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Alginate-based cancer-associated, stimuli-driven and turn-on theranostic prodrug nanogel for cancer detection and treatment

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ABSTRACT

Alginate-based cancer-associated, stimuli-driven and turn-on theranostic prodrug nanogels were designed for the tumor diagnosis and chemotherapy, by crosslinking the folate-terminated poly(ethylene glycol) (FA-PEG-NH_2) and rhodamine B (RhB)-terminated poly(ethylene glycol) (RhB-PEG-NH_2) modified oxidized alginate (OAL-*g*-PEG-FA/RhB) with cystamine (Cys), followed covalent conjugation of doxorubicin (DOX) via acid-labile Schiff base bond. Owing to the surface folic acid (FA) groups, disulfide crosslinking structure and Schiff base conjugation for DOX, the folate receptor (FR)-mediated targeting and pH/reduction dual responsive intracellular triggered release of DOX was achieved. The cytotoxicity and cellular uptake results clearly illustrated that most DOX was released and accumulated in the cell nuclei and killed the cancer cells efficaciously, due to the desirable targeting intracellular triggered release. Furthermore, the theranostic nanogels could be used for the real-time and noninvasive location tracking to cancer cells, owing to the pH-modulated fluorescence property of the pendant RhB groups.

1. Introduction

Theranostic nanoparticles, which combine both therapeutic and diagnostic functions in one dose, has promise to propel the biomedical field toward personalized medicine, especially in cancer treatment (Ma, Huang, Song, Chen, & Zhang 2016). As natural biological macromolecules, besides the benefits of diversity in size and charge, biodegradable and abundant and low toxicity in vivo (Uthaman, Maya, Jayakumar, Cho, & Park, 2014), polysaccharides have numerous advantages for theranostics: the integrated approach of therapeutics and diagnostics. Their derivable reactive groups can be leveraged for functionalization with a nanoparticle-enabling conjugate, therapeutics (small molecules, proteins, peptides, photosensitizers) and/or diagnostic agents (imaging agents, sensors) (Swierczewska, han, Kim, Park, & Lee, 2016). Furthermore, their stimuli sensitivity makes the engineering polysaccharide-based nanogels smart theranostic nanomedicine (Maya et al., 2013).

Natural polysaccharides could easily form biodegradable nanogels, owning good stability in biological fluids due to the existence of the low driving forces for their aggregation. Compared to other nanocarriers, nanogels usually have good biocompatibility, high aqueous dispersibility, and well-defined stable structure (Li, Maciel, Rodrigues, Shi, & Tomas 2015).Their flexibility and softness may allow their easier penetration ability through human skin while maintaining the bioactivity of the therapeutics in comparison with those of the corresponding rigid nanoparticles. Additionally, their flexibility can also help prolong the circulating lifetime through reducing the possibility of their entrapment by macrophages. Nanogels have been proven to be more efficiently taken up by cells than conventional nanocarriers such as liposomes, which are less stable, resulting in an improved bioavailability and safety of the therapeutics in vivo.

Alginate is one of the most abundant biodegradable natural linear polysaccharide derived from gulfweed, bacteria or seaweed of brown algae, composed of 1–4 linked α-L-guluronic (G) and β-D-mannuronic (M) acid residues. It has been recognized in biomedical applications, owing to its non-antigenicity, nontoxicity, satisfactory biocompatibility, favorable biodegradability, and pH sensitivity (Lee & Mooney, 2012). The engineering alginate-based nanogels have been reported as smart theranostic nanomedicine in the most recent years. For examples, Su and Cheng designed the highly biocompatible and safe nanocomposites composed of alginate and iron oxide nanoparticles to encapsulate doxorubicine for brain tumor therapy, which also allowed for magnetic resonance imaging (MRI) (Su & Cheng, 2015). Podgorna et al. prepared the gadolinium alginate gel nanoparticles (GdNG) by the reverse microemulsion and physical crosslinking method as the vehicles able to carry hydrophilic drugs and to be traced by the MRI (Podgorna et al.,

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2017). Lengert et al. fabricated silver alginate hydrogel microcapsules serve as microcontainer with a functional substance payload, which also demonstrated the possibility to enhance the Raman signal of up to 105 fold: a magnitude which is in the range for applications in theranostics (Lengert et al., 2017).

