Acta Biomaterialia 54 (2017) 69–80

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Photoluminescent and biodegradable polycitrate-polyethylene glycol-polyethyleneimine polymers as highly biocompatible and efficient vectors for bioimaging-guided siRNA and miRNA delivery

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ARTICLE INFO

Article history:
Received 17 October 2016
Received in revised form 15 February 2017
Accepted 16 February 2017
Available online 20 February 2017

Keywords:
Poly(1,8-octanedio-citric acid)-co-poly (ethylene glycol)
Polyethylenimine
Photoluminescence
Non-virus vectors
Bioimaging
Gene delivery

ABSTRACT

Development of biodegradable and biocompatible non-viral vectors with intrinsical multifunctional properties such as bioimaging ability for highly efficient nucleic acids delivery still remains a challenge. Here, a biodegradable poly (1,8-octanedio-citric acid)-co-polyethylene glycol grafted with polyethyleneimine (PEI) (POCG-PEI) polymers with the photoluminescent capacity were synthesized for nucleic acids delivery (siRNA and miRNA). POCG-PEI polymers can efficiently bind various nucleic acids, protect them against enzymatic degradation and release the genes in the presence of polyanionic heparin. POCG-PEI also showed a significantly low cytotoxicity, enhanced cellular uptake and high transfection efficiency of nucleic acids, as compared to commercial transfection agents, lipofectamine 2000 (Lipo) and polyethylenimine (PEI 25K). POCG-PEI polymers demonstrate an excellent photostability, which allows for imaging the cells and real-time tracking the nucleic acids delivery. The photoluminescent property, low cytotoxicity, biodegradation, good gene binding and protection ability and high genes delivery efficiency make POCG-PEI highly competitive as a non-virus vector for genes delivery and real-time bioimaging applications. Our results may be also an important step for designing biodegradable biomaterials with multifunctional properties towards bioimaging-guided genes therapeutic applications.

Statement of Significance

Here, a biodegradable poly (1,8-octanedio-citric acid)-co-polyethylene glycol grafted with polyethyleneimine (PEI) (POCG-PEI) polymers with controlled photoluminescent capacity were synthesized for nucleic acids delivery (siRNA and miRNA). POCG-PEI polymers can efficiently bind various nucleic acids, protect them against enzymatic degradation and release the genes in the presence of polyanionic heparin. POCG-PEI also showed a significantly low cytotoxicity, enhanced cellular uptake and high transfection efficiency of nucleic acids, as compared to commercial transfection agents, lipofectamine 2000 (Lipo) and polyethylenimine (PEI 25K). POCG-PEI polymers demonstrate an excellent photostability, which allows for imaging the cells and real-time tracking the nucleic acids delivery. Our results may be also an important step for designing biodegradable biomaterials with multifunctional properties towards bioimaging-guided genes therapeutic applications.

1. Introduction

The occurrence of diseases is usually closely related with the change of gene structure or its function, and gene-based therapies have become one of the attractive methods to restore tissue

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http://dx.doi.org/10.1016/j.actbio.2017.02.034
1742-7061/

functions (tissue regeneration) and treat some diseases such as cancers [1]. Antisense DNA, small interfering RNA (siRNA) and microRNA (miRNA) have been widely investigated in cancer therapy and tissue regeneration [2–4]. Gene therapy is carried out by delivering the therapeutic genes into the specific target cells for correcting the genetic defects or promote/inhibit the expression of the target protein [5]. The main obstacles to prevent the successful applications of gene therapy include poor delivery and transfection, cytotoxicity, and the rapid degradation under serum nucleases [6]. Therefore, the development of suitable vectors for highly efficient gene delivery is very necessary.

Ideal gene vectors should possess biodegradability, good biocompatibility, high transfection efficiency and target specificity [7]. Conventional viral vectors showed high efficiency in delivering and transfecting genes, but suffered from some risks of tumorigenesis, inflammatory response and mutagenesis [8,9]. Thus, non-viral vectors including cationic polymers and liposomes and inorganic particles, have been developed, due to their safety and easy fabrication [10–12]. However, these conventional non-viral vectors often showed low delivery efficiency, high cytotoxicity and non-biodegradability [13]. On the other hand, most of current gene vectors do not have the imaging ability which allows them real-time tracking the materials and gene delivery [14]. In addition, the non-biodegradation of cationic polymers and inorganic particles may prevent them large-scale *in vivo* application because of their potential long-term tissue toxicity [15,16]. Therefore, there is an urgent need for developing biodegradable novel vectors with imaging ability and high delivery efficiency [17].

