Transfection of mammalian cell lines using Metafectene Reagent

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Transfection of mammalian cell lines with plasmid DNA is one of the standard procedures in modern cell- and molecular biology. In the post-genome aera, validation of numerous potential target genes through the expression of genes and ESTs in a variety of cell lines has become a central task for the establishment of novel therapeutic strategies.
For the transfection of standard cell lines such as COS-7 or Hela, several efficient and well tolerated transfection lipids have been developed. The situation is more difficult when immortalized or even primary human cell lines are used. These cells become more and more important in these validation tests because in contrast to the SV40-transformed or tumorigenic cell lines they represent the normal situation, however in many cases they can be transfected only to very limited extend.

Metafectene was tested in the adherent, epithelial human cell line SKOV-3 (ATCC No. HTB-77), the African green monkey cell line COS-7 (ATCC No. CRL-1651) and in human HOSE cells. SKOV-3 is an ovarian carcinoma cell line, HOSE is a non-tumorigenic, immortalized ovarian epithelial cell line (Tsao et al.). Whereas SKOV-3 is derived from a tumour, HOSE represents the normal ovarian epithelium, however, this cell line is extremely hard to transfect. Our results show, that using Metafectene, COS-7 cells can be transfected with very high efficiencies (up to 75%). In HOSE cells we reached transfection efficiencies between 5 and 11%, dependeding on the experimental conditions.
Two experimental parameters are considered significant, *a.* cell density per cell culture flask or 6-well plate and *b.* the lipid:DNA mixture. In addition, we noticed that the time the cells were exposed to the lipid was important for long-term survival of the cells.

Most of the experiments were performed with an Enhanced Green Fluorescent Protein expression plasmid (EGFP) using the following scheme:

Day1: Plating of cells. Day 2: 24 hours later, cells were transfected with the various lipid:DNA mixtures. In the first two experiments lipid:DNA mixtures of 3:1, 5:1 and 7:1 were tested. In all experiments, the DNA concentration was 1 µg/µl and in each transfection, 2 µg DNA were used. Using a lipid:DNA mix of 3:1 means that 100 µl Optimem Medium were mixed with 2µl DNA (Mix A) and 100µl Optimem Medium were mixed with 6µl Metafectene (Mix B). A and B were mixed together, incubated at room temperature for twenty minutes and added to the cells in 2ml medium. In all further experiments, the amount of DNA was kept constant, but alternative Metafectene concentrations were tested. Day 4: 48 hours later, positive cells were counted and survival of the cells was evaluated. We used cell densities between $1x10^5$ and $3x10^5$ cells per $25cm^2$ cell culture flask (T25) or per well of a 6-well plate. COS-7 and SKOV-3 cells were transfected in 6 well plates, HOSE cells grew better in T25 flasks. In all experiments, also a “mock-transfection” using only Metafectene without DNA was used to control for potential toxicity of the lipid.

The first two transfection experiments already revealed clear differences in transfection efficiency between the different cell lines and after using various lipid:DNA mixtures (Table 1):

Table 1

Experiment	Cell type	Cell No.	3:1	5:1	7:1	5:2	4:1	6:1
1	HOSE	$2x10^5$	0	0	0			
1	SKOV-3	$2x10^5$	6	14	6			
1	COS-7	$2x10^5$	52	29	27			
2	HOSE	$1x10^5$				5	2	6
2	SKOV-3	$1x10^5$				8	7	14
2	COS-7	$1x10^5$				32	44	63

Table 1: Numbers represent EGFP-positive cells and are given as mean numbers from 4 microscopic fields counted.

Using the conditions given above in the second experiment, HOSE cell could be transfected for the first time (Fig.1). In addition to the obvious role of cell density and lipid:DNA mix, a limited toxicity was visible in the HOSE cell cultures. In the first experiment, EGFP-positive cell were present, however, these cells were no longer adherent and therefore presumably not living cells. Therefore, this experiment was counted as negative (0). In a third experiment, HOSE cell densitiy was again varied, the lipid:DNA mix of 6:1 was kept constant:

Table 2

Eperiment	Cell type	Cell No.	6:1	6:1	6:1
3	HOSE	$3x10^5$	11	6	4
3	SKOV-3	$1x10^5$	17	15	15
3	COS-7	$1x10^5$	75	59	59

Table 2: Numbers represent EGFP-positive cells and are given as mean numbers from 4 microscopic fields counted. For each cell line 3 independent transfections with the same cell density and lipid:DNA mixture were performed.

