Title of Experiment:

Trasfection of murine macrophage RAW264.7 cells with METAFECTENE PRO.

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Transient transfection: RAW264.7 cells (1.2 x 10^5 cells/well) were seeded in 1 ml of DMEM/F12 in 24-well culture one day before transfection, so that were approximately 70% confluent at the time of transfection. The day of transfection the pEGFP-N1 plasmid (4 μg) (Clontech Laboratories) was diluted in 30 μl of Opti-MEM I (Invitrogen) Medium without serum and antibiotics; Metafectene-PRO (from 4 to 8 μl) was gently mixed before use, then diluted in 30 μl of Opti-MEM I.. The diluted DNA was combined with Metafectene-PRO after 5 minutes of incubation at room temperature, without any mixture procedure and incubated for another 20 minutes at room temperature. The mixture was added drop by drop to the cells and incubated at 37°C in a CO_2 incubator for 4 hours prior to change the medium with 1 ml of fresh serum-containing medium (DMEM/F12). Gene activity was tested 36 hours after transfection.
The ratios DNA/metafectene-PRO used were respectively: 4μg:4μl; 4μg:6μl; 4μg:8μl (Figure 1).

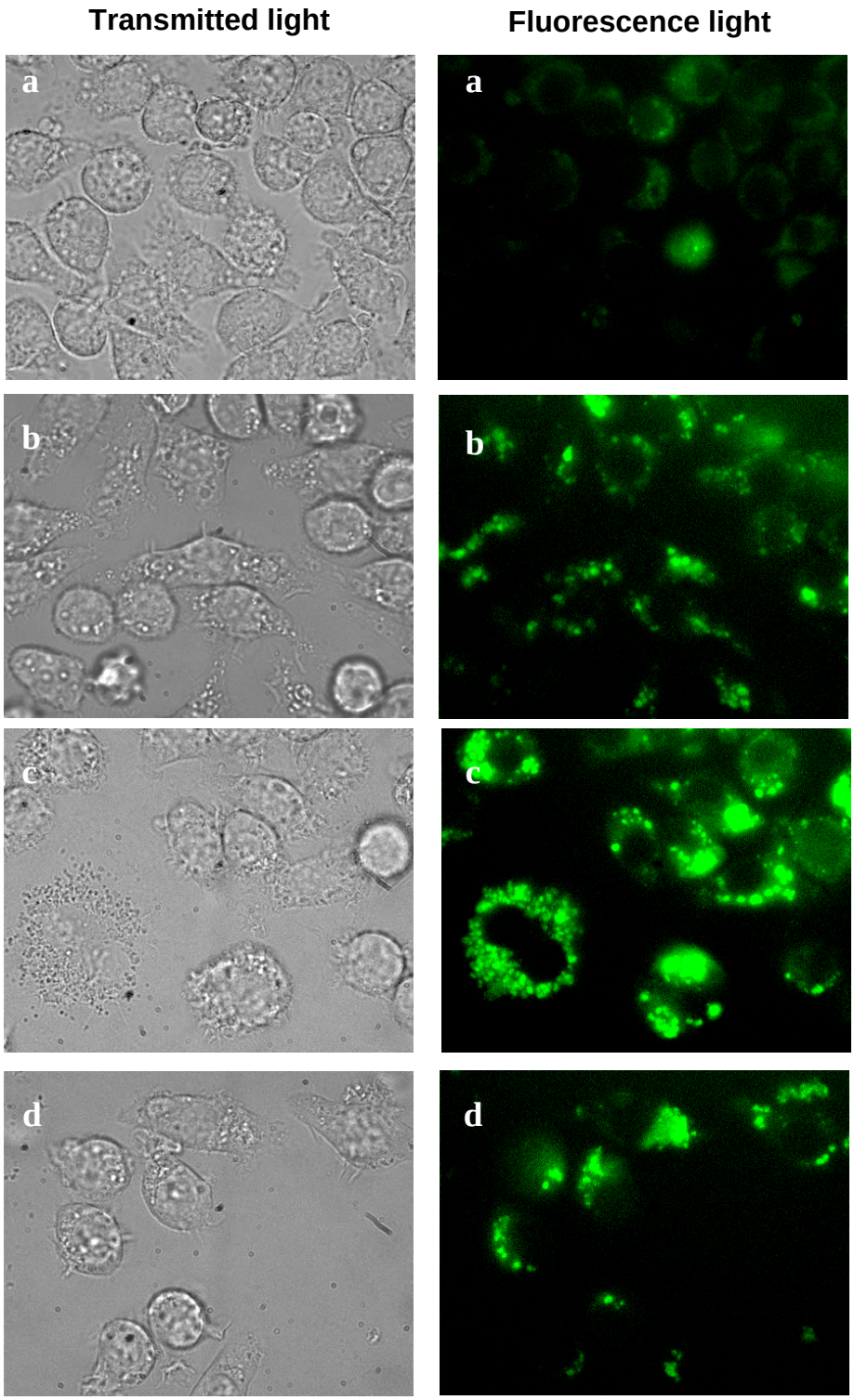


Figure 1.
a. RAW264.7 wild type;
b. RAW264.7 transiently transfected with 4 μg of pEGFP-N1 and 4 μl of metafectene-PRO;
c. RAW264.7 transiently transfected with 4 μg of pEGFP-N1 and 6 μl of metafectene-PRO;
d. RAW264.7 transiently transfected with 4 μg of pEGFP-N1 and 8 μl of metafectene-PRO.

