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# Ultrasound responsive block copolymer micelle of poly(ethylene glycol)–poly(propylene glycol) obtained through click reaction

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ABSTRACT

The well-defined amphiphilic poly(ethylene glycol)-block-poly(propylene glycol) copolymer containing 1, 2, 3-triazole moiety and multiple ester bonds (PEG-click-PPG) was prepared by click reaction strategy. The PEG-click-PPG copolymer can self-assemble into spherical micelles in aqueous solution. It is found that high intensity focused ultrasound (HIFU) can open the copolymer PEG-click-PPG micelles and trigger the release of the payload in the micelle. The multiple ester bonds introduced in the junction point of the copolymer chain through click reactions were cleaved under HIFU, and leads to the disruption of the copolymer micelle and fast release of loaded cargo. The click reaction provides a convenient way to construct ultrasound responsive copolymer micelles with weak bonds.



## 1. Introduction

The molecular design for new stimuli-responsive amphiphilic block copolymer has gained a great interest in recent years [1,2]. When copolymer micelles suffer certain stimuli such as thermal [3], pH [4], light [5], magnetic [6] and ultrasound [7–10], the micelle structure can be broken physically or chemically, leading to the release of the encapsulated hydrophobic drugs. The synthesis of novel stimulus responsive copolymer and the design of new stimuli-responsive means are of equal importance. The major challenge is the realization of the optimized coupling interaction between physical or chemical stimulus means and the copolymer micelle microcontainer.

Ultrasound triggered drug release was firstly investigated by Pitt [7], Rapoport [11] and Hussein [12]. It was found that ultrasound could physically break the micelle and trigger the drug release. High intensity focused ultrasound (HIFU) is another promising external trigger for the drug release from polymer, which has the advantages such as focused tiny area, deep penetration, non-invasiveness and remote controllable properties [13–15]. Previously Xia and Zhao et al. [13–17] investigated HIFU induced release behavior of payload entrapped in polymer micelles containing weak bonds, and proposed a novel mechanism, i.e. breaking the copolymer micelle under HIFU by a mechanochemical way. In order to develop block copolymer micelles that can be rapidly and efficiently disrupted by HIFU, the copolymer should contain weak bonds, ideally mechano-labile ones that are sensitive to the mechanical effects associated with the ultrasonic cavitation. On the other hand, for drug delivery, HIFU intensity should be low to be acceptable by human body and HIFU time should be as short as possible.

Recently, a wide variety of mechanochemical reactions have been demonstrated through the deliberate incorporation of mechanophores into polymer chain, with the aim of developing mechanoresponsive polymers [18–25]. The exploring in new mechanophore structure and the routes to introduce the mechanophore into the polymer are the main directions [26,27]. The mechanophore concept could provide a novel approach to fabricate ultrasound responsive copolymer and its micelle system.

Click chemistry has emerged as an established robust and efficient method to link functional moieties with each other, which is especially interesting for the preparation of functional materials such as block copolymers. The orthogonal 1, 3-dipolar cycloaddition click reaction of azides and alkynes provides efficient route to synthesize amphiphilic block copolymer containing 1, 2, 3-triazole ring [28,29], such as $(PCL)_2$-$(PEG)_2$ miktoarm star copolymer [30] and PCL-*g*-PEG [31]. Click chemistry is also tried to introduce the mechanophore into the homopolymer, however, the mechanochemical ring-opening for the 1, 2, 3-triazole moiety under low frequency ultrasound in organic solvent is not successful [32].

Herein, we utilized the mechanophore-functionalization through click reaction of azides and alkynes for amphiphilic block copolymer to develop ultrasound responsive micelle. Previously we have confirmed that the irreversible release mechanism

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resulting from the cleavage of ester bonds as the "mechanophore" at the junction points of PEG and PPG blocks based on our designed Pluronic type copolymer PEG-COO-SS-PPG [17]. The robust click reaction route can introduce more ester bonds, i.e. mechanophore, into the junction points of PEG and PPG blocks, which may endow the copolymer rapid and efficient ultrasound responsiveness. Meanwhile, it is necessary to check whether HIFU can unlock the 1, 2, 3-triazole ring embedded in copolymer micelle in aqueous solution, and lead to the disruption of micelles and release of encapsulated hydrophobic drug.

In this study, the amphiphilic Pluronic type block copolymer PEG-click-PPG containing 1, 2, 3-triazole moiety and four ester bonds in the junction point was synthesized by click chemistry as shown in Scheme 1a, using azide-terminated PEG as the hydrophilic block and alkyne-terminated PPG as the hydrophobic block. Pluronic copolymer was selected because it is one of the few copolymer micelles which have been used as a FDA approved drug delivery system for the last decades. Base on this new copolymer, the HIFU triggered release of the copolymer micelle was expected. The proposed mechanism as illustrated in Scheme 1b includes HIFU-induced site-specifically mechanochemical degradation of the PEG-click-PPG chain containing weak bonds, and consequently the micelle disruption and controlled payload release.

## 2. Materials and methods

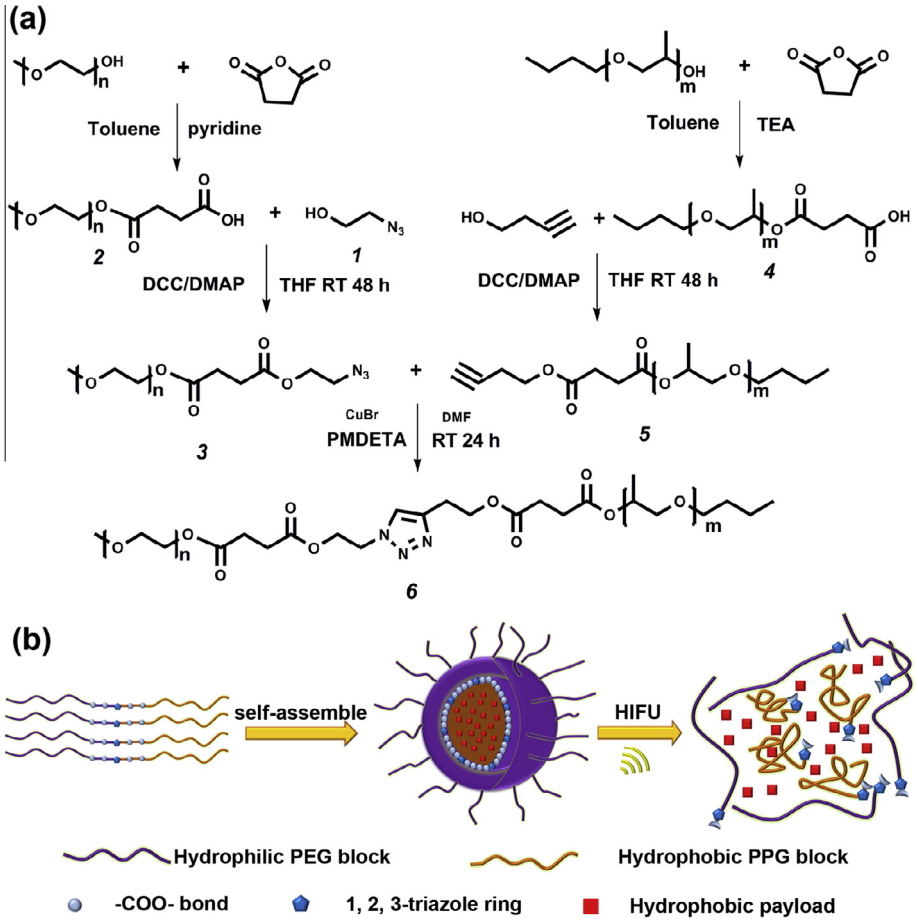
### 2.1. Raw materials

Poly(ethylene glycol) methyl ether (mPEG) ($M$n = 2000), Poly(propylene glycol) monobutyl ether (PPG) ($M$n = 2500), Succinic anhydride, dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), and Nile Red were obtained from Sigma–Aldrich Chemical Company and used without further purification. CuBr (99%; Maya) was purified by stirring overnight in acetic acid. Sodium azide ($NaN_3$), 3-Butyn-1-ol, Pentamethyldiethylenetriamine (PMDETA), 2-Bromoethanol and Sodium azide were purchased from Maya-Reagent. Diethyl ether ($Et_2O$), N, N-Dimethylformamide (DMF), Tetrahydrofuran (THF), dichloromethane (DCM), $CaH_2$, methanol, ethanol, and sodium were purchased from Chengdu Kelong Chemical Reagents Institute. THF and DCM were dried by refluxing over sodium wire and $CaH_2$ respectively, and distilled prior to use to remove the moisture and oxidative impurity. All other chemicals and solvents were used as received unless stated.

