Optimization of an electroporation protocol using acute myeloid leukemia cell line as a model: role of the pulse strength, duration and concentration of plasmid

6th Kaunas / Lithuania International
Hematology / Oncology Colloquium
28 May 2021

Roberta Vadeikienė[1δ], Baltramiejus Jakštys[2δ], Saulius Šatkauskas[2], Rasa Ugenskienė[1, 3], Elona Juozaitytė[4]

[1]Oncology Research Laboratory, Oncology Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania. [2]Biophysical Research Group, Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania. [3]Department of Genetics and Molecular Medicine, Lithuanian University of Health Sciences, Kaunas, Lithuania. [4]Department of Oncology and Hematology, Hospital of Lithuanian University of Health Sciences Kauno klinikos, Kaunas, Lithuania. [δ]These authors contributed equally to this work.

Objective

Transfer of biomacromolecules, such as mRNA, DNA or proteins, into cells is essential for cellular manipulation, genome editing or medical applications. Non-adherent cells are difficult to transfect with standard chemical-mediated delivery methods. Therefore, electroporation (EP) is becoming the most commonly used strategy to introduce a molecule of interest into suspension cell lines. It is well known that particular care must be taken with the viability of the transfected cells, since parameters, which increase transfection efficiency result in higher cell death rates.

In this work, we describe the methodologies that we have developed for optimization of EP settings to balance the highest possible transfection efficiency with robust UT-7 (acute myeloid leukemia) cell line viability and growth post-electroporation.

Methods

- The UT-7 human cell line was purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Cell line authentication was done by DSMZ.
- These cells were used for square-wave electroporation experiments in which the following parameters were tested systematically: pulse strength, duration and different concentrations of the plasmid DNA.
- The pEGFP (3400 bp) plasmid encoding a green fluorescent protein was used as an indicator for the assessment of transfection efficiency.
- Cells were transfected with pEGFP by EP at various conditions using the EP system BTX T820 (Harvard Apparatus).
- The transfection efficiency was determined by the counting pEGFP positive cells 48 h after transfection. Additionally, cells were observed with propidium iodide (PI) viability assay 48 h post-transfection for discrimination between live/dead transfected cells. Fluorescence of the cells was analysed with BD Accuri C6 (USA) flow cytometer.

Results

- Firstly, different EP conditions were assessed, including variations in pulse strength (1200, 1400, 1600, 1800 V/cm) and pulse duration (100, 250, 500 and 750 µs). The entire experiment was performed in laboratory-made EP buffer containing 100 µg/ml final concentration of pEGFP plasmid. One high voltage electric pulse was applied. The best results were obtained applying 1200 V/cm pulse with duration of 500 µs and yielded 11 ± 1.61% pEGFP-positive live cells with total 42.3 ± 5.4 % viable cells in the sample compared to untreated cells. Further increase of pulse strength or pulse duration resulted in decrease of overall cell viability and amount of transfected viable cells. The intensity of fluorescence under these conditions was 19 % lower compared to that at 1400 V/cm 750 µs pulse where only 3.6 ± 0.5 % pEGFP-positive live cells with total 11.11 ± 1.2 % of viable cells were obtained (Figure 1).
- Furthermore, various plasmid concentrations (20, 50, 100, 200 µg/ml) were used for transfection using 1200 or 1400 V/cm 250 µs pulse. The optimal plasmid amount with respect to viability and pEGFP-positive live cells was 200 µg/ml when a single pulse of 1400 V/cm for 250 µs was used (Figure 2).

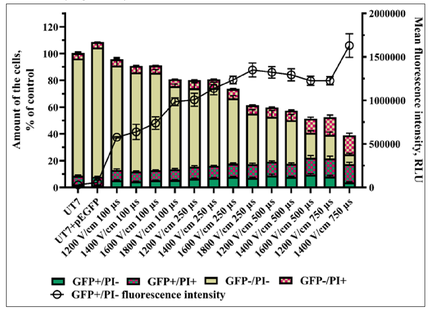

Figure 1. Optimization of electroporation parameters for the delivery of pEGFP into UT-7 cell line. The effect of different pulse strength and duration. Cells were measured 48 h after transfection. The green bars show viable cells (no permeability to PI) with transient pEGFP expression. The yellow bars represent dead cells (membranes are permeable to PI) without pEGFP expression. The green-pink bars show dead cells with pEGFP expression. The yellow-pink bars represent viable cells without pEGFP expression. The line shows fluorescence intensity on UT-7 cells transfected by pEGFP. Each column is the average of three independent experiments. Data is showed as the mean ± standard deviation.

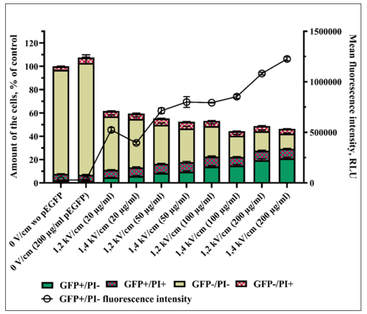

Figure 2. Optimization of electroporation conditions for expression of pEGFP in UT-7 cell line. A pilot experiment varying the concentration of plasmid DNA. Cells were measured 48 h after electroporation. The green bars show viable cells (no permeability to PI) with transient pEGFP expression. The yellow bars represent dead cells (membranes are permeable to PI) without pEGFP expression. The green-pink bars show dead cells with pEGFP expression. The yellow-pink bars represent viable cells without pEGFP expression. The line shows fluorescence intensity on UT-7 cells transfected by pEGFP. Each column is the average of three independent experiments. Data is showed as the mean ± standard deviation.

Conclusions

Transfection efficiency in difficult to transfect myeloid non-adherent cells like UT-7 can be improved by testing and adjusting various electroporation parameters. The use of optimal pulse strength, electric pulse duration and concentration of plasmid greatly determines the transfection efficiency. This study demonstrates that electroporation can be a competitive transfection tool for difficult to transfect cell lines compared to conventional plasmid delivery methods.

Key words

Electroporation; acute myeloid leukemia; UT-7 cell line; gene transfer.