



Gene delivery in adherent and suspension cells using the combined physical methods

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Received: 10 October 2021 / Accepted: 25 January 2022 / Published online: 3 February 2022
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Abstract Physical methods are widely utilized to deliver nucleic acids into cells such as electro-transfection or heat shock. An efficient gene electro-transfection requires the best conditions including voltage, the pulse length or number, buffer, incubation time and DNA form. In this study, the delivery of pEGFP-N1 vector into two adherent cell lines (HEK-293 T and COS-7) with the same origin (epithelial cells), and also mouse bone marrow-derived dendritic cells (DCs) was evaluated using electroporation under different conditions alone and along with heat treatment. Our data showed that the highest green fluorescent protein (GFP) expression in HEK-293 T and COS-7 cells was observed in serum-free RPMI cell culture medium as electroporation buffer, voltage (200 V), the pulse number (2), the pulse length (15 ms), the circular form of DNA, and 48 h after electro-transfection. In addition, the highest GFP expression in DCs was detected in serum-free RPMI, voltage (300 V), the pulse number (1), the pulse length (5 ms), and 48 h after electro-transfection. The use of sucrose as electroporation buffer, the pulse number (2), and the

pulse length (25 ms) led to further cytotoxicity and lower transfection in HEK293T and COS-7 cells than other conditions. Moreover, the high voltage (700 V) increased the cell cytotoxicity, and decreased electro-transfection efficiency in DCs. On the other hand, the best conditions of electroporation along with heat treatment could significantly augment the transfection efficiency in all the cells. These data will be useful for gene delivery in other cells with the same properties using physical methods.

Keywords Transfection · Non-viral delivery system · Physical delivery · Electroporation · Heat · Cytotoxic effect

Introduction

Effective and safe delivery of DNA, RNA, and protein is a critical issue in biomedical and clinical studies (Zheng et al. 2017; Wang and Bodovitz 2010; Carlo and Lee 2006). Although, viruses are considered as a gold standard for gene delivery, but they possess various disadvantages, e.g., cytotoxicity, high cost, the potential of mutagenesis/tumorigenesis/immunogenicity, and size restriction (Kim and Eberwine 2010; Roth et al. 2018; Gallego-Pérez et al. 2017). These drawbacks led to the development of non-viral techniques. In the last four decades, electroporation was suggested as an effective, easy, low cost and safe strategy for delivery of different exogenous molecules

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10616-022-00524-4>.

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into tissues and cells compared to viral vector-mediated gene delivery (Bolhassani et al. 2014; Kotnik et al. 2019; Neumann et al. 1999; Jung et al. 2014). Gene electro-transfer was utilized mainly for DNA-based vaccines against inflammation, infectious diseases, multiple sclerosis and cancer, and also gene therapy (Sardesai and Weiner 2011; Zhao et al. 2010; Heller and Heller 2010).

Electroporation is generally performed through delivery of single or sequential electrical pulses to a cuvette containing biological sample suspended in electroporation buffer (Kotnik et al. 2019; Pucihar et al. 2011). The electro-transfection efficiency (eTE) is usually determined based on the percentage of cells receiving biological molecules and also cell viability. In general, two different wave forms of a pulse including exponential decay and square-wave can be produced in electroporation setting. The square-wave electroporation was more suitable for biomolecule delivery in mammalian cell lines due to the control of voltage, duration of pulses, and generation of fast repeating pulses. Various factors influence the efficacy of electro-transfection including cuvette type, the number and amplitude of pulses, intervals between multiple pulses, cell type, conductivity and composition of electroporation buffer (Bolhassani et al. 2014; Kotnik et al. 2019; Pucihar et al. 2011; Yao et al. 2009; Djuzenova et al. 1996; Jordan et al. 2008; Cemazar and Sersa 2007; Escoffre et al. 2009). Electroporation buffer is an important factor responsible for cell viability after electroporation (Sherba et al. 2020). Thus, electroporation conditions should be optimized for different cell types. On the other hand, Takizaki et al. reported that gene transfection could be enhanced by heat treatment as a physical method (2017).

Among various mammalian cell lines, human embryonic kidney 293 T (HEK-293 T) cells are widely applied to express the recombinant proteins, anticancer agents, and vaccine constructs (Graham et al. 1977; Hu et al. 2018; Lin et al. 2014). HEK-293 T cell line has the potency of effective transfection of plasmid DNA, translation, and processing of the recombinant proteins (Thomas and Smart, 2005). In addition, African green monkey kidney (COS-7) cell line was applied for propagation of the recombinant SV40 viruses, rotavirus and polyomavirus (Asano et al. 1985; Gluzman 1981; Díaz et al. 2012; Prezioso et al. 2017), and also biological,

immunological and cell signaling studies (D'Agostino et al. 2014; Valizadeh et al. 2016; Sakurai et al. 2017). On the other hand, dendritic cells (DCs) are the most powerful antigen presenting cells (APCs) in immune system (Cohn and Steinman 1973; O'Neill 2004). DCs are divided into three groups based on their differentiation stage such as precursors, immature, and mature DCs (Maraskovsky et al. 2000; Inaba et al. 1992a, b; Scheicher et al. 1992; Inaba et al. 1992a, b). DCs loaded with proteins or peptides and/or transfected with plasmid DNA were widely utilized in cell-based vaccines (Bolhassani et al. 2019; Soleymani et al. 2019). Thus, optimizing the expression efficiency of a recombinant protein in these cells is critical using different gene delivery systems.

