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Gene electrotransfer using locally enhanced electric field

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Duration of the experiment: day 1: 60 min; day 2: 60 min
Max. number of participants: 4
Location: Cell Culture Laboratory 3
Level: Basic

PREREQUISITES

Participants should be familiar with the Safety rules and Rules for sterile work in cell culture laboratory. No other specific knowledge is required for this laboratory practice.

The aim of this laboratory practice is to perform gene electrotransfer on attached cells using classical and localized electroporation

THEORETICAL BACKGROUND

Gene electrotransfer is a safe non-viral method used to transfer genes into living cells with the use of high-voltage electric pulses that cause reversible electroporation [1]. Nucleic acids such as DNA are large molecules that cannot cross an intact plasma membrane. However, if we expose the cells to electric pulses, the DNA can be transported across the electroporated membrane and enters the cell in a process termed gene electrotransfer.

In typical *in vitro* experiments, gene electrotransfer is performed by placing cells in suspension or attached to a surface between two parallel electrodes and exposing them to high-voltage electric pulses. The DNA is in the medium surrounding the cells. While this classical gene electrotransfer can be quite efficient, it can also be damaging to the cells, resulting in a considerable decrease in cell viability. These damaging effects are sometimes attributed to the large surface area of the plasma membranes that becomes electroporated in such experimental configuration [1].
In localized electroporation, nanostructured elements such as nanochannels, nanostraws, or nanopores are used to achieve localization of the electric field over small parts of the plasma membrane, which enhances gene electrotransfer efficiency and improves cell survival [2-5]. Various experimental configurations for localized electroporation have been developed. One of the simplest configurations uses commercially available cell culture inserts containing polyethylene-terephthalate (PET) porous substrate with pore size of 400 nm [2]. The configuration consists of an insert, a multiwell plate, and a pair of platinum/iridium wires as electrodes, one placed above and one below the porous substrate. The cells are grown attached to the upper side of the substrate, whereas the DNA solution is placed below the substrate.

When applying electric pulses, the pores within the substrate assist in DNA transfer in two ways: 1. they amplify the electric field and enable one to localize electroporation only to small parts of the plasma membrane which are in direct vicinity of the pores; 2. since the electric field within the pores is amplified, the pores act as tubes that electrophoretically pull DNA molecules towards and into the cells. Because the electric field is greatly amplified around the pores of the porous substrate, we can use a much lower pulse voltage for localized electroporation than for the classical one. In fact, application of too high voltage in localized electroporation results in cell damage as well, since electroporation is no longer localized under such conditions.

EXPERIMENT

On day one, we will transfect Chinese hamster ovary cells (CHO) with a plasmid DNA (pEGFP-N_1) encoding green fluorescent protein (GFP) using classical and localized electroporation. On day two, we will determine gene electrotransfer efficiency and cell viability using fluorescence microscopy.

Protocol Day 1: Gene electrotransfer

Classical electroporation: CHO cells will be plated to 24-well plates 24 h before the experiment in concentration of 10^5 cells per well. Just before the experiment, culture medium will be removed and replaced with 150 µl of electroporation medium (10 mM phosphate buffer Na_2HPO_4/NaH_2PO_4, 1 mM $MgCl_2$, 250 mM sucrose; pH = 7.2) containing plasmid DNA with concentration 100 µg/ml. Prior to gene electrotransfer, cells will be incubated with plasmid DNA for 2 minutes at room temperature. Afterwards, the Pt-Ir wire electrodes with inter-electrode distance of d = 2 mm will be placed into the well. BetaTech Electro cell B10 generator will be used to deliver a train of eight rectangular pulses with duration of 5 ms, U = 140 V (U/d = 700 V/cm), and 1 Hz. Immediately after pulse exposure, 37.5 µl of fetal calf serum will be added to well. The pulse application will be repeated in the next well but with only 20 V (the same as in localized electroporation). After treatment, the cells will be incubated for 5 min at 37°C. Afterwards, 1 ml of culture medium will be added and cells will be incubated for 24 hours in a CO_2 incubator 37°C.

