Polymer 134 (2018) 53–62

Contents lists available at ScienceDirect

Polymer

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

Ring-opening polymerization of diepoxides as an alternative method to overcome PEG dilemma in gene delivery

Ya-Ping Xiao [a], Ji Zhang [a, *], Yan-Hong Liu [a], Xiao-Chuan Chen [b], Qing-Ying Yu [a], Chao-Ran Luan [a], Ju-Hui Zhang [a], Xi Wei [b, **], Xiao-Qi Yu [a, ***]

[a] Key Laboratory of Green Chemistry and Technology (Ministry of Education), College of Chemistry, Sichuan University, Chengdu 610064, China
[b] Department of Operative Dentistry and Endodontics, Guanghua School of Stomatology, Institute of Stomatological Research, Sun Yat-sen University, Guangzhou 510055, China

ARTICLE INFO

Article history:
Received 2 June 2017
Received in revised form
12 September 2017
Accepted 22 November 2017

Keywords:
Non-viral gene vector
Diepoxide ring-opening polymerization
PEG dilemma
Polyethylenimine
Biocompatibility

ABSTRACT

Gene therapy has shown great potential in the treatment of various diseases, and the development of low-toxic and efficient gene carriers is still one of the burning issues for gene therapy. PEGylation is generally regarded as an efficient strategy to improve the biocompatibility of non-viral vectors. However, PEGylation also led to several problems such as lower cellular uptake, difficult endosomal escape and much reduced transfection efficiency, which was described as “PEG dilemma”. We herein devote to the development of polymeric gene vectors that can overcome such dilemma via diepoxide ring-opening polymerization (DEROP) of low molecular weight PEI, and unmodified PEI and PEG-grafted PEI were used for comparison. Transfection experiments with various concentrations of serum revealed that the DEROP polymers gave much higher transfection efficiency together with better serum tolerance than PEI and PEGylated ones. Protein adsorption and flow cytometry assays further proved their better serum resistance. Besides, confocal microscopy suggested that the polyplexes formed from these polymers could escape from endosome/lysosome more efficiently than those derived from PEGylated material. *In vivo* biochemical studies also reflect good biocompatibility of the DEROP polymers. Results demonstrate that such polymer construction strategy gave cationic materials with both high transfection efficiency and improved biocompatibility, and afforded an effective way to overcome PEG dilemma.

1. Introduction

Since the first use of virus infection aiming to cure dementia in 1975, gene therapy has been brought into clinical application [1]. In the past few decades, gene therapy has made more excellent successes in curing hereditary diseases compared with traditional therapy and incrementally takes the place of traditional treatment of cancer [2]. To achieve better treatment effects such as enrichment of therapeutic genes in targeted areas, residence time, activity in harsh conditions, and high-level expression, one of the essential factors is to develop efficient gene carriers (vectors) [3], which were commonly divided into two categories: recombinant viruses and synthetic non-viral vectors. Virus vectors always have high transfection efficiency, however, some issues such as low DNA loading, immunogenicity, teratogenicity and production problems limit their application. Therefore, many non-viral vectors as alternatives have been developed and studied in the past few decades [4–8].

Among numerous types of non-viral vectors, cationic polymers have attracted more and more attentions on account of several unique features including facile manufacturing, high capacity of nucleic acids, good stability, easily modification, high permeability and low immunogenicity [9–13]. As one of the most studied cationic polymers, polyethylenimine (PEI) has been known for its high transfection efficiency, which was largely attributed to the “proton sponge effect” [14]. However, PEI is also facing many biosecurity challenges, especially for the *in vivo* application. For example, after systemic intake, polyplexes formed by PEI would inevitably encounter abundant blood components, such as albumin, antibodies and complements, and be easily recognized and excluded by the reticuloendothelial systems [15]. Besides, the

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: jzhang@scu.edu.cn (J. Zhang), weixi@mail.sysu.edu.cn (X. Wei), xqyu@scu.edu.cn (X.-Q. Yu).

https://doi.org/10.1016/j.polymer.2017.11.059
0032-3861/

polyplexes contact with cells with poor selectivity, leading to non-effective cellular uptake and reduced transfection efficiency [16,17]. Further, high charge density of PEI also leads to increased cytotoxicity via both necrotic and apoptotic mechanisms [18–20]. To overcome above shortages, various biodegradable linkages such as ester, amide, disulfide and acetal were introduced to conjugate low molecular weight PEI for the purpose of degradation into less toxic species [15,21,22]. However, the concern about serum stability still exists.

One popular strategy to solve this problem is to conjugate negatively charged hydrophilic polymers to PEI to provide the polyplexes with a biologically inert surface, which affords so-called "stealth ability". Among various hydrophilic polymers including natural polysaccharides and synthetic polymers, polyethylene glycol (PEG) has been generally regarded as a golden standard polymer for the stealth ability [23–26]. Polyplexes with PEG chains on the surface can significantly avoid the aggregation with blood components because of the steric repulsive effect [27,28]. However, on the other hand, the PEGylation seriously suppresses the interaction of polyplexes with the negatively charged cell membrane, resulting in much reduced cellular uptake. Moreover, PEGylation provides the polyplexes with better stability and leads to poor endosomal escape [29,30]. The double-edged property of PEG is regarded as "PEG dilemma" [31,32]. To a certain extent, the introduction of biodegradable linkers between PEG and cationic polymers may enhance the cellular uptake and endosomal escape by unloading PEG shell via degradation [33–36]. However, the transfection efficiency, which is essential for non-viral vectors, yet remained to be a problem.

