Synthesis and Characteristics of PEI-based Copolymers and Nanoparticles for Potential Gene Delivery Applications

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Introduction: Gene therapy holds great promise for treating cancer and genetic disorders. Viral vectors are known for high gene transfection efficiency. But safety concerns of viral vectors have greatly limited their clinical applications. Non-viral vectors such as cationic polymers are investigated owing to their ease of preparation, manipulable structures, large DNA loading capability and absence of immune response [He CX, et al., International Journal of Pharmaceutics, 2010, 386 (1-2), 232-242.]. Among these polymers, poly(lactic-*co*-glycolic acid) (PLGA) and chitosan are extensively used for making nanoparticles. Poly(ethylenimine) (PEI) with abundant cationic charges has been widely employed for transfection of cells. However, its molecular weight-dependent cytotoxicity limits its applications [Wang MX, et al., Journal of Materials Chemistry, 2012, 22 (13), 6038-6046]. Poly(ethylene glycol) (PEG) modification of delivery vehicles is a useful strategy for providing shielding and ensuring long-circulation and can also serve as active sites for targeting ligand via covalent bonding. Folate is a known targeting ligand for most tumors and covalent conjugation of folate to designed polymer can promote cell transfection in folate expressing cells. Therefore, biodegradable copolymer nanoparticles with buffering capacity, hydrophilic modification and targeting ligand are promising developments for efficient gene delivery. In this investigation, several types of copolymers and nanoparticle, including PEI/PLGA, PEI-g-PLGA, PEI-g-chitosan and FOL-PEG-g-PEI-g-PLGA, were synthesized and studied for potential gene delivery applications.

Methods: For synthesizing different copolymers, PLGA (MW: 36kDa) with acid end group, low molecular weight chitosan, PEI (MW: 1.8kDa, 25kDa) and PEG (COOH-PEG-NH_2, MW: 3kDa) were used. For making nanoparticles, the stabilizer-free method and double emulsion evaporation method were employed. The chemical composition of copolymers was studied using FTIR and ^{1}H-NMR. The buffering capacity of copolymers was determined by titration experiments. The morphology and structure of nanoparticles and nanocomplexes were examined using SEM and TEM. Their particle size and surface charge were characterized. Plasmid EGFP encoding enhanced green fluorescence protein and plasmid GL3-control encoding luciferase were employed as model genes to evaluate the copolymers as potential non-viral vector. DNA condensation effect of copolymers and nanoparticles was studied using agarose gel electrophoresis. HepG2 cells were used to evaluate the cytotoxicity of copolymers and nanoparticles and also to assess the transfection efficiency.

Results: The chemical composition of copolymers was confirmed by FTIR and 1H-NMR, indicating successful synthesis of desired copolymers (Fig.1). Acid titration experiments showed that copolymers possessed considerable buffering capacity, which could help in polyplexes release from endosomes and lysosomes. SEM examination revealed that both nanoparticles and polyplexes were spherical in shape and uniform in size (less than 200nm in diameter and with low polydispersity) (Fig.2). TEM result showed that a core-shell structure existed in polyplexes (Fig.2). Both nanoparticles and polyplexes were positively charged, indicating DNA was shielded. Surface charge of polyplexes increased with the increase of N/P ratio. Gel electrophoresis of polyplexes indicated successful condensation of DNA at N/P ratio of 5 (Fig.3). The amphiphilic FOL-PEG-g-PEI-g-PLGA copolymer exhibited the lowest cytotoxicity and best transfection efficiency.

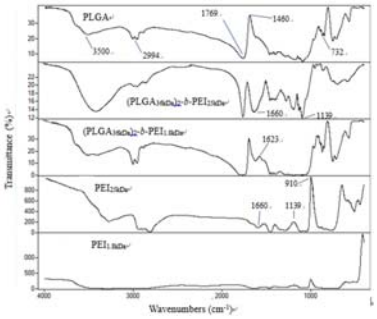

Fig.1. FTIR spectra of PEI-g-PLGA copolymers.

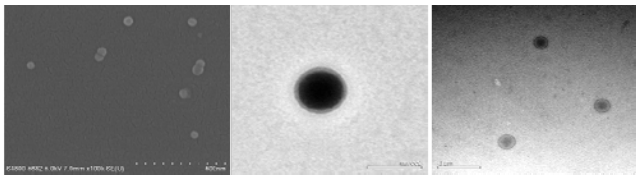

Fig.2. Morphology and structure of nanoparticles and polyplex (Left: PLGA NPs; middle: a PEI/PLGA NP; right: DNA/PEI/PLGA polyplexes).

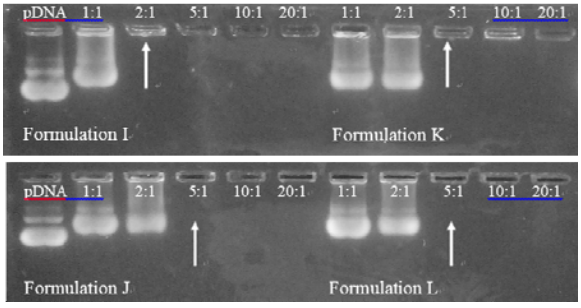

Fig.3. Gel electrophoresis of the DNA/PEI/PLGA system with different formulations.

Conclusions: Biodegradable copolymers with desired composition and nanoparticle and polyplexes with designed structures were synthesized. Nanoparticles and polyplexes were positively charged. Above a particular N/P ratio, DNA was shielded and condensed. Cytotoxicity and transfection efficiency of copolymers and nanoparticles were evaluated.