

Electronic Supplementary Information

High Intensity Focused Ultrasound and Redox Dual Responsive Polymer Micelles

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1. Experiment Section

Materials

3,3'-dithiodipropionic acid (DTPA), Thiodiglycolic acid (TDA), dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), Dichloromethane (DCM), L-Glutathione reduced (GSH) and pyrene were purchased from Sigma-Aldrich Chemical Company and used as received. Polyethylene glycol (Mn=2000) were obtained from Tianjin Chemical Reagents Institute.

Synthesis of DTPA-PEG-DTPA

As shown in Scheme 2a, 1 mmol of PEG and 5 mmol of DTPA were added into 10 ml of tetrahydrofuran (THF). The reaction mixture was stirred to obtain a clear solution. To this, 4.0 mmol of DCC and 30 mg of DMAP in 5 ml of THF were added. After stirring 5 minutes, the white precipitate started to appear. The reaction mixture was stirred at room temperature for 2 days and then filtered to remove the precipitated dicyclohexylurea (DCU). The clear solution was concentrated by vacuum distilling. Then 100 ml of DCM was added and allowed to stand for 1 hour to precipitate the remaining traces of DCU. 250 ml of cold diethyl ether was added into the solution to precipitate the DTPA-PEG-DTPA. The obtained products were dried in vacuum for 40 hours at 40 °C. DTPA-PEG-DTPA (1.9 g, 77%). The TDA-PEG-TDA was obtained by the same procedure by using TDA instead of DTPA.

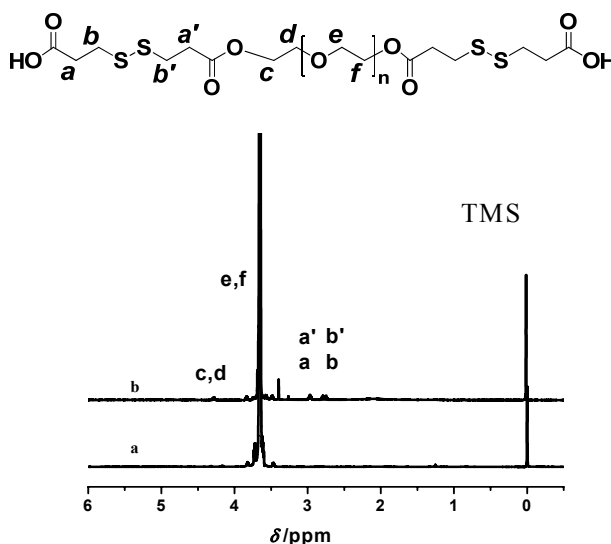
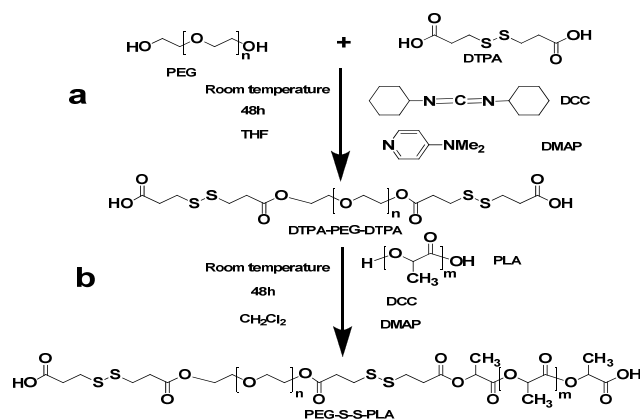


Fig. 1S ^1H NMR spectra of PEG (a) and DTPA-PEG-DTPA (b) in CDCl_3 .

