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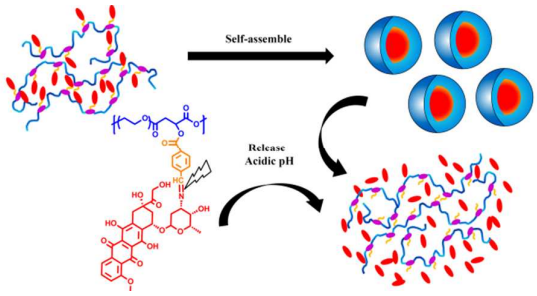
Graphic abstract

We prepared pH-sensitive amphiphilic oligo(ethylene glycol)-doxorubicin alternative conjugates for controlled release of doxorubicin.



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ARTICLE

Acid-Triggered Drug Release from Micelles Based on Amphiphilic Oligo(ethylene glycol)-Doxorubicin Alternative Copolymer†

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We report a facile strategy to synthesize pH-sensitive amphiphilic oligo(ethylene glycol) (OEG)-doxorubicin (DOX) alternative conjugates. Poly[oligo(ethylene glycol) malicate] (POEGM) with numerous pendent hydroxyl groups was first synthesized by direct polycondensation of oligo(ethylene glycol) (OEG) with malic acid under mild conditions. Then benzaldehyde groups were introduced into the POEGM backbone via esterification between the pendant hydroxyl groups and 4-formylbenzoic acid. DOX moieties were finally attached to the polymeric backbone via benzoic imine linkages, to give OEG-DOX conjugates. Attributing to the high molecular weight and alternate architecture, this kind of amphiphilic OEG-DOX alternative conjugates can form stable micelles in aqueous solution with high DOX loading content (38.2 wt-%) and low critical micelle concentration (0.021 mg/mL). Due to the pH-sensitive benzoic imine linkages between DOX moieties and polymeric backbone, DOX could be released rapidly from micelles at pH 5.8, whereas only a minimal amount of DOX was released at pH 7.4 under the same conditions. Cytotoxicity assay indicates that OEG-DOX conjugates show cytotoxic effects to MCF-7 tumor cells, while the corresponding polymer material POEGM-CHO exhibits great biocompatibility for MCF-7 tumor cells. These pH-sensitive and high drug loading nano-carriers based on the OEG-DOX alternative conjugates provide a promising platform for targeted cancer therapy.

