DNA-transfection of murine kidney podocytes (SVI cells) using "Biontex K2®Transfection System".

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Materials and Methods

Murine kidney podocytes (SVI cells) were cultured in 75 cm² CELLSTAR® Cell Culture Flasks (Greiner Bio-One GmbH, Germany) in 10 ml growth medium: RPMI 1640 Medium with L-Glutamin (Lonza, Belgium) supplemented with 10% fetal calf serum (pH 7.0-7.5; GIBCO™ Invitrogen Corporation, Germany) and 1% penicillin-streptomycin (GIBCO™ Invitrogen Corporation, Germany). Cell culture dishes (60x15mm; Greiner Bio-One GmbH, Germany) with 12 round slides (12 mm diameter; Carl Roth GmbH & Co. KG, Germany) were coated with mouse collagen type IV (BD Bioscience, Germany) for 30 minutes and subsequently washed two times with 1x PBS Dulbecco without Ca^{2+} and Mg^{2+} (Biochrom, Germany). Cells were trypsinated and seeded on coated slides in 4 ml medium. After 3 days slides were transferred into 4- and 24-well cell culture plates (Greiner Bio-One GmbH, Germany), respectively, and medium per slide was added. The transfection was performed on 60-70 % confluent cells.

Cell transfection

(Please find accurate reagent amounts in the table below)

Prior to application stock solutions were brought to room temperature and agitated gently. Cells were treated with K2® Multiplier 2 hours before transfection. The Multiplier was added slowly into the medium and mixed gently by circular swirling. K2® Transfection Reagent and siRNA were both mixed with RPMI 1640 Medium with L-Glutamin without supplements (FBS or penicillin-streptomycin). SiRNA-solution (solution A) was added to K2® Transfection Reagent-containing solution (solution B) – please note this order! Solutions were mixed by gently pipetting up and down once and afterwards incubated for 20 minutes at room temperature. Mixture was added dropwise to K2® Multiplier-treated cells and mixed gently by circular swirling. Cells were incubated with transfection reagents at 38°C and 5% CO^2 for 24 hours. The next day the medium was removed, fresh growth medium was added and cells were cultivated again for 24 hours. Following cells were fixed. Since siRNA was not labeled, an immune fluorescence staining was performed to visualize transfected cells and estimate transfection efficiency.

Dish size: (4- or 24-well plate)

		Solution A		Solution B	
Growth medium per well	K2® Multiplier per well	RPMI 1640 Medium with L-Glutamin without supplements	siRNA (60-80% confluent cells)	RPMI 1640 Medium with L-Glutamin without supplements	K2® Transfection reagent
500 µl	10 µl	30 µl	30 nM	30 µl	1.6 µl

