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# Preparation, characterization and transfection efficiency of cationic PEGylated PLA nanoparticles as gene delivery systems

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**Abstract**

The cationic polylactic acid (PLA) nanoparticle has emerged as a promising non-viral vector for gene delivery because of its biocompatibility and biodegradability. However, they are not capable of prolonging gene transfer and high transfection efficiency. In order to achieve prolonged delivery of cationic PLA/DNA complexes and higher transfection efficiency, in this study, we used copolymer methoxypolyethyleneglycol-PLA (MePEG-PLA), PLA and chitosan (CS) to prepare MePEG-PLA-CS NPs and PLA-CS NPs by a diafiltration method and prepared NPs/DNA complexes through the complex coacervation of nanoparticles with the pDNA. The object of our work is to evaluate the characterization and transfection efficiency of MePEG-PLA-CS versus PLA-CS NPs. The MePEG-PLA-CS NPs have a zeta potential of 15.7 mV at pH 7.4 and size under 100 nm, while the zeta potential of PLA-CS NPs was only 4.5 mV at pH 7.4. Electrophoretic analysis suggested that both MePEG-PLA-CS NPs and PLA-CS NPs with positive charges could protect the DNA from nuclease degradation and cell viability assay showed MePEG-PLA-CS NPs exhibit a low cytotoxicity to normal human liver cells. The potential of PLA-CS NPs and MePEG-PLA-CS NPs as a non-viral gene delivery vector to transfer exogenous gene in vitro and in vivo were examined. The pDNA being carried by MePEG-PLA-CS NPs, PLA-CS NPs and lipofectamine could enter and express in COS7 cells. However, the transfection efficiency of MePEG-PLA-CS/DNA complexes was better than PLA-CS/DNA and lipofectamine/DNA complexes by inversion fluorescence microscope and flow cytometry. It was distinctively to find that the transfection activity of PEGylation of complexes was improved. The nanoparticles were also tested for their ability to transport across the gastrointestinal mucosa in vivo in mice. In vivo experiments showed obviously that MePEG-PLA-CS/DNA complexes mediated higher gene expression in stomach and intestine of BALB/C mice compared to PLA-CS/DNA and lipofectamine/DNA complexes. These results suggested that MePEG-PLA-CS NPs have favorable properties for non-viral gene delivery.


*Keywords:* Gene delivery; Nanoparticle; PEGylated; Polylactic acid (PLA); Chitosan

## 1. Introduction

Gene therapy is of increasing interest because it holds exciting promise in treating many disorders. The two main systems for DNA delivery are viral and non-viral vectors. Although, the efficiency of gene delivery by non-viral-based vectors is less than that by the viral vectors, non-viral systems are preferred in terms of safety, stability, the relative ease of large-scale production and characterization, and the lack of immunogenicity (Felgner, 1997; Pouton and Seymour, 2001). To date, an ideal gene vector with high efficacy of gene transfer, targeting ability and good biocompatibility, especially high stability and the ability to prolong gene transfer, is thought to be an important feature for pharmaceutical application.

Among synthetic vectors, the use of nanoparticles or microparticles prepared with biocompatible and biodegradable poly(lactide) (PLA) polymers has become one of the most successful methods for in vivo DNA delivery. Several authors have reported gene delivery system using cationic PLA microspheres

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including polylysine, polyethyleneimine, chitosan, etc. (Ravi Kumar et al., 2004; Kim et al., 2005; Munier et al., 2005). Kumar et al. (Kumar et al., 2004; Ravi Kumar et al., 2004) reported using polyvinyl alcohol (PVA)-chitosan to prepare cationic poly-(D-lactide-co-glycolide) (PLGA) nanoparticles, with defined size and shape, as DNA carriers by cationic modification of a PVA-chitosan blend by emulsion solvent evaporation technique. Munier et al. described a versatile approach for the elaboration of cationic PLA cNP based on the use of pre-formed particles by the emulsification–diffusion method in the presence of Pluronic F68 surfactant and subsequent adsorption of three different cationic polymers (chitosan, PEI, pDMAEMA). The three types of cationic nanoparticles (PLA-PEI, PLA-chitosan and PLA-pDMAEMA) were compared with regards to DNA binding and release properties and in vitro transfection efficiency (Munier et al., 2005). Chitosan has been considered as a promising carrier because it can be biocompatible in vivo and has minimal toxicity (Mansouri et al., 2006). Nevertheless, chitosan (CS) protonated in acidic condition can form complexes with anionic DNA by electrostatic interaction and these complexes are appropriated to be endocytosed by cells because chitosan has a better ability to go straight through cell membrane. Subsequently, these complexes also can be released from endosomes and enter nucleus (Borchard, 2001). Plasmid DNA (pDNA) in these complexed forms is protected from enzymatic degradation, thus increasing the efficiency of gene delivery within a cell. Chitosan has been used widely in the agricultural and pharmaceutical industries, such as a dietary supplement, a wound healing biomaterial and a pharmaceutical excipient (Illum, 1998).

