TY. Electroporation of cell membranes. Biophys J. 1991; 60(2): 297–306.
16. Weaver JC, Chizmadzhev YA. Theory of electroporation: A review. Bioelectrochem Bioenerg. 1996; 41(2): 135–60.

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LĄSTELIŲ ELEKTROTRANSFEKCIJOS IR GYVYBINGUMO PRIKLAUSOMYBĖ NUO ELEKTROPORACIJOS TERPIŲ

Santrauka

Elektroporacija – tai toks fizikinis metodas, kai naudojant elektrinius laukus galima padidinti ląstelių membranos pralaidumą. Elektroporacijos metodas šiuo metu plačiai taikomas vaistų ir genų pernašai į ląsteles ir audinius. Šiame darbe siekėme įvertinti elektroporacijos terpių poveikį ląstelių elektrotransfekcijos efektyvumui ir ląstelių gyvybingumui.

Tyrimai buvo atlikti naudojant dvi skirtingas ląstelių linijas: Kininio žiurkėno kiaušidžių ląsteles (CHO) ir Kininio žiurkėno plaučių fibroblastus (DC-3F) bei dvi skirtingas elektroporacijos terpes: SMEM ir laboratorijoje pagamintą žemo laidumo (0,1 S/m) elektroporacijos terpę. Vienoje iš šių terpių paruošta ląstelių suspensija buvo papildyta liuciferazę koduojančia plazmide (10 μg/ml) ir veikta 1, 5 ar 10 aukštos amplitudės (1200 V/cm, 100 μs, pasikartojančios 1 Hz dažniu) elektrinių laukų impulsais. Transfekcijos efektyvumas buvo vertintas pagal liuciferazės aktyvumą praėjus 24 val. po poveikio, o ląstelių gyvybingumas – taikant ląstelių kolonijų formavimo testą.

Gauti rezultatai rodo statistiškai patikimus transfekcijos skirtumus tarp ląstelių linijų, priklausančius nuo elektroporacijos terpių. CHO ląstelių transfekcijos efektyvumas buvo didesnis EP terpėje. Žemas šių ląstelių transfekcijos efektyvumas SMEM terpėje yra susijęs su sumažėjusiu šių ląstelių gyvybingumu po jų elektroporacijos SMEM terpėje. Priešingai nei CHO ląstelių atveju, DC-3F ląstelių transfekcijos efektyvumas buvo didesnis SMEM terpėje. Straipsnyje aptariamos galimos šių skirtumų priežastys.

Raktažodžiai: genų elektropernaša, elektroporacijos terpė, žemo laidumo terpė, SMEM, CHO, DC-3F