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Reduction/pH dual-sensitive PEGylated hyaluronan nanoparticles for targeted doxorubicin delivery

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ABSTRACT

To minimize the side effect of chemotherapy, a novel reduction/pH dual-sensitive drug nanocarrier, based on PEGylated dithiodipropionate dihydrazide (TPH)-modified hyaluronic acid (PEG-SS-HA copolymer), was developed for targeted delivery of doxorubicin (DOX) to hepatocellular carcinoma. The copolymer was synthesized by reductive amination via Schiff's base formation between TPH-modified HA and galactosamine-conjugated poly(ethylene glycol) aldehyde/methoxy poly(ethylene glycol) aldehyde. Conjugation of DOX to PEG-SS-HA copolymer was accomplished through the hydrazone linkage formed between DOX and PEG-SS-HA, and confirmed by FTIR and ^{1}H NMR spectra. The polymer–DOX conjugate could self-assemble into spherical nanoparticles (~150 nm), as indicated by TEM and DLS. In vitro release studies showed that the DOX-loaded nanoparticles could release DOX rapidly under the intracellular levels of pH and glutathiose. Cellular uptake experiments demonstrated that the nanoparticles could be efficiently internalized by HepG2 cells. These results indicate that the PEG-SS-HA copolymer holds great potential for targeted intracellular delivery of DOX.

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1. Introduction

Liver cancer is the fifth most common cancer worldwide but the third most frequent cause of cancer death (Jemal et al., 2011). According to the statistics (Ferlay et al., 2010), 784,300 new liver cancer cases and 695,900 cancer deaths occurred in the world in 2008, and half of these cases and deaths appeared in China. 60–70% of all liver cancer patients are diagnosed at advanced stage or with progression (Llovet et al., 2008). Chemotherapy, one of the main approaches to cancer treatment, is often limited by the toxicity of anticancer drugs to normal tissues and cells (Cho et al., 2012; Dong et al., 2010). To reduce the side effect and increase therapeutic efficacy of anticancer drugs, various drug delivery vectors have been developed, such as polymersomes (Cerritelli, Velluto, & Hubbell, 2007), nanoparticles (Sun et al., 2009), microspheres (Kawaguchi, 2000), and nanogels (Lee, Mok, Lee, Oh, & Park, 2007). In recent years, polymeric nanoparticles have been extensively investigated for targeted drug delivery (Duncan, 2003; Fox, Szoka, & Frechet, 2009; Gu et al., 2007).

Polymeric nanoparticles are usually associated with several merits including largely enhanced drug solubility in water, prolonged circulation time, passive targeting to the tumor tissues via the enhanced permeability and retention effect (Maeda, Wu, Sawa, Matsumura, & Hori, 2000), decreased side effect, and improved drug bioavailability. To enhance the targeting ability of drug nanocarriers for liver cancer, galactosamine has been employed as an active targeting moiety because it is able to recognize and bind to the asialoglycoprotein (ASGP) receptors on the surfaces of hepatoma cells (Han, Oh, Kim, & Kim, 1999; Wu et al., 2009). Moreover, poly(ethylene glycol) (PEG) has been extensively used for the development of tumor-targeting nanoparticles, because it allows a long circulation of nanoparticles in the bloodstream and their passive accumulation in tumor tissues (Min et al., 2010). Hyaluronic acid (HA), a naturally biodegradable and linear polysaccharide, has been investigated as an efficient targeting moiety in drug delivery system (Stern, Asari, & Sugahara, 2006), because it can bind to the CD44 receptor which is over-expressed in various specific tissues such as liver, kidney, and most of tumor tissues (Choi et al., 2011; Engstromlaurent, Loof, Nyberg, & Schroder, 1985). It has been known that more than 90% of HA in the blood stream is taken up and metabolized by sinusoidal endothelial cells of liver tissue (Asayama, Nogawa, Takei, Akaike, & Maruyama, 1998), so HA may be an ideal carrier for liver-targeted drug delivery.

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Some studies have revealed that the rapid drug release from nanocarriers arriving at the pathological sites can enhance the therapeutic efficacy and reduce probability of drug resistance of cancer cells (Cheng et al., 2011; Li et al., 2012). However, the conventional drug delivery systems based on biodegradable polymers such as aliphatic polyesters and polycarbonates usually exhibit gradual degradation kinetics inside body, which leads to sustained release of drugs over a period of days to weeks and reduced drug efficacy (Li et al., 2011; Shuai, Ai, Nasongkla, Kim, & Gao, 2004). Recently, stimuli-sensitive nanoparticles that can rapidly degrade by acidic or enzymic hydrolysis have been extensively studied for the rapid "burst" release of antitumor drugs (Li et al., 2012; Meng, Hennink, & Zhong, 2009). It was reported that the micelles formed by polymer–DOX conjugates bearing hydrazone linkages could stably preserve drugs under physiological conditions (pH 7.4) and selectively release them by sensing the intracellular pH decrease in endosomes/lysosomes (pH 5–6) (Bae, Fukushima, Harada, & Kataoka, 2003; Bae et al., 2005). Disulfide bonds, which are stable in the mildly oxidizing extracellular milieu, may be prone to rapid cleavage through thiol-disulfide exchange reactions with intracellular reducing molecules, especially glutathione (GSH). The intracellular GSH concentration (2–10 mM) is known to be substantially higher than extracellular one (2–20 μM) (Jones et al., 1998; Koo et al., 2008; Kuppusamy et al., 2002). The significant difference in GSH level is the basis for designing reduction-sensitive nanocarriers. To the best of our knowledge, there is still no report on reduction/pH dual-sensitive drug nanocarriers based on hyaluronic acid. In addition, DOX is one of the most potent anticancer drugs and widely used in the treatment of different types of solid malignant tumors (Adams, Lavasanifar, & Kwon, 2003; Akinc, Anderson, Lynn, & Langer, 2003). It is known that DOX interacts with DNA by intercalation and inhibition of macromolecular biosynthesis (Gewirtz, 1999). It is crucial, therefore, to deliver and release DOX in the cytoplasm and/or right into the cell nucleus. The aim of this study is to develop a triggered intracellular DOX delivery system, which is expected to enhance the efficiency of cancer chemotherapy.