Poly (1,8-octanedio-co-citric acid) (POC)-based polymers have been extensively investigated for tissue regeneration and bioimaging applications due to their linear biodegradation, high biocompatibility and low cost [18]. In previous studies, our group developed a series of highly elastomeric POC-Siloxane hybrid polymers and demonstrated their excellent biodegradation and biocompatibility *in vitro* and *in vivo* [19–21]. However, conventional POC-based polymer is not water-soluble and does not show any characteristics of positive charges, which limit their promising applications in gene delivery. As a biocompatible polymer, polyethylene glycol (PEG) has been widely used to modify hydrophobic polymers for improving their solubility and the circulation time *in vivo* [22,23]. On the other hand, polyethyleneimine (PEI)-based polymers have been regarded as one of effective gene vectors among various cationic polymers, due to their high surface positive charge density and escape ability from endosomes via the "proton sponge effect" [24,25]. Despite the high transfection efficiency of PEI with high molecular weights in gene delivery, the strong charge density and poor biodegradation make them high cytotoxicity [26]. PEI with the low molecular weight exhibited low cytotoxicity but presented low gene transfection ability. Therefore, in recent years, developing novel biodegradable copolymers based on PEI with low molecular weight has shown much interest in non-viral gene therapy [27–29].

In this study, we report the synthesis of POC-co-PEG grafted with PEI (POCG-PEI) and investigate their performance as novel gene vectors and bioimaging agents. The synthesis conditions and physicochemical structure of POCG-PEI were studied in detail. Furthermore, the buffering capacity, photoluminescent ability, cytotoxicity of POCG-PEI and their transfection efficiency for various genes (siRNA and miRNA) were demonstrated particularly. To demonstrate the potential applications in cancer therapy and bone tissue regeneration, herein, a siRNA targeting VEGF and miRNA for osteogenic differentiation was employed respectively. We also present the potential cellular imaging application of POCG-PEI to show their non-invasive monitoring ability for gene delivery.

2. Materials and methods

2.1. Materials

Citric Acid (99%), 1,8-Octanediol (98%), polyethylene glycol (PEG) (1 kDa), branched polyethylenimine (PEI) (600 Da, 1.8 kDa, 10 kDa and 25 kDa), tetrahydrofuran (THF), sodium hydroxide (NaOH), hydrochloric acid (37%) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Sigma-Aldrich. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 99%), N-Hydroxysuccinimide (NHS, 98%) and 2-(N-Morpholino) ethanesulfonic Acid (MES, 99%) were obtained from J&K Scientific. Phosphate buffered saline (PBS), Dulbecco's Modified Eagle Medium (DMEM), 4′-6-diamidino-2-phenylindole (DAPI), LIVE/DEAD staining kit, Alamar Blue kit and Lipofectamine™ 2000 (Lipo) were bought from Invitrogen. The DNA from herring sperm was supplied from Sigma-Aldrich. The negative siRNA and Cy3-siRNA (Cy3-labeled) were made by RiboBio Co., Ltd. The miRNA and Fam-miRNA (Fam-labeled) were supplied from GenePharma. The cells used in this study were obtained from cell bank in Chinese Academy of Sciences. The primary rat preadipocytes (rPA) were purchased from Pricells Biotechnology in China. All chemicals were used as received without further treatment.

2.2. Synthesis of POCG

The POCG was synthesized using citric acid (CA), 1,8-octanediol (OD) and polyethylene glycol (PEG) by a melt-derived polymerization at the different molar ratios (Table S1). Briefly, monomers of CA, OD and PEG were melted at 160 °C for 20 min with a constant flow of nitrogen gas and reacted at 140 °C for another 5 h under vacuum with stirring. The resulted POCG was purified through dialysis of 2 days using a dialysis tube (MWCO 3500). The final POCG was freeze-dried and stored for the subsequent experiments. To ensure the water solubility of POCG, POCG polymer with 60 wt% PEG was used to synthesize POCG-PEI.

2.3. Synthesis of POCG-PEI

The POCG-PEI with different molecular weights of PEI were synthesized using POCG and branched polyethylenimine (PEI) under catalytic reaction of EDC and NHS in MES buffer (50 mM, pH 5–6). The various samples were denoted as POCG-PEI 600, POCG-PEI 1.8K and POCG-PEI 10K respectively. Briefly, carboxyl groups of POCG were activated by EDC in MES buffer for 30 min, and then reacted with the amine group of PEI under stirring for 24 h at room temperature. The resulted POCG-PEI was purified by dialyzed using a dialysis tube (MWCO 3500) for 2 days, followed by freeze-drying.

2.4. Characterizations of POCG and POCG-PEI

The chemical structure of POCG, POCG-PEI 600, POCG-PEI 1.8K and POCG-PEI 10K were characterized by the ^{1}H nuclear magnetic resonance (^{1}H NMR) instrument (Ascend 400 MHZ, Bruker) and Fourier transformation infrared (FT-IR) spectroscopy (NICOLET 6700, Thermo). Briefly, the polymers were dissolved in heavy water (D_2O) with a concentration of 40 mg/mL and ^{1}H NMR spectra were reported at 25 °C. The actual monomers ratios of polymers were determined from ^{1}H NMR. FT-IR was analyzed using a KBr slice method and the spectra were obtained from 4000 to 400 cm^{-1} at a scan resolution of 4 cm^{-1}.