Stable transfection: 3.5 x 10^6 RAW264.7 cells were plated in 10 cm petri dishes and incubated overnight. The next day, 24 µg of pmeLUC cDNA (a cDNA engineered in our laboratory) and 40 µl of Metafectene-PRO (Figure 2) (the same amount of LIPOFECTAMINE 2000 (Invitrogen) (Figure 3) and ARREST IN (Open biosystems) (figure 4) was used as comparison) were resuspended in 700 µl of Opti-MEM I respectively. After 5 min incubation at room temperature, they were mixed and re-incubated for other 20 min. The mix was added to the dish dropwise and gently swirled. The medium was changed after 4 hours of incubation at 37°C in a CO_2 incubator. On the second day post-transfection, 0.8 mg/ml of G418 (Calbiochem) was added to selecte transfected clones. The amount of G418 was reduced to 0.2 mg/ml after 20 days of transfection and stable clones were maintained in culture medium containing G418 at the same concentration.

Conclusions: For transient transfection there is a significant increase in the intensity of the fluorescence of the RAW264.7 transfected with Metafectene-PRO, but at 4µg:8µl ratio, the polycationic liposomal become toxic for the cells.
For stable transfection, Metafectene-Pro has significative higher tansfection efficiency than Arrest-In, but the same efficiency of Lipofectamine 2000 in transfecting RAW264.7 cells. Arrest-In has displayed low toxicity despite Metafectene-Pro and Lipofectamine 2000, but no stable clones were obtained from the polymer.

