Short Communication

Conditions for Gene Transfection into the HL-60 Human Leukaemia Cell Line by Electroporation

(leukaemia / electroporation / HL-60)

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Abstract. Electroporation represents a powerful technique for cell transfection; however, its efficiency in haemopoietic cells (~1%) is largely unsatisfactory. Biological processes in haemopoietic cells are often studied using leukaemia cell line HL-60. For this reason we developed conditions for efficiently introducing plasmids to HL-60 cells by electroporation, as an alternative to other techniques. This technique employs the electric pulse (250–270 V; 1000 μF) followed by separation of living cells on a Ficoll-Paque discontinuous gradient. Using 10–20 μg of plasmid, we routinely achieve 12–14% of transfectants.

The immortal HL-60 cell line derived from a patient with acute promyelocytic leukaemia (type M2) provides a useful model for investigation of the biochemical events in human leukaemia cells (Yen, 1990). For this reason genetic modification of HL-60 cells may enrich our knowledge of both proliferation and differentiation processes in myeloid leukaemia cells. Electroporation has proved to be one of the suitable methods. However, the efficiency of gene transfection into leukaemia cell lines by electroporation is generally very unsatisfactory (low). Lipofection also gives only about 0.5% of positive HL-60 cells (Kusumawati et al., 1999). Adding dimethyl-sulphoxide (DMSO) into the electroporation mix may improve the transfection efficiency (Melkonyan et al., 1996). However, DMSO induces differentiation in many cell types including HL-60, which makes its use impossible (Yen, 1990).

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Abbreviations: DMSO – dimethyl sulphoxide.

Here we describe the electroporation conditions for the gene transfer into HL-60 cells, which give 12–14% of positively transfected cells, and the subsequent application of Ficoll-Paque discontinuous gradient centrifugation for post-pulse separation of both dead and living cells.

Material and Methods

The HL-60 cells (ECACC; Porton Down, Salisbury, UK) were grown in RPMI 1640 medium (Sigma, Prague, Czech Republic) supplemented with 10% foetal calf serum (PAN, Trasadingen, Switzerland) and gentamicin (50 μg/ml, Sigma) under standard conditions (37°C, 5% CO_2, 95% humidity). Viability was assigned by propidium iodide (2 μg per ml) exclusion after 5 min of incubation and fluorescence was measured by flow cytometry (FCM; FACSCalibur equipment, Becton-Dickinson, San Jose, CA). For the experiments cells growing exponentially for 3 days were collected by centrifugation (200 *g* for 5 min at room temperature (R.T.)) and resuspended in serum-free RPMI medium to the required cell concentration (2 x 10^7 per ml). Plasmids were extracted and purified through a Qiagen Plasmid Mega Kit (Qiagen, Valencia, CA), dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), and used in the circular form. The cell suspension was mixed with the plasmid of the appropriate concentration, and let stand for 5 min at R.T. This suspension (400 μl) was transferred into an electroporation cuvette (0.4 cm gap, Bio-Rad, Hercules, CA) and pulsed (Gene Pulser II Apparatus and Capacitance Extender Plus, Bio-Rad). Five minutes after the pulse the cuvette content was rinsed in fresh RPMI medium with 10% FCS, and seeded to Petri dishes for continuous cultivation. After 24 h and 48 h the cultivated cells were taken for FCM analysis. The quantitative efficiency of the transfection was evaluated using plasmid encoding continuous expression of green-fluorescent protein (GFP; phGFP-S65T, Clontech 6088-1). GFP expression was analysed by FCM and checked by fluorescence microscopy. For comparison, a plasmid encoding β-galactosidase (CMV-βgal, given from Dr. J.

Šmarda, Masaryk University Brno) was used. β-galactosidase activity was monitored by adding X-Gal solution (1 mg/ml, Molecular Probes, Eugene, OR) to formaldehyde-fixed cells, and by overnight incubation.

Results and Discussion

On the basis of our preliminary experiments, the RPMI medium and R.T. were chosen for electroporation. Low temperature and simple low-salt buffers are recommended by many authors (Chang et al., 1992), but these conditions rapidly decreased cell viability in our experiments. Similarly, chilling the cell suspension (4°C) before, during or after the pulse extremely decreased the HL-60 cell viability (data not shown). It is important that the use of cells from higher passages (more than 25) also attenuated electroporation efficiency. This fact may be connected with significant changes of the physiological properties of HL-60 cells from more than 30 passages (Parmley et al., 1987).

