Comparison of DNA-transfection efficiency in HeLa cells using K4 Transfection System and Lipofectamine 3000.

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Introduction and aims.

HeLa (human cervix adenocarcinoma) cells represent a useful cell model widely used in scientific research. A large number of experimental techniques, including transient or stable transfection, are commonly used with this cell model. The aim of this work is to analyze transfection efficiency of K4 Transfection System and Lipofectamine 3000 in HeLa cells.

Cells and Plasmids.

HeLa 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); Lipofectamine™ 3000 Reagent (Invitrogen); MTT-Thiazolyl Blue Tetrazolium Bromide (Sigma-Aldrich).

Transient transfection.

Cell transfection with K4 Transfection System and Lipofectamine™ 3000 was performed as follows. One day before transfection HeLa (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 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 for K4 Transfection System and for 3 hours for Lipofectamine ™ 3000. 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 analysis of green fluorescent cells (GFP) positive cells using flow cytometry (FACs).

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 HeLa cells for the two different transfection reagents were determined 48 and 72 hours after transfection. After 48h the highest transfection efficiency was measured for K4 Transfection System (67.73%) followed by Lipofectamine 3000 (41.03%). Similar results were obtained after 72h of transfection, with K4 Transfection System showing the highest transfection efficiency (63.17%) followed by Lipofectamine 3000 (36.57%) (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 at 48 and 72 hours compared to control. On the other hand, in cells transfected with K4 Transfection System, cell viability was slight reduced compared to control at 48 and 72 hours after transfection against to that observed for Lipofectamine 3000 (Figure 2).

