Transfection of human intestinal CaCo-2 cells with METAFECTENE® EASY

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Introduction

The human epithelial cell line CaCo-2 is derived from a colorectal adenocarcinoma and serves as a common *in vitro* model in basic and clinical research. CaCo-2 W, a subclone of the CaCo2 BBe cell line, shows a well differentiated phenotype and was used in this experimental set up. The efficiency of METAFECTENE® EASY in the transfection of Caco-2 cells was evaluated in comparison to Lipofectamine™2000 which had worked best in past transfection experiments.

Materials and Methods

Reagents

METAFECTENE® EASY was obtained from Biontex Laboratories GmbH (München, Germany). Lipofectamine™2000 was purchased from Invitrogen (Karlsruhe, Germany) and plasmid pEGFP-N1 was obtained from Clontech (Mountain View, CA).

Cells

Cells of the human intestinal cell line Caco-2 W were provided by J.R. Turner (University of Chicago). Cells were grown at 37°C and 5 % CO_2 in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin, 1x non-essential amino acids, 1 mM sodium pyruvate and 1x GlutaMAX-I.

Transient Transfection

Transient transfection with METAFECTENE® EASY was carried out according to the manufacturer's instructions. Briefly, for transfection in a 12 well plate for each well to be transfected 10 µl METAFECTENE® EASY were mixed with 200 µl 1x reaction buffer. 10 µg DNA were added and the transfection mix was incubated at room temperature for 15 min. CaCo-2 W cells were trypsinized and diluted to 3.5 to 7 x 10^5 cells/ml. The transfection mix was added and 1200 µl cell suspension per well was plated. Optional, fresh medium was applied 6 to 8 hours after transfection.
For transfection with Lipofectamine™2000 50.000 cells/well were seeded on a 12 well plate one day prior to transfection. The following day cells were incubated in medium without antibiotics while the transfection mix was prepared. For each well to be transfected 2 µg DNA in 100 µl OptiMEM as well as 4 µl Lipofectamine™2000 in 100 µl OptiMEM were incubated at room temperature for 5 min, mixed and incubated for another 20 min at room temperature. The reaction mix was administered and cells were incubated at 37°C and 5 % CO_2. After 4 to 6 hours fresh medium was applied.

Fluorescence Microscopy

24 to 72 hours after transfection cells were analysed by fluorescence microscopy. Pictures were edited with Adobe Photoshop 6.0.

Results

The use of both transfection reagents, METAFECTENE® EASY as well as Lipofectamine™2000, resulted in a transfection rate of approximately 30 % as analysed by fluorescence microscopy. No significant differences could be observed concerning cell survival as both methods were well tolerated by the CaCo-2 W cells.

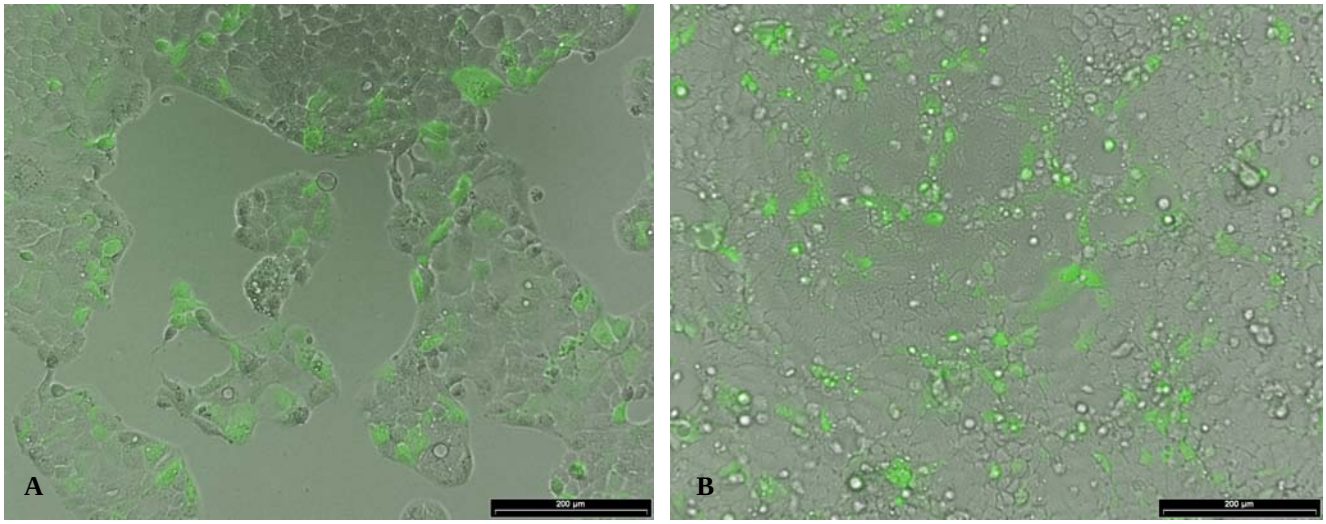

Figure 1: Transfection efficiency of MEATFECTENE® EASY compared to Lipofectamine™2000 in CaCo-2 W cells
CaCo-2 W cells were transfected with A) Lipofectamine™2000 or B) MEATFECTENE® EASY and the transfection rate was analysed by fluorescence microscopy. Both transfection reagents were similarly efficient.

Discussion

Transfection with METAFECTENE® EASY was faster and equally efficient as with Lipofectamine™2000, which required an additional day. However, the shorter time period when using METAFECTENE® EASY was obtained on expense of the DNA amount which was 5 times higher than needed for transfection with Lipofectamine™2000. As METAFECTENE® EASY has not been fully optimized yet the required amount of DNA might be further reducible.
Altogether, it can be concluded that both transfection methods work equally well in CaCo-2 cells but do fulfil different needs (shorter time frame as opposed to smaller DNA amount).