Comparison of DNA-transfection efficiency in SH-SY5Y cells using K4 Transfection System, METAFECTENE PRO and Lipofectamine 3000

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Introduction and aims.
SH-SY5Y are human neuroblastoma cells that can be differentiated into neuron-like cells showing many features of mature neurons and represent a useful *in vitro* model in neuroscience research. The aim of this work is to compare transfection efficiency of K4 Transfection System, METAFECTENE PRO and Lipofectamine 3000 in SH-SY5Y cells.

Cells and Plasmids.
SH-SY5Y cells were grown in DMEM supplemented with 10% FBS, 2 mM glutamine, 100 U/ml penicillin and 10 µg/ml streptomycin, in a 5% CO_2 air/incubator at 37°C. Cells were transfected with the mammalian expression vector pCMV3-C-GFPSpark supplied by Sino Biological Inc.

Reagents.
K4 Transfection System (Biontex); METAFECTENE PRO (Biontex); Lipofectamine™ 3000 Reagent (Invitrogen); MTT-Thiazolyl Blue Tetrazolium Bromide (Sigma-Aldrich).

Transient transfection.
Cell transfection with K4 Transfection System, METAFECTENE PRO and Lipofectamine™ 3000 was performed as follows. One day before transfection SH-SY5Y (2 x 10^4 cells/well) were seeded in 0.5 ml of DMEM supplemented with serum and antibiotics in 24 well culture plates and were grown overnight at 37°C.
To produce reagent-DNA complexes to transfect cells, for each well, 0.5 µg of plasmid DNA was mixed with one of the different transfection reagents and incubated at room temperature for 20 minutes following manufacturer's instructions. In particular, for K4 Transfection System and METAFECTENE PRO, 0.5 µg of DNA in 30 µl of serum/antibiotic-free medium was added to 2 µl of transfection reagent in 30 µl serum/antibiotic-free medium; for Lipofectamine™ 3000 0.5 µg of DNA and 1 µl of P3000 Reagent in 25 µl of serum/antibiotic-free medium was added to 1.5 µl of Lipofectamine 3000 in 25 µl of serum/antibiotic-free medium. Cells transfected with the K4 Transfection System were pre-incubated for two hours, with 5µl of K4 Multiplier.
For transfection, the reagent-DNA complex mixtures were applied dropwise to cells and incubated for 5 hours. After the incubation, transfection medium was removed and complete medium was added to each well.
Transfection efficiency was determined after 48 and 72 hours by counting GFP expressing cells using a fluorescence microscope.

MTT assay.
Toxicity of transfection procedures was measured by MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma Aldrich]. Briefly, cell culture medium was discarded and 500 µl of complete medium with MTT solution (500µg/ml) was added into each well. After incubation at 37°C for 3 hours, medium assay was removed and replaced by isopropanol. The solubilized formazan was determined spectrophotometrically at 570 nm.

Results.
The transfection efficiency and cell viability in SH-SY5Y cells for the three different transfection reagents were determined 48 and 72 hours after transfection. After 48h the highest transfection efficiency was measured for K4 Transfection System (34.91%) followed by METAFECTENE PRO (29.68%) and Lipofectamine 3000 (13.65%). After 72h of transfection METAFECTENE PRO showed the highest transfection efficiency (24.50%) followed by K4 Transfection System (23.22%) and Lipofectamine 3000 (13.96%) (Figure 1). MTT assay was performed at 48 and 72 hours after transfection to evaluate toxicity of transfection reagents. Significant reduction of cell viability was observed for cells transfected with Lipofectamine 3000 and METAFECTENE PRO at 48 and 72 hours compared to control. On the other hand, in cells transfected with K4 Transfection System, cell viability was similar to control at 48 hours after transfection while at 72 hours a reduction of cell viability comparable to that observed for Lipofectamine 3000 and METAFECTENE PRO was observed (Figure 2).

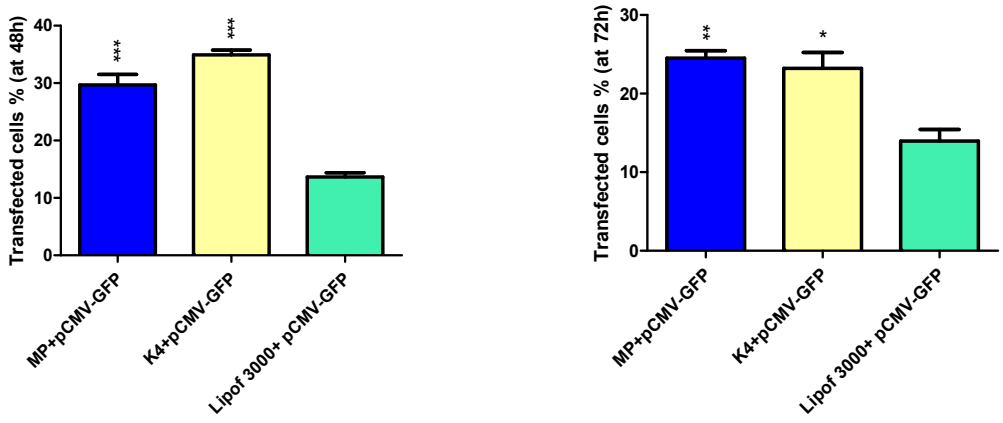

Fig.1. Transfection efficiency was measured using fluorescence microscope. After 48h from transfection quantification showed highest transfection efficiency with K4 Transfection Reagent and METAFECTENE PRO against Lipofectamine 3000. Data were collected from three independent experiments. (***P < 0.01 vs. Lipofectamine3000+pCMV-GFP group). In cells transfected for 72h quantification showed highest transfection efficiency in cells transfected with METAFECTENE PRO and K4 Transfection System. Data were collected from three independent experiments (**P < 0.03 vs. Lipofectamine3000 group; * P < 0.05 vs. Lipofectamine3000 group).

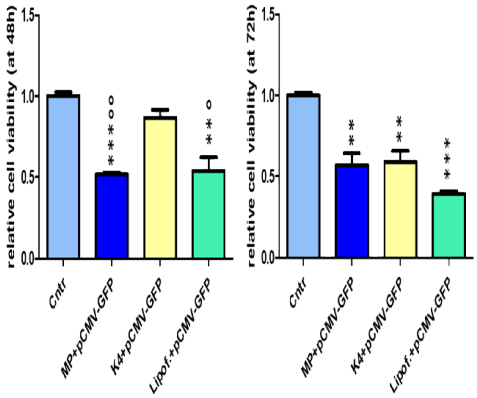

Fig.2. Cell viability was measured with MTT assay. In cells transfected for 48h quantification showed a decreased cell viability in cells transfected using Lipofectamine 3000 and METAFECTENE PRO. Data were collected from three independent experiments. (***P < 0.01 vs. control group; **P < 0.03 vs. control group; °°P < 0.03 vs. K4+pCMV-GFP; ° P < 0.05 vs K4+pCMV-GFP). In cells transfected for 72h quantification showed a decrease in cell viability for cells transfected with all transfection reagents. Data were collected from three independent experiments (***P < 0.01 vs. control group; ** P < 0.03 vs. control group).

Conclusions.

Comparison of the transfection efficiency in SH-SY5Y using the K4 Transfection System, METAFECTENE PRO and Lipofectamine 3000 reveals that K4 Transfection System is the most effective transfection reagent for SH-SY5Y cells followed by METAFECTENE PRO and Lipofectamine 3000. However, regardless the transfection reagents the overall transfection efficiency was low and in SH-SY5Y cells (<40%) and further experiments are needed to improve the transfection efficiency of the different reagents.