Synthesis of PEG-S-S-PLA copolymer

DCM was dried over CaH_2 and distilled. The reaction of DTPA-PEG-DTPA and PLA was carried out using the above method. DCC (0.5 g) as the dehydrolyzing agent, DMAP (0.02 g) as the catalyst and DTPA-PEG-DTPA (1.25 g) were dissolved in 20 ml DCM and the mixture was stirred at room temperature for 5 minutes. To this, the poly (L-lactic acid) (0.5 g) in 10 ml DCM were added (The PLA was synthesized according to our previous paper^[30]). The solution was added into the flask and stirred for another 48 hours. After that, the reaction mixture was filtered to remove the DCU. Then, 150 ml of cold diethyl ether was added into the concentrated solution to precipitate the PEG-S-S-PLA. At last, the product was dissolved into water and centrifuged (10000 rpm $\times$ 10 min) to remove the excess DTPA-PEG-DTPA. The precipitate was dried in vacuum for 40 hours at room temperature. The white wax solid was obtained. The synthesis process and structure of the copolymer are shown in Scheme 2b. PEG-S-S-PLA (0.632 g, 84%). The PEG-S-PLA copolymer was obtained by the same procedure by using TDA-PEG-TDA instead of DTPA-PEG-DTPA.

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Scheme 1S Synthetic route to diblock copolymer of the PEG-S-S-PLA.

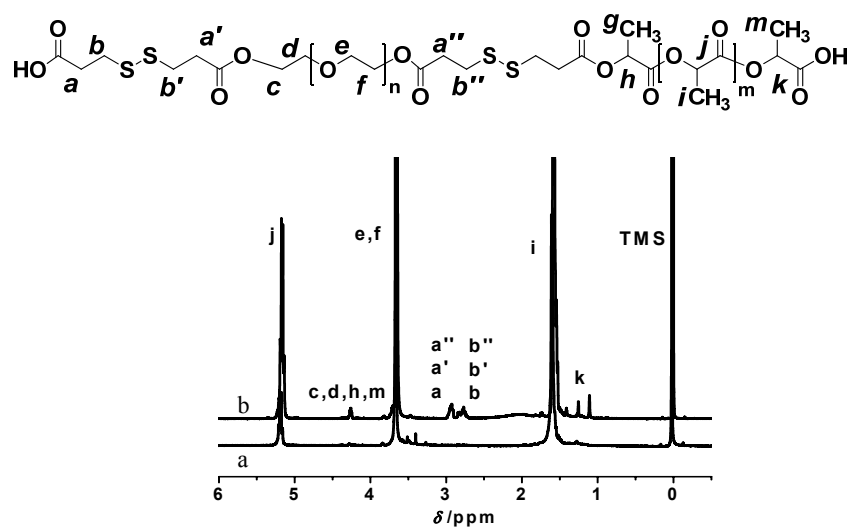


Fig. 2S ^1H NMR spectra of PEG-b-PLA (a) and PEG-S-S-PLA (b) in CDCl_3 .

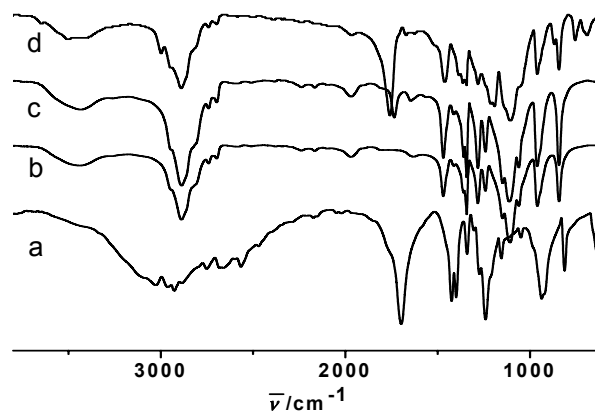


Fig. 3S FTIR spectra of DTPA (a), PEG (b), DTPA-PEG-DTPA (c) and PEG-S-S-PLA (d).

