J Membrane Biol (2012) 245:789–795
DOI 10.1007/s00232-012-9480-0

# Optimization of Electrotransfection Conditions of Mammalian Cells with Different Biological Features

**Huichen Guo · Rongzeng Hao · Yanquan Wei · Dehui Sun · Shiqi Sun · Zhencang Zhang**

Received: 21 January 2012 / Accepted: 30 June 2012 / Published online: 27 July 2012


**Abstract** We introduced eukaryotic expression plasmid pEGFP-N1 encoding green fluorescent protein (GFP) genes into cells with different biological features through electroporation. The effects of conditions, including voltage, capacitor flow, pulse cycle, DNA dosage and buffer, on transfection efficiency were investigated based on fluorescent microscopy and posttransfection survival rate of cells by staining with trypan blue. Better electrotransfection outcomes were achieved in the following epithelial cells: Vero cells at 300 V/850 μF, PK15 cells at 300 V/500 μF, MDCK cells at 200 V/600 μF, F81 cells at 200 V/500 μF, cancer cells MB49 at 300 V/400 μF, Hela cells at 200 V/450 μF, HF-29 cells at 300 V/800 μF and B16F1 cells at 200 V/650 μF. Among fibroblast cells, better electrotransfection was achieved in BHK21 cells at 300 V/600 μF and ST cells at 200 V/750 μF. RPMI-1640 medium without antibiotics and serum demonstrated higher electrotransfection efficiency and cell survival rate than other cell culture media as electroporation buffer. Our findings further prove that electroporation transfection is an effective method for genetic transfection. Cells with different biological features require varying transfection conditions to obtain higher transfection efficiency of target genes.

**Keywords** Electroporation transfection · Mammalian cell · Parameter optimization

## Introduction

Cell transfection is an important technique in cell biology and molecular biology. At present, common transfection methods include calcium phosphate sedimentation, liposome transfection, gene gun and electroporation. The calcium phosphate sedimentation method involves combining calcium phosphate and plasmid DNA into a sediment that is not easily dissolvable, which will be adsorbed onto the surface of the cell membrane. The combination of calcium phosphate sediment and plasmid DNA enters cells through endocytosis. This method could be used for plasmid DNA of large molecular weight but is quite noxious to cells (Kingston et al. 2001). The liposome transfection method requires wrapping of the positively charged liposome around the negatively charged DNA through electrostatic interaction to form DNA–lipid compounds. These compounds are then adsorbed by negatively charged cell membranes and enter cells through cell infusion or endocytosis. This method is disadvantageous because of the high cost of liposomes, toxicity to cells and wide variations in transfection outcomes for different cell types (Kawakami et al. 2008; Gao et al. 2007). The gene gun method involves immersion of 4 μm tungsten or gold powder in donor DNA and injection of the particles into cells, tissues or organs using the gene gun. Its disadvantages include the need for special equipment, high cost and lower transfection efficiency than other transfection methods (Robinson

H. Guo and R. Hao contributed equally to this work.

H. Guo · R. Hao · Y. Wei · D. Sun · S. Sun (✉)
State Key Laboratory of Veterinary Etiological Biology and National Foot and Mouth Disease Reference Laboratory, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Xujiaping 1, Lanzhou 730046, Gansu, People's Republic of China
e-mail: shiqisun21@hotmail.com

H. Guo
e-mail: ghch-2004@126.com

Z. Zhang
Yangling Vocational and Technical College, Yangling 712100, Shanxi, People's Republic of China

and Pertmer 2001). The electroporation method has received extensive attention as an ideal method to achieve higher transfection efficiency in the laboratory. In this method, cells are placed in a transient high-pressure current pulse environment. Cell membranes develop nanometer-level micropores due to shock and drugs, genetic substance, protein and other large biological molecules; and liposomes are able to enter the cytoplasm through such micropores or due to the redistribution of cell membrane components during closure of micropores. This method has the advantage of being simple, fast, reproducible, safe and suitable for a wide spectrum of cells. In addition, it has higher transfection efficiency, particularly for suspension culture cells, which are generally believed to be difficult for transfection (Baum et al. 1994). However, the efficiency of electroporation transfection and the survival rate of cells after transfection are affected by many factors. Thus, there is a need to individually optimize transfection conditions for specific cellular strains.