In the present study, reduction/pH dual-sensitive nanoparticles formed by PEGylated TPH-modified hyaluronic acid (PEG-SS-HA) containing disulfide and hydrazone bonds are developed for targeted intracellular delivery of DOX. The galactosamine-decorated PEG-SS-HA nanoparticles are expected to be stable under physiological conditions, can be selectively taken up by liver tumor cells via ASGP receptor-mediated endocytosis, and then rapidly release DOX. The structure, drug-loading capacity and physicochemical characteristics of the PEG-SS-HA nanoparticles were investigated in detail. The in vitro evaluation of reduction/pH dual-sensitive drug releasing behavior was carried out in different reducing acidic environments. Cellular uptake of the PEG-SS-HA nanoparticles was observed by fluorescence microscopy using human hepatocellular carcinoma cell line (HepG2).

2. Materials and methods

2.1. Materials

Sodium hyaluronic acid (HA, molecular weight: 300 kDa) was purchased from Freda Biochem Co., Ltd. (Shandong, China). Hydrazine hydrate, 3,3′-dithiodipropionic acid and N-hydroxysuccinimide (NHS) were purchased from Aladdin Reagent Inc. (Shanghai, China). Doxorubicin (DOX) was purchased from Hisun Pharmaceutical Co., Ltd (Zhejiang, China). Pyrene, DL-dithiothreitol (DTT), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl), sodium cyanoborohydride ($NaBH_3CN$), galactosamine hydrochloride, 2-(4-morpholino)ethanesulfonic acid (MES), poly(ethylene glycol) and methoxy poly(ethylene glycol) (molecular weight: 2 kDa) were purchased from Sigma–Aldrich. 4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI) was obtained from Beijing Fanbo Science and Technology Co., Ltd. (Beijing, China). All other chemicals were of analytical grade and were used without further purification. All aqueous solutions were prepared using ultrapure water with a resistance of 18.25 MΩ.

2.2. Synthesis of PEG-SS-HA copolymer

2.2.1. Synthesis of dithiodipropionate dihydrazide modified HA (HA-TPH)

TPH was synthesized according to the procedure of Sakamoto, Takeda, Yamada, and Tonami (1970); Teramura, Kaneda, and Iwata (2007). ^{1}H NMR (DMSO-d6, Bruker 400 MHz, δ_{ppm}): 2.53 (t, 2H, —NH—C**H$_2$**—), 2.85 (t, 2H, —S—C**H$_2$**—), 4.2 (s, 2H, —N**H$_2$**), 9.1 (s, H, —N**H**—). Yield 70%, m.p. 131–132 °C.

HA-TPH was synthesized via carbodiimide reaction between the carboxyl groups of HA and the hydrazide groups of TPH following a reported method with some modifications (Pouyani & Prestwich, 1994). In brief, HA (300 mg, 0.75 mmol repeating units) and TPH (802.4 mg, 3.37 mmol) were first dissolved in MES solution (60 mL, 10 mM), and the pH of the solution was adjusted to 5.0 with 1 M hydrochloric acid. After EDC·HCl (43 mg, 0.23 mmol) and NHS (26 mg, 0.23 mmol) were added, the mixture solution was stirred for 24 h at room temperature. The resulting solution was dialyzed (MWCO 3500) against distilled water for 3 days and lyophilized. The degree of substitution of HA was determined to be about 30% by ^{1}H NMR spectroscopy using the ratio of the methylene protons of TPH to the acetyl methyl protons of HA (Luo & Prestwich, 1999).

