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Redox-responsive core cross-linked micelles of poly(ethylene oxide)-*b*-poly(glycidyl methacrylate) by click chemistry

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ABSTRACT

Redox-responsive core cross-linked polymeric micelles were prepared by using azide-alkyne click chemistry. Poly(ethylene oxide)-b-poly(glycidyl methacrylate) (PEO-b-PGMA) copolymers were prepared by the atom transfer radical polymerization, and the PGMA parts were subsequently functionalized with azido groups. The micelles of azido-functional PEO_{5k}-b-$PGMA_{1.1k}$-N_3 were cross-linked using dipropargyl 3,3′-dithiodipropionate as a disulfide-containing cross-linking agent. The block copolymers and polymeric micelles were characterized by using GPC, FT-IR, ^{1}H NMR, TEM, and DLS analyses. The nanoparticle micelles showed much improved structural stability under physiological condition, while they were rapidly dissociated in the 1,4-dithio-D,L-threitol reducing environment.

KEYWORDS

Click chemistry; ATRP; Core cross-linked micelles; Redox-responsive

Introduction

The polymeric micelles formed via self-assembly of block copolymer chains have been extensively studied due to the hydrophobic drug solubility, prolonged circulation time, and specific tissue targeting [1, 2]. They present a core-shell structure wherein the hydrophobic core serves as a microenvironment for the incorporation of drugs while the hydrophilic shell stabilizes colloid against aggregation. However, the instability of micelles is a significant obstacle for many applications. Under certain conditions, the polymeric micelles dissociate into unimers which cause non-targeted drug release and toxicity [3]. The cross-linking of micelles is one of the most powerful tools to stabilize the self-assembled structure [4]. Significant advances in this method do not only enhance the stability of the nanocarriers but also enable controllable tuning of drug release in micelles. Cross-linked hydrophilic shells may affect the stealthiness of the drug delivery systems and the mobility of the hydrophilic moiety [5], and they are unable to avoid undesired inter-micellar cross-linking. Meanwhile, cross-linked hydrophobic core became more favorite strategy, which could be reversed or degraded in a redox circumstance [6, 7]. Core cross-linked (CCL) micelles could be prepared by various methods including near-infrared light-responsive of photo-sensitive groups and metal-ligand coordination [8].

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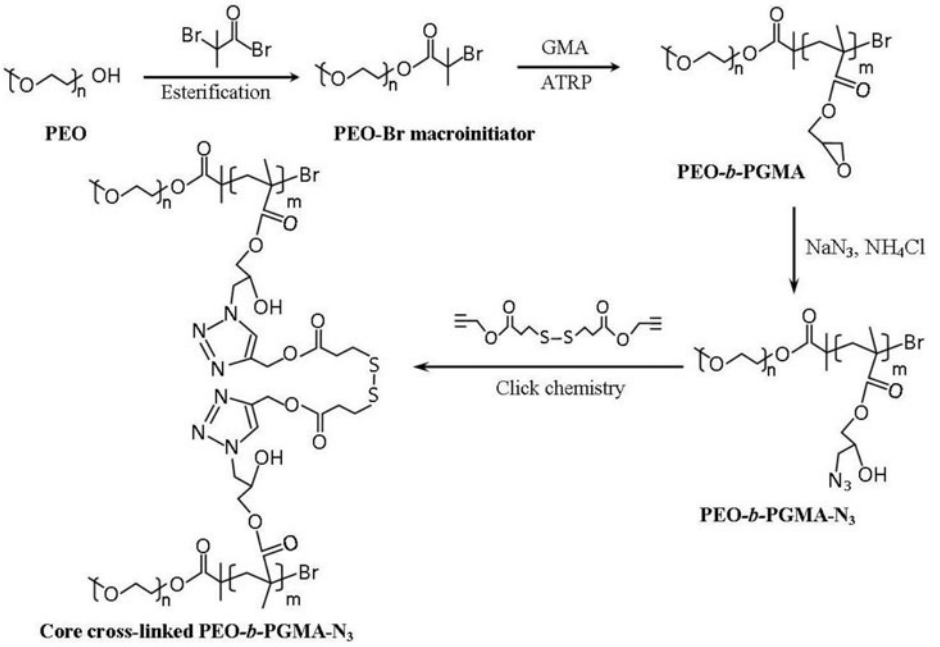

Scheme 1. Schemetic representation of the synthesis of PEO_{5k}-*b*-$PGMA_{1.1k}$-N_3 block copolymers followed by core cross-linking of their micelles.

