Video Article

Changing the Direction and Orientation of Electric Field During Electric Pulses Application Improves Plasmid Gene Transfer *in vitro*

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Abstract

Gene electrotransfer is a physical method used to deliver genes into the cells by application of short and intense electric pulses, which cause destabilization of cell membrane, making it permeable to small molecules and allows transfer of large molecules such as DNA. It represents an alternative to viral vectors, due to its safety, efficacy and ease of application. For gene electrotransfer different electric pulse protocols are used in order to achieve maximum gene transfection, one of them is changing the electric field direction and orientation during the pulse delivery. Changing electric field direction and orientation increase the membrane area competent for DNA entry into the cell. In this video, we demonstrate the difference in gene electrotransfer efficacy when all pulses are delivered in the same direction and when pulses are delivered by changing alternatively the electric field direction and orientation. For this purpose tip with integrated electrodes and high-voltage prototype generator, which allows changing of electric field in different directions during electric pulse application, were used. Gene electrotransfer efficacy is determined 24h after pulse application as the number of cells expressing green fluorescent protein divided with the number of all cells. The results show that gene transfection is increased when the electric field orientation during electric pulse delivery is changed.

Video Link

The video component of this article can be found at http://www.jove.com/video/3309/

Protocol

1. Cell culture, plasmid and buffer preparation for the experiment

1. In this experiment Chinese hamster ovary cells (CHO-K1) are used. Cells are grown in a nutrient mixture HAM-F12 (PAA) supplemented with 2 mM L-glutamine, 10% fetal bovine serum, 400 µl/l gentamicin (all from Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany), and 1 ml/l crystacilin (Pliva, Zagreb, Croatia). Cells are kept at 37°C in a humidified 5% CO_2 atmosphere in the incubator for 24h.
2. Amplify plasmid pEGFP-N1 (Clontech Laboratories Inc., Mountain View, CA, USA) encoding green fluorescent protein (GFP) in DH5α strain of Escherichia coli and isolate it with HiSpeed Plasmid Maxi Kit (Qiagen, Hilden, Germany). Plasmid DNA concentration (plasmid dissolved in TE buffer) should be spectrophotometrically determined at 260 nm and confirmed by gel electrophoresis.
3. Prepare isoosmolar sodium phosphate buffer (10 mM Na_2HPO_4, 10 mM NaH_2PO_4, 1 mM $MgCl_2$, 250 mM sucrose, pH 7.4).
4. On the day of experiment prepare cell suspension by trypsinization with 0.25% trypsin/EDTA solution (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany). Centrifuge cells for 5 min at 1000 rpm (180 x g) at 4°C (Sigma, Germany) and resuspend cell pellet in isoosmolar sodium phosphate buffer to a cell density of 5×10^6 cells/ml.

2. Hardware equipment

1. Cells are exposed to electric field in pipette tip (Figure 1) with integrated electrodes connected to a high-voltage prototype generator. The tip and electrode geometry allows application of relatively homogeneous electric field and the generator allows delivery of electric pulses in different directions. The tip and the generator were developed at Laboratory of Biocybernetics, Faculty of Electrical Engineering, University of Ljubljana [1].

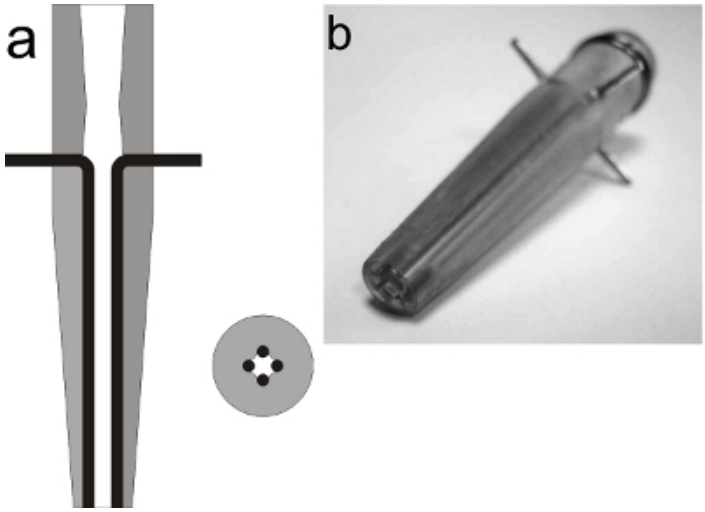

Figure 1. Vertical and horizontal (a) cross section and photograph (b) of pipette tip with integrated electrodes. In the cross section grey color is used for the plastic housing and black for the electrodes. The pipette tip with integrated electrodes consists of four cylindrical rod electrodes. The electrodes are made of stainless steel; their diameter is 1.4 mm, adjacent electrodes are 1 mm apart, and opposite electrodes are 2 mm apart. The electrodes are glued into the plastic tip in parallel and their applicable length is 30 mm [2].

3. Gene electrotransfer protocol

1. Add plasmid pEGFP-N1 to a cell suspension in concentration 10 µg/ml.
2. Incubate the mixture for 2-3 minutes at room temperature, before applying electric pulses.
3. Aspire 100 µl of cell suspension into the pipette tip with integrated electrodes.
4. To achieve best gene electrotransfer efficacy and maintain cell viability, optimal parameters of electric pulses should be used. In this experiment a train of 8 rectangular pulses (each with duration of 1 ms, amplitude 225 V at 1 Hz repetition frequency) is applied to each sample, using high-voltage prototype generator. Two different electric field protocols (Figure 2) are used: in the first protocol all pulses are delivered in the same direction, whereas in the second protocol pulses are delivered by changing alternatively the electric field direction and orientation. The second protocol can only be used with appropriate pulse generator, which allows application of electric pulses in different directions.
5. Immediately after the pulse application transfer the cells from pipette tip into 6 well plate and add fetal calf serum (FCS-Sigma, USA) (25% of sample volume).
6. Incubate cells for 5 min at 37°C to allow cell membrane resealing.
7. Add 2 ml of HAM-F12 to each sample in 6 well and incubate cells for 24h at 37°C in a humidified 5% CO_2 atmosphere in the incubator.

