Comparison of different DNA transfection protocols with HEK293 cells in suspension cultures.

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Material and methods

Transfection reagents tested

3 different transfection reagents were tested:

- Commercial Lipid based reagent
- K2 Transfection System (Biontex)
- 25kD Linear Polyethylenimine (PEI, Sigma-Aldrich). PEI efficiency was tested in two different culture mediums, SFM4Transfex (Hyclone) and Freestyle (Gibco)

Cell Culture

Human embryonic kidney cells (HEK293) were cultured and transfected in suspension cell cultures. HEK 293 cells were cultured in a 125mL Shake Flask (Corning) containing 20mL of SFM4Transfex Medium (Hyclone) supplemented with 5% Fetal Bovine Serum –FBS- (Sigma-Aldrich) and 4mM Glutamax (Gibco). The seeding cell density was about 2.5 $x10^5$ cells/mL and shakes were cultured for 48 hours into a CO_2 incubator at 110 rpm. The transfection experiment was carried out when cell density approximately reached $1x10^6$ cell/mL. Cell density and cell viability was determined by means of cell counting in a Neubauer hemocytometer chamber (Brand) using the trypan blue (Sigma-Aldrich) exclusion method. The cultures viability at time of transfection was over 90% for all experimental conditions.

Cell Transfection

1 mL of each culture was placed in a 12-well tissue culture plate (Corning) for DNA transfection. Transfection was conducted in suspension cell cultures at 37ºC in a humidified atmosphere with 5% of CO2. Two replicates and negative controls for each condition were tested (experimental conditions are compiled in table 1).

The manufacturer instructions were followed when the Commercial reagent was used for transfection, being as follows: 1µg of plasmid DNA encoding enhanced Green Fluorescent Protein (eGFP) was dissolved in SFM4Transfex medium without FBS up to 416µL of final volume. Separately, 3µL of the transfection reagent was dissolved in 208µL of the same medium. Same volumes of Reagent and DNA solutions were mixed (1:1 v/v ratio) and 600 µL of the final mixture was added to each well containing $1x10^6$cells suspended in 400µL of medium. The final total volume was about 1mL.

Transfection with 25kDa Linear PEI was performed as described in the following conventional protocol. Briefly, 1µg of plasmid DNA was dissolved in 100µL of media without FBS, either SFM4Transfex or Freestyle, and vortex for 10s. Then, 4 µL of PEI stock solution (1mg/mL) was added to each media/DNA mixture and vortexed for 5 seconds (x 3 cycles of homogenization). Finally, the mixture was incubated for 15min at room temperature (RT) and dispensed into each well containing $1x10^6$cells. Final volume per well was about 1mL.

The transfection with Biontex K2 Transfection System was conducted as follows (all quantities are referred to a single well): $1x10^6$cells were harvested from the pre-culture, centrifuged (5min, 800xg), washed once with 900µL of SFM4Transfex without FBS and resuspended in 900µL of fresh media. The cell suspension was dispensed homogeneously into each well and 20µL of K2 Multiplier was added. The plate was homogenized gently by swirling and then incubated for 2h. In the meantime, transfection solutions were prepared by mixing 1µg of plasmid DNA and 50µL of SFM4Transfex medium without FBS (Solution A), and 50µL of the same media with 4µL of K2 transfection reagent (Solution B). The solutions were mechanically homogenized by pipetting using sterile and DNAse free filter tips. Same volumes of Solution A and B were mixed (1:1 v/v ratio) and then incubated for 20min at RT. Finally, 100µL of DNA/transfection reagents solution was dispensed into each well containing the cells and the plate was swirled gently.