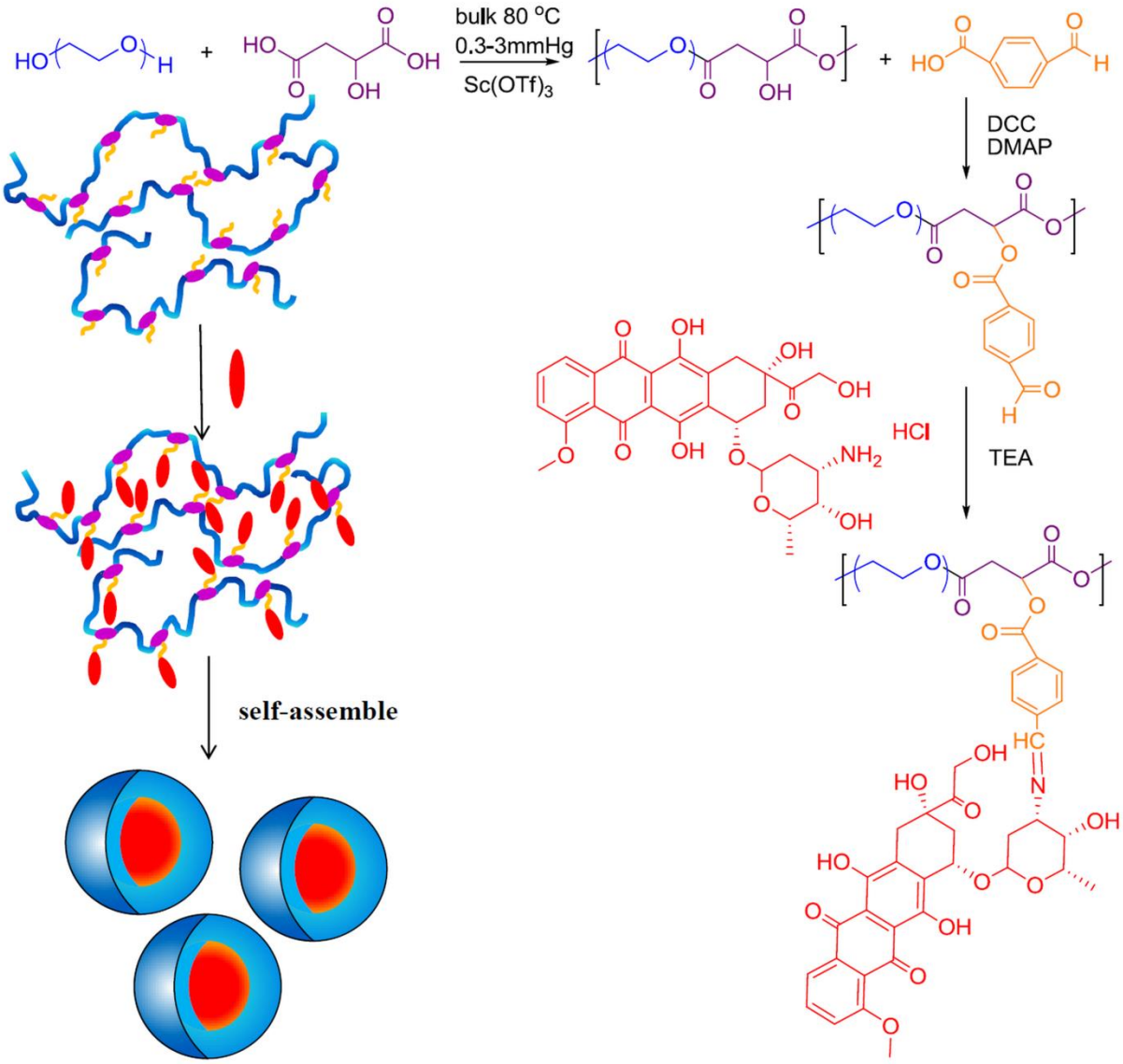
Introduction

Numerous anticancer drugs have been discovered for cancer therapy nowadays.[1-3] However, clinical outcomes still have been discouraging because of the poor solubility, low tumor selectivity and high systemic toxicity of the anticancer drugs.[4-6] Therefore, seeking for a new and effective treatment strategy is still urgent and necessary. In recent years, tremendous efforts have been focused on the development of various nanocarriers for targeted delivery of anticancer drugs, including liposomes,[7, 8] dendrimers,[9-12] micelles,[13-15] nanogels,[16-18] which may greatly enhance therapeutic efficacy while minimizing side effects of the drugs. Polymeric micelle, as one kind of antitumor drug nanocarriers based on amphiphilic polymers, is particularly appealing and has emerged as one of the most promising deliveries for various hydrophobic drugs owing to its nanoscale size, thermodynamic stability and the unique core-shell-type architecture, which can mimic naturally occurring transport systems, such as lipoproteins and viruses.[19-21] Hydrophobic anticancer drugs can be well encapsulated into the hydrophobic core of micelle carriers. However, for only physical encapsulation, the weak interactions between the drug and nano-carriers, may lead to premature release and low drug loading capacity,[22-24] and a great quantity of carriers had to be used to administer a needed dose of drug, which may give rise to systemic toxicity and impose an extra burden to excrete the carriers. One strategy to overcome such limitation is to convert drug into an inactive but more stable polymer-drug conjugates by convalently bonding the drugs onto polymer backbone, which can also revert back to the pharmacologically active drug triggered by unique biological stimuli such as pH,[25] redox,[26] or enzyme.[27] Meanwhile, the stimuli responsive site is usually introduced at the linker unit where the drug is conjugated to the polymer. Taking advantage of the pH differences in certain pathological sites (pH 7.4) and intracellular compartments (pH 5.0-6.5), various pH-responsive polymeric prodrug designs have been explored by covalent attachment of drug onto polymer via amide bond,[28, 29] hydrazone bond[30] and benzoic-imine bond[31] for intelligent drug delivery.

PEG-drug conjugates, so called PEGylated prodrugs, are particularly appealing and being widely investigated as a biocompatible drug carrier for the reason that PEG can avoid renal excretion and reduce nonspecific interactions with proteins and cells, which has been approved by U.S. Food and Drug Administration (FDA).[32-35] However, the preparation of PEGylated prodrugs still remain challenging due to a lack of active sites for drug conjugation, and for PEG terminal conjugation, it is easy to prepare but may result in low drug loading content and high solubility. Shen's group reported that a PEGylated prodrug realized high drug loading content[36, 37] through conjugating anticancer drugs to oligo(ethylene glycol) (OEG) terminal, but the obtained low molecular weight OEGylated prodrugs exhibit poor stability because of high critical

micellar concentration (CMC). Recently, Jing's group reported on developing PEGylated prodrugs with high stability, based on amphiphilic copolymer with pendant functional groups for drug attachment.[38, 39] But in this system, the preparation is difficult and complicated with protection/deprotection; moreover, comonomers without functional groups are required to reduce the steric hindrance, resulting in low drug loading content. [40]