### 2.2. Sample preparation

#### 2.2.1. Synthesis 2-Azidoethanol (1)

According to the reference [33], 2-bromoethanol (7.51 g, 60.5 mmol), $NaN_3$ (5.13 g, 122 mmol), and Tetrabutylammonium bromide (500 mg, 1.5 mmol) were added to a 50 mL flask, and stirred for 15 h at 110 °C. Then the mixture was cooled and the product was taken up with $Et_2O$ (20 mL), and the precipitate was removed by filtration. The precipitate was washed with $Et_2O$ (~20 mL). Evaporation of the solvents gave a yellow residue that was purified by distillation (bp 35 °C/1 Torr) to yield 5.0 g (95%) of 2-Azidoethanol as a colorless liquid. $^1$H NMR (400 MHz, $CDCl_3$, δ): 2.06 (m, —OH), 3.45 (m, —$CH_2$—$N_3$), 3.78 (m, —$CH_2$—OH). FTIR: $\nu$ = 3377.5, 2937.5, 2880.2, 2110 vs (—$N_3$), 1442.5, 1296.3, 1070.0 cm$^{-1}$.




**Scheme 1.** (a) Synthetic route of PEG-click-PPG copolymer. (b) Schematic illustration of HIFU responsive process of copolymer micelle containing mechano-labile ester bonds.

*2.2.2. Synthesis of carboxyl-terminated PEG (2)*

The mPEG (10.0 g, 5 mmol) was dissolved in toluene and refluxed to remove the water, then succinic anhydride (2.5 g, 25 mmol), and pyridine was added under anhydrous conditions and refluxed for 24 h under vigorous stirring. The solvent was evaporated completely using a rotary evaporator. The residue was dissolved in tetra chloromethane and the excess succinic anhydride was removed by filtration. The solution was concentrated and cold diethyl ether was added to precipitate the carboxyl-terminated PEG (mPEG-COOH) (yield: ~9.89 g, ~90%). $^1$H NMR (400 MHz, $CDCl_3$, δ): 4.27(t, —$COOCH_2$—), 3.63 (m, —$OCH_2$-$CH_2O$—), 3.35 (s, $CH_3O$—), 2.64 (m, —$OCOCH_2CH_2OCO$—). FTIR (KBr): $v$ = 3431.5, 2886.4, 1735.1 vs (—COO—), 1468.4, 1291.3, 1113.6 $cm^{-1}$.

*2.2.3. Synthesis azide-terminated PEG (3)*

2-Azidoethanol (25 mmol, 2.18 g) and mPEG-COOH (5 mmol, 10 g) were dissolved in anhydrous THF (50 ml) in a 100 ml flask. The mixture was stirred for several min to obtain a clear solution. To this, DCC (25 mmol, 5.16 g) and DMAP (2.5 mmol, 0.31 g) were added in. After a few min, white precipitate appeared and the reaction mixture was stirred for another 48 h at room temperature. The obtained reaction mixture was filtered to remove the precipitate N, N′-dicyclohexylure (DCU). The filtrate was concentrated by rotary evaporation. The azide-terminated PEG (mPEG-$N_3$) was precipitated by addition of cold diethyl ether and dried in vacuum to constant weight (yield: ~8.04 g, ~76%). $^1$H NMR (400 MHz, $CDCl_3$, δ): 4.27 (t, —$COOCH_2$—), 4.24 (t, —$CH_2OCO$—), 3.65 (m, —$OCH_2CH_2O$—), 3.38 (s, $CH_3O$—), 2.77 (t, —$CH_2N_3$), 2.60 (m, —$OCOCH_2CH_2OCO$—). FTIR (KBr): $v$ = 3441.9, 2887.0, 2105.7 vs (—$N_3$), 1737.8, 1477.3, 1116.9 $cm^{-1}$.

*2.2.4. Synthesis of carboxyl-terminated PPG (4)*

The PPG (10.0 g, 4 mmol) was dissolved in toluene and refluxed to remove the water, then succinic anhydride (2.0 g, 20 mmol), and triethylamine was added under anhydrous conditions. The mixture was stirred at 80 °C for 24 h. Toluene was evaporated using a rotary evaporator. The residue was dissolved in tetrachloromethane and the excess succinic anhydride was removed by filtration. The tetrachloromethane was evaporated completely using a rotary evaporator, and the residue was dissolved in dichloromethane. The obtained clear solution was washed with 0.5 N HCl, saturated $NaHCO_3$ and saturated NaCl solutions for three times, and then dried over anhydrous $MgSO_4$. The solvent was then removed by evaporation and the product carboxyl-terminated PPG (PPG-COOH) was obtained (yield: ~7.50 g, ~72%). $^1$H NMR (400 MHz, $CDCl_3$, δ): 3.55 (t, —CH—O—), 3.40(s, —$CH_2$—O—), 2.62 (m, —$OCOCH_2CH_2OCO$—). 1.14 (—$CH_3$). FTIR ($CH_2Cl_2$): $v$ = 3516.2, 3260.0, 2930.0, 2870.8, 1736.6 vs (—COOH), 1455.5, 1107.3 $cm^{-1}$.

*2.2.5. Synthesis of alkyne-terminated PPG (5)*

The obtained PPG-COOH (4 mmol, 10 g) and 3-Butyn-1-ol (20 mmol, 1.4 g) were dissolved in anhydrous THF (50 ml) in a 100 ml flask. The mixture was stirred for several min to obtain a clear solution. To this, DCC (20 mmol, 4.13 g) and DMAP (2.0 mmol, 0.24 g) were added in. After a few min, white precipitate appeared and the reaction mixture was stirred for another 48 h at room temperature. The obtained reaction mixture was filtered to remove the precipitate N, N′-dicyclohexylure (DCU). The filtrate was concentrated by rotary evaporation, and the residue was dissolved in dichloromethane The obtained clear solution was washed with 0.5 N HCl, saturated $NaHCO_3$ and saturated NaCl solutions for three times, and then dried over anhydrous $MgSO_4$. The solvent was then removed by evaporation and the obtained product Alkyne-terminated PPG (PPG-Alkyne) (yield: ~9.29 g, ~90%). $^1$H NMR (400 MHz, $CDCl_3$, δ): 4.20 (t, —$CH_2OCO$—), 3.55 (t, —CH—O—), 3.40(s, —$CH_2$—O—), 2.66 (s, —C≡CH), 2.64 (m, —$OCOCH_2CH_2$-OCO—), 2.53 (t, —$CCH_2$—), 1.14 (—$CH_3$). FTIR ($CH_2Cl_2$): $v$ = 3516.2, 3260.0 (—C≡CH) and, 2935.0, 2123.0 (C≡C), 1745.8, 1458.9, 1111.5 $cm^{-1}$.

