NANO-MICELLES BASED ON STEARIC ACID-GRAFTED POLYETHYLENEIMINE AS NONVIRAL GENE CARRIER

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Introduction

Gene therapy has been considered as a promising approach for correcting deficient genes and modulating gene expression that account for genetic diseases [1]. It refers to transmit the DNA that encodes a therapeutic gene into the targeted cells and consequently express the specific protein in tissue. In the past years, numerous researches have been focused on the development of gene therapy based on viral and non-viral carriers. Viral vectors constructed from adenovirus, retrovirus, adeno-associated virus, or herpes simplex virus-1 all showed high efficiency to transfer genes into cells. However, safety concerns such as inflammatory, immunogenicity, oncogenicity, and other toxic side effects remain crucial issues yet to be resolved [2, 3]. Therefore, non-viral vectors composed of biodegradable polymers or lipids have been developed as safer alternatives.

Comparing to other cationic polymers, polyethyleneimine (PEI) possesses high pH- buffering capacity which serves as a "proton sponge", that is, buffering of the endosomes by accumulation of ions within the vesicles. However, it has been reported that cytotoxicity of PEI depends on the molecular weight; and that lower molecular weight PEI has advantages for *in vivo* application over higher molecular weight PEI owning to its nature of low cytotoxicity [4-6]. Therefore, modifications of PEI structure for clinical application have been investigated in order to reduce the cytotoxicity of PEI associated with higher molecular masses and high doses, or improve the insufficient transfection efficiency of lower molecular weight PEI.

In this study, PEI was modified by grafting stearic acid and formulated to polymer micelles with positive surface charge in aqueous environment. The amine group on PEI was crosslinked with the carboxylic group of stearic acid by 1-ethyl-3-(3-dimethylamino- propyl) carbodiimide (EDC) to increase hydrophobic segments for facilitating micelles formation. Based on PEI with high pH-buffering capacity and high transfection efficiency, the PEI-SA micelles can be a promising gene carrier candidate in genetic and chemotherapeutic therapy.

Experimental

Materials

PEI (MW 10K) was purchased from Wako, Osaka, Japan. Steric acid (SA), and EDC (1-ethyl-3-(3-dimethylamino-propyl) carbodiimide), were purchased from Aldrich-Sigma. Plasmid encoding enhanced green fluorescent protein was amplified in *Escherichia coli*, extracted by alkalilysis technique, and purified by QIAGEN kit (Chatsworth, CA, USA) following manufacture's protocol. The plasmid concentration and purity were then determined by electrophoresis with 1% agarose gel and UV spectroscopy.

Apparatus and Porcedures

SA grafted PEI was synthesized by reacting the carboxyl group of SA with the amine group of PEI using 1-ethyl-3-(3-dimethylaminopropyl) carbodimide (EDC) as a linker. PEI-SA micelles were prepared using o/w solvent evaporation method. The final solution was then dialyzed against 10% ethanol solution using a dialysis membrane (MWCO: 8000 dalton, spectrum Laboratories, CA, USA) for 16 hr to remove by-products and unreacted reagents, followed by dialysis against distilled water for 24 hr with frequent exchange of fresh distilled water.

The hydrodynamic diameters and zeta potentials of PEI-SA micelles were determined by photon correlation spectroscopy using dynamic laser light scattering (Zetasizer Nano ZS90, Worcestershire, UK) with He-Ne ion laser (633nm) as the incident beam.

The binding efficiency of PEI-SA/pDNA micelles was evaluated by gel retardation assay by 1% agarose gel. Cytotoxicity was analyzed by Cell Titer 96 A_{queous} One Solution Cell Proliferation Assay (Promega, Wisconsin, USA) according to manufacturer's protocol.

Gene transfection efficiency was evaluated by Flow Cytometry in the selected gate region ($2x10^2 < FL1\text{-}H < 10^4$) normalized with untreated EGFP expressing Huh-7 cells.

Results and Discussion

The physical characteristics of the formulated 10k PEI-SA micelles were obtained by photon correlation spectroscopy with averaged diameter of 149.6 ± 1.2 nm and zeta potential of 64.1±1.5 mV. Fig. 1 showed complex formulated by 10k PEI-SA/pDNA was completely retarded in agarose gel electrophoresis for weight ratios higher than 0.7, indicated an effective binding efficiency as a gene carrier.