Tested reagent	Cell density at transfection	DNA	Transfection reagent	K2 Multiplier	Media for transfection/DNA	Replicate number	No DNA Control
Commercial	$1x10^6$	1µg	3µL	-	208µL/416µL	2	1
K2 System	$1x10^6$	1µg	4µL	20µL	50µL/50µL	2	1
PEI	$1x10^6$	1µg	4µL	-	100µL	2	1

Table 1: Experimental setup (Quantities per well)

Results:

Evaluation of the transfection efficiency

Efficiency of transfection was assessed by Fluorescence-activated cell sorting (FACS) with a FACS Canto BD Flow Cytometer (BD Bioscience) using an end-point analysis method. The end-point samples were taken 16h after transfection. The efficiency of transfection was assumed as the percentage of GFP(+) cells from the viable cell population. Cell viability of samples was determined by means of propidium iodide staining, being for all conditions around 70-80% of the total cell density. Figure 1 shows the FACS analysis obtained for each reagent: A) Commercial Lipid base reagent, B) K2 tranfection system, C) 25kDa Linear PEI with Freestyle medium , D) 25kDa Linear PEI with SFM4Transfex medium and E) Negative control (untransfected cells). The cell fluorescence distribution is plotted in the figure: red dots represent the untransfected cells, while orange dots belong to GFP(+) cells. Negative controls were used to set the basal cell fluorescence of untransfected cells (Fig.1E). As it can be seen in Figure 1B, the K2 transfection system achieved not only a higher GFP expression level , but also, the highest ratio of GFP(+) cell population (meaning a higher transfection efficiency) (Fig.2).