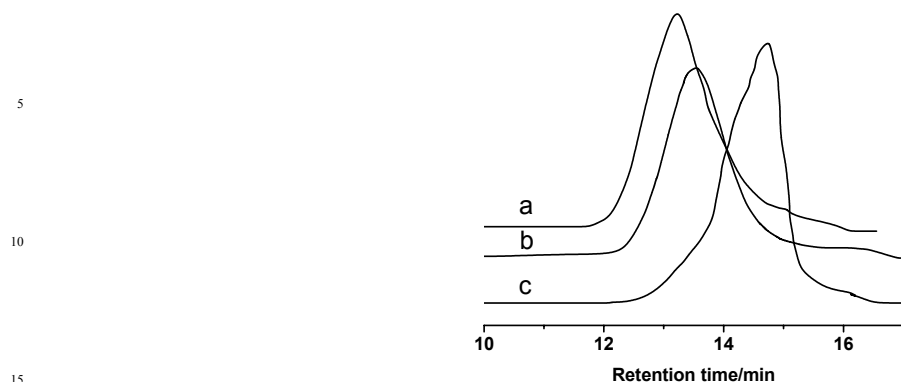


Fig. 4S GPC traces of PEG-S-S-PLA (a), PLA (b) and DTPA-PEG-DTPA (c).

Table 1 The molecular weight and the molecular weight distribution (D).

Polymer	Mn	Mw	D
DTPA-PEG-DTPA	2600	3700	1.41
PLA	5100	7000	1.37
PEG-S-S-PLA	8000	11000	1.39

Table 2 The element analysis result of PEG-S-S-PLA copolymer.

Element	C	H	S
Practical content (%)	46.45	6.20	1.620
Theoretic content (%)	50.03	6.44	1.732

Polymer micelle preparation

Typically, PEG-S-S-PLA (50 mg) was allowed to evaporate by heating to 45 °C for 24 h. The solution was then removed by filtration through 0.45 μm membrane. The initial mg) was introduced to THF solution (10 mL), and then water (40 mL) was added to induce the formation of micelles, while THF w polymer concentration was 0.5 mg/mL. To load fluorescent pyrene, which is a model hydrophobic compound, the same procedure was used except that the THF solution contained both polymer and pyrene. Upon addition of water, the aggregation of the hydrophobic block allowed some pyrene molecules to be solubilized by polymer chains forming the core of micelle.

HIFU irradiation of polymer micelles

HIFU apparatus is shown in the supporting information (Fig. 5S). Typically, 8 mL pyrene/PEG-S-S-PLA micelle solution was placed into cuvette reactor, which was sealed by latex membrane and immersed in a water tank. The focused beams of ultrasound penetrate through latex membrane and act on the PEG-S-S-PLA micelle solution. In all HIFU irradiation experiments, unless otherwise stated, the focal spot of the beams was set at the center of the micelle solution. The HIFU power output was set at 0-150 W. After a certain time of HIFU irradiation, the cuvette reactor was removed from the water tank and the micelle solution was used for characterizations at room temperature.

Glutathione treatment of polymer micelles

Glutathione (10 mg) were added into the pyrene/PEG-S-S-PLA micelle solution (10 mL) and stirred for few seconds and then kept in an oven at 37 °C for a certain time. The micelle solution at different time was used for characterizations at room temperature.

