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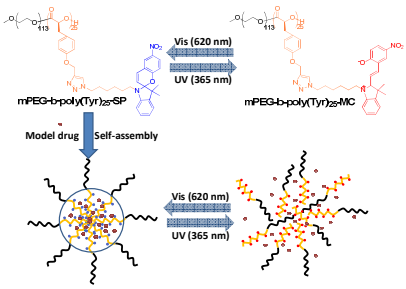
Graphic Abstract

Spiropyran-decorated light-responsive amphiphilic poly(α-hydroxy acids) micelles constructed via CuAAC reaction

Yile Niu, Yefei Li, Yanbing Lu* and Weijian Xu



Light-responsive amphiphilic poly(α-hydroxy acids) with pendent spiropyran chromophore was synthesized and the resultant micelles assembled in aqueous solution presented excellent light-response.

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Light-responsive amphiphilic poly(α-hydroxy acids) mPEG-b-poly(Tyr)-SP was prepared by introduction of spiropyran chromophore into the side chain of poly(ethylene glycol)-modified poly(α-hydroxy acids) (mPEG-b-poly(Tyr)) via copper-catalysed azide-alkyne cycloaddition (CuAAC) reaction. The resultant copolymer can self-assemble into spherical micelles with an average diameter of 222.7 nm and a critical micelle concentration of 0.0085 mg mL^{-1}. The micelles showed reversible self-assembly and disassembly in aqueous solution under the alternative UV and visible light irradiation. Model drug coumarin 102 was then encapsulated into the micelles successfully. Light-controlled release and re-encapsulation behaviours were demonstrated by fluorescence spectroscopy. MTT assay revealed that the mPEG-b-poly(Tyr)-SP micelles exhibited excellent cell compatibility. This study provides a convenient way to construct smart poly(α-hydroxy acids)-based nanocarriers for controlled release and re-encapsulation of hydrophobic drugs.

Introduction

Polymeric micelles self-assembled from amphiphilic polymers have attracted more and more attention in the field of drug delivery due to their distinct advantages including improved solubility of hydrophobic anticancer drugs and prolonged circulation time.[1-4] Controlled drug release can be achieved by molecular design to prepare stimuli-responsive micelles. The most frequently used stimuli are pH, temperature, light, redox potential, ultrasound, charge, gases, biomolecules and enzymes.[5-14] Compared with other stimuli-responsive systems, light-responsive polymeric micelles don't need any changes in the surroundings. In addition, the wavelength and intensity of illumination can be adjusted accurately and the time, direction and area of illumination are easy to be controlled.[15-18] Light-responsiveness is usually provided by photochromic molecules attached to the polymers. Spiropyran (SP), as one of the well-investigated photochromic compounds, adopts the colorless SP form in the dark or under visible light, whereas it can convert to the colored and fluorescent merocyanine (MC) form upon UV irradiation.[19-21] This isomerization is accompanied by a very large change in polarity, which is reflected by changes in hydrophilicity/hydrophobicity.[22-25] Chen synthesized amphiphilic triblock copolymer covalently attached with spiropyran moieties. This triblock copolymer micelle formed a fluorescence resonance energy transfer system in water and displayed reversible fluorescence modulation through energy transfer. [23] Recently, Kim prepared a series of hyperbranched polyglycerols (SP-*hb*-PG), which were obtained from the ring-opening multibranching polymerization of glycidol using spiropyran as initiator. The resulting polymeric micelles showed successful self-assembly and disassembly behaviors via irradiating with UV and visible light.[25]

Poly(α-hydroxy acids), such as poly(lactic acid) (PLA), poly(glycolic acid) and poly(lactic-co-glycolic acid), are widely used in controlled release, drug delivery and tissue engineering because of their excellent biocompatibility and biodegradability.[26-29] One drawback of conventional poly(α-hydroxy acids) is their lack of side-chain functionalities, which has prevented structural alteration via side-chain modifications and thus limited poly(α-hydroxy acids) applications, particularly in situations requiring postmodification of poly(α-hydroxy acids) side chains. Ring-opening polymerization (ROP) of O-carboxyanhydrides (OCAs), a class of five-membered ring compounds derived from amino acids, has recently emerged as a viable method to prepare side-chain functionalized poly(α-hydroxy acids).[30-37] Bourissou prepared an gluOCA from glutamic acid. Well-controlled poly(α-hydroxy acids) featuring pendant carboxylic acid groups were obtained via ROP of gluOCA followed by deprotection of the benzyl ester side groups.[31] Dove synthesized a malOCA from L-malic acid. After ROP of malOCA and deprotection of the benzyl ester side

groups, the resulting hydrophilic poly(α-malic acid) was observed to fully degrade within 7 days in aqueous solution.[32] In 2012, Cheng reported the synthesis of Tyr(alkynyl)-OCA, an OCA bearing alkyne group derived from tyrosine.[35] A side-chain aminated poly(α-hydroxy acids) was achieved through the ROP of Tyr(alkynyl)-OCA, followed by thiol-yne "click" photochemistry with 2-aminoethanethiol hydrochloride(AET). The poly(α-hydroxy acids) showed excellent cell penetration and gene delivery properties. Wang developed the synthesis of an OCA bearing carbobenzyloxy (Cbz)-protected amino groups derived from lysine. After ROP of the OCA and removal of Cbz protecting group, poly(α-hydroxy acids) with pendant amino group was obtained. The poly(α-hydroxy acids) exhibited excellent cell compatibility.[38]