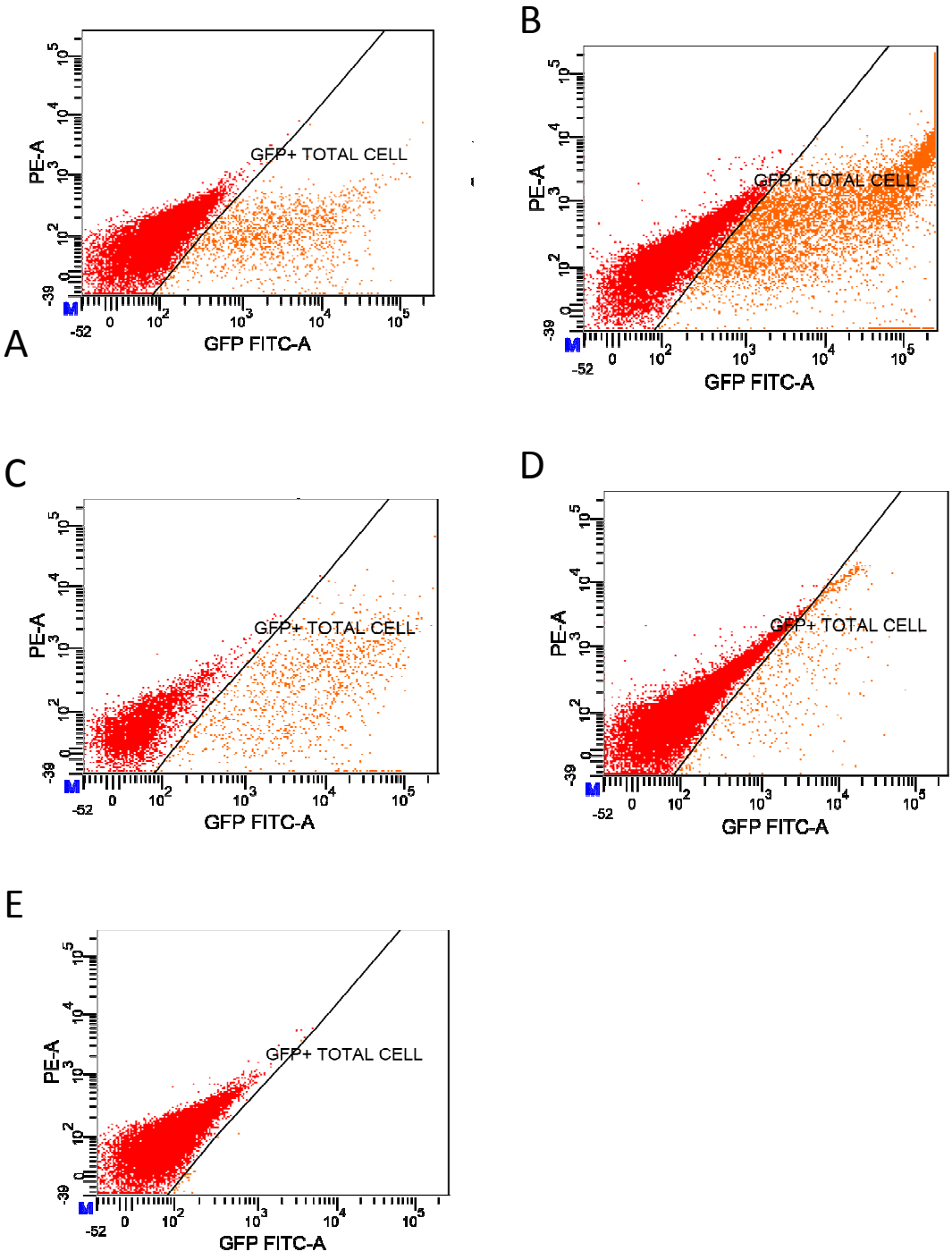

Figure 1: Efficiency of transfection of HEK293 cells using a reporter GFP assessed by FACS analysis. Different transfection protocols were compared: A) Commercial Lipid base reagent, B) K2 transfection system, C) 25kDa Linear PEI with Freestyle medium , D) 25kDa Linear PEI with SFM4Transfex medium and E) Negative control (untransfected cells)

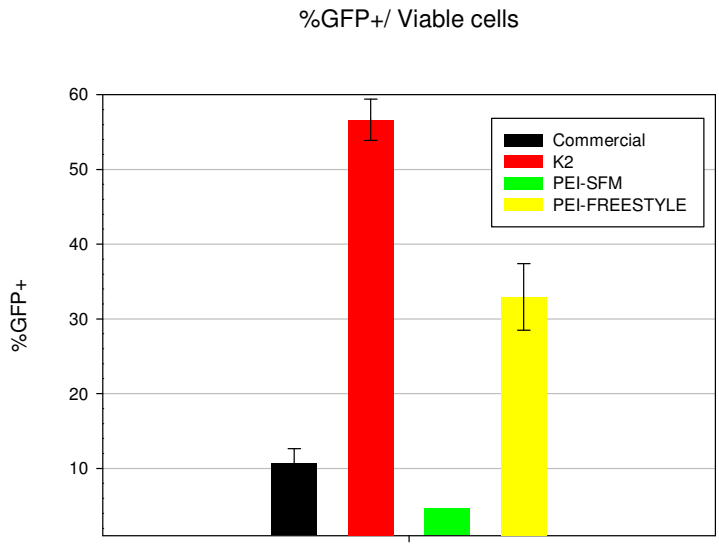

Figure 2: Percentage of GFP positive cells 16h post-transfection obtained by Fluorescence-activated cell sorting (FACS) for all transfection reagents tested.

Conclusions

Biontex K2 ® Transfection System was found, by far, the most efficient reagent for DNA transfection of suspended HEK cells in SFM4 transfex medium. The efficiency of transfection achieved was about 55% of GFP(+) cells from the viable cell population. The efficiency of transfection obtained with the Biontex K2 ® reagent was about 2-fold increase compared with 25KDa Linear PEI in the Freestyle medium (Figure 2).

Moreover, the fluorescence intensity per cell, which is indicative of the amount of eGFP accumulated into the cells, was also higher when K2 ® Transfection system was used, as it is shown in Figure 1B. Taking a closer look to these results, we hypothesize that not only a larger number of cells were transfected, but also the amount of DNA transfected per cell could be also higher.