Cell Internalizing and pH-responsive Chitosan Nanoparticles for Improved Delivery of DNA Biopharmaceuticals

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Abstract

Non-viral gene therapy currently arises as an exceptionally promising approach for the treatment of a wide spectrum of incurable pathologies that have striking worldwide occurrence, such as cancer or HIV. However, regardless of its unique therapeutic potential, the translation of nucleic acid-based pharmaceuticals onto realistic clinical applications remains largely hindered. Such fact, is a direct consequence of a rather inefficient ability of the available nanoparticulated delivery systems to tackle major cellular barriers and deliver the genetic material into the nucleus. Hence, the design and development of improved nanocarrier systems remains a key challenge to be overcome when therapeutic application is envisioned. In order to surpass these limitations, herein we synthesised a novel biocompatible and bioresorbable chitosan nanoparticulated gene delivery carrier functionalized with amino acid moieties. This bioinspired conjugation takes advantage not only of chitosan complexation of nucleic acids [1], but also, of the amino acid specific DNA binding [2] and biological activity at the nano-bio interface. The functionalization of chitosan was promoted by the selective amidation of the primary amine residues of the polymer backbone, thus allowing its coordination with two amino acid residues. The amino acid conjugation with the polymer backbone was confirmed by Fourier transform infrared and ^{1}H NMR spectroscopy (Figure 1). Moreover, under precise formulation conditions the synthesized polymer had the ability to condense plasmid DNA biopharmaceuticals and spontaneously assemble into stable nanoparticulated polyplexes with 105 nm, positive surface charge density and spherical morphology (Figure 2), characteristics that are crucial to improve cellular uptake and transfection. In fact, the obtained flow cytometry data also revealed that the nano carriers were efficiently internalized by malignant cells, a fact that is associated with the grafting of cell-penetrating amino acid residues in the polymeric chain (Figure 3). Additionally, the characterization of nanoparticle cytotoxic profile showed that cellular transfection and gene delivery occurs without eliciting any deleterious effect on normal cellular metabolism and cell morphology. After cellular entry, the carriers demonstrated their ability to escape from degradative endosomal/lysosomal trafficking pathways and promoted a remarkable increase in therapeutic transgene expression in comparison to the native material. Collectively, these important findings emphasise the relevance of amino acids as novel biocompatible functionalization materials for gene delivery systems, and thus opening the possibility to design a new generation of specifically tailored and proficient nanoparticles for gene-based therapies.

References

[1] Gaspar et al., J. Control. Release, 2011, **156:** 212–222.
[2] Sousa et al, J. Gene Med 2009; **11:** 79–88.

Figures

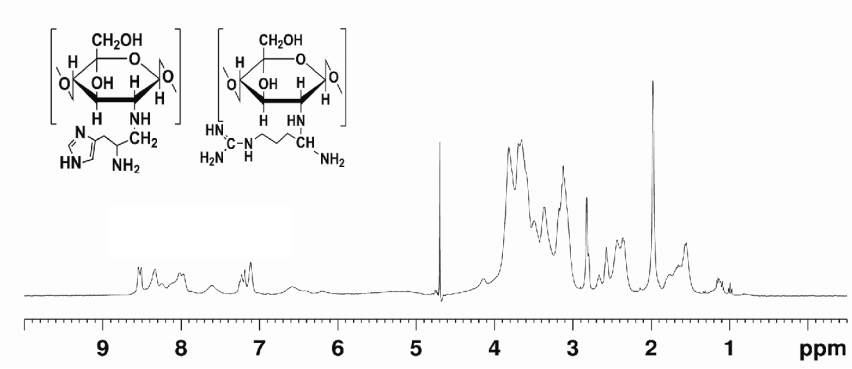

Figure 1. ^{1}H 1D NMR spectra of chitosan-Hist-Arg.

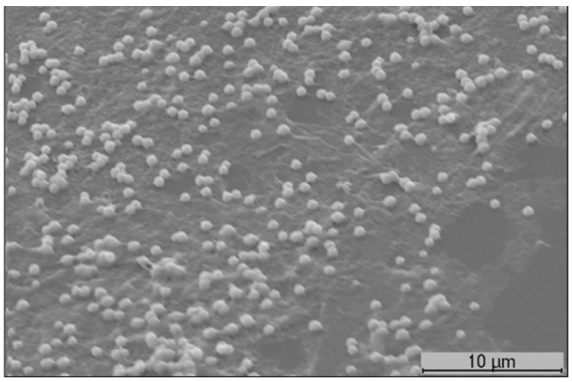

Figure 2. Scanning electron microscopy image of chitosan-amino acid nanoparticles.

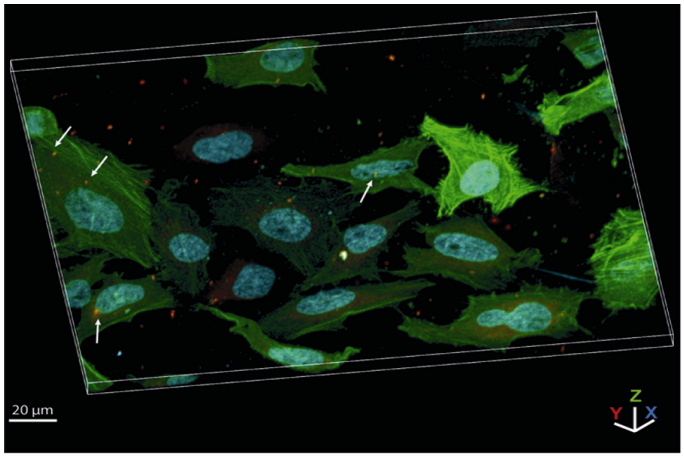

Figure 3. Confocal laser scaning microscopy analysis of nanoparticle cellular uptake