In this study, we electro-transfected the green fluorescent protein (GFP)-expressing plasmid (pEGFP-N1) in two adherent cell lines (HEK-293 T and COS-7) with the same origin and immature DCs, and optimized some electroporation conditions for achieving the highest level of GFP expression, and also cell viability. These optimized conditions can be applied to express biologically active molecules in the cells for different purposes. Moreover, the heat effects were investigated to increase the transfection efficiency of plasmid DNA into cells after electroporation.

Materials and methods

Plasmid preparation

At first, the *E. coli* DH5 α strain was transformed with pEGFP-N1 vector. Then, the single clone was grown in Luria–Bertani (LB) medium, and the plasmid was purified by ion exchange chromatography using DNA extraction mini-kit (Qiagen) according to the manufacturer's instructions. Next, the purity and concentration of pEGFP-N1 vector was estimated by NanoDrop spectrophotometer. To attain the linearized pEGFP-N1 vector, this plasmid was digested by *NotI* restriction enzyme, and purified from agarose gel using gel extraction mini-kit (Qiagen).

Preparation of HEK-293 T and COS-7 cells

Human embryonic kidney (HEK-293 T; ATCC: CRL-3216TM), and COS-7 (CRL-1651) cell lines were prepared from the cell bank at Pasteur Institute

of Iran. HEK-293 T and COS-7 cell lines were cultured in RPMI 1640 medium (Gibco) containing 10% heat-inactivated fetal bovine serum (FBS, Gibco), and penicillin (100 U/ml)/streptomycin (0.1 mg/ml). Both cell lines were cultured at 37 °C with 95% relative humidity in a 5% CO₂ incubator.

Preparation of mouse bone marrow-derived DCs

For extraction of DCs from mouse bone marrow, inbred BALB/c male mice were provided from the breeding stocks maintained at Pasteur Institute of Iran under specific pathogen-free conditions. All procedures were performed according to approved protocols and in accordance with recommendations for the proper use and care of laboratory animals (Approval ID: IR.PII.REC.1398.061; Approval Date: 2020-02-18). Mice were sacrificed and their bone marrows were extracted. After washing and lysis of red blood cells using ACK buffer, the cells were cultured in RPMI 1640 medium supplemented with FBS 10%, GM-CSF (20 ng/ml), and IL-4 (10 ng/ml). The culture medium containing cytokines was refreshed every two days. The cells were harvested on day 5. DCs were identified by a FACScan Flow Cytometer (Becton Dickinson) using anti-CD86, anti-CD11c and anti-CD83 antibodies (BD Pharmingen; Strome et al. 2002).

Gene delivery in HEK-293 T and COS-7 cells using electroporation

Various parameters such as electroporation buffer, voltage, the pulse length, and the form of plasmid DNA (linear or circular) were studied to optimize gene delivery along with cell viability in two individual experiments. Electroporation buffer and the pulse length conditions were evaluated in the first experiment (Table 1), and voltage and the form of plasmid DNA (pDNA) conditions were investigated in the second experiment (Table 2) as follows.

In the first experiment, the efficiency of two different buffers including serum-free RPMI 1640 and sucrose was evaluated to determine the best buffer conditions. The 300 mM saccharose (sucrose) solution was prepared by dissolving pure saccharose powder in sterile water and kept at 4 °C. The cell density, voltage and pDNA concentration were set on 2×10^6 cells/ml, 200 V and 5 µg, respectively. Furthermore, two pulses of 15 ms (2×15 ms) and of 25 ms (2×25 ms) with one second interval were investigated. Before electro-transfection using Gene Pulser II Electroporation System (Bio-Rad, Richmond, CA), the mixture of cells and pDNA was transferred into the electroporation cuvette (0.4 cm, BioRad) and the cuvette was incubated on ice for 5 min. After pulsing, the cuvette was incubated on ice for 10 min. Next, fresh RPMI 1640 culture medium containing

Table 1 Evaluation of buffer and pulse length in the first experiment (for HEK-293 T and COS-7 cells)

Treatment	Plasmid form	Concentration of DNA (µg)	Buffer	Cell density (cells/ml)	Voltage (V)	Pulse length (ms)	Intervals (second)
1	Circular	5	RPMI	2×10^6	200	2×15	1
2	Circular	5	RPMI	2×10^6	200	2×25	1
3	Circular	5	Sucrose	2×10^6	200	2×15	1
4	Circular	5	Sucrose	2×10^6	200	2×25	1

Table 2 Evaluation of voltage and plasmid form in the second experiment (for HEK-293 T and COS-7 cells)