*2.2.6. Synthesis of PEG-click-PPG (6)*

PEG-click-PPG copolymers were synthesized by "click" reaction between mPEG-$N_3$ and PPG-Alkyne. As an example, mPEG-$N_3$ (2 g, 1 mmol of azide group) and 2 equiv of PPG-Alkyne (5 g, 2 mmol of alkyne group) were dissolved in 15 ml dry DMF. Then, CuBr (72 mg, 0.5 mmol) and PMDETA (87 mg, 0.5 mmol) were added into the flask sequentially. The reaction mixture was degassed by three freeze–pump–thaw cycles, left in $N_2$, and stirred at 25 °C for 24 h. The solution was passed through an alumina column to remove the copper salt. The solvent was then removed by evaporation and the obtained crude product was purified by silica gel column chromatography (using ethanol as the eluent to remove unreacted PPG-Alkyne and other impurities, and then using 10:1 DCM/methanol to obtain the pure product (yield: ~2.96 g, ~66%).

*2.3. Preparation of blank micelle solutions*

The obtained block copolymer was firstly dissolved in the THF. The copolymer micelles were formed by adding phosphate buffer solution (PBS) dropwise to the solution. Typically, 50 mg of PEG-click-PPG was dissolved in 10 ml of THF, and then 40 ml of PBS was added drop by drop under vigorous stirring to induce the aggregation of hydrophobic PPG blocks forming the micelle core. After that, another 50 ml PBS was added rapidly to stabilize the micelle morphology. THF was removed by evaporation at 40 °C for 24 h. The initial polymer concentration was 0.5 mg $ml^{-1}$.

*2.4. Preparation of micelle solutions containing Nile Red*

The micelle solutions containing Nile Red were prepared using a similar procedure to the blank micelle solutions. For instance, Nile Red was firstly dissolved in THF with an initial concentration of 0.5 mg $ml^{-1}$, and then 50 mg of PEG-click-PPG was introduced to 10 ml of Nile Red/THF solution, 40 ml PBS was then added dropsies to induce the formation of the micelles and simultaneously the encapsulation of Nile Red. After that, another 50 ml PBS was added rapidly to stabilize the micelle morphology. THF was allowed to evaporate by heating to 40 °C for 24 h. The unencapsulated Nile Red was deposited and then removed by filtration using 450 nm filters.

*2.5. HIFU treatment of PEG-click-PPG copolymer micelles*

Typically, 5 mL micelle solution was placed into glass cuvette reactor, which was sealed by latex membrane and immersed in a water tank (37 °C). The focused beams of ultrasound penetrate through latex membrane and act on the micelle solutions. In all HIFU treatment experiments, the focal spot of the beams was set at the center of the solution. After HIFU treatment for a certain time at a certain power output, the cuvette reactor was removed from the water tank and the sample was taken out for characterization. The fluorescence intensity of the copolymer micelles before and after HIFU treatment was detected after filtered with 450 nm filter.

*2.6. Characterization*

FTIR analysis of the samples was performed on a Nicolet 560 Fourier transform infrared (FTIR) spectrometer. Proton nuclear magnetic resonance $^1$H NMR spectra were recorded at room temperature with a Bruker spectrometer operating at 400 MHz using

$CDCl_3$ as the solvent and tetramethylsilane as an internal reference. Molecular weight was measured with gel permeation chromatography (GPC, TOSOH, HLC-8320GPC) with THF as the eluent at a flow rate of 0.6 ml·min$^{-1}$ at 40 °C. Molecular weight was calibrated with polystyrene standard. Dynamic light scattering (DLS) was performed on a Brookhaven BI-200 goniometer with vertically polarized incident light of wavelength $\lambda = 532$ nm supplied by an argon laser operating at 200 mW and a Brookhaven BI-9000 AT digital autocorrelator. Measurements were made at 25.0 °C and at the detect angle of 90°. The autocorrelation functions from DLS were analyzed by using the non-negatively constrained least square algorithm (NNLS) method to obtain the diameter distributions. Micellar morphology was observed with Scanning Electron Microscopy (SEM, Inspect F, Fei Company, USA). Specimens for SEM observations were prepared by depositing several drops of micellar solutions onto silicon wafers and were dried by lyophilization. Steady-state fluorescence emission spectra of the micelle solutions were recorded on the 970CRT spectrophotometer (Shanghai Precision & Scientific Instrument Co., Ltd). The excitation wavelength was 600 nm.

## 3. Results and discussion

### 3.1. Design and synthesis of PEG-click-PPG copolymer

As shown in Scheme 1a, the PEG-click-PPG block copolymer was synthesized by click reaction of self-made azide-terminated PEG and alkyne-terminated PPG in aqueous media and catalyzed by the copper (I) species in-situ generated through the catalyst of CuBr/PMDETA. The precursors, azide-terminated PEG and alkyne-terminated PPG, were firstly synthesized by esterification reaction.

#### 3.1.1. Synthesis and characterization of mPEG-$N_3$

The azide-terminated PEG (mPEG-$N_3$) was synthesized from commercially available PEG with monohydroxyl end group (mPEG) in two steps. First, carboxyl-terminated mPEG (mPEG-COOH) was prepared from the reaction of mPEG-OH with succinic anhydride. Then, 2-Azidoethanol was coupled with mPEG-COOH at room temperature in the presence of DCC and DMAP.

Fig. 1 shows the $^1$H NMR spectra of mPEG-COOH and mPEG-$N_3$. For mPEG-COOH, resonance at 4.26 ppm (Hc) is the characteristic signal of the methylene protons conjoint with ester group (—COO—), and compared with carboxyl-terminated precursor, it is notable that a new signal at 2.77 ppm corresponding to the methylene proton conjoint with azide group appears. Furthermore, Fig. 2b shows the FTIR spectra of mPEG-$N_3$. Compared with mPEG-COOH precursor, a new absorption peak related to azide group at 2110 cm$^{-1}$ appears which indicates the successful synthesis of mPEG-$N_3$.

#### 3.1.2. Synthesis and characterization of alkyne-terminated PPG

The synthesis of alkyne-Terminated PPG was similar to mPEG-$N_3$. First, carboxyl-terminated PPG (PPG-COOH) was prepared from the reaction of PPG-OH with succinic anhydride in the presence of triethylamine (TEA). Then, 3-Butyn-1-ol was coupled with PPG-COOH at room temperature in the presence of DCC and DMAP. In the $^1$H NMR (Fig. 3) of PPG-Alkyne, The characteristic signal peaks at 4.20 and 2.53 ppm are assigned to the methylene protons of butyne group, while the resonance of the alkyne proton is also detected at 2.66 ppm. In the FTIR spectrum of PPG-Alkyne (Fig. 2d), alkyne group was evidenced by the characteristic bands of HC≡C and C≡C at 3260 and 2123 cm$^{-1}$, respectively, which all confirms the successful synthesis of PPG-Alkyne.

