Increasing the siRNA knockdown efficiency of lipid nanoparticles by morphological transformation with the use of dihydrosphingomyelin as a helper lipid

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Supplementary Figures

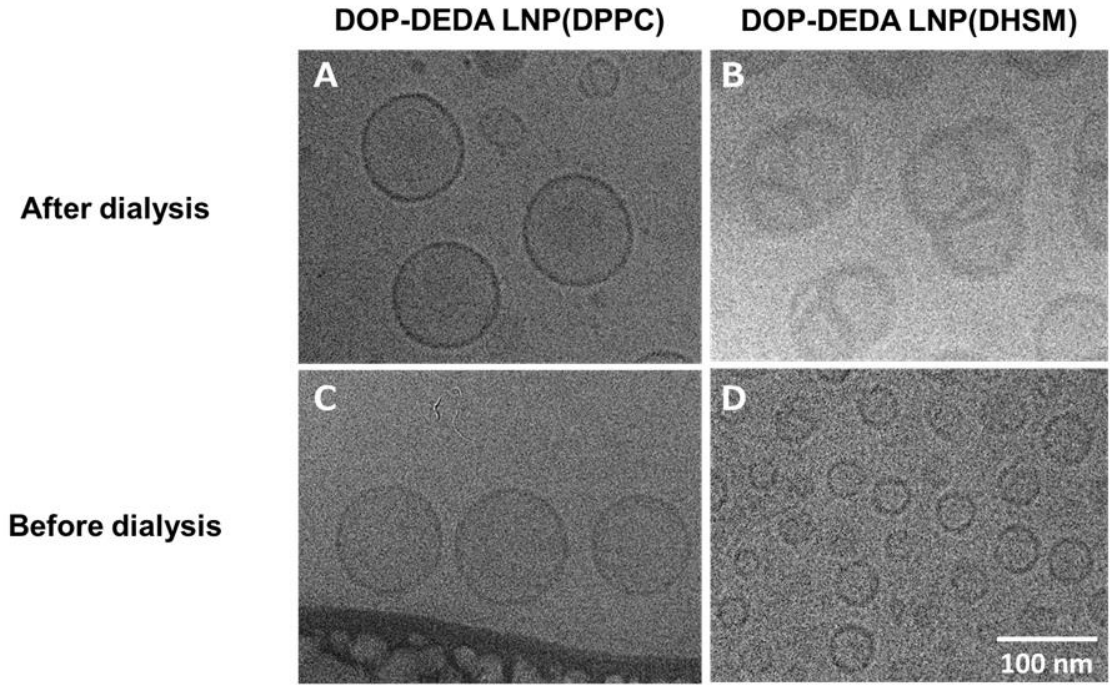


Figure S1. The morphology of siRNA-free DOP-DEDA LNPs generated from different lipid compositions. DOP-DEDA LNPs (DPPC) composed of DOP-DEDA/DPPC/Chol (45/10/45 mol%) after dialysis (A) DOP-DEDA LNPs (DHSM) composed of DOP-DEDA/DHSM/Chol (45/10/45 mol%) after dialysis (B). DOP-DEDA LNPs (DPPC) before dialysis (C). DOP-DEDA LNPs (DHSM) before dialysis (D). Scale bar = 100 nm.

