Biomaterials 47 (2015) 41–50

Contents lists available at ScienceDirect

Biomaterials

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

Multi-functional self-fluorescent unimolecular micelles for tumor-targeted drug delivery and bioimaging

Guojun Chen [a, b], Liwei Wang [b, c], Travis Cordie [c], Corinne Vokoun [d], Kevin W. Eliceiri [d], Shaoqin Gong [a, b, c, e, *]

[a] Materials Science Program, University of Wisconsin–Madison, Madison, WI 53715, USA
[b] Wisconsin Institutes for Discovery, University of Wisconsin–Madison, Madison, WI 53715, USA
[c] Department of Biomedical Engineering, University of Wisconsin–Madison, Madison, WI 53715, USA
[d] Laboratory for Optical and Computational Instrumentation, University of Wisconsin–Madison, Madison, WI 53706, USA
[e] University of Wisconsin Carbone Cancer Center, University of Wisconsin–Madison, Madison, WI 53792, USA

ARTICLE INFO

Article history:
Received 21 October 2014
Accepted 15 January 2015
Available online 4 February 2015

Keywords:
Self-fluorescence
Unimolecular micelles
Drug nanocarrier
Drug delivery
Bioimaging probe
Cancer theranostics

ABSTRACT

A novel type of self-fluorescent unimolecular micelle nanoparticle (NP) formed by multi-arm star amphiphilic block copolymer, Boltron® H40 (H40, a 4th generation hyperbranched polymer)-**b**iodegradable **p**hoto-**l**uminescent **p**olymer (BPLP)-poly(ethylene glycol) (PEG) conjugated with cRGD peptide (i.e., H40-BPLP-PEG-cRGD) was designed, synthesized, and characterized. The hydrophobic BPLP segment was self-fluorescent, thereby making the unimolecular micelle NP self-fluorescent. cRGD peptides, which can effectively target $\alpha_v\beta_3$ integrin-expressing tumor neovasculature and tumor cells, were selectively conjugated onto the surface of the micelles to offer active tumor-targeting ability. This unique self-fluorescent unimolecular micelle exhibited excellent photostability and low cytotoxicity, making it an attractive bioimaging probe for NP tracking for a variety of microscopy techniques including fluorescent microscopy, confocal laser scanning microscopy (CLSM), and two-photon microscopy. Moreover, this self-fluorescent unimolecular micelle NP also demonstrated excellent stability in aqueous solutions due to its covalent nature, high drug loading level, pH-controlled drug release, and passive and active tumor-targeting abilities, thereby making it a promising nanoplatform for targeted cancer theranostics.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Multifunctional nanoparticles (NPs) integrating therapeutic agents, imaging probes, and tissue/cell specific targeting ligands are desirable for a wide range of biomedical applications including targeted cancer theranostics [1–5]. Many different types of NPs have been reported as therapeutic and/or imaging agent nanocarriers, among which liposomes, polymer micelles, and vesicles have been the most widely studied [6–8]. Liposomes, polymer micelles, and vesicles are typically formed by a large number of linear amphiphilic molecules via a self-assembly process. Thus, their *in vitro* and *in vivo* stabilities are susceptible to a number of factors including the concentration of amphiphilic linear molecules, flow stress, and interactions with serum proteins, which often lead to insufficient *in vivo* stability [9–11]. Premature rupture of such self-assembled multi-molecular NPs during circulation can cause a burst release of high concentration anti-cancer drugs and/or imaging probes into the bloodstream, which can not only can lead to potential systemic toxicity, but can also undermine their tumor-targeting and imaging abilities [9–11]. To improve the *in vitro* and *in vivo* stability of drug/agent nanocarriers, we developed a series of unimolecular micelles made of judiciously engineered multi-arm star amphiphilic block copolymers [3, 4, 12–17]. Since each unimolecular micelle NP is formed by a single multi-arm star amphiphilic block copolymer molecule consisting only of covalent bonds, it possesses excellent stability. Unimolecular micelles also provide a high drug loading capacity, possess a narrow nanoparticle size distribution, and offer excellent chemical versatility that allows for further surface modification such as ligand conjugation [3, 4, 13–18].

Fluorescent drug nanocarriers are highly desirable for both *in vitro* and *in vivo* applications as the fluorescence property allows for easy tracking of the nanocarriers using a variety of microscopy

* Corresponding author. Department of Biomedical Engineering, University of Wisconsin–Madison, Madison, WI 53715, USA.
E-mail address: sgong@engr.wisc.edu (S. Gong).

http://dx.doi.org/10.1016/j.biomaterials.2015.01.006
0142-9612/© 2015 Elsevier Ltd. All rights reserved.

imaging techniques [19–23]. For instance, cellular internalization and intracellular trafficking of fluorescent NPs, as well as *in vivo* biodistribution of fluorescent NPs, can be conveniently carried out using fluorescence microscopy [21–24]. Current strategies to create fluorescent NPs include conjugating or encapsulating organic dyes or utilizing inorganic fluorescent NPs such as quantum dots (QDs) or other metallic particles [25–27]. However, there are various limitations to these common approaches. For instance, the organic dyes conjugated onto or encapsulated into the NPs may dissociate from the NPs. Moreover, organic dyes often exhibit low photostability [24, 28]. Meanwhile, inorganic fluorescent NPs such as QDs may possess high cytotoxicity and can limit the design of drug nanocarriers that may also require complicated synthesis processes [29–31].