2.2.2. Grafting of PEG onto HA

Poly(ethylene glycol)-dialdehyde (OHC-PEG-CHO) and methoxy poly(ethylene glycol)-aldehyde (mPEG-CHO) were prepared by the oxidation of PEG with dimethylsulfoxide/acetic anhydride according to the reported procedure (Bhattarai, Ramay, Gunn, Matsen, & Zhang, 2005; Harris et al., 1984). Galactosamine-conjugated PEG-aldehyde (Gal-PEG-aldehyde) was prepared by Schiff's base formation. Briefly, galactosamine hydrochloride (172.4 mg) was dissolved in water (2 mL) and neutralized with 0.1 M NaOH solution. The solution was added dropwise to 10 mL of aqueous solution of OHC-PEG-CHO (2 g). After the mixture was stirred for 12 h at room temperature, an aqueous $NaBH_3CN$ solution (0.8 mg, 4 mL) was added and stirred for further 24 h at room temperature. The product was extracted with dichloromethane for three times. The collected organic phase was concentrated and precipitated with excess diethyl ether. After drying under vacuum, Gal-PEG-CHO as a pale yellow powder was obtained. IR data (IR Prestige-21 FTIR spectrometer, Shimadzu, Japan): 1736 cm^{-1} for C=O stretching vibration mode of OHC-PEG-CHO; 1728 cm^{-1} for C=O stretching vibration mode of Gal-PEG-CHO, 1060 cm^{-1} for C—N stretching vibrations (Pham, Oulahyane, Mostefai, & Chehimi, 1998; Renuga Devi & Gayathri, 2010).

PEG-SS-HA copolymer was also prepared via Schiff's base formation. HA-TPH (0.1 g) was dissolved in water, and mPEG-CHO and Gal-PEG-CHO (40 mg/5 mg) were added. The solution pH was adjusted to 6.5 by 0.1 M NaOH solution. After the solution was stirred for 12 h at room temperature, $NaBH_3CN$ (10 eq, with respect to aldehyde groups) was added and stirred for 24 h. The solution was dialyzed with dialysis membrane (MWCO 12000) against distilled water for 2 days to remove the unreacted PEG. After freeze-drying, PEGylated hyaluronic acid (PEG-SS-HA) was obtained.

2.3. Conjugation of DOX onto PEG-SS-HA copolymer

DOX was conjugated onto PEG-SS-HA copolymer via hydrazone linkages formed between the ketone groups of DOX and the

hydrazide side groups of PEG-SS-HA. In a typical synthesis, PEG-SS-HA copolymer (30 mg) was dissolved in 8 mL of PBS buffer (2 mM, pH 6.5), and then DOX·HCl (1 mg) in 6 mL of water was added dropwise during 1 h. The solution was adjusted to pH 6.5 with 0.1 M NaOH and stirred for 2 h, and then was dialyzed against 2 mM PBS buffer (pH 7.8) until no further color change was observed. The solutions were protected from light at all times. The degree of conjugation was determined by UV/Vis spectrophotometry at 485 nm using a standard calibration curve (1–100 μg/mL).

2.4. Preparation and characterization of PEG-SS-HA and DOX-loaded PEG-SS-HA nanoparticles

The particle size and zeta potential were measured by dynamic light scattering (DLS) using a Malvern Zetasizer Nano-ZS90 (Malvern instruments, UK). The DLS measurements were performed at a fixed scattering angle of 90°. The morphology and size of the nanoparticles were observed by transmission electron microscopy (TEM, JEM-2000CX, JEOL, Japan).

To prepare nanoparticles, PEG-SS-HA copolymer or DOX-conjugated PEG-SS-HA (3 mg) was added to 3 mL of H_2O, and the system was sonicated for 10 min using a probe-type ultrasonicator (JY 92-2D; Ningbo Scientz Biotechnology Co., Ltd, Nanjing, China) at 100 W in an ice bath.

The critical micelle concentration (CMC) of PEG-SS-HA copolymer was evaluated by fluorescence spectroscopy (F-7000 Hitachi, Japan), using pyrene as the probe. Briefly, 1 mL of pyrene solution in acetone (6.0×10^{-6} M) was added to a series of 10 mL volumetric flasks, and then acetone was evaporated. A series of PEG-SS-HA copolymer solutions (10 mL) with different concentrations (1×10^{-4} to 0.5 mg/mL) were added to the volumetric flasks, followed by sonication for 30 min. The samples were incubated at 50 °C for 1 h, and left to cool down overnight. The fluorescence spectrum of pyrene was obtained with an emission wavelength of 390 nm. The CMC was estimated as the crosspoint when extrapolating the intensity ratio I_{338}/I_{333} at low and high concentration regions.

2.5. In vitro drug release triggered by DTT and pH

The in vitro release profiles of DOX from the nanoparticles were studied using a dialysis method in six different media: PBS buffer (pH 7.4, 6.0 and 5.0) with and without 10 mM DTT. Briefly, 5 mg of DOX-loaded nanoparticles suspended in 5 mL of water were placed in a dialysis tube (MWCO 3500), and the tube was immersed in PBS buffer and shaken at a speed of 150 rpm at room temperature. At desired time intervals, 100 μL aliquots from the bags were analyzed by fluorescence measurement, and the DOX concentration was calculated based on the absorbance intensity at 485 nm. The release experiments were conducted in triplicate, and the results presented the average data.