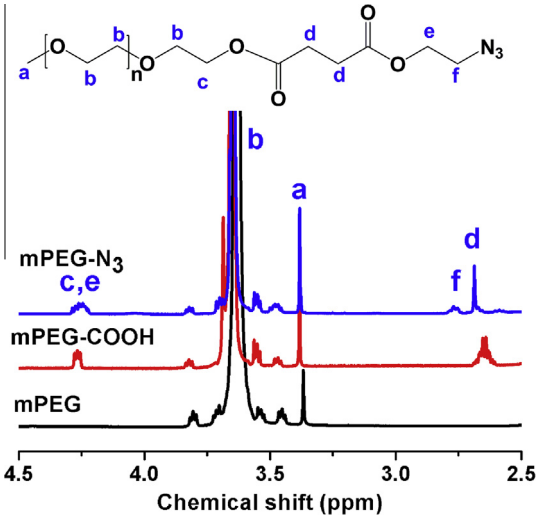



**Fig. 1.** $^1$H NMR spectra (400 MHz, $CDCl_3$) of mPEG, mPEG-COOH and mPEG-$N_3$.

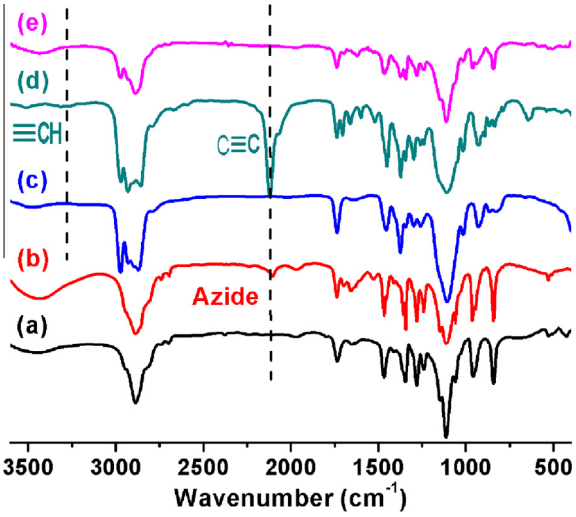



**Fig. 2.** FTIR spectra of (a) mPEG-COOH, (b) mPEG-$N_3$, (c) PPG-COOH, (d) PPG-Alkyne and (e) PEG-click-PPG.

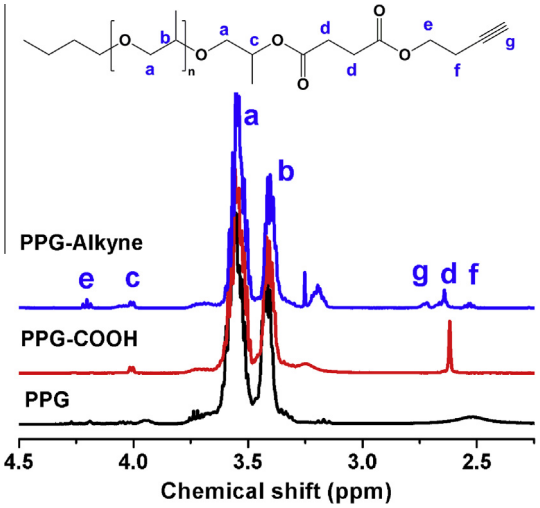



**Fig. 3.** $^1$H NMR spectra (400 MHz, $CDCl_3$) of PPG, PPG-COOH and PPG-Alkyne.

### 3.1.3. Synthesis and characterization of PEG-click-PPG by "click" chemistry

"Click" chemistry strategy was employed to synthesize PEG-click-PPG copolymer between mPEG-$N_3$ and PPG-Alkyne using CuBr/PMDETA as catalyst in DMF at room temperature. In the $^1$H NMR of PEG-click-PPG (Fig. 4), the signal at 7.50 ppm due to the proton on the triazole ring was clearly detected, indicating that the "click" reaction was achieved successfully. Fig. 2 shows the FTIR spectra of mPEG-$N_3$, PPG-Alkyne and PEG-click-PPG. Compared with the mPEG-$N_3$ and PPG-Alkyne precursors, the characteristic absorption peaks at 2110 $cm^{-1}$ assigned to azide group of mPEG-$N_3$, and the peaks at 3260 and 2123 $cm^{-1}$ assigned to alkyne group of PPG-Alkyne completely disappear, which also confirms the successful synthesis of PEG-click-PPG copolymer.

Fig. 5 shows the GPC traces of PEG-click-PPG in comparison with those of the corresponding mPEG-$N_3$ and PPG-Alkyne precursors. Table 1 lists the molecular weight and its distribution. It is clear that after "click" reaction, the *M*n of PEG-click-PPG reaches ~7600, which is nearly the sum of mPEG block and PPG block, indicates that well defined PEG-click-PPG copolymer was successfully prepared.

### 3.2. HIFU induced site-specifically mechanochemical degradation for the PEG-click-PPG copolymer in the micelle

The "click" strategy not only introduces 1, 2, 3-triazole moiety in the copolymer chain but also provides an efficient route to synthesize amphiphilic block copolymer with multiply ester bonds with HIFU responsibility. As mentioned above, it is interesting to check whether HIFU unlocks the 1, 2, 3-triazole ring, or breaks the ester bonds in the junction point of copolymer chain in the aqueous micelle solution. In order to conduct this investigation, the amphiphilic PEG-click-PPG copolymers micelles were firstly prepared by adding PBS buffer aqueous solution dropwise to the copolymer/THF solution and then the obtained micelle solutions were subject to HIFU treatment. The solid samples used for $^1$H NMR and FTIR characterization were obtained as follows: the PEG-click-PPG micelles solution before and after HIFU treatment were dried to obtain the solid PEG-click-PPG copolymer, which was further purified by dissolving it into dichloromethane and filtering the un-dissolved salt.

The FTIR results are shown in Fig. 6a. It is clear that the absorption of carbonyl groups in ester bonds (~1739 $cm^{-1}$) decreases and a new absorption peak of hydrogen-bonded carbonyl acid dimers appears (~1646 $cm^{-1}$) after HIFU treatment for 10 min, indicating

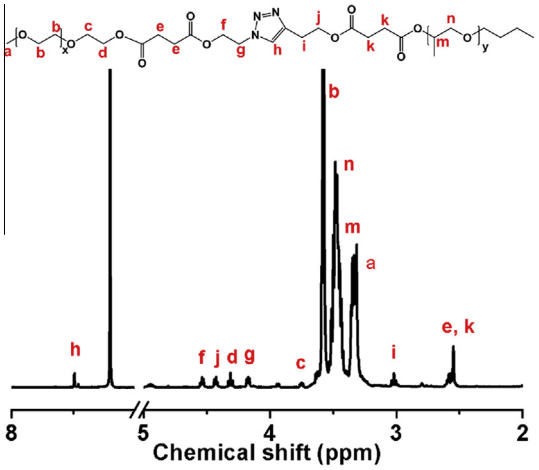



**Fig. 4.** $^1$H NMR spectra (400 MHz, $CDCl_3$) of PEG-click-PPG copolymer.

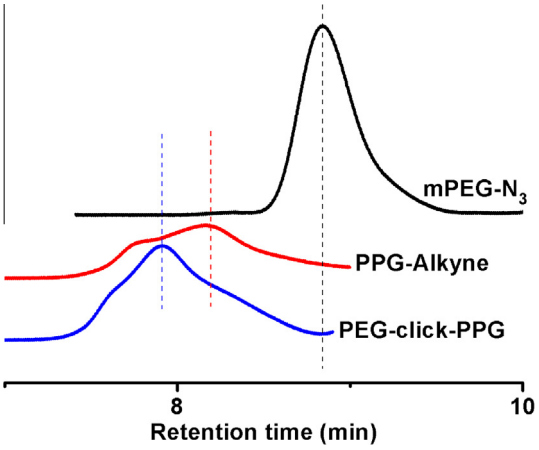



**Fig. 5.** GPC traces of mPEG-$N_3$, PPG-Alkyne and PEG-click-PPG copolymer.

**Table 1**
The number average molecular weight (*M*n), weight average molecular weight (*M*w) and polydispersity index (*D*p) of the mPEG-$N_3$, PPG-Alkyne and PEG-click-PPG copolymer.

