Proceedings of the 3rd World Congress on Recent Advances in Nanotechnology (RAN'18)
Budapest, Hungary – April 10 - 12, 2018
Paper No. NDDTE 111
DOI: 10.11159/nddte18.111

Characterization and Stability of Long siRNA Loaded-Chitosan Nanoparticles

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Extended Abstract

RNA interference (RNAi) has the potential for application in a gene therapy [1]. Small interfering RNAs (siRNAs) are ~21-23 bp long double-stranded RNA molecules which trigger RNAi mechanism [2]. It was reported that increasing the length of the siRNA may enhance its potency [3], [4]. The main problems in the therapeutic use of siRNAs are related to rapid degradation and poor intracellular uptake of siRNAs. Chitosan is a cationic biopolymer and has been shown to be effective for delivery of siRNAs [5].

The aim of this study is to compare the physicochemical properties and stability of the both, after preparing lyophilized chitosan nanoparticles containing long siRNAs (35 bp) and short (21 bp) siRNAs.

Chitosan nanoparticles containing 21 and 35 bp siRNA were prepared separately by ionic gelation method using TPP as the crosslinking agent, and then lyophilized. The nanoparticles were compared to the physicochemical properties including encapsulation efficiency, particle size, zeta potential, morphology. Serum stability was performed in 2% agarose gel by electrophoresis. Lyophilized and non-lyophilized siRNA-loaded chitosan nanoparticles were incubated at 4 and 25°C for 60 days to investigate storage stability by measuring particle size and zeta potential of the nanoparticles at the pre-determined time. After 60 days, in vitro release studies of lyophilized nanoparticles were performed and it was determined whether there are any changes in the release characteristics of the nanoparticles.

21 bp siRNA loaded- chitosan nanoparticles with the Z-average size of 139.2 ± 51.5 nm and zeta potential of 26.5 ± 1.4 mV were produced at 1:1 chitosan to TPP w/w ratio, while 35 bp siRNA loaded-chitosan nanoparticles have Z-average size of 145.1 ± 10.8 nm and zeta potential of 23.8 ± 2.1 mV. After lyophilization, size of 21 bp and 35 bp siRNA-loaded chitosan nanoparticles increased to 268.2 ± 47.3 nm and 311.8 ± 3.14, respectively. Zeta potential of lyophilized 21 bp siRNA-chitosan nanoparticles was 9 ± 0.6 mV and that of lyophilized 35 bp siRNA-chitosan nanoparticles was 12.6 ± 1.3 mV. 21 bp and 35 bp siRNA loaded-chitosan nanoparticles have high encapsulation efficiency of 95% and 89%, respectively. TEM micrographs indicated spherical and irregular morphology of both chitosan nanoparticles. The chitosan nanoparticles effectively protected siRNA against serum nucleases up to 48h. Storage of the non-lyophilized nanoparticles for 60 days resulted in a significant increase in average size. The particle size of lyophilized nanoparticles increased moderately compared to non-lyophilized nanoparticles for 60 days when stored at 4 and 25°C. The least change in particle size was observed in the storage at 4°C (21 bp siRNA-chitosan nanoparticle sizes reached to 497.2 ± 83.8 nm compared to 552.3 ± 86.7 nm for 35 bp siRNA-chitosan nanoparticle). Zeta potential values of lyophilized nanoparticles remained relatively constant during storage at both temperatures. They showed sustained release of siRNAs for up to 1 month even after 60 days. Consequently, improvement of nanoparticle stability after lyophilization was observed.

As a result of comparing with short siRNA-loaded chitosan nanoparticles, it has been shown that length of siRNA doesn't significantly affect the formulation properties and chitosan is a suitable carrier for long siRNAs.

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