Treatment	Plasmid form	Concentration of DNA (µg)	Buffer	Cell density (cells/ml)	Voltage (V)	Pulse length (ms)	Intervals (second)
1	Circular	5	RPMI	2×10^6	200	2×15	1
2	Circular	5	RPMI	2×10^6	100	2×15	1
3	Linear	5	RPMI	2×10^6	200	2×15	1
4	Linear	5	RPMI	2×10^6	100	2×15	1

10% FBS, and penicillin (100 U/ml)/streptomycin (0.1 mg/ml) was added to the samples in 6-well plate. Finally, the cells were incubated in a 5% CO₂ incubator at 37 °C for 48 h. In the second experiment, based on the above results, other electroporation conditions such as voltage (200 V or 100 V) and plasmid DNA form (circular or linear) were studied. Herein, the pulse length and electroporation buffer were considered 2×15 ms and RPMI medium, respectively. Transfection efficiency was investigated by measuring the percentage of GFP-expressing cells using flow cytometry (Partec, Germany) and fluorescent microscopy.

Gene delivery in mouse bone marrow-derived DCs using electroporation

DCs were only transfected with the circular form of pDNA. Some parameters such as voltage, number of pulses, and incubation time were optimized for gene delivery in DCs. The effects of voltage and number of pulses were evaluated for transfection efficiency in the first experiment (Table 3). After obtaining the results, the effects of voltage and incubation times were studied in the second experiment (Table 4) as follows.

In the first experiment, after five days of culturing DCs, the immature DCs were harvested, and

centrifuged at 200 × g for 5 min. The cells were resuspended in 200 µl of serum-free RPMI-1640 medium at a density of 2×10⁶ cells/ml, and added to the cuvettes after mixing with 2 µg of pDNA. The pulse length was set on 5 ms with one second interval. The effects of voltage (700 V and 300 V), and number of pulses (one or two times) were studied on the transfection efficiency. The electroporation was performed using Gene Pulser II Electroporation System (Bio-Rad, Richmond, CA). Then, the transfected DCs were diluted in 2 ml RPMI 1640 supplemented with 10% FBS and penicillin (100 U/ml)/streptomycin (0.1 mg/ml), and transferred into a 12-well plate. Finally, the cells were incubated in a humidified 5% CO₂ incubator at 37 °C for 48 h. In the second experiment, based on the above results, the effects of voltage and incubation time after electroporation were studied on transfection efficiency. Hence, the cell density, pDNA concentration, buffer and pulse length were set on 2×10⁶ cells/ml, 2 µg, serum-free RPMI 1640 and 1×5 ms, respectively. The voltages were 300 V and 400 V. The electroporation procedure was performed similar to the first experiment. The cells were incubated under standardized conditions (5% CO₂, 37 °C, and 95% relative humidity) for 24 h and 48 h after electroporation. Transfection efficiency was investigated using flow cytometry and fluorescent microscopy.

Table 3 Evaluation of voltage and number of pulses in the first experiment (for dendritic cells)

Treatment	Concentration of DNA (µg)	Buffer	Cell density (cells/ml)	Voltage (V)	Pulse length (ms)	Intervals (second)
1	2	RPMI	2×10 ⁶	700	1×5	1
2	2	RPMI	2×10 ⁶	700	2×5	1
3	2	RPMI	2×10 ⁶	300	1×5	1
4	2	RPMI	2×10 ⁶	300	2×5	1

Table 4 Evaluation of voltage and incubation time after electro-transfection in the second experiment (for dendritic cells)

Treatment	Concentration of DNA (µg)	Buffer	Cell density (cells/ml)	Voltage (V)	Pulse length (ms)	Intervals (second)	Incubation time (hours)
1	2	RPMI	2×10 ⁶	300	1×5	1	24
2	2	RPMI	2×10 ⁶	300	1×5	1	48
3	2	RPMI	2×10 ⁶	400	1×5	1	24
4	2	RPMI	2×10 ⁶	400	1×5	1	48

The heat effects on efficiency of electro-transfection

The cell plate was covered with parafilm and fully incubated in a water bath at 42 °C for 2 h after electro-transfection (with the optimized conditions). Then, the cell plate was incubated at 37 °C for 48 h. Transfection efficiency was investigated using flow cytometry and fluorescent microscopy.

Cell viability

The cytotoxic effects of electroporation and heat treatment on HEK-293 T cells, COS-7 cells, and DCs were investigated using MTT assay (Davoodi et al. 2019).

Statistical analysis

The data were analyzed by Prism software using *t*-test and at statistical significance of 0.05. Data were represented as mean \pm standard deviation (SD). Two independent experiments were performed to obtain reproducibility. Indeed, each experiment such as gene delivery or MTT was performed two times. Moreover, we used two replicates (duplicates) for each condition (in gene delivery) or MTT in each independent experiment.