| Samples | *M*n | *M*w | *D*p |
|---|---|---|---|
| mPEG-$N_3$ | 2700 | 2800 | 1.05 |
| PPG-Alkyne | 4900 | 6700 | 1.36 |
| PEG-click-PPG | 7600 | 8900 | 1.17 |

the cleavage of ester bonds. The peaks assigned to the azide (~2110 $cm^{-1}$) and terminal alkyne (3295 and 2123 $cm^{-1}$) functionalities are not present in the FTIR spectrum of the PEG-click-PPG after HIFU treatment for 10 min, which indicates the ring-opening of 1, 2, 3-triazole in the PEG-click-PPG copolymer chain does not happen under HIFU interaction. Fig. 6b shows the number average molecular weight *M*n of PEG-click-PPG copolymer decreases with HIFU time. After HIFU treatment for 10 min, the *M*n decreases from ~7600 to ~3400. This proves the cleavage of copolymer chains after HIFU treatment.

Fig. 7 shows the $^1$H NMR of PEG-click-PPG copolymer in the micelle before and after HIFU treatment. The chemical shift of peak d belongs to the resonance of protons in methylene groups of the terminal repeat units of PEG connected with ester groups (—$CH_2$—O—CO—), and the decrease in the intensity of peak d indicates the cleavage of ester groups. After HIFU treatment, the peak (d) intensity of chemical shift at ~4.3 decreases for the copolymer, and the changes of related peaks e, f, j and k have the same trend. The peak area ratios were listed in Table 2. The results show that the peak area ratios of the every hydrogen (d, f, j, e and k) in ester bond to the all hydrogens (b, n, m) in the PEG and PPG chains decrease after HIFU treatment, suggesting the cleavage of ester bonds. The signal at 7.50 ppm due to the proton on the triazole ring was also existed, which again confirms the ring-opening of 1, 2, 3-triazole does not happen under HIFU.

When the copolymer micelle is subjected to HIFU in aqueous solution, the formation and collapse of ultrasonic cavitation bubbles produces solvodynamic shear forces [34], which are exerted to polymer chains and leads to the cleavage of the ester bond as the mechanophore in the polymer chain. In our previous study [17], the mechanochemical cleavage mechanism for the ester bond in the copolymer chain was confirmed, while the possibility of the thermally induced the cleavage of the ester bond was ruled out. Boulatov et al. [35,36] theoretically investigated the mechanochemical scission of ester bonds under stretching force. The cleavage possibility for 1, 2, 3-triazole ring was also examined. The above FTIR and $^1$H NMR results confirm that the 1, 2, 3-triazole

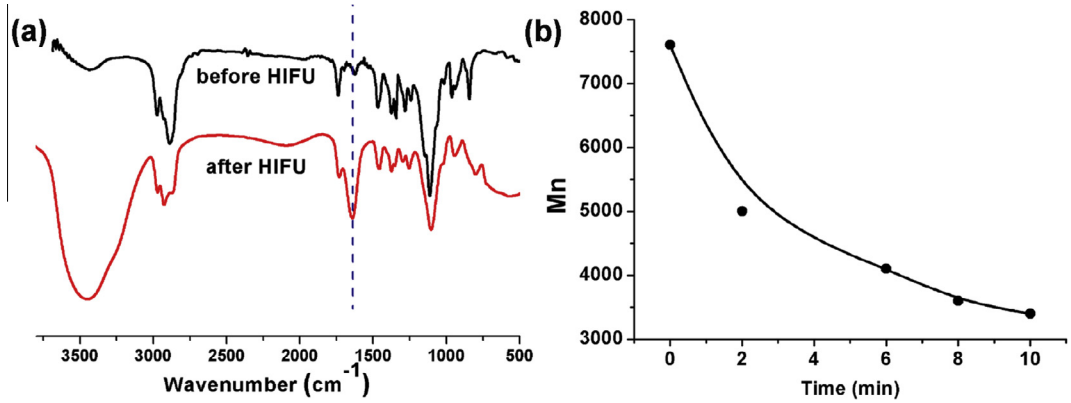



**Fig. 6.** (a) FTIR spectra of PEG-click-PPG micelle before and after HIFU treatment for 10 min. (b) The change in weight average molecular weight (*M*n) of copolymer PEG-click-PPG obtained after HIFU treatment at different time, determined by GPC (HIFU power output: 70 W).

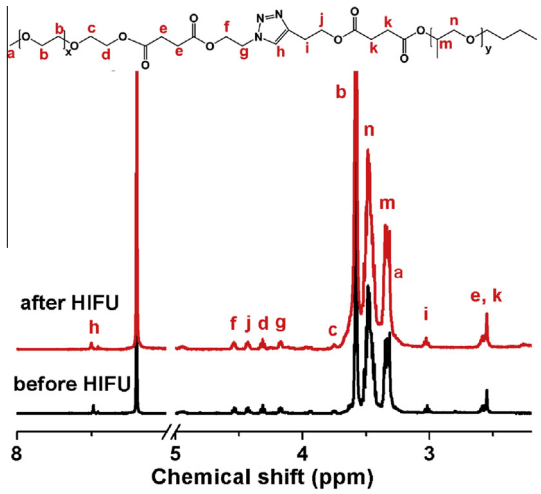



**Fig. 7.** $^1$H NMR spectra of PEG-click-PPG copolymer in the micelle before and after HIFU treatment (70 W, 10 min).

bonds are not broken by HIFU. The Cu(I)-catalyzed alkyne–azide cycloaddition process is thermodynamically favored ($\Delta H \sim -45$ to $-55$ kcal mol$^{-1}$), an essential feature for click reactions [37]. As a result, the triazole ring is stable toward normal thermal treatment and is inert in aqueous or biologically relevant environments [38,39]. The HIFU induced changes in the molecular structures for our copolymer as stated above confirm the preferential cleavage of ester bonds not the triazole ring. Previously we investigated the PEO-PPO-PEO micelle system without ester bonds under HIFU, and the results suggested that the PEO-PPO-PEO chains cannot be degraded by HIFU [11,13].

### 3.3. HIFU induced PEG-click-PPG micelle structure change

The HIFU-induced site-specific degradation for the copolymer chain provides a basis for the copolymer micelle disruption and payload release as shown in Scheme 1b. When the PEG-click-PPG copolymer micelle is subjected to HIFU, the micelle structure will be disrupted due to the mechanochemical cleavage of PEG-click-PPG copolymer chains in the micelle. This was confirmed by DLS and SEM.

The DLS data (Fig. 8a) show that the diameter of the blank PEG-click-PPG micelles is ~26 nm with a narrow size distribution. Fig. 8b shows that after HIFU treatment for 10 min at a power of 70 W, the original micelle structure nearly disappears, the particles with an average diameter of ~90 nm appears, which should be the aggregates of the disrupted PPG block resulted from HIFU-induced site-specific degradation. To further confirm the disruption of PEG-click-PPG micelles, the morphology of micelle before and after HIFU was observed by SEM. A large amount of spherical micelles with a mean diameter of ~35 nm can be observed before HIFU treatment. The size observed by SEM is a little bit larger than that measured by DLS, which is attributed to the slight collapse of micelles after drying, since the polymer is soft (Fig. 8c). After HIFU treatment, a large number of micelles are destroyed and the new irregular aggregated structure are formed (Fig. 8d). The SEM images are consistent with the DLS results.