In this study, we utilized plasmids with green fluorescent protein (GFP) encoding genes as a model in order to optimize transfection conditions and parameters commonly used by laboratories for electroporation of cell strains with different biological features. Voltage, capacitance, degree of shock, concentration of DNA and buffer components were controlled to provide reference for optimization or improvement of the transfection efficiency of various cells.

## Materials and Methods

### Cell Strains

Vero, PK15, MDCK, F81, MB49, Hela, HT-29, B16F1, BHK21 and ST strains were all purchased from ATCC (USA) and preserved in our laboratory. MB49 and Hela cells were cultured in RPMI-1640 medium (GIBCO, Grand Island, USA) containing 10 % fetal bovine serum (FBS; Hyclone, Victoria, Australia), 100 U/ml penicillin and 0.1 mg/ml streptomycin. Other cell strains were cultured in DMEM (GIBCO, Grand Island, USA) medium containing 10 % FBS, 100 U/ml penicillin and 0.1 mg/ml streptomycin, . All cells were cultured at 37 °C in a 5 % $CO_2$ incubator.

### Plasmid Purification

#### *Escherichia coli*

DH5a transfected with pEGFP-N1 plasmid (Clontech, Palo Alto, USA) were inoculated into LB medium (ampicillin 100 μg/ml) and cultured overnight at 37 °C. Plasmids were purified using a Midiprep plasmid kit (Invitrogen, Carlsbad, USA). DNA concentration was estimated with a UV–Visible spectrophotometer. Plasmids were stored at –20 °C for future use.

### Design of Electroporation Transfection Experiments

#### *Different Electroporation Buffer*

Voltage was set to 200 V, capacitance flow to 850 μF, cell concentration to $1 \times 10^6$/ml and DNA dosage to 4 μg. Different electroporation buffers were used: 1× PBS (GIBCO), OPTI-MEM (Invitrogen), RPMI-1640 (GIBCO) without antibiotics and serum for electroporation (Gene Pulser II Electroporation System; Bio-Rad, Richmond, CA). The volume of the electrotransfection cup was 0.8 ml. Cell suspension (0.3 ml, $1 \times 10^6$/ml) and pEGFP-N1 plasmid were mixed evenly for electrotransfection. After electroporation, fresh DMEM culture medium containing 10 % FBS was added. Cells were placed at 37 °C in a 5 % $CO_2$ incubator for 24 h, after which transfection efficiency and cell survival rate were measured. Nontransfected cells were used as reference.

#### *Different Voltage*

Capacitance flow rate was set to 850 μF, cell concentration to $1 \times 10^6$/ml and DNA dosage to 4 μg. RPMI-1640 culture solution was used as electroporation buffer. Voltage was varied at 200, 300 and 400 V. The steps were as described above (see "Different Electroporation Buffer" section).

#### *Different Capacitance Flow*

The optimal voltage for each cell as determined in the previous experiment was used. Cell concentration was fixed to $1 \times 10^6$/ml and DNA dosage to 4 μg. RPMI-1640 culture solution was used as electroporation buffer. Capacitance flow was varied at 450, 650 and 850 μF. The steps were as described above (see "Different Electroporation Buffer" section).

#### *Different Pulsated Shocks*

Transfected cells were given one, two and three doses of pulsated shocks with 1-min intervals under the most suitable voltage and capacitance flow for electroporation. The steps were as described above (see "Different Electroporation Buffer" section).

#### *Different DNA Dosage*

Transfected cells underwent electroporation with the most suitable voltage and capacitance flow for electroporation

and cell concentration of $1 \times 10^6$/ml. DNA was varied at 4, 6 and 8 μg.

### Calculation of Electrotransfection Efficiency and Cell Survival Rate

Total counts of cells were determined under the count vision with a fluorescent microscope (Leica CTR 6000). Fluorescent light source was used, and cells expressing GFP were included in the count. Transfection efficiency (%) was calculated as the number of cells with green fluorescence under the fluorescent microscope divided by the total number of cells under visible light, with that value multiplied by 100. Survival rate of cells (%) was determined after staining with 0.4 % trypan blue as total number of live cells divided by total number of live and dead cells, with that value multiplied by 100.

