Transfection of MeWo-Zellen using Metafectene

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The possibility to transfect eucaryotic cells opens a wide and commonly used field for the analyses and characterisation of regulatory elements of cellular genes, functions of defined genes as well as signal transduction pathways connected with those genes. Furthermore, this is an excellent technique to study viral genes and their promoters or to investigate the virus-host-interaction on the level of gene expression by transfection of plasmids containing knockout-constructs. With all these experiments it is of great importance to bring the DNA molecules efficiently into a high number of cells without any damage or destruction of the cells as a consequence of the transfection reagent used.
In this study presented here, we established and optimised the transfection of MeWo cells (ECACC 93082609), which is a human melanoma cell line. This cell line is of some interest, because it is one of the few lines which can be infected with the Varicella-zoster virus (VZV), a member of human-pathogenous α-herpes viruses. Until now a method to transfect MeWo cells efficiently is missing.

In a first experimental approach $1{,}5x10^5$ cells were seeded in each well of a 12-wells plate. MeWo cells were cultivated in EMEM, supplemented with 10% FCS, non-essential aminoacids and penicillin/streptomycin. Transfection was performed at the following day when the cells had a confluency around 60%. The plasmid pCMV-EGFP was used for transfection. 1,5 µg plasmid DNA was mixed in the ratio of 1:3 with Metafectene according to the instructions of Biontex and added to the cells. Before the transfection procedure began, cells were washed with PBS and covered with 1 ml of normal medium or with 1 ml of serum-free medium. The same conditions were used to perform a transfection using another commercial reagent according to manufacturer's instructions. After an incubation period of 4 hours, all wells were filled with additional 2 ml of normal medium. Evaluation was done 24 hours post transfection by counting green fluorescing cells. Results are shown in figure 1: MeWo cells can be transfected, but serum-free conditions have to be used. The use of serum-containing conditions resulted in a extremely low transfection efficiency of 0,27%. Compared to the other transfection reagent tested, Metafectene showed a three fold higher efficiency under serum-free conditions.

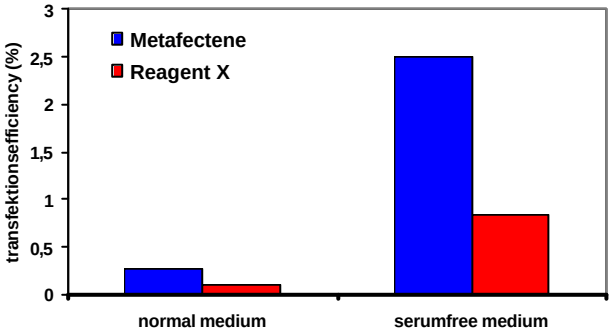

Fig. 1:
Transfection of MeWo cells using Metafectene or another commercial transfection reagent.
Those cells, which were cultivated under serumfree conditions showed a clearly higher transfection efficiency.

In the following experimental series the use of Metafectene was optimised. For that purpose 1) we tested different ratios of DNA to Metafectene using both, serum-free and serum-containing conditions and 2) we analysed increasing amounts of DNA using a constant ratio of DNA to Metafectene again using both, serum-free and serum-containing conditions. For the first series, 1,5 µg of DNA was chosen in the ratios of 1:2, 1:3 and 1:4; the evaluation was done after 24, 48 and 72 hours (Fig. 2). Again, cells cultivated under serum-free conditions showed a clearly higher transfection efficiency with all DNA-Metafectene ratios tested than those cultivated under serum-containing conditions. The highest transfection efficiency was found when a ration of 1:4 was used. Additionally, we observed a increase of green-fluorescing cells over the time with a maximum at 72 hours post transfection.

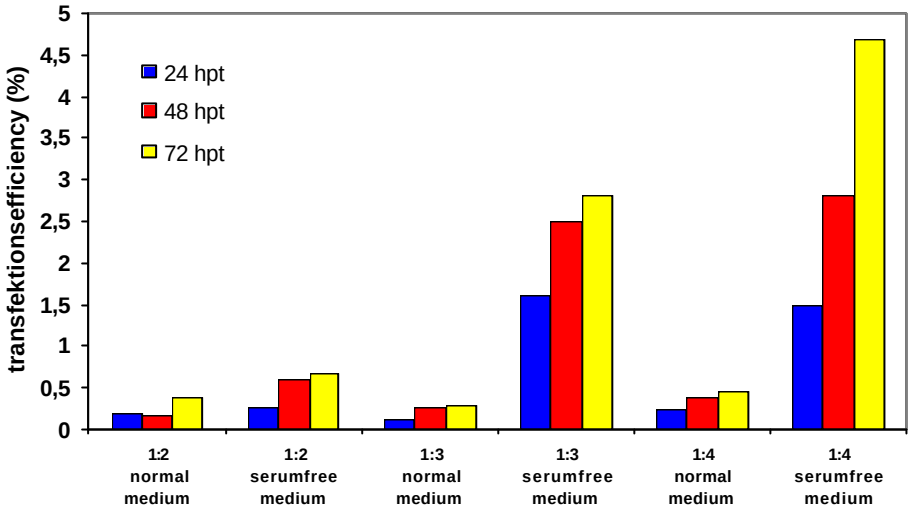

Fig. 2:
Optimisation of transfection, part I: Different DNA-Metafectene ratios were tested (using 1,5 µg DNA and evatuated over a period of 72 hours in 24 h intervalls. Cells were cultivated under both, serum-free and serum-containing conditions. The plasmid pCMV-EGFP was used for transfection.
A ration of DNA to Metafectene 1:4 seemed to be the most efficient one, if cells were cultivated in serum-free medium.

In the second part of the optimisation series, a constant ration of 1:4 of DNA to Metafecene was used with increasing amounts of DNA (2,3 and 4 µg of DNA). Evaluation was done by counting GFP-expressing cells after 24 and 48 hours. In general, the use of increasing amounts of DNA resulted in an increase of GFP-expressing cells, even if we found a slight decrease of fluorescing cells when 4 µg of DNA were used (Fig. 3). In addition, we found that there seemed to be a kind of saturation effect when higher amounts of DNA were used. In all 4 samples, the number of GFP-expressing cells remained on a constant level over the period of time tested.

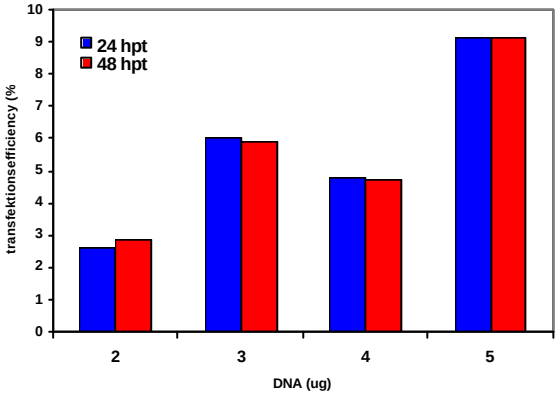

Fig. 3:
Optimisation of transfection, part II: Using the ration of DNA to Metafectene of 1:4, we transfected increasing amounts of DNA and evaluated the expression of GFP inside the cells after 24 and 48 hours.
We observed a saturation effect, because the GFP expression did not increase over the period of time tested.

In summary, MeWo cells are transfectable with Metafectene. Results are much better, when cells are cultivated under serum-free conditions during the entire transfection procedure. The ration of DNA to Metafectene of 1:4 seems to be the one resulting in the highest transfection efficiency, even if this is obviously a saturation effect when increasing amounts of plasmid-DNA are used.