Transfection Method Comparison Study Against Neuronal Like Cell Line

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Abstract:

We have studied different methods for efficient DNA transfection in neuroblastoma cell line. Here we report that the optimum transfection mode by nucleofection method yielded the maximum efficiency for SK-N-SH cells. To optimize transfection we selected different methods like electroporation, liposome mediated and PEI transfection. The DNA that was transfected was pmaxGFP plasmid (3.49kb). Our results suggest that the most efficient method for DNA transfection is very important to study gene expression and regulation by finding ways to deliver DNA into the difficult to transfect mammalian cells. The transfection efficiency by electroporation was ~60-70%, Lipofection was ~ 18-20% and PEI yielded <1%.

Keywords: SK-N-SH, transfection, electroporation, neuroblastoma

I. Introduction

Neurodegenrative diseases like Alzheimers Disease (AD) and Parkinson's Disease (PD) are incurable devastating disorders characterized by progressive loss of memory and cognitive ability due to multiple intertwined mechanisms. It is the most common cause of dementia, accounting for an estimated 60% to 80% of cases. These disorders affect millions of people who eventually require long-term nursing care.

SK-N-SH is a neuroblastoma cell line that displays epithelial morphology and grows in adherent culture. Treatment with all-trans-retinoic acid causes these cells to differentiate and adopt a neuronal phenotype, characterized by extensive neurite outgrowth. This makes them particularly useful for delineating signaling pathways involved in neuronal differentiation.

They been used to test neuroprotective compounds against some AD and PD relevant features. This cell line is frequently chosen because of its human origin, neuronal properties, ease of maintenance and it being a suitable transfection host. This established cell line is used as valuable preclinical model for neurological research that provides scientists with a better understanding of the biology of the disease and could lead to the development of therapeutics. In addition, the SK-N-SH cells are known to form tumors in immunocompromised mice.

The SK-N-SH cell line was isolated by J.L. Biedler in 1970 from brain tissue of a 4-year-old female patient during a bone marrow biopsy of a metastatic neuroblastoma. This cell line displays epithelial morphology and grows in adherent culture. Treatment with all-trans-retinoic acid causes these cells to differentiate and adopt a neuronal phenotype, characterized by extensive neurite outgrowth. This makes them particularly useful for delineating signaling pathways involved in neuronal differentiation. [1,2]

For the human neuroblastoma (SK-N-SH) cells the optimum and efficient condition for DNA transfection needs to be studied as they are difficult to transfect cell line hence yielding very low transfection efficiency. For this study we compared three different methods of transfection i.e. electroporation, liposome mediated lipofction and cationic polymer transfection using PEI (polyethylenimine).

1) Electroporation

Electroporation is a physical method of gene delivery. For this reason it is widely applicable to a variety of cell types, including animal, plant, and microbial [6] During electroporation, cells are exposed to a high-voltage pulse in the presence of exogenous nucleic acid. The high voltage causes the cellular membrane to be transiently permeabilized, allowing the foreign nucleic acids to enter the cell.[6-8] Every cell type requires slightly different electroporation conditions that must be determined experimentally. [9]

2) Lipofection

Lipofectamine LTX Reagent transfection reagent is based on the lipofection method, in which the negatively charged vector DNA is trapped in a cationic lipid vacuole, the liposome, formed by the reagent. The liposome fuses with the cell membrane and thereby releases vector DNA into the cell. [11-12]

3) PEI

DNA can be introduced into a host cell by transfection with polyethylenimine (PEI), a stable cationic polymer.[3] PEI condenses DNA into positively charged particles that bind to anionic cell surfaces. Consequently, the DNA:PEI complex is endocytosed by the cells and the DNA released into the cytoplasm (4). PEI is usually preferred over other cell transfection reagents because of its low cost. [5]

We have optimized the transfection protocol to allow us to vary several of the paramaters that would prove beneficial for typically difficult to transfect cell line.

II. Research Materials and Method

Cell line and culture conditions

Human SK-N-SH, obtained from the American Type Culture Collection (ATCC) were grown as a monolayer in the Eagle's Minimum Essential Medium (EMEM) media supplemented with 10% of heat-inactivated Foetal Bovine Serum (FBS), penicillin (20 units/ml) and streptomycin (20 mg/ml) at 37°C in a saturated humid atmosphere with 5% CO_2. As the cells became confluent, they were split at 1:3 ratio after treatment with Trypsin-EDTA.