Recently, our group developed linear or branched polymers by diepoxide ring-opening polymerization (DEROP), which afforded the cationic polymers with oxygen atoms from the inside out [37,38]. These oxygen atoms on the polymer backbone and hydroxyl groups can screen the positive charge of these polymers, and effectively weaken the electrostatic interactions toward serum components and avoid blood toxicity. Besides, cytotoxicity via necrotic and apoptotic mechanisms could also be reduced as a result of decreased positive charge [39,40]. Thus these materials were believed to have the ability to enhance the biocompatibility. To further confirm such advantage of materials from DEROP, i.e., the "stealth ability" together with enhanced transfection efficiency, we herein elaborately design PEI analogs from low molecular weight PEI via DEROP strategy, and unmodified PEI and PEG-grafted PEI were used for comparison. Such materials are endowed with the following characteristics: (1) reduced positive charge density without decrease of DNA condensation ability; (2) high serum stability; (3) alleviated damage on cell vitality and excellent biocompatibility; (4) increased gene transfection efficiency. We consider that such strategy is an efficient method to obtain non-viral gene vectors with enhanced properties and the ability to overcome PEG dilemma.

2. Experimental section

2.1. Materials

All chemicals and reagents unless specially noted were obtained commercially and used without further purification. Anhydrous chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2) and ethanol (CH_3CH_2OH) were dried and purified according to standard methods and were distilled immediately before use. Column chromatography was performed using 200–300 mesh silica gel. All aqueous solutions were prepared from deionized or distilled water. LMW-PEI (branched, average molecular weight 600 Da) was purchased from Aladdin (Shanghai, China). HMW-PEI (branched, average molecular weight 25 kDa) and MTS (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) were purchased from Sigma-Aldrich (St Louis, MO, USA). pGL-3 (Promega, Madison, WI, USA) coding for luciferase DNA as the plasmids and pEGFP-N1 (Clontech, Palo Alto, CA, USA) coding for eGFP DNA, were used in the study. Cy5™ was obtained from Molecular Probe (Mirus, Madison, WI, USA). The Dulbecco's Modied Eagle's Medium (DMEM), 1640 Medium and fetal bovine serum (FBS) were purchased from Invitrogen Corporation. A549 (human lung cancer cell lines), HEK293 (human embryonic kidney cell lines), and HepG2 (human hepatoma cell lines) were purchased from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. MicroBCA protein assay kit was obtained from Pierce Biotechnology (Rockford, IL, USA). The luciferase assay kit was purchased from Promega (Madison, WI, USA). The endotoxin-free (EndoFree) plasmid purification kit was purchased from TIANGEN Biotechnology (Beijing, China).

^{1}H NMR spectra were obtained on a Bruker AV400 spectrometer. The molecular weights of polymers were determined by gel permeation chromatography (GPC), which consisted of a Waters 515 isocratic HPLC pump, and Waters 2410 Refractive Index Detector. A filtered mixture of 0.5 mol L^{-1} HAc/NaAc buffer was used as the mobile phase, with a flow rate of 0.5 mL min^{-1}. Molecular weights were calculated against polyethylene glycol standards, with average molecular weights ranging from 200 to 80000.

Some of the experiments, including dynamic light scattering (DLS) assay for the measurement of particle size and zeta potential, transmission electron microscopy (TEM), ethidium bromide (EB) exclusion assay, amplification and purification of plasmid DNA, were carried out according to previously reported procedures [37].

2.2. Synthesis and characterization of target polymers

2.2.1. Synthesis and characterization of ***P1-P3***

P1-P3 were obtained according to the method previously reported [41]. 2 g of mPEG 2 kDa was dissolved in 20 mL anhydrous $CHCl_3$, which contained 4 g of isoporon diisocyanate (IPDI), and 0.36 g of dibutyltin dilaurate (DBTL). The mixture was refluxed at 75 °C for 8 h. After the reaction, the crude product was precipitated by the addition of petrol. The precipitate was collected and dried in a vacuum to get the product as white waxy solid. For synthesis of **P1**-**P3**, activated mPEG 2 kDa (approximately 1 g for **P1**, 0.4 g for **P2**, 0.2 g for **P3**) and PEI 25 kDa (about 1 g) were dissolved in anhydrous $CHCl_3$. The reaction mixture was further stirred at 65 °C for 16 h. After the reaction, the crude products were precipitated by diethyl ether. The precipitates were collected and dried by using vacuum to get the product as white waxy solid or semi-solids. The number of PEG modified on each PEI 25 kDa were determined from ^{1}H NMR spectra using integral values obtained from the number of -CH_2CH_2O- protons of PEG and -CH_2CH_2NH- protons of PEI.

P1. 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ: 3.57 (s, 1.2H, -CH_2CH_2O-), 2.48–2.73 (m, 1H, -CH_2CH_2NH-). Average PEG units on each PEI molecule: 14.6. GPC: Mw = 30 kDa, PDI = 1.90.
P2. 78.5% yield. ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ: 3.57 (s, 0.19H, -CH_2CH_2O-), 2.48–2.77 (m, 1H, -CH_2CH_2NH-). Average PEG units on each PEI molecule: 2.2. GPC: Mw = 28.2 kDa, PDI = 1.48.