Transfection of HepG2 cells with METAFECTENE

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The import of DNA in cells of higher organisms (transfection) is a suitable method to investigate biological and medical questions. Under proper requirements the imported DNA is expressed and therefore the transfected cells can be used to investigate the properties of exo- and endogenous proteins, to analyse intracellular processes, to determine the activity of gene promoters and to work on many other purposes. The requirements to a transfection method are a low consumption of DNA, a low cytotoxicity and of course a high efficiency.
For the transfection of the human hepatoma cell line HepG2 a non-liposomal lipid, an activated dendrimer and METAFECTENE were compared with respect to transfection efficiency. The HepG2 cells were cultivated in RPMI medium containing 10% FCS, penicillin and streptomycin and then transfected with the plasmid pSV ß-Gal (Promega) that encodes ß-galactosidase. The transfections were performed according to the standard protocols given in the manuals without optimization. After an incubation of 24 to 48 hours the transfection efficiency was photometrically determined with a ß-Gal assay (Promega). The HepG2 cells transfected with METAFECTENE showed a much higher activity of ß-galactosidase and therefore a much higher transfection efficiency than the cells transfected with the other methods.

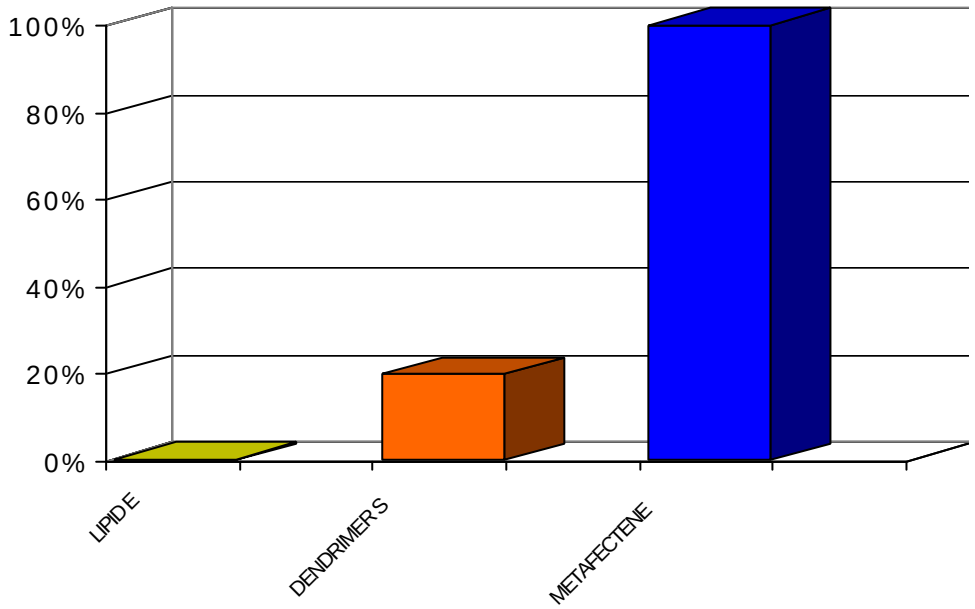

Fig. 1: Transfection of HepG2 cells with different methods

In the next step the optimisation protocol for 12-well dishes was performed to transfect the HepG2 cells with METAFECTENE. For this purpose the corresponding amounts of DNA and volumes of METAFECTENE were diluted in serum- and antibiotics-free RPMI medium, mixed, incubated for 20 minutes at room temperature and then added to 1.5 x 10^5 HepG2 cells in 1 ml serum- and antibiotics-free RPMI medium. After a cultivation period of 24 hours in the incubator the transfection efficiency was determined with the ß-Gal assay by the activity of the ß-galactosidase.

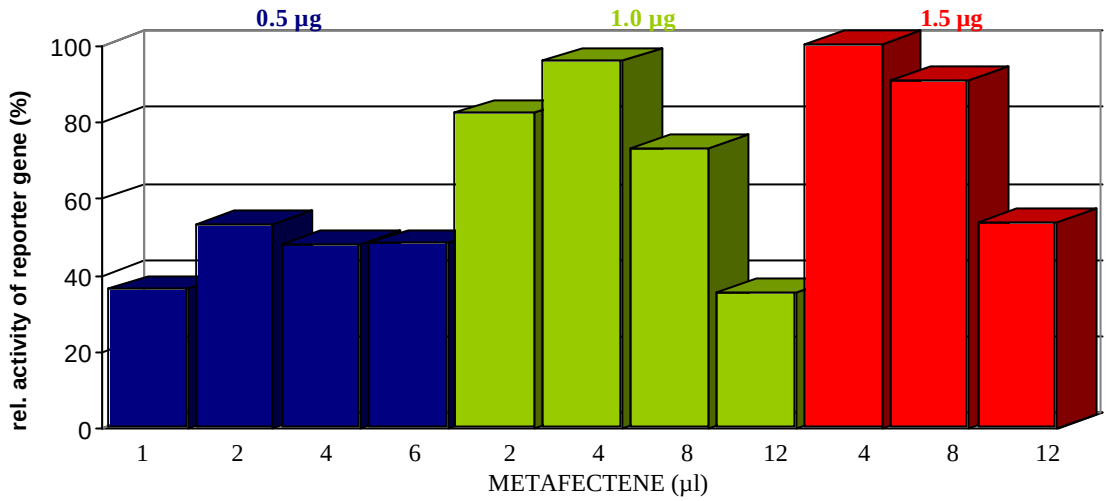

Fig. 2: Optimisation of the transfection of HepG2 cells with METAFECTENE

The best transfection efficiency was achieved with 1.5 µg pSV ß-Gal plasmid and 4 µl METAFECTENE. In the transfections with 0.5 and 1.0 µg plasmid DNA best results were observed with 2 respectively 4 µl METAFECTENE indicating an optimal relation of METAFECTENE to DNA of 4. For this reason a further improvement of the efficiency is probable achievable by the use of 6 µl METAFECTENE in combination with 1,5 µg DNA (METAFECTENE : DNA = 4).