Effect of pressure treatment on lipoplex formation and their transfection

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Statement of Purpose: Cationic lipid/DNA complexes (lipoplexes) are a promising candidate alternative to viral vector for cell transfection *in vitro* and *in vivo* [1]. Lipoplex is formed through electrostatic interaction and significantly introduced into cells because their stable complex formation could protect DNA from nuclease attack. However, the gene expression efficiency is lower than viral vector. One possible reason is that the stable complex formation inhibits the transcription and translation of the delivered DNA into cells [2]. Therefore, it is important to improve the transcription efficiency of the delivered DNA into cell. We have developing the novel DNA compaction method using ultra-high pressure technology with and without cationic compounds. Previously, we reported that DNA compaction was induced by high pressurization [3]. In the present study, to apply pressure technology for lipoplex-mediated transfection, physicochemical property and gene expression of lipoplexes, which were treated with different pressurization process, were investigated.

Methods: Plasmid DNAs encoding enhanced green fluorescent protein gene (pEGFP) or luciferase gene (pGL3) were used. Commercially available cationic lipids, lipofectamine and lipofectamine 2000, were used. COS7 cells and HeLa cells were used. The lipoplexes of cationic lipids and plasmid DNA were prepared by three procedures described below. As control (c-lipoplex), lipoplexes were prepared according to manufacturer's instructions. As pre-compacted lipoplex (pc-lipoplex), the plasmid DNA was pressurized at 10,000 atmospheres and 40 ºC for 15 minutes, and then mixed with cationic lipids. As pressurized lipoplex (p-lipoplex), the lipoplex of cationic lipid and plasmid DNA was pressurized on the condition described above. The hydrodynamic diameter of the obtained lipoplexes was measured by dynamic light scattering (DLS). The obtained lipoplexes were added to the cells ($1x10^5$ cells). After 8, 20 hours, the transfected cells were observed using a fluorescent microscope. The fluorescent intensity of expressed EGFP and chemiluminescence of expressed luciferase were also measured with a fluorescent spectrometer (Ex 488nm, Em 508 nm) and luminometer at 24, 48 and 72 hours later.

Results: For DLS measurement of the prepared lipoplexes, the size of lipoplex was changed by the pressurization. This result suggests that the lipoplex formation was affected by pressurization. Cellular toxicity and transfection efficiency of the prepared lipoplexes were elucidated *in vitro*. There was no change in the viability of cells transfected with the prepared lipoplexes. The transfection efficiency of lipoplexes was examined by the fluorescent microscopic observation after 8, 20 hours. In all lipoplexes, EGFP positive cells were observed at both times. The number of cells expressing EGFP was increased with increasing incubation time. Using the pc-lipoplex, the number of cells expressing EGFP was increased compared to the c-lipoplex. Furthermore, the number of EGFP positive cells was significantly increased for the p-lipoplex compared to the others. The fluorescent intensity of the EGFP positive cells of the p-lipoplex is also higher than the others. To quantify the transfection efficiency of lipoplexes, the fluorescent intensity of the cellular lysates was measured with a fluorescent spectrometer (Figure 1). The fluorescent intensity of the pc-lipoplex was slightly increased comparison with c-lipoplex. The highest fluorescent intensity of expressed EGFP was measured for p-lipoplex. In the both cases of lipofectamine and lipofectamine 2000, the p-lipoplex had tendency to enhance the gene transfection efficiency. These results indicate that the pressurization process of lipoplex preparation strongly affected on the gene transfection. We assume that the change of lipoplex formation affect some kind of gene delivery stages, such as cellular uptake, transfer to nucleus and transcription in nuclei.

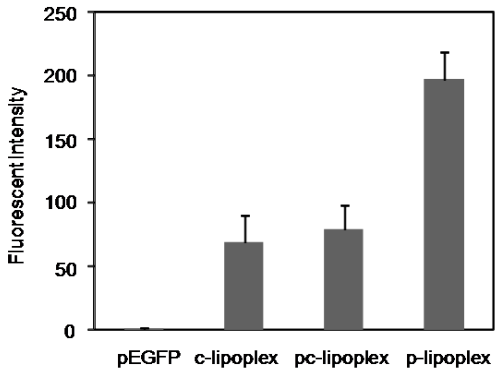

Figure 1. Transfection efficiency of various lipoplexes.

Conclusions: We have successfully improved the transfection efficiency of lipoplex using high pressure technology.

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