To dissipate these defects, we herein designed a new type of pH-sensitive PEGylated prodrug nano-carrier with high stability and high drug loading content based on OEG-DOX alternative conjugates. According to our previous researches, multifunctional PEG derivatives could be synthesized in a facile "one-pot" strategy by polycondensation of OEG diols with functional diacids, such as malic acid,[41] mercaptosuccinic acid [42-45] and maleic anhydride[46] owing to the chemoselectivity of $Sc(OTf)_3$ catalyst.[47, 48] In this study, as shown in Scheme 1, poly(oligo(ethylene glycol) malate) (POEGM) containing mutiple hydroxyl groups was first synthesized according to our previous reports. Then benzaldehyde groups were introduced into the POEGM backbone by esterification reaction, resulting in OEG-benzaldehyde alternative copolymer (POEGM-CHO). DOX, one of the most effective and widely used chemotherapeutic agents with high activity in different types of tumors,[2, 3, 49] was selected as a poorly water-soluble model drug and covalently bound to POEGM-CHO via a pH-sensitive benzoic imine linkage, which showed desired stability at physiological pH (7.4) and lability under acidic conditions.[50-52] As the steric hindrance was much reduced from the neighboring pendent benzaldehyde groups in POEGM-CHO, OEG-DOX alternative conjugates (POEGM-DOX) with high drug loading content was thus prepared. Amphiphilic POEGM-DOX can form stable micelles in aqueous media with low CMC, presenting a minimized release in circulation (pH 7.4) and a quick release in tumor cells (pH 5.8). This kind of biocompatible amphiphilic polymeric prodrug with high drug loading content, low CMC and stimuli responsive properties is expected to be a potential nano-carrier for cancer therapy.



Scheme 1 Synthetic routes of POEGM-DOX

Experimental

Materials

Tetrahydrofuran (THF), toluene and triethylamine (TEA) were dried over CaH_2 and distilled just prior to use. Oligo(ethylene glycol) diols (OEG_{13}, M_n = 600 and OEG_{22}, M_n = 1000, Aladdin, China) were dried by azeotropic distillation in the presence of dry toluene. Doxorubicin hydrochloride (DOX·HCl) was purchased from Shanxi Sciphar Hi-tech Industry Co., Ltd, China. Dicyclohexylcarbodiimide (DCC; 99 %; Aladdin, China), 4-dimethylaminopyridine (DMAP; 99 %; Aladdin, China), DL-malic acid (MA; 99 %; Aladdin, China), 4-Formylbenzoic acid (FBA, 98 %, TCI, Japan) and other reagents were used as received. $Sc(OTf)_3$ was synthesized according to our previous report.[53]

Preparation of POEGM

27.0 g of OEG_{13} (45 mmol), 6.03 g of MA (45 mmol) and 0.22 g of $Sc(OTf)_3$ (0.45 mmol) were stirred in a 250 mL round-bottom reactor at 80 °C under argon atmosphere for 4 h. Then the pressure was gradually decreased to 0.3-3 mmHg and stirred for 24 h. The crude product was purified by neutral alumina column chromatography with methylene choloride as the eluent. After concentration, the solution was poured into ethyl ether to precipitate $POEG_{13}M$, which was dried in vacuum overnight to constant weight. Yield: 30.2 g (96 %). Meanwhile, $POEG_{22}M$ was synthesized from 30.0 g of OEG_{22} (30 mmol), 4.02 g of MA (30 mmol) and 0.15 g of $Sc(OTf)_3$ (0.3 mmol) in the same manner as described above. Yield: 33.1 g (97 %).

Synthesis of POEGM-CHO

POEGM-CHO was prepared as follows: $POEG_{13}M$ (3.0 g, 4.29 mmol of OH group), 4-formylbenzoic acid (0.71 g, 4.71 mmol), DCC (1.77 g, 8.58 mmol) and DMAP (0.05 g, 0.43 mmol) were dissolved in 50 mL of anhydrous THF. The mixture was stirred at room temperature for 48 h under argon atmosphere, and the precipitation was removed by filtration. After concentrating by rotatory evaporator, $POEG_{13}M$-CHO was recovered by adding cold ethyl ether, and dried in vacuum to constant weight. Yield: 3.21 g (90 %). $POEG_{22}M$-CHO was synthesized based on 5.0 g of $POEG_{22}M$ (4.55 mmol of OH group), 0.76 g of 4-formylbenzoic acid (5.04 mmol), 1.88 g of DCC (9.1 mmol) and 0.05 g of DMAP (0.43 mmol) in the same manner as described above. Yield: 5.21 g (92 %).

Synthesis of POEGM-DOX

Conjugation of DOX to POEGM-CHO was accomplished through Schiff's reaction between the benzaldehyde group of POEGM-CHO and the amino group of DOX. $POEG_{13}M$-CHO (180 mg, 0.22 mmol of CHO group), DOX·HCl (118 mg, 0.22 mmol) and TEA (184 μL, 1.32 mmol) were dissolved in 30 mL of anhydrous dimethyl sulfoxide (DMSO) and stirred in dark at room temperature for 48 h. The obtained $POEG_{13}M$-DOX was purified by dialysis against DMSO and distilled water sequentially (MWCO 3500, Fisher Scientific). After filtration through a 0.45 μm syringe filter, $POEG_{13}M$-DOX was finally recovered by lyophilization. Yield: 276 mg (93 %). $POEG_{22}M$-DOX was also prepared in the same manner as described above. Yield: 364 mg (94 %). The DOX loading content, defined as the weight percentage of DOX in POEGM-DOX, was determined by UV-vis spectrophotometer at the absorbance of 479 nm, by treating POEGM-DOX with 1 N HCl for 24 h to cleave the benzoic imine bonds and diluting with acetate buffer (0.01 M, pH 5.0). Serially diluted concentrations of DOX·HCl in acetate buffer (0.01 M, pH 5.0) were used to construct a calibration curve.

