■ **DNA-transfection of primary human mesenchymal stem cells (hMSCs) using "Biontex K2® Transfection System".**

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Materials and Methods

Cell culture

Human mesenchymal stem cells (hMSCs) were cultured in α-MEM supplemented with 10% FBS, 1% penicillin-streptomycin solution, at 37 °C in a humidified atmosphere containing 5% CO_2. Cells of passage seven were used for the transfection studies.

Cell transfection

5×10^4 human hMSCs were plated in a single well of a 24-well dish in 1 ml medium. On the day of transfection, the cell confluence was 70-90%. Two hours before DNA transfection, 15 µl K2® Multiplier was dripped to each well. The following solutions were prepared in a polypropylene vessel.

Solution A: 37.5 µl serum-free medium with 1 µg DNA plasmid.

Solution B: 37.5 µl serum-free medium with 2 µl K2® Transfection Reagent.

Solution A was added to Solution B. The mixture was incubated at room temperature for 20 min. The mixture was then immediately added to the cells. Transfections were conducted at 37 °C and 5% CO_2 for 24 hours. Cell viability and transfection efficiency were estimated.

Dish size	▪ -MEM	K2® Multiplier	K2® Transfection Reagent	Serum-free medium for DNA/K2 reagent	DNA plasmid
24 well	1 ml	15 µl	2 µl	37.5 µl	1 µg

Results

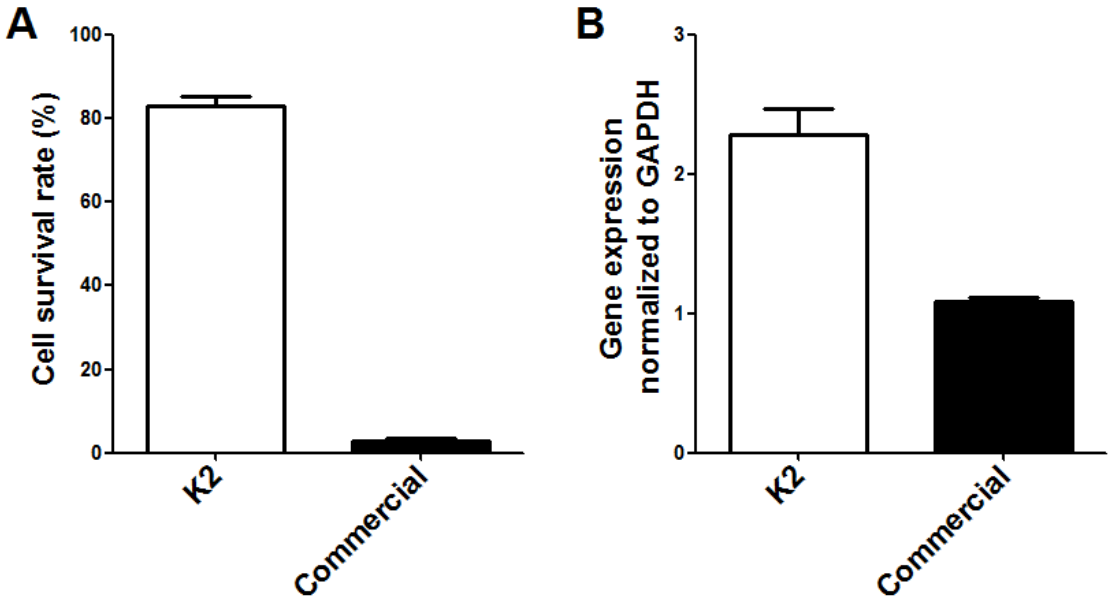
Viability of hMSCs

To determine the cell survival rate, cell pellets were resuspended in PBS containing 1 µl of propidium iodide (PI) and subjected to flow cytometric analysis. The viability of hMSCs transfected by K2 transfection reagent was higher than that by a dendrimer-based commercial transfection reagent (Figure 1A).

Transfection efficiency of hMSCs

Real-time RT-PCR was used to analyze the expression of exogenous (transfected)

gene. The mRNA expression of the transfected gene by K2 was about 1.5-fold higher than that by a dendrimer-based commercial transfection reagent (Figure 1B).



Conclusions

The data shows that K2® Transfection System has low cytotoxic effects compared to another dendrimer-based commercial transfection reagent. Besides, the transfection efficiency for hMSCs evaluated by mRNA expression is also higher by K2® Transfection System.