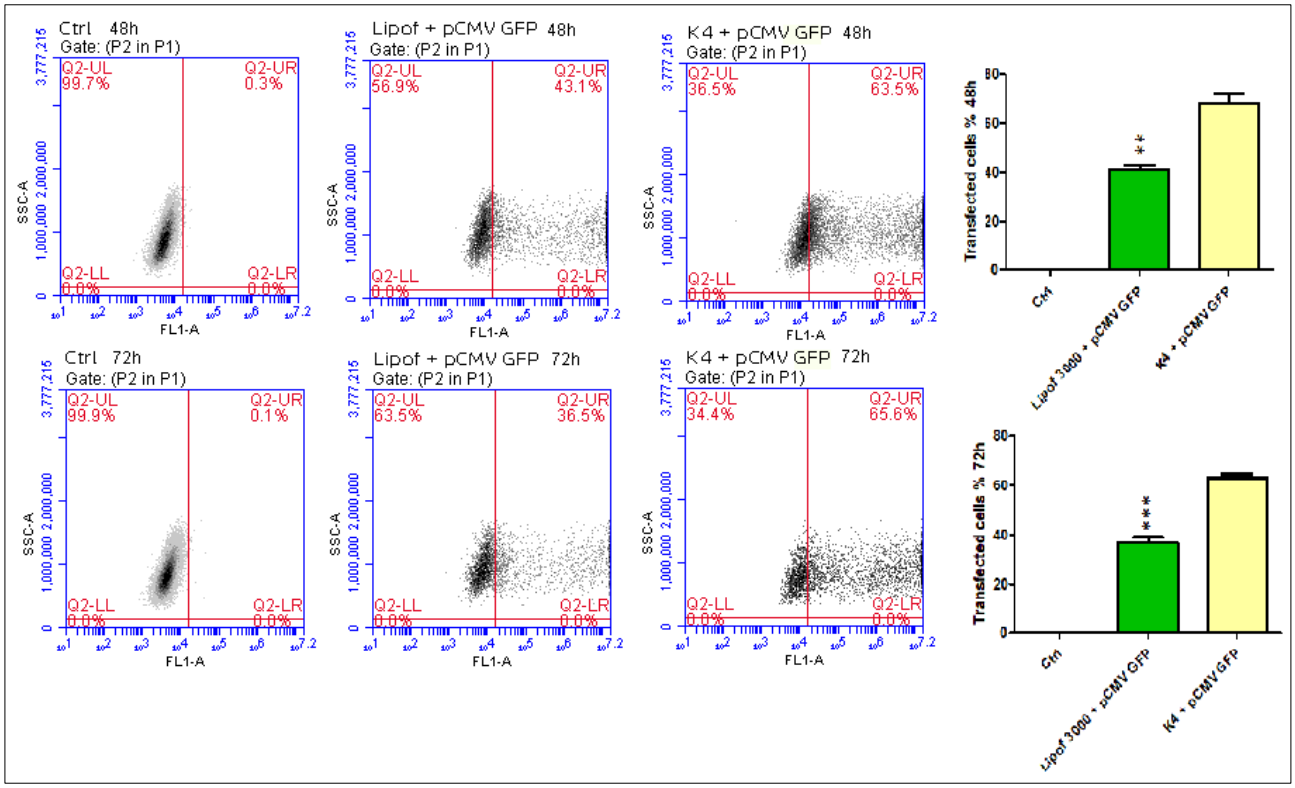

Fig.1. Transfection efficiency was measured using flow cytometry. Both after 48h and 72h from transfection quantification showed highest transfection efficiency with K4 Transfection System against Lipofectamine 3000. Data were collected from three independent experiments. (**P < 0.03 vs. K4 Transfection System group +pCMV-GFP group; ***P < 0.01 vs. K4 Transfection System +pCMV-GFP group). The cytograms represent samples taken from one of the three experiments for each of the three experimental conditions.

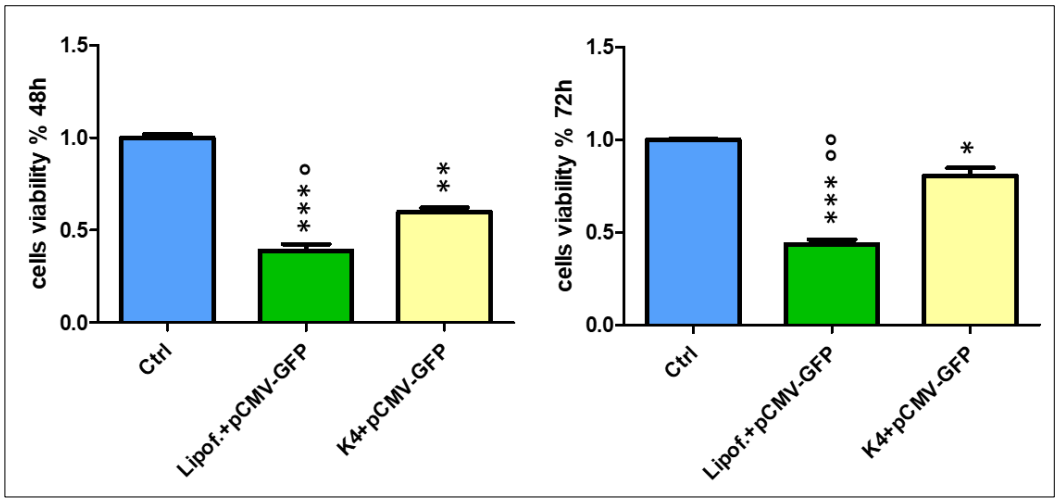

Fig.2. Cell viability was measured with MTT assay. In cells transfected for 48h and 72h quantification showed a sharp decrease in cell viability in cells transfected using Lipofectamine 3000. Data were collected from three independent experiments. (***P < 0.01 vs. control group; **P < 0.03 vs. control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

Conclusions.

Comparison of the transfection efficiency in HeLa cells using the K4 Transfection Reagent and Lipofectamine 3000 reveals that K4 Transfection System is a good transfection reagent for HeLa cells. K4 Transfection System allows to obtain high percentage of transfected cells with reduced toxic effects due to transfection itself.