However, there have been limited reports of the synthesis of stimuli-responsive poly(α-hydroxy acids). Cheng reported a redox-responsive core cross-linked (CCL) poly(α-hydroxy acids) micelle obtained through ROP of Tyr(alkynyl)-OCA with monomethoxy poly(ethylene glycol) (mPEG), followed by cross-linking the polyester alkynyl side chains in the hydrophobic cores with bis(azidoethyl) disulfide.[34] The CCL micelles not only improved the structural stability, but also allowed the controlled release of cargo molecules in response to the reducing reagent.[36, 37]

In this study, we developed a facile method to prepare spiropyran-decorated light-responsive amphiphilic poly(α-hydroxy acids). The spiropyran chromophore was introduced into the side chain of the hydrophobic poly(α-hydroxy acids) via copper catalyzed azide-alkyne cycloaddition (CuAAC) reaction between azide-functional spiropyran (SP-N_3) and amphiphilic mPEG-b-Poly(Tyr) (Scheme 1). Owing to the reversible SP-ME photoisomerization upon UV/vis irradiation, the resultant amphiphilic copolymers showed good light-response. Under visible light, spiropyran chromophore was in SP form and the copolymer can self-assemble into micelles. However, when the micelles were irradiated with UV light, the chromophore converted into MC form and the micelles would disassemble.

Experimental Section

Materials

N-(tert-butyloxycarbonyl)-(S)-tyrosine (Boc-L-tyr) was purchased from Shanghai Hanhong chemical Co. Ltd. and used as received. Monomethoxy poly(ethylene glycol) (MWs= 5 kDa, mPEG5k), nile red (NR), coumarin 102, 5-nitro-salicylaldehyde and 2,3,3-trimethyl-indolenine were purchased from Sigma-Aldrich and used as received. Propargylbromide, sodium nitrite, triphosgene, 1,6-dibromohexane, and 4-dimethylaminopyridine (DMAP) were purchased from Sinopharm Chemical Reagent Co. Ltd. Dichloromethane (DCM) was dried over calcium hydride for 24 h at room temperature and distilled under reduced pressure. Dimethylformamide (DMF) was dried by columns packed with 4 Å molecular sieves. Tetrahydrofuran (THF) was distilled in the presence of sodium and benzophenone before use. The monomer Tyr(alkynyl)-OCA was synthesized from Boc-L-tyr according to the strategy reported in the literatures.[34,35] Other solvents were purified by standard procedures.

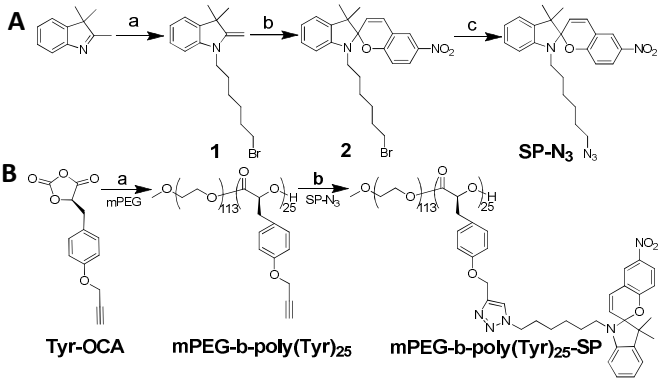
Characterization

FT-IR spectra of the monomer and polymers were obtained with a WQF-410 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on an INOVA-400 instrument using TMS as an internal standard. Gel permeation chromatography (GPC) of the obtained diblock copolymer was performed on a Waters 1515 GPC instrument in THF calibrated with standard polystyrene. Fluorescent spectra were recorded at room temperature with a HITACHI F-4600 fluorescence spectrophotometer with the excitation and emission slit widths at 5.0 and 5.0 nm respectively. Scan electron microscopes (SEM) were performed on a JSM-6700F SEM at 5.0 kV. Dynamic light scattering (DLS) measurements were carried out using a Nano-ZS90 zeta-potential and particle analyzer. The UV-Vis absorption spectra were recorded on a UV Bluestar Plus UV-Vis spectrophotometer.

Synthesis of azide-containing spiropyran derivative SP-N_3

The azide-containing SP-N_3 was synthesized according to the route shown in Scheme 1. A mixture of 2, 3, 3-trimethylindolenine (3.18 g, 0.02 mol) and excess 1,6-dibromohexane (14.7 g, 0.06 mol) in acetonitrile was heated for 24 h under reflux and N_2. After cooling down to room temperature, the solvent was distilled off under reduced pressure. The residue was stirred with aqueous NaOH solution at room temperature for 3 h and extracted with DCM. The organic phase was concentrated to afford compound **1** (5.87 g, 91%).

