Electroporation Parameters for Transfection of HL-60 Leukocytic Cell Line With siRNA Using the Gene Pulser MXcell™ System

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Introduction

HL-60 cells are a leukemic suspension cell line that can be terminally differentiated over the course of several days into neutrophil-like cells. Neutrophils are widely studied leukocytes that are responsible for the response to acute inflammation and for combating bacterial infection. However, neutrophils do not survive more than 2 days outside of the body and, hence, are of limited use for in vitro studies. Differentiated HL-60 cells have been invaluable as a cell line model of neutrophil behavior because they exhibit several unique qualities of mature neutrophils, including clearance of bacteria. Differentiation of HL-60 cells into neutrophil-like cells is typically induced by supplementing the growth medium with retinoic acid or dimethyl sulfoxide. Unfortunately, HL-60 cells are notoriously difficult to transfect, often requiring fluorescence-activated cell sorting to enrich a successfully transfected population. Therefore, optimization of multiple conditions for HL-60 cell transfection efficiency and cell survival are important for assays that require a high proportion of transfected cells, such as siRNA assays.

Methods

HL-60 cells were cultured in GIBCO Advanced RPMI Medium 1640 (Invitrogen Corporation) containing 10% fetal bovine serum at 37°C, and given fresh medium 24 hr prior to transfection. Cells were washed twice in 30 mM HEPES-buffered saline, then centrifuged and resuspended at 1 x 10^6–1 x 10^7 cells/ml in Gene Pulser® electroporation buffer (Bio-Rad Laboratories, Inc.). Aliquots of the cell suspension (200 µl) were transferred into the wells of a 96-well electroporation plate (Bio-Rad) and electroporated with the Gene Pulser MXcell system (Bio-Rad). Following electroporation, cells were diluted 1:10 in GIBCO Advanced RPMI Medium 1640 (Invitrogen) and grown for 24 hr. The median fluorescent intensity (MFI) of 10,000 cells transfected with siRNA conjugated to Alexa Fluor 488 (Invitrogen), then washed with nonfluorescent salt solution, was measured by flow cytometry. Nontransfected cells treated in this manner showed a negligible fluorescent signal and, therefore, MFI was recorded as an index of transfection efficiency. Survival was assessed by measuring the concentration of cells excluding trypan blue in culture 24 hr after transfection. This concentration was divided by the cell concentration that was initially electroporated and put into culture the previous day to yield a survival percentage. Each data point shown in figures is the average of three wells.

Results and Discussion

To optimize conditions for transferring siRNA into HL-60 cells by electroporation, the Gene Pulser MXcell system was used to examine several parameters simultaneously. Parameters examined included voltage, cell density, and RNAi concentration. For each parameter tested, transfection efficiency and cell viability were recorded.

Effect of Voltage

Voltage conditions between 175 and 250 V were applied to HL-60 cells at a density of 2 x 10^6 cells/ml and 50 nM RNAi-Alexa Fluor 488 (Figure 1). Cell survival, measured 24 hr postelectroporation, declined sharply with increased voltage while transfection efficiency improved with increased voltage. A sharp increase in siRNA delivery was observed between 175 and 200 V. These results show that the choice

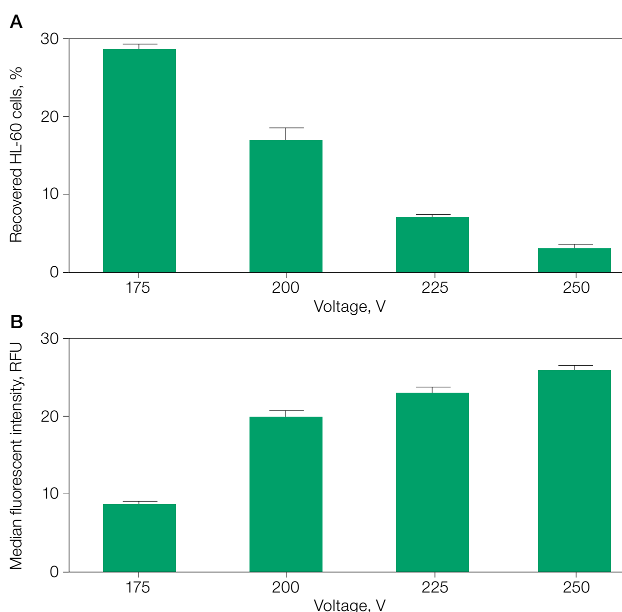

Fig. 1. Effect of voltage on HL-60 cell survival and transfection efficiency. HL-60 cells were suspended in electroporation buffer at a density of 2 x10^6 cells/ml in the presence of 50 nM RNAi-Alexa Fluor 488. Cells were subjected to an exponential waveform pulse at 500 µF capacitance, 1,000 Ω resistance, and different voltage settings. **A**, cell survival; **B**, transfection efficiency as an index of MFI measured by flow cytometry. HL-60 cells in the absence of labeled RNAi had minimal autofluorescence, but nonelectroporated controls in the presence of labeled RNAi were not included, and it is possible RNAi adhered to the cell surface to some extent. RFU, relative fluorescence units.

of the voltage to apply to the cells is a trade-off between cell survival and nucleotide delivery. The optimal voltage to apply will depend on the number of living cells required in the downstream experimental assay and on the intracellular siRNA concentration required for a robust genetic effect.

