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A tumor-targeting nano doxorubicin delivery system built from amphiphilic polyrotaxane-based block copolymers

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ABSTRACT

Amphiphilic polyrotaxane (PR)-based block copolymers are synthesized by end-capping polypseudorotaxanes (PPRs) formed from distal 2-bromopropionyl terminated Pluronic F68 and a varying amount of β-cyclodextrins (β-CDs) using hydrophilic polymeric blocks of poly(ethylene glycol) methyl ether methacrylate (PEGMA) yielded *via* the *in situ* ATRP. To gain a tumor-targeting nano doxorubicin (DOX) delivery system for cancer chemotherapy, an active tumor-targeting ligand, folic acid (FA), is conjugated to the two ends of the resulting copolymers through "azide-ethylene click chemistry". The conjugated copolymers enable to self-assemble into unique core–shell structured micelles in aqueous solution and to load DOX into the hydrophobic core. The drug loading content is increased from 2.0 wt% to 25.5 wt% with respect to the blank block copolymer most likely due to the hydrogen bond interaction between DOX and β-CDs threaded. After drug loading, the size of the micelles is enlarged from 120 nm to 220 nm in diameter as determined by dynamic light scattering (DLS) analysis. Moreover, these tumor-targeted polymeric micelles exhibit a slower and sustained DOX release behavior. The cell uptake and distribution, as well as the cytotoxicity of the polymeric micelles are also evaluated toward the MDA-MB-231 cells. The FA-conjugated PR-based block copolymer micelles appear to be internalized by the cancer cells *via* FA receptor mediated endocytosis; thus, they present enhanced cytotoxicity to the selected breast cancer cells.

1. Introduction

Cancer treatment has being a global problem for a long time due to the fact that most of the chemotherapeutic drugs not only have low water solubility and short blood half-lives, but also lack of the specificity of the pharmacological effect toward the target cells. The severe side effects of cytotoxic agents to implement conventional chemotherapy in the treatment of many cancers usually lead to limited clinical success. Alternatively, polymeric core–shell micelles or nanoparticles, self-assembled from amphiphilic block copolymers in aqueous solution, have recently received increasing attention as drug carriers for cancer chemotherapy because they could increase the aqueous solubility of chemotherapeutic agents and prolong their in vivo half-lives with lessened systemic toxicity [1]. Furthermore, when the size of these unique nanoparticles is around 100 nm or below in diameter, they could target the tumor tissue through the enhanced permeability and retention (EPR) effect [2]. As these antitumor drugs are physically encapsulated into the core of nanoparticles *via* the hydrophobic interaction, the drug loading content and encapsulation efficiency become key indicators of a drug loading controlled release system.

Cyclodextrins (CDs) constituting a series of cyclic oligosaccharides composed of 6, 7 and 8 D-glucose units linked by α-1,4 bonds (α-, β- and γ-CDs) have been broadly investigated as host molecules in supramolecular chemistry [3,4]. The geometry of CDs is like a hollow truncated cone enabling to form a hydrophobic cavity, and consequently they can include not only small molecules, but also polymers by the host–guest interaction to give rise to novel polypseudorotaxanes (PPRs) and polyrotaxanes (PRs) after end-capping with bulky stoppers. Since CDs are nontoxic and biodegradable, the CD-based PRs have been widely explored for polymer therapeutics, especially for carriers of the controlled drug release owing to their fascinating structural character [5,6]. Besides the free moving and rotating of CDs along the polymer main chains, a large number of hydroxyls located on the CD rings can be chemically modified to allow conjugating bioactive agents to them [7]. Although several studies on drug conjugated nanocarriers constructed from CD-based PRs were reported in literature [8–12], they generally suffered from the lower loading contents of anticancer drugs. In our previous report, it was found that β-CD built PRs could increase the drug

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loading content of Amphotericin B (AmB) presumably due to the hydrogen bonding between AmB and β-CDs regularly stacked along polymer chains [13]. As a result these β-CD containing PRs show the great potential to be used as carrier with a high drug loading ability for the drugs having a lot of hydroxyls in the molecules.

Although the nanoparticles could be passively accumulated toward a tumor site through the EPR effect, passive targeting is still restricted in its ability to eradicate the tumor because the nanoparticles may release a considerable portion of drugs before being taken up by tumor cells [14]. To further improve the delivery efficiency and cancer specificity, active tumor-targeting ability is highly desirable. Active targeting could be achieved by functionalizing the nanocarriers with targeting ligands, such as small molecules (e.g., folic acid (FA)), peptides (e.g., cRGD), galactoses and antibodies, etc [15–17]. Among these ligands, FA is widely used for active targeting because it is inexpensive, nontoxic, nonimmunogenic, and easy to conjugate to carriers, retains high binding affinity toward the FA receptor (FR) and is stable during storage and in circulation [18]. Furthermore, FR is overexpressed in many tumor organs and cells, such as ovarian, lung and breast cancer cells. Thus, combing with EPR effect, actively targeted delivery system conjugated with FA could achieve better pharmacological efficacy by an enhancement of cellular uptake.