Compound **1** (1.69 g, 5.24 mmol) and 5-nitrosalicylaldehyde (0.96 g, 5.67 mmol) were refluxed in ethanol (20 mL) for 24 h. On cooling, a precipitate was formed which was washed with cold ethanol. Recrystallization of the precipitate from ethanol



Scheme 1 Synthesis route for (A) azide-containing spiropyran derivative (a: 1, 6-dibromohexane, CH_3CN, aqueous NaOH solution; b: 5-nitrosalicylaldehyde, ethanol; c: sodium azide, DMF) and (B) mPEG-b-poly(Tyr)$_{25}$-SP (a: mPEG5k, DMAP, DCM; b: sodium ascorbate, copper sulphate, DMF).

gave **2** (1.48 g, 60%) as a green powder.
^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ 1.11-1.79 (m, 14H, alkyl), 3.02-3.15 (m, 2H, NC**H$_2$**), 3.29-3.32 (t, 2H, J=6.8 Hz, C**H$_2$**Br), 5.77-5.80 (d, 1H, J=10.4 Hz, vinyl), 6.48-6.50 (d, 1H, J=7.6 Hz, aromatic), 6.66-6.68 (d, 1H, J=8.4 Hz, aromatic), 6.78- 6.82 (t, 1H, J=7.2 Hz, aromatic), 6.89-6.92 (d, 1H, J=10.4 Hz, vinyl) ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ 1.11-1.79 (m, 14H, alkyl), 3.02-3.15 (m, 2H, NC**H$_2$**), 3.29-3.32 (t, 2H, J=6.8 Hz, C**H$_2$**Br), 5.77-5.80 (d, 1H, J=10.4 Hz, vinyl), 6.48-6.50 (d, 1H, J=7.6 Hz, 7.00-7.03 (d, 1H, J=8.4 Hz, aromatic), 7.10-7.14 (t, 1H, J=8.2 Hz, aromatic), 7.93 (s, 1H, aromatic), 7.95-7.96 (d, 1H, J=2.4 Hz, aromatic).
^{13}C NMR (400 MHz, $CDCl_3$): δ 19.71, 26.11, 26.32, 27.83, 28.78, 32.53, 33.88, 43.68, 52.57, 106.64, 106.74, 115.64, 118.49, 119.40, 121.69, 122.07, 122.83, 126.05, 127.52, 128.25, 135.94, 140.86, 147.08, 159.68.
A mixture of compound **2** (1.32 g, 2.8 mmol) and sodium azide (0.47 g, 7 mmol) in DMF was stirred for 24 h under room temperature. Thereafter, 100 mL H_2O was added and the mixture was extracted with DCM. The organic phase was dried over $MgSO_4$ and the solvent was distilled off under reduced pressure to yield SP-N_3 (1.08 g, 89%).
^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ 1.10-1.59 (m, 14H, alkyl), 3.01-3.11 (m, 2H, NC**H$_2$**), 3.14-3.17 (t, 2H, J=6.8 Hz, C**H$_2$**N_3), 5.77-5.79 (d, 1H, J=10.4 Hz, vinyl), 6.48-6.49 (d, 1H, J=7.6 Hz, aromatic), 6.64-6.66(d, 1H, J=9.6 Hz, aromatic), 6.76- 6.80 (t, 1H, J=7.4 Hz, aromatic), 6.82-6.84 (d, 1H, J=10.0 Hz, vinyl), 6.99-7.01 (d, 1H, J=8.4 Hz, aromatic), 7.08-7.12 (t, 1H, J=8.2 Hz, aromatic), 7.91 (s, 1H, aromatic), 7.93-7.94 (d, 1H, J=2.4 Hz, aromatic).
^{13}C NMR (400 MHz, $CDCl_3$): δ 19.85, 26.03, 26.56, 26.87, 28.79, 28.86, 43.63, 51.35, 52.65, 106.64, 106.76, 115.53, 118.50, 119.36, 121.72, 122.05, 122.74, 125.93, 127.76, 128.15, 135.95, 140.93, 147.10, 159.68.

Synthesis of mPEG-b-poly(Tyr)$_{25}$

The amphiphilic diblock copolymer mPEG-b-poly(Tyr)$_{25}$ was synthesized via ROP of Tyr(alkynyl)-OCA with DMAP as catalyst and $mPEG_{5k}$ as initiator.[34,35] In a glovebox, Tyr(alkynyl)-OCA (615mg, 2.5mmol) in DCM (10 mL) was added to a DCM solution (1 mL) of DMAP (12.2 mg, 0.1mmol) and $mPEG_{5k}$ (500mg, 0.1mmol) at room temperature. The complete monomer consumption was verified by measuring the intensity of the anhydride peak of OCA at 1810cm^{-1} by FT-IR. After the polymerization was complete, the diblock copolymer mPEG-b-poly(Tyr)$_{25}$ was precipitated with ether, collected by centrifugation, and the precipitate was dried under vacuum (843mg, 93.3% yield).