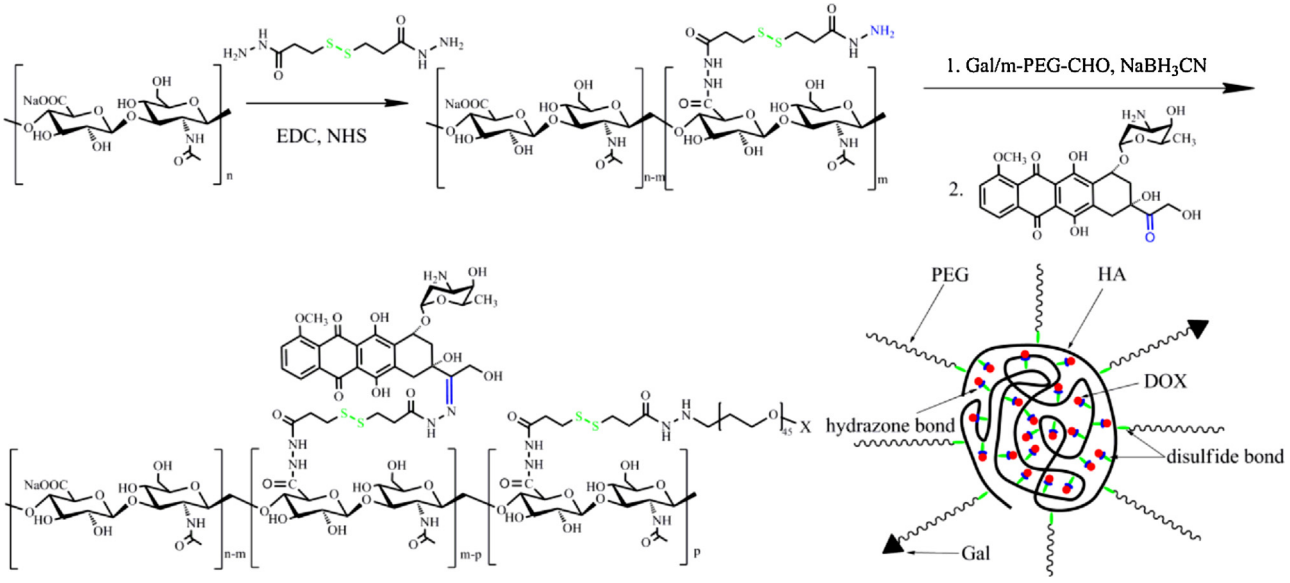
2.6. Cellular uptake study

HepG2 cells were seeded in a 6-well plate at a density of 1×10^5 cells/well, and cultured in DMEM with 10% FBS for 24 h in a humidified atmosphere with 5% CO_2 at 37 °C. The media were then replaced with 2 mL of fresh media containing 2 mg of DOX-conjugated PEG-SS-HA nanoparticles with or without Gal. After incubating at 37 °C for 1, 4 and 24 h, the culture media were discarded, and the cells were rinsed with PBS for three times to remove the nanoparticles that were not ingested by the cells. After that, the cells were fixed with 4% paraformaldehyde, and 200 μL of DAPI solution were added to stain cell nuclei before the fluorescent imaging. The cellular uptake of DOX-conjugated polymer nanoparticles was imaged using fluorescence microscopy.

3. Results and discussion

3.1. Synthesis of DOX-conjugated PEG-SS-HA copolymer

The synthetic route of DOX-conjugated PEG-SS-HA copolymer was shown in Scheme 1. HA was first modified with TPH via carbodiimide reaction between the carboxyl groups of HA and the hydrazide groups of TPH, and then grafted with poly(ethylene glycol) by reductive amination via Schiff's base formation between (Gal)-PEG-aldehyde and HA-TPH. Finally, DOX was conjugated onto the as-synthesized PEGylated TPH-modified HA through hydrazone linkages formed between the ketone groups of DOX and the hydrazide side groups of PEG-SS-HA. The synthesis of DOX-conjugated PEG-SS-HA copolymer was characterized by FTIR and ^{1}H NMR spectra, as shown in Figs. 1 and 2. As revealed in Fig. 1b, compared with HA (Fig. 1a), the appearance of characteristic peak of hydrazide groups at 1594 cm^{-1} suggests the successful



Scheme 1. Synthetic scheme of DOX-conjugated PEG-SS-HA copolymer and illustration of reduction/pH dual-sensitive nanoparticles. X denotes CH_3— or Gal—.

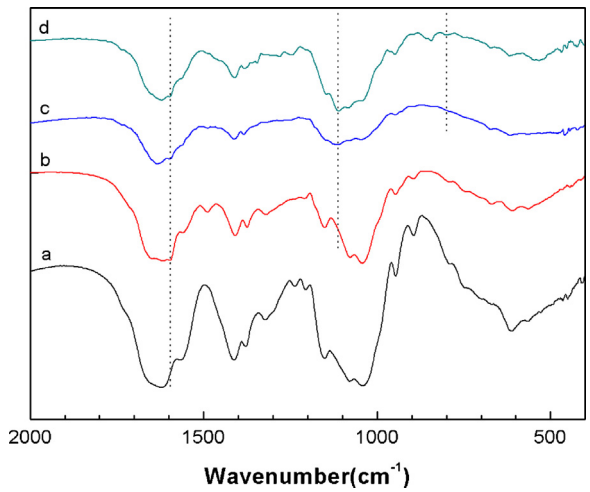


Fig. 1. FTIR spectra of (a) HA, (b) HA-TPH, (c) PEG-SS-HA copolymer, and (d) DOX-conjugated PEG-SS-HA copolymer.

modification of HA with TPH. The new peak at 1102 cm^{-1} in Fig. 1c is assigned to the ether bonds of PEG, verifying the successful grafting of PEG onto HA. One characteristic absorption peak of DOX at 803 cm^{-1} is observed in Fig. 1d (Zhang & Misra, 2007), which confirms the formation of DOX-conjugated PEG-SS-HA copolymer.