Chemical Reagents and Equipment

1) Lonza Kit V was purchased from Amaxa Cell Line Nucleofector **kit V** (**Lonza** Ltd.) for electroporation The kit came with Nucleofector™ Solution V and Supplement.
2) Lipofectamine® LTX Reagent, PLUS™ Reagent, Opti-MEM® Medium
3) Linear PEI (polyethyleimine) 25 kDa was purchased from Polysciences Inc.(2g # 23966-2)
4) EMEM + 10%FBS

Plasmid for transfection

pmaxGFP™ vector provided in Lonza kit V

Transfection Methods

In order to make all transfection experiments as comparable as possible, the number of cells transfected were approximated in the same range. The plasmid used was from a single batch. While it was not possible to carry out all transfections simultaneously, strict monitoring ensured that all transfections were maintained under the same environmental conditions.

Electroporation:

1) The optimal confluency before Nucleofection was ~80–90%. A 6-well plate was prepared by filling appropriate number of wells with 1.4 ml of EMEM + 10% FBS and pre-incubated in a humidified 37°C/5% CO2 incubator. 293T cells were harvested by trypsinization and an aliquot of trypsinized cells were counted using hemocytometer.
2) 1 x 10^6 cells/ sample were centrifuged at 200g for 10 mins at room temperature. The cell pellet was resuspended carefully in 100 µl room temperature Nucleofector™ Solution. For each 100 µl Nucleofector™ Solution, 82 µl of Nucleofector™ Solution V was added to 18 µl of supplement to make 100 µl of total reaction volume. To this cell suspension 2 µg pmaxGFP™ vector was added and mixed gently.
3) The cell/DNA suspension into certified cuvette; sample must cover the bottom of the cuvette without air bubbles. Cuvette cap was closed carefully.
4) The appropriate N.ucleofector™ Program (U-29) for Nucleofector™ I Device.
5) The cuvette with cell/DNA suspension was inserted into the Nucleofector™ Cuvette Holder and the selected program was applied. The cuvette was removed from the holder once the program was finished.

6) ~500 µl of the pre-equilibrated culture media was added to the cuvette and the sample was gently transferred immediately into the 6-well plate (final volume 2 ml media per well). The supplied pipettes that came along with the kit were used to avoid repeated aspiration of the sample.
7) The cells were incubated in a humidified 37°C/5% CO2 incubator until analysis.

Lipofection:

1) SK-N-SH cells were seeded in 6-well plate so that they were at 50-60% confluency at time of transfection
2) 15ul Lipofectamine LTX Reagent was diluted in 135ul Opti-MEM Medium
3) 2.5 µg pmaxGFP plasmid vector was diluted in Opti-MEM® Medium to reach a final volume of 136ul and then 14ul PLUS™ Reagent was added to it.
4) The diluted DNA was gently added to diluted Lipofectamine LTX Reagent.
5) The mix was incubated for 5 minutes at room temperature.
6) The DNA-lipid complex was gently added to cells dropwise.
7) The cells were incubated in a humidified 37°C/5% CO2 incubator until analysis.

PEI Transfection

1) SK-N-SH cells were seeded in 6-well plate so that they were at 50-60% confluency at time of transfection
2) 15ul PEI was diluted in 70ul Opti-MEM Medium
3) 4.7 µg pmaxGFP plasmid vector was diluted in Opti-MEM® Medium to reach a final of 85ul
4) The PEI-mix was added to DNA-mix and immediately vortexed gently at low speed
5) The DNA:PEI transfection cocktail was incubated for 15 minutes at room temperature and then added dropwise to the cells.
6) The cells were incubated in a humidified 37°C/5% CO2 incubator until analysis.

III. Results

We studied to determine the optimal conditions for transfection of neuroblastoma cell line SK-N-SH. This cell line is difficult to transfect because of its neuronal behavior so for obtaining the highest transfection efficiency, we optimized the transfection conditions by varying SK-N-SH cell density, mode of transfection to obtain the highest efficiency.

