Preparation of aptamer-functionalized lipid nanoparticles (LNPs) encapsulating siRNAs

Lingqiang Zhang (zhanglq@nic.bmi.ac.cn)
State Key Laboratory of Proteomics, Beijing Proteome Research Center, Beijing Institute of Radiation Medicine, Beijing, China

Aiping Lu (aipinglu@hkbu.edu.hk)
Institute for Advancing Translational Medicine in Bone & Joint Diseases, Hong Kong Baptist University, Hong Kong SAR, China

Ge Zhang (zhangge@hkbu.edu.hk)
Institute for Advancing Translational Medicine in Bone & Joint Diseases, Hong Kong Baptist University

Heng Wu
Institute for Advancing Translational Medicine in Bone & Joint Diseases, Hong Kong Baptist University, Hong Kong SAR, China

Chao Liang
Institute for Advancing Translational Medicine in Bone & Joint Diseases, Hong Kong Baptist University, Hong Kong SAR, China

Baosheng Guo
Institute for Advancing Translational Medicine in Bone & Joint Diseases, Hong Kong Baptist University, Hong Kong SAR, China

Method Article

Keywords: Lipid nanoparticles, aptamers, siRNAs

Posted Date: December 16th, 2014

DOI: https://doi.org/10.1038/protex.2014.053

Abstract

Briefly, lipid nanoparticles were prepared by spontaneous vesicle formation after a lipid-ethanol solution was slowly injected into siRNA buffer solution and then dialyzed. The 3’ thiol-modified aptamers were first activated and then conjugated to DSPE-PEG2000-Mal to form aptamer-PEG2000-DSPE. Finally, aptamer-PEG2000-DSPE in the form of micelles was inserted into the surface of the LNPs.

Procedure

Synthesis of aptamer-PEG2000-DSPE. 1. Dissolve the lipids of DSPE-PEG2000-Mal in chloroform. 2. Dry into a thin film and hydrate with 20 mM HEPES buffer \(pH 6.5). 3. Meanwhile, activate 3’ thiol-modified aptamer in 100 mM Tris-\(2-carboxyethyl) phosphine \(TCEP) solution at 4 °C for 30 min. 4. Then, add the freshly prepared aptamers to DSPE-PEG2000-Mal solution at a lipid/aptamer molar ratio of 5:1. 5. Perform the coupling reaction overnight at 4 °C with gentle stirring. 6. Evaluate the conjugation by polyacrylamide gel electrophoresis \(PAGE). 7. Purify by ultracentrifugation \(10,000 g, 15 °C, 15 min) in centrifugal filter tubes \(MWCO 10,000). **Preparation of LNPs.** The LNPs were prepared by spontaneous vesicle formation. 1. Dissolve approximately 5 mg of lipids, including Dlin-KC2-DMA, DPPC, cholesterol and C16 Ceramide-PEG2000, in 350 μl of ethanol at a molar ratio of 48:10:38:4. 2. Slowly add the lipids to 650 μl of 0.62 mg ml−1 siRNA solution \(50 mM citrate buffer at pH 4) under a strong vortex. 3. Perform dialysis in PBS \(155 mM NaCl, 3 mM Na2HPO4 and 1 mM KH2PO4 at pH 7.4) through a regenerated cellulose tubular membrane \(MWCO 10,000) for 5 h to remove the ethanol. Increase the external pH by exchanging the buffer at intervals. **Post-insertion of aptamer into the surface of LNPs and purification.** 1. Add 4 mol% aptamer-PEG2000-DSPE \(relative to the total lipids) to the LNPs suspension and incubate in a water bath at 37 °C overnight. 2. After incubation, purify the aptamer-LNPs by size exclusion chromatography on a Sepharose CL-4B column using HBS \(pH 7.4) as a running buffer to remove external siRNA, unconjugated micelles and chemical reagents.