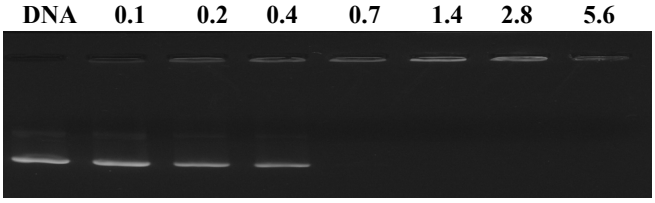

Fig. 1. Gel retardation analysis of 10k PEI-SA/pDNA complex in various weight ratios from 0 to 5.6.

Cytotoxicity of 10k PEI, 10k PEI-SA, and lipofectamine was evaluated at concentration of 10 and 20 μg/ml using MTS assay at 20, 65, 115 hours (Fig. 2). For 10 μg/ml treatment, 10k PEI resulted in significant low cell viability against Huh-7 cells compared with control and lipofectamine. In contrast, SA grafted PEI showed reduced cytotoxicity that the cell viability of 10K PEI-SA treatment was comparable to control. For treatment concentration 20 μg/ml, both 10K PEI and lipofectamine showed low relative cell viability compare to 10K PEI-SA and control.

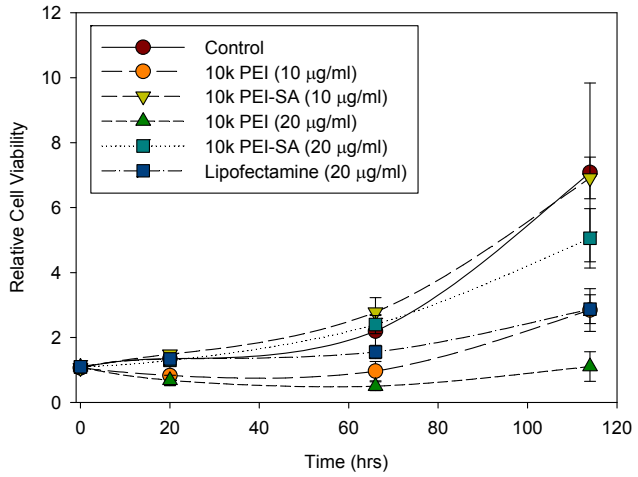

Fig. 2 Cytotoxicity of 10k PEI, 10k PEI-SA and lipofectamine evaluated at concentration of 10 and 20 μg/ml.

Plasmid encoding EGFP was then used to investigate tranfection efficiency of PEI-SA/pDNA micelles at weight ratio of 4.5. Based on flow cytometry (Fig. 3), Huh-7 cells transfected with 10k PEI-SA/pEGFP complex resulted in an average of 62.9±2.2% EGFP expression in 2.5 days comparing with treatment with pEGFP only.

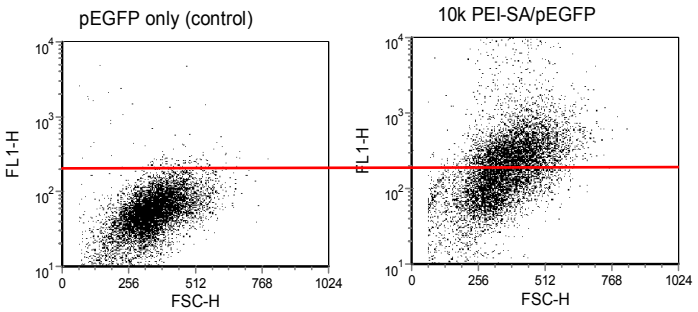

Fig. 3 Flow cytomerty analysis for DNA (pEGFP) expression in Huh-7 cells by transfected with free plasmid DNA and 10k PEI-SA/pEGFP micelles

Conclusion

In this study, PEI was modified by grafting stearic acid and formulated to polymer micelles with positive surface charge in the aqueous environment. Gel retardation assay demonstrated effective binding of pDNA and also excellent transfection efficiency based on flow cytometry results. PEI-SA can also reduce the cytotoxicity of PEI and still provide high transfection efficiency. This nano-sized micelles complex can further possess multifunctional capability as drug carrier in cancer therapy, or as carrier candidate in genetic and chemotherapeutic therapy.

References

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