Additionally, the *cis*-diol groups in alginate could be oxidized, usually resulting into the cleavage of glucosidic bonds, and the resultant lower molecular-weight alginate is good for excretion (Yang, Xie, & He, 2011). In the oxidation process, two aldehyde groups are simultaneously created, enabling more modification feasibilities (Pawar & Edgar, 2012). Furthermore, the created acetal groups are susceptible to hydrolysis, endowing the oxidized alginate (OAL) tunable degradation rates (Boontheekul, Kong, & Mooney, 2005). So the OAL, which could be designed with controllable chain length and more derivable reactive groups (inherent carboxyl and hydroxyl groups, as well as created aldehyde group), shows promising potential in smart theranostic nanomedicine. Our group has designed mPEG-OAL-DOX/Cdots theranostic nanoparticles for imaging-guided drug delivery in tumor treatment, by conjugating DOX via acid-labile Schiff base linkage onto mPEG-OAL/Cdots nanoparticles that were prepared by cross-linking the PEGylated oxidized alginate (mPEG-OAL) with fluorescent carbon dots (Cdots) (Jia et al., 2016a). In these reported theranostics nanoparticles based on alginate or OAL, the diagnostic agents could generate signals in both physiological and tumor media.

Nowadays, the cancer-associated, stimuli-driven and turn-on nanotheranostics have been proposed with the capability of selectively delivering therapeutic reagents into cancer cells or tumor tissues while simultaneously generating unique signals that can be readily monitored under both in vitro and in vivo conditions (Li, Kim, Yoon, & Chen, 2017). That is to say, the diagnostic agents could only generate unique signals in the tumor tissues, which could be readily used for the real-time monitoring of the tumor tissues. For example, the nanotheranostics bearing pH-dependent fluorescent Rhodamine 6G group only possessed strong fluorescence in acidic tumor microenvironment, with very weak intensity in the physiological medium (Zhao et al., 2016). Besides, it has been demonstrated that the cancer-targeted nanotheranostics could enhance the selective targeting to tumors expressing high levels of folate receptor (FR), by conjugation of folate to the nanotheranostics (Badruddoza et al., 2013; Jia et al., 2016b).

Based on the multi-functionalities of OAL, we hypothesize that novel promising cancer-associated, stimuli-driven and turn-on theranostic prodrug nanogel could be designed via all-in-one functionalization of OAL. Inspired by the hypothesis, alginate-based multi-functional theranostic prodrug nanogels were designed for the first time in the present work by crosslinking the folate-terminated poly(ethylene glycol) (FA-PEG-NH_2) and rhodamine B (RhB)-terminated poly(ethylene glycol) (RhB-PEG-NH_2) modified oxidized alginate (OAL-*g*-PEG-FA/RhB) with cystamine (Cys), followed covalent conjugation of DOX via acid-labile Schiff base bond (Scheme 1). In the hypothesis, the designed theranostic prodrug nanogels should be stable in blood stream with low toxic and side effect because of their minimal DOX-leakage owing to the PEGylation and the acid-cleavable covalent conjugation of DOX. And they could express very weak fluorescence in such neutral media. Then they targeted in tumor tissues via EPR effect, and could be uptaken by tumor cells via FR-mediated endocytosis. As in the reductant and acidic media in the tumor microenvironment, both the disulfide crosslinking structure and Schiff base conjugation for DOX should be cleaved off, realizing the pH/reduction dual responsive intracellular triggered DOX release. Furthermore, the pendant RhB groups would express strong fluorescence in such acidic media, which could be used the real-time and noninvasive location tracking to cancer cells. Therefore, the cancer-associated, stimuli-driven and turn-on theranostic prodrug nanogels showed promising potential for cancer detection and treatment.

2. Materials and methods

2.1. Materials

Sodium alginate (AL, Chemical pure, M/G = 0.50, M_n = 35,000 and M_w = 218,000) was purchased from Xudong Chem. Co. Ltd., Beijing, China. Poly(ethylene glycol) diamine (H_2N-PEG_{2000}-NH_2, M_n = 2000, 99%) was provided by Beijing Kaizheng Biotech Development Co. Ltd.