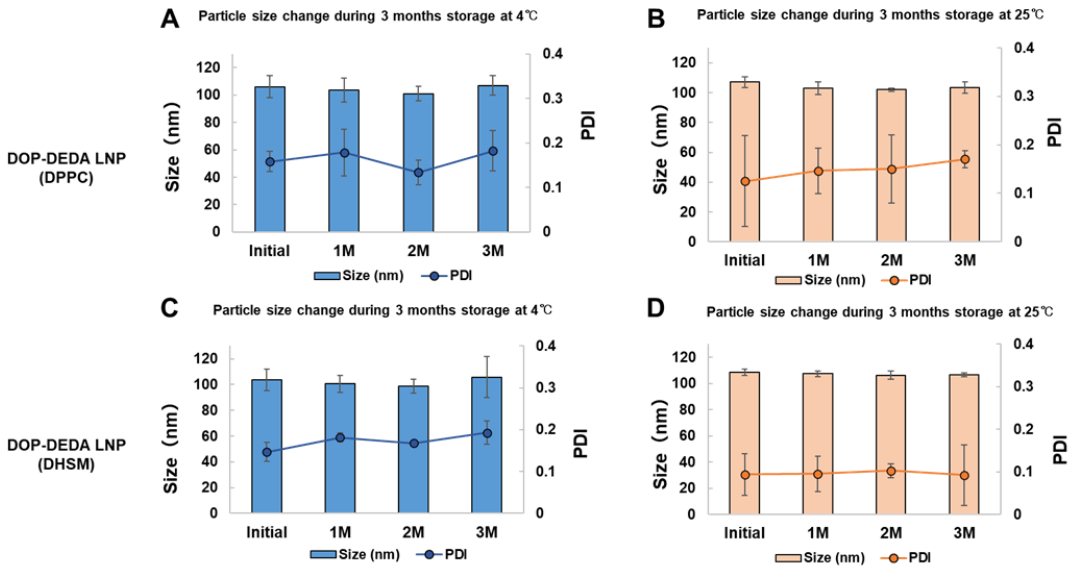


Figure S2. The size change of DOP-DEDA LNPs during the storage at different temperature conditions. DOP-DEDA LNPs were stored at 4 or 25°C for 3 months. At the indicated time points, particle size and PDI were measured by a Zetasizer Ultra. Three batches for each temperature condition were prepared to confirm reproducibility. The size change of DOP-DEDA LNPs composed of DOP-DEDA/DPPC/Chol (45/10/45 mol%) at 4°C (A) and 25°C (B). The size change of DOP-DEDA LNPs composed of DOP-DEDA/DHSM/Chol (45/10/45 mol%) at 4°C (C) and 25°C (D).

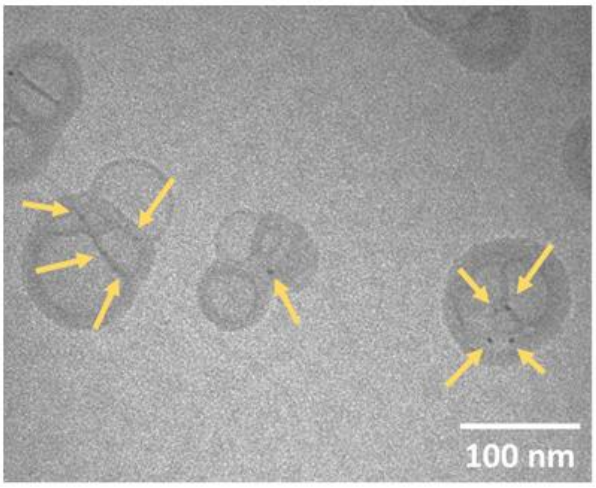
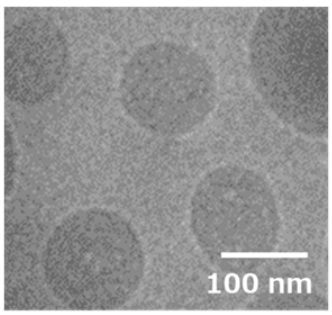


Figure S3. The cryo-TEM image of DOP-DEDA LNPs (DHSM) prepared with negatively charged gold nanoparticles. The molar ratio of total lipid:siRNA was 7000:0.9 and gold nanoparticles to total lipids was 8.9×10^{17} particles/mol lipid. Yellow arrows indicate gold nanoparticles. Scale bar = 100.

Zeta potential(mV)
+ 25.1 ± 4.2

Figure S4. The zeta potential of DOP-DEDA LNPs composed of DOP-DEDA/DHSM/Chol (45/10/45 mol%) in 1 mM citrate solution (pH 4.5). The measurement was performed using a Zetasizer Ultla.