The synthesis of DOX-conjugated PEG-SS-HA copolymer was also investigated by ^{1}H NMR spectroscopy. Compared with HA (Fig. 2a), the signals at 2.53 and 2.85 ppm from the protons of TPH appears in Fig. 2b, indicating the successful synthesis of HA-TPH. As expected, the degree of substitution (DS, defined as the number of TPH per 100 sugar residues of HA) (Oh et al., 2008) increased as the feed ratio of EDC to the carboxyl groups of HA increased. In the present study, the DS is controlled to 30 considering the solubility of HA-TPH in water. The grafting of PEG onto HA is confirmed by the appearance of the shift at 3.6 ppm of PEG, as shown in Fig. 2c. However, the signals assigned to the protons of DOX are not observed in Fig. 2d, and the possible reason is that DOX was embedded into the inner cores of nanoparticles.

3.2. Fabrication and characterization of the nanoparticles

The particle size distribution and zeta potential of nanocarriers play important roles in intravenous administration (Dong & Feng, 2004). Fig. 3 shows the DLS results and TEM images of PEG-SS-HA and DOX-conjugated PEG-SS-HA nanoparticles. DLS data (Fig. 3A and C) indicate that the average size of DOX-conjugated PEG-SS-HA nanoparticles (150 nm) is smaller than that of PEG-SS-HA nanoparticles (240 nm). The reason is that the skeletons of DOX-conjugated PEG-SS-HA copolymers are more hydrophobic than those of PEG-SS-HA copolymers due to the introduction of hydrophobic DOX molecules on HA skeletons. The zeta potential values of PEG-SS-HA and DOX-conjugated PEG-SS-HA nanoparticles are −31.2 mV and −32.3 mV, respectively. The negative surface charges arise from the deprotonation of the carboxyl side groups of HA. Because the cells possess negative-charged surfaces, the as-prepared nanoparticles will hinder the non-specific cellular uptake due to the electrostatic repulsion (Shen et al., 2011). The TEM images of PEG-SS-HA and DOX-conjugated PEG-SS-HA nanoparticles reveal their spherical morphology (Fig. 3B and D). It is quite obvious that the average diameters measured from TEM (~25 nm) is much smaller than those from DLS results. This means that the nanoparticles are partially swollen in water. TEM images show the size of dried nanoparticles, while DLS

