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Well-defined reducible cationic nanogels based on functionalized low-molecular-weight PGMA for effective pDNA and siRNA delivery

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ABSTRACT

Nucleic acid-based gene therapy is a promising treatment option to cure numerous intractable diseases. For non-viral gene carriers, low-molecular-weight polymeric vectors generally demonstrate poor transfection performance, but benefit their final removals from the body. Recently, it was reported that aminated poly(glycidyl methacrylate) (PGMA) is one potential gene vector. Based on ethylenediamine (ED)-functionalized low-molecular-weight PGMA (denoted by PGED), a flexible strategy was herein proposed to design new well-defined reducible cationic nanogels (denoted by PGED-NGs) with friendly crosslinking reagents for highly efficient nucleic acid delivery. α-Lipoic acid (LA), one natural antioxidant in human body, was readily introduced into ED-functionalized PGMA and crosslinked to produce cationic PGED-NGs with plentiful reducible lipoyl groups. PGED-NGs could effectively complex plasmid DNA (pDNA) and short interfering RNA (siRNA). Compared with pristine PGED, PGED-NGs exhibited much better performance of pDNA transfection. PGED-NGs also could efficiently transport MALAT1 siRNA (siR-M) into hepatoma cells and significantly suppressed the cancer cell proliferation and migration. The present work indicated that reducible cationic nanogels involving LA crosslinking reagents are one kind of competitive candidates for high-performance nucleic acid delivery systems.

Statement of Significance

Recently, the design of new types of high-performance nanoparticles is of great significance in delivering therapeutics. Nucleic acid-based therapy is a promising treatment option to cure numerous intractable diseases. A facile and straightforward strategy to fabricate safe nucleic acid delivery nanovectors is highly desirable. In this work, based on ethylenediamine-functionalized low-molecular-weight poly(glycidyl methacrylate), a flexible strategy was proposed to design new well-defined reducible cationic nanogels (denoted by PGED-NGs) with α-Lipoic acid, one friendly crosslinking reagent, for highly efficient nucleic acid delivery. Such PGED-NGs possess plentiful reducible lipoyl groups, effectively encapsulated pDNA and siRNA and exhibited excellent abilities of nucleic acid delivery. The present work indicated that reducible cationic nanogels involving α-lipoic acid crosslinking reagents are one kind of competitive candidates for high-performance nucleic acid delivery systems.

1. Introduction

Nucleic acid-based gene therapy is one promising method to treat numerous intractable diseases such as cancers [1,2]. Plasmid DNA (pDNA)-based therapy functions through precisely controlling the biosynthesis of curative proteins, while short interfering RNA (siRNA)-based therapy involves the silence of objective messenger RNA or mutation of genes related to cancer cell proliferation and vasculogenesis [3–7]. Polycations as the major non-viral vectors had been widely used to protect nucleic acid (NA) and improve NA delivery efficiency [8,9]. Diverse methods brought about abundant kinds of NA delivery carriers including polyethylenimine (PEI) derivatives [10,11], polysaccharide-based polycations [12–14],

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nanostructured candidates [15–17], and other vectors [18–22]. However, the design of ideal polycation-based vectors with high transfection efficiency and low toxicity is still challenging. One way was to build up cationic nanogels (NGs) [23]. NGs could be loaded with proteins [24], small molecules [25], and carbohydrates [26]. PEI-based NGs with biscarbamate crosslinkages exhibited enhanced transfection activities [27].

It was well known that the redux potential between extracellular and intracellular milieu differs considerably. The concentration of glutathione in intracellular milieu is 50–1000 folds as high as that in extracellular milieu, readily inducing the cleavage of disulfide linkages in cells [28,29]. The degradable NGs containing disulfide bonds were synthesized for unhindered release of pDNA [28], siRNA [30] or drugs [31]. α-Lipoic acid (LA) with one disulfide bond is one natural antioxidant with beneficial anti-obesity properties in human body [32]. The lipoylation of PEI distinctly promoted the ability to compress pDNA and cellular internalization [33]. The hydrophobic lipoyl groups easily turned into hydrophilic dihydrolipoyl groups under the intracellular reductive environment, facilitating pDNA release. LA also could be used as a friendly crosslinking reagent. The reversibly crosslinked nanoparticles from lipoylated dextran could efficiently deliver the entrapped anticancer drugs [31]. The design of reducible cationic NGs by using LA crosslinking reagents is worth being explored for improving gene delivery systems.

Recently, it was reported that aminated-functionalized poly (glycidyl methacrylate)s (PGMAs) with plentiful cationic and hydroxyl groups are the promising gene vectors [17,34–36]. It was noted that similar to other polycationic vectors, the transfection performances of PGMA-based vectors were also dependent on the high molecular weights. However, linear PGMA-based vectors were non-degradable, and relatively high toxicity could be induced because of high molecular weight. Thus, low-molecular-weight PGMA-based vectors with good transfection performances are highly desirable. In this work, based on ethylenediamine (ED)-functionalized low-molecular-weight PGMA (denoted by PGED), new well-defined reducible cationic nanogels (PGED-based NGs, denoted by PGED-NGs) were flexibly designed for highly efficient nucleic acid delivery (Fig. 1). The simple consecutive reduction and oxidation reactions were used to crosslink PGED with LA reagents, producing reducible PGED-NGs with plentiful disulfide linkages. The proposed PGED-NGs were used to deliver pDNA and siRNA and characterized through series of experiments including NA condensation ability, cytotoxicity, transfection efficiency, cellular uptake, quantitative-polymerase chain reaction (qPCR), and cell migration assays.