Preparation of POEGM-DOX micelles

POEGM-DOX (25 mg) was dissolved in 5 mL of DMSO, and then 5 mL of PBS (0.01 M, pH 7.4) was added dropwise to the solution under vigorous stirring. The resulting solution was stirred for 2 h and dialyzed (MWCO 3500, Fisher Scientific) against PBS (0.01 M, pH 7.4) over 24 h to completely remove DMSO and form micelle structure. The final concentration of the micellar solution was adjusted to 1.0 mg/mL.

In vitro DOX release from POEGM-DOX micelles

As the benzoic-imine linkage connecting DOX to POEGM backbone is known for its lability at low pH and stability at neutral pH,[54] the pH-dependent release of DOX from POEGM-DOX nanocarriers was carried out as below: 4 mL of dispersed POEGM-DOX micellar solutions (1.0 mg/mL) were transferred into a dialysis membrane bag (MWCO 3500, Fisher Scientific), which was immersed in 40 mL of PBS (0.01 M) at pH 5.8 and 7.4 at a constant temperature of 37 °C in a water bath with horizontal shaking. At a predetermined time interval, 5 mL of incubated solution was taken out and replenished with an equal volume of corresponding fresh PBS. DOX release profiles were characterized by measuring the UV absorbance of the solutions at 479 nm with the help of a calibration curve of DOX in the same PBS. The release measurements were conducted in triplicate, and the results presented are the average data.

Cell viability assays

The *in vitro* cytotoxicity of POEGM-CHO was evaluated against MCF-7 cells (human breast cancer, obtained from Meihua Sui Group) by CCK-8 (Dojindo, Japan) assay. POEGM-CHO was dissolved in PBS (0.01 M, pH 7.4) and sterilized by filtration (0.22 μm). The cells were pre-incubated in a 96-well plate (1×10^4 cells/well for MCF-7 cells) with culture medium 10 % FBS/α-MEM (Invitrogen Co., Carlsbad, CA) in a humidified 5 % CO_2-containing atmosphere at 37 °C for 24 h to allow the cells to adhere to plate. Then cells were further incubated with POEGM-CHOs with followed concentrations, 0.1 mg/L, 1 mg/L, 10 mg/L, 100 mg/L and 1000 mg/L for 48 h. Subsequently, media was aspirated and replenished with 100 μL of fresh culture medium and 10 μL CCK-8 reagents were added into each well. The cells were incubated at dark for another 1 h. Absorbance was measured at 450 nm using a microplate reader (Sunrise™ Basic; TECAN, Zurich, Switzerland). Non-treated cells were used as a negative control, wells without cell but culture medium was used as blank. The relative cell viability was calculated as follows:

$$\text{Cell viability}(\%) = [(OD_{450\text{sample}} - OD_{450\text{blank}})/(OD_{450\text{control}} - OD_{450\text{blank}})] \times 100\%$$

Data are presented as average ± SD (n = 3).

The cytotoxicity of POEGM-DOX against MCF-7 cells was also characterized by CCK-8 assay. Similarly, cells were seeded in 96-well plates with a density of 1×10^4 cells/well and incubated for 24 h. Then the cells were exposed to free DOX and polymeric prodrug POEGM-DOXs with different DOX concentrations (0.01–10 mg/L DOX) at 37 °C. After incubated for 48 h, media was aspirated and replenished with 100 μL of fresh culture medium, 10 μL of CCK-8 reagents were added into each well and the cells were incubated at dark for another 1 h. Absorption was detected at a wavelength of 450 nm in a microplate reader. Non-treated cells were used as a negative

control, and wells without cell but culture medium was used as blank.

Characterization

^{1}H NMR spectra were recorded using a Bruker Avance DMX500 spectrometer in $CDCl_3$ or DMSO-d_6 with tetramethylsilane as internal standard. The molecular weight and molecular weight distribution were determined by gel permeation chromatography (GPC) on a Waters degasser, a Waters 1515 Isocratic HPLC pump, and columns: PLgel 5 μm MIXED-C, 300×7.5 mm. THF was used as the mobile phase with a flow rate of 1.0 mL/min at 60 °C and standard narrow PDI polystyrene (PS) was used for calibration. The hydrodynamic diameter and size distribution of micelles were determined by dynamic light scattering (DLS) at 90° angle to the incident beam on a Brookhaven 90 Plus particle size analyzer. All micellar solutions had a final polymer concentration of 1.0 mg/mL and were filtered through a 0.45 μm filter. TEM images were obtained using JEM-1230 operating at an acceleration voltage of 60 kV. A drop of 1.0 mg/mL micellar solution was put onto the surface of formvar-carbon film-coated copper grids. Excess solvent was quickly removed away with a filter paper and then stained by 2 wt % phosphotungstic acid aqueous solution. The critical micelle concentration (CMC) was estimated by fluorescence measurement using pyrene as a fluorescent probe. The pyrene concentration was fixed at 6.0×10^{-7} M with the concentration of block copolymer was varied from 1.0×10^{-6} to 1 mg/mL. Fluorescence excitation spectra (300-360nm) of the solutions were recorded using Hitachi F-4500 fluorescence spectrometer at 390 nm emission wavelength, with the excitation and emission bandwidths set at 5 nm slit width. The concentration of DOX was determined by measuring the absorbance at a wavelength of 479 nm using a Shimadzu UV2550 UV-vis spectrophotometer. Calibration curves were established from known concentrations of DOX in corresponding solution as shown in Fig. S2.