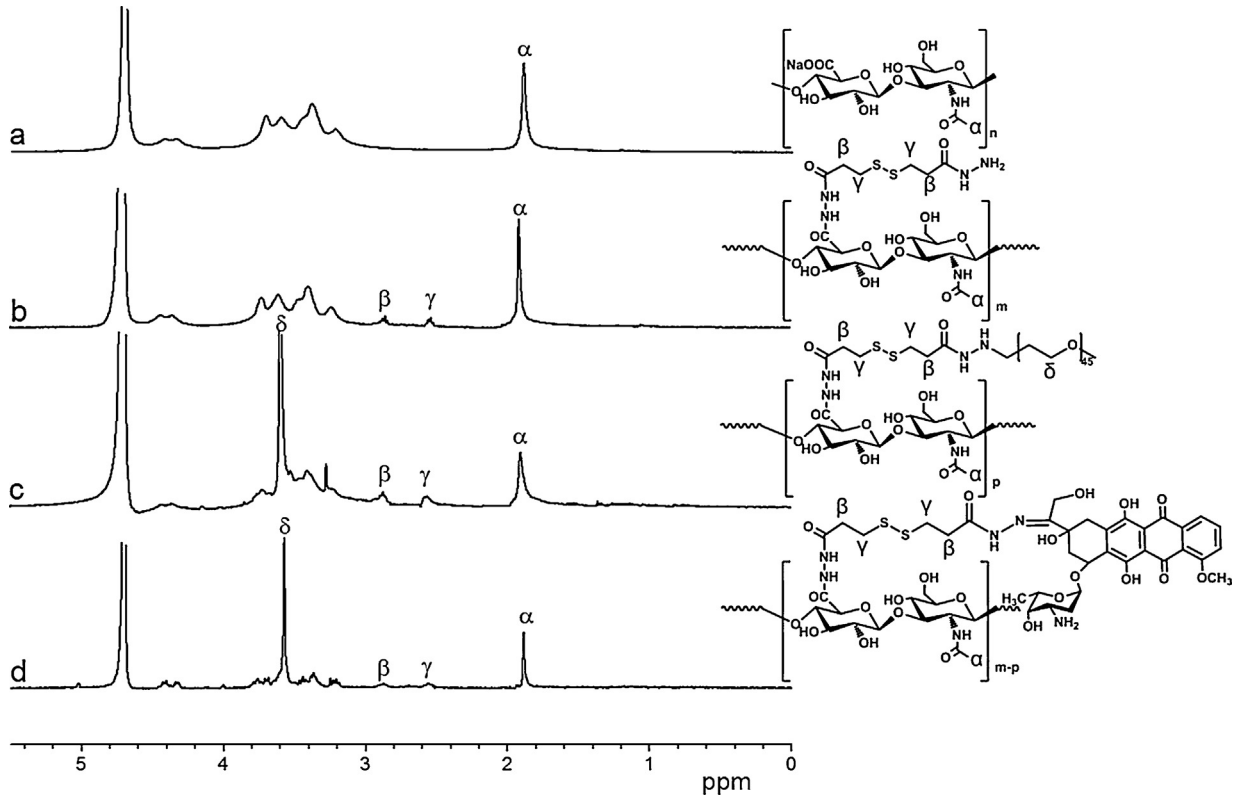


Fig. 2. ^{1}H NMR spectra (in D_2O) of (a) HA, (b) HA-TPH, (c) PEG-SS-HA copolymer, and (d) DOX-conjugated PEG-SS-HA copolymer.

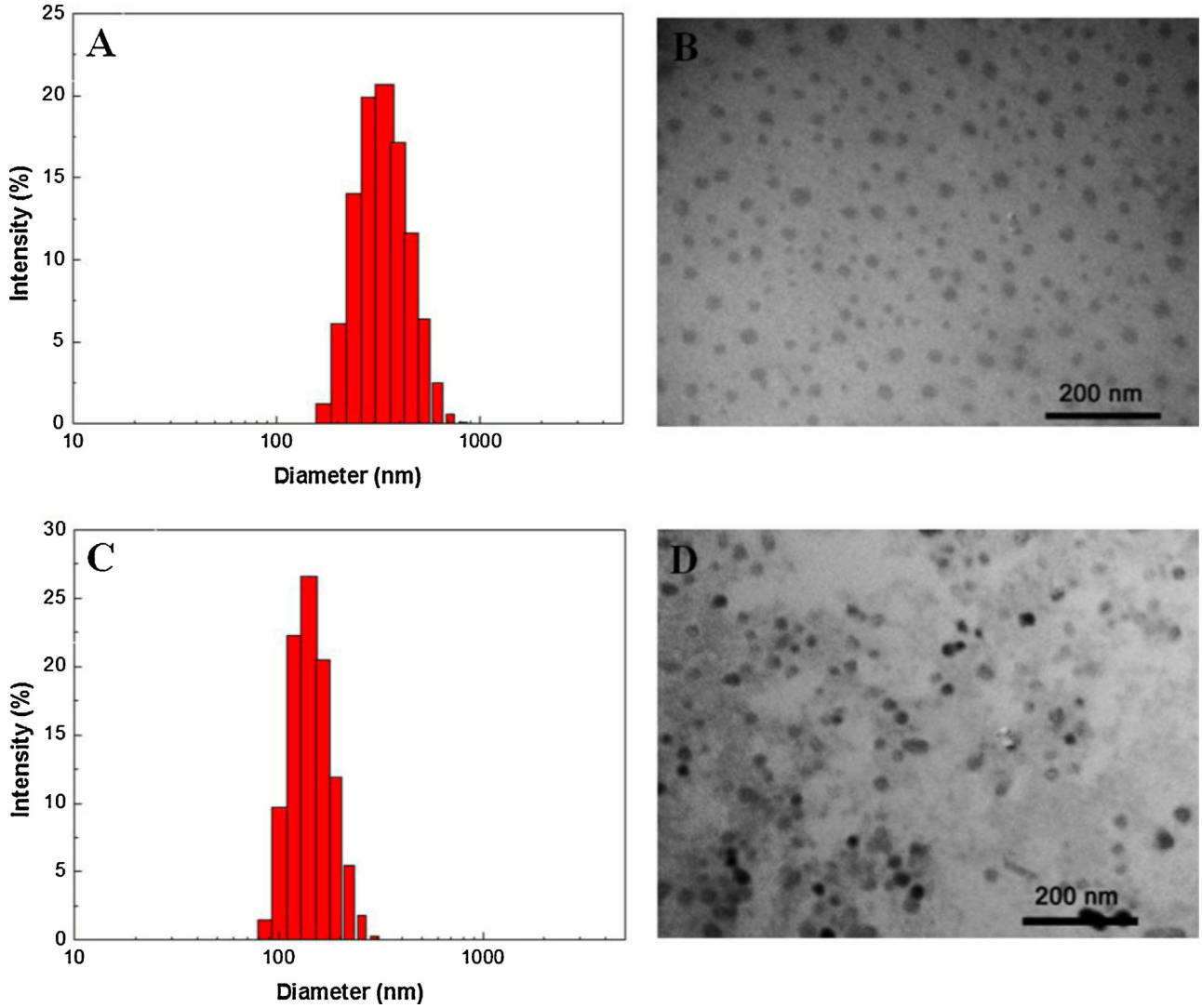


Fig. 3. (A and C) DLS results and (B and D) TEM images of (A and B) PEG-SS-HA and (C and D) DOX-conjugated PEG-SS-HA nanoparticles.

data reflect the hydrodynamic size. It has been reported that the nanoparticles with the sizes of <200 nm tended to accumulate in tumor sites due to the EPR effect (Parveen, Misra, & Sahoo, 2012; Peer et al., 2007). Therefore, the nanoparticles synthesized in this study might be used as the potential vectors for anticancer drug delivery.