5 Apparatus

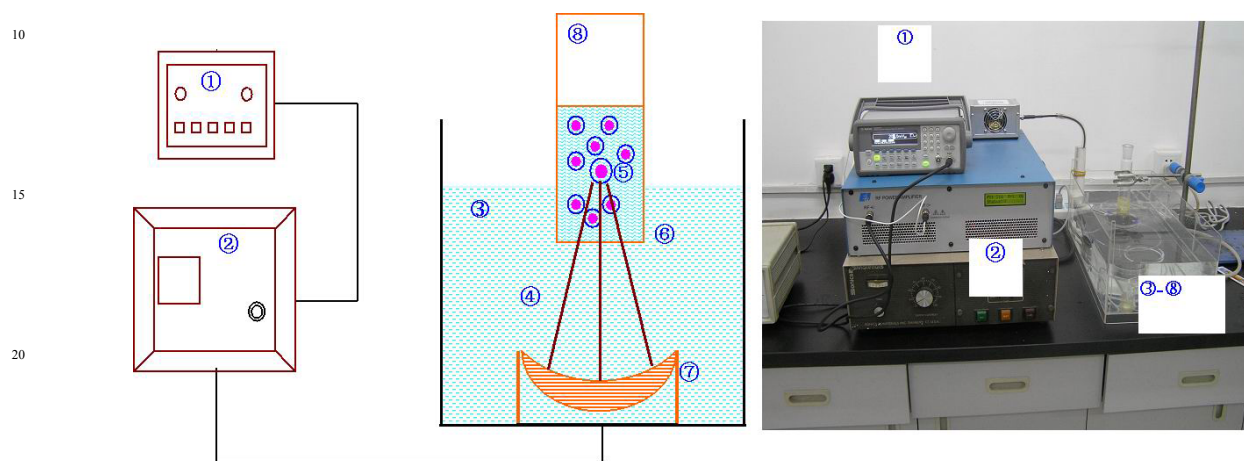


Fig. 5S The schematic illustration for experimental setup (left) and high intensity focused ultrasound apparatus (right). 1: Arbitrary waveform generator, 2: RF power amplifier, 3: Water bath, 4: Ultrasonic beam, 5: Micelles, 6: Latex membrane, 7: Acoustic lens transducer, 8: Cuvette reactor.

Characterizations

FTIR analysis of the samples was performed on a Nicolet 560 Fourier transform infrared (FTIR) spectrometer. Proton nuclear magnetic resonance ^1H NMR spectra were recorded at room temperature with a Bruker spectrometer operating at 400 MHz by using CDCl_3 as solvent and tetramethylsilane as an internal reference. The molecular weight was measured with gel permeation chromatography (GPC, Agilent 1100 Series) with tetrahydrofuran (THF) as the eluent at a flow rate of 1 ml min^{-1} at 35°C . The molecular weight was calibrated with polystyrene standard. The content of each element was measured by elemental analyzer (EA; Euro EA 3000 Series). Dynamic light scattering (DLS) was performed on a Brookhaven BI-200 goniometer with vertically polarized incident light of wavelength $\lambda = 532 \text{ nm}$ 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 an 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. The micellar morphology was observed with scanning electron microscopy (SEM, Inspect F, Philips). The specimens for SEM observations were prepared by depositing several drops of micellar solutions onto silicon wafers and were dried in a vacuum at room temperature. Steady-state fluorescence emission spectra of pyrene/PEG-S-S-PLA micelle solution were recorded on an 970CRT spectrophotometer (Shanghai Precision & Scientific Instrument Co. Ltd). The excitation wavelength was 337 nm. The transmittance of pyrene/PEG-S-S-PLA solution in the presence of GSH was monitored by UV-vis spectrum (UV-vis3010, Hitachi, Japan).

2. Pyrene/PEG-S-S-PLA micelle stability

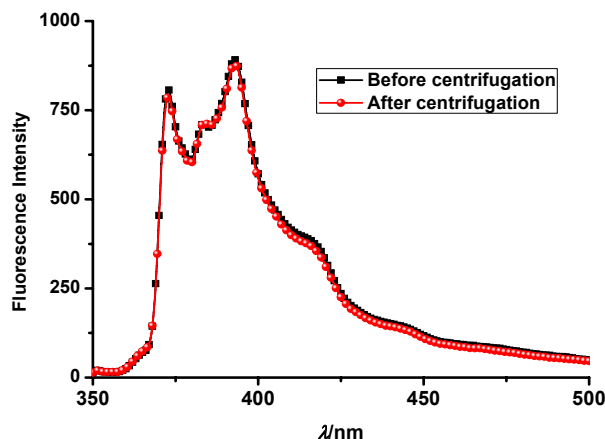


Fig. 6S The fluorescence emission spectra of pyrene/PEG-S-S-PLA micelle solution before and after centrifugation for 10 min at 10000 rpm. No decrease in the fluorescence emission intensity was observed, suggesting that the pyrene/PEG-S-S-PLA micelle is stable.

