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Reduction-sensitive fluorescence enhanced polymeric prodrug nanoparticles for combinational photothermal-chemotherapy

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ABSTRACT

In this study, a reduction-sensitive supramolecular polymeric drug delivery system was developed for combinational photothermal-chemotherapy of cancer. The multifunctional system was self-assembled by specific host–guest interactions between hydrophilic β-cyclodextrin functionalized hyaluronic acid and adamantane linked camptothecin/dye conjugate, where a near-infrared (NIR) absorbing dye IR825 was loaded. The hydrophilic hyaluronic acid shell endows the assembly with excellent colloidal stability and biocompatibility. The embedded disulfide bond in the camptothecin/dye conjugate was cleaved under reducing environment, leading to the release of the conjugated drug and the recovery of fluorescence emission. Meanwhile, the dye IR825 could efficiently transfer the absorbed light into local heat, making the nanoplatform an effective system for photothermal therapy. As evident by confocal microscopy images, the nanoplatform was quickly internalized by HeLa, MCF-7, and U14 cancer cells and released drug molecules inside the cells. *In vitro* cell viability assays confirmed that the cancer cells were efficiently killed by the treatment of the nanoplatform under NIR light irradiation. Significant tumor regression was also observed in the tumor-bearing mice upon the administration of the nanoplatform through combinational photothermal-chemotherapy therapy. Hence, this nanoplatform presented a great potential in site-specific combined photothermal-chemotherapy of tumor.

1. Introduction

The design of multifunctional therapeutic nanosystems for the treatment of cancer has always been a challenging task [1]. With the advancement of nanoscience and materials chemistry, new opportunities have arisen for the development of next generation nanoscale drug delivery platforms, creating smart nanovehicles that are stable, environment-responsive, and tumor site-selective [2–4]. Given biodegradable merits, organic nanocarriers especially polymeric nanoparticles, have shown a great clinical translation potential, and a certain number of them have already been investigated in different stages of clinical trials or employed for real clinical uses [5,6]. So far, the majority of reported polymeric nanoparticles for drug delivery were prepared by the self-assembly of amphiphilic copolymers. On the other hand, the preparation of multifunctional polymeric nanoparticles is still a tough task [7], since the amphiphilic polymers have to be prepared *via* complicated synthetic routes and multi-stepped separations. Such process will undoubtedly compromise the homogeneity, controllability and repeatability of the resultant products.

To simplify the preparation procedures, supramolecular polymeric nanoparticles have been synthesized through various non-covalent interactions [8–10]. Among these interactions, the

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utilization of host–guest complexation is frequently preferred, because of its good selectivity and convenience. Cyclodextrin (CD) has been widely used as biodegradable amphiphilic host molecule, with hydrophobic inner cavity that can accommodate various hydrophobic guest molecules having specific size/shape [11]. For the preparation of supramolecular polymeric nanoparticles, two complementary group-functionalized homopolymers could be easily complexed in water to form amphiphilic polymeric aggregates [12–14]. It is well known that some drugs can form the inclusion complexes with CD, which could significantly increase the aqueous solubility of the included drugs [15,16]. In particular, the adamantane group serves as specific and effective guest molecule to complex with β-CD with a high association constant. Thus, some anticancer drugs have been modified with adamantane group, and the obtained drug-adamantane conjugates could specifically complex with β-CD functionalized polymer for drug delivery applications [17–20].

Hyaluronic acid (HA), a water-soluble natural polysaccharide, has been extensively explored as a hydrophilic building block of nanoscale drug carriers [21–23]. It has several excellent features for pharmaceutical and biomedical applications, such as good biocompatibility, biodegradability and easy chemical functionalization. In particular, HA could selectively bind to some specific receptors, such as CD44 and RHAMM, that are over-expressed on the surface of various malignant tumor cells [24,25]. Taking these advantages, β-CD has been modified on HA and then complexed with adamantane linked therapeutic agents, forming supramolecular polymeric nanoparticles for the drug delivery [17,19].