Furthermore, varieties of modifications have been incorporated into the polymer by recent studies. Polyethylene glycol (PEG) (Ross and Hui, 1999) is expected to be a good candidate as the soluble polymeric modifier in organic synthesis or a pharmacological polymer due to its high hydrophilicity, low cytotoxicity and high cell permeability. It has been proved that PEG could diminishes non-specific interactions with serum proteins and decreases the uptake of cationic polymer–DNA complex by the macrophages in the liver and spleen leading to an increased blood circulation time, which ultimately improve the transfection efficiency of polycationic polymers (Plank et al., 1996; Hu et al., 2003; Jaiswal et al., 2004; Aktas et al., 2005). PEG-PLA or PLGA nanoparticles were studied in many reports (Yun et al., 2005; Gao et al., 2006). Gao et al. (2006) explored the possibility of Lectin-conjugated PEG-PLA nanoparticles for brain drug delivery after intranasal administration. Yun et al. (2005) have formulated microspheres by physically combining poly(ethylene glycol)-grafted chitosan (PEGg-CHN) with poly(lactide-co-glycolide) (PLGA) using a modified conventional in-emulsion solvent evaporation method and the microspheres are bioactive and show enhanced transfection in vitro or in vivo over DNA released from conventional PLGA microspheres containing no PEG-g-CHN.

With these considerations, to develop and characterize a novel cationic PEGylated PLA nanoparticle for gene delivery, in this study, we used copolymer methoxypolyethyleneglycol-PLA (MePEG-PLA), PLA and chitosan (CS) to prepare MePEG-PLA-CS NPs and PLA-CS NPs by a diafiltration method (Kim et al., 2005). After that, the morphology and particle size of the NPs were surveyed by the atomic force microscope (AFM) and zeta potentials were determined with the laser grain analyzer. The cytotoxicities of the MePEG-PLA-CS NPs to liver cancer cell lines (HepG2 cells) and normal liver cell line (L-02 cells) were examined by the MTT assay. DNase I was used to study the gene protection effect of NPs to pDNA. Finally, the transfection activities of the MePEG-PLA-CS NP and PLA-CS NP were qualitatively assessed in vitro and in vivo by pEGFP-C1 plasmid and luciferase (pGL-2 plasmid) determination assay.

## 2. Materials and methods

### 2.1. Materials

The COS7, HepG2 and L-02 cells were obtained from the cell bank of Xiangya School of Medicine, Central South University (Changsha, China). COS7, HepG2 and L-02 cell lines were maintained in medium supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamine at 37 °C in 5% $CO_2$.

Poly(ethylene glycol)-block-polylactide copolymer (MePEG-PLA) (linear formula: $CH_3O(CH_2CH_2O)_x(COCHCH_3O)_yH$, PEG average Mn 350, PLA average Mn 1000), the ethidium bromide, agarose, fetal bovine serum (FBS), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), dimethylsulfoxide (DMSO), chitosanase and DNase I were purchased from Sigma–Aldrich; chitosan ($M = 7000$, degree of acetylation 4.23%) was purchased from Fluka (Switzerland). Opti-MEMR I reduced serum medium from Gibco-BRL Life Technologies; QIAGEN Plasmid Purification Kit (QIAGEN), Trizol (GIBCO BRL), Bright-Glo™ Luciferase Assay System was from Promega. BCA™ Protein Assay Kit was from Pierce Biotechnology. Commercially available transfection reagent lipofectamine from Invitrogen™. Dichloromethane (DCM) of HPLC grade was used in this study.

During this study, two plasmids were used. pEGFP C1 was a 4.7 kb cDNA encoding the enhanced green fluorescence protein which was purchased from Clonetech of USA. pGL-2 was a 6046 bp cDNA encoding luciferase gene with polyadenylation signal from Promega of USA.

Six-to-8-week-old BALB/C mice were obtained from the Department of Laboratory Animal Science of Xiangya School of Medicine. Mice were maintained at room temperature under 40% humidity. The Medical Laboratory Animal Management Committee of Xiangya School of Medicine, Central South University, approved all animal procedures.

All chemicals, reagents and solvents in the present study were the highest grade available and used as directed. The distilled water used was obtained by an ion exchanging and distillation process.

### 2.2. Preparation and characterization of nanoparticles

The PLA-CS and MePEG-PLA-CS nanoparticles were prepared by a diafiltration method (Kim et al., 2005). Either PLA or MePEG-PLA (25 mg) dissolved in 10 ml of dimethylsulfoxide