2. Experimental section

2.1. Materials

Ethyl 2-bromoisobutyrate (EBA, 98.0%), anhydrous dimethyl sulfoxide (DMSO, 99.8%), copper(I) bromide (CuBr, 99%), glycidyl methacrylate (GMA, 97%), triethylamine (TEA, 99%), ethylenediamine (ED, 99%), *N, N, N′, N′, N″*-pentamethyl diethylenetriamine (PMDETA, 99%), α-lipoic acid (LA, 99%), 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide hydrochloride (EDCI, 98%), 4-dimethylaminopyridine (DMAP, 99%), sodium borohydride ($NaBH_4$, 98%), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), penicillin, and streptomycin were purchased from Sigma-Aldrich Chemical Co. HEK293, COS7, and HepG2 cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD). Plasmid pRL-CMV encoding Renilla luciferase and plasmid pEGFP-N1 encoding enhanced green fluorescent protein (EGFP) were obtained from Promega Co., Cergy Pontoise, France and BD Biosciences, San Jose, CA, respectively. The both pDNA were amplified in *Escherichia coli* and purified according to the supplier's protocol (Qiagen GmbH, Hilden, Germany). Diethyl pyrocarbonate (DEPC), negative control siRNA (denoted as siR-NC: sense sequence, UUCUCCGAACGUGUCACGUTT; antisense sequence, ACGUGACACGUUCGGAGAATT) and metastasis-associated lung adenocarcinoma transcript 1 (MALAT1)-Homo-6108 siRNA (denoted as siR-M: sense sequence, CCCUCUAAAUAAGGAAUAATT; antisense sequence, UUAUU CCUUAUUUAGAGGGTT) were bought from GenePharma Co., Shanghai, China.

2.2. Synthesis of PGMA and its amination

Linear PGMAs with two molecular weights were prepared via atom transfer radical polymerization (ATRP) according to our earlier work [37]. Briefly, EBA (180 μL) was added into a 50 mL round-bottom flask containing 10 mL of DMSO. Then, GMA, PMEDTA and CuBr were successively dissolved into the above solution under the degassing conditions to achieve the feed molar ratio [EBA, 180 μL (or 90 μL)]:[GMA]:[CuBr]:[PMDETA] of 1:45:0.2:0.4 for PGMA1 (or 1:90:0.4:0.8 for PGMA2). The polymerization proceeded under nitrogen atmosphere for 4 h, followed by precipitation with methanol and desiccation in vacuum. The corresponding final yields were 2.8 g for PGMA1 and 3.0 g for PGMA2.

PGED was prepared as follows. The above obtained PGMA (500 mg) was first dissolved in DMSO, and then, excess ED (20 times the molar amount of the epoxy groups of PGMA) was added. The reaction proceeded at 80 °C for 40 min under nitrogen atmosphere. The reaction solution was diluted with excess deionized (DI) water (10 times amount of reaction solution) and dialyzed against DI water (6 × 5 L) for 48 h with dialysis membranes (MWCO 3.5 kDa). The final PGED (~530 mg) was obtained via lyophilization.

2.3. Synthesis of cationic nanogels

PGED was first dissolved into anhydrous DMSO via ultrasound. Then, the predetermined amount of LA was dissolved into the above DMSO containing EDCI (equivalent to the molar amount of LA) and catalytic amount of DMAP. After 24 h under continuous stirring at 60 °C, one portion of the system was extracted for intermediate product LA-functionalized PGED (PGED-LA), and the remaining was stirred for additional 2 h in the presence of $NaBH_4$ (8 times the molar amount of LA) and bubbling oxygen for another 2 h at room temperature to produce PGED-NG. The purified PGED-LA and PGED-NG were obtained via dialysis and lyophilization similar to the procedures of PGED.

2.4. Polymer characterization

Gel permeation chromatography (GPC) was used to determine the molecular weight of PGMA. The GPC system was equipped with a Waters 1515 isocratic HPLC pump, two phenogel columns containing DMSO eluent and a Waters 2414 refractive index detector. The calibration curve was generated with series of monodispersed polyethylene glycol (PEG) standards. The ^1H nuclear magnetic resonance (NMR) spectra of polymers were obtained with a Bruker ARX 400 MHz spectrometer for the chemical structures by using $CDCl_3$ (for PGMA) and D_2O (for PGED and PGED-LA) as solvents.

2.5. Biophysical characterization of complexes

For bulk solutions, PGED and PGED-NG were respectively dissolved in sterile water to achieve 10 mM of nitrogen (N) moiety. The bulk pDNA solution was at the concentration of 0.1 μg/μL, and the corresponding phosphate (P) moiety concentration was

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