Colloids and Surfaces B: Biointerfaces 136 (2015) 562–569

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

A pH- and thermo-responsive poly(amino acid)-based drug delivery system

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ARTICLE INFO

Article history:
Received 29 July 2015
Received in revised form
18 September 2015
Accepted 27 September 2015
Available online 1 October 2015

Keywords:
Stimuli-responsive
pH-sensitive
Thermo-responsive
Drug delivery
Poly(amino acid)

ABSTRACT

A pH- and thermo-responsive poly(amino acid)-based amphiphilic copolymer was developed, functioning as a tumour targeting drug delivery system with good biocompatibility and biodegradability. To provide multi-stimuli sensitivity characteristics to the poly(amino acid)s, the polyaspartamide scaffold has been functionalized with *N*,*N*-diisopropylamide groups via aminolysis reaction of polysuccinimide. PEG chains have also been chemically grafted to the poly(amino acid) backbone through acid-labile hydrazone linkages, providing a removable shield for the poly(amino acid) based nanoparticles. Furthermore, doxorubicin was chemically linked to the copolymer chain via hydrazone bonds, acting as the hydrophobic moiety to drive the polymeric self-assembly. Free doxorubicin molecules could be encapsulated into the self-assembled nanoparticles via hydrophobic interactions and molecular π–π stacking. The results obtained show that the drug release can be triggered by the temperature with a significantly increased release being observed under acidic conditions. The cytotoxicity behaviour of the copolymers and drug-loaded nanoparticles was investigated in vitro at varying pH values and different temperatures. In doing so, superior characteristics concerning compatibility and anti-cancer activity could be observed.

1. Introduction

In the past decades, stimuli-responsive drug delivery systems [1] have emerged and attracted considerable interest due to the fact that the release of drugs can be modulated in response to internal or external stimuli [2–4]. Among other nano-assemblies, stimulus-responsive polymeric nanoparticles provide the most promising candidates [5] for the production of drug delivery systems. This latter hypothesis is most notably due to the core–shell structures [6] exhibited by such systems and features that may enhance accumulation characteristics in tumour sites by encapsulation of a drug, protected from degradation and exhibiting a prolonged blood circulation time. Furthermore, the cytotoxicity behaviour towards healthy cells and tissues may be reduced with simultaneous advantages of enhanced delivery and therapeutic efficacy [7,8].

Select examples of stimuli include internal (e.g. pH [9], redox microenvironment [10], glucose [11]) and external stimuli (e.g. temperature [12], light [13], magnetic [14], and ultrasound [15]). The development of dual-stimuli responsive polymeric nanoparticles [5] is intriguing to further fine-tune the drug release in order to maximize the therapeutic potential and minimize potential side-effects. PH- and temperature-sensitive nanoparticles [16–18] have been the most extensively studied dual-responsive nanoparticles. Here, the pH represents one of the first and most evaluated stimuli, taking advantage of the fact that a variety of different pH gradients can be found in the body [19]. The temperature provides another stimulus that is easy to control, with practical advantages both in vivo and in vitro.

Poly(*N*-isopropylacrylamide) (PNIPAAm), able to undergo rapid and reversible thermo-responsive hydration/dehydration changes through a lower critical solution temperature (LCST) of 32 °C, has been extensively studied. Although PNIPAAm and other acrylamide related polymer species exhibit reasonably responsive behaviours in response to changes in temperature, the toxicity and non-biodegradability features under physiological conditions limit their applications in gene and drug delivery fields [20]. Efforts have been made to replace PNIPAAm with biocompatible thermo-sensitive materials. For instance, graft or block PNIPAAm based on natural polymers like pullan [20], chitosan, or poly(amino acid) moieties such as poly(L-glutamic acid) [21], and poly(L-alanine) [22], have been developed to reduce potential side effects and enhance biodegradability. Here, most of the thermo-responsive scaffolds are composed of carbon–carbon linked chains as backbones that

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http://dx.doi.org/10.1016/j.colsurfb.2015.09.057
0927-7765/

exhibit poor hydrolytic biodegradability features and therefore prove to be toxic to a variety of organisms [23].

Poly(amino acid) s represent a class of biodegradable macromolecular materials with low toxicity and good biocompatibility. This type of polymer may be able to degrade into short peptide chains or amino acids via cleavage of peptides bonds through microbial cleavage and/or enzymatic reactions [22]. Poly(aspartic acid) (PASP) and its derivatives represent a new class of biodegradable peptide materials that can be prepared in a simple fashion, by hydrolysis and aminolysis of polysuccinimde (PSI) [24–26] instead of carrying out complex ring-opening polymerization reactions of amino acid *N*-carboxyanhydrides (NCA). PSI can be directly synthesized by thermal bulk polycondensation of aspartic acid and a variety of functional groups can be introduced into the side chains of PASP via aminolysis reactions. This method has also been investigated to produce poly(amino acid) s and the thermo-responsive behaviour associated with this material has also been studied [23,27,28]. We have developed a series of thermo-responsive materials based on poly(amino acid)s through functionalization of isopropylamide and hydroxyalkylamide groups onto the polyaspartamide scaffold via aminolysis reaction of PSI [27]. Additionally, Kim et al. have also prepared a pH- and thermo-responsive polyaspartamide hydrogel with diisopropylamine and hydroxyethyl pendants [29].