In this study, we present the synthesis of CCL micelles by using alkyne-azide click chemistry. The block copolymer of poly(ethylene oxide)-*b*-poly(glycidyl methacrylate) (PEO-b-PGMA) was prepared by the atom transfer radical polymerization (ATRP) using a PEO-based macroinitiator followed by the azidation process. The click reaction was then allowed to take place between azide groups located along the poly(glycidyl methacrylate) backbone (PGMA-N_3) and the disulfide cross-linker to produce redox-responsive CCL micelles (scheme 1).

Experimental

Materials

2-Bromoisobutyryl bromide (Alfa) and triethylamine were distilled prior to use. 4-(Dimethylamino)pyridine (Aldrich) was recrystallized in toluene. Poly(ethylene oxide) methyl ether (PEO, M_n = 5000 g/mol, Aldrich) were dried by azeotropic distillation using anhydrous toluene. Glycidyl methacrylate (GMA) (≥97%, Sigma-Aldrich) was passed through a neutral alumina column. 1,4-dithio-D,L-threitol (DTT) (Aldrich) and other solvents of analytical grade were used as received. The PEO-Br macroinitiator was prepared according to the previous literature [9]. Dipropargyl 3,3′-dithiodipropionate was prepared as described in previous work [10].

Preparation of PEO_{5k}-b-$PGMA_{1.1K}$

In a typical procedure, PEO_{5k}-Br (0.52 g, 0.1 mmol), CuBr (0.014 g, 0.1 mmoL), and anisole (0.75 mL) were introduced into a round-bottom flask equipped with a magnetic bar under nitrogen. Then, GMA (0.25 g, 1.76 mmol) and PMDETA (0.0174 g, 0.1 mmol) were added to

the mixture under nitrogen. The flask was stirred for 6 h at room temperature. The product was diluted in CH_2Cl_2 and passed through a basic alumina column to remove the catalyst. The solution was precipitated in an excess amount of cold ethyl ether. The final product was dried under vacuum.

Preparation of PEO_{5k}-b-$PGMA_{1.1k}$-N_3 block copolymer

A mixture of PEO_{5k}-*b*-$PGMA_{1.1k}$ (0.10 g, 0.016 mmol of epoxide moieties), NaN_3 (19.5 mg, 0.3 mmol), NH_4Cl (16 mg, 0.3 mmol) and DMF (1 mL) were placed in a round-bottomed flask and stirred at 50°C for 24 h. After removing insoluble salts by filtration, the filtrates were dialyzed (MW cutoff, 1 kDa) against deionized water for 24 h. Fresh water was replaced approximately every 4 h. The final product was obtained by freeze-drying [12].

Preparation of CCL micelles of PEO_{5k}-b-$PGMA_{1.1k}$-N_3 block copolymer

PEO_{5k}-*b*-$PGMA_{1.1k}$-N_3 (5.0 mg, 19.5×10^{-3} mmol of azide group) and dipropargyl 3,3′-dithiodipropionate (3.5 mg, 13×10^{-3} mmol) were dissolved in anhydrous DMF (3 mL). Afterward, 30 mL of DI was added dropwise to the solution under vigorous stirring for one day. $CuSO_4.5H_2O$ (4.5 mg, 18 μmol) in water (200 μL) and sodium ascorbate (4 mg, 20 μmol) in water (200 μL) were added in order. The mixture was stirred at room temperature for 24 h. After that the solution was transferred into a dialysis bag (MW cutoff, 1 kDa), and subjected to dialysis against 2 L of distilled water, which was renewed every 4 h during the course of 2 days. The micelle solution was passed through 0.45 μm filter and then freeze-dried.

Reactivity of CCL micelles stabilization

The CCL micelles were mixed with a large amount of deionized water in the presence or absence of 10 mM DTT to examine the effect of reducing environment to the micelles. The general procedure was as follows. To 0.6 mL of the aqueous micellar solution, a predetermined amount of deionized water was added and the solution was shaken for 5 min. The solution was kept overnight prior to analysis.