METAFECTENE-PRO

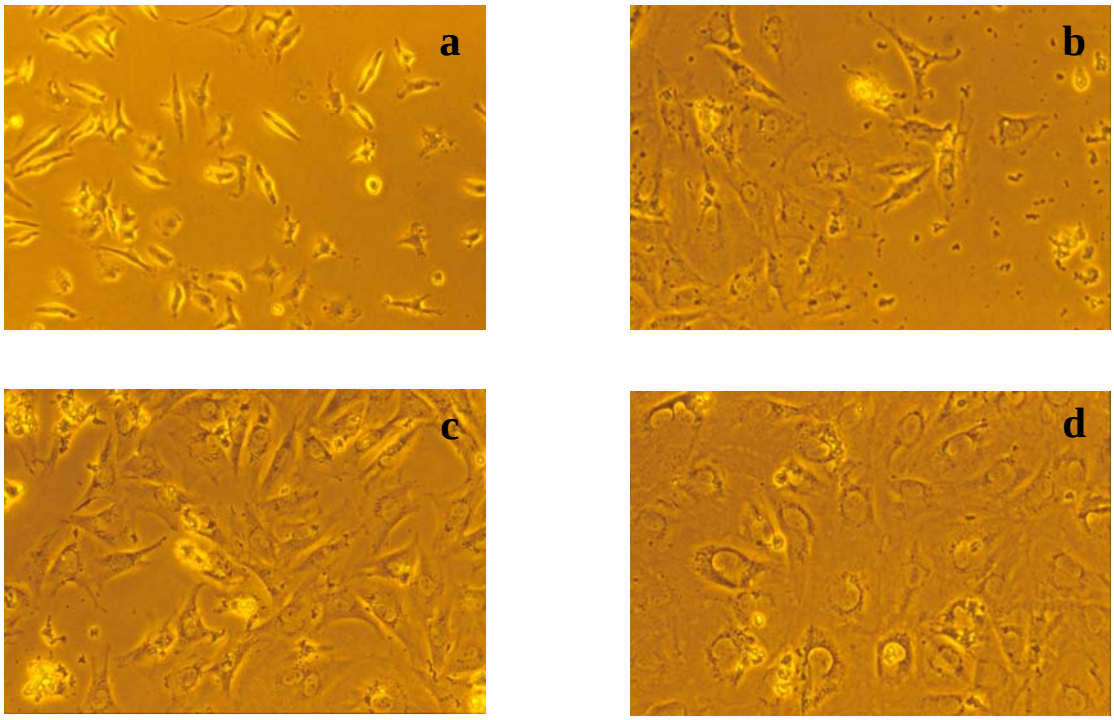


Figure 2.
a. RAW264.7 before transfection;
b. RAW264.7 1 day after transfection with METAFECTENE PRO;
c. RAW264.7 under G418 selection (15 days after transfection);
d. RAW264.7 stable clone (1 month after transfection).

