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Biodegradable gadolinium-chelated cationic poly(urethane amide) copolymers for gene transfection and magnetic resonance imaging

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ABSTRACT

Theranostic nano-polyplexes containing gene and imaging agents hold a great promise for tumor diagnosis and therapy. In this work, we develop a group of new gadolinium (Gd)-chelated cationic poly(urethane amide)s for gene delivery and T_1-weighted magnetic resonance (MR) imaging. Cationic poly(urethane amide)s (denoted as CPUAs) having multiple disulfide bonds, urethane and amide linkages were synthesized by stepwise polycondensation reaction between 1,4-bis(3-aminopropyl)piperazine and a mixture of di(4-nitrophenyl)-2, 2′-dithiodiethanocarbonate (DTDE-PNC) and diethylenetriaminepentaacetic acid (DTPA) dianhydride at varied molar ratios. Then, Gd-chelated CPUAs (denoted as GdCPUAs) were produced by chelating Gd(III) ions with DTPA residues of CPUAs. These GdCPUAs could condense gene into nanosized and positively-charged polyplexes in a physiological condition and, however, liberated gene in an intracellular reductive environment. In vitro transfection experiments revealed that the GdCPUA at a DTDE-PNC/DTPA residue molar ratio of 85/15 induced the highest transfection efficiency in different cancer cells. This efficiency was higher than that yielded with 25 kDa branched polyethylenimine as a positive control. GdCPUAs and their polyplexes exhibited low cytotoxicity when an optimal transfection activity was detected. Moreover, GdCPUAs may serve as contrast agents for T_1-weighted magnetic resonance imaging. The results of this work indicate that biodegradable Gd-chelated cationic poly(urethane amide) copolymers have high potential for tumor theranostics.

1. Introduction

Nucleic acid-based cancer therapy is an alternative strategy to routine chemotherapy [1]. For successful nucleic acid-based cancer therapy, highly efficient delivery of nucleic acids into targeted somatic cells in a bio-safe way is still a challenge. Although engineered viral vectors encoded with nucleic acids can robustly infect human cells, they are associated with a few uncertain bio-safety issues such as uncontrolled mutagenesis, immunogenicity and oncogenicity, thus impeding their clinical translations [2]. Alternatively, non-viral vectors based on cationic polymers reveal high potential for relatively safe gene delivery as a result of their low toxicity, facile synthesis and high nucleic acid-carrying capacity [3]. Accordingly, over the past two decades, a number of polymeric vectors for non-viral gene delivery have been widely studied [4]. A lot of studies have confirmed a massive promise of cationic polymer-nucleic acid assembled complexes (also called as polyplexes) for cancer gene therapy [5].

The development of nano-carriers for tumor imaging and therapy has received increasing interest in recent years [6]. From clinical point of view, such dual-function nano-carriers allow clinical physicians to accurately evaluate anti-cancer efficacy and choose balanced drug dose in time. As such, a variety of theranostic nano-carriers have been designed which combine both therapeutic functions (chemotherapy and gene therapy) and single-/multi-imaging modalities [7]. It should be however noted that most of these theranostic nano-carriers reported are based on inorganic nanoparticles [8]. Only few literatures have focused on theranostic polymers for gene therapy and imaging such as magnetic resonance (MR) imaging. For example, Bryson et al. reported on Gd-chelated polycations containing oligoamines for gene delivery and MR imaging [9]. In another work, Wu et al. designed polymethacrylamide-based cationic polymers having europium-labeled residues for MR imaging [10]. Recently, Xue et al. showed a group of Gd-chelated polycations for siRNA delivery [11]. However, these theranostic polymers usually lack biodegradability, thus probably hampering their potential of clinical translations.