Results and discussion

Synthesis and characterization of POEGM

POEGM was synthesized via the direct polycondensation of OEG diols with equimolar amount of malic acid under reduced pressure in bulk using $Sc(OTf)_3$ as chemoselective catalyst. Fig. 1A shows a typical ^{1}H NMR spectrum of $POEG_{13}M$, in which all of the relevant signals were well labeled. The signal centered at 4.55 ppm (H^d) is attributed to the protons of methylidyne group in the malate unit, which strongly implies the acquisition of the PEG derivative with pendent secondary hydroxyl groups via chemoselective esterification of primary hydroxyl groups with carboxyl groups. Notably, the molar ratio of OEG/MA in the copolymer is in great agreement with the feeding molar ratio (1: 1), according to the integrals of corresponding protons. Meanwhile, the molecular weights of POEGMs were determined by GPC (Fig. 2); as a result, the unimodal spectrum further confirms the successful synthesis of POEGMs via polycondensation.

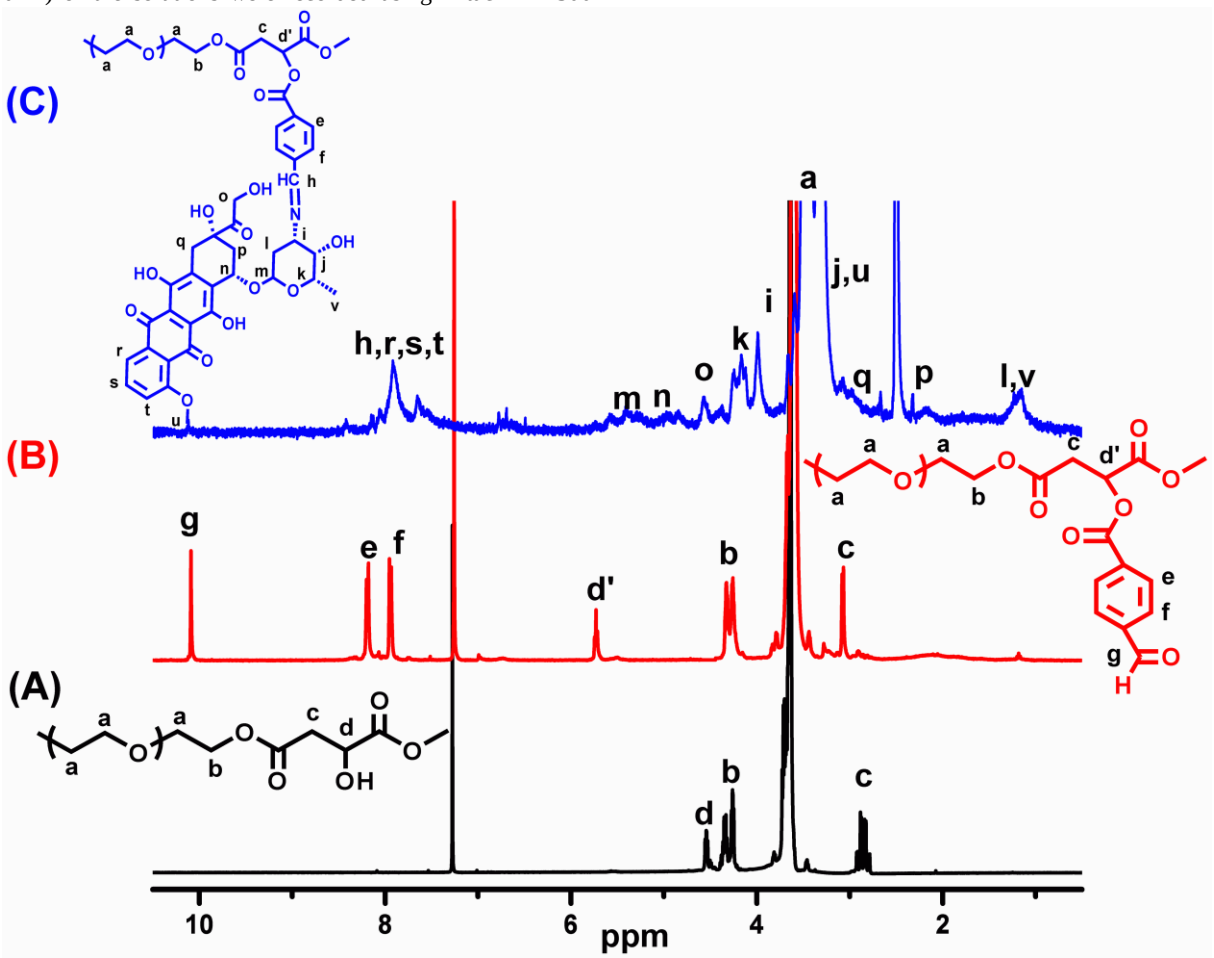


Fig. 1 ^{1}H NMR spectra of $POEG_{13}M$ (A), $POEGM_{13}$-CHO (B) in $CDCl_3$ and $POEGM_{13}$-DOX (C) in DMSO-d_6.