The expression of GFP was examined at 24 hr and 48 hr post transfection. At both points, comparable levels of GFP expression relative to living cells were observed. We were able to achieve ~60-70% transfection efficiency thus illustrating that the optimized electroporation conditions gave efficient results [Figure 1 (A-D)] as compared to Lipofectamine LTX Reagent [Figure1 (E-H)] and PEI [Figure 1 (I-L)].

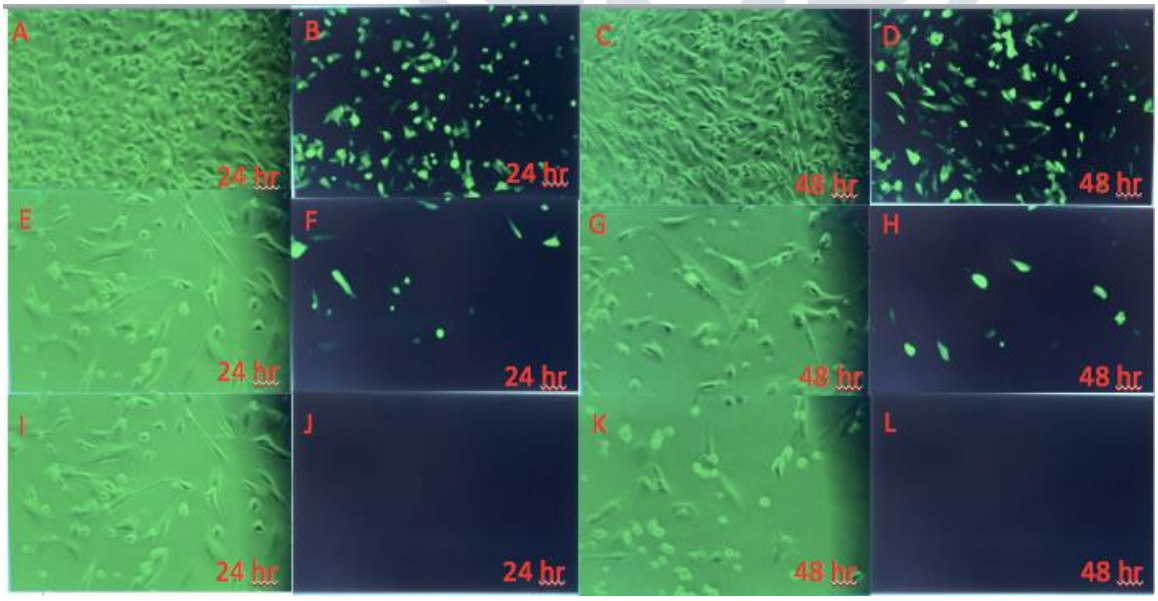

Figure 1: Microscopic observation of SK-N-SH cells after transfection. Cells were observed 24 hr and 48 hr post transfection. Images of light (A, C, E, G, I, K) and corresponding fluoresce microscopy images of cells expressing GFP (B, D, F, H, J, L).

Cell density played a critical role because for electroporation. It was observed that too high density did yield in lot of cell death causing cellular toxicity in the culture media. The density in a range on 0.75 – 1 x 10^6 cells/ sample at the time of transfection gave better cell viability and efficacy. It was observed that Lipofectamine LTX Reagent and PEI was somewhat toxic to the cells as there were lot of dead cells floating 24 hrs and 48 hrs post transfection (Figure 2).

