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A biodegradable poly(ester amine) based on polycaprolactone and polyethylenimine as a gene carrier

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Abstract

The aim of research was to develop and optimize delivery systems for plasmid DNA (pDNA) based on biodegradable polymers, in particular, poly(ester amine)s (PEAs), suitable for non-viral gene therapy. Poly(ester amine)s were successfully synthesized by Michael addition reaction between polycaprolactone (PCL) diacrylate and low molecular weight polyethylenimine (PEI). PEA/DNA complexes showed effective and stable DNA condensation with the particle sizes below 200 nm, implicating its potential for intracellular delivery. PEAs showed controlled degradation and were essentially non-toxic in all three cells (293 T: Human kidney carcinoma, HepG2: Human hepatoblastoma and HeLa: Human cervix epithelial carcinoma cell lines) at higher doses in contrast to PEI 25 K. PEAs also revealed much higher transfection efficiencies in three cell lines as compared to PEI 25 K. The highest reporter gene expression was observed for PCL/PEI-1.2 (MW 1200) complex having transfection efficiency 15–25 folds higher than PEI 25 K in vitro. Also PEA/DNA complexes successfully transfected cells in vivo after aerosol administration than PEI 25 K. These PEAs can be used as most efficient polymeric vectors which provide a versatile platform for further investigation of structure property relationship along with the controlled degradation, significant low cytotoxicity and high transfection efficiency.

Keywords: Gene delivery; Degradable polymers; Poly(ester amine)s; Polycaprolactone; Polyethylenimine

1. Introduction

Gene therapy comprises a novel form of molecular medicine that will have a major impact on human health in next century. The need for safe and efficient methods for gene delivery still remains a critical obstacle to the routine clinical implementation of human gene therapy [1]. While recombinant viruses are the most efficient gene delivery vectors currently available, polymeric vectors have several advantages that make them a promising alternative. Over the past decade, many researches focused to design breakthrough non-viral vectors that could not only achieve the level of gene expression and specificity shown by viral ones, but also allow greater flexibility in cDNA size and bypass the immune responses. It will potentially revitalize the development of synthetic vectors for gene therapy in terms of improvement in cellular delivery and also exploit vector's intrinsic biological activities for synergistic therapeutic effect [2,3].

Polyethylenimine (PEI) is one of the successful and widely used gene delivery polymers [4] because of 'proton sponge effect' [5]. However, it has been reported by many researchers that PEI has high cytotoxicity in many cell lines. Additionally, transfection efficiency and cytotoxicity of PEI depends on molecular weight and it is accepted that PEI with high molecular weight (PEI 25 kDa) shows high transfection efficiency and cytotoxicity [6,7].

Various biodegradable cationic polymers such as, linear poly(ester amine)s (PEAs) [8–11] and PEAs networks [12] have been investigated as non-viral gene carriers. Recently Langer group reported a large library of linear PEAs and

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screened for in vitro gene transfection [8,9,11]. Several biodegradable PEI [13], cross-linked low molecular weight PEI [14], and PEI-g-Chitosan [15] have been investigated as gene carriers to reduce cytotoxicity. Recently, Park et al. [16] synthesized degradable PEAs comprising of low molecular weight of PEI and poly(ethylene glycol) (PEG) with biodegradable linkages. They reported that the transfection was decreased with increasing PEG molecular weight. In correlation to that, our approaches to reduce cytotoxicity and increase transfection efficiency are based on the synthesis of copolymers containing low molecular weight PEI and biodegradable, hydrophobic moiety such as polycaprolactone (PCL).

PCL is biocompatible and semi-crystalline linear resorbable aliphatic polyester. It has been reported that the PCL, being hydrophobic, has slower degradation kinetics than other polyester family members such as poly (lactide), poly (glycolide) and poly (lactide-*co*-glycolide) thereby increases circulating half-life [17]. It is expected that PEAs prepared by copolymerization of PCL and PEI will be non-toxic along with increased transfection efficiency due to the hydrophobicity of PCL.

In this context, a novel biodegradable PEA based on PCL and PEI was prepared and characterized. Also DNA condensation ability, protection ability and in vitro degradability of PEAs were checked. Furthermore, we observed remarkable transfection efficiency and significant low cytotoxicity as compared to PEI 25 K.