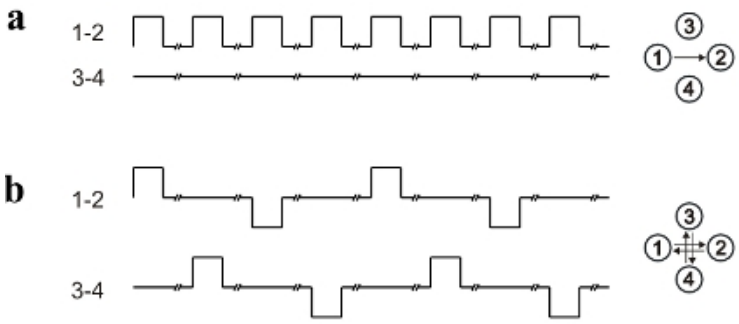

Figure 2. Electric field protocols: (a) all pulses are delivered in the same direction, (b) pulses are delivered by changing alternatively the electric field direction and orientation.

4. Image acquisition and determination of gene electrotransfer efficacy

1. Efficacy of gene electrotransfer is determined as the percentage of cells expressing GFP 24h after the pulse application.
2. The cells are observed using a fluorescence microscope (in our case Zeiss 200, Axiovert, ZR Germany) with excitation light at 488 nm generated with a monochromator system (PolyChrome IV, Visitron, Germany) and emission is detected at 507 nm. The images are recorded using imaging system (MetaMorph imaging system, Visitron, Germany), but other similar acquisition software can also be used.
3. Acquire at least five images (phase contrast and green fluorescence) at 20x objective magnification.

4. Count cells in phase contrast image and cells that are expressing GFP in green fluorescence image. Determine the percentage of gene electrotransfer efficacy by dividing the number of cells that are expressing GFP with the number of all cells in each corresponding image (Figure 3).

5. Representative results:

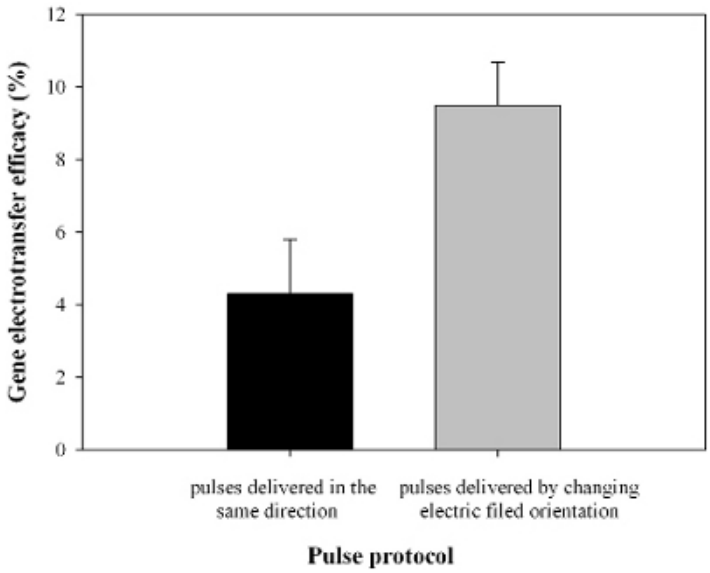

Figure 3. The percentage of cells expressing GFP when all pulses are delivered in the same direction and when pulses are delivered by changing alternatively the electric field direction and orientation is presented. Cells were exposed to a train of eight pulses with amplitude 225 V, duration 1 ms and repetition frequency of 1 Hz. Results were obtained by means of fluorescence microscopy. Each value in the graph represent mean of three independent experiments ± standard deviation. By changing the electric field direction and orientation the percentage of cells expressing GFP increases.

Discussion

Gene electrotransfer is a versatile biotechnology technique that enables transfer of DNA into cells by means of applying short, high voltage electric pulses [3] and represents a safer alternative to viral vectors due to its safety, efficacy and ease of application. Although today gene electrotransfer is widely used to transfect all types of cells and first phase I clinical trial using this method has been reported [4], the underlying mechanisms are still not completely understood. It is known, that application of electric pulses of sufficient strength to the cell causes an increase in the transmembrane potential, which induces the membrane destabilization [5]. Cell membrane permeability is increased and otherwise nonpermeant molecules enter the cell. Many parameters have been described [6-9], which influence the efficacy of gene electrotransfer, especially application of different pulse parameters were studied to enable better gene transfer [10-12]. Changing the electric field direction and orientation during the pulse delivery increases the area of the permeabilized cell membrane [13] therefore increases competent area available for transfer of DNA molecules. It was shown, that the percentage of cells expressing transferred gene increases when electric field direction and orientation is changed during the application [14]. For this purpose electric pulse generator, which allows application of electric pulses in different directions has to be used [1]. We are using such pulse generator and pipette tip with integrated electrodes in order to demonstrate the difference in gene electrotransfer efficacy, when all pulses are delivered in the same direction or when pulses are delivered by changing alternatively the electric field direction and orientation. The percentage of cells expressing transferred GFP gene was assessed 24h after pulse application and the increase of successfully transfected cells when the pulses were delivered by changing alternatively the electric field direction and orientation (survival rate 80,8% ± std 12%) in comparison when all pulses were delivered in the same direction (survival rate 76% ± std 16,2%) was obtained.

Disclosures

No conflicts of interest declared.

Acknowledgements

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