Post-functionalization via CuAAC reaction

mPEG-b-poly(Tyr)$_{25}$ (100mg, 10.4 mmol), SP-N_3 (equiv with respect to acetylene groups), and 400 μL of 5 mg mL^{-1} sodium ascorbate were dissolved in DMF. The resulting solution was transferred to a Schlenk flask and deoxygenated. After the solution had warmed to room temperature, a 0.1 M solution of $CuSO_4{\cdot}5H_2O$ in deoxygenated DMF (5 mol % with respect to the acetylene groups) was added under nitrogen, and the reaction mixture was then stirred at room temperature for 10 h. At the end of the reaction, the solids in the reaction mixture were removed by filtration. The reaction mixture was isolated by dialysis (MWCO of 1000) in water overnight and then dried under vacuum to yield a pale yellow solid.

Preparation of micelles

Micelles of the spyropyran-containing copolymer mPEG-b-poly(Tyr)$_{25}$-SP were prepared by a solvent exchange method. 10.0 mg copolymer was dissolved in DMF (2 mL), then DI water (20 mL) was slowly added under vigorous stirring. After vigorous stirring for another 2 h at room temperature, the micelles were obtained and further dialyzed against DI water for 24 h to remove DMF (MWCO of 1000 Da). The final polymer concentration was adjusted by adding DI water to 0.2 mg mL^{-1}.

Determination of critical micelle concentration (CMC)

The CMC of the micelle was determined using Nile Red (NR) as a fluorescence probe. NR in THF (0.1 mg mL^{-1}, 30 μL) was added to a glass vial via a microsyringe. After THF was evaporated, a micellar solution (2 mL) was added. The concentration of the micellar solution was varied from 0.2 to 5 × 10^{-4} mg mL^{-1}. Then the solution was stirred for 5 h. Finally, fluorescence measurements were taken at an excitation wavelength of 557 nm and the emission monitored from 580 to 720 nm.

Light-responsive experiments

For light-responsive experiments, the mPEG-b-poly(**Tyr**)$_{25}$-SP micelles were irradiated with UV light at 365 nm or visible light at 620 nm. The micelles (0.05 mg mL^{-1}) were exposed to UV light for 5 min, followed by visible light for 30 min. UV-vis spectroscopy measurement was used to monitor the whole process. Then, the samples were alternatively irradiated with UV light and visible light to confirm the reversible light-responsiveness of the micelles.

In vitro cytotoxicity assay

The HeLa cells were used for studying the cytotoxicity of the micelles. They were seeded in a 96-well plate at the density of 9.6 × 10^3 cells per well and incubated in DMEM at 37 °C in 5% CO_2 for 24 h. Then, the medium was removed and replaced with 200 μL polymer micelles. The aggregate concentrations of each formulation were prepared by serial dilution with DMEM medium. After treatment for 24 h, 20 μL of fresh medium containing 10% of MTT of 5 mg mL^{-1} stock was replaced to each well. The plates were incubate d for 4 h, and then 230 μL of DMSO was added to each well to dissolve intracellular MTT formazan crystals, followed by absorbance at 490 nm using a

microplate reader (Varioskan Flash, Thermo Scientific). Experiments were done in triplicate.

Light-triggered release and re-encapsulation of coumarin 102

Coumarin 102 was selected as hydrophobic model drug to be encapsulated into the mPEG-b-poly(Tyr)$_{25}$-SP micelles and fluorescence spectroscopy was used to investigate the release and re-encapsulation behaviors of the micelles. 2.0 mL coumarin 102 solution in acetone (0.05 mg mL^{-1}) was added into a 10 mL vessel. After the acetone dried out, 0.2 mg mL^{-1} micellar solution (5 mL) was added dropwise into the vessel under vigorous stirring. After stirring for 24 h, the fluorescence spectrum of the micelles was recorded immediately. The micelles were then exposed to 365 nm UV light for 10 min and subsequently irradiated with 620 nm visible light for 2 h. Fluorescence measurements were taken at an excitation wavelength of 420 nm and the emission spectrum monitored from 440 to 650 nm.

Results and discussion

Synthesis and Characterization of Block Copolymers

To achieve the light-responsive polyester, amphiphilic diblock copolymer mPEG-b-Poly(Tyr)$_{25}$ bearing pendent propargyl group was readily prepared via living ROP of Tyr(alkynyl)-OCA at the monomer to initiator of 25 using DMAP as catalyst and mPEG$_{5k}$ as macroinitiator. The structures of the copolymer were demonstrated by ^{1}H NMR and FT-IR, which were in accordance with those reported in reference 34-35. Calculating from the ^{1}H NMR (Figure S2A), the degree of polymerization of polyester backbone was determined to be 23, and the number average molecular weight (M_n) value of mPEG-*b*-Poly(Tyr)$_{25}$ was 9.6 kDa, which were close to the theoretical values. GPC analysis showed the obtained copolymer had a narrow polydispersity index of 1.09 (Figure S3).