2. Materials and methods

2.1. Materials

Branched PEI (0.6, 1.2, 1.8 and 25 K), PCL diol (Mn: 530), triethylamine, acryloyl chloride and anhydrous methanol were purchased from Sigma- Aldrich. MTS reagent for cell viability, Luciferase Reporter 1000 Assay System for in vitro transfection assay, and pGL3-control vector with SV-40 promoter were obtained from Promega. Plasmid pEGFP-N_2, which has the early promoter of CMV and enhanced green fluorescence protein (EGFP) gene, was obtained from Clontech.

2.2. Synthesis of PCL diacrylate (PCLDA)

PCL diacrylate (PCLDA) was synthesized by end capping the terminal hydroxyl groups of PCL diol (Mn: 530) with acrylated groups as described previously by our group [18].

2.3. Synthesis of PEAs

We succeeded in the synthesis of PEAs by Michael addition reaction. In a typical polymerization procedure, branched PEI (MW: 600, 1200 and 1800 Da) and PCLDA (Mn: 530) were separately dissolved in anhydrous methanol in three different stoichiometric ratios as 1:1, 2:1 and 4:1 of PEI to PCLDA. The solutions of PCLDA were slowly added to solutions of PEI with continuous stirring. The reaction mixtures were maintained at 40 °C with constant shaking for 24 h. After completion of reaction, the reaction mixtures were dialyzed using Spectra/Por® membrane (MWCO = 6000–8000) against distilled water at 4 °C for 24 h and lyophilized.

2.4. Characterizations of PEAs

Synthesized polymers were characterized by Nuclear Magnetic Resonance (^{1}H-NMR) (Avance™ 500, Bruker). Molecular weights of the polymers were determined by gel permeation chromatography with multi angle laser light scattering (GPC–MALLS) (Dawn Eos, Wyatt, USA) (690 nm laser wavelength) using Sodex OHpack SB-803 HQ (Phenomenox, USA) column, (temperature 25 °C) operated at a flow rate of 0.5 ml/min. Ammonium acetate (0.5 M, pH 5.5) was used as a mobile phase.

2.5. Degradation of PEAs

In a typical study, polymers were dissolved in PBS (0.1 g/ml) and incubated at 37 °C with constant shaking at 100 rpm for a specified time. After incubation solutions were lyophilized and the molecular weights of lyophilized samples were measured by SEC–MALLS.

2.6. Complexation study

All polymer/DNA complexes were prepared freshly before use by gently vortexing a mixture of DNA (pGL3-control, 0.1 μg) the solutions of polymers (10 μl) at various N/P ratios. The complexes were incubated at room temperature for 30 min, and a 12 μl volume, including loading dye mixture, was loaded on 0.8% agarose gel with ethidium bromide (0.1 μg/ml) and run with tris-acetate (TAE) buffer (100 V, 40 min). The gel was analyzed on UV illuminator to show the location of the DNA.

2.7. Particle sizes and zeta potential measurement

The particle sizes and zeta potential measurements of polymer/DNA complexes were made using an Electrophoretic Light Scattering Spectrophotometer (ELS8000, Otsuka Electronics, Osaka, Japan), with 90 and 20° scattering angles, respectively. Polymer/DNA complexes were prepared in water from N/P ratios 1–30.

2.8. Atomic force microscopy (AFM)

The morphologies of the PEA/DNA complexes were analyzed using scanning probe microscopy (Autoprobe CP™, PISA, USA). The samples were prepared by mixing 0.1 μg of plasmid DNA with aqueous polymer solutions at various N/P ratios. After 30 min incubation complex solutions were placed on freshly cleaved mica surface and allowed to stick for specified time, followed by drying at room temperature for 24 h. Non-contact mode was used for the imaging along with V-shaped cantilevers and silicon pyramidal tip.

2.9. Protection and release assay of DNA

Polymer/DNA complexes and free DNA (0.2 μg) were separately incubated with DNase-I (1 unit) in DNase/Mg^{2+} digestion buffer consisting of 50 mM Tri-Cl, pH 7.6 and 10 mM $MgCl_2$ at 37 °C for 30 min as previously described [19]. DNase-I digestion was followed by treatment of complexes with 4 μl of 250 mM EDTA for 10 min to inactivate DNase-I and samples were mixed with sodium dodecyl sulfate (SDS) in 0.1 M NaOH (pH 7.2) at a final concentration of 1 wt%. Finally, samples were incubated at room temperature for 2 h and were run electrophoretically using 0.8% agarose gel in TAE running buffer at 100 V for 1 h.

2.10. Cell viability assays

Cell viability of PEA/DNA complexes was investigated by Cell Titer 96® AQueous One Solution Cell Proliferation Kit (Promega). Cells seeded