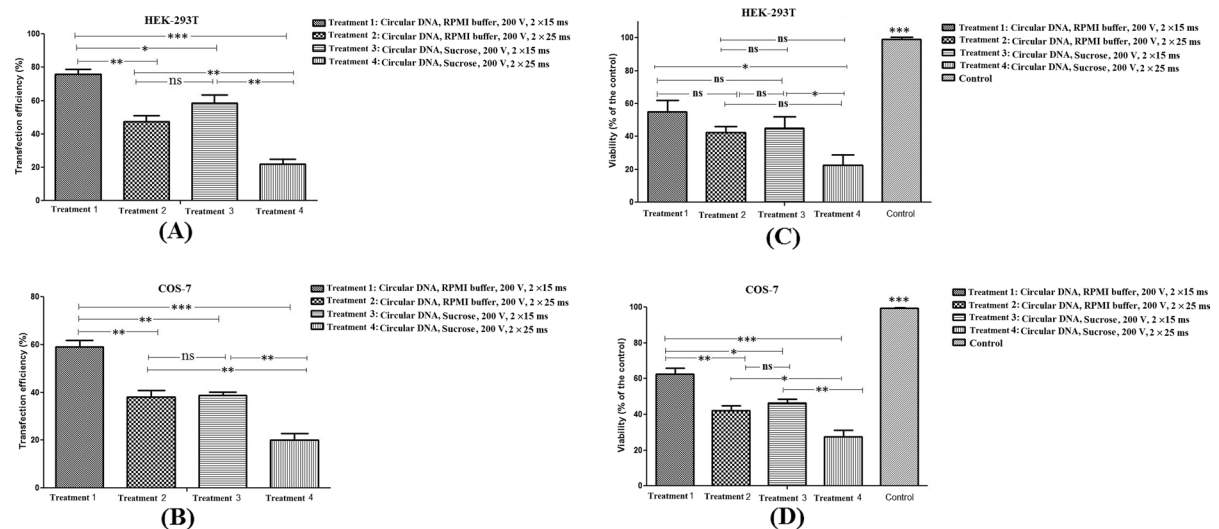


Fig. 1 Evaluation of electroperoration buffer and pulse length in HEK-293 T and COS-7 cells using flow cytometry (A, B); The cell viability after electroperoration under different conditions

Results

Preparation of pDNA

The pEGFP-N1 eukaryotic vector was prepared with high purity. Moreover, the pEGFP-N1 vector was correctly linearized by digestion with *NotI* restriction enzyme as a clear band of ~4700 bp on agarose gel (Supplementary Fig. 1). The concentration of the purified circular and linear pDNA was determined by NanoDrop spectrophotometry.

Electro-transfection of pDNA into HEK-293 T and COS-7 cells

In the first experiment, the serum-free RPMI medium and 2 pulses of 15 ms (2 × 15 ms) showed higher DNA delivery than sucrose and 2 pulses of 25 ms (2 × 25 ms), respectively. The percentage of GFP expression was shown in Fig. 1A and B for HEK-293 T and COS-7 cells, respectively. In the second experiment, the circular form of pDNA and 200 V indicated higher GFP expression than the linear form of pDNA and 100 V, respectively. The percentage of GFP expression was shown in Fig. 2A and B for HEK-293 T and COS-7 cells, respectively. Generally, the best transfection efficiency (under electroporation conditions: circular DNA, RPMI

for HEK-293 T and COS-7 cells using MTT assay (C, D); *ns* non-significant; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

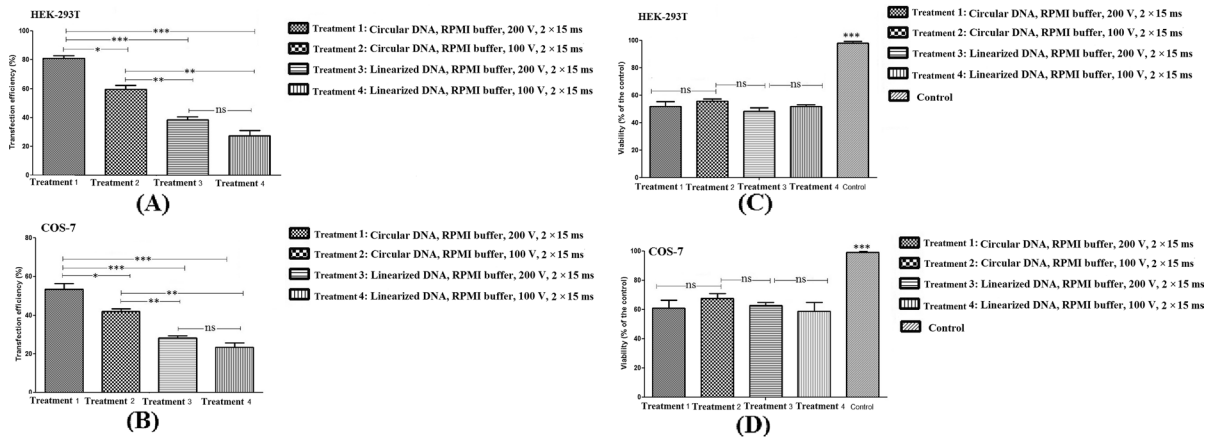


Fig. 2 Evaluation of voltage and DNA form in HEK-293 T and COS-7 cells using flow cytometry (A, B); The cell viability after electroporation under different conditions for HEK-

293 T and COS-7 cells using MTT assay (C, D); *ns* non-significant; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

buffer, 200 V & 2 × 15 ms) was about 77.91% ± 5.05 and 60.04% ± 3.23 for HEK-293 T and COS-7 cells, respectively. The fluorescent microscopy image was shown for the best electroporation conditions, as well (Fig. 3A and B).