3. HIFU induced pyrene/PEG-S-S-PLA micelle disruption

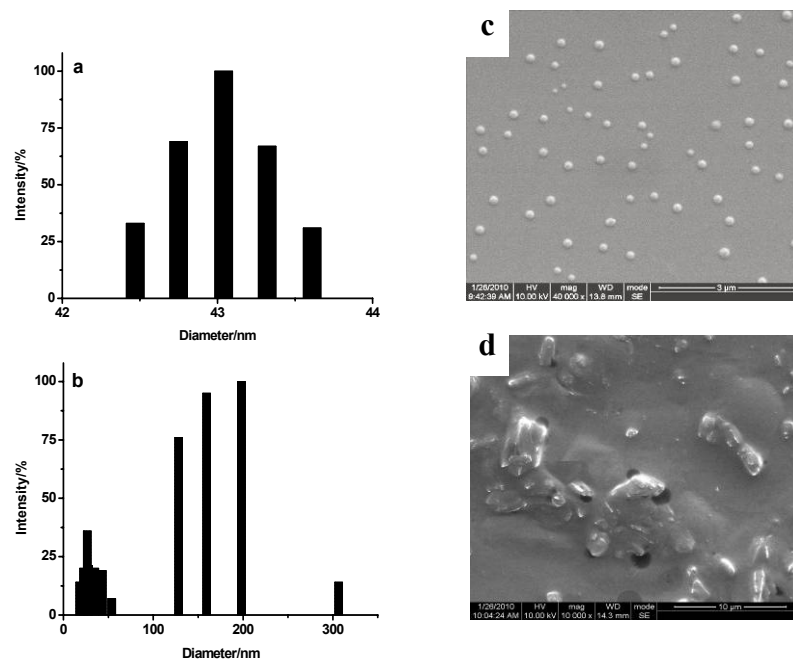
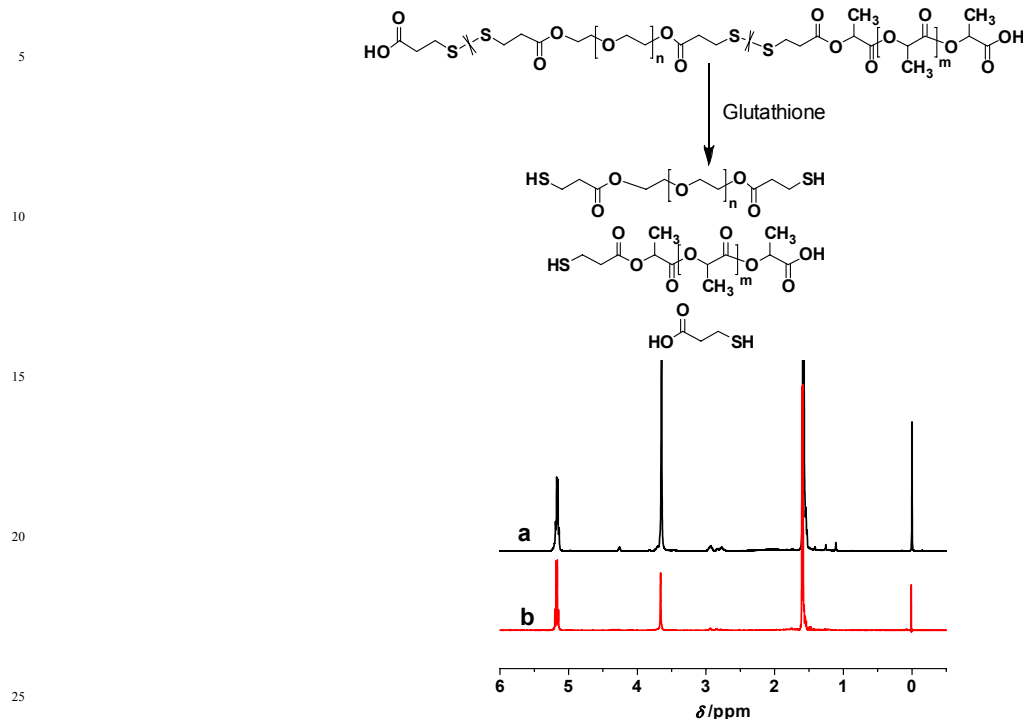