An ideal theranostic polymer should offer efficient gene transfection and accurate imaging. Importantly, such polymer should be essentially

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low toxic against the cells and the body. In order to generate efficient and low-toxic polymers for gene delivery, disulfide-containing (reducible) cationic polymers for gene delivery have recently received rapid growing interest [12]. A lot of studies confirm that reducible polyethylenimine, polylysine and polyamides may induce superior transfection efficacy to their non-reducible counterparts and, meanwhile, are associated with a minor cytotoxicity [12]. This low toxicity is due to intracellular cleavaging of disulfide linkage by glutathione (0.5–10 mM) [13], leading to intracellular degradation of reducible polymers and thus reduced toxicity. Reducible polymers thus represent suitable non-viral carriers for safe and efficient gene delivery. However, to our best knowledge, no report have presented on reducible cationic polymers for gene delivery and MR imaging. It would be thus meaningful to develop such reducible cationic polymers and evaluate their feasibility for theranostics.

We recently reported on reducible cationic polyurethanes synthesized by non-isocyanate chemistry and found that these polyurethanes possessed low toxicity and high ability for gene transfection towards tumor cells [14]. Herein, we aim to design and prepare theranostic reducible cationic polyurethanes for gene delivery and imaging. To this end, a group of linear reducible cationic poly(urethane amide)s (denoted as CPUAs) are designed and prepared that comprise multiple disulfide bonds, tertiary amine groups and diethylenetriaminepentaacetic acid (DTPA) residues. The DTPA residue allows for complexation with Gd(III) ions to generate Gd-chelated CPUAs (denoted as GdCPUAs). We hypothesized that GdCPUAs would be practical as non-viral vectors for gene delivery and MR imaging. We present synthesis and characterization of GdCPUAs and the properties of GdCPUA-based polyplexes in terms of particle size, surface charge, magnetism, and gene release ability. Transfection ability and cytotoxicity of GdCPUA polyplexes are evaluated in vitro against cancer cells. Moreover, T_1-weighted MR imaging by using GdCPUAs as contrast agents is investigated.

2. Materials and methods

2.1. Materials

All chemicals were used directly as received unless otherwise statement. 2,2′-Dithiodiethanol (DTDE), gadolinium chloride ($GdCl_3$), 4-nitrophenyl chloroformate (PNC), dithiothreitol (DTT), 2-[4-(2-hydroxyethyl)piperazin-1-yl] ethane sulfonic acid (HEPES), branched polyethylenimine (BPEI, M_w = 25 kDa), 1,4-diethylenetriaminepentaacetic acid (DTPA) dianhydride, and bis(3-aminopropyl)piperazine (BAP) were ordered from Sigma-Aldrich Co. (USA). Lipofectamine 2000 transfection agent was ordered from Life Technology (USA). The plasmids encoding for green fluorescent protein (GFP) and luciferase (Luc), also termed as pCMV-GFP and pCMV-Luc, respectively, were obtained from plasmid factory (Bielefeld, Germany). shRNA-VEGF plasmid was purchased from InvivoGen (USA). Di(4-nitrophenyl)-2,2′-dithiodiethanocarbonate (DTDE-PNC) was prepared according to our previous report [14].

2.2. Synthesis and characterization of Gd-chelated polymers

Gd-chelated reducible cationic poly(urethane amide) (GdCPUA) polymers was obtained by a two-step procedure. First, a group of cationic poly(urethane amide)s (CPUAs) were obtained by polymerization reaction between BAP and a mixture of DTDE-PNC and DTPA dianhydride at varied molar ratios (i.e. 85/15, 70/30, 50/50). In a typical procedure for the synthesis of CPUA30 at a DTDE-PNC/DTPA anhydride ratio of 70/30, DTDE-PNC (0.5 g, 1.03 mmol), BAP (0.30 g, 1.47 mmol) and DTPA anhydride (0.17 g, 0.44 mmol) were dissolved in anhydrous DMSO (5 mL) as a solvent in a brown-colour round flask. After running for 5 days at 40 °C in nitrogen atmosphere, the reaction was run for another 2 days after adding an excess amount of BAP (0.03 g). Finally, resulting solution was purified by ultrafiltration process (3 kDa cut-off) with acidic deionized water (3 × 5 L, pH 4.5). CPUA30 was obtained as a white solid after freeze-drying (yield: 0.3 g, 45%). Chemical composition of CPUAs was tested by 1H NMR spectrum meter (Varian, USA). Next, GdCPUAs were yielded by Gd(III) chelation with DTPA residues in CPUAs. As an example for the preparation of GdCPUA30, CPUA30 (50 mg) was dissolved in 20 mL of deionized water. Next, $GdCl_3$ (~4 mmol) in deionized water (5 mL) was dropwise added into CPUA30 solution. During mixing process, the solution became turbid but transparent again after pH was set to pH 6.5 with 4 M HCl. The resulting solution was stirred for 4 h and then transferred in dialysis bag (1 kDa cut-off) for exhaustive dialysis in deionized water at pH ~6. GdCPUA30 was obtained as a solid powder after freezing-drying (yield: 0.35 g, 67%). GdCPUAs were analyzed by inductively coupled plasma (ICP) system to determine Gd content after dissolving these polymers in 4 M $H2NO_3$.