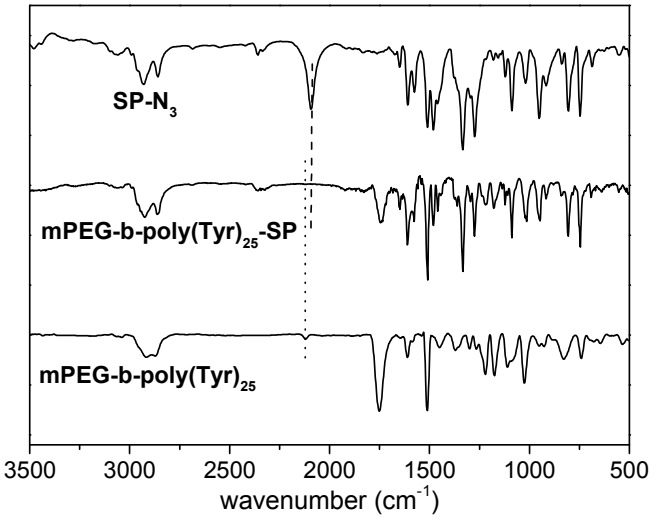


Figure 1 FT-IR spectra of SP-N$_3$ (A), mPEG-*b*-poly(Tyr)$_{25}$-SP (B) and mPEG-*b*-poly(Tyr)$_{25}$ (C).

The azide-containing SP-N$_3$ was synthesized according to the route shown in Scheme 1 and its structure was verified by ^{1}H NMR and FTIR. The prepared SP-N$_3$ exhibited a clear color transition from colorless to pink upon 365 nm UV light irradiation, displaying the desired UV-responsive character. The light-responsive amphiphilic polyester mPEG-b-poly(Tyr)$_{25}$-SP was thus achieved by introduction of the spiropyran chromophore into the hydrophobic block of mPEG-b-poly(Tyr)$_{25}$ using highly specific and efficient CuAAC click reaction. The complete disappearance of characteristic azide and alkynyl absorption peaks at ~2100 cm^{-1} in FTIR spectrum confirmed the complete click reaction (Figure 1). ^{1}H NMR was further used to demonstrate the functionalization of mPEG-*b*-poly(Tyr)$_{25}$ by the appearance of new resonances consistent with the triazole proton at 8.16 ppm (Figure S2B).

Micelle preparation and characterization of mPEG-b-poly(Tyr)$_{25}$-SP

It is known that amphiphilic polymers can self-assemble into micelles, polymersomes, or other assembies in selected solvents. In our work, we synthesized amphiphilic polymer mPEG-b-poly(Tyr)$_{25}$-SP which contains a hydrophilic PEG chain and a hydrophobic poly(Tyr). The micelles were prepared from the amphiphilic copolymer by initial dissolution in DMF followed by the slow addition of excess amounts of DI water with subsequent dialysis. The self-assembly behavior of mPEG-b-poly(Tyr)$_{25}$-SP micelles was investigated by fluorescence spectroscopy study with NR as fluorescence probe.[24, 39] It can be seen from Figure 2A that NR exhibited a relatively low fluorescence intensity at low concentrations (e.g., 0.001 mg mL^{-1}), indicating that the NR was in water, and few micelles were present. With increasing concentration of micellar solution, the fluorescence intensity increased dramatically. The result indicated that NR was encapsulated into a hydrophobic

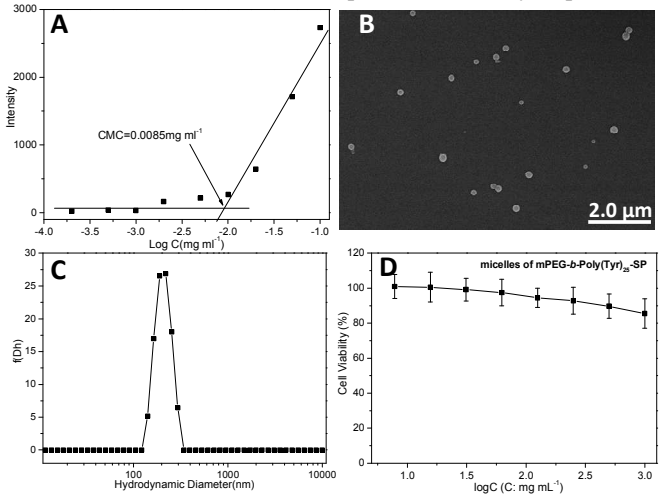


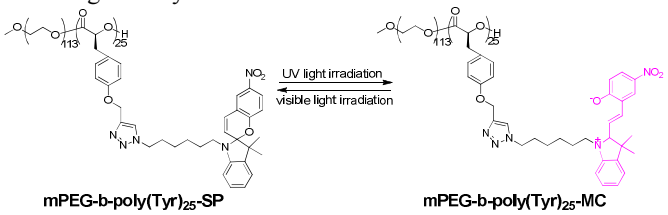
Figure 2 Characterization of mPEG-*b*-poly(Tyr)$_{25}$-SP micelles: plot of the fluorescence intensity at 631 nm versus the log of micelle concentration (A), SEM image (B), DLS size distribution (C) and plot of cell viability against HeLa cell determined by MTT assay (D).