A similar HIFU-induced structure change for PEG-click-PPG micelle containing the payload Nile Red was observed (Fig. 9). The DLS data show that the diameter of the PEG-click-PPG/Nile Red micelles is ~37 nm (Fig. 9a), which is a little bit larger than blank micelles. This may be caused by the encapsulated Nile Red into the core of PEG-click-PPG micelles. After HIFU treatment, the particles with an average particle size of ~190 nm appears (Fig. 9b), which should be the mixed aggregates of the degraded PPG block by HIFU together with the released Nile Red. The SEM images are consistent with the DLS results. The original PEG-click-PPG micelles containing Nile Red is also very spherical with a size of ~66 nm (Fig. 9c). The irregular aggregated particles formed by mixed aggregates of degraded PPG block and Nile Red after HIFU treatment can be observed by SEM (Fig. 9d).

### 3.4. HIFU-responsive release behavior of the PEG-click-PPG copolymer micelles containing Nile Red

To verify the HIFU responsive process shown in Scheme 1b, the HIFU stimulus responsive payload release from PEG-click-PPG

**Table 2**
$^1$H NMR peak area ratio of the hydrogen in ester bond to hydrogens in PEG and PPG chains for PEG-click-PPG copolymer in the micelle before and after HIFU treatment (70 W, 10 min).

| Sample | d/(b + m + n) | f/(b + m + n) | j/(b + m + n) | (e + k)/(b + m + n) |
|---|---|---|---|---|
| Before HIFU | 0.60% | 0.57% | 0.59% | 0.25% |
| After HIFU | 0.41% | 0.38% | 0.40% | 0.23% |

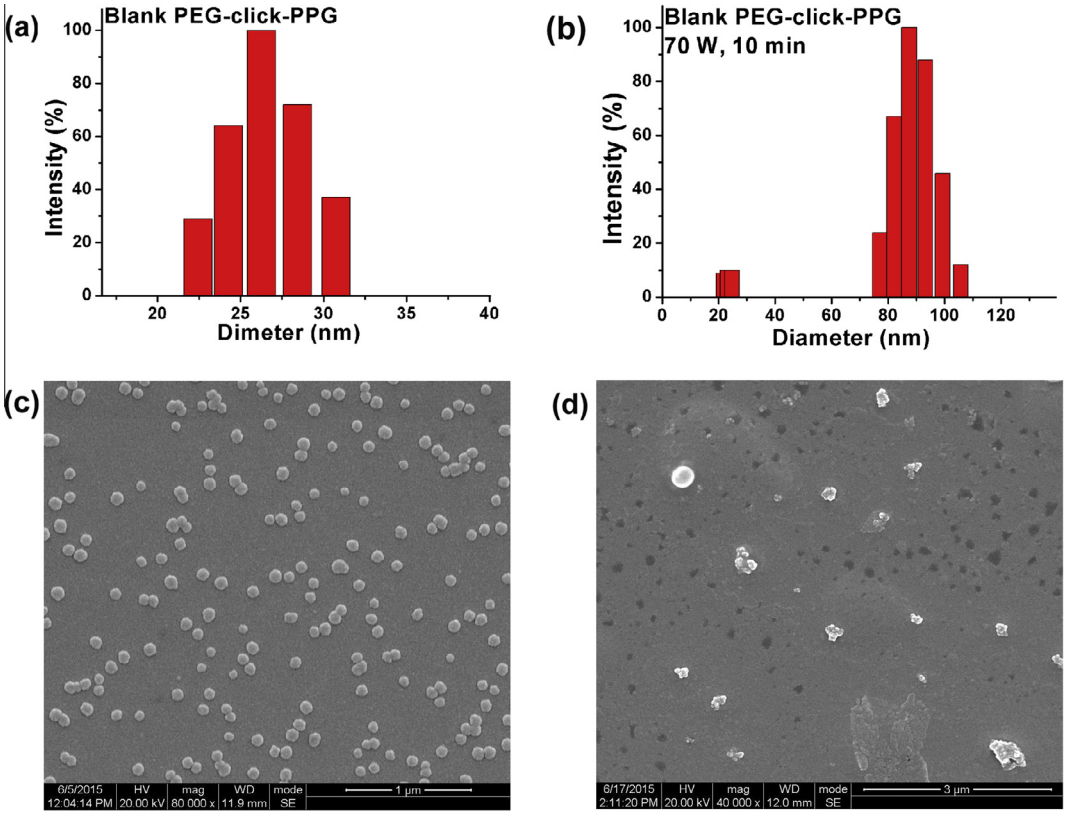



**Fig. 8.** (a, b) DLS curves and (c, d) SEM images of blank PEG-click-PPG micelles (a, c) before and (b, d) after HIFU treatment (70 W, 10 min).

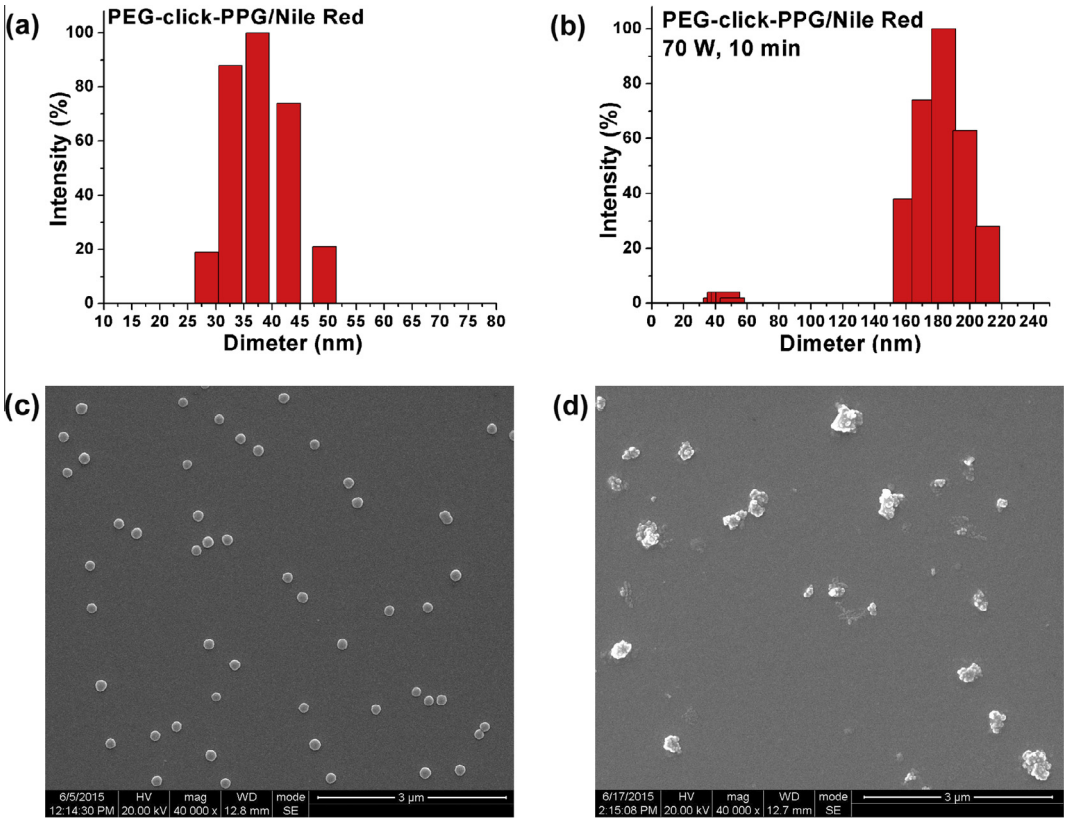



**Fig. 9.** (a, b) DLS curves and (c, d) SEM images of PEG-click-PPG/Nile Red micelles before (a, c) and after (b, d) HIFU treatment (70 W, 10 min).