Figure 1 shows the influence of voltage and capacitance on transfection efficiency. The cells were prepared as described above and 20 μg of the plasmid were used per pulse. As shown, the highest transfection efficiency (about 12–14%) was reached using 250–275 V and capacitance near to 1000 μF. In addition, the effects of low capacitance (about 25–100 μF) combined with a different voltage setting were also tested, but the yield of the positive cells did not exceed 2% of the living cells per sample.

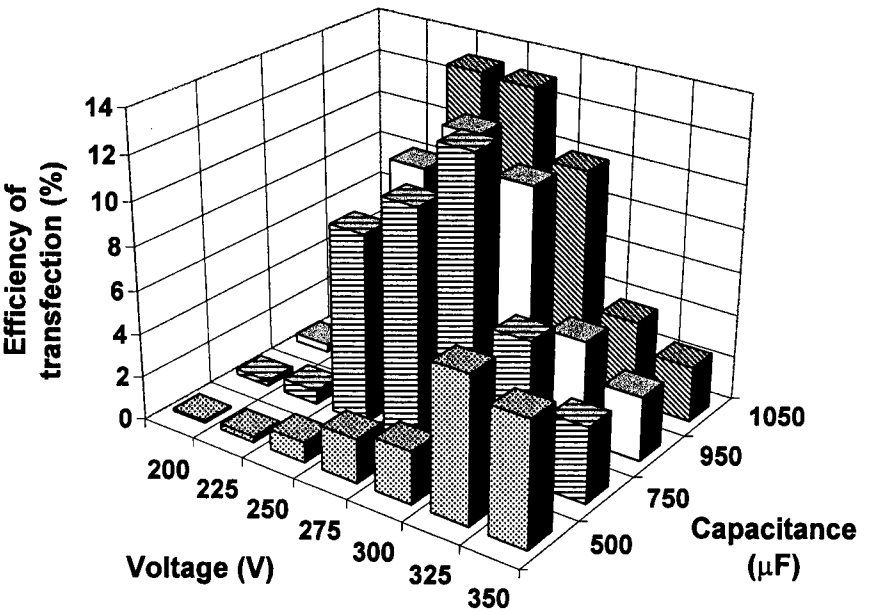

Fig. 1. Dependence of electroporation transfection efficiency on voltage and capacitance settings. 8 x 10^6 HL-60 cells were electroporated with 20 μg of plasmid phGFP-S65T in 400 μl RPMI. The GFP expression was measured 24 h after electroporation using FCM. The data were collected from living cells only. The cell viability was determined using propidium iodide exclusion and FCM analysis as described in the text. The data shown represent the mean from at least two independent experiments.

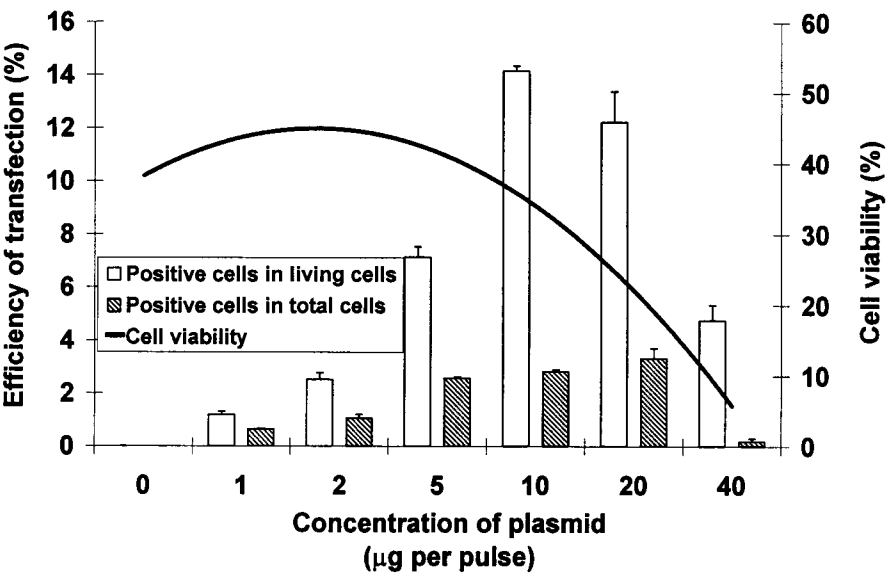

Fig. 2. Dependence of electroporation transfection efficiency on plasmid concentrations. 8 x 10^6 HL-60 cells were electroporated with various concentrations of plasmid phGFP-S65T in 400 μl RPMI under the following conditions: 950 μF capacitance and 250 V voltage. The GFP expression and viability were determined as in Fig. 1. The data shown represent the mean from three independent experiments ±SEM.

