DNA-transfection of human adipose-derived stem cells (hADSC) with the Biontex K2® Transfection System

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This series of experiments aimed at the transfection of rather hard-to-transfect primary human adipose-derived stem cells. To this end, a luciferase-encoding plasmid DNA was employed and transfection efficacies were determined by luciferase activity. Different ratios / amounts of Biontex K2® Transfection Reagent and K2® Multiplier were compared.

Materials and Methods

Cell culture

Human adipose-tissue derived stem cells (passage 9) were cultured under 5 % CO_2 at 37 °C in a humid atmosphere in Dulbecco's Modified Eagle Medium (high glucose) containing 10 % FCS without penicillin/streptomycin.

For transfection experiments, 20,000 cells / well were seeded in 48-well plates one day prior to transfection. The transfections were carried out in 0.25 mL Dulbecco's Modified Eagle Medium (high glucose) containing 10 % FCS without antibiotics.

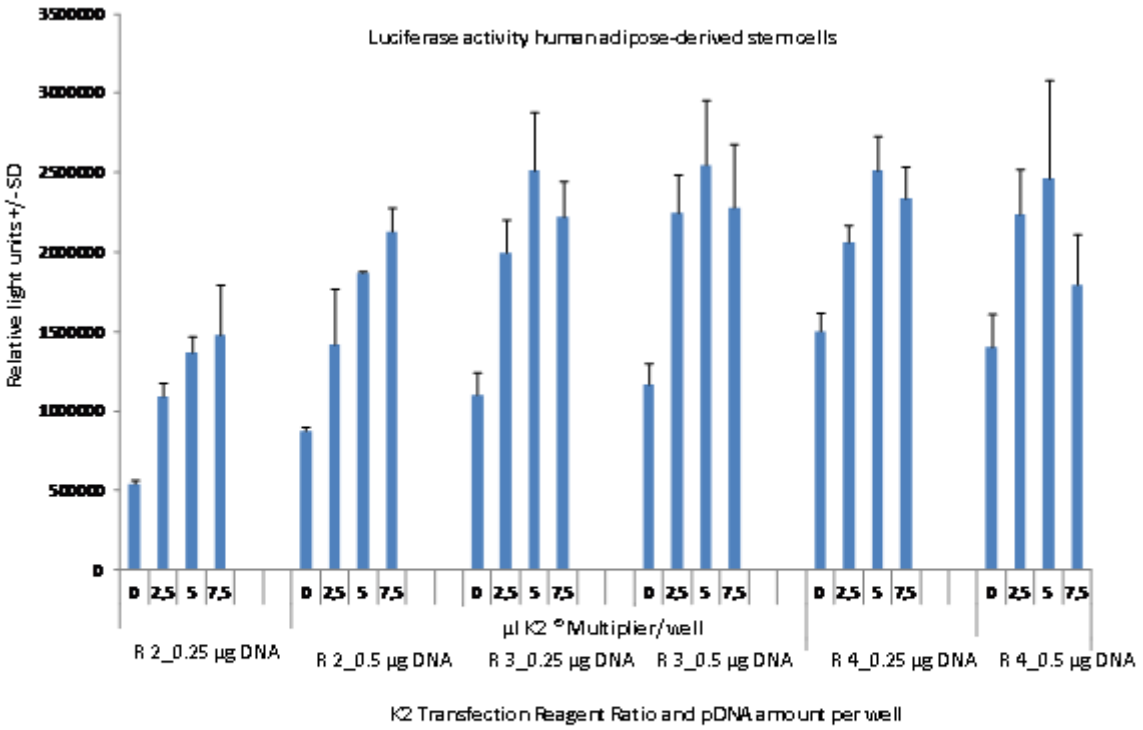
Cell transfection

The cells were treated with the K2® Multiplier 2 hours prior to transfection according to the manufacturer's protocol. To this end, different volumes of the K2® Multiplier were added to the wells as indicated in the figure. The plates were gently shaken to distribute the Multiplier homogenously.

The lipoplexes were prepared following the protocol provided in the manual. Briefly, an appropriate amount of the luciferase-encoding plasmid DNA (pGL3-Control; Promega) was mixed with serum-free DMEM. The K2® Transfection Reagent was separately diluted in DMEM at different ratios (µg pDNA/µl transfection reagent = 'Ratio' (R)) as detailed in the figure. The pDNA solution was added to the diluted Transfection Reagent and mixed by gentle pipetting up and down. After 15 minutes incubation at room temperature, the lipoplexes containing 0.25 µg (30 µL) or 0.5 µg (60 µL) pDNA were added to the wells, followed by gentle shaking of the well plates. The medium was changed the next day.

The determination of luciferase activity was performed at 72 h after transfection, using the Beetle-Juice kit from PJK (Kleinblittersdorf, Germany). Briefly, the medium was aspirated and the cells were lysed in 50 µL lysis buffer (Promega). In an appropriate tube, 25 µL luciferin

substrate was mixed with 10 µL lysate and chemiluminescence was immediately determined in a luminometer (Berthold, Bad Wildbad, Germany).



R2 = 1:2 [µg DNA]/[µl K2 Transfection reagent]
R3 = 1:3 [µg DNA]/[µl K2 Transfection reagent]
R4 = 1:4 [µg DNA]/[µl K2 Transfection reagent]

Results and Conclusions

For the transfection of human adipose-derived stem cells with a luciferase-encoding plasmid DNA, the K2® Transfection System was used at three different reagent ratios and increasing volumes of the K2® Multiplier. Furthermore, two amounts of pDNA were tested and compared.

Profound luciferase expression was observed. A trend towards higher luciferase expression was detected with increasing transfection reagent ratios, independent of pretreatment with K2® Multiplier. The Multiplier treatment enhanced the luciferase expression at all transfection reagent ratios and DNA amounts. Optimum conditions for transfection were ratio 3; 5 µL Multiplier and 0.25 µg pDNA. The double amount of pDNA (0.5 µg) per well showed a slight advantage only at ratio 2, while at higher ratios the cell growth was rather inhibited. At ratio 4, 0.25 µg pDNA led to cell growth inhibition and cytotoxicity was observed with 0.5 µg pDNA.

We conclude that the K2® Transfection System has shown promising results for pDNA transfection of hard-to-transfect primary human adipose-derived stem cells and that the multiplier further increases transfection efficacy.