Supporting Information

Biocompatible ionic liquid-based nanoparticles for effective skin penetration and intracellular uptake of antisense oligonucleotides

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1. Optimization of reaction conditions

1-1. Reaction time

Formulations were prepared by shaking the reaction time, and carrier formation was confirmed by DLS measurement.

Experimental manipulation

Trabedersen was selected as the encapsulant and IL [EDMPC][Lin] as the surfactant to prepare the complexes.

(1) Formation of IL-nucleic acid complex: 1 mg of Trabedersen (ssDNA) was dissolved in 5 mL of Milli-Q water. Also, 50 mg of IL [EDMPC][Lin] was dissolved in 1 mL of 99.5% ethanol. These aqueous and ethanol solutions were mixed to form IL-nucleic acid complexes spontaneously by (1) freezing in liquid nitrogen upon addition, (2) stirring with a vortex mixer for 10 seconds, and (3) stirring gently with a stirrer for 10 minutes / (4) 6 hours. All reactions were performed at room temperature.

(2) Removal of internal aqueous and oil phases: The IL-nucleic acid complexes were prepared by freezing the above water/ethanol solution in liquid nitrogen for 20 minutes, and then removing the internal aqueous and oil phases by freeze-drying overnight.

(3) Dispersion in oil: The above surfactant-nucleic acid complex was dispersed in 1 mL of IPM, an oil base with high transdermal penetration promoting effect, to obtain a nucleic acid-incorporated formulation with a final concentration of 1 mg/mL.

(4) The complex solution was diluted 100-fold and particle size and PDI were measured by DLS.

Results and Discussion

The results of particle size and PDI are shown in Fig. S1.

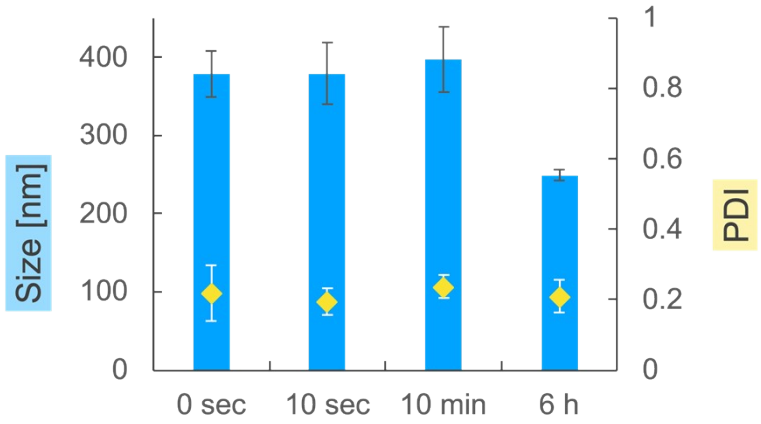

Fig. S1 Reaction time and particle size, PDI. *N* = 3, mean ± SD.

2-2. Dilution factor

In the ethanol dilution method, the water/ethanol ratio (dilution factor) is an important factor. In this section, we examine whether similar complexes are formed when water / ethanol = 1 / 5 or 1 / 10.

Experimental manipulation

Trabedersen was selected as the endosorbent and IL [EDMPC][Lin] was selected as the surfactant to prepare the complexes (Table S1).

(1) Formation of IL-nucleic acid complex: 1 mg of Trabedersen (ssDNA) was dissolved in 5 mL and 10 mL of Milli-Q water. Also, 50 mg of IL [EDMPC][Lin] was dissolved in 1 mL of 99.5% ethanol. These aqueous and ethanol solutions were mixed and stirred gently at room temperature for 6 hours to spontaneously form IL-nucleic acid complexes.

(2) Removal of the inner aqueous and oil phases: The IL-nucleic acid complexes were prepared by freezing the above aqueous/ethanol solutions in liquid nitrogen for 20 minutes, and then removing the inner aqueous and oil phases by placing them in a freeze-drying machine overnight.

(3) Dispersion in oil: The above surfactant-nucleic acid complex was dispersed in 1 mL of IPM, an oil base with high transdermal penetration promoting effect, to obtain a nucleic acid-incorporated formulation with a final concentration of 1 mg/mL. The composition of each sample is shown in Table 3-5.

(4) The complex solution was diluted 100-fold and the particle size and PDI were measured by DLS.

Results and Discussion

The results of particle size and PDI are shown in Fig. S2.

Table S1. Composition of samples with different dilution ratios (water / ethanol = 1 / 5, 1 / 10)

Sample	Trabedersen / Water	Surfactant / Ethanol	IPM
W/E = 1/5	1 mg / 5mL	50 mg / 1mL	1 mL
W/E = 1/10	1 mg / 10mL	50 mg / 1mL	1 mL

Sample	Size (nm)	PDI
W/E = 1/5	253.6	0.176-0.226
W/E = 1/10	288.8	0.164-0.247

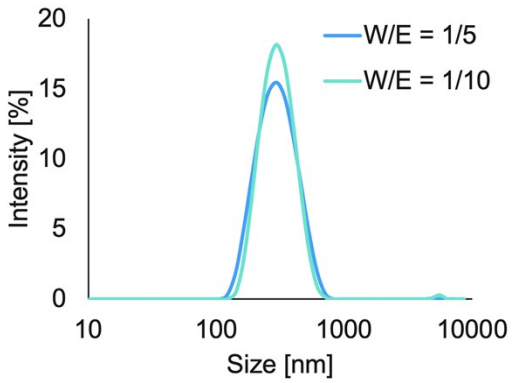

Fig. S2 Dilution Magnification and Particle Size Distribution