In further experiments we studied the effects of plasmid concentration on the efficiency of transfection and cell viability. Figure 2 shows that a concentration higher than 20 μg per pulse decreased the viability of cells and thus the efficiency of transfection. In conclusion, plasmid concentrations in the range from 10 to 20 μg are an optimal compromise between cell viability and transfection efficiency. Besides, optimal efficiency was achieved with about 30% of cell viability after the pulse. The toxic effect of

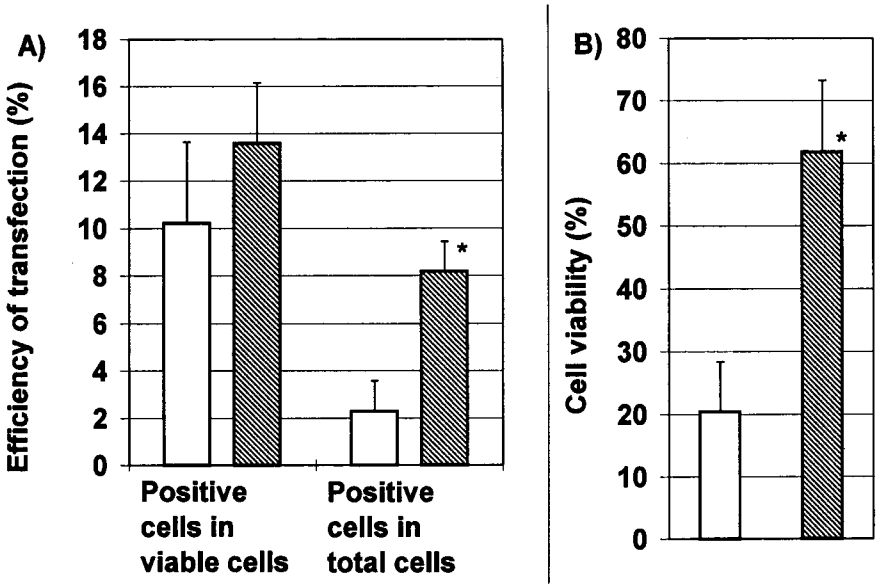

Fig. 3. Efficiency of transfection and cell viability after post-pulse separation of dead and viable cells. 24 h after electroporation with phGFP-S65T the HL-60 cells were overlaid onto a Ficoll-Paque discontinuous gradient and centrifuged at 350 x *g* for 30 min at R.T. The cells were collected and transferred to growth medium for continuous cultivation followed by FCM analysis. A) Efficiency of transfection before (void column) and after post-pulse separation (hatched column), B) cell viability before (void column) and after post-pulse separation (hatched column). The cell viability was determined as in Fig. 1. The data shown represent the mean from six independent experiments ± SEM.
*$P < 0.01$ as compared with not post-pulse separated cells (Student's t-test).

GFP on HL-60 cells was detected 48 h after electroporation as a decrease of transfection efficiency determined by GFP. These conclusions may also be supported by the corresponding appearance of blisters on the GFP-positive cell surface (data not shown).

One of the general problems of transient gene transfection of cells growing in suspension is a high percentage of dead cells in this suspension after the pulse. To increase the yield of viable cells in the sample we used gradient centrifugation with Percoll (according to the manufacturer's instruction for blood cells) or Ficoll-Paque (both from Pharmacia, Little Chalfont, Buckinghamshire, UK). Figure 3 compares the results of the analysis of crude and separated populations 24 h after the pulse. It is shown that separation using the Ficoll-Paque gradient increases the yield of transfection-positive (4 times; Fig. 3A) and viable (3 times; Fig. 3B) cells per total cells. However, using the Percoll gradient we did not obtain applicable results (data not shown).

In summary, in this report we studied the different electroporation conditions for HL-60 cells. We concluded that the best efficiency of transfection (about 12–14% of positive cells) could be reached in the following conditions: exponentially growing cells, 250–270 V and about 1000 μF in RPMI medium as the electroporation buffer, and 10–20 μg plasmid per pulse. Finally, an electroporated suspension of HL-60 cells may be efficiently separated into living and dead cells using the Ficoll-Paque reagent.

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