environment in the interior of the micelles, suggesting formation of self-assemblies. The CMC was obtained from the intersection of two straight lines: the base line and the tangent of the rapid rising curve. The value of the CMC was about 0.0085 mg mL^{-1}, which was much lower than that of mPEG-b-poly(Tyr)$_{25}$.[34,35] It was because the introduction of the spiropyran increased the hydrophobicity of the hydrophobic chain. The morphology of the self-assembled micelles was measured by SEM. SEM image in Figure 2B revealed that the micelles were formed with a spherical morphology and the average size was about 200 nm. To accurately measure the diameter of the micelles, DLS was further conducted and the result was shown in Figure 2C. The figure showed that mPEG-b-poly(Tyr)$_{25}$-SP formed micelles with a number-average hydrodynamic diameter (Dh) of 222.7 nm and a polydispersity of 0.168, indicating that the micelles had a narrow size distribution. The bigger diameter of the micelles determined by DLS than that by SEM was probably due to shrinkage of the PEG shell upon drying.[40-43]

To evaluate the potential toxicity of polymeric materials, the in vitro cytotoxicity of mPEG-b-Poly(Tyr)$_{25}$-SP toward HeLa cells was assessed by MTT assay. As shown in Figure 2D, the viability of HeLa cells after treating the mPEG-b-Poly(Tyr)$_{25}$-SP micelles still kept about 90% when the micellar concentration reached at 500 mg L^{-1}, indicating that the prepared mPEG-b-Poly(Tyr)$_{25}$-SP has low cytotoxicity. It was mainly attributed to the excellent biocompatibility of the PEG and poly(α-hydroxy acids). It is reasonable that the amphiphilic copolymer mPEG-b-Poly(Tyr)$_{25}$-SP in this study has potential to be an ideal drug carrier system.

Light-Response of the Micellar Aggregates

As shown in Scheme 2, under UV light irradiation and visible light irradiation, the spiropyran chromophore proceeds photoisomerization between hydrophobic SP and hydrophilic MC. When the spiropyran chromophore was introduced into the side chain of amphiphilic mPEG-b-poly(Tyr)$_{25}$, light-responsive polymer was obtained. In order to investigate light-responsive behavior, the prepared micelles was irradiated with 365 nm UV light and subsequent 620 nm visible light. UV-vis spectroscopy was employed to monitor the micellar aggregation behavior. The photo-isomerization process for the block copolymer mPEG-*b*-Poly(Tyr)$_{25}$-SP was shown in Figure 3. Before UV irradiation, no absorption peak was found at 550 nm and the solution was colorless. After UV irradiation, the peak at 550 nm gradually increased as a function of the UV irradiation



Scheme 2 Reversible photoisomerization of mPEG-*b*-poly(Tyr)$_{25}$-SP

time (Figure 3A), indicating that colorless SP changed to colored MC. The micellar solution became pink. Figure 3B showed the back photoisomerization process upon subsequent exposure to 620 nm visible light. After 120 min of irradiation, a colorless solution appeared, in which the spiropyran closed form is the principal component. During this process, the absorption peak of MC at 550 nm decreased and at last

