Gene transfection using PVA/HAp/DNA nanoparticles prepared by high hydrostatic pressurization

Tsuyoshi Kimura[1)], Kwangwoo Nam[1)], Tsutomu Furuzono[2)], Masahiro Okada[2)], Akio Kishida[1)*]
[1)] Institute of Biomaterials and Bioengineering, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo, Japan
[2)] Department of Biomedical Engineering, NCVC Research Institute, Osaka, Japan

Statement of Purpose: Non-viral gene delivery systems are of great interest in gene therapy. In many cases, cationic compounds, such as cationic polymers and cationic lipids, were used to introduce DNA into cells because the stable and small complexes were formed with DNA via electrostatic interaction [1]. However, the intrinsic cytotoxicity of them is essential problem [2]. Therefore, we have developing a formulation method of DNA complex with non-ionic, water soluble polymers via hydrogen bonds using high hydrostatic pressure technology. Previously, we reported that polyvinyl alcohol (PVA) was utilized as the model hydrogen bonding polymers, and PVA/DNA nanoparticle was obtained by high hydrostatic pressurization [3]. The PVA/DNA nanoparticle was up-taken by cells with non-toxicity, whereas no significant gene expression was showed.

In this study, we hypothesize that hydroxyapatite (HAp) promote the endosomal escape of transferred DNA because HAp is dissolved under low pH condition (<5.5) in endosome vesicles and then the rupture of endosome is induced by osmotic shock. We investigated the preparation of PVA/HAp/DNA nanoparticle using high hydrostatic pressure technology and its transfection efficiency *in vitro*.

Methods: Plasmid DNA encoding luciferase gene under SV40 promoter was used. HAp having the average diameter of 50nm was synthesized by modified micro-emulsion method. The mixture solution of PVA, HAp and DNA at various concentrations was hydrostatically pressurized at 10,000 atm and 40 °C for 10 min using high pressure machine. After removal of pressure, the obtained PVA/HAp/DNA complexes were subjected to SEM observation and DLS measurement. COS-7 cells were used. The PVA/HAp/DNA complexes were added to COS-7 cells. After several hours incubation, the luciferase activity was measured by using luminometer.

Results: Figure 1 shows SEM image of PVA/HAp/DNA complexes prepared by high hydrostatic pressurization at higher PVA concentration. The irregular surface of particles was observed without significant absorbing HAp on particles. On the other hand, in the case of PVA/DNA complex, the surface was smooth. This result indicates that HAp particles were encapsulated in the PVA/HAp/DNA complexes. The size of PVA/DNA and PVA/HAp/DNA composites at various concentrations of PVA and HAp were measured by DLS measurement. With decreasing the PVA concentration, the particle size of PVA/DNA and PVA/HAp/DNA complexes was decreased. At the PVA concentration of 0.001%, the diameters of PVA/DNA and PVA/HAp/DNA complexes were about 300 and 350 nm, respectively.

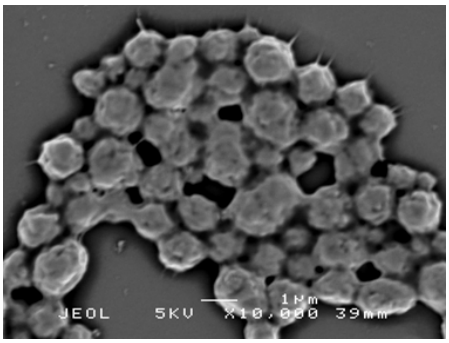

Figure 1 PVA/HAp/DNA complexes obtained by high hydrostatic pressure treatment.

When the PVA/DNA and PVA/HAp/DNA nanoparticles were added to COS-7 cells, the high viability of COS-7 cells (>90%) was exhibited in the both cases. In order to investigate the cellular uptake of PVA/DNA and PVA/HAp/DNA nanoparticles, rhodamine-labeled DNA was used. By fluorescent microscope observation, the quick and effective cellular uptake of HAp/PVA/DNA nanoparticle was exhibited compared to PVA/DNA nanoparticle. Further, 10 fold luciferase activity of PVA/HAp/DNA nanoparticle was showed compared to that of PVA/DNA nanoparticle. These results suggest that the effective DNA release from endocytosis was achieved by using PVA/HAp/DNA nanoparticle.

Conclusions: We successfully obtained PVA/HAp/DNA nanoparticle, in which nano-scaled HAp particles were encapsulated, using high hydrostatic pressure technology. The PVA/HAp/DNA nanoparticle enhanced the efficiency of gene transfection with non-cytotoxicity *in vitro*.

Acknowledgement: This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology and the ministry of Health, Labour and Welfare.

References:

[1] CC Conwell, L Huang, *Non-viral Gene therapy*, 2003, 3-11, Springer
[2] D Fischer, et al. *Biomater.*, 2003, 24, 1121-1131
[3] Kimura, T. et al., *Mater. Sci. Eng. C*, 2004, 24, 797-801.