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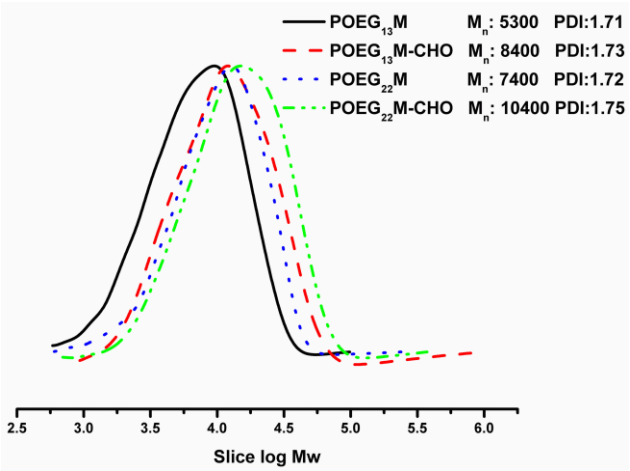

Fig. 2 GPC curves of $POEG_{13}M$, $POEGM_{13}$-CHO, $POEG_{22}M$ and $POEG_{22}M$-CHO.

Synthesis and characterization of POEGM-CHO

POEGM-CHO was synthesized via esterification between hydroxyl groups of POEGMs and the carboxyl groups of 4-formylbenzoic acid. A representative ^1H NMR spectrum of $POEG_{13}M$-CHO was presented in Fig. 1B. All the peaks can be well assigned with their chemical structure. Importantly, compared with its precursor ($POEG_{13}M$, Fig. 1A), the shift of the signal from δ 4.55 ppm (H^d) to δ 5.46 ppm ($H^{d'}$) clearly indicates the complete esterification. Moreover, the presence of the signal at 10.1 ppm (H^g) fairly exhibited the presence of the benzaldehyde groups in POEGM-CHO. POEGM-CHOs were further characterized by GPC measurements. As shown in Fig. 2, compared with their corresponding precursors, the GPC curves of POEGM-CHOs significantly shifted to high molecular weight region, but still maintained unimodal, signifying the successful preparation of POEGM-CHOs without any byproducts.

Synthesis and characterization of POEGM-DOX

POEGM-DOX was prepared via Schiff's reaction between POEGM-CHO and DOX·HCl in the presence of TEA. The molecular structure of the $POEG_{13}M$-DOX was confirmed by ^1H NMR (Fig. 1C). The presence of proton signal at δ 8.4 ppm (H^h) and the absence of the aldehyde proton (10.1 ppm, H^g in Fig. 1B) intelligibly confirm the formation of benzoic-imine bond, which clearly indicates the successful synthesis of OEG-DOX alternative copolymers without free aldehyde groups. The amount of conjugated DOX was determined by UV-vis spectrometry after treating POEGM-DOX with 1N HCl. The results show that the drug loading contents of $POEG_{13}M$-DOX and $POEG_{22}M$-DOX are 38.2 wt % and 30.4 wt % respectively, which are very close to the theoretic value.

CMC of POEGM-DOX micelles

Amphiphilic POEGM-DOX can self-assemble into micelles in aqueous solution by dialysis method. The CMC of POEGM-DOX micelles were determined using pyrene as a fluorescent probe. The intensity ratio of bands at 338 and 333 nm (I_{338}/I_{333}) was calculated and plotted versus the polymer concentrations, presenting a sigmoid curve as shown in Fig. 3. The sharp rise in intensity ratio of peaks at 338 and 333 nm of pyrene in the excitation spectra points the on-set of micellization for amphiphilic copolymer. Herein, the CMCs of $POEG_{13}M$-DOX and $POEG_{22}M$-DOX are about 0.021 mg/mL and 0.053 mg/mL, intelligibly indicating that POEGM-DOX can self-assemble into stable micelles in aqueous solution with the conjugated hydrophobic drug (DOX) being sufficiently protected by the OEG shell. Moreover, the CMC value of $POEG_{13}M$-DOX is lower than that of $POEG_{22}M$-DOX, probably due to the higher DOX substitution and shorter OEG chain length of $POEG_{13}M$-DOX.

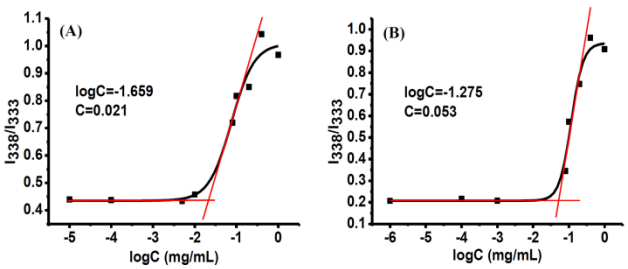

Fig. 3 Plots of fluorescence intensity ratio I_{338}/I_{333} from pyrene excitation spectra *vs.* log C for $POEG_{13}M$-DOX and $POEG_{22}M$-DOX.