Localized electroporation: CHO cells will be plated into cell culture inserts, adapted for 24-well plates, and containing a PET substrate with pores with a diameter of 400 nm. Two days prior to gene electrotransfer, 0.8×10^4 cells will be plated in 300 µL of culture medium in each insert. The inserts will then be placed into a 24-well plate containing 700 µL of culture medium/well. On the day of experiment, the inserts will be moved to a 24-well Falcon plate with 500 µl of electroporation medium (LCIS) per well containing plasmid DNA at a final concentration of 100 µg/ml. To enable electrophoretic transfer of the negatively charged DNA across the substrate into the cells, the negative electrode should be placed below the substrate and the positive electrode above the substrate, as shown in Fig. 2. Following the experimental setup, a train of eight rectangular pulses with duration of 5 ms, 20 V, 1 Hz will be applied. After 1 min, the insert will be transferred to a new multiwell plate with fresh culture medium and incubated for 24 hours in a CO_2 incubator at 37°C. To test the role of electrophoresis, cells in another insert will be treated in the same way, but with switched polarity of the electrodes (negative electrode above and positive below the substrate).

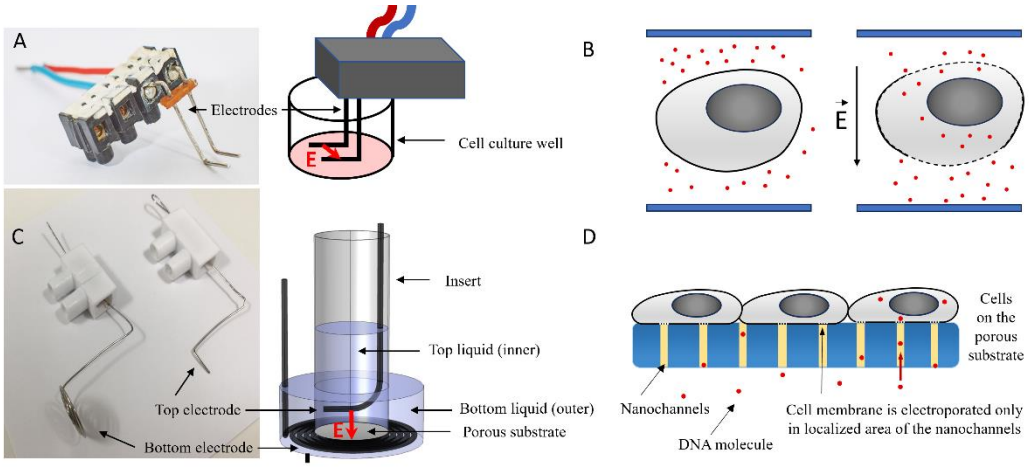

Figure 1. Gene electrotransfer using two methods. (A) Electrodes used for classic electroporation and their position in a well of a cell culture multiwell plate. (B) Bulk electroporation mechanism and pore formation in the presence of electric field. (C) Electrodes used for localized electroporation and their position in a well of a cell culture multiwell plate with porous substrates. (D) Mechanism of localized electroporation on the porous substrate.

Protocol Day 2: Determining cell viability and gene electrotransfer efficiency

After 24 h incubation you will determine the difference in cell viability and gene electrotransfer efficiency for both classical and localized electroporation by fluorescence microscopy (Leica Thunder, Wetzlar, Germany) at 20x magnification using GFP filter with excitation at 488 nm.

For classical gene electrotransfer protocol you will determine cell viability as the ratio between the number of viable cells in the treated sample and in the control sample. Gene electrotransfer efficiency will be determined from the ratio between the number of green fluorescent cells (successfully transfected) and the total number of viable cells counted under the brightfield microscopy. You will determine overall gene electrotransfer efficiency from the ratio between the number of green fluorescent cells in treated sample and the total number of viable cells in control sample. Number of green fluorescent cells will be compared between two presented methods – classical and localized gene electrotransfer.

REFERENCES:

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NOTES & RESULTS