Characterization

^{1}H NMR spectra were recorded on a JNM-ECP 400 (JEOL) instrument. Gel permeation chromatography (GPC) was performed using a HP 1100 apparatus with THF as a solvent. The columns were calibrated with polystyrene standards. Fourier transform infrared (FTIR) spectra were measured on a JASCO FT/IR-4100 spectrometer with DLATGS detector. For transmission electron microscopy (TEM; JEOL JEM-2010), the samples were prepared by depositing a drop of dispersed nanoparticles in acetonitrile (upper portion) on copper grids. Dynamic laser light scattering (DLS) measurements for determining the average hydrodynamic diameter of micelles were performed using an electrophoretic light scattering instrument (ELS-8000, Otsuka Electronics Corporation), equipped with an ELS controller and a He-Ne laser at wavelength of 632.8 nm. The intensity of scattered light was detected at 90° to an incident beam. All the analyses were performed at 25°C.

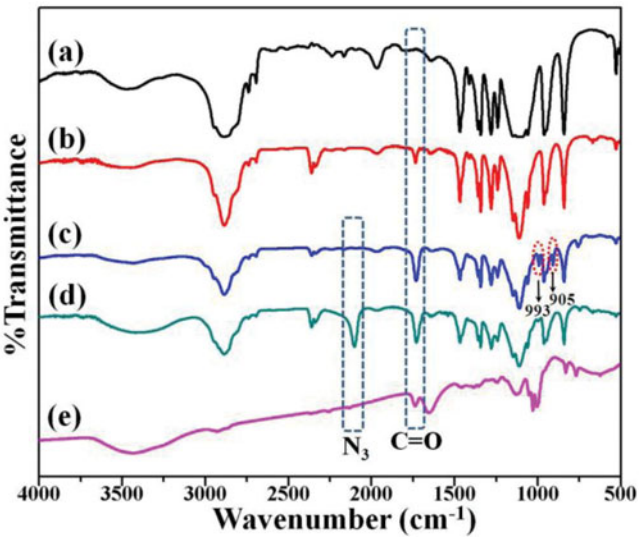

Figure 1. FT-IR spectra of (a) PEO_{5k}, (b) PEO_{5k}-Br macroinitiator, (c) PEO_{5k}-*b*-$PGMA_{1.1k}$, (d) PEO_{5k}-*b*-$PGMA_{1.1k}$-N_3, and (e) CCL micelles of PEO_{5k}-*b*-$PGMA_{1.1k}$-N_3.

Results and discussion

PEO_{5k}-*b*-$PGMA_{1.1k}$ copolymers were prepared by ATRP of glycidyl methacrylate using the PEO_{5k}-Br macroinitiator. The controlled molecular weight ($M_n = 6100$) and narrow polydispersity (PDI = 1.23) of the block copolymers indicated a good level of control in the ATRP-mediate reaction, as evidenced by GPC analysis. FT-IR spectrum analysis was used to confirm the formation of polymers and CCL micelles. In Figure 1(b), the presence of initiator moieties was identified by the carbonyl vibration band at 1730 cm^{-1}. The epoxy ring vibration bands of PEO_{5k}-*b*-$PGMA_{1.1k}$ copolymers were shown at 905 and 993 cm^{-1} (Fig. 1(c)). The absorption bands at 2800–3000 cm^{-1} could be ascribed to the vibration of methylene and methyl protons of the PGMA segment. However, these characteristic bands overlapped with the methylene vibration of the PEO-Br macroinitiator (Fig. 1(b)), so that they could not be seen independently in the FT-IR spectrum. The azidation of PEO_{5k}-*b*-$PGMA_{1.1k}$ was demonstrated by the new absorption band at 2095 cm^{-1} which was implied to the valence vibration of azide functions in PGMA-N_3 moieties (Fig. 1(d)). In addition, the opened epoxy rings have generated a stronger and broader band of hydroxyl groups at 3450 cm^{-1}, whereas the signal of the ring at 905 and 993 cm^{-1} disappeared. After click reaction, the FT-IR spectrum of nanoparticle micelles showed the reduced intensity of the characteristic band of azides, confirming their reaction with alkyne groups of the cross-linker (Fig. 1(e)).