Electro-transfection of pDNA into DCs

The mouse bone marrow-derived DCs were successfully harvested after 5-day culture in medium containing GM-CSF and IL-4 cytokines for gene electro-transfection. The expression levels of CD86, CD11c, and CD83 in immature DCs were 53.3%, 60.9%, and 15.6%, respectively (as previously described by our group: Bolhassani et al. 2019). In the first experiment, the 300 V and one pulse of 5 ms showed higher DNA delivery than the 700 V and two pulses of 5 ms, respectively (Fig. 4A). In the second experiment, the 300 V and incubation time of 48 h indicated higher GFP expression than the 400 V and incubation time of 24 h, respectively (Fig. 4B). As observed, the voltage change showed a significant effect on transfection efficiency (700 V vs 300 V; $p < 0.05$) as compared to the number of pulse (two pulses vs one pulse, $p > 0.05$) in different conditions. The best transfection efficiency was about 30.15% ± 5.21 for DCs. The fluorescent microscopy image was shown for the best electroporation conditions, as well (Fig. 3C).

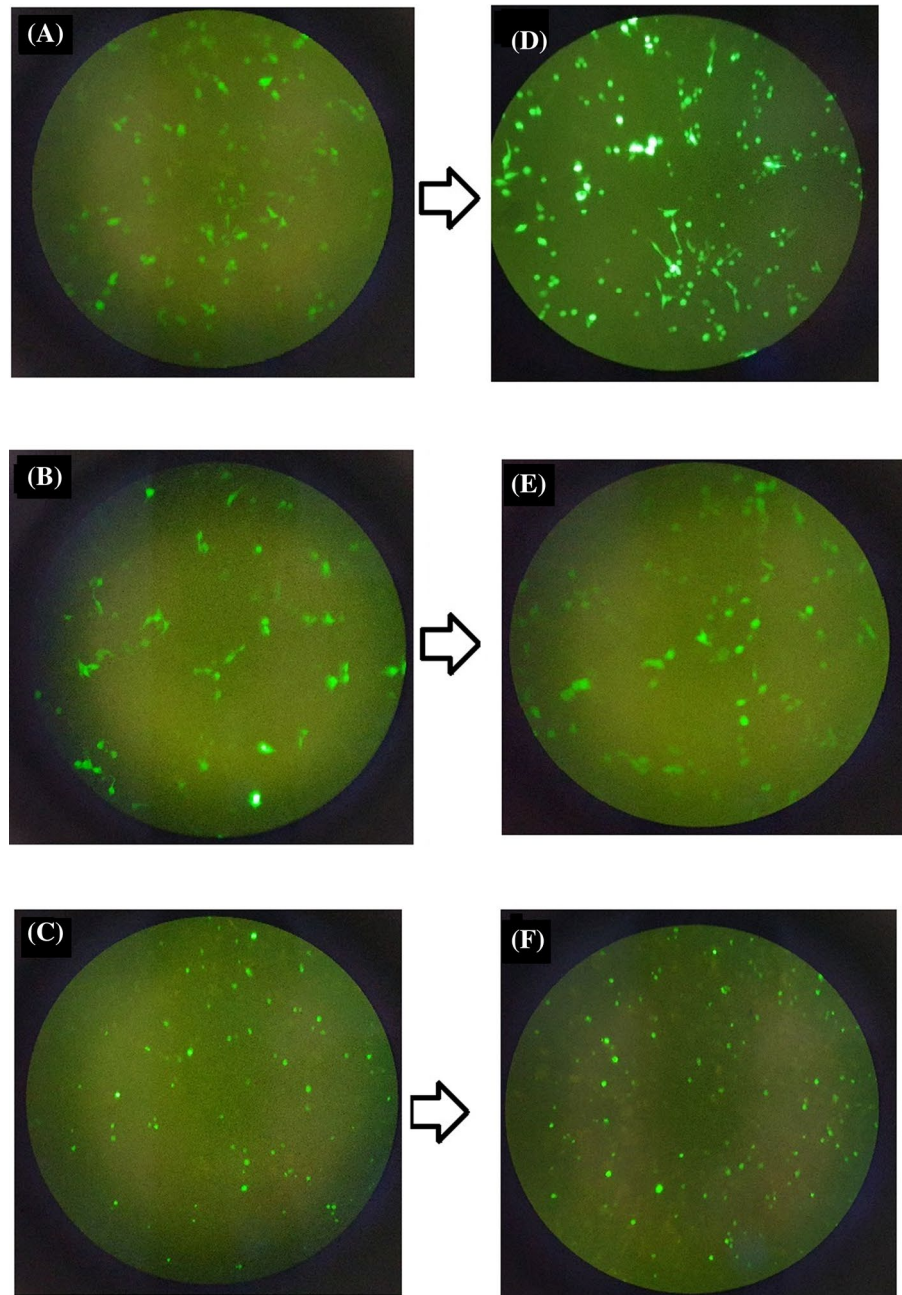
Heat treatment after electroporation

After determination of the optimal electroporation conditions for DNA delivery in HEK-293 T cells, COS-7 cells and DCs, the cells were incubated at 42 °C for 2 h after electroporation. The transfection efficiency was about 90.13% ± 2.06, 71.02% ± 2.80 and 43.25% ± 3.11 for HEK-293 T cells, COS-7 cells and DCs, respectively (Fig. 5A). The transfection efficiency was significantly higher in all the cells under the combined electroporation and heat treatment than the electroporation conditions, alone ($p < 0.05$; Fig. 5A). The GFP expression was higher in HEK-293 T cells than that in COS-7 cells ($p < 0.05$; Fig. 5A). Moreover, the GFP expression was higher in COS-7 cells than that in DCs ($p < 0.01$; Fig. 5A). The fluorescent microscopy image was shown for the combined electroporation and heat treatment (Fig. 3D–F).