Particle size and morphology of POEGM-DOX micelles

The morphology and particle size of $POEG_{13}M$-DOX and $POEG_{22}M$-DOX micelles were characterized by TEM and DLS, as shown in Fig. 4. $POEG_{13}M$-DOX has a diameter of 130 nm with narrow size distribution (PDI = 0.106) in aqueous solution, which is in good accordance with that detected by TEM. However, no signal of any particle could be detected by DLS for $POEG_{13}M$-CHO, which implies that $POEG_{13}M$-CHO is entirely water-soluble, whereas $POEG_{13}M$-DOX can form stable micelles due to the chemically bonding of hydrophobic DOX moieties on the $POEG_{13}M$-CHO backbone. Similarly, $POEG_{22}M$-DOX can also self-assemble into uniform micelles with smaller particle size (33 nm, PDI = 0.103) than those by $POEG_{13}M$-DOX. This phenomenon could be attributed to the increase of the weight fraction of hydrophilic PEG segment in polymer-drug conjugates, which might evade the hydrophobic interaction and van der Waals interaction of hydrophobic domains of the micelles.[55] Just as reported in our previous research,[56, 57] the micelle sizes of amphiphilic polymer could be controlled by the ratio of hydrophobic segments to hydrophilic parts, and the decrease of the weight fraction of PEG segment may lead to form large aggregates. Importantly, all these results from TEM and DLS demonstrate that the dimension of these prodrug micelles could be controlled by the length of OEG segments, providing POEGM-DOX nanocarriers with a tunable diameter.

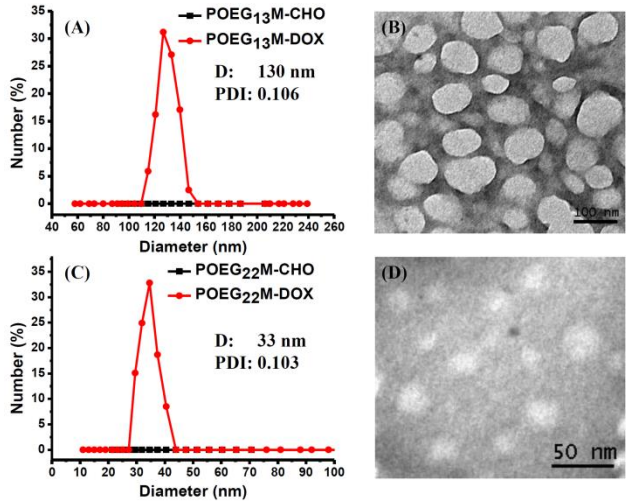

Fig. 4 Particle size, distribution of $POEG_{13}M$-DOX micelles (A) and $POEG_{22}M$-DOX micelles (C) measured by DLS; TEM images of $POEG_{13}M$-DOX micelles (B) and $POEG_{22}M$-DOX micelles (D).

In vitro DOX release from POEGM-DOX micelles

The pH-dependent release of DOX from POEGM-DOX micelles was investigated using dialysis methods under two different pH values (5.8 and 7.4) at 37 °C. The solutions taken out at predetermined intervals were measured by UV-vis spectrometry at 479 nm to determine the amount of DOX that has been released. The *in vitro* DOX release profiles from $POEG_{13}M$-DOX and $POEG_{22}M$-DOX micelles both showed faster release of DOX under acidic environments than that at neutral pH (Fig. 5). For $POEG_{13}M$-DOX micelles, almost 45 % of DOX were released within 32 h at pH 5.8. However, only 8 % of DOX was released at pH 7.4 under the same conditions. Similar DOX release profiles were also observed from $POEG_{22}M$-DOX micelles. Notably, the pH change from 7.4 to 5.8 corresponds to the pH change from the normal physiological environment in body circulation to intracellular tumor tissue. So these nanocarriers based on amphiphilic OEG-DOX alternative conjugates are expected to be a promising drug delivery system for tumor-targeted delivery of DOX.

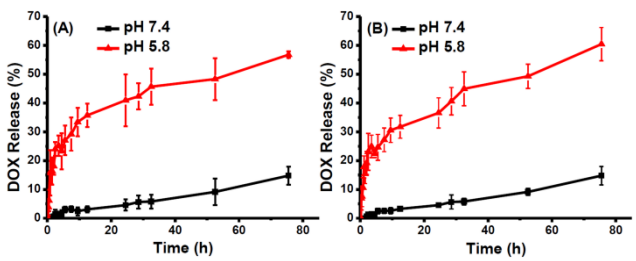

Fig. 5 *In vitro* DOX release profiles from $POEG_{13}M$-DOX micelles (A) and $POEG_{22}M$-DOX micelles (B).

In vitro cytotoxicity studies.