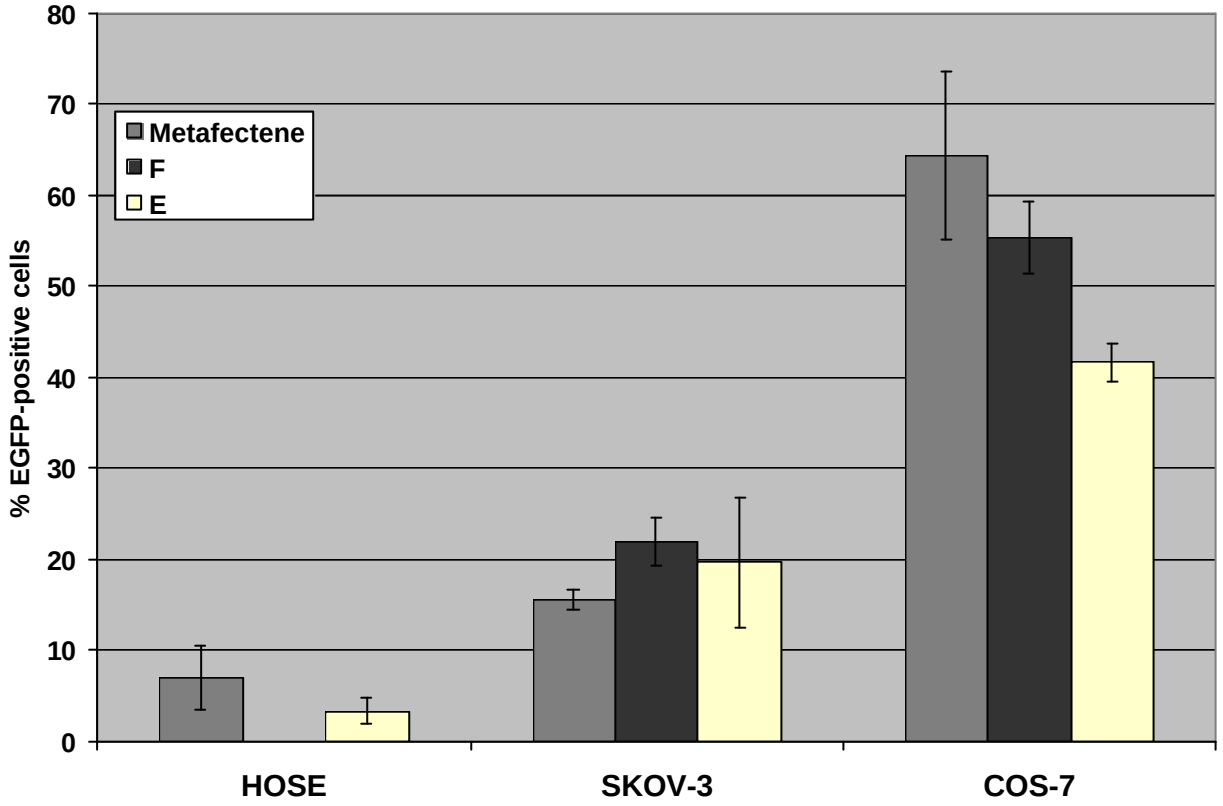
These experiments revealed a high reproducibility in all three cell lines and a remarkably narrow range for optimal transfection efficiencies concerning cell density and lipid:DNA mixture.

When compared to two other transfection reagents (E, F), the highest transfection efficiencies in COS-7 cells (60-75%) where reached with Metafectene. SKOV-3 cells could be transfected with all three transfection reagents, Lipid E was the best choice here with a maximal efficiency of 36%. In HOSE cells, similar efficiencies between 2 and 11 % could be obtained with Metafectene and Lipid E, no EGFP-positive cells where detected after repeated transfection with Lipid F (Fig. 2). In the meantime, further experiments in rat cell lines have resulted in a 50% transfection efficiency using Metafectene (data not shown).

With all three reagents we observed a limited toxicity towards the cells. These effects are clearly dependent on cell density and the proliferative capacity of the cells. For example, the

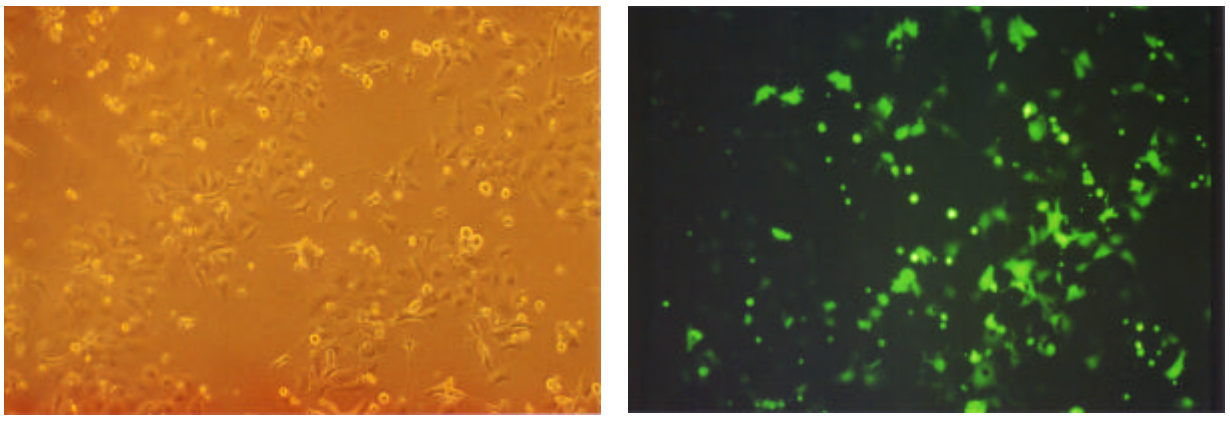
slowly growing and hardly transfectably HOSE cells could be transfected to higher efficiencies and where more resistant towards lipid-induced toxicity when transfected at $3x10^5$ cells per T25 cell culture flask as compared to the fast growing COS-7 cells, where the best results where obtained at $1x10^5$ cells. When these parameters have been established for a given cell line in one or two preliminary experiments together with the optimal lipid:DNA mix, Metafectene is a useful tool for testing plasmid DNA in cell lines which are hard to transfect.