Fig. 7S (1) The change in the size distribution of pyrene/PEG-S-S-PLA micelles in solution before (a) and after (b) HIFU irradiation for 10 min, determined by DLS; (2) SEM images of pyrene/PEG-S-S-PLA micelles before (c) and after (d) HIFU irradiation for 10 min

4. More evidences for redox-responsive behavior of polymer micelles



Scheme 2S The schematic illustration for the decomposition of PEG-S-S-PLA in the presence of GSH and ¹H NMR spectra of solid samples recovered from the PEG-S-S-PLA micelle solution in the presence of glutathione: (a) 0 min; (b) 480 min. The ratio of PEG protons to methyldyne protons in PLA decreased significantly. The lower ratio of peak area is indicative of the decreasing amount of PEG block in the sample. The decreasing of a peak at 2.95 and 2.77 ppm confirmed that the glutathione induced the cleavage of disulfide.

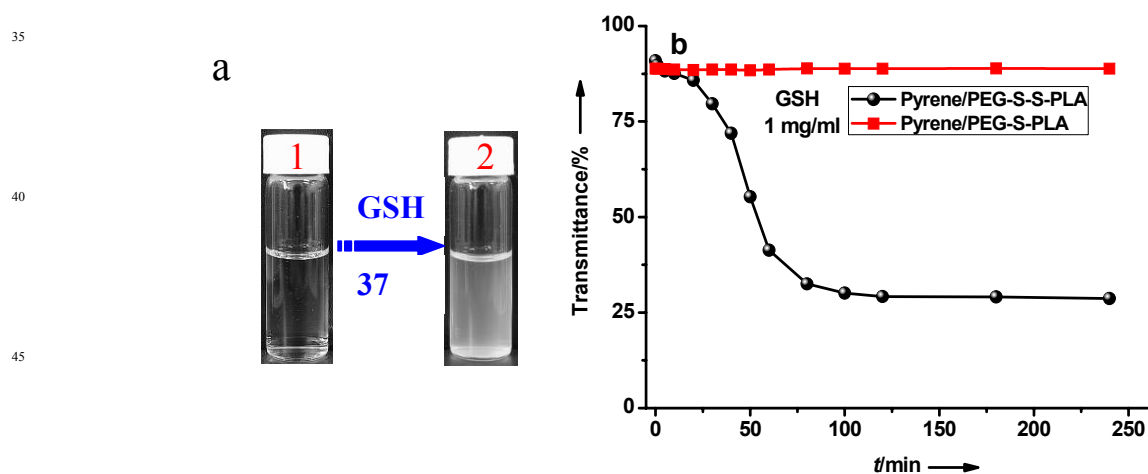


Fig. 8S (a) Color change of the pyrene/PEG-S-S-PLA micelle solution: before (1) and after (2) treating with GSH for 240 minutes; (b) The UV-Vis transmittance of PEG-S-S-PLA and PEG-S-PLA micelle solution at 500 nm with time in the presence of GSH. Compared to a significant reduction for the transmittance of PEG-S-S-PLA micelle solution, there is no change for that of PEG-S-PLA without disulfide bond.