The structure of polymers was further confirmed by ^1H NMR spectra. The substitution of the hydroxyl groups was proved by new signals at 1.93 and 4.32 ppm, which implied the methylene group adjacent to the ester linkage and methyl groups of α-bromoisobutyryl bromide, indicating the formation of the PEO-Br macroinitiators (Fig. 2(a)). The methylene protons in the main chain of PEO at 3.51 ppm were visualized, while the peaks at 3.21, 2.81, and 2.67 ppm were associated to the methylene proton (s, t) of the epoxy ring as well as signals at 4.31, 3.73, 1.90, 1.82, 1.20, 0.98, and 0.81 ppm contributed to methylene (r, p) and methyl (q) protons of PGMA moieties (Fig. 2(b)). The ring-opening process was confirmed by the change of the proton bearing epoxy ring in the spectroscopy, which was depicted in Fig. 2(c). The characteristic peaks of the PEO part were maintained even though the signals of the

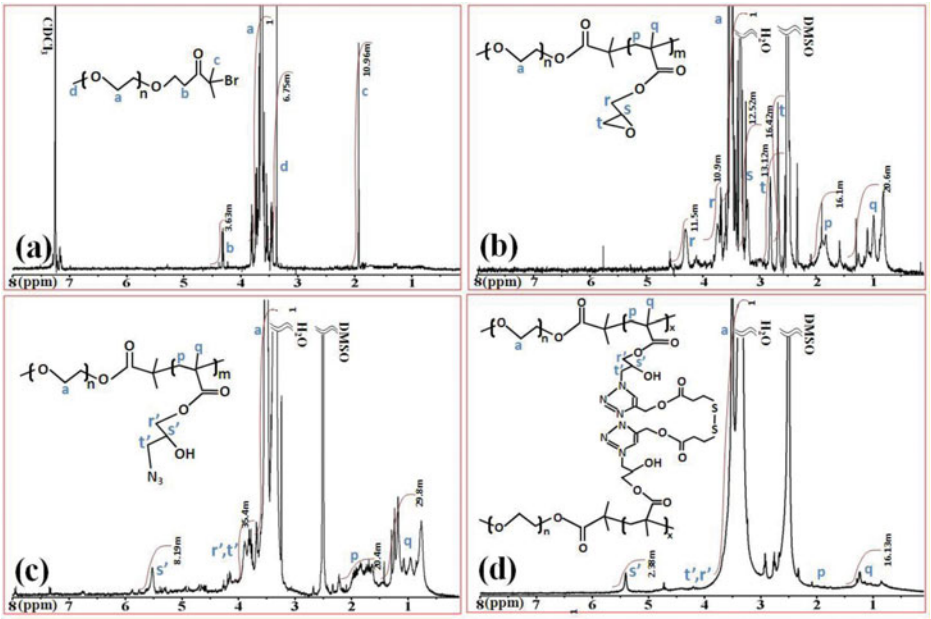

Figure 2. ^{1}H NMR spectrum of (a) PEO-Br in $CDCl_3$, (b) PEO_{5k}-*b*-$PGMA_{1.1k}$, (c) PEO_{5k}-*b*-$PGMA_{1.1k}$-N_3, and (d) CCL micelles of PEO_{5k}-*b*-$PGMA_{1.1k}$-N_3 in DMSO-d_6.

PGMA moiety have already changed. The new peaks of the opened ring appeared at 5.51, and 3.88 ppm, which implied to the proton of CH-O (s′), CH_2-O (r′), and CH_2-N_3 (t′) whereas the epoxy ring protons at 4.31, 3.73, 3.21, 2.81, 2.67 ppm disappeared. The result demonstrated that azide groups were anchored on the backbone of the PGMA part. The click reaction between the dialkyne cross-linker and the azide groups in the PGMA-N_3 parts induced CCL micelles which consisted of the PEO shell and the core of cross-linked PGMA-N_3. The ^{1}H NMR spectrum indicated the depression of the signal pertinent to the PGMA-N_3 block whose protons were shielded from magnetic resonance by PEO coronas. As a result, the proton signal of cross-linked PGMA-N_3 for r′, s′, t′, p, and q decreased significantly in comparison with the non-cross-linked PGMA-N_3 segment (Fig. 2(d)).

