Supporting Information

Intracellular delivery of CII TA genes by polycationic liposomes for suppressed immune response of dendritic cells

Lin Su[a†], Gang Liu[b†*], Shuangnan Zhang[a], Hanjie Wang[a], Sheng Wang[a], Xue Li[a], and Jin Chang[ac*]

a School of Materials Science and Engineering , School of Life Sciences, Tianjin University, 92 Weijin Road, Nankai District, Tianjin 300072, P.R. China

b Department of General Surgery, Tianjin Medical University General Hospital, 154 Anshan Road, Heping District, Tianjin 300052, P.R.China

c Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin 300072, China

†These authors contributed equally to this paper.

*Corresponding author. Tel: +86 022 87898212.

E-mail: jinchang@tju.edu.cn; lg1059@sina.com.

1. Experiment section

1.1 Synthesis and characterization of OQLCS

Firstly, Chitosan powder (0.11 g) was dissolved in 10 mL deionized water, followed by addition of 5 mL HCl (3 mol/L). After BOC-lysine, EDC, and DMF were successively added, the solution was under stirring. The reaction was performed at 25 °C for 12 h. Then the butyloxycarbonyl groups (BOC) on the lysine was removed by adding tri-fluoroacetic acid (TFA) into the reaction system. Finally, the precipitate was washed twice with ethanol thrice, followed by dialyzing in water. The solution was lyophilized to get a light white powder.

LCS (5 g) was 100 mL of deionized water and mixed with QA (10g) within 50 mL isopropanol. After 30 mL NaOH solution (42%, w/w) was added, the solution was performed at 80 °C for 24 h under stirring. Finally, the solution was dialyzed and lyophilized to get OQLCS white powder. The structure of the chitosan derivatives have been tested using FTIR spectra and 1H NMR spectroscopy.

1.2 Preparation of polymer liposomes

The synthesized OQLCS was dissolved in 4 mL chloroform with cholesterol at room temperature (30 g for total lipids). The nanoliposomes structures were prepared by a gentle hydrophobic interaction. Briefly, OQLCS and Chol (mass ratio x) were dissolved in 4 ml chloroform at room temperature. Chloroform was then evaporated using a vacuum rotary evaporator, and a thin film of cationic polymer liposomes formed on the wall of a 50-ml round-bottled flask. Then the lipid film was dispersed in 10 ml distilled water and sonicated at room temperature for 10 min. Subsequently,

SPDP was added to the lipid solution to yield a 2-pyridyldithiol-end group on the liposomes' surface, followed by addition of TAT solution. The mixture was incubated overnight at 4 ℃ to a disulfide linkage between the surface of liposomes and TAT peptide. The morphology of the cationic polymer liposome was characterized by transmission electronic microscope. The average particle size and size distribution were determined by dynamic light scattering.

1.3 The preparation and characterization of the pCIITA/liposomes complex

After the redundant TAT removed, the pCIITA were incubated with the liposomes at different mass ratio (1:0,1:0.5,1:1,1:2,1:2.5,1:3,1:3.5), respectively. The complex with different mass ratio were detected and screened by agarose gel electrophoresis (AGE). The particle size and zeta potential was characterized by dynamic light scattering.

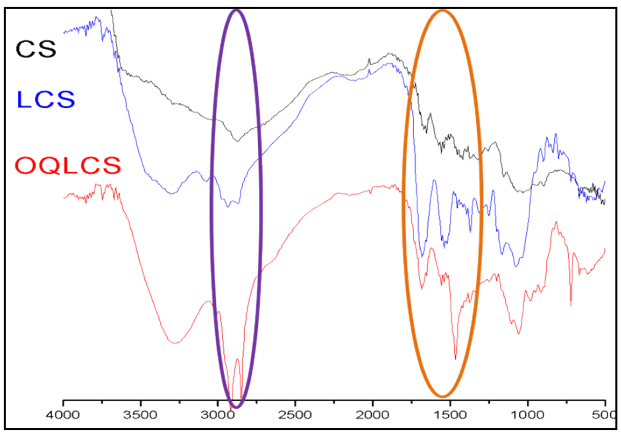

Fig. S1 The FT-TR spectra of CS, LCS and OQLCS.

