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pH-sensitive micelles self-assembled from multi-arm star triblock co-polymers poly(ε-caprolactone)-b-poly(2-(diethylamino)ethyl methacrylate)-b-poly(poly(ethylene glycol) methyl ether methacrylate) for controlled anticancer drug delivery

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ABSTRACT

A series of amphiphilic 4- and 6-armed star triblock co-polymers poly(ε-caprolactone)-b-poly(2-(diethylamino)ethyl methacrylate)-b-poly(poly(ethylene glycol) methyl ether methacrylate) (4/6AS-PCL-b-PDEAEMA-b-PPEGMA) were developed by a combination of ring opening polymerization and continuous activators regenerated by electron transfer atom transfer radical polymerization. The critical micelle concentration values of the star co-polymers in aqueous solution were extremely low (2.2–4.0 mg l^{-1}), depending on the architecture of the co-polymers. The self-assembled blank and doxorubicin (DOX)-loaded three layer micelles were spherical in shape with an average size of 60–220 nm determined by scanning electron microscopy and dynamic light scattering. The in vitro release behavior of DOX from the three layer micelles exhibited pH-dependent properties. The DOX release rate was significantly accelerated by decreasing the pH from 7.4 to 5.0, due to swelling of the micelles at lower pH values caused by the protonation of tertiary amine groups in DEAEMA in the middle layer of the micelles. The in vitro cytotoxicity of DOX-loaded micelles to HepG2 cells suggested that the 4/6AS-PCL-b-PDEAEMA-b-PPEGMA micelles could provide equivalent or even enhanced anticancer activity and bioavailability of DOX and thus a lower dosage is sufficient for the same therapeutic efficacy. The results demonstrate that the pH-sensitive multilayer micelles could have great potential application in delivering hydrophobic anticancer drugs for improved cancer therapy.

1. Introduction

Due to the heterogeneity and adaptations of cancerous cells cancer therapy remains a tremendous challenge and great efforts have been devoted to cancer therapy over the past several decades. Chemotherapy plays an important role in cancer therapy and has become one of the most widely used tools in combating cancer. However, there are still various intrinsic limitations of anticancer agents, such as poor water solubility, uncontrolled pharmacokinetic processes (short duration in the circulation and improper biodistribution), and the possible occurrence of severe side-effects, which will considerably decrease the therapeutic efficacy [1–3].

Recently novel drug delivery approaches (e.g., nanoparticles, liposomes, hydrogels and polymeric micelles) have been investigated to obtain higher antitumor efficiency with reduced toxicity by altering the biodistribution of anticancer drugs [4–9]. In addition, nanoparticles with a hollow structure have also been attracting attention because they can encapsulate drugs with high efficacy [10–13]. Polymeric micelles with nanoscopic core–shell structures formed by amphiphilic co-polymers demonstrate a series of attractive properties as anticancer drug carriers, providing a high loading capacity of poorly water soluble drugs, improving the apparent water solubility, providing both passive and active targeting capabilities, altering the pathways of drug biocirculation, reducing uptake by the reticuloendothelial system (RES), prolonging the in vivo circulation duration and increasing specific accumulation within tumor tissues, thus affording enhanced therapeutic efficacy and negligible adverse effects [14–17].

However, the formation of polymeric micelles is thermodynamically favorable only above the critical micelle concentration (CMC) of the amphiphilic molecules and they are relatively unstable in infinitely dilute environments. Once introduced into the

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bloodstream by intravenous administration polymeric micelles become thermodynamically unstable when the concentration of amphiphilic polymers drops below the CMC by severe dilution. The disruption of micellar structures might lead to the burst release of physically encapsulated drugs, which may cause serious side-effects, mostly due to large fluctuations in the drug concentration [18,19]. All these characteristics may reduce the effectiveness of drug delivery and limit the in vivo application of polymeric micelles.

In contrast to the classical linear polymers, one promising approach to improving the thermodynamic stability of micelles is to develop star-shaped amphiphilic polymers which mimic the morphology of polymeric micelles. Star-shaped polymers, a form of dendritic polymer, present unique properties and advantages, such as a small hydrodynamic radius, a large number of arms, which are capable of forming a stable micelle structure, a low intrinsic viscosity and crystallinity, high functionality and simple synthesis, owing to their particular well-defined architecture with multiple polymer chains radiating from the central core [2,20–23]. Thus amphiphilic star-shaped co-polymers could be promising candidates for anticancer drug delivery [24–29].

Considering the tumor targeting drug delivery field, an ideal anticancer drug carrier should retain the drug molecules in the micellar core in the bloodstream and normal tissues and release them at the specific tumor sites. So the production of pH-responsive star polymers could significantly expand the application of these polymers [26,30–33]. Compared with the normal physiological environment of pH 7.4, the extracellular pH values in tumorous tissues have been determined to be around 6.5–7.0, and the intracellular pH of the endosomal and lysosomal environments is typically acidic (pH 5.0 and 4.5–5.0) [34–36]. Poly(2-(diethylamino)ethyl methacrylate) (PDEAEMA), a cationic polyelectrolyte with a pK_b of 6.9, making it soluble in acidic solution by protonation of the pendant amine groups but hydrophobic at around neutral pH, is a promising pH-sensitive material for tumor-targeted drug delivery [34,37]. Well-defined four arm poly(ethylene oxide)-b-poly(2-(diethylamino)ethyl methacrylate) (four arm PEO-b-PDEAEMA) has been synthesized and its pH-responsive self-assembly examined. The results showed that the micelles shrank due to electrostatic charge screening of the protonated DEAEMA groups at high pH and dissociated into unimers at low pH [38]. Furthermore, four arm PEO-b-PDEAEMA micelles possessed a high gene transfection efficiency for the delivery of DNA [39]. A co-micelle drug delivery system of a star block co-polymer poly(ε-caprolactone)-block-poly(diethylamino)ethyl methacrylate (S(PCL-b-PDEAEMA)) and a linear block cpolymer methoxy poly(ethylene glycol)-block-poly(ε-caprolactone) (mPEG-b-PCL) was developed to enhance the micelle stability and improve the pH-sensitive release behavior [40].

