



# Development of an Efficient Targeted Gene Delivery Vector Based on Mannose-Histidine Grafted Chitosan

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## ABSTRACT

**Introduction:** The application of non-viral vectors shows promise in the safe delivery of therapeutic genes. Among non-viral vectors, chitosans are suggested as alternative, biocompatible cationic polymers for this purpose. Despite their many advantages, chitosans exhibit low transfection efficiency due to the limited buffering capacity, which results in poor endosomal escape and low cell specificity. Research has demonstrated that incorporating an imidazole ring can enhance transfection efficiency by facilitating escape from endo-lysosomal compartments through the proton-sponge effect. Furthermore, mannosylated chitosan, a type of ligand-conjugated chitosan, is recognized as a gene carrier that specifically targets antigen-presenting cells (APCs) due to the interactions between mannose moieties and mannose receptors. Mannosylated chitosan-grafted histidine shows significant potential as a safe gene carrier aimed at targeting APCs in vaccine development. This study aimed to develop an efficient targeted gene delivery vector based on mannose-histidine grafted chitosan.

**Methods and Materials:** Histidine-conjugated chitosan polymers were synthesized by reacting the reactive carboxylic acid group in histidine with the available primary amino groups in chitosan, utilizing EDC and NHS as coupling agents. L-histidine was gradually added to the chitosan solution (1 mg/ml in 1% acetic acid). Subsequently, 0.5 g of EDC crosslinker was incorporated while continuously stirring the mixture. Mannose conjugation to the histidine-conjugated chitosan was achieved through a reductive amination process in the presence of sodium triacetoxyborohydride. The in vitro transfection efficiency of mannosylated chitosan-graft histidine polymer was evaluated in Raw 264.7 and HEK293 cells at various N/P ratios by transfecting the cells with polymer/pEGFP-N1 complexes. Transfection efficiency was evaluated using fluorescence microscopy and flow cytometry.

**Results:** After 48 hours, the transfection of mannosylated chitosan-graft histidine polymer complexing 1 µg of pEGFP-N1 at different N:P ratios (ranging from 4 to 32) was evaluated using a fluorescence microscope and flow cytometry, with lipofectamine 3000 as a control group. The results showed that the most efficient formulation for DNA transfection was at an N:P ratio of 12. The flow cytometry results revealed that approximately 70% of the cell population expressed GFP, while cells transfected with lipofectamine 3000 had 82% transfection efficiency.

**Conclusion and Discussion:** The study successfully developed and assessed a new gene carrier targeting APCs named mannosylated chitosan-graft histidine polymer. This polymer exhibited excellent DNA complex formation capabilities and appropriate physicochemical characteristics for gene delivery systems. Hence, the mannosylated chitosan-graft histidine polymer shows promise as a safe and effective gene carrier targeting APCs.

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