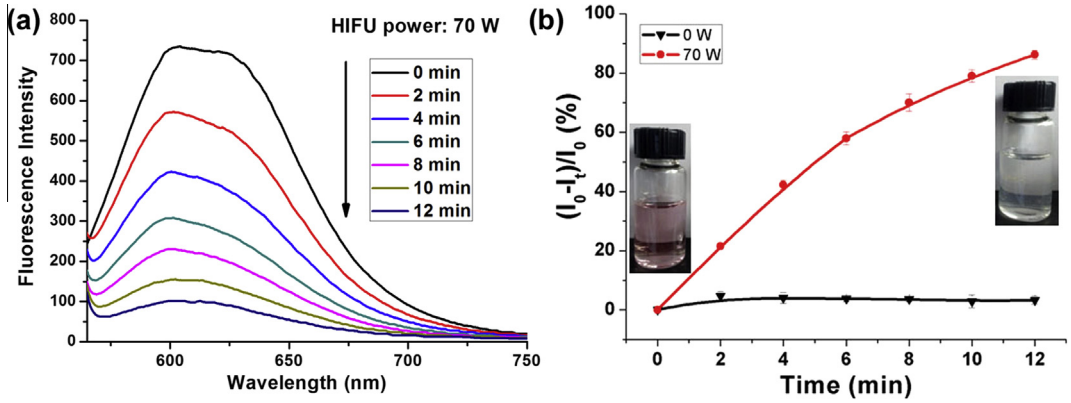



**Fig. 10.** (a) Variation of fluorescence emission spectra of PEG-click-PPG/Nile Red solutions ($\lambda$ex = 600 nm) with different time at a HIFU power output of 70 W. (b) Variation of $(I_0 - I_t)/I_0$ of PEG-click-PPG/Nile Red micelles under HIFU treatment (70 W).

micelle was investigated. Nile Red, as a model payload, is used for the investigation on the controlled release behavior, particularly for the dilute micelle solution [40–42]. The release of Nile Red can be monitored and revealed by the changes in their fluorescence emission spectra. The fluorescence emission spectra of the PEG-click-PEG/Nile Red micelle solution at different HIFU time were measured at 37 °C. The release percentages were evaluated using the following equation: % release = $(I_0 - I_t)/I_0$, where $I_0$ and $I_t$ is the fluorescence emission peak intensity at ~600 nm measured before and after HIFU treatment for $t$ min, at a power output of 70 W, respectively.

Fig. 10a shows that with increasing HIFU time, the fluorescence emission intensity decreases rapidly. The fluorescence peaks nearly disappear in 12 min under a HIFU power output of 70 W. Fig. 10b shows that under HIFU treatment at a power output of 70 W, the ~50% of Nile Red is released from PEG-click-PPG/Nile Red micelles at only 4 min, and the released percentage of Nile Red reaches about 90% at 12 min, while the fluorescence intensity of PEG-click-PPG/Nile Red micelles remains nearly no change without HIFU treatment. For our previously investigated HIFU responsive poly(ethylene glycol) methyl ether (mPEG) and poly (propylene glycol) monobutyl ether (PPG) block copolymer PEG-COO-PPG micelle containing two ester bonds in the central junction point, the ~20% of payload release occurs after HIFU treatment for 4 min at a power output of 70 W [17]. This suggests that PEG-click-PPG with four ester bonds in the central junction points introduced by click reaction has a more sensitive and rapid ultrasound responsiveness than PEG-COO-PPG, which is important for controlled drug release. The PEG-click-PPG micelle could achieve quicker release in a short time because of the more HIFU responsive ester bonds in the junction point of the PEG-click-PPG chains.

The digital pictures of PEG-b-PPG/Nile Red micelle solutions before and after HIFU treatment for 12 min were also shown in Fig. 10b. After HIFU treatment for 12 min, the pink color of micelle solution became colorless, which provides direct evidence that Nile Red encapsulated in the hydrophobic core of micelle was released into aqueous solution under HIFU treatment. These results suggest that HIFU could be a promising trigger for the cargo release from Pluronic type copolymer micelle containing the mechanophore.

## 4. Conclusions

In summary, a novel HIFU responsive amphiphilic copolymer was successfully synthesized by the "click" chemistry based on the widely used Pluronic type copolymer. The copolymer could self-assemble into micelles in aqueous solution, and HIFU stimulus was used to break the micelle by a mechanochemical way. The copolymer micelles could be rapidly disrupted in several min by HIFU in a remote way and present a faster release rate. The release behavior was attributed to a dynamic micelle disruption process resulted from the HIFU induced sites-specific cleavage of the multiple ester bonds in the junction points of PEG-click-PPG chain. It is confirmed that the mechanochemical cleavage occurs preferentially at the central ester bond rather than at the 1, 2, 3-triazole ring in the micelle aqueous solution, which can guide the design of novel HIFU responsive copolymer micelles as carriers for drug delivery. The click reaction provides a convenient way to construct ultrasound responsive copolymer micelles.

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## References

[1] J.R. Kumpfer, J. Jin, S.J. Rowan, Stimuli-responsive europium-containing metallo-supramolecular polymers, J. Mater. Chem. 20 (2010) 145–151.
[2] A. Klaikherd, C. Nagamani, S. Thayumanavan, Multi-stimuli sensitive amphiphilic block copolymer assemblies, J. Am. Chem. Soc. 131 (2009) 4830–4838.
[3] C.M. Schilli, M. Zhang, E. Rizzardo, S.H. Thang, Y.K. Chong, K. Edwards, G. Karlsson, A.H.E. Müller, A new double-responsive block copolymer synthesized via RAFT polymerization: poly(N-isopropylacrylamide)-block-poly(acrylic acid), Macromolecules 37 (2004) 7861–7866.
[4] Y. Bae, S. Fukushima, A. Harada, K. Karaoka, Design of environment-sensitive supramolecular assemblies for intracellular drug delivery: polymeric micelles that are responsive to intracellular pH change, Angew. Chem. Int. Ed. 42 (2003) 4640–4643.
[5] J.F. Gohy, Y. Zhao, Photo-responsive block copolymer micelles: design and behavior, Chem. Soc. Rev. 42 (2013) 7117–7129.
[6] J. Park, G. Maltzahn, E. Ruoslahti, S.N. Bhatia, M. Sailor, Micellar hybrid nanoparticles for simultaneous magnetofluorescent imaging and drug delivery, Angew. Chem. Int. Ed. 47 (2008) 7284–7288.
[7] W.G. Pitt, G.A. Husseini, B.J. Staples, Ultrasonic drug delivery – a general review, Expert Opin. Drug Deliv. 1 (2004) 37–56.
[8] N.S.M. Yusofa, M. Ashokkumar, Ultrasonic transformation of micelle structures: effect of frequency and power, Ultrason. Sonochem. 24 (2015) 8–12.
[9] H.J. Kim, H. Matsuda, H.S. Zhou, I. Honma, Ultrasound-triggered smart drug release from a poly(dimethylsiloxane)-mesoporous silica composite, Adv. Mater. 18 (2006) 3083–3088.
[10] H. Hasanzadeha, M. Mokhtari-Dizajia, S.Z. Bathaieb, Z.M. Hassan, Effect of local dual frequency sonication on drug distribution from polymeric nanomicelles, Ultrason. Sonochem. 18 (2011) 1165–1171.
[11] N. Rapoport, W.G. Pitt, H. Sun, J.L. Nelson, Drug delivery in polymeric micelles: from in vitro to in vivo, J. Control. Release 91 (2003) 85–95.
[12] G.A. Husseini, W.G. Pitt, Micelles and nanoparticles for ultrasonic drug and gene delivery, Adv. Drug Deliv. Rev. 60 (2008) 1137–1152.
[13] H.J. Zhang, H.S. Xia, J. Wang, Y.W. Li, High intensity focused ultrasound-responsive release behavior of PLA-b-PEG copolymer micelles, J. Control. Release 139 (2009) 3–391.