It is important and necessary to evaluate the biocompatibility of polymeric nanocarriers for drug delivery. The *in vitro* cytotoxicities of POEGM-CHOs with a series of polymer concentrations toward MCF-7 cells were evaluated using CCK-8 assay. As shown in Fig. 6, after 48 h of incubation, $POEG_{13}M$-CHO and $POEG_{22}M$-CHO both showed great biocompatibility to MCF-7 cells, even with high polymer concentration up to 1 mg/mL. The results imply the low cytotoxicity of POEGM-CHOs, which would be potential biocompatible materials for efficient intracellular drug delivery.

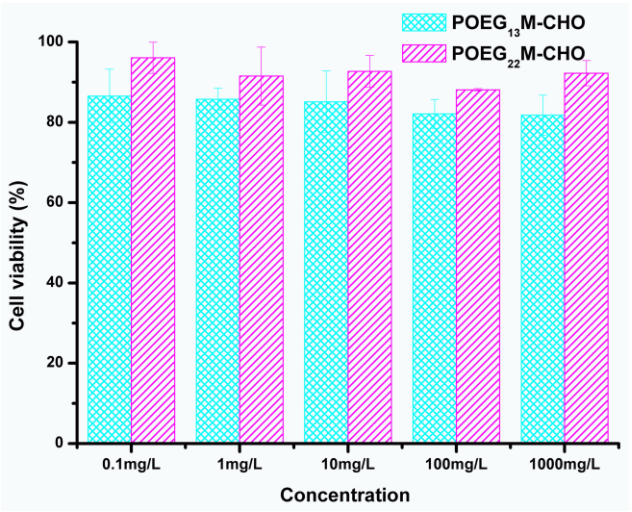

Fig. 6 *In vitro* cytotoxicities of $POEG_{13}M$-CHO and $POEG_{22}M$-CHO to MCF-7 cells after incubation for 48 h.

After conjugating DOX to POEGM-CHO via benzoic-imine linker, the obtained POEGM-DOX could form stable micelles, from which free DOX molecules could be quickly released under acidic condition. Accordingly, the ability to inhibit the cell proliferation of POEGM-DOX was evaluated using MCF-7 cells with different DOX concentrations (0.01 to 10 mg/L). The MCF-7 cells incubated with free DOX were also studied as a comparison (Fig. 7). Unlike biocompatible POEGM-CHO precursor, POEGM-DOX exhibits significant cytotoxicity toward MCF-7 cells. Free DOX shows slightly higher cytotoxicity to MCF-7 cells than that by POEGM-DOX at the equivalent DOX dosage, probably owing to the rapidly diffusion of small molecules (free DOX) into cell nuclei once inside the cells to effectively inhibit the DNA replication. However, POEGM-DOX micelles were endocytosed to enter the cells, and activated in acidic endosomal compartments for DNA inhibition.

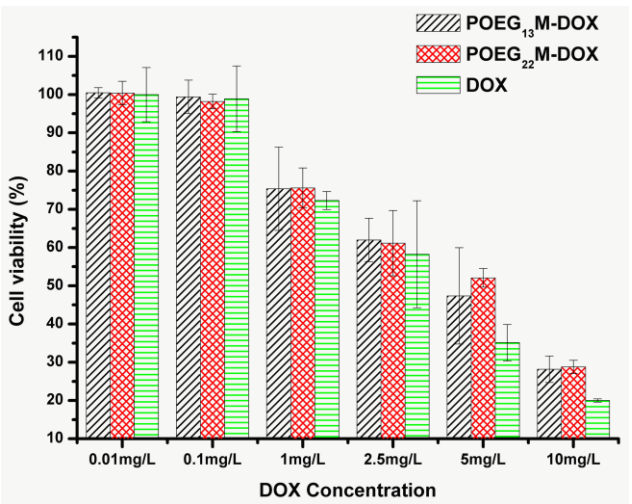

Fig. 7 *In vitro* cytotoxicity of $POEG_{13}M$-DOX, $POEG_{22}M$-DOX and free DOX with different DOX concentrations against MCF-7 cells after 48 h incubation.

Conclusions

In this study, we developed a facile strategy to synthesize amphiphilic OEG-DOX alternative conjugates (POEGM-DOX), which can form stable micelles in aqueous solution with high drug loading contents and low critical micelle concentration. The diameter of POEGM-DOX micelles could be well controlled by the length of the OEG segment. Due to the acid-sensitive benzoic-imine linker between POEGM backbone and DOX moieties, POEGM-DOX micelles present a pH-stimuli drug release behavior. The POEGM-DOX micelles is expected to be sufficiently stable during blood circulation, effectively delivered into tumor cells by EPR effect, and quickly activated by the release of conjugated DOX at endo/lysosomal compartments to improve the therapeutic outcome of the chemotherapy.

Acknowledgements

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Notes and references

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† Electronic Supplementary Information (ESI) available: ESI includes the ^{1}H NMR characterization of $POEG_{22}M$, $POEGM_{22}$-CHO and $POEGM_{22}$-DOX and the calibration curves of DOX in PBS (0.01 M, pH = 7.4, 5.8, 5.0). See DOI: 10.1039/b000000x/

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