Fig. 1

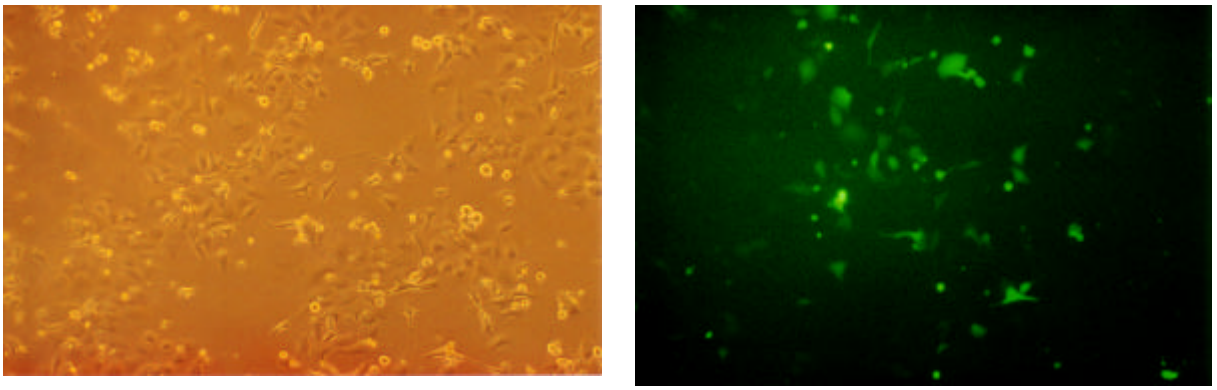


Transfection efficiency in HOSE ($3x10^5$), SKOV-3 ($1x10^5$) and COS-7 ($1x10^5$). The mean values from three independent transfections using a Metafectene Lipid:DNA mix of 6:1 are shown.

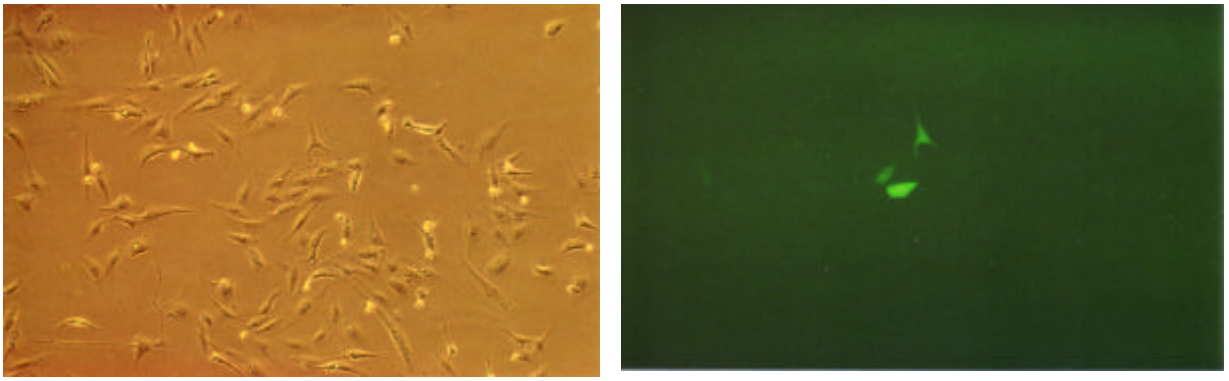
Picture 1 Transfected Cos-7 Cells



Picture 2 Transfected SKOV-3 Cells



Picture 3 Transfected HOSE Cells



Reference:

Tsao, S.-W., Mok, S.C., Fey, E.G., Fletcher, J.A., Wan, T.S.K., Chew, E.-C., Muto, M.G., Knapp, R.C., and Berkowitz, R.S. Characterization of human ovarian epithelial cells immortalized by human papilloma viral oncogenes (HPV-E6E7 ORFs). Exp.Cell Res., *218*: 499-507, 1995.