### Statistical Analysis

All data were analyzed by SPSS 13.0 (SPSS, Inc., Chicago, IL) and expressed as mean with standard deviation. The transfection efficiency of electroporation groups of each type cell was compared by *t*-test. $p < 0.05$ was considered statistically significant.

## Results

### Influence of Electrotransfection Buffer on Transfection Efficiency and Cell Survival Rate

We determined the suitable electrotransfection buffer among three types of common buffer liquid for electroporation. Transfection efficiency and cell survival rate of Vero cells in different shock buffers were observed under a fluorescent microscope. As Fig. 1 shows, the transfection efficiencies in the OPTI-MEM (Fig. 1a, b) and 1× PBS (Fig. 1c, d) groups were very low, so they were difficult to evaluate correctly. However, the transfection of cells in RMPI-1640 exceeded 50 % (Fig. 1e, f), indicating successful introduction of pEGFP-N1 plasmids into cells. Hence, RMPI-1640 was selected as optimal for use in all subsequent experiments.

### Influence of Voltage on Transfection Efficiency and Cell Survival Rate

Under fixed capacitance conditions, varying voltages had different influences on the expression rate of pEGFP-N1 plasmids and the survival rate of cells (Figs. 2, 3). When capacitance flow was fixed and voltage was changed from 200 to 300 V, there was no significant difference in transfection efficiency in all groups. When voltage exceeded 300 V, transfection efficiency decreased significantly. Cell death rate increased as voltage increased, indicating that the intensity of the electric field has a great influence on cell survival and transfection efficiency. The optimal voltages for the electroporation of different cells determined through the experiments were as follows: Vero, 300 V; PK15, 300 V; MDCK, 200 V; F81, 200 V; MB49, 300 V; Hela, 200 V; HT-29, 300 V; B16F1, 200 V; BHK21, 300 V; and ST, 200 V.

### Influence of Capacitance Flow on Transfection Efficiency and Cell Survival Rate

Under the optimal voltage for different cells, the capacitance strength was varied: 450, 650 and 850 μF. Varying capacitance values had different influences on the transfection efficiency of pEGFP-N1 plasmids in cells, indicating that the specific capacitance should be determined for different cells in order to achieve optimal transfection efficiency (Figs. 4, 5). The results demonstrate that cell death rate increases with higher capacitance values. Based on transfection efficiency and cell death rate under different capacitance conditions, the optimal capacitance values for different cells were as follows: Vero, 850 μF; PK15, 500 μF; MDCK, 600 μF; F81, 500 μF; MB49, 400 μF; Hela, 450 μF; HT-29, 800 μF; B16F1, 650 μF; BHK21, 600 μF; and ST, 750 μF.

### Influence of Dose of Pulsated Shocks on Transfection Efficiency and Cell Survival Rate

We observed cells under the fluorescence microscope after electroporation with several doses of pulse. There were no significant differences in transfection efficiency between groups (Figs. 6, 7). Cell survival rate decreased as pulse dose was increased so that the highest survival rate was observed in cells that received only one dose of shock.

### Influence of DNA Dosage on Transfection Efficiency and Cell Survival Rate

As shown in Figs. 8 and 9, there were no significant differences in transfection efficiency of cells after transfection with different pEGFP-N1 plasmids. Cell activity decreased with higher DNA dosage.

## Discussion

The cell membrane is comprised of a lipid bilayer and has as its main function separating the internal microenvironment from the external environment of the cell. It allows