Recently, a family of biodegradable photo-luminescent polymers (BPLPs) has been reported by Yang et al.[21, 23, 32, 33]. The reactants used to synthesize BPLPs, including citric acid, amino acids, and aliphatic diols, are all compounds used in many Food and Drug Administration-regulated devices [21]. In contrast to organic dyes or QDs, BPLPs have demonstrated excellent photostability and biocompatibility [21, 33]. Due to their polymeric nature, BPLPs can be conveniently used to fabricate NPs or scaffolds [21, 23].

Here we report the first self-fluorescent unimolecular micelle NP that exhibits excellent aqueous stability and photostability, low cytotoxicity, and a pH-controlled drug release profile. Furthermore, this unique unimolecular micelle NP is conjugated with cRGD peptides that can effectively target $\alpha_v\beta_3$ integrin-expressing tumor neovasculature and/or cells [3, 34–36]. $\alpha_v\beta_3$ integrin plays an important role for both tumor development and tumor metastasis, and is over-expressed on both the tumor cells and the angiogenic endothelial cells of many types of solid tumors (e.g., glioblastoma, breast, prostate, ovarian cancer, and melanoma) [37, 38]. In addition, $\alpha_v\beta_3$ integrin is up-regulated in tumors following radiotherapy [34]. The self-fluorescent unimolecular micelle NP is formed by a multi-arm star amphiphilic block copolymer molecule, Boltron® H40 (H40, a 4th generation hyperbranched polymer)-**b**iodegradable **p**hoto-**l**uminescent **p**olymer (BPLP)-poly(ethylene glycol) (PEG) conjugated with cRGD peptide (i.e., H40-BPLP-PEG-cRGD). This unique self-fluorescent unimolecular micelle NP exhibits excellent aqueous stability and photostability, low cytotoxicity, high drug loading level capacity, and pH-controlled drug release, making it an extremely promising nanoplatform for various biomedical applications including targeted cancer theranostics.

2. Materials and methods

2.1. Materials

Boltorn® H40 (a hyperbranched polyester with hydroxyl terminal groups) was provided by Perstorp Polyols Inc. The heterobifunctional poly(ethylene glycol) (PEG) derivatives, COOH-PEG-maleimide (Mw = 5000 g/mol) and COOH-PEG-OCH_3 (Mw = 3500 g/mol), were acquired from JenKem Technology. Citric acid, 1,8-octanediol, and L-cysteine were purchased from Sigma–Aldrich. Succinic anhydrous, 4-dimethylamino pyridine (DMAP), and 1,3-dicyclohexylcarbodiimide (DCC) were purchased from ACROS and used without further purification. Triethylamine (TEA), anhydrous dimethyl sulfoxide (DMSO), anhydrous dimethylformamide (DMF), and tris(2-carboxyethyl)phosphine (TCEP) were purchased from Sigma–Aldrich. All other chemicals and reagents used were of analytical reagent grade. The anti-cancer drug, doxorubicin hydrochloride (DOX·HCl) was purchased from Beijing Mesochem Technology Co., Ltd. Cyclo (Arg-Gly-Asp-D-Phe-Cys) (cRGD) peptide was purchased from Peptides International. Ultrapure deionized water (DI water, Milli-Q Water Systems) was used for all buffer solutions and experiments. Dulbecco's phosphate-buffered saline (DPBS, pH 7.4), Dulbecco's Modified Eagle Medium (DMEM, high glucose, pyruvate), Trypsin-EDTA (0.25%), and Fetal Bovine Serum (FBS) were purchased from Invitrogen, USA. The U87MG human glioblastoma cells (expressing high levels of intergrin $\alpha_v\beta_3$) were purchased from ATCC and cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin.

2.2. Synthesis of BPLP polymers

BPLP polymers were synthesized following a literature protocol with slight modifications [32]. Briefly, citric acid, 1, 8-octanediol, and L-cysteine with a molar ratio of 1:1.2:0.2 were added into a 100 mL two-neck flask and dried under vacuum for 2 h. After the mixture was melted at 160 °C in an oil bath under continuous stirring, the temperature was lowered to 120 °C for another 75 min. Next, the polymer was dissolved in 1,4-dioxane and the resulting polymer solution was added dropwise into DI water under constant stirring. The BPLP polymer was obtained after lyophilization.

2.3. Synthesis of COOH-PEG-cRGD

COOH-PEG-cRGD was prepared via a thiol-maleimide coupling reaction. Briefly, the COOH-PEG-Mal and cRGD-SH with a molar ratio of 1:1.2 were added in a 50 mL two-neck flask and dissolved in DMSO. TECP was added to avoid disulfide formation among cRGD peptides. The mixture was stirred under argon gas at room temperature for 24 h and followed by dialysis against DI water to remove impurities using a cellulose dialysis membrane (molecular weight cut-off, 2 kDa). After 48 h dialysis, the product was dried by lyophilization.