LIPOFECTAMINE 2000

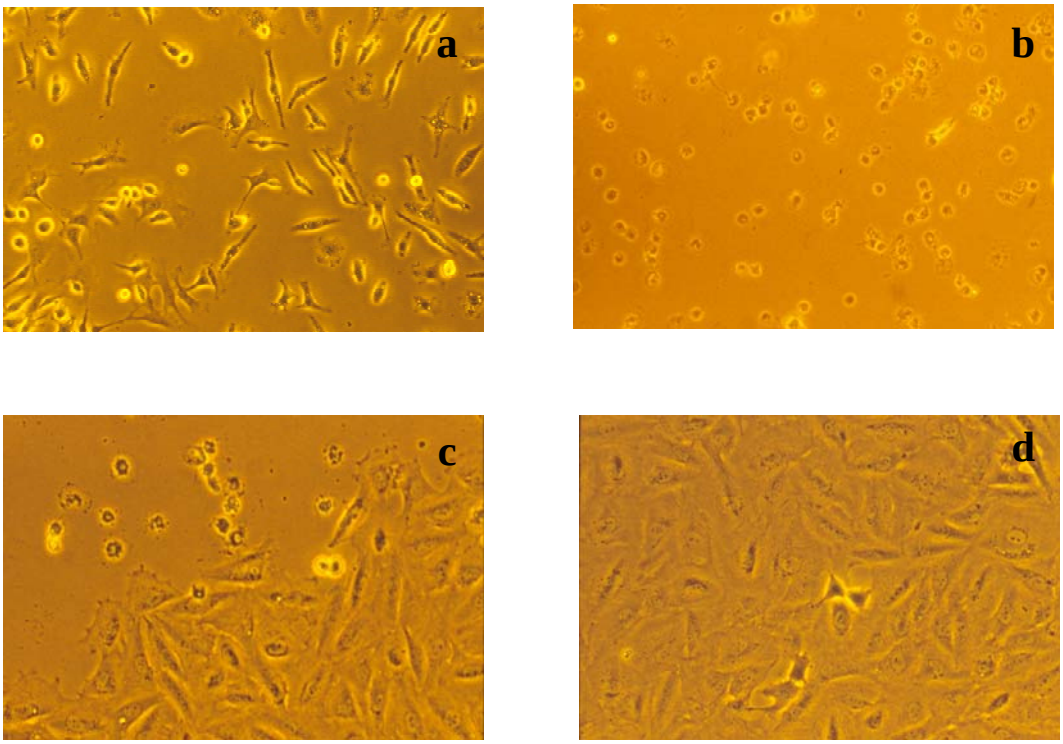


Figure 3.
a. RAW264.7 before transfection;
b. RAW264.7 1 day after transfection with LIPOFECTAMINE 2000;
c. RAW264.7 under G418 selection (15 days after transfection);
d. RAW264.7 stable clone (1 month after transfection) .

ARREST IN

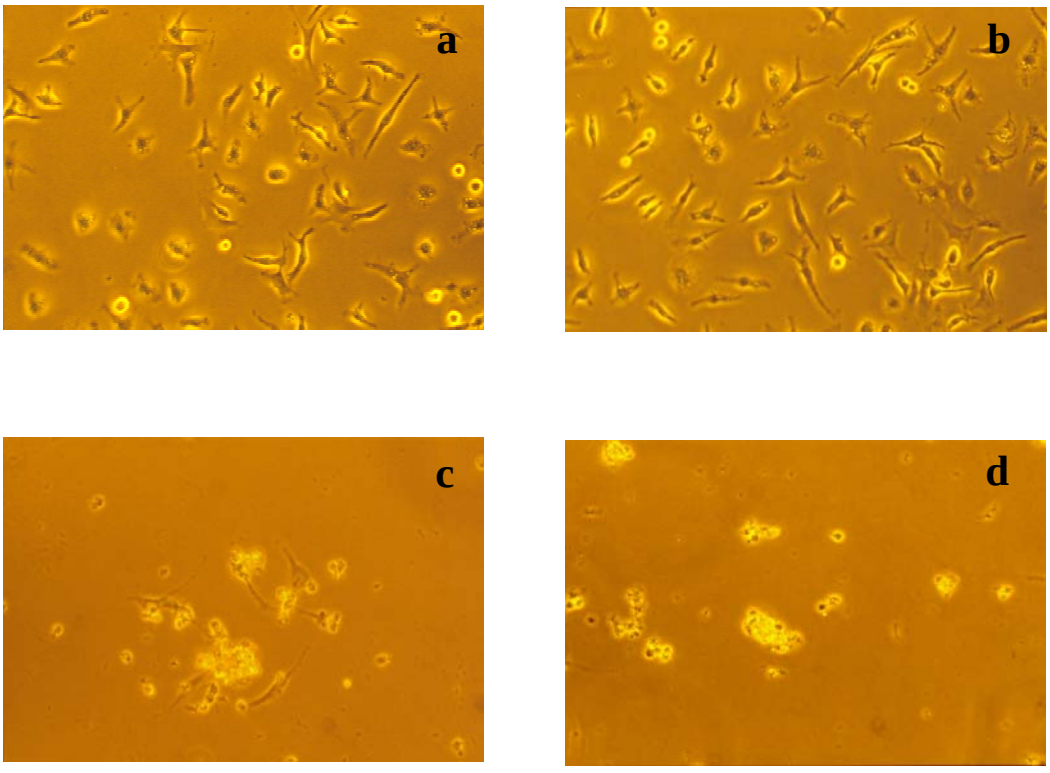
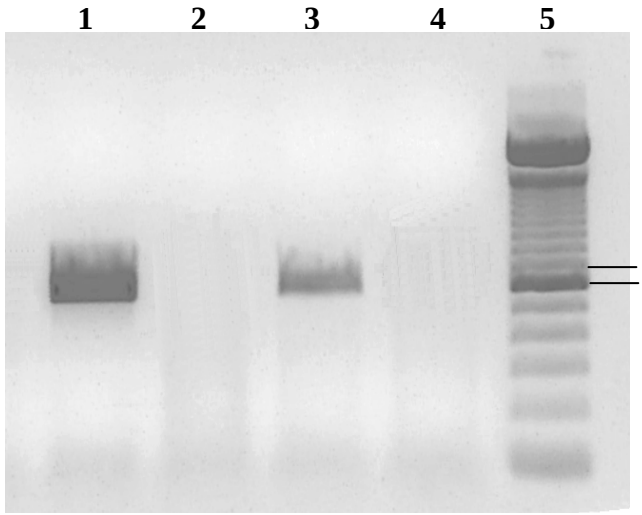