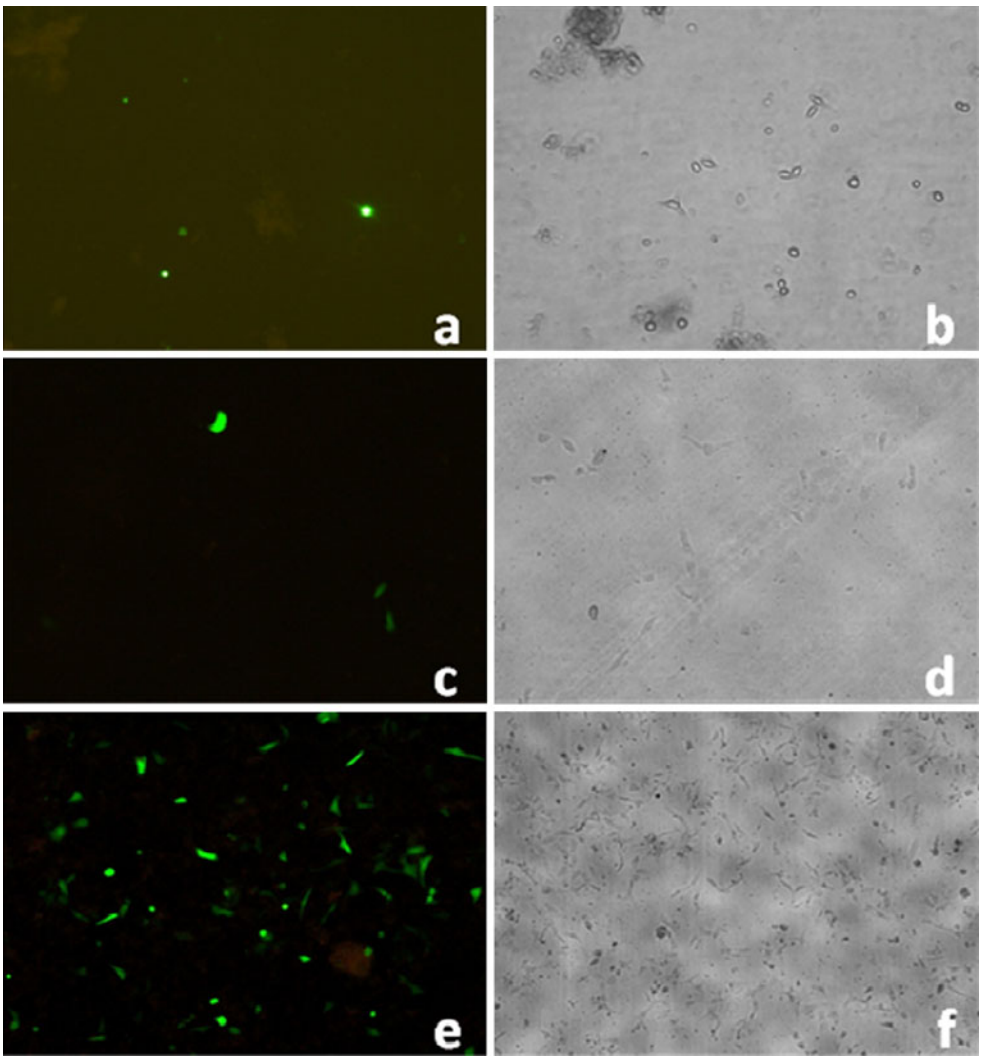


**Fig. 1** Transfection efficiency of Vero cells in different media using an inverted fluorescence microscope: **a** cells in OPTI-MEM under a fluorescence microscope and **b** corresponding phase-contrast photo; **c** cells in PBS under a fluorescence microscope and **d** corresponding phase-contrast photo; **e** cells in RPMI-1640 under a fluorescence microscope and **f** corresponding phase contrast photo

chemical materials to pass through selectively; large molecules generally cannot pass through the cell membrane. The introduction of larger molecules, such as a genetic carrier in cell genetic engineering and medicine, into cells is an extremely necessary and important means for the study of life sciences.

Electroporation transfection is a physical method of introducing exogenous substances into cells that was invented in the mid-1980s (Canatella et al. 2001). Micropores appear on cell membranes under high-voltage pulse, thus allowing for the introduction of exogenous genes into cells. It can be used to transfer DNA, RNA, antibiotics, enzymes and other biologically active molecules into bacterial, enzyme, animal and plant cells. Electroporation transfection is a genetic transfer method with the advantages of simplicity, convenience, reproducibility, high transfection efficiency and suitability to many cell lines that are incomparable to those of other transfection methods (Cegovnik and Novaković 2004).

