Non-Viral Delivery of Gene HPV-16 E7 into Mammalian Cells

M. Dinarvand¹ A. Tahamtan², A. Ghaemi², F. Atyabi³* mdinarvand@hotmail.com

¹ Nanotechnology Research Centre, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran14171, Iran, ²Infectious Diseases Research Center, Department of Microbiology, Golestan University of Medical Sciences, Golestan, Iran

ABSTRACT SUMMARY
Chitosan nanoparticles (CS NPs) were prepared as a carrier for HPV-16 E7 gene and its gene transfection ability was evaluated in vitro. The plasmid expressing green fluorescent protein (pEGFP) was used as a reporter gene. Gel electrophoresis demonstrated full binding of CS NPs with the pDNA by electrostatic interaction. The transfection of chitosan-pEGFP NPs was efficient in CHO cells and the expression of green fluorescent proteins was well observed. The expression of E7 proteins was confirmed under SDS-PAGE and western blot analysis. As a conclusion CS NPs have potential as an effective non-viral carrier for gene delivery.

INTRODUCTION
Human papillomavirus type 16 (HPV-16) infections in humans is associated with most cervical cancers and expression of the early oncogenic proteins E6 and E7 is required to maintain the transformed state of the tumor cell. Therefore, E7 is an attractive target not only for development of cancer vaccine to prevent cervical tumors, but also a good model for exploration of immunotherapeutic approaches to stimulate immune responses against existing HPV-16-associated malignancy. Gene therapy, offers the potential for permanent cure of cancers¹. The success in gene therapy depends on an efficient strategy for the delivery of nucleic acid into the target cells. Positively charged polycations such as chitosan are promising carriers among non-viral vectors. In this study chitosan nanoparticles were acquired by the process of ionotropic gelation of positive charge of chitosan and the negatively charged DNA into compact structures, giving them protection during cellular trafficking and promoting its uptake to cells.

EXPERIMENTAL METHODS
Plasmid Propagation and Extraction; Bacterial strain Escherichia coli DH5α was used for propagation and preparation of the pEGFP-N1 and pCDNA3.1 Plasmid pCDNA3.1 containing E7 were constructed by cloning the PCR products of the E7 gene from the HPV-16. Chitosan nanoparticles were prepared according to the ionotropic gelation technique with pentasodium tripolyphosphate (TPP) cross linker. Size distribution and zeta potential of NPs were measured by nanosizer. CS/pDNA complexes were developed at different N/P ratios through incubation of NPs and plasmid and confirmed by electrophoresis. The in vitro transfection studies were performed. Cells transfected with lipofectamin and naked plasmid were used as controls and GFP expression in transfected cells was analyzed by fluorescence microscopy. Cytotoxicity evaluation was performed with MTT assay. The expression of HPV-16 E7 proteins from CS-pDNA complexes in CHO cells was performed by SDS-PAGE and western blot analysis. The extracted total proteins lysed in SDS-PAGE sample loading buffer, and lysates separated by SDS-PAGE and staining by coomassie blue R250. For blotting separated proteins by SDS-PAGE, transferred onto polyvinylidene difluoride membranes and hybridized with the monoclonal HPV-16 E7 mouse antibody, and detection performed with goat anti-mouse secondary antibody conjugated to alkaline phosphate as in secondary antibody
solution. Color was developed by incubating the membrane in Di Amino Banzedin (DAB).

RESULTS AND DISCUSSION

Prepared nanoparticles showed a narrow size distribution, positive zeta potential with a mean diameter of about 70 nm. SEM images shows prepared nanoparticles are spherical with smooth surfaces (Fig. 1). Very high loading efficiency yield was further observation. This can be explained by the high positive charge presenting on the surface of nanoparticles (+20 mV) which attracts negatively charged pDNA on the surface.

Figure 1. SEM image of NPs

The gel retarding analysis of complex formation between chitosan nanoparticles and pDNA at different N/P ratios (0.1 to 16) showed the naked pDNA could migrate in gel while the pDNA loaded in chitosan nanoparticles was immobile and remained in the loading wells when N/P ratio was above 1 (Fig. 2). EGFP expression was observed in CHO cells transfected with CS-pEGFP nanoparticles in a similar extent to the control cells which were transfected with lipofectamine. The results are shown in figure 4

Figure 2: Agarose gel electrophoresis analysis of CS/pDNA, pDNA (1), CS/pDNA complexes at N/P ratios of 0.1, 0.5, 1, 2, 4, 6, 8 and 16 (2–8).

Figure 3: EGFP expression in CHO cell lines transfected by a) nanoparticles and b) lipofectamin

CHO cell line is proved to be highly efficient in transfection with gene material. The green fluorescence observed by florescent microscope is an indication of successful transfection of pEGFP inside the cells (fig.3). The cytotoxicity assay revealed NPs have reserve about 85% of their viability in comparison with cells being in contact to naked DAN (fig.4) showing acceptable safety for NPs.

Figure 4: Cell viability assay of NPs in CHO cells.

CONCLUSION

In this study, a polycation vector with improved transfection efficiency to eukaryotic cells was prepared. Its transfection efficiency and cell viability evaluated showed NPs may serve as an effective non-viral carrier for delivery of nucleotides into eukaryotic cells

REFERENCES