

Target Specific Gene silencing of Hyaluronic Acid – siRNA Conjugates Using Cationic Solid Lipid Nanoparticles

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Statement of purpose: Despite wide investigation on siRNA therapeutics, there is no clinically available product, most likely due to difficulties in the delivery of siRNA. The ineffective siRNA delivery might be caused by low negative charge density, high stiffness, and fast degradation in the serum of siRNA [1]. The negatively charged hyaluronic acid (HA) can prevent the nonspecific interaction with serum proteins and facilitate the target specific delivery of siRNA to the liver with abundant HA receptors [2]. Furthermore, cationic solid lipid nanoparticles (CSLN) were also used for target specific delivery of siRNA to the liver [3]. In this work, reducible HA-siRNA conjugate was successfully synthesized and used for the formation of HA-siRNA/CSLN complex for target specific gene silencing applications.

Methods:

Synthesis of reducible HA-siRNA conjugate: For target specific delivery of siRNA, slightly modified HA-DAB (10 mol%) - SPDP (10 mol%) conjugate was prepared and conjugated with thiol-siRNA to synthesize cleavable (reducible) HA-SS-siRNA conjugate. We blocked the remaining 2-pyridyldithio groups of HA chains with cysteine for the prevention of adverse effect of unreacted 2-pyridyldithio groups.

Preparation of CSLN: Cholesterol, cholesterol oleate, triolein, DOPE, DC-Chol, and DSPE-PEG 2k were mixed to prepare a lipid suspension in the co-solvent of chloroform/methanol. After addition of DI water, the solvent was rapidly evaporated. The resulting CSLNs were purified by extensive dialysis against DI water.

Cytotoxicity and gene silencing efficiency: *In vitro* cytotoxicity of HA-siRNA/CSLN complex was assessed in mFVII expressing-Hela cells by MTT assay. *In vitro* gene silencing efficiency was evaluated in the mFVII expressing-Hela cells by measuring the mFVII enzyme concentration of the supernatants.

Results: Figure 1A shows a schematic illustration for the synthesis of reducible HA-siRNA conjugate. HA-DAB-SPDP was conjugated to thiolated siRNA and the remaining SPDP was blocked with cysteine to prevent the adverse effect of unreacted SPDP groups. CSLNs were prepared by the modified emulsification and solvent evaporation method. They were prepared as a biomimetic system by reconstituting the composition of natural apolipoprotein-free LDLs. HA-siRNA/CSLN complexes were prepared by mixing HA-siRNA conjugate solution with CSLN solution at the weight ratio of 20. The complex formation was characterized by gel electrophoresis, DLS, and AFM. Figure 2 shows *in vitro* cytotoxicity and *in vitro* gene silencing efficiency of siRNA/CSLN and HA-siRNA/CSLN complexes in the mFVII expressing-Hela cells. The therapeutic indexes

(LC₅₀/IC₅₀) of siRNA/CSLN and HA-siRNA/CSLN complexes were determined as 6.94 and 9.58, respectively.

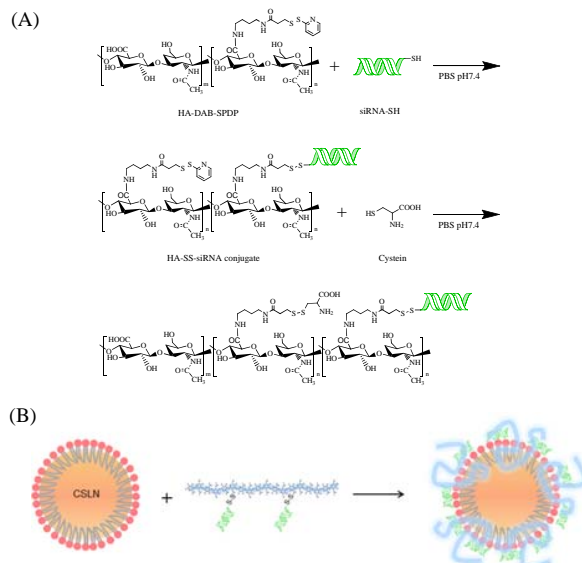


Figure 1. Schematic representations of (A) reducible HA-siRNA conjugate and (B) HA-siRNA/CSLN complex.

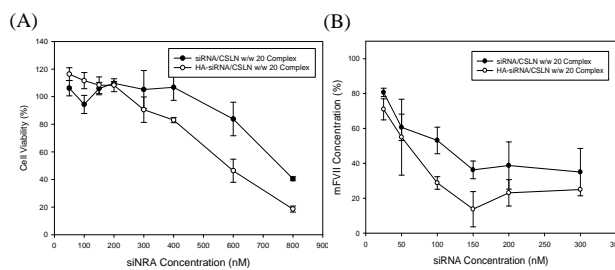


Figure 2. (A) *In vitro* cytotoxicity and (B) *in vitro* gene silencing efficiency of HA-siRNA/CSLN complex in mFVII expressing-Hela cells.

Conclusions: We successfully synthesized reducible HA-siRNA conjugate for the formation of HA-siRNA/CSLN complex for target specific intracellular delivery of siRNA. The cytotoxicity and gene silencing efficiency of HA-siRNA/CSLN complex were determined in mFVII expressing-Hela cells. The therapeutic index (LC₅₀/IC₅₀) of HA-siRNA/CSLN complex was much higher than that of siRNA/CSLN complex. The novel HA-siRNA/CSLN complex will be investigated further for the treatment of various liver diseases.

References:

1. Mok H. Nature Materials. 2010;9:272–278.
2. Oh EJ. J. Control. Rel. 2010;141: 2-12.
3. Kong WH. Biomaterials. 2013;34:542-551.