During application, we found that disturbance of the proteins in the cell membrane and membrane skeleton with a strong electrical field reduces the stability of cells, facilitates cell transformation, allows formation of pseudopods and may even lead to cell death. To overcome this shortcoming, transfection of eukaryotes is usually performed at low voltage (200–300 V) and high capacitance (900–1,000 μF). High transfection efficiency and reproducibility could be obtained through optimization of conditions since different cell strains require different transfection conditions (Yao et al. 2001). Transfection conditions and maximal transfection efficiency are not necessarily the same for all cell strains because of different growth states, cell sizes and cell membrane structures of eukaryotes.

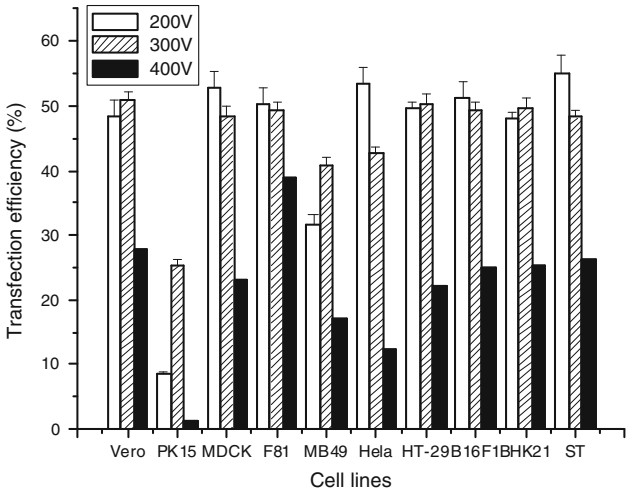


**Fig. 2** Transfection efficiency of different cell lines using different voltages

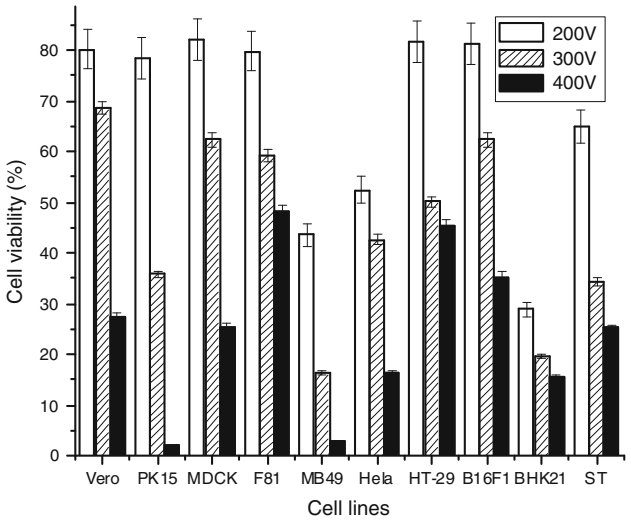


**Fig. 3** Rate of survival of cells using different voltages

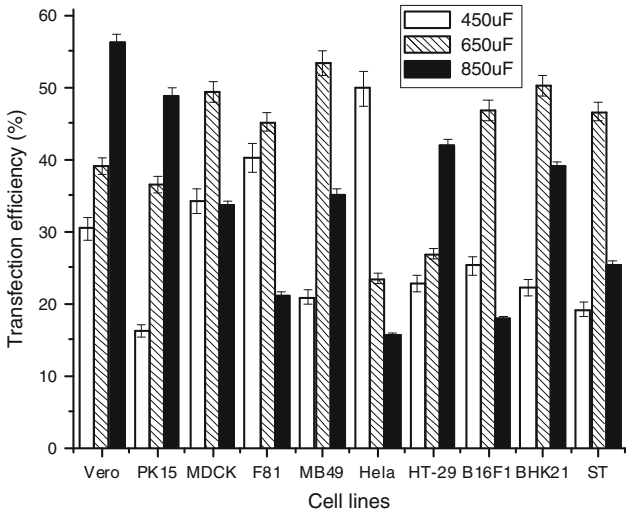


**Fig. 4** Transfection efficiency of different cell lines using different capacitance flows

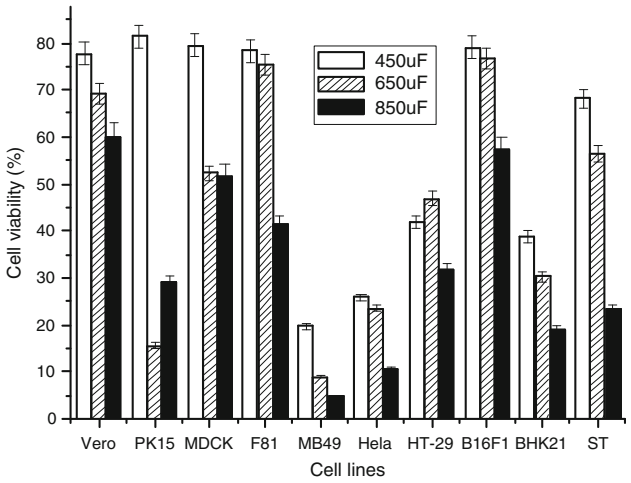


**Fig. 5** Rate of survival of cells using different capacitance flows

The major factors affecting electroporation transfection efficiency and cell survival rate include the intensity of electrical fields (i.e., voltage), pulse interval (i.e., capacitance flow), temperature, buffer ingredients, cell state and volume and the concentration and conformation of exogenous genes. Among them, pulse amplitude is the key factor affecting transfection efficiency. Different cells have different degrees of adaptation to the intensity of the electrical field and the interval of the electric pulse. When voltage is too low and pulse electroporation conditions are insufficient for a specific cell strain, the cell membranes will not be altered, exogenous genes will not be able to enter cells easily and transfection efficiency will not be maximized. On the other hand, excessive voltage will cause irreversible damage to the cells, so survival and transfection efficiency will be extremely affected (Hui 1995). At present, the main disadvantage of the electroporation transfection method is the high cell toxicity (50–90 %) (Baron et al. 2000). In general, electroporation can be optimized to control survival rate within 30–40 % and to maximize transfection efficiency (Zhou et al. 1999).

Temperature and buffer ingredients influence transfection efficiency. Among the other transfection methods, phosphate buffer without calcium and magnesium ions or buffer with low osmotic pressure are the most extensively used. These buffers do not require serum, especially for gene transfection mediated by cationic liposome. Serum is regarded as the most important factor affecting transfection efficiency. To measure the influence of shock buffer on the cell survival rate, we selected RPMI-1640 cell culture medium without serum and antibiotics, PBS and OPTI-MEM shock buffer. Electroporation was performed at

