Transfection efficiency and cytotoxicity of commonly used siRNA transfection reagents

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Introduction

The delivery of oligonucletides such as siRNA into eukaryotic cells has become a powerful tool to study and control gene expression. The choice of transfection reagent strongly influences transfection results. Generally transfection reagent should give high transfection efficiencies and reproducible results, and cause minimal cytoxicity.

Materials and methods:

MCF-7 and HeLa cells were plated into 96 wells plates in density 8x10^3 cells per well in Opti-MEM medium 24h before experiments. Cells were transfected using 50nM of scrambled siRNA, 50nM siControl TOX (control of transfection efficiency) and FAM-labeled GAPDH siRNA for 48h according to the manufacturer's protocols. Viability/cytotoxicity tests and confocal microscopy were used for evaluation of the cell cytotoxicity and transfection efficiency. The results were statistically evaluated by ANOVA and Tukey's multiple range tests using Graph Pad Prism® 4.0. P ≤ 0.05 was regarded as significant, at P ≤ 0.01 as highly significant and p≤0,001 as very high significant.

Aim:

Present study was focused on the comparison of efficiency and cytotoxicity of five most popular transfection reagents: Lipofectamine 2000, Lipofectamine RNAi MAX (Invitrogen), HyPerFect (Qiagen), DharmaFECT 1 (Dharmacon), INTERFERin (PolyTransfection) and X-tremeGENE (Roche).

Results

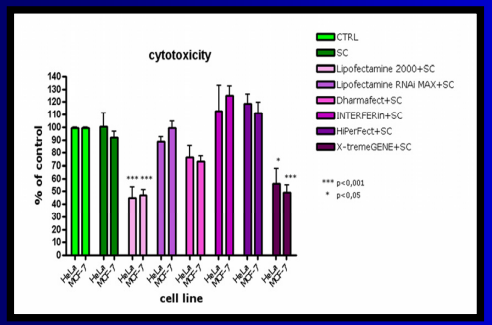


Fig. 1 Cytotoxic effect of common used transfection reagents on MCf-7 and HeLa cells transfected with 50nM scrambled (SC) siRNA for 48h. Cell viability was measured using MTS test (CellTiter 96 AQueous One Solution Proliferation Assay, Promega)

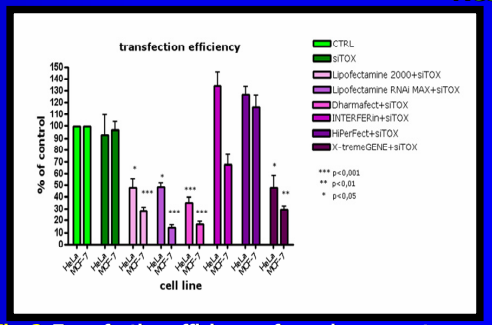
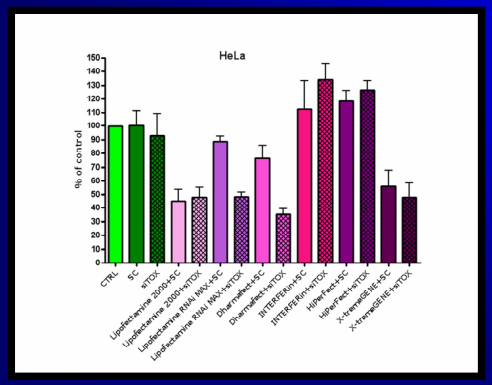


Fig. 2 Transfection efficiency of popular reagents was measured using 50nM siControl TOX (Dharmacon). Cell viability after transfection with siControl TOX which induced cell death after 48h of efficient transfection was measured using MTS test.



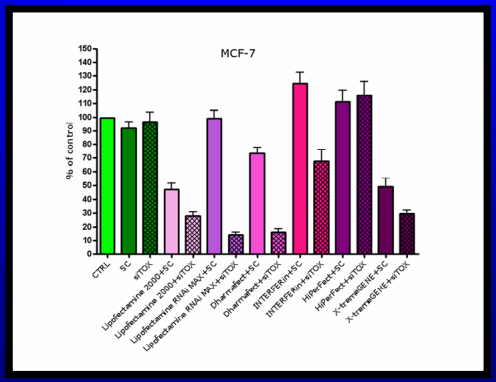


Fig. 3 Comparison of cytotoxicity and efficiency of transfection reagents after 50nM scrambled siRNA and siControl TOX transfection. Cell cytotoxicity/viability was measured using MTS test 48h after transfection.

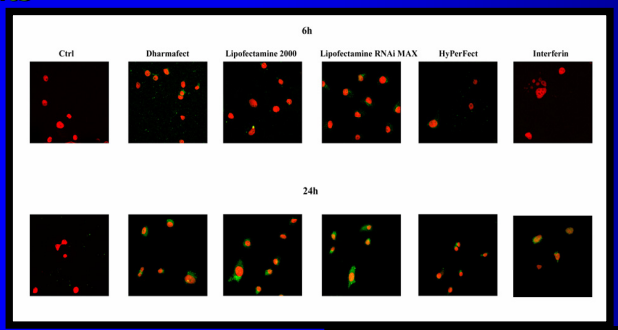
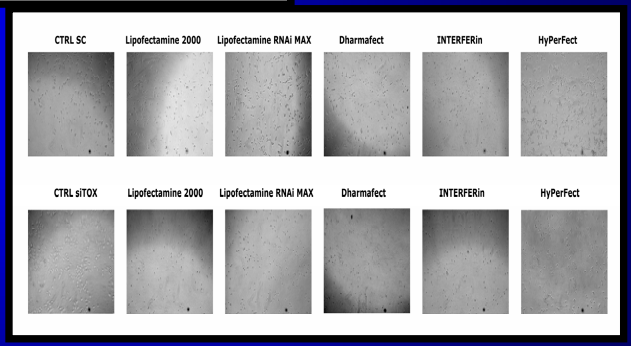


Fig. 4 Effect of breast cancer MCF-7 cells transfection with 50nM GAPDH siRNA labeled with FAM (green fluorescence) and common used transfection reagents. Images were made using confocal microscopy 6h and 24h after transfection. For counterstaining nuclei were stained with 7-AAD (red fluorescence)

Fig. 5 Morphological changes after transfection of MCF-7 cells with 50nM scrambled siRNA and siControl TOX . Cytotoxicity of transfection reagents after 50nM scrambled siRNA transfection and transfection efficiency using siControl TOX were manifested as mass cell apoptosis/necrosis and were observed using light microscope.



Conclusions:

- **Except Lipofectamine RNAi MAX and DharmaFECT, other reagents have relatively high cytotoxicity and low transfection efficiency in MCF-7 and HeLa cells**
- **These results suggest that transfection reagents differ significantly in their profile and choice of transfection reagent may influence outcome of the transfection experiments**