Figure 4.
a. RAW264.7 before transfection;
b. RAW264.7 1 day after transfection with ARREST IN;
c. RAW264.7 under G418 selection (15 days after transfection);
d. All cells died following G418 selection (1 month after transfection) .

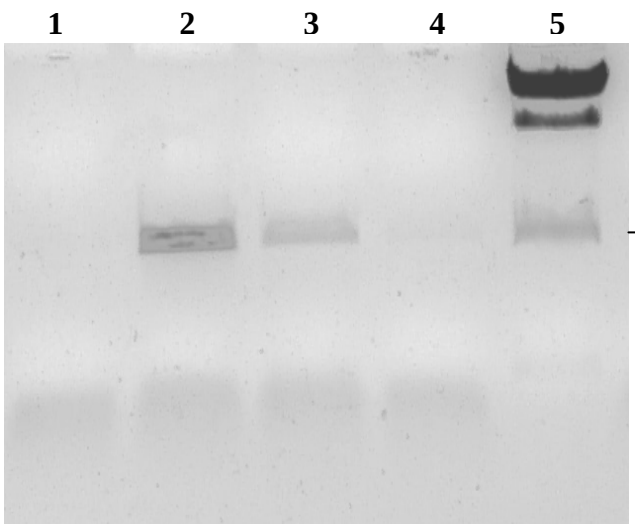
Analysis of pmeLUC/mRNA Expression - Total RNA was extracted from RAW 264.7-pmeLUC stable clone (obtained with METAFECTENE PRO and LIPOFECTAMINE 2000 methods) and HEK293-pmeLUC (HEK293 stably expressing pmeLUC already present in our laboratory, as a control) using RNAspin Mini Isolation Kit (GE Healthcare) as described by the manufacturer. Primers for pmeLUC were designed, based on sequence data: forward 5'-ATATGTGGATTTCGAGTCGTC-3' and reverse 5'-GATGGATTCCAATTCAGCGGG-3' (PCR product, 597 bp). To obtain cDNA, 200 ng of total RNA were transcribed with Access RT-PCR System (Promega). RT-PCR was performed using 10 µl of buffer AMV/Tfl Reaction buffer, 30 µl H_2O DNAse/RNAse free, 2 µl of each primer (final concentration 20 picoM each), and 2 µl of $MgSO_4$ (stock, 25 mM). Retrotranscriptions conditions were: 2 min at 95°C, then 1 cycle of 48°C for 55 min and 2 min at 95 °C; followed 35 cycles of amplification: 94°C for 1 min, 56°C for 1 min, 72°C for 2 min) and a final elongation at 72°C for 8 min. PCR products were resolved by electrophoresis on a 1% agarose gel.

LIPOFECTAMINE 2000



1. HEK293-pmeLUC
2. HEK293 wild type
3. RAW264.7-pmeLUC
4. RAW264.7 wild type
5. Marker

METAFECTENE PRO



1. HEK293 wild type
2. HEK293-pmeLUC
3. RAW264.7-pmeLUC
4. RAW264.7 wild type
5. Marker