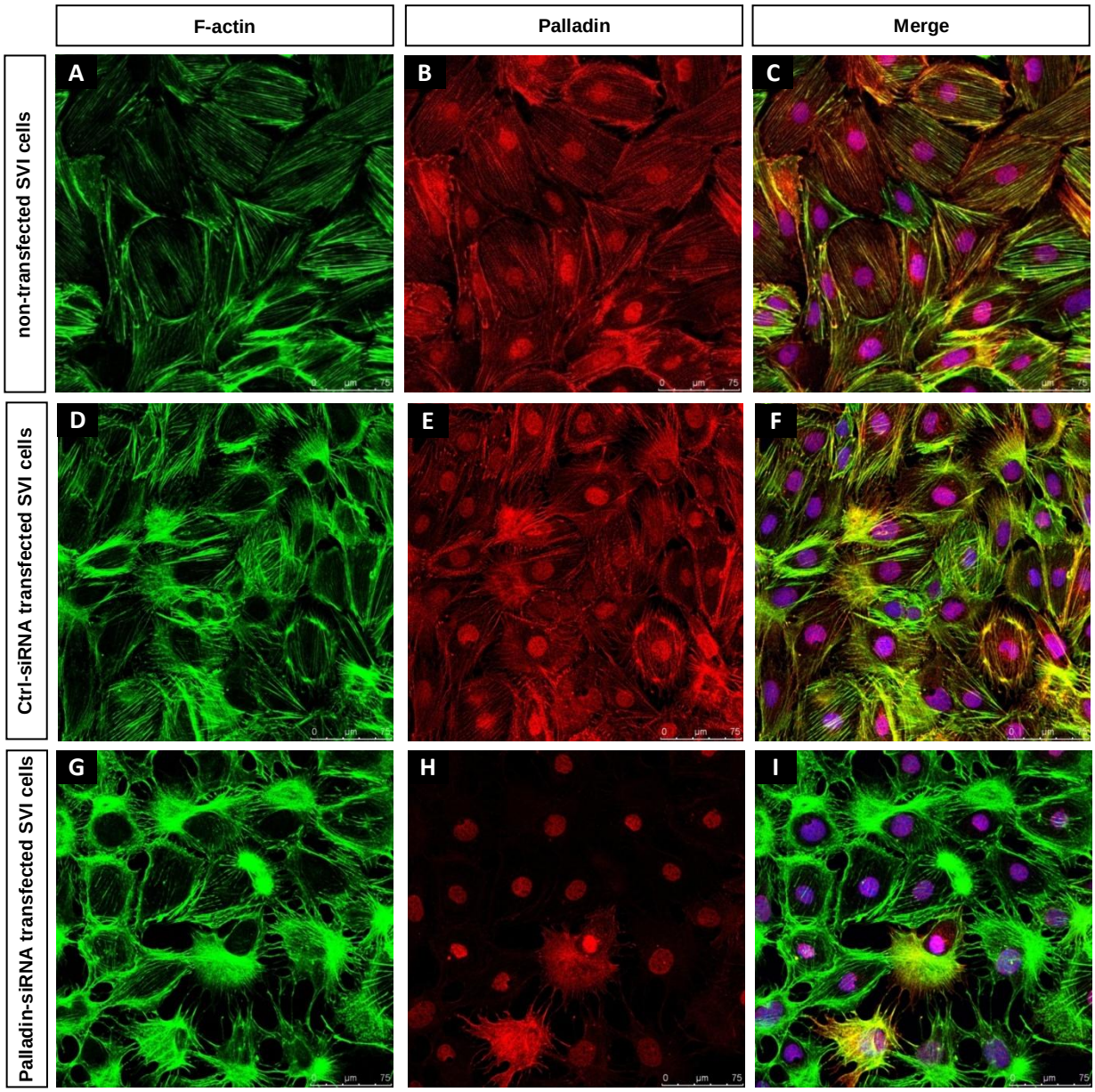

Figure 1 | Immune fluorescence staining of non-transfected and transfected SVI cells. Cultured SVI cells were stained to visualize F-actin (green, Alexa Fluor® 546-Phalloidin), palladin (red, anti-rabbit Palladin) and nuclei (blue, DAPI). Non-transfected cells show normal morphology. Palladin is co-localize with F-actin in stress fibers and focal adhesions (A-C). Transfection System induces no toxic effect on cells, so Ctrl-siRNA transfected cells have no morphological changes (D-F). Palladin-siRNA transfected cells show a high transfection rate. Transfected cells have no signal and only a few non-transfected cells are stained red, respectively (G-I).

Conclusions

K2® Transfection System transfected cells have a very low death rate. 24 h after transfection, cells have the same confluence at the day before. In comparison to control cells (non-transfected cells), their cell growth rate is slightly reduced, whereas all other parameters remain at a comparable level.

To investigate cell morphology and transfection rate, an immune fluorescence staining was performed after transfection. Transfected cells show the same normal morphology as non-transfected cells do. Hence, cell physiology and morphology are not negatively affected by this Transfection System. The transfection rate was not found to be dependent on cell density. Cells with 60% confluence had the same transfection rate as more confluent cells. Furthermore, fluorescence stained transfected cells gave a strong and non-transfected cells gave no signal, respectively. Interestingly, no intermediate or weak signal intensities were observed, what indicates, that cells are either transfected or not, but not partially.

Taken together, application of Biontex K2® Transfection System gives high transfection rates (70–90%) without any cytotoxic effects of the transfection system.