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Study of Chitosan-Arginine with Different Degrees of Substitution at Chitosomes Nanoparticles Formulation for Gene Delivery

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Extended Abstract

Development of efficient and safe gene delivery systems is still a big challenge for human and animal gene therapy. Nanoparticles are safer than viral vectors for gene delivery in terms of virus-related concerns, but transfection efficiency and toxicity of nanocarriers still need to be improved for clinical applications. Many categories of nanoparticles have been synthesized based on the physicochemical properties of building-blocks. Chitosan is derived from the chitin, and it is already available for medical and biomedical purposes, and liposomes are largely used for drug and gene delivery.[1] Therefore, in general, these materials are considered biocompatible, biodegradable, less toxic and immunogenic. As these nanoparticles have distinct physicochemical properties, a new category of nanoparticle was created complexing chitosan and liposome: chitosome. Recently, we synthesized chitosomes with DOPE/DOTAP surfactants and arginine-modified chitosan (CH-Arg) which showed a very high transfection rate in HEK293 cells (86% ± 3).[2] In this study we investigated the effect of CH-Arg with different degrees of substitution (DS), in chitosome formulation and their interaction with plasmids. The CH-Arg with low and high DS were synthesized by a method previously described,[3] and chitosomes were synthesized by the association of DOPE/DOTAP (L) and CH-Arg High or Low by reverse phase evaporation technique. Elemental analysis, fourier transform infrared spectroscopy (FTIR) and proton nuclear magnetic resonance (H1-NMR) spectroscopies were performed for CH-Arg characterization. Dynamic light scattering (DLS) and zeta potential (ZP) were performed for chitosome characterization and measures were made periodically for 1 year to assess the aggregation and sedimentation behaviours after storing it at 25ºC and 4ºC. Chitosomes were complexed with the plasmid pEGFP-N3 and *in vitro* transfection efficacy was assessed by the fluorescent green protein (GFP) expression in HeLa cells by fluorescence microscopy. Elemental analysis estimated that the DS of CH-Arg-High was 5,7% while CH-Arg Low was 3,4%. CH-Arg High was completely soluble in water, but CH-Arg Low was only partially soluble. FTIR and H1-NMR spectra showed that arginine was chemically coupled with chitosan and a higher peaks intensity was observed in CH-Arg High, confirming a higher degree of substitution in this synthesis. DLS and ZP measurements showed that chitosomes did not aggregate for 9 months at 25ºC and 4ºC. Both chitosomes' nanoparticles had similar small sizes (88,8 ± 13,4 nm), low polydispersity indexes (0,390 ± 0,077) and positive charges (+58,1 ± 6,9 mV). The chitosomes were more efficient in transfection, especially at lower concentrations, when compared to L. Chitosomes had a transfection rate ranging from10-35%, depending on the concentration, while lipofectamine had an efficiency of 40%. Collectively, these results show that chitosomes' nanoparticles are good carriers for gene delivery and chitosomes with different DS did not affect transfection efficiency. CH-Arg Low is interesting for laboratory work because it is efficient, cheaper, and simpler for preparation, however, the highly water-soluble CH-Arg High has great advantages when preparing in large scale, since it is easier and faster for preparation, and stable for long time.

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