Cell viability

MTT assay was performed to investigate the viability of electroporated cells versus non-electroporated cells (control) in both experiments for three cell types, individually. In both experiments, the cell viability rate was between 20 and 60% as compared to control (95–100%; $p < 0.001$, Figs. 1, 2, and 4C, D). The cell viability was almost constant after heat treatment, as well ($p > 0.05$; Fig. 5B).

Fig. 3 The fluorescent microscopy image of the cells under the best electroporation conditions (A–C), and the combined electroporation and heat conditions (D–F): HEK-293 T cells (A, D), COS-7 cells (B, E) and DCs (C, F)



Discussion

Electroporation is a non-viral delivery system for increasing the cellular uptake of exogenous biomolecules (RNA, DNA and proteins) in vitro and in vivo (Latella et al. 2016; Thakore et al. 2015; Son et al. 2016; Liu et al. 2015; Daud et al. 2008; Greaney et al. 2020; Ogunremi et al. 2013; De Keersmaecker

et al. 2020; Jansen et al. 2020). About four decades ago, Neumann and colleagues showed the first electro-transfection of herpes simplex thymidine kinase (TK) gene into mouse lyoma cells (Neumann et al. 1982). In 2004, the first clinical trial using electroporation was started to deliver interleukin-12 pDNA in metastatic melanoma cells (Daud et al. 2008). This approach was successful in a wide-range of clinical

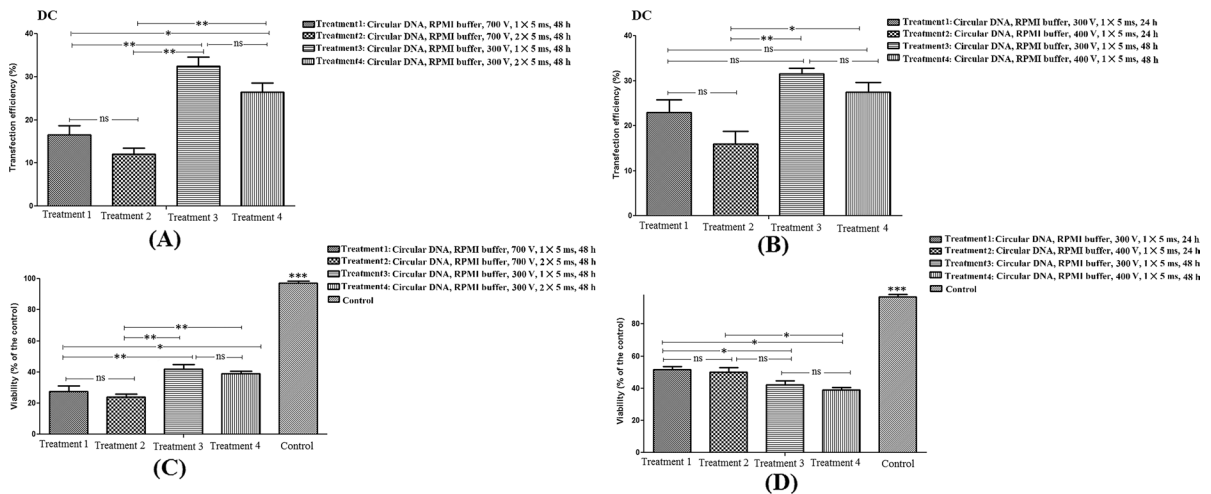


Fig. 4 Evaluation of pulse number & voltage (A), and voltage & incubation time after electroporation (B) in DCs using flow cytometry; The cell viability after electroporation under

different conditions for DCs using MTT assay (C, D); ns non-significant; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