[14] R. Tong, H.S. Xia, X.L. Lu, Fast release behavior of block copolymer micelles under high intensity focused ultrasound/redox combined stimulus, J. Mater. Chem. B 1 (2013) 886–894.
[15] J. Wang, M. Pelletier, H.J. Zhang, H.S. Xia, Y. Zhao, High-frequency ultrasound-responsive block copolymer micelle, Langmuir 25 (2009) 13201–13205.
[16] Y.W. Li, R. Tong, H.S. Xia, H.J. Zhang, J. Xuan, High intensity focused ultrasound and redox dual responsive polymer micelles, Chem. Commun. 46 (2010) 7739–7741.
[17] R. Tong, X.L. Lu, H.S. Xia, A facile mechanophore functionalization of an amphiphilic block copolymer towards remote ultrasound and redox dual stimulus responsiveness, Chem. Commun. 50 (2014) 3575–3578.
[18] C.R. Hickenboth, J.S. Moore, S.R. White, N.R. Sottos, J. Baudry, S.R. Wilson, Biasing reaction pathways with mechanical force, Nature 446 (2007) 423–427.
[19] M.M. Caruso, D.A. Davis, Q. Shen, S.A. Odom, N.R. Sottos, S.R. White, J.S. Moore, Mechanically-induced chemical changes in polymeric materials, Chem. Rev. 109 (2009) 5755–5798.
[20] Z.S. Kean, S.L. Craig, Mechanochemical remodeling of synthetic polymers, Polymer 53 (2012) 1035–1048.
[21] Z.S. Kean, A.L.B. Ramirez, S.L. Craig, High mechanophore content polyester-acrylate ABA block copolymers: synthesis and sonochemical remodelling, J. Polym. Sci., Part A: Polym. Chem. 50 (2012) 3481–3484.
[22] J.M.J. Aulusse, R.P. Sijbesma, Selectivity of mechanochemical chain scission in mixed palladium(II) and platinum(II) coordination polymers, Chem. Commun. 37 (2008) 4416–4418.
[23] S. Karthikeyan, S.L. Potisek, A. Piermattei, R.P. Sijbesma, Highly efficient mechanochemical scission of silver-carbene coordination polymers, J. Am. Chem. Soc. 130 (2008) 14968–14969.
[24] D.A. Davis, A. Hamilton, J.L. Yang, L.D. Cremar, D. Van Gough, S.L. Potisek, M.T. Ong, P.V. Braun, T.J. Martínez, S.R. White, J.S. Moore, N.R. Sottos, Force-induced activation of covalent bonds in mechanoresponsive polymeric materials, Nature 459 (2009) 68–72.
[25] S.L. Potisek, D.A. Davis, N.R. Sottos, S.R. White, J.S. Moore, Mechanophore-linked addition polymers, J. Am. Chem. Soc. 129 (2007) 13808–13809.
[26] M.K. Beyer, H. Clausen-Schaumann, Mechanochemistry: the mechanical activation of covalent bonds, Chem. Rev. 105 (2005) 2921–2948.
[27] J.N. Brantley, K.M. Wiggins, C.W. Bielawski, Polymer mechanochemistry: the design and study of mechanophores, Polym. Int. 62 (2013) 2–12.
[28] J.E. Hein, V.V. Fokin, Copper-catalyzed azide–alkyne cycloaddition (CuAAC) and beyond: new reactivity of copper (I) acetylides, Chem. Soc. Rev. 39 (2010) 1302–1315.
[29] H. Nulwala, K. Takizawa, A. Odukale, A. Khan, R.J. Thibault, B.R. Taft, B.H. Lipshutz, C.J. Hawker, Synthesis and characterization of isomeric vinyl-1,2,3-triazole materials by azide−alkyne click chemistry, Macromolecules 42 (2009) 6068–6074.
[30] P. Gou, W. Zhu, N. Xu, Z. Shen, Synthesis, self-assembly and drug-loading capacity of well-defined drug-conjugated amphiphilic $A_2B_2$ type miktoarm star copolymers based on poly(ε-caprolactone) and poly(ethylene glycol), J. Polym. Sci., Part A: Polym. Chem. 47 (2009). 6962-6076.
[31] K. Zhang, Y. Wang, W. Zhu, X. Li, Z. Shen, Synthesis, characterization, and micellization of PCL-g-PEG copolymers by combination of ROP and "click" chemistry via "graft onto" method, J. Polym. Sci., Part A: Polym. Chem. 50 (2012) 2045–2052.
[32] M. McNutt, Editorial retraction, Science 344 (2014) 834.
[33] H.R. Pfaendler, V. Weimar, Synthesis of racemic ethanolamine plasmalogen, Synthesis 11 (1996) 1345–1349.
[34] H.S. Xia, Q. Wang, Y.Q. Liao, X. Xu, S.M. Baxter, R.V. Slone, S.G. Wu, G. Swift, D. G. Westmoreland, Polymerization rate and mechanism of ultrasonically initiated emulsion polymerization of *n*-butyl acrylate, Ultrason. Sonochem. 9 (2002) 151–158.
[35] S. Akbulatov, Y. Tian, E. Kapustin, R. Boulatov, Model studies of the kinetics of ester hydrolysis under stretching force, Angew. Chem. Int. Ed. 52 (2013) 6992–6995.
[36] T.J. Kucharski, Z. Huang, Q. Yang, Y. Tian, N.C. Rubin, C.D. Concepcion, R. Boulatov, Kinetics of thiol/disulfide exchange correlate weakly with the restoring force in the disulfide moiety, Angew. Chem. Int. Ed. 48 (2009) 7040–7043.
[37] Q. Wang, S. Chittaboina, H. Barnhill, Advances in 1,3-dipolar cycloaddition reaction of azides and alkynes – a prototype of click chemistry, Lett. Org. Chem. 2 (2005) 293–301.
[38] Y. Lin, Q. Wang, Unlocking the 1,2,3-triazole ring using mechanical force, Angew. Chem. Int. Ed. 51 (2012) 2006–2007.
[39] D. Schütze, K. Holz, J. Müller, M.K. Beyer, U. Lüning, B. Hartke, Pinpointing mechanochemical bond rupture by embedding the mechanophore into a macrocycle, Angew. Chem. Int. Ed. 54 (2015) 2556–2559.
[40] E.R. Gillies, T.B. Jonsson, J.M.J. Frechet, Stimuli-responsive supramolecular assemblies of linear-dendritic copolymers, J. Am. Chem. Soc. 126 (2004) 11936–11943.
[41] J.Q. Jiang, X. Tong, D. Morris, Y. Zhao, Toward photocontrolled release using light-dissociable block copolymer micelles, Macromolecules 39 (2006) 4633–4640.
[42] A. Klaikherd, S. Ghosh, S. Thayumanavan, A facile method for the synthesis of cleavable block copolymers from ATRP-based homopolymers, Macromolecules 40 (2007) 8518–8520.