A



B

Size(nm)	PDI	Zeta potential(mV)	Encapsulation efficiency of siRNA(%)
79.4 ± 1.8	0.23 ± 0.03	+ 7.2 ± 0.4	98.3 ± 0.39

Figure S5. The morphology and physicochemical properties of DOP-DEDA LNPs (DHSM) dialyzed against a solution of ultrapure water/ethanol (volume ratio was 4.15:1) and then re-dialyzed against ultrapure water. The morphology was obtained by cryo-TEM (A). The size, PDI and zeta potential were measured using a Zetasizer Ultla. DOP-DEDA LNPs (DHSM) was diluted in RNase-free water (for size and PDI) or Tris-HCl buffer (pH 7.4) (for zeta potential) prior to measurement. Encapsulation efficiency of siRNA was evaluated by RiboGreen Assay（B）.

Zeta potential(mV)
- 19.0 ± 0.94

Figure S6. The zeta potential of DHSM-based liposome composed of DHSM/Chol (55/45 mol%) generated by a microfluidic mixing method in Tris-HCl buffer (pH7.4). The measurement was performed using a Zetasizer Ultla.

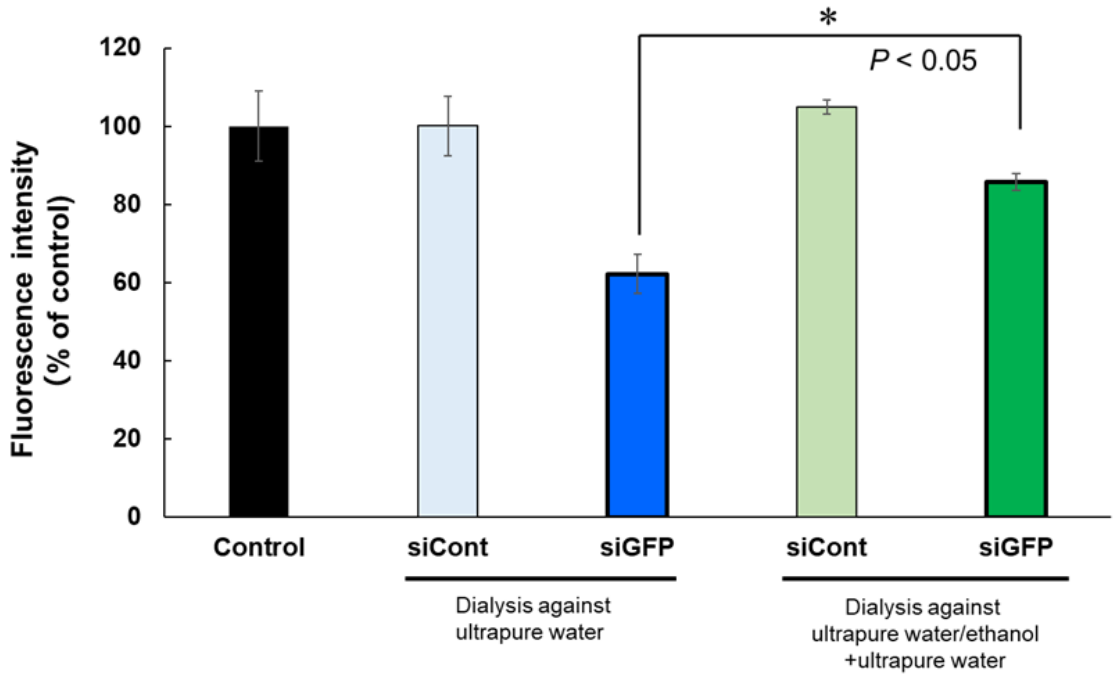


Figure S7. *in vitro* siRNA knockdown efficiency of two kind of DOP-DEDA LNPs (DHSM). Reduction of EGFP fluorescence intensity in HT1080-EGFP cells after the treatment with RNase-free water (control), DOP-DEDA LNPs (DHSM) encapsulating siGFP or siControl. Mean values are shown with SD (n=3). Asterisks indicate significant differences (*P < 0.05).