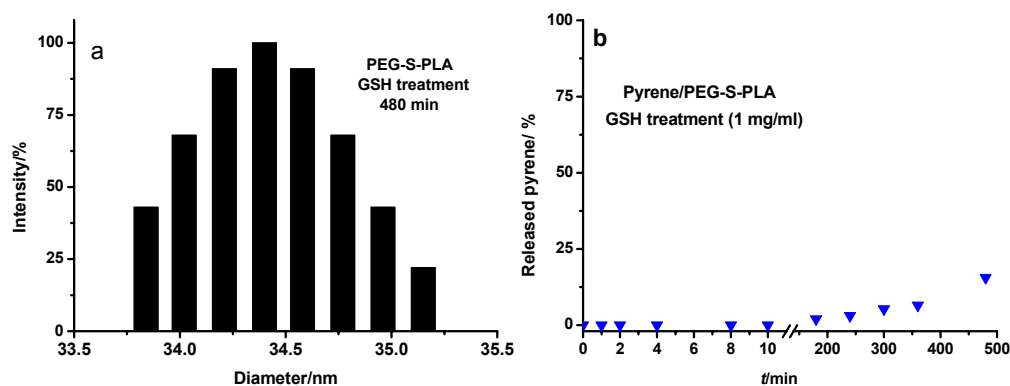


Fig. 9S (a) Particle size distribution of PEG-S-PLA after 480 min in the presence of GSH. The control experiment suggests that there is no effect of GSH on the morphology of PEG-S-PLA micelle without the disulfide bond; (b) The released percentage of pyrene from PEG-S-PLA micelle with time in the presence of GSH. Compared to that for PEG-S-S-PLA micelle, a much lower released percentage of pyrene for PEG-S-PLA micelle under GSH treatment can be noted.

5. A control experiment: the release of pyrene from pyrene/PEG-S-PLA micelle

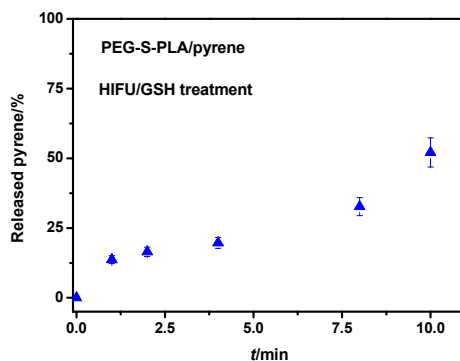


Fig. 10S The calculated released percentage of the pyrene from pyrene/PEG-S-PLA micelle solutions with the time ($\lambda_{ex}=337$ nm). The data shows a slower release rate for pyrene/PEG-S-PLA micelle compared to that of pyrene/PEG-S-S-PLA, suggesting the PEG-S-S-PLA has a quick response to HIFU because the labile disulfide bond is more easily to be broken by ultrasonic cavitation.

6. A control experiment: the examination of pyrene degradation

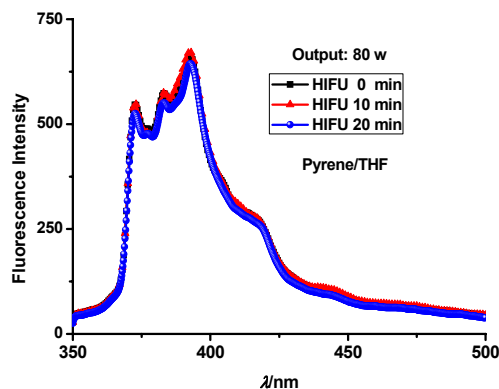


Fig. 11S The fluorescence emission spectra of pyrene/THF solutions ($\lambda_{\text{ex}}=337$ nm) before and after HIFU. This control experiment shows that pyrene can not be degraded by HIFU, suggesting that the decrease in the fluorescence emission intensity is not attributed to the degradation of pyrene, but the release of pyrene from the copolymer micelle.

7. Change in the fluorescence emission of PEG-S-S-PLA micelle solutions with time

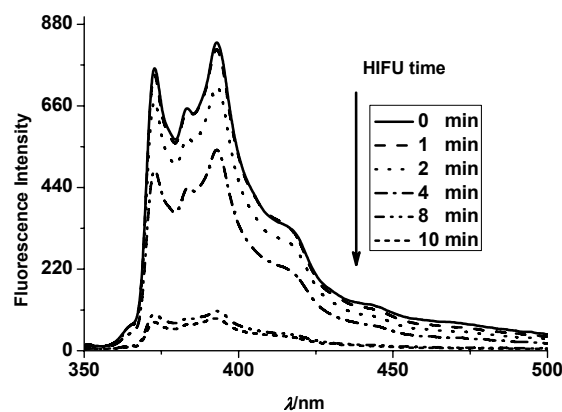


Fig. 12S Variation of fluorescence emission spectra of pyrene/PEG-S-S-PLA micelle solutions ($\lambda_{\text{ex}}=337$ nm) with time at a power output of 80 W.