Transfection of Suspension cell line: THP-1 cells using METAFECTENE

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We recently evaluated the transfection efficiency of METAFECTENE in the suspension cell line: the human leukemic T cell line Jurkat. Another suspension cell line, THP-1, derived from human monocytic carcinoma represents an *in vitro* model, critical as well for studying the features of interesting genes. However, until now a method to transfect THP-1 cells efficiently is missing. For this purpose, we tested the new transfection reagent MEFAFECTENE, in comparison with another commercial liposome-based reagent: lipid F. Both of them are based on cationic liposome-mediated transfection techniques, which incorporate the gene into liposomes that fuse with the cell membrane and induce a high expression of the reporter gene within a short time after transfection.
The following experiments, we analyzed: i) the transfection rates of a suspension cell line THP-1 utilizing METAFECTENE reagent following the supplier's directions without optimization; ii) the optimal ratio of METAFECTENE to DNA; iii) the transfection efficiency with optimized METAFECTENE compared to that obtained using lipid F, which is commonly chosen as a high-level transfection system.

Materials:
Sterile 6-well tissue culture plates
75 ml cell culture flasks
Sterile Eppendorf tubes

Medium:
RPMI + 10% fetal bovine serum + 1% penicillin
Serum-free RPMI
Sterile water

Cells:
Human derived monocytic cell line, THP-1 (ATCC TIB 202)

Plasmid:
The green fluorescence protein (GFP)-expressing reporter plasmid pEGFP-N1

Transfection reagents:
METAFECTENE (Biontex)
Lipid F

Transfection protocol:

Cells were seeded 24h before transfection (1×10^6cells/well) and incubated overnight

in RPMI with 10% serum and antibiotics. The transfection procedure on the next morning was carried out as following: for each transfection, the indicated amount of transfection reagent was added to 50 µl of serum-free RPMI in a 1.5 ml Eppendorf centrifuge tube; in a separate tube, 1 or 2 µg of the plasmic DNA was mixed with 50 µl of serum-free medium; adding media containing lipid into DNA and mixing them gently; the tubes then were allowed to stand at room temperature for 20 min to allow lipid-DNA formation; at the end of this incubation, the lipid-DNA complexes was added onto the cells drop by drop. The cells therefore were incubated at 37˚C degree overnight.

Measurement of the transfection efficiency:

24h after transfection, the cells were washed once with PBS. Each sample (1×10^6cells) was subsequently resuspended in 1 ml PBS and analyzed by flowcytometry- CYAN (Dako Cytomation). Results below are shown as histograms of GFP fluorescence versus cell number.

TEST 1: Examining the Transfection Rates of THP-1 Cells by utilizing METAFECTENE and lipid F.