In general, the critical micelle concentration (CMC) is determined using pyrene as a probe (Hanson, Li, & Deming, 2010). In Fig. 4, the fluorescence intensity ratio ($\lambda_{338\,nm}/\lambda_{333\,nm}$) at λ_{em} = 390 nm vs. the PEG-SS-HA concentration is plotted, and the CMC is estimated from the threshold concentration of self-assembled nanoparticles. The PEG-SS-HA nanoparticles show a low CMC of 20 mg/L, which is lower than the CMC of HA nanoparticles reported in the literature (Cho et al., 2011; Li et al., 2012). Such low CMC value guarantees the nanoparticles to retain their original morphology under highly diluted conditions before reaching the targeting sites. This feature is very important for drug delivery applications.

3.3. Drug loading and in vitro drug release

To conjugate DOX to PEG-SS-HA copolymer, PEG-SS-HA and DOX·HCl were dissolved in PBS buffer (pH 6.5) and dialyzed against 2 mM of PBS buffer (pH 7.8) to remove unconjugated DOX. The drug loading content, defined as the weight percentage of DOX based on the total nanoparticle weight, is 3.3 wt%. The low drug loading content should be the result of DOX loading in the form of polymer–DOX conjugates.

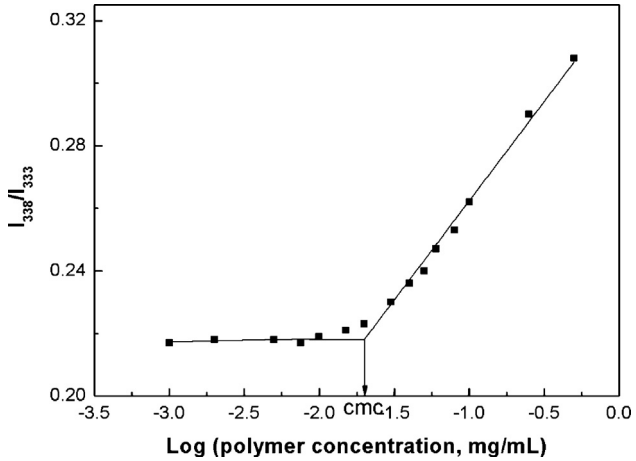


Fig. 4. Determination of CMC from the fluorescence intensity ratio ($\lambda_{338\,nm}/\lambda_{333\,nm}$) at λ_{em} = 390 nm.

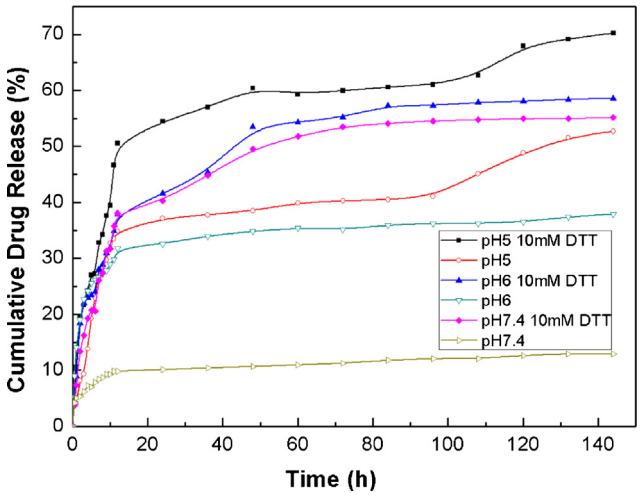


Fig. 5. In vitro reduction/pH-triggered release profiles of DOX from DOX-conjugated PEG-SS-HA nanoparticles in PBS buffer (pH 7.4, 6.0, and 5.0) with and without 10 mM DTT.

The release of DOX from the nanoparticles was investigated using a dialysis tube in PBS buffer (pH 7.4, 6.0 and 5.0) in the presence or absence of 10 mM DTT, and the results are shown in Fig. 5. As indicated in Fig. 5, drug release rates increase with decreasing pH value of PBS buffer no matter whether DTT is present or not (i.e., DOX release rate: pH 5.0 > pH 6.0 > pH 7.4), which is due to the acidic hydrolysis of the hydrazone linkages between DOX and polymer. On the other hand, DOX is released more rapidly in the presence of DTT than in the absence of DTT. For example, more than half of total drug loading content under acidic reducing conditions (pH 5.0, 10 mM DTT) was released within the first 12 h. In contrast, a minimal drug release (<15%) was observed within the first 144 h in a neutral environment. Therefore, fast drug release can be triggered by GHS and acidness. These results suggest that the DOX-conjugated PEG-SS-HA nanoparticles are likely to be stable in the plasma after intravenously administered, whereas rapid drug release can be achieved in the intracellular environment in tumor. Therefore, the PEG-SS-HA copolymer is highly promising for targeted delivery and rapid intracellular release of DOX.