2.4. Synthesis of BPLP-PEG-cRGD and BPLP-PEG-OCH_3

BPLP-PEG-cRGD was prepared via an esterification process in the presence of DCC and DMAP. Briefly, COOH-PEG-cRGD (5000 g/mol), BPLP, DCC, and DMAP with a molar ratio of 1.1:1:1.2:0.24 were dissolved in 10 mL of DMSO. The reaction was carried out at room temperature for 48 h. Next, the dicyclohexylurea (DCU) precipitated in the reaction solution was filtered out and the remaining solution was added dropwise into cold ethyl ether to obtain the crude polymers. The crude polymers were redissolved in DMSO and dialyzed against DI water for 48 h using a cellulose dialysis membrane (molecular weight cut-off, 5 kDa). The purified polymer was dried via lyophilization. BPLP-PEG-OCH_3 was prepared following a similar procedure by using COOH-PEG-OCH_3 (3500 g/mol) instead.

2.5. Synthesis of H40-COOH

To purify H40-OH, it was dissolved in acetone overnight and was then precipitated in cold ethyl ether. H40-COOH was prepared by converting the hydroxyl terminal groups into carboxyl terminal groups in the presence of succinic anhydrous. Briefly, H40-OH, succinic anhydrous, and DMAP with a molar ratio of 1:70:7 were dissolved into 10 mL of CH_2Cl_2. The reaction was carried out at room temperature for 48 h and the product was precipitated using diethyl ether and vacuum-dried.

2.6. Synthesis of H40-BPLP-PEG-OCH_3/cRGD

H40-BPLP-PEG-OCH_3/cRGD was synthesized by reacting H40-COOH with BPLP-PEG-cRGD and BPLP-PEG-OCH_3 in 10 mL of DMF in the presence of DCC and DMAP. The molar ratio of reactants (H40-COOH:BPLP-PEG-cRGD:BPLP-PEG-OCH_3) was 1:7:28. The reaction mixture was stirred at room temperature for 48 h and the by-product, DCU, was removed by filtration. The impurities were removed by dialysis against DMF for 12 h and against DI water for another 36 h using a cellulose dialysis membrane (molecular weight cut-off, 15 kDa). The resulting polymer H40-BPLP-PEG-OCH_3/cRGD was obtained after lyophilization and used to prepare targeted unimolecular micelles. Multi-arm star amphiphilic block copolymer H40-BPLP-PEG-OCH_3 (without cRGD conjugation) was also prepared following a similar procedure and was used to prepare non-targeted unimolecular micelles.

2.7. Preparation of DOX-Loaded unimolecular micelles

DOX·HCl (4 mg) was dissolved in 4 mL of anhydrous DMSO and treated with 2 mol excess of TEA for 2 h. Subsequently, the multi-arm star amphiphilic block copolymer H40-BPLP-PEG-cRGD or H40-BPLP-PEG-OCH_3 (20 mg) was added to this solution. Thereafter, 12 ml of DI water was added dropwise into the solution under constant stirring. The resulting solution was stirred using a magnetic stirring bar for 4 h and then dialyzed against DI water using a cellulose dialysis membrane (molecular weight cut-off, 2 kDa) for 24 h followed by freeze-drying.

2.8. Characterization

^{1}H NMR spectra of all intermediate and final polymer products were recorded on a Varian Mercury Plus 300 spectrometer using DMSO-d6 as a solvent at 25 °C. Molecular weights (M_n and M_w) and polydispersity indices (PDI) of the polymers were determined by gel permeation chromatography (GPC) equipped with a refractive index detector, a viscometer detector, and a light scattering detector (Viscotek, USA). DMF with 0.1 mmol of LiBr was used as a mobile phase with a flow rate of 1 mL/min. Fluorescent spectra of the unimolecular micelle solutions were acquired on a Nanolog FL3-2iHR spectrofluorometer (HORIBA Jobin Yvon Inc., USA). The sizes and morphologies of the unimolecular micelles were determined by dynamic light scattering (DLS, ZetaSizer Nano ZS90, Malvern Instrument, USA) and transmission electron microscopy (TEM, FEI Tecnai G^2 F30 TWIN 300 KV, E.A. Fischione Instruments, Inc. USA) at a polymer concentration of 0.05 mg/ml. The TEM sample was prepared by depositing a drop of the copolymer solution (0.05 mg/ml) containing 1 wt% of phosphotungstic acid onto a 200 mesh copper grid coated with carbon. The DOX loading level, defined as the weight percentage of DOX in the DOX-loaded unimolecular micelle NPs, was measured by a Cary 500 UV–Vis-NIR spectrophotometer based on a standard calibration curve of DOX at 485 nm.

Download English Version:

https://daneshyari.com/en/article/5733

Download Persian Version:

https://daneshyari.com/article/5733

Daneshyari.com