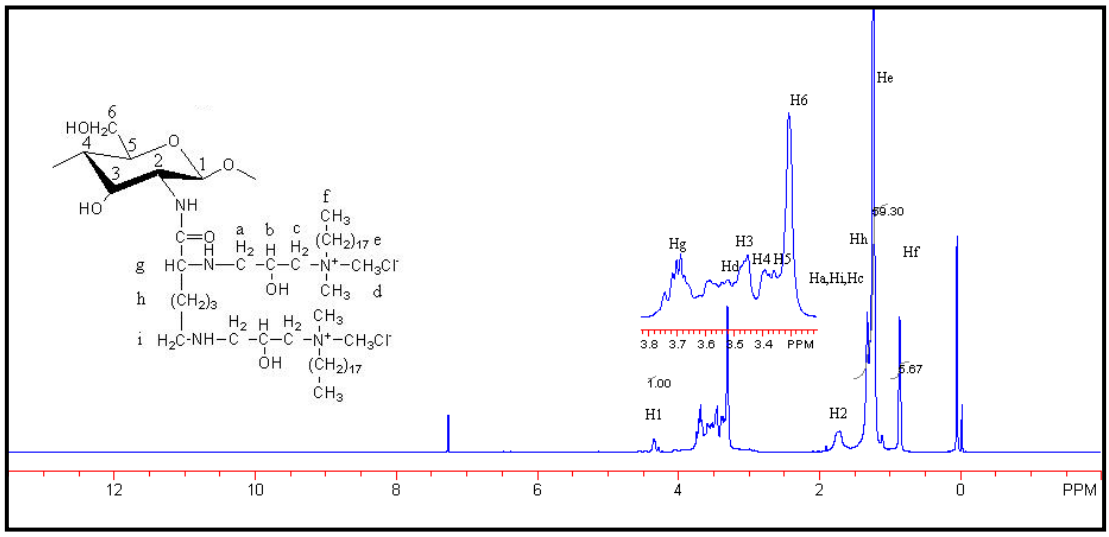

Fig. S2 ^{1}H NMR spectrum of OQLCS.

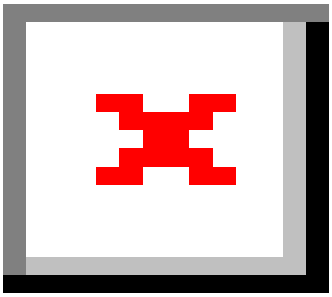

Fig. S3 Analysis of intracellular delivery efficiency by flow cytometry. Cells transfected with a) blank, b) pCIITA, c) lipofectamine and d) pCIITA/liposomes nanocomplexes.

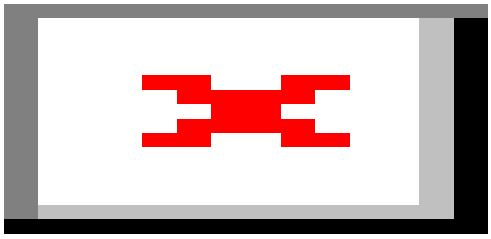

Fig. S4 Transcription level of genes a) CII TA mRNA and b) MHC II mRNA.

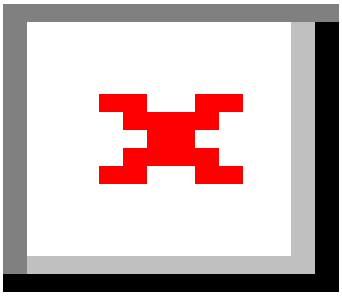

Fig. S5 Analysis of MHC II proteins expression by flow cytometry. Cells transfected with a) blank, b) pCIITA, c) lipofectamine and d) pCIITA/liposomes nanocomplexes.