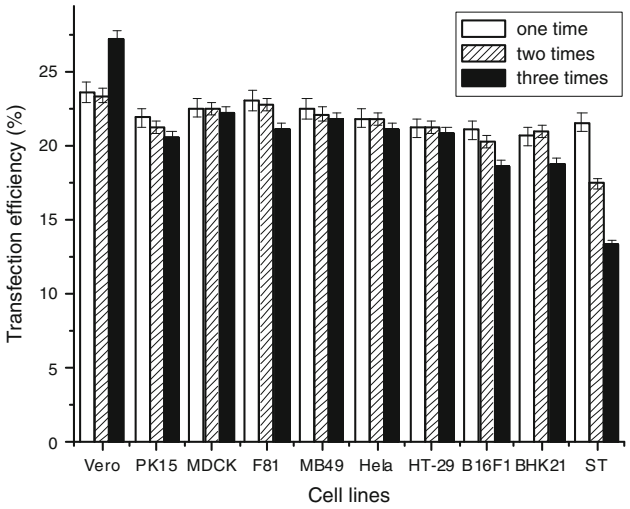


**Fig. 6** Transfection efficiency of different cell lines using different doses of pulsed shocks

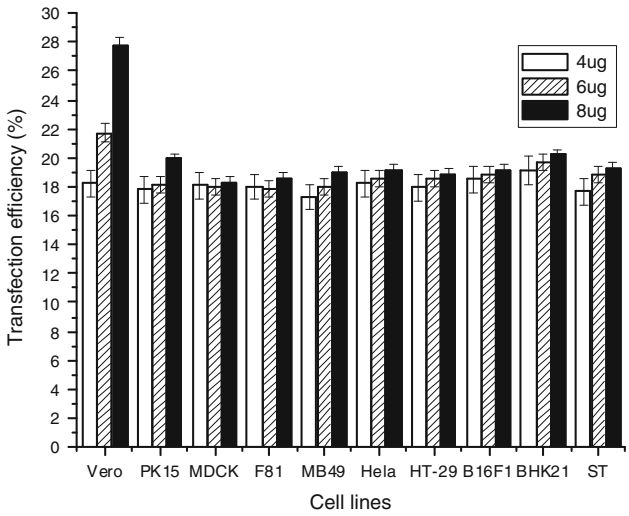


**Fig. 8** Transfection efficiency of different cell lines using different DNA dosages

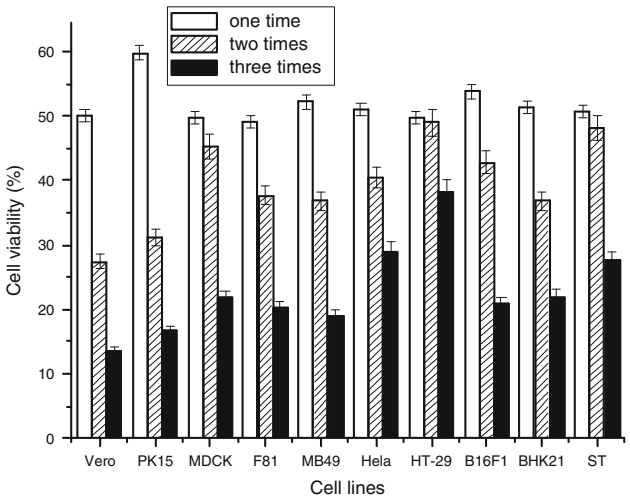


**Fig. 7** Rate of survival of cells using different doses of pulsed shocks

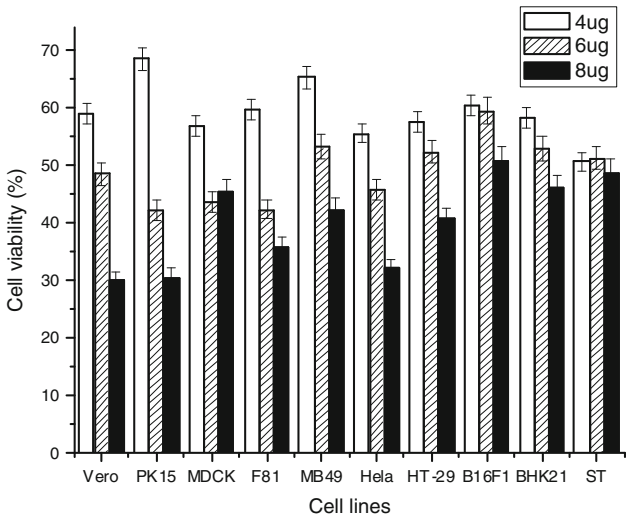


**Fig. 9** Rate of survival of cells using different DNA dosages

200 V and 850 μF, after which the cell survival rate was compared among the different buffer groups. RPMI-1640 without serum and antibiotics had a higher cell survival rate after shock than other buffers. Even for cells cultured with other media, transfection and survival rate were not affected when RPMI-1640 was used as transfection buffer. Our findings indicate that RPMI-1640 culture medium can be used as the shock buffer of various cell strains to further simplify the operation and reduce cell damage and death after transfection. Moreover, placing the cells in an ice bath for 10 min before and after shock leads to slow closure of holes on the cell membrane, thus allowing the entry of more plasmids into cells and improving transfection efficiency. However, when shock is delivered in a low-temperature environment, eukaryotes are more easily damaged, survival rate drops and transfection efficiency is affected. Under room temperature, holes on the cell membrane close quite quickly and plasmids enter the cells through electric internalization, thus guaranteeing the survival of cells. Transfection efficiency is not lower than that at 4 °C (Yang et al. 2009).

High voltage helps to perforate cells and improve transfection efficiency. When capacitance flow is set at 850 μF, transfection efficiency at 400 V decreases significantly because the excessively high intensity of the electric field causes cell damage during perforation. As a result, a large amount of cells die and transfection efficiency is reduced. In addition to voltage, strength, survival and transfection efficiency are affected by capacitance flow. Capacitance flow influences transfection in the same manner as voltage since both are factors of cell electroporation. Capacitance flow is

related to the length of the pulsated shock. As the pulse cycle is prolonged, cell perforation is reinforced, plasmids enter cells and more cells will have irreversible damage and die. Therefore, electrotransfection conditions can be optimized by bidirectional adjustment of the strength of the electric field and pulse period. The results of this study demonstrate that various cells can achieve good electrotransfection outcomes under individually optimized conditions through bidirectional adjustment of the intensity of the electric field and pulse interval.

