A Multifunctional Gene Delivery Nanovector for Cancer Treatment

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Introduction: Gene therapy holds great promise to treat diseases, including cancer, at their genetic roots [Thomas CE. Nature Rev Genet. 2003;4:346-358.]. This is achieved by utilizing therapeutic genes to replace defective genes that cause disease or silence the expression of mutated genes. Prior to reaching the targeted sites of action, therapeutic genes must overcome various biological barriers at different levels. Developing safe and efficient gene carriers is crucial for the success of gene therapy. Viral vectors show high gene transfection efficiency but there are great safety concerns. Non-viral vectors have advantages over viral vector such as low immune response and large DNA loading ability. Nanotechnology now offers unprecedented opportunities for the development of non-viral vectors [Panyam J. Adv Drug Deliv Rev. 2003;55:329-347.]. A variety of nanoparticle (NP)-based gene delivery vehicles have emerged, using cationic polymers such as polyethyleneimine and chitosan. Although great efforts have been made to develop various polymeric vectors, very few of them are employed for clinical applications due to their low transfection efficiency and non-specific delivery. In this study, multifunctional nanovectors which were composed of a bimetallic nanoparticle core and a polymer shell were investigated. The bimetallic core can be used as the imaging moiety for real-time cancer detection, while the polymer shell can be tasked for cancer cell targeting and gene delivery.

Methods: A facile method was employed to fabricate the multifunctional nanovectors [Li SY. *IET Nanobiotechnol.* 2012; 6:136-143.]. Chitosan was firstly conjugated with folic acid through EDC/NHS catalysis. Folic acid-conjugated chitosan (CS-FA) was then used as both reducing agent and structure-directing agent for synthesizing Au-Ag NPs. Au and Ag precursors were added in CS-FA solution for forming Au-Ag NPs with a CS-FA shell (Au-Ag@CS-FA NPs, the nanovectors). For cancer detection, rhodamine B (RhB, a Raman reporter) was embedded in NPs. Plasmid DNA encoding enhanced green fluorescent protein with a CMV promoter (pDNA) was used as a model gene to evaluate the NPs as potential non-viral nanovectors. Au-Ag@CS-FA/pDNA complex was made using a complex coacervation technique with different NP to pDNA ratios. Electrophoresis was carried out to assess binding of pDNA with NPs. Various techniques (SEM, TEM, etc.) were used to evaluate NPs.

Results: Folate receptors are overexpressed in human epithelial cancer cells and hence many researchers use FA-conjugation to improve NP internalization through receptor-mediated endocytosis in targeted cancer cells. As shown in Fig.1a, characteristic absorption peaks of FA at 1412 cm^{-1} and 1603 cm^{-1} were observed in the CS-FA spectrum, indicating successful FA to CS conjugation. Synthesized Au-Ag@CS-FA NPs (Fig.1b) had highly branched structures with sizes around 90 nm. Selected area electron diffraction (SAED) pattern indicated NPs were crystalline with random orientation. NPs with such branched structures could generate strong surface plasmon resonances for local electromagnetic enhancement, which would greatly increase the intensity of Raman signals from the Raman molecules embedded in the nanodevices. The CF-FA polymer shell provided the NPs with high stability in medium. The amount of FA on the surface of NPs was measured to be 1.798×10^{-5} mol, which is sufficient for targeting folate-receptor overexpressed cancer cells. The NPs containing RhB that are internalized in cancer cells can provide strong Raman signals for the detection of cancer. The Au-Ag@CS-FA NPs were assessed as potential gene nanocarriers. The zeta potential of NPs was +37.1 mv in deionized water, suggesting cationic polyelectrolyte nature of NPs which could provide bonding with negatively charged DNA. The binding ability of plasmid DNA with NPs was studied with NP:pDNA molar ratio from 1:1 up to 10:1 using gel electrophoresis. As shown in Fig.2, no successful retardation of pDNA could be realized when the molar ratio was 1:1 and 2:1. The complete retardation of pDNA was observed when the ratio was above 5:1. These results showed that the condensation of plasmid DNA by Au-Ag@CS-FA NPs could be achieved.

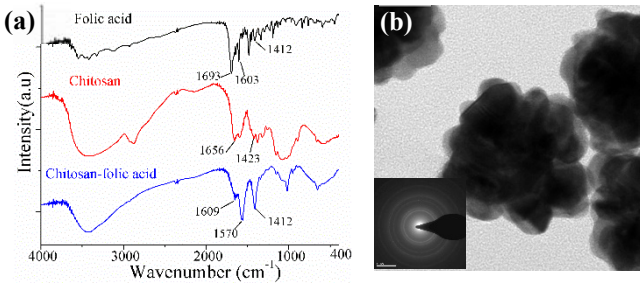

Fig. 1 Synthesis of nanovectors: (a) FTIR spectrum of CS-FA, (b) TEM image and SAED of Au-Ag@CS-FA.

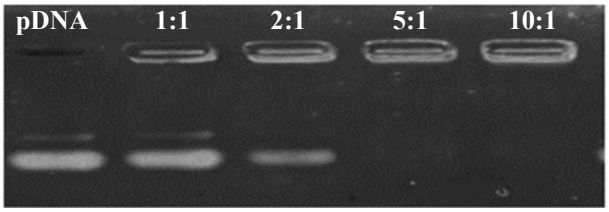

Fig.2. Gel electrophoresis analysis of pDNA and NP/pDNA complexes with different NP:pDNA ratios.

Conclusions: Polymer-metal NPs as multifunctional nanovectors could be synthesized using a facile method. The Au-Ag@CS-FA NPs based on highly branched bimetallic Au-Ag NPs were stable and exhibited strong DNA binding ability in the acidic condition. FA molecules in CS-FA conjugate on NPs would equip NPs with cancer cell-targeting ability. Au-Ag@CS-FA NPs are promising as a new gene delivery vehicle.