DNA : Lipid = 1 µg : 2 µl

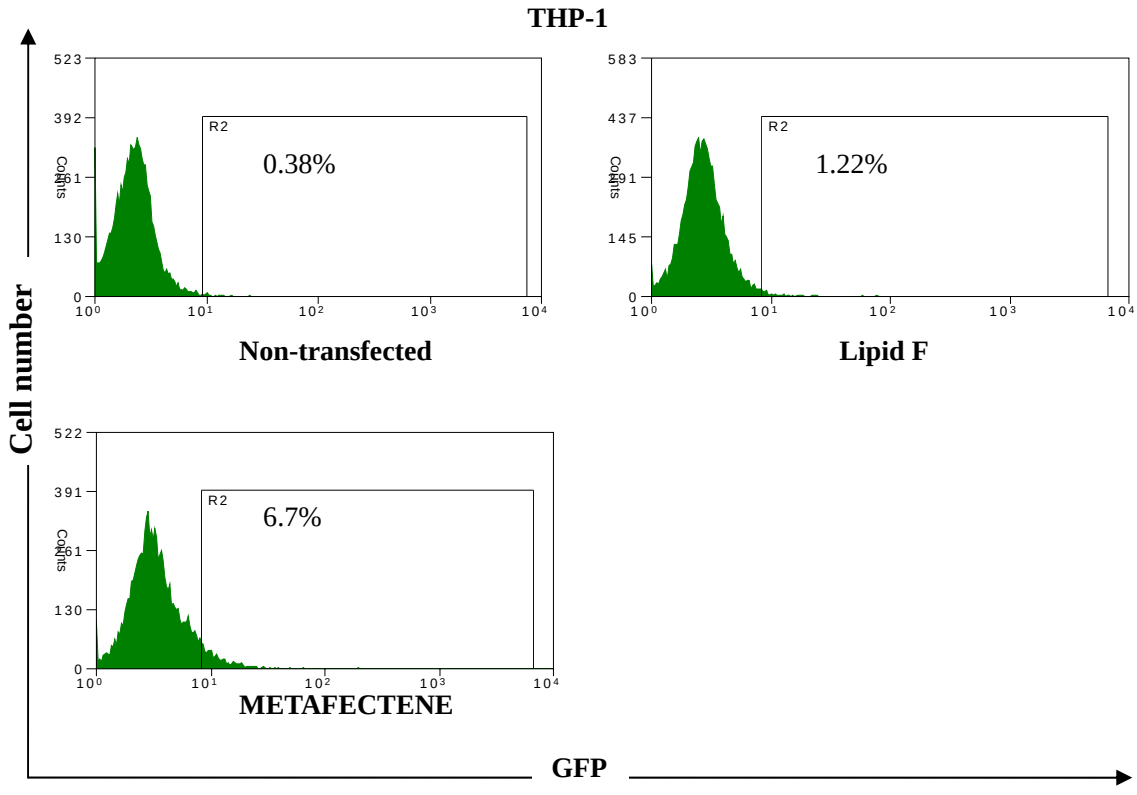

Fig. 1. The transfection rates of THP-1 cells are compared using lipid F (the middle panel) and METAFECTENE (the bottom panel). The results are shown as histograms

of GFP fluorescence versus cell number. The percentage of viable cells was determined by gating on the population with GFP-fluorescence.

TEST 2: Optimization of the Transfection for THP-1 cells.

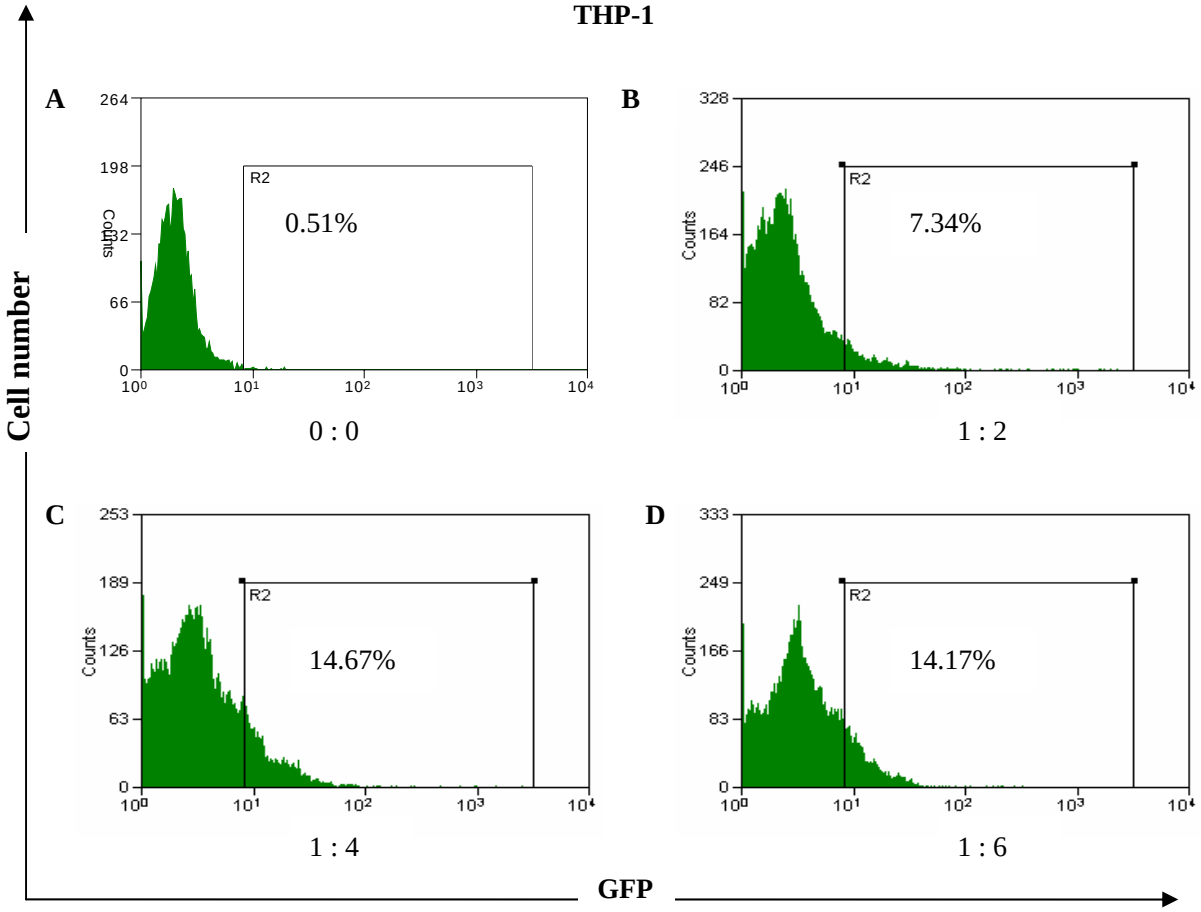

Fig. 2. In order to analyze the influence of different ratios of METAFECTENE to DNA, the experimental conditions are given: (A) Non-transfected THP-1 cells B) DNA : METAFECTENE = 2 µg : 4 µl; (C) DNA : METAFECTENE = 2 µg : 8µl; (D) DNA : METAFECTENE = 2 µg : 12 µl. When 2 µg of DNA was used in the ratio of 1 : 4, we already achieved a clearly high transfection efficiency, 14.67%.