2.3. Preparation and characterization of GdCPUA based polyplexes

The polyplexes of GdCPUA copolymers were prepared by gently mixing plasmid DNA with GdCPUAs at N/P ratios in HEPES buffer (20 mM, pH 7.4) [15]. Particle size and surface charge of GdCPUA polyplexes were determined by dynamic light scattering (DLS) analysis (Nanosizer NS90, Malvern, UK) at 25 °C. Besides, the polyplex solution was analyzed by agarose gel electrophoresis using the method reported previously [16]. To characterize gene release behavior in a reductive environment, the polyplexes were co-incubated with DTT (5 mM) in the HEPES buffer for 30 min, analyzed by the gel electrophoresis, DNA band was visualized by TanonGel Image system (Shanghai, P.R. China).

Relaxation time (T_1) of GdCPUAs and their polyplexes was measured using a contrast agent relaxation rate analysis system (PQ100, NIUMAG, Shanghai, P.R. China) according to the manufacture's protocol. A clinical T_1-contrast agent, Magnevist (Gd-DTPA complex), was used as a positive control.

T_1-weighted MR imaging with the polyplexes of GdCPUA copolymers was performed by running Siemens Magnetom Verio 3.0 T MR instrument. In brief, the polyplexes were prepared in HEPES buffer (pH 7.4) in EP tube at different Gd concentrations ranging from 0.1 mM to 0.8 mM. MR imaging was obtained by placing the tubes in an in-house built 10-cm RF coil installed in the instrument according to the manufacturer's instruction.

2.4. Cell culture, transfection test and cytotoxicity assay in vitro

SKOV-3 cells (ATCC, USA) were cultured in Mccyo's-5α medium culture medium (GIBCO) having 10% FBS, 100 U/mL penicillin and streptomycin (GIBCO). MCF-7, HepG2 and A549 cells were cultured in DMEM complete medium. The cells were grown at 37 °C in cell incubator (Thermo Scientific, Waltham, MA, USA) supplied with 5% CO_2.

Transfection activity of GdCPUA copolymers in tumor cells was evaluated according to our previous transfection protocol [16]. In brief, the cells ($5–7 \times 10^4$ cells/well) were cultured in a 24-well plate for 24 h until 60–70% cell confluence was reached. GdCPUA polyplexes containing pCMV-GFP plasmid (1 μg) in a HEPES buffer (20 mM, pH 7.4) were then prepared at varied N/P ratios. The transfection tests were performed by adding the polyplex solution in each well and co-incubating with the cells for 1 h in the absence or presence of 10% FBS. The cells were cultured in complete medium for another 24 h. Besides, a formulation of BPEI polyplexes at an N/P ratio of 10/1 and lipofectamine 2000 were used as positive controls. Those cells without transfection (untreated cells) were considered as a blank (negative) control. The same protocol was applied in the transfection with GdCPUA polyplexes containing shRNA-VEGF plasmid. VEGF protein and mRNA level were analyzed by using ELISA kit (Abcam, USA) and RT-PCR, respectively.

Transfection efficiency was determined by testing relative fluorescence intensity of GFP expressed in the cells according to our previous