However, the relationship between the molecular structure of the star polymers and the drug release performance has still not been clearly elaborated and the drug delivery performance of these pH-sensitive star polymeric micelles was still far from satisfactory. Thus enhancing the accuracy of the response to a stimulus and the drug delivery effectiveness and bioavailability, and forming an integrated understanding of the mechanism of drug release from star polymeric micelles are imperative [41]. We herein report pH-sensitive three layer micelles self-assembled from amphiphilic 4- and 6-arm star triblock poly(ε-caprolactone)-b-poly(2-(diethylamino)ethyl methacrylate)-b-poly(poly(ethylene glycol) methyl ether methacrylate) (4/6AS-PCL-b-PDEAEMA-b-PPEGMA) for efficient intracellular anticancer drug delivery. These micelles have a hydrophobic poly(ε-caprolactone) (PCL) to encapsulate the anticancer drug, a pH-responsive middle poly(2-(diethylamino)ethyl methacrylate) (PDEAEMA) layer and a poly(ethylene glycol) (PEG) outer layer. The pH-responsive PDEAEMA layer is hydrophobic and collapses on the core at physiological pH (7.4), which can prevent premature burst drug release, but becomes highly positively charged by protonation of the pendant tertiary amine groups and leads to the micelles being adsorbed onto negatively charged cell membranes and subsequently endocytosed by tumor cells at tumor extracellular pH. Once internalized and transferred to lysosomes the further charged PDEAEMA leads to faster release of the entrapped drug into the cytoplasm and nucleus [34]. The hydrophilic PPEGMA with short side-chains, known to be non-immunogenic, non-antigenic and non-toxic, is distributed on the surface of the self-assembled micelles and provides a compact protective layer maintaining the stability of the micelles in the circulation [42,43]. Doxorubicin (DOX), one of the most potent anticancer drugs [44], was used as a model drug encapsulated in the 4/6AS-PCL-b-PDEAEMA-b-PPEGMA micelles. The influence of the PCL and PDEAEMA contents, and the 4- or 6-arm topological structure on the micellar physico-chemical properties and release performance, as well as the final anticancer activity, were explored in depth.

2. Materials and methods

2.1. Materials

ε-Caprolactone (ε-CL) (99% pure, Aldrich) was dried over calcium hydride and distilled under reduced pressure before use. 2-(Diethylamino)ethyl methacrylate (DEAEMA) (TCI-EP) was distilled from calcium hydride, and stored under argon at −20 °C. Poly(ethylene glycol) methyl ether methacrylate (PEGMA) (M_n 475 Da, 99% pure, Aldrich) was purified by passage through a column filled with neutral alumina to remove inhibitor. Pentaerythritol and dipentaerythritol was dried under reduced pressure overnight prior to use. Tetrahydrofuran (THF) was dried over sodium using benzophenone as a dryness indicator and distilled under nitrogen prior to use. Toluene was distilled from calcium hydride. Pyrene (99% pure Aldrich), 2-bromoisobutyryl bromide (98% pure, Alfa Aesar), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA) (99% pure, Aldrich), $CuBr_2$, methanol, stannous octoate ($Sn(Oct)_2$), triethylamine (TEA), dimethyl sulfoxide (DMSO), acetone, and all other reagents were used as received. Doxorubicin hydrochloride (DOX·HCl) was purchased from Wuhan Yuancheng Gongchuang Technology Co. Ltd and used as received. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin and streptomycin were all purchased from Invitrogen. HepG2 cells were purchased from the American Type Culture Collection (ATCC) and cultured under the recommended conditions according to the supplier. 3-(4,5-Dimethyltlliazol-2-yl)-2,5-diphenyltetrazoxium bromide (MTT) was purchased from Sigma Chemical Co.

2.2. Measurements

The number average molecular weight (M_n) and polydispersity index (M_w/M_n) were determined by gel permeation chromatography (GPC) using an Agilent 1200 series GPC system equipped with a LC quant pump, 5 μm PL-Gel™ 500 Å, 10,000 Å and 100,000 Å pore size columns in series, and a refractive index detector. The column system was calibrated with a set of monodisperse polystyrene standards using HPLC grade THF as the mobile phase with a flow rate of 1.0 ml min^{-1} at 30 °C. ^1H NMR spectra were recorded in d-$CDCl_3$ at 25 °C using a Bruker Avance III 400 operating at 250 MHz. Fluorescence spectra were obtained using a F-4500 fluorescence spectrophotometer (Hitachi, Japan). The micelle size and distribution (PDI) were determined by dynamic light scattering (DLS) (Malvern Zetasizer Nano S, Malvern, UK). The zeta potentials

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