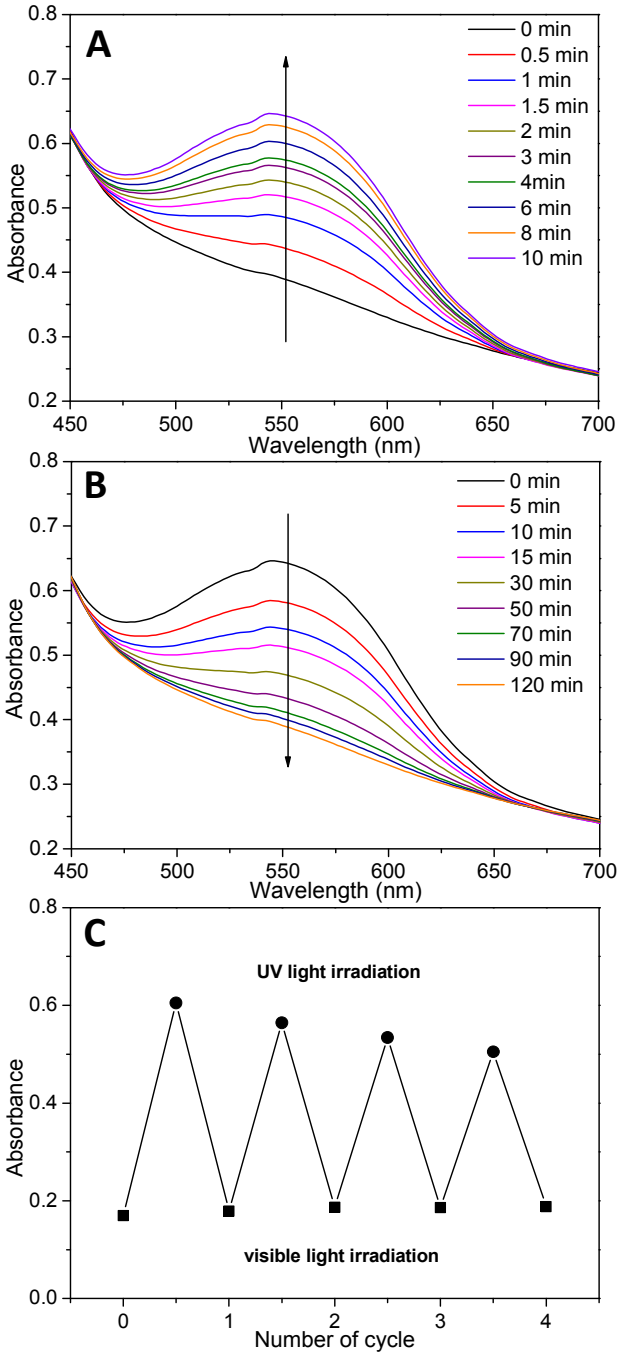


Figure 3 Reversible photoisomerization process of mPEG-*b*-poly(Tyr)$_{25}$-SP micelles (0.05 mg mL^{-1}) after irradiating with different light monitored by UV-vis spectroscopy: 365 nm UV light for 10 min (A), subsequent 620 nm visible light for 2 h (B) and alternatively irradiating with UV light (10 min) and visible light (2 h) for several cycles (C).

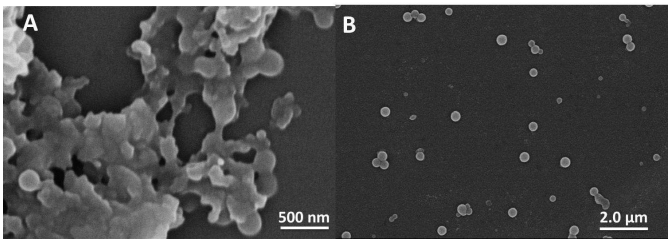


Figure 4 SEM images of mPEG-b-Poly(Tyr)$_{25}$-SP micelles (0.05 mg mL^{-1}) after UV light irradiation for 10 min (A) and subsequent visible light irradiation for 2 h (B)

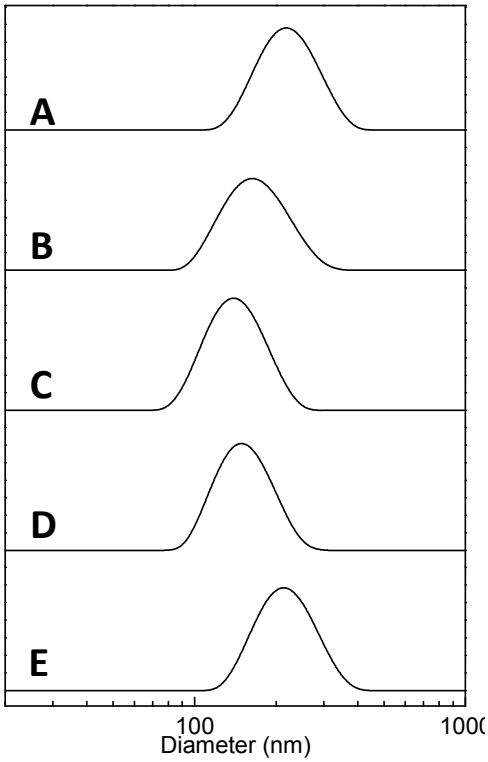


Figure 5 Size distributions after irradiation with different lights for different times: before UV irradiation (A); 365 nm UV light irradiation for 2 min (B); 365 nm UV light irradiation for 10 min (C); 620 nm visible light irradiation for 30 min (D) and 620 nm visible light irradiation for 120 min (E).

disappeared with the increase of the visible light irradiation time. To test the cyclic reversible response ability of the micelles, the sample was irradiated with alternative 365 nm UV light and 620 nm visible light and the results were shown in Fig. 3C. The change of UV absorbance at 550 nm demonstrated the reversible isomerization can take place very well, indicating the micelles obtained in this study can fulfil the requirement of reversible responsiveness when they deliver drugs.[24]

SEM and DLS were also used to monitor the morphology and size change during the irradiation process. As shown in Figure 4A, after the micellar solution was irradiated under 365 nm UV light for 10 min, most of the spherical micellar aggregations disrupted, indicating that hydrophilic-hydrophobic balance of the micelles destroyed due to conversion from hydrophobic SP form to hydrophilic MC form. [28-30] After 120 min of 620 nm visible light irradiation, spherical micelles aggregated again and the size was close to that observed prior to UV exposure (Figure 4B). Figure 5 shows the diameter change of the micelles after irradiating with different lights for different times. The average diameter decreased from 222.7nm before irradiation to 169.3 nm after irradiating with UV light for 2 min, and then to 143.5 nm after irradiating with UV light for 10 min. Meanwhile, after exposure to 620 nm visible light for 30 min and 120min, the average diameter gradually increased to 158.4 nm and 215.6 nm, respectively. These results further demonstrated that the mPEG-b-Poly(Tyr)$_{25}$-SP micelles could undergo photo-induced reversible self-assembly and disassembly due to the isomerization transformation between hydrophobic SP and hydrophilic MC.