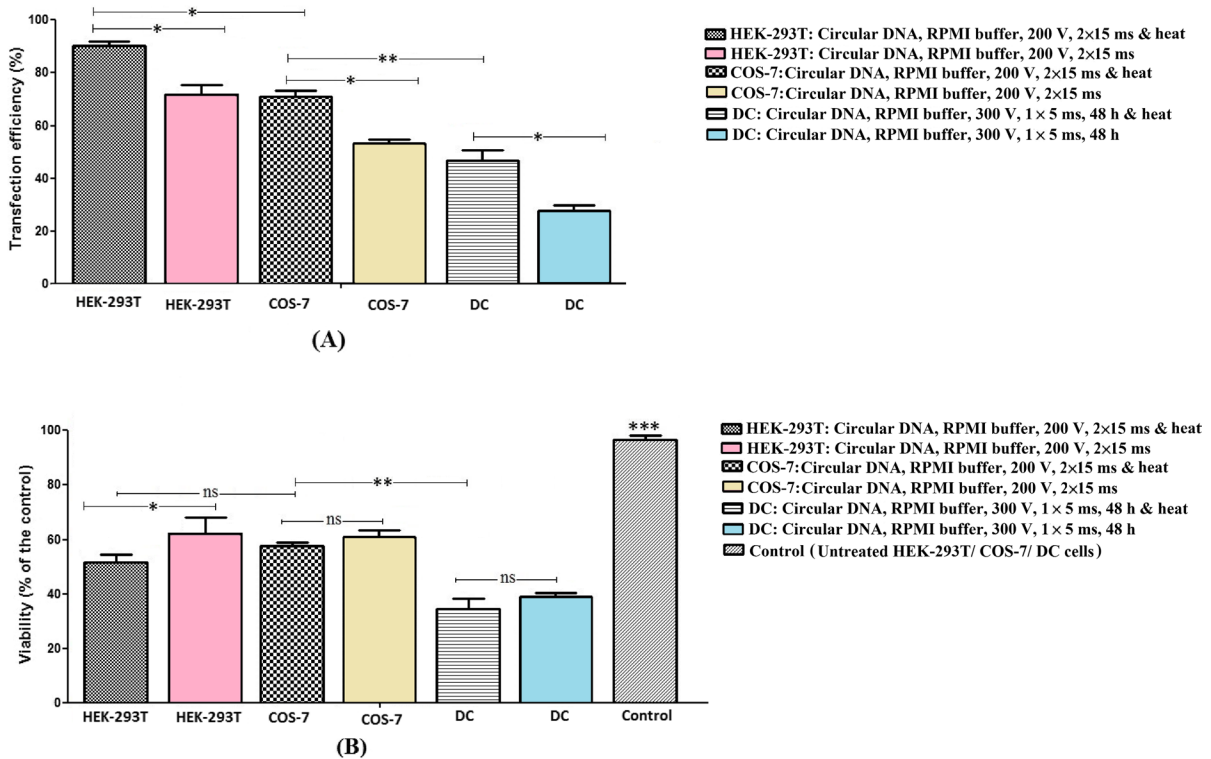


Fig. 5 The transfection efficiency of plasmid DNA into HEK-293 T cells, COS-7 cells and DCs using the combined electroporation and heat treatment as compared to electroporation,

alone (A); Cell viability after the combined electroporation and heat treatment as compared to electroporation, alone (B); ns non-significant; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

trials (Heller and Heller 2015; Meijerink et al. 2021; Mpendo et al. 2020). Recently, the efficacy of therapeutic DNA vaccine (GX-188E) injected intramuscularly by electroporation was proved for inducing regression of cervical intraepithelial neoplasia (CIN3) in patients (Choi et al. 2020). However, every cell type needs different electro-transfection conditions that should be optimized experimentally (Ovcharenko et al. 2005). For instance, the delivery of large biomolecules (e.g., nucleic acids) was dependent on electrical forces established during pulsing (Venslauskas and Šatkauskas 2015). Our study describes the optimization of pEGFP-N1 electro-transfection into HEK-293 T cells, COS-7 cells and DCs under different conditions including strength of electric field, duration and number of electric field, and electroporation buffer. The *egfp* reporter gene has been extensively utilized to investigate the efficiency of gene delivery (Soleymani et al. 2019). Our data showed that the rate of DNA transfection was dependent on various conditions of the selected square-wave pulse and the electroporation buffer. The culture medium (pH 7.5) showed an important role in increasing the transfection efficiency of pDNA into the cells compared to sucrose buffer. Potter and Heller showed the effect of electroporation buffer related to pH in protocols (2017). Guo et al. (2012) showed that RPMI-1640 without serum and antibiotics as electroporation buffer was more effective than phosphate-buffered saline (PBS 1X) buffer. Moreover, incubating the cells on ice prior and after pulsing enhances electroporation efficiency (Guo et al. 2012). Indeed, incubation of cells on ice usually leads to higher transfection rate particularly at high voltage due to heat generation (Potter et al. 1984). Another critical factor in electroporation is the pulse length and voltage. The studies showed that the millisecond pulses are more desirable for enhancing the cell uptake than the microsecond pulses (Lucas and Heller 2001). In our study, the 15 ms pulse was more effective than the 25 ms pulse for DNA uptake in both HEK-293 T and COS-7 cells. The longer exposure time to voltage led to the reduction of electroporation efficiency which may be due to heat generation during the pulse. The reports showed that the optimal voltage for electro-transfection had an inverse relationship with the cell size (Chu et al. 1987). It was previously confirmed that two pulses are sufficient for electro-transfection of DNA into most cell types (Jianqiong et al. 2000).