Folic acid (FA, 97%) and rhodamine B (AR) were got from Tianjin Guangfu Technology and Development Co. Ltd. N-Hydroxysuccinimide (NHS, 98%) was obtained from Aladdin Chemistry Co. Ltd. Low molecular weight 1-(3-dimethylaminoproyl)-3-ethylcarbodiinide hydrochloride (EDC·HCl, 99%) was purchased from J& K Chemical Co. Ltd. Doxorubicin hydrochloride (DOX, 99.4%) was provided from Beijing Huafang United Technology Co. Ltd. Glutathione (GSH, 97%) was obtained from Shanghai Aladdin Reagent Co., Shanghai, China. All other reagents were analytical grade and used directly. Deionized water was used throughout the experiments.

2.2. Synthesis procedure

2.2.1. Synthesis of oxidized alginate (OAL)

Oxidized alginate (OAL) was synthesized by the periodate oxidation method as reported previously (Mu, Lu, & Liu, 2011). Typically, sodium alginate (3.000 g) was dissolved in 300 mL deionized water with vigorous stirring in the dark for 12 h to obtain a homogeneous solution. 30 mL sodium periodate ($NaIO_4$, 2.000 g, 9.35 mmol, 62 mol% relative to the alginate units) aqueous solution was added into and stirred for 24 h in the dark at room temperature. The product was separated though dialysis (MWCO of 14000) against water for 3 days in the dark and subsequently lyophilization. The degree of oxidation (DO%, defined as the number of oxidized alginate units per 100 alginate units) of OAL was analyzed as about 20%, by determining the concentration of unconsumed periodate after 6 h by iodometry (Luan et al., 2017).

2.2.2. Synthesis of rhodamine B-terminated poly(ethylene glycol) (RhB-PEG-NH_2)

Rhodamine B (0.2395 g, 0.50 mmol) was dissolved in 15 mL of anhydrous dichloromethane. After phosphorus oxychloride (0.14 mL, 1.49 mmol) was added within 5 min, the mixture was refluxed with stirring for about 4 h until colorless. The compound was obtained via rotary evaporation and dissolved in 35 mL acetonitrile. The solution was added in 25 mL NH_2-PEG_{2000}-NH_2 (1.000 g, 0.50 mmol) solution in acetonitrile containing drops of tirethylamine with vigorous stirring within 2 h, and then the reaction mixture was stirred for 24 h at room temperature. The red solid was obtained via precipitation in anhydrous ether in ice both. The product was purified though three cycles of dissolution in dichloromethane and precipitation in anhydrous ether, finally dried in vacuum at 40 °C (Li, Zhang, Hu, Cheng, & Liu 2010). ^{1}H NMR (DMSO-*d*6, δ, ppm) 8.62 (N=C***H***-), 6.63-7.66 (C***H*** of aromatic ring), 3.50 (PEG, $-CH_2C\boldsymbol{H}_2O$-), 3.34 (PEG, $-C\boldsymbol{H}_2CH_2O$-), 4.48 (-N***H***CH_2-), 2.69 (-NHC$\boldsymbol{H}_2$-), 0.99–1.73 (-C$\boldsymbol{H}_2CH_2$-,-N$\boldsymbol{H}_2$) (Fig. S1). The graft degree of RhB-PEG-NH_2 was determined as 42.3% from the ^{1}H NMR analysis.

2.2.3. Synthesis of folate-terminated poly (ethylene glycol) (FA-PEG-NH_2)

Triethylamine (0.11 mL, 0.79 mmol) and folic acid (0.2207 g, 0.5 mmol) were dissolved in 10 mL anhydrous DMSO with vigorous stirring. Then, DCC (0.1032 g, 0.5 mmol) and NSH (0.0575 g, 0.5 mmol) were added in the solution and the mixture was stirred for 8 h at room temperature in the dark. After removing the generated precipitate (dicyclohexylurea, DCU) by the filtration, 10 mL DMSO solution of NH_2-PEG_{2000}-NH_2 (2.000 g, 0.50 mmol) was added into the filtrate and the reaction was preceded for 48 h at room temperature. Subsequently, the product was purified via dialysis (MWCO of 1000)