Light-controlled release and re-encapsulation of model drug coumarin 102

Coumarin 102, a hydrophobic model drug, was chosen to demonstrate the concept of light-controlled drug release. 0.1 mg of coumarin 102 was used to encapsulate into 5 mL of 0.2 mg mL^{-1} micellar solution by solvent exchange method. The

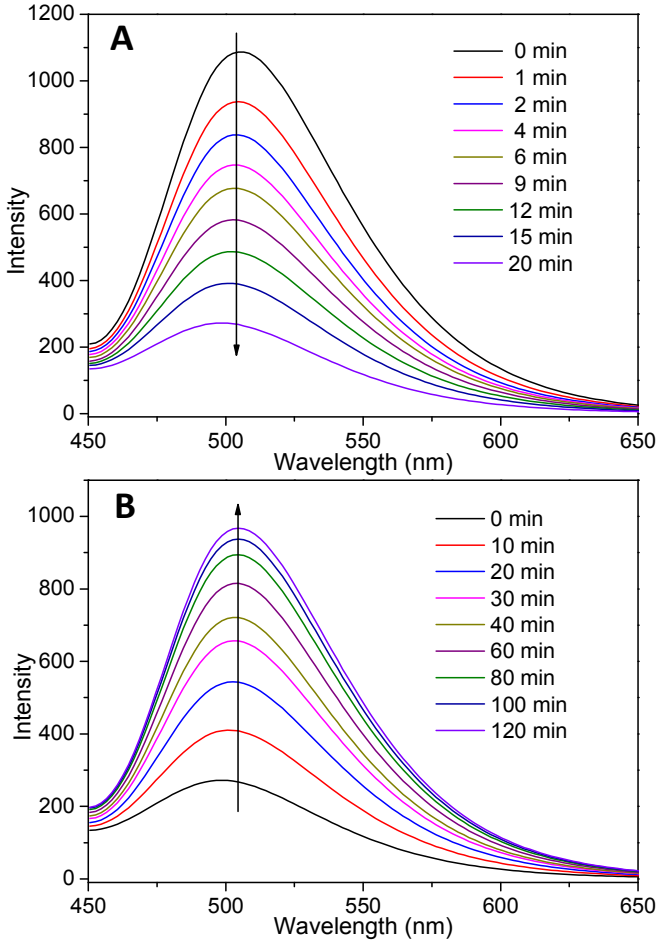


Figure 6 Fluorescence emission spectra of mPEG-b-Poly(Tyr)$_{25}$-SP micelles (0.05 mg mL^{-1}) with encapsulated coumarin 102 after irradiation with 365 nm UV light for 20 min (A) and subsequent 620 nm visible light for 120 min (B).

loading capacity and loading efficiency was found to be 0.087 mg mg^{-1} and 87%, respectively. The release and re-encapsulation process of the coumarin 102 loaded micelles was investigated by fluorescence spectrometry. As shown in Figure 6A, without irradiation, a strong emission peak at the wavelength around 500 nm was shown, suggesting that coumarin 102 was indeed encapsulated into the hydrophobic core of the micelles. During the 365 nm UV light irradiation process, the emission intensity of coumarin 102 gradually decreased with the increase of irradiation time, indicating that the model drug coumarin 102 was released into water from the disintegration of micelles. It is believed that this phenomenon happened because of the isomerization of hydrophobic SP to hydrophilic MC under UV irradiation.

Conversely, when the micellar solution was irradiated subsequently by 620 nm visible light, the characteristic peak of coumarin 102 increased gradually in Figure 6B, suggesting that some model drug molecules were re-encapsulated into hydrophobic cores of the micelles. It is noted that the emission peak did not increase back to the original level before UV irradiation, indicating that not all coumarin 102 molecules were encapsulated in the process. The reason for the incomplete re-encapsulation might be the rate of micelle formation is higher than that of drug loading.[24] All these results in this part demonstrated that the drug release and re-encapsulation behaviors of the micelles can be controlled by irradiating with UV or visible light.

Conclusions

In summary, we reported a synthetic method for the preparation of spiropyran-decorated light-responsive amphiphilic diblock copolymers composed of a hydrophilic block of PEG and a hydrophobic block of poly(α-hydroxy acids). The amphiphilic diblock copolymer mPEG-b-poly(Tyr)$_{25}$-SP can self-assemble into spherical micelles in aqueous solution and show good light response. SEM and DLS showed reversible changes in morphology and size of the micellar aggregates under alternating UV and visible light irradiation. Release and re-encapsulation of model drug coumarin 102 was demonstrated to be controlled by irradiating with UV or visible light. The light-responsive poly(α-hydroxy acids) micelles with biocompatibility, biodegradability are promising as smart carriers for controlled delivery of hydrophobic molecules for biomedical applications.

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Notes and references

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