Moreover, the cell distance from electrodes plays a crucial role in electro-transfection efficiency. The 0.4 cm cuvette showed better results in comparison with 0.2 cm cuvette due to greater cell distance from electrodes (Geng and Lu 2013; Grys et al. 2017; Hyder et al. 2020). We also used the 0.4 cm cuvette for electro-transfection. On the other hand, our study showed that the linearized pDNA led to a decreased expression of EGFP protein. Other studies demonstrated that the circular DNA is more effective than the linearized DNA for transient gene expression (Potter and Heller 2017).

In current study, electroporation was utilized to deliver pDNA into DCs. DCs were widely used for development of cell-based vaccines in infectious diseases and cancer. DCs as an antigen presenting cell (APC) are responsible for antigen uptake, their processing and presentation to the major histocompatibility complex (MHC) molecules (Yi and Appel 2013). In various studies, antigen-pulsed DCs could stimulate tumor-specific immune responses (Porgador et al. 1996; Gabrilovich et al. 1996). Some findings showed the gene transfer into DCs through electro-transfection (Lenz et al. 2003; Artusio et al. 2006). Our data showed that lower voltages (300 or 400 V) were more efficient than high voltage (700 V) in electro-transfection efficiency ($p < 0.05$), but the number of pulses did not influence the cell uptake ($p > 0.05$).

On the other hand, heat treatment could alter the structure of the cell membrane in various cell types. Heat-induced changes in the membrane potential were determined in normal and transformed hamster lymphocytes. Incubation for 1–2 h at temperatures between 38 and 42 °C resulted in a depolarization of normal cells and a hyperpolarization of SV40-transformed cells (Mikkelsen and Koch 1982). In 2017, Tkizaki et al. reported that gene transfection could be enhanced by heat treatment (2017). Heat shock likely influences the cells through an increase in the number of cells that uptake the plasmid, and/or an increased stable integration rate (Pipes et al. 2005). In our study, heat treatment of the cells at 42 °C for 2 h after electroporation could increase transfection efficiency and gene expression in both adherent and suspension cells ($p < 0.05$).

Generally, in our study, different parameters were studied to determine the best conditions of DNA electro-transfection into adherent cells (HEK-293 T and COS-7 cells). The RPMI buffer, circular form of DNA, two pulses of 15 ms and 200 V conditions were

more effective than the sucrose buffer, linear form of DNA, two pulses of 25 ms and 100 V conditions for DNA delivery into both HEK-293 T and COS-7 cells. In all experiments, the viability was significantly reduced in electro-transfected cells as compared to untransfected cells. However, the sucrose buffer, 200 V and two pulses of 25 ms led to further cytotoxicity than other conditions. Moreover, the electro-transfection of DCs with the plasmid DNA was studied. Our data indicated that serum-free RPMI buffer, circular form of DNA, 300 V, and one pulse of 5 ms and incubation time of 48 h were the most effective conditions for DNA delivery into DCs. Moreover, the voltages of 300 and 400 (300 V and 400 V) showed the same cell viability, but the voltage of 700 (700 V) indicated high cell cytotoxicity with low transfection. On the other hand, the best conditions of electroporation along with heat treatment could significantly augment the transfection efficiency in all the cells.

In summary, our study showed that DNA delivery can be successfully performed in various cell types through electroporation using the optimized parameters such as buffer, electric field, number of pulsing, pulse length, DNA form and incubation time after electro-transfection. The low voltage, low pulse length, and cell culture medium as electroporation buffer were important parameters in cell uptake and also cell viability. Significant differences were observed between DCs and adherent cells (HEK-293 T and COS-7 cells) in electro-transfection efficiency. However, HEK-293 T and COS-7 cells showed the same conditions in electro-transfection parameters likely due to their same origin (epidermal tissue). These findings can be used for DNA delivery through electroporation in other cells. Moreover, heat treatment along with electroporation could significantly increase the transfection efficiency in the cells. Further studies will be required to optimize other electroporation conditions (e.g., DNA concentration, buffer, etc.) as well as mechanism of heat effects on electroporation.

Acknowledgements Authors acknowledge the financial support by Pasteur Institute of Iran for experimental works (Grant No. 1135).

Data availability All data are available in the manuscript.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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