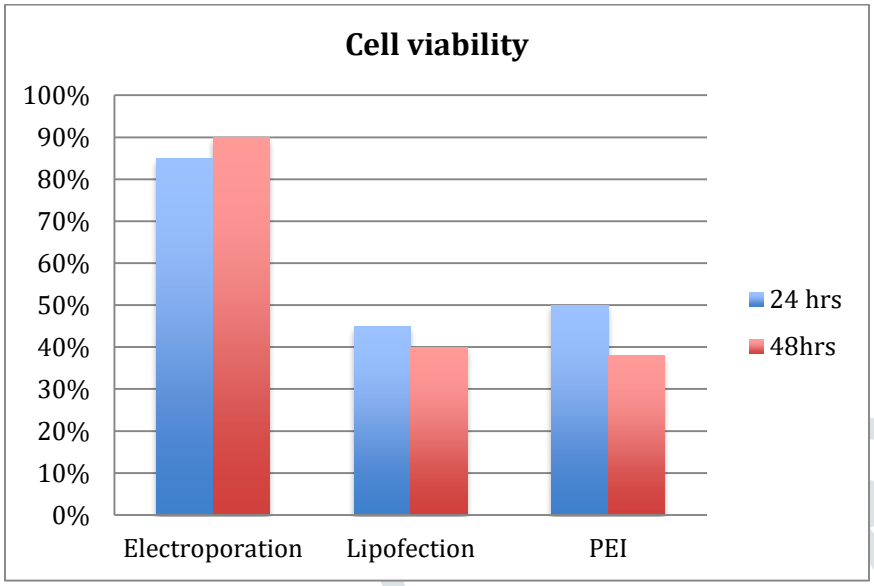

Figure 2: Cell Viability was measured by using the Trypan Blue dye.

IV. Conclusion

We were able to show that electroporation can be a very efficient method for introducing foreign DNA into cells of interest, including those that are often considered very difficult to transfect. Even though electroporation is not the most cost efficient method for transfection as compared to other methods, it is critical to figure out the purpose of our study and to find the best approach as to what you want to do with your cells. Will they be used for biochemical approach or for imaging? SK-N-SH cell lines can be used in vitro to overcome the limitation of testing therapeutic drugs directly on brain cells (neurons). It plays a critical role in preclinical model against neurodegenerative diseases.

High transfection efficiency for cells like SK-N-SH was achieved by first identifying the most favorable electroporation waveform, and then by refining the parameters related to that waveform. We optimized the Nucleofector® Program to modify the voltage and pulse duration, so that we could make adjustments to the voltage and capacitance settings. We assessed the results to confirm that our conditions maximized both transfection efficiency and cell viability

References

1. Biedler JL et al. (1973) Morphology and growth, tumorigenicity, and cytogenetics of human neuroblastoma cells in continuous culture. *Cancer Research* 33: 2643-2652 (PubMed ID: 4748425)

2. Helson L et al. (1975) Human neuroblastoma in nude mice. *Cancer Research* 35: 2594-2599 (PubMed ID: 167965)

3. (Boussif et al., 1995) A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine (PubMed ID: 7638184)

4. (Sonawane et al., 2003) Chloride accumulation and swelling in endosomes enhances DNA transfer by polyamine-DNA polyplexes (PubMed ID: 12944394)

5. (Patti A. Longo, Jennifer M. Kavran, Min-Sung Kim, and Daniel J. Leahy[1,]
2014)Transient Mammalian Cell Transfection with Polyethylenimine (PEI) (PubMed ID: 24011049)

6. (*Shigekawa K, Dower WJ, 1988*) Electroporation of eukaryotes and prokaryotes: a general approach to the introduction of macromolecules into cells (PubMed ID: 3273636)

7. (Tsong TY, 1991). Electroporation of cell membranes. *Biophys J.* 1991;60:297–306.
8. Rols M-P, Golzio M, Delteil C, Teissie J. In vitro delivery of drugs and other molecules to cells. In: Jaroszeski MJ, Heller R, Gilbert R, editors. *Methods in Molecular Medicine.* Vol. 37. Totowa, NJ: Humana Press; 2000. pp. 83–97. [PubMed] [Google Scholar]
9. Rols MP. Electropermeabilization: A physical method for the delivery of therapeutic molecules into cells. *Biochim Biophys Acta.* 2006;1758:423–428.
10. Elizabeth T. Jordan, Michelle Collins, Joseph Terefe, Luis Ugozzoli, and Teresa Rubio, 2008 Optimizing Electroporation Conditions in Primary and Other Difficult-to-Transfect Cells. (PubMed ID: 19183796)
11. Felgner PL. Gadek TR. Holm M, et al. Lipofection—A highly efficient, lipid-mediated DNA-transfection procedure. *Proc Natl Acad Sci USA.* 1987;84:7413–7417.
12. Felgner PL. Ringold GM. Cationic liposome-mediated transfection. *Nature.* 1089;337:387–388.