TEST 3: Comparison of METAFECTENE with lipid F by utilizing the optimized Transfection Condition.

DNA : Lipid = 2 µg : 8 µl

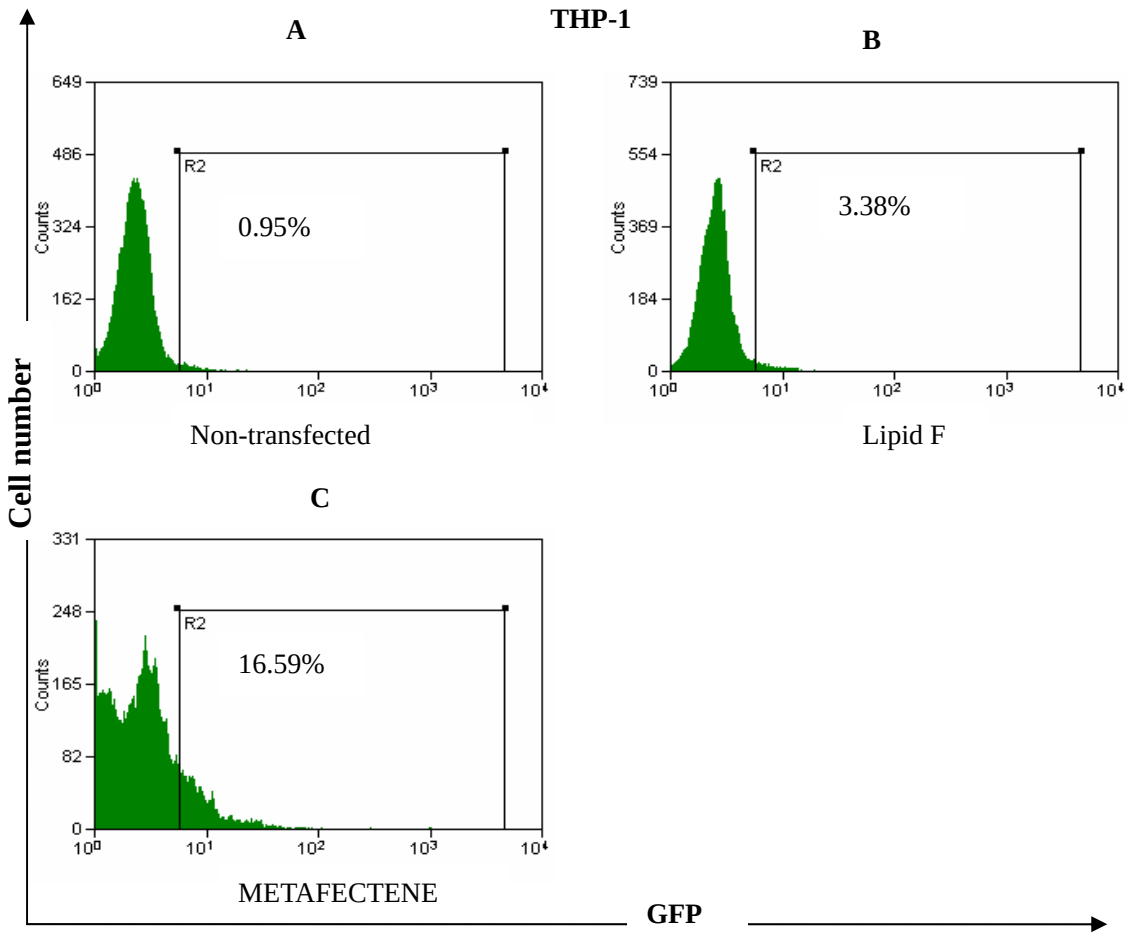

Fig. 3. Using the optimized transfection efficiency for THP-1 cells with (C) the METAFECTENE Reagent, a noticeably high transfection rate of more than 16.59% is reached. Compare to (B) the lipid F Reagent, which only shows around 3.38% efficiency.

Conclusion:

Our experiments once again indicate that METAFECTENE can be used for the *in vitro* gene transfection in human suspension cell lines especially the monocytic cell line THP-1, which is notorious for its low efficiency of transfection.
THP-1 cells transfected with the lipid F showed considerably lower transfection efficiencies than those transfected with METAFECTENE.
In THP-1 cells, ratios of Reagent : DNA = 1 : 4 is optimal for gene incorporation and protein expression.