DNA-transfection of human BeWo cells using "Biontex K2® Transfection System"

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Materials and Methods:

Cell culture

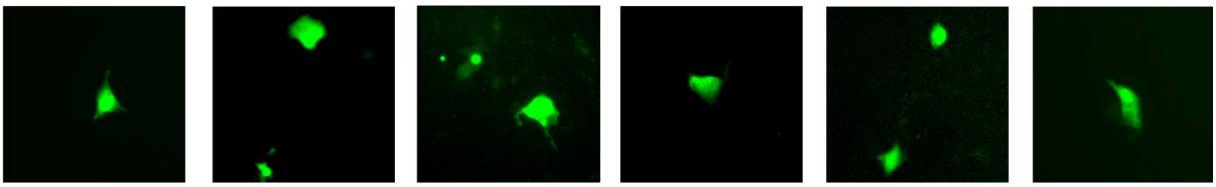
Human BeWo cells were cultured in cell culture flasks (Sarstedt) in phenolred–free Dulbecco's modified eagle medium, F-12 Nutrient Mixture (Ham) 1:1 (Gibco) containing 10% fetal calf serum (Gibco) and 100 U/ml Penicillin and 0,1 mg/ml Streptomycin.
Before transfection $5x10^5$ cells were seeded in 6-well Plates in DMEM/F12, 3% Charcoal/Dextran treated FBS (Hyclone, Thermo scientific) was tested versus 10% FCS (Gibco)

Cell transfection

Some wells were treated with K2® Multiplier, 2 hours before DNA transfection. For this K2® Multiplier was dripped slowly onto the medium and mixed by gently swaying the dishes. K2® Transfection Reagent was mixed with PBS and left on room temperature during preparation of the DNA. Plasmid-DNA was mixed with PBS. DNA solution was added to the solution containing the K2® Transfection reagent and mixed by tapping the tubes, followed by 15 minutes incubation at room temperature. Transfection solution was applied to cells by slow dropwise addition to the medium followed by gently swaying the dishes to achieve mixing. Transfections were incubated at 37 °C and 5% CO2 for 24 hours. Transfection efficiency was estimated by transfection of Plasmid-DNA for Green Fluorescent Protein (GFP) and fluorescence microscopy.
Parallel Luciferase-Assay was performed.

Results:

	Transfection reagent	Serum	Surviving cells	Transfection efficiency (GFP)
I	1x PEI	10% FCS	90%	+
II	1x PEI	3% C/D FBS	70%	++
III	K2®	10% FCS	90%	++
IV	K2®	3% C/D FBS	90%	+
V	K2® Multiplier	10% FCS	70%	+++
VI	K2® Multiplier	3% C/D FBS	50%	+



I II III IV V VI

Conclusions:

Plasmid-GFP-DNA was transfected into human BeWo cells using the K2® Transfection system and the K2® Transfection system in combination with K2® Multiplier. Results were compared to transfections using 1x PEI transfection reagent.

Transfecting the BeWo cells in medium containing 3% C/D FBS led to a lower survival rate of the cells than using 10% FCS, independently from the transfection reagent. Only the K2® system did not affect the cell survival. The living cells showed no morphological abnormalities.

Successful transfection and transfection efficiency were evaluated by the number of GFP expressing cells and analyzed via fluorescence microscopy. Using the K2® system we could observe a higher GFP-expression with 10% FCS, than in cells cultured with 3% C/D FBS. This observation was confirmed in transfections using K2® and the Multiplier system. In contrast, transfections using 1x PEI reagent with 3% C/D FBS achieved a higher transfection rate in comparison with 1x PEI and 10% FCS.

Because of studies of the PPARγ activity it was used 3% C/D FBS.