3.4. Cellular uptake study

It has been well-documented that the ASGP receptor is abundantly expressed on the surfaces of various hepatoma cell lines including HepG2 cell line (Ong et al., 1991). To visually investigate the cellular uptake of DOX-conjugated PEG-SS-HA nanoparticles, the cell nuclei were fluorescently stained with DAPI. DAPI-stained cell nuclei are blue in color in the fluorescent images. To assess the internalization capacity of the nanoparticles in HepG2 cells, HepG2 cells were incubated with Gal-decorated DOX-conjugated PEG-SS-HA nanoparticles for 1, 4 and 24 h, using the nanoparticles without Gal as a control group, and the corresponding fluorescence microscopy images are shown in Fig. 6. As revealed in Fig. 6, in either the experimental group or the control group, the fluorescence intensity of DOX (red) in the cytoplasm appreciably increases with the prolongation of incubation time, but DOX fluorescence in the control group cells is much weaker than that in the experimental group cells at the same culture time. Such results indicate that ASGP receptors do express on the surface of HepG2 cells. One specific characteristic of ASGP receptors is that they are able to recognize galactose and galactosamine. Once a galactose or galactosamine ligand binds to ASGP receptor, the ligand-receptor complex is rapidly internalized by HepG2 cell, and the receptor recycles back to the surface of cells (Neradovic, van Nostrum, & Hennink, 2001). The above results demonstrate that the Gal-decorated PEG-SS-HA nanoparticles are promising to deliver DOX into HepG2 cells.

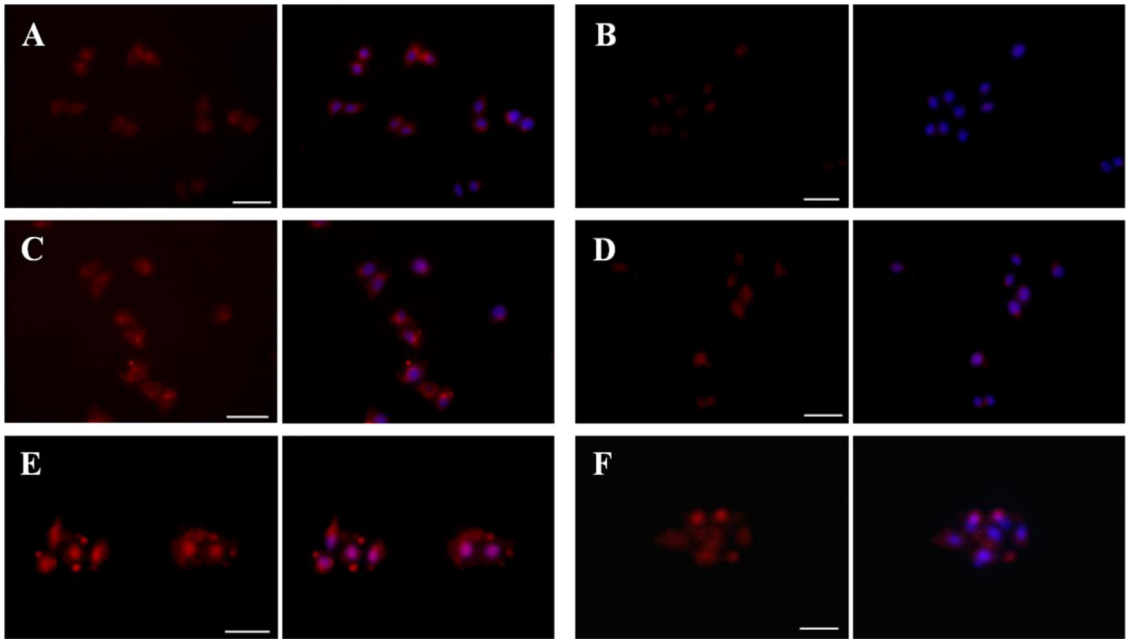


Fig. 6. Fluorescence microscopy images of HepG2 cells incubated with (left two rows) Gal-decorated DOX-conjugated PEG-SS-HA and (right two rows) DOX-conjugated PEG-SS-HA nanoparticles for (A and B) 1 h, (C and D) 4 h and (E and F) 24 h. In each panel, the two images from left to right show the red fluorescence of DOX and the overlaying fluorescence of DOX and DAPI, respectively. Scale bars correspond to 50 μm in all the images.

4. Conclusions

In this study, a novel reduction/pH dual-sensitive nanocarrier based on PEG-SS-HA copolymer is successfully synthesized. DOX molecules are conjugated onto PEG-SS-HA copolymer via hydrazone bonds between the ketone groups of DOX and the pendant hydrazide groups of PEG-SS-HA copolymer. DOX-conjugated PEG-SS-HA copolymer can self-assemble in aqueous solutions into spherical nanoparticles with an average diameter of 150 nm, with a low CMC of 20 mg/L. The release of DOX from the nanoparticles at neutral pH exhibit a slow and sustained behavior, while more than 50% of total drug content can be released within the first 12 h under acidic reducing conditions mimicking the tumor environment. The cellar uptake studies confirm that the Gal-decorated nanoparticles can specifically interact with HepG2 cells and be internalized into HepG2 cells via receptor-mediated endocytosis. Therefore, the novel reduction/pH dual-sensitive nanoparticles have great potential for liver tumor-targeted intracellular delivery of DOX and fast intracellular release, leading to enhanced anticancer efficiency.

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