The atom transfer radical polymerization (ATRP) is a living/controlled radical polymerization technique, which allows the preparation of well-defined polymers with predictable chain length, narrow molecular weight distribution, controlled microstructure, defined chain ends and controlled architecture [19]. In recent years, the range of possibilities of ATRP has been further broadened by click chemistry, which is well-known for high selectivity, excellent reliability, high yield and mild conditions [20,21]. In this paper, to create a tumor-targeted drug delivery system from amphiphilic PR-based block copolymers, PPRs self-assembled from distal 2-bromopropionyl terminated Pluronic F68 and a varying amount of β-CDs were end-capped and transformed into PRs by using hydrophilic polymeric blocks of poly(ethylene glycol) methyl ether methacrylate (PEGMA) formed *via* the *in situ* ATRP. Active tumor-targeting ligand, FA, was then conjugated to the distal ends of the resulting PR-based block copolymers through "azide-alkyne click chemistry". The conjugated copolymers enabled to self-assemble into unique core–shell polymeric micelles in aqueous solution. As a model antitumor drug, doxorubicin (DOX) was loaded into the FA attached polymeric micelles. Both transmission electron microscopy (TEM) and dynamic light scattering (DLS) were performed to characterize the change of morphology and size of micelles before and after DOX encapsulation. The drug release profile, cell uptake and distribution, and cytotoxicity toward to the MDA-MB-231 cells were also assessed in vitro.

2. Experimental section

2.1. Materials

β-CD was supplied by Sinopharm Chemical Reagent Company, China, and recrystallized three times before use. Pluronic F68 comprising a central block of 30 PPO units and two PEO blocks of 70 units ($M_n = 8400$), poly(ethylene glycol) methyl ether methacrylate (PEGMA) (M_n = 300 g/mol), *N,N,N′,N″,N‴*-pentamethyldiethylenetriamine (PMDETA) and *N*-phenyl-1-naphthylamine (PNA) were purchased from Sigma, USA. PEGMA was passed over a short basic alumina column to remove inhibitor before polymerization. Both 2-bromopropionyl bromide and 4-dimethylaminopyridine (DMAP) were available from Alfa Aesar, USA. Propargylamine, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and folic acid (FA) were purchased from J&K Scientific Ltd., China. Doxorubicin·HCl (DOX·HCl) was obtained from HuaFeng United Technology Co., Ltd, Beijing, China. All other solvents and reagents were of analytical grade.

2.2. Synthesis of 2-bromopropionyl terminated Pluronic F68 (BrP–F68–PBr)

Pluronic F68 was converted to the corresponding ATRP macroinitiator through the end-capping reaction with a four-fold molar excess of 2-bromopropionyl bromide in CH_2Cl_2. In brief, in a 100 mL three-neck round-bottom flask, Pluronic F68 (8.4 g, 1 mmol) was dissolved in distilled CH_2Cl_2 (20 mL). Then DMAP (122 mg, 1 mmol), TEA (0.42 mL, 3 mmol) and 10 mL dry CH_2Cl_2 containing 2-bromopropionyl bromide (0.92 g, 4 mmol) were added dropwise under nitrogen. The reaction continued for 2 h at 0 °C and for another 24 h at room temperature under stirring. Finally the mixture was filtered to remove the precipitated salts. The product was purified by precipitation into 500 mL anhydrous ether at 5 °C. The sequence was repeated three times. ^{1}H NMR analysis was carried out to determine the degree of esterification (higher than 98%). ^{1}H NMR (DMSO-d6) (δ, ppm): 4.22–4.24 (t, 4 H, $-CH_2-O-C(=O)-$), 1.02–1.04 (d, 90 H, $-O-CCH_3-C-O-$), 1.73–1.76 (d, 3 H, CH_3-C-Br), 4.67–4.72 (m, 1 H, $-CH-Br$), 3.40–3.50 (m, CH_2CH_2O of PEG and CH_2CHO of PPG) ppm.

2.3. Synthesis of amphiphilic PR-based block copolymer via ATRP of PEGMA

A typical protocol for the synthesis of amphiphilic PR-based block copolymers *via* the *in situ* ATRP of PEGMA was as follows. In a sealable Pyrex reactor, an aqueous solution containing a predetermined amount of β-CDs was added to 1 mL aqueous solution of BrP–F68–PBr (0.24 g, 0.03 mmol), followed by vigorous stirring at room temperature for 24 h to create PPR. PEGMA (0.27 g, 0.9 mmol) and PMDETA (12.6 mg, 0.072 mmol) were then added to the resulting suspension of PPR. After quenched in the liquid nitrogen, Cu(I)Cl (6.0 mg, 0.06 mmol) was added, followed by three times of degassing using a nitrogen purge. The reactor was sealed under vacuum and the reaction started and maintained for 6.0 h at 25 °C. The polymerization stopped after breaking the Pyrex reactor. The crude product was directly freeze-dried before dissolved in 15 mL DMF, and then the solution was dialyzed using a dialysis bag (MWCO 3500) for 48 h with water changing every 12 h, in which the whole dialyzing bag was put into a 60 °C water bath for 16 h. All the content was freeze-dried. The crude product was again dissolved in DMF and fractionally precipitated with anhydrous ether. The purified product was dried under vacuum, yield 25.6%. For the convenience of expression, the obtained PR-based block copolymers were designated as PR-nβ-m,where PR means polyrotaxane, *n* stands for the feed molar ratio of β-CD to Pluronic F68 and *m* represents the feed molar ratio of PEGMA to Pluronic F68, respectively.

2.4. Preparation of PPEGMA-F68-PPEGMA (PR-0β-20)

To compare the ability of β-CDs threaded onto the Pluronic F68 main chain for the drug loading, a pentablock copolymer without adding β-CDs was synthesized as a control *via* the *in situ* ATRP of PEGMA initiated with BrP–F68–PBr at 25 °C as described above. The feed molar ratio of BrP–F68–PBr to Cu(I)Cl to PMDETA was kept at 1:2:2.4. The product was dialyzed using a cellulose bag (MWCO 3500) for 48 h with water changing every 12 h, and the whole content was freeze-dried. The crude product was dissolved in DMF and fractionally precipitated with anhydrous ether. The purified product was finally dried under vacuum, yield 58.4%. ^{1}H NMR (DMSO-d_6) (δ, ppm): 1.02–1.04 (d, 90 H, $-OCCH_3-CO-$),