The size and morphologies of the non-cross-linked and CCL micelles could be obtained by TEM measurements as shown in Figure 3. The images in both case showed fine spherical

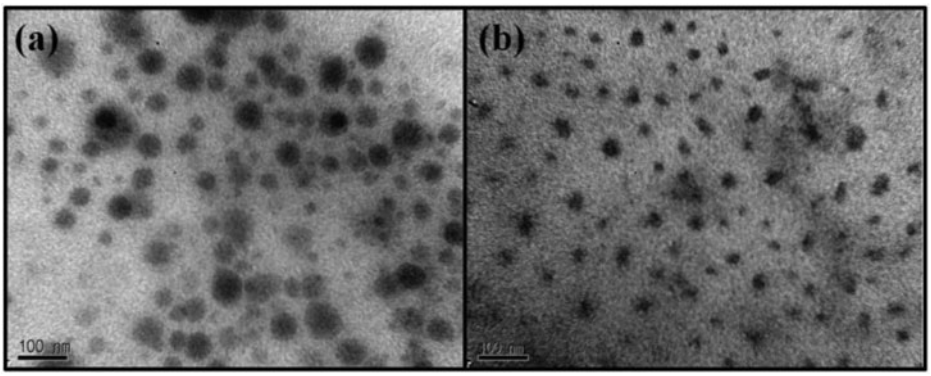

Figure 3. TEM images of (a) non-cross-linked micelles and (b) CCL micelles of PEO_{5k}-*b*-$PGMA_{1.1k}$-N_3 block copolymers.

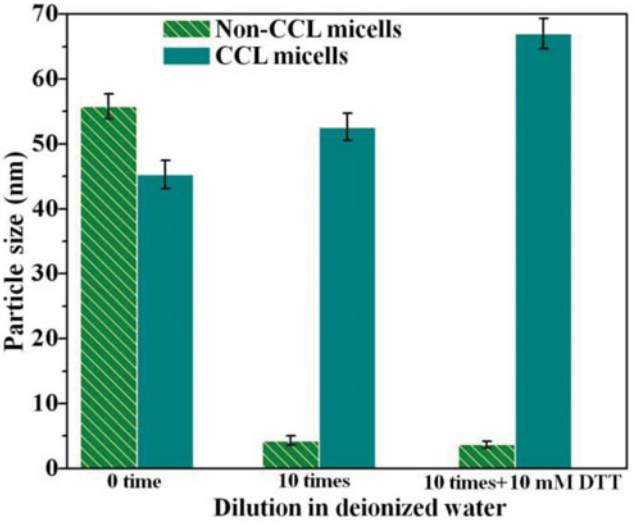

Figure 4. The stability of non-cross-linked and CCL micelles in deionized water after 10 times of dilution in the absence and presence of 10 mM DTT.

distributions. The average diameter of non-cross-linked micelles was about 46 nm (Fig. 3(a)) while the average diameter of CCL micelles was about 33 nm (Fig. 3(b)). The particle size after cross-linking slightly decreased owing to the enhanced compactness and tightness of the core-shell structure.

To investigate the stability and reactivity of polymeric micelles under physiological conditions, the non-cross-linked and CCL micelles were diluted with 10-fold of deionized water. As shown in Figure 4, the size of non-cross-linked micelles completely changed upon dilution with deionized water. The average hydrodynamic diameter of non-cross-linked micelles was below 5 nm indicating unimer solutions whereas the particle sizes of CCL micelles slightly increased from 45 to 53 nm. In other words, cross-linked micelles showed an insoluble swollen core that still preserved the core-shell structure and prevented the disintegration from dilution. To investigate whether the disulfide cross-linker in the micellar core was decomposed in a redox environment, DTT (10 mM) was added to the CCL micelles, and the particle sizes were measured. The hydrodynamic diameter of the micelles increased significantly up to 67 nm. This result can be explained by the fact that the S-S bridges of the cross-linked micelles were susceptible to the reducing reagent, DTT and cleaved into thiols. The cleavage rate of the cross-link in the micelles increased upon time and the core structure of the micelles became loose. Consequently, the size of the aggregates increased due to the formation of larger hydrophobic segments [13].

Conclusions

A system of redox-responsive CCL micelles based on PEO_{5k}-*b*-$PGMA_{1.1k}$ copolymers has been constructed by the combination of ATRP and alkyne-azide click chemistry. The block copolymers were characterized by GPC, FT-IR, ^{1}H NMR, and TEM images. The CCL micelles showed the fine spherical distribution with 33 nm of diameter. The CCL micelles were more stable than non-cross-linked micelles under large volume dilution and could be de-cross-linked in a reducing environment. The redox-responsive CCL micelles promise bright prospects for drug delivery and therapy.

Acknowledgment

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