Examination of the relationship between DNA dosage and transfection outcomes revealed no significant differences in transfection efficiency among cells administered with 4, 6 and 8 μg DNA. Changes in DNA concentration apparently do not influence the survival rate of cells, although cell death rate increased with higher DNA dosage. Previous studies have found that at a cell density of $1 \times 10^6$/ml, transfection efficiency is highest when DNA dosage is 2–5 μg/M. Transfection efficiency increases linearly with higher plasmid concentration to a peak, after which it gradually decreases with further increases of DNA dosage. This effect can be attributed to the saturation of DNA absorption; excessive adsorption will result in toxicity and lower survival rate, which decrease transfection rate. For most cells the ideal dose of pulse is three to four times (Zhang et al. 2000). In theory, two pulses are enough to transfect DNA into most cells, and an increased number of pulses helps to improve transfection efficiency. However, if more than five pulses are applied, the resulting damage or even death of cells will offset the improvement of transfection efficiency (Zhang et al. 2000). Our experiments revealed that there is no significant difference in transfection efficiency after one, two or three doses of shock and that cell survival rate decreases as pulse dose is increased.

The electroporation transfection method has been extensively applied and has provided a convenient method for genetic transfection and other work. The optimized electrotransfection conditions obtained in this study may serve as reference for other researchers.

**Acknowledgements** This work was supported by the National Natural Science Foundation of China (Grants 31100688 and 31101838), the Foundation of Science and Technology Committee of Gansu (Grants 1102NKDA034 and 1102NKDA033) and the International Science and Technology Cooperation Program of Gansu (Grant 1104WCGA185).

## References

Baron S, Poast J, Rizzo D, McFarland E, Kieff E (2000) Electroporation of antibodies, DNA, and other macromolecules into cells: a highly efficient method. J Immunol Methods 242:115–126

Baum C, Forster P, Hegewisch-Becker S, Harbers K (1994) An optimized electroporation protocol applicable to a wide range of cell lines. Biotechniques 17:1058–1062

Canatella PJ, Karr JF, Petros JA, Prausnitz MR (2001) Quantitative study of electroporation-mediated molecular uptake and cell viability. Biophys J 80:755–764

Cegovnik U, Novaković S (2004) Setting optimal parameters for in vitro electrotransfection of B16F1, SA1, LPB, SCK, L929 and CHO cells using predefined exponentially decaying electric pulses. Bioelectrochemistry 62:73–82

Gao X, Kim K-S, Liu D (2007) Nonviral gene delivery: what we know and what is next. AAPS J 9:E92–E104

Hui SW (1995) Effects of pulse length and strength on electroporation efficiency. Methods Mol Biol 55:29–40

Kawakami S, Higuchi Y, Hashida M (2008) Nonviral approaches for targeted delivery of plasmid DNA and oligonucleotide. J Pharm Sci 97:726–745

Kingston RE, Chen CA, Okayama H (2001) Calcium phosphate transfection. Current protocols in immunology. Wiley, New York

Robinson HL, Pertmer TM (2001) Nucleic acid immunizations. Current protocols in immunology. Wiley, New York

Yang W, Wang D, Cheng K, Wang M, Wang G, Hu J, Wang J (2009) Selection of electroporation condition of plasmid. Acta Medicinae Universitatis Scientiae et Technologiae Huazhong 38: 858–860

Yao Y, Zhang D, Luo Y, Zhang D, Huang A, Zhou W, Ren H (2001) Influence of electroporation on the biological activities of primary rat hepatocytes. Chin J Hepatol 9:178–180

Zhang J, Zhanag X, Xie W, Shan Xiang, Lin L (2000) Survey of the highest of transformation efficiency of the competent cell. J Nanjing Normal Univ 23:72–75

Zhou Z, Zhang D, Ren H (1999) The optimal electroporation condition of recombinant plasmid. Acta Universitatis Scientiae Medicinae 24:130–132