By anchoring drug molecules on the polymer substrate *via* a cleavable covalent linkage, polymeric prodrug nanoparticles are very advantageous for cancer therapy [26,27]. Such polymer-drug conjugates could be stable during the blood circulation, but quickly release the drug molecules inside specific cellular compartments after cellular uptake and subsequent linker cleavage. In addition, the assembled core-shell structures possess hydrophobic domains to retain other therapeutic agents inside, achieving the combinational treatment of cancer [28–30]. In comparison with chemotherapy alone, the additional treatment helps in overcoming multi-drug resistance accompanied by the conventional chemotherapy, leading to synergistic effects for improved therapeutic efficacy of cancer [31–33]. In order to maximize the antitumor efficacy, it is of great significance to develop smart multifunctional nanoparticles combining several therapeutic agents with different action mechanisms for controlled drug delivery [34,35]. Generally, anticancer drugs and photothermal agents are loaded or conjugated to conventional block copolymer based nanocarriers for combined photothermal-chemotherapy. For example, Ge and coworkers prepared reduction-responsive cross-linked micelles conjugated with cypate and cisplatin prodrugs for synergistic photothermal-chemotherapy [36]. In addition, acid-labile doxorubicin prodrug polymeric nanoparticles encapsulating photothermal dye IR825 dye were developed by us for combination therapy [30]. Recently, Cai et al. used the disulfide linked block polymer micelles to incorporate semiconducting polymer dots and doxorubicin simultaneously for the combination of photothermal therapy with chemotherapy [37]. On the other hand, the drug release process has hardly been monitored in these conventional delivery systems, which is unfavorable for the realization of precise and effective drug delivery to cancer cells. Thus, it is highly desirable to develop multifunctional therapeutic platforms with self-reporting drug release for the combination therapy.

Based on above considerations, we aimed to develop a novel type of multifunctional supramolecular nanoparticles encapsulating IR825 dye for the combined photothermal-chemotherapy. As shown in Scheme 1, the nanocarrier was assembled by a host-guest complexation between adamantane-modified camptothecin/naphthalimide conjugate (Nap-CPT-Ad) and β-CD decorated HA (HA-CD). Both camptothecin (CPT) and naphthalimide (Nap) were linked to adamantane bearing a nitrobenzene moiety by the reduction-cleavable disulfide bond, affording efficient fluorescence quenching of the two chromophores due to photoinduced electron transfer mechanism [38–40]. During the self-assembly process, the photothermal dye IR825 was retained inside hydrophobic cores of the nanocarrier.

The resultant multifunctional IR825 loaded nanoparticles could efficiently transfer the absorbed NIR light energy into heat for the photothermal therapy, while CPT was released under a reduction environment for chemotherapy. It should be noted that when the disulfide linkage was cleaved in response to the endogenous reducing agents. Meanwhile, the fluorescence of the drug and dye was recovered upon their release, which present obvious fluorescence changes for monitoring intracellular drug release [41,42]. On account of the exterior HA polymer shell, the prepared nanoparticles exhibited an excellent stability in aqueous physiological condition, and could be efficiently internalized by cancer cells through HA-receptor mediated endocytosis. This work is expected to provide a facile approach to prepare fluorescence "turn-on" polymeric prodrug system for combinational photothermal-chemotherapy of cancer.

2. Materials and methods

2.1. Materials and reagents

Sodium hyaluronic (molecular weight 9.5 kDa) was purchased from Freda Biochem Co., Ltd. (Shandong, China). Ethyl-3-(3-dimethyl aminopropyl)carbodiimide (EDC) (167.7 mg, 0.875 mmol), *N*-Hydroxysuccinimide (NHS), *DL*-Dithiothreitol (DTT), pyrene, *N*-ethylmaleimide (NEM), glutathione monoester (GSH-OEt) and indocyanine green (ICG) were all obtained from Sigma-Aldrich and used as received. Other salt reagents and solvents were all also purchased from Sigma-Aldrich and used directly. Mono-6-deoxy-6-ethylenediamino-β-CD and IR825 was prepared according to the literature report, respectively [43,44].

2.2. Instruments

^{1}H NMR spectra were recorded on a Bruker BBFO-400 spectrometer, and D_2O or $CDCl_3$ was as the solvent. Transmission electron microscopy (TEM) images were obtained on JEM-1400 (JEOL) at an acceleration voltage of 100 kV. The UV–vis–NIR absorption and fluorescence emission spectra were performed on a Shimadzu UV-3600 and Shimadzu RF5301PC spectrophotometer, respectively. Hydrodynamic diameters and zeta potential values were determined by a Malvern Zetasizer Nano-S dynamic light scattering (DLS) system at 25 °C. High performance liquid chromatography (HPLC) analysis was performed with Shimadzu LC2010 using the Waters XSelectTM HSS C18 (5 μm, 4.6 × 250 mm) column with UV detection at 360 nm. Acetonitrile/water binary system containing 0.5% trifluoroacetic acid was used as the mobile phase with flow rate at 1 mL min^{-1}. The analysis was performed with gradient elution method starting with 95% water to 5% water and back to 95% water at 30 °C over a period of 40 min. Confocal laser scanning microscopy (CLSM) images were acquired by a Leica TCS confocal microscope with a Nikon Eclipse TE2000-S objective (60 × oil).

2.3. Synthesis of Nap-CPT-Ad

The detailed synthetic procedure of adamantine modified