The phase transition temperature of the copolymers has been manipulated by changing the ratio between hydrophobic and hydrophilic moieties with pH variations. Typically, polymeric micelles are created by self-assembly from amphiphilic copolymers to form a core–shell structure with a hydrophobic inner core. The latter unit serves as an encapsulation site for a given drug via hydrophobic interactions while the hydrophilic outer shells ensure stability of the assembly in aqueous media. For most of the polymeric drug nanocarriers, the hydrophobic segments on the polymer are mostly inert and have therefore no effect on therapeutic efficacy. Various efforts have been directed to the reduction of the inert component and increase the compatibility between a drug and core-forming segment. Zhuo et al. used hydrophobic drug molecules as hydrophobic moiety for the preparation of the nanoparticles [30]. Kataoka et al. have covalently conjugated doxorubicin molecules to the copolymer side chains, resulting in an improved drug encapsulation efficiency due to the hydrophobicity and intermolecular interaction between the conjugate and doxorubicin [31]. However, cytotoxicity tests confirmed that doxorubicin molecules covalently linked to the copolymers through non-cleavable bonds potentially lose their bioactivity entirely.

Hydrazone bonds represent a type of reversible and cleavable linkage, which proves to be stable under physiological conditions but cleavable under acidic conditions. This bond type has been investigated to produce pH-sensitive drug conjugates without reducing bioactivity due to slight pH differences between the blood stream and solid tumours [32,33].

In this paper we describe the development of a drug-conjugate copolymer based on the biodegradable and biocompatible poly(amino acid) as illustrated in Scheme 1. The *N,N*-diisopropylaminoethyl aminegroups are conjugated to PASP via PSI ring opening reaction to furnish poly(amino acid) featuring thermo-responsive properties. The doxorubicin (DOX) molecules are chemically conjugated to the polymer chain via acid-labile hydrazone bonds and serve as hydrophobic moiety to facilitate the self-assembly of the polymer. Furthermore, the high affinity of bound DOX to free DOX molecules presumbly increases the drug-load capacity via hydrophobic interactions and molecular π–π stacking[34]. In this system, hydrophilic PEG chains have also been chemically grafted to the polymer chain via hydrazone bonds to form a removable, protective functionality. In blood vessels, PEG shells have been demonstrated to reduce the premature clearance of nanoparticles by the reticuloendothelial system and therefore extend the blood lifetime of nanoparticles. When targeting the tumour location, the PEG-removable nanoparticles are able to shed their outer layer, facilitating the drug release. As a novel drug carrier, the drug release behaviour at various pH ranges and temperature conditions as well as the cytotoxicity characteristics of the nanoparticles have been evaluated. The stimuli-responsive anticancer effect of the nanoparticles has also been a subject of these studies.

2. Experiment

2.1. Materials

L-Aspartic acid (L-Asp) and *N,N*-diisopropylaminoethyl amine (DIPAE) were purchased from Aladdin Reagent Company. DOX hydrochloride was obtained from Beijing Huafeng United Technology Co. and was used as received. Monomethoxy poly(ethylene glycol) (mPEG) (M_n = 1000) was obtained from Alfa Aesar. An aqueous solution of hydrazine hydrate (80%) and other reagents were purchased from Tianjin Chemical Reagent.

2.2. Synthesis of DIPAE and hydrazide grafted PASP (PADHy)

PSI, the precursor polymer (M_n = 13.7 kDa, PDI = 1.5), was prepared using a previously reported procedure [35]. *N,N*-diisopropylaminoethyl amine (1.3 g) was added dropwise to a vigorously stirred solution of PSI (0.5 g) in 5 mL of DMF at 0 °C. The mixture was stirred in a heated water bath at 50 °C for 10 h. Upon cooling, an excess amount (based on succinimide) of an aqueous solution of hydrazine hydrate was added to the solution at 0 °C and the resulting reaction mixture was stirred at 25 °C for 4 h. The mixture was poured into diethyl ether and the resulting precipitate was washed with diethyl ether and dried at 25 °C in vacuo.

2.3. Synthesis of methoxy poly(ethylene glycol) benzaldehyde (mPEG-CHO)

MPEG-CHO was prepared using a method reported previously [36]. Briefly, mPEG-OH (3.0 g) was dissolved in a mixture of 25 mL dichloromethane (DCM) and 1 mL pyridine, followed by the addition of p-$C_6H_4MeSO_3Cl$ (2.3 g). The mixture was stirred at room temperature for 12 h and then poured into excess diethyl ether. The resulting precipitate was washed with diethyl ether and dried at 25 °C in vacuo. The precipitate (1.3 g) was then dissolved in 16 mL acetone and potassium carbonate togetner with 4-hydroxybenzaldehyde (4.6 g) were added. The mixture was refluxed for 25 h. Upon removal of the acetone in vacuo, the residue was dissolved in 30 mL water and extracted with DCM (3 × 5 mL). The solvent of the collected organic phase was removed in vacuo, and addition of an excess of diethyl ether resulted in precipitation. The solid was filtered off and was dried in vacuo.

2.4. Synthesis of mPEG grafted PADHy (mPEG-hyd-PADHy)

PDAHy (0.2 g) was dissolved in 8 mL of DI water. The pH value of the solution was adjusted to 5.0 with 0.1 N HCl, followed by the addition of mPEG-CHO (80 mg) to the reaction mixture. After stirring at room temperature for 24 h, the solution was dialyzed and subsequently lyophilized to dryness.

2.5. Grafting of DOX onto mPEG-hyd-PADHy and preparation of DOX-loaded nanoparticles

DOX·HCl (10 mg) and mPEG-*hyd*-PADHy (30 mg) were dissolved in DMSO and stirred in the dark for 24 h. Two equivalents of tri-