Effect of Cell Density

When transfection efficiency is at a premium over cell survival, the use of higher voltage is advantageous. In order to compensate for cell death caused by high voltage, a larger number of cells (>1 x 10^6 cells/ml) can be electroporated. We tested a range of cell densities using high voltage conditions (Figure 2). While cell survival appeared to be higher at a lower cell density (1 x 10^6 cells/ml), transfection efficiency appeared to be largely independent of cell density; at densities up to 1 x 10^7 cells/ml, little impact was observed in transfection efficiency. This indicates that at nucleotide concentrations as low as 50 nM, the siRNA may have been in excess, and was transferred efficiently into the cells. The increase in cell death observed as cell density was increased may have resulted from an increased number of lysed cells following electroporation and, hence, an increase in potentially cytotoxic cell content released from these cells.

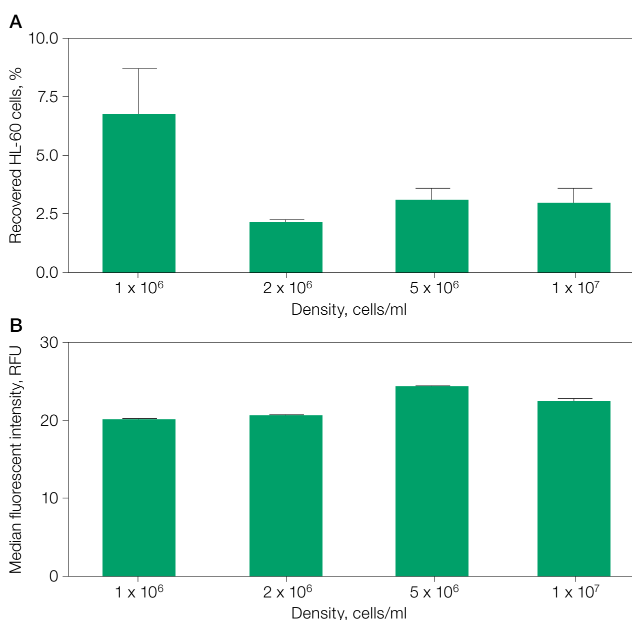

Fig. 2. Effect of cell density on transfection efficiency and cell survival. Different densities of HL-60 cells were electroporated at 250 V with 50 nM siRNA. **A**, cell survival; **B**, transfection efficiency as an index of MFI measured by flow cytometry. RFU, relative fluorescence units.

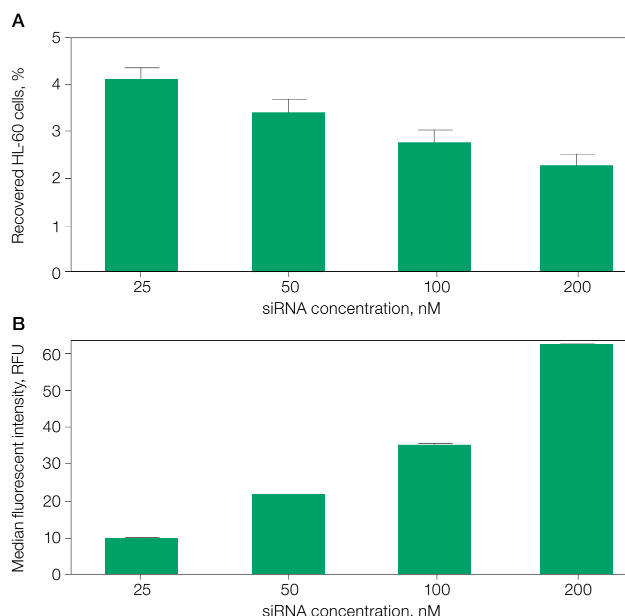

Fig. 3. Effect of RNAi concentration on HL-60 cell survival and transfection efficiency. HL-60 cells were electroporated (at 250 V, 500 µF, and 1,000 Ω) with a range of siRNA concentrations. **A**, increased siRNA concentrations corresponded with decreased fractions of cells surviving after 24 hours; **B**, delivery of labeled siRNA into the cells increased with concentration, and was roughly linear. RFU, relative fluorescence units.

Effect of RNAi Concentration

Cell survival and transfection efficiency were monitored at concentrations of siRNA ranging between 25 and 200 nM. The results presented in Figure 3 show that cell survival decreased approximately 25% with increased concentrations of RNAi over the range tested, while transfection efficiency increased linearly with the RNAi concentrations. Decreased cell survival at higher RNAi concentrations suggests that the RNAi may exert a cytotoxic effect on cells at these concentrations. If conservation of precious cells is a major consideration, increasing the concentration of siRNA in solution may be preferable to higher electroporation voltages. siRNA delivery is essentially linear, as twice the nucleotide concentration will transfect approximately twice the siRNA into the cell. Nucleotides and especially double